Part 4000 INORGANIC NONMETALLIC CONSTITUENTS

4010 INTRODUCTION

The analytical methods included in this part make use of classical wet chemical techniques and their automated variations and such modern instrumental techniques as ion chromatography. Methods that measure various forms of chlorine, nitrogen, and phosphorus are presented. The procedures are intended for use in the assessment and control of receiving water quality, the treatment and supply of potable water, and the measurement of operation and process efficiency in wastewater treatment. The methods also are appropriate and applicable in evaluation of environmental water-quality concerns. The introduction to each procedure contains reference to special field sampling conditions, appropriate sample containers, proper procedures for sampling and storage, and the applicability of the method.

4020 QUALITY ASSURANCE/QUALITY CONTROL

4020 A. Introduction

Without quality control results there is no confidence in analytical results reported from tests. As described in Part 1000 and Section 3020, essential quality control measurements include: method calibration, standardization of reagents, assessment of individual capability to perform the analysis, performance of blind check samples, determination of the sensitivity of the test procedure (method detection level), and daily evaluation of bias, precision, and the presence of laboratory contamination or other analytical interference. Details of these procedures, expected ranges of results, and frequency of performance should be formalized in a written Quality Assurance Manual and Standard Operating Procedures.

For a number of the procedures contained in Part 4000, the traditional determination of bias using a known addition to either a sample or a blank, is not possible. Examples of these procedures include pH, dissolved oxygen, residual chlorine, and carbon dioxide. The inability to perform a reliable known addition does not relieve the analyst of the responsibility for evaluating test bias. Analysts are encouraged to purchase certified ready-made solutions of known levels of these constituents as a means of measuring bias. In any situation, evaluate precision through analysis of sample duplicates.

Participate in a regular program (at a minimum, annually, and preferably semi-annually) of proficiency testing (PT)/performance evaluation (PE) studies. The information and analytical confidence gained in the routine performance of the studies more than offset any costs associated

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with these studies. An unacceptable result on a PT study sample is often the first indication that a test protocol is not being followed successfully. Investigate circumstances fully to find the cause. Within many jurisdictions, participation in PT studies is a required part of laboratory certification.

Many of the methods contained in Part 4000 include specific quality-control procedures. These are considered to be the minimum quality controls necessary to successful performance of the method. Additional quality control procedures can and should be used. Section 4020B describes a number of QC procedures that are applicable to many of the methods.

4020 B. Quality Control Practices

1. Initial Quality Control

See Section 3020B.1.

2. Calibration

See Section 3020B.2. Most methods for inorganic nonmetals do not have wide dynamic ranges. Standards for initial calibration therefore should be spaced more closely than one order of magnitude under these circumstances. Verify calibration by analyzing a midpoint or lower calibration standard and blank as directed. Alternatively, verify calibration with two standards, one near the low end and one near the high end, if the blank is used to zero the instrument.

3. Batch Quality Control

See Section 3020B.3*a* through *d*.

4110 DETERMINATION OF ANIONS BY ION CHROMATOGRAPHY*#(1)

4110 A. Introduction

Because of rapid changes in technology, this section is currently undergoing substantial revision.

Determination of the common anions such as bromide, chloride, fluoride, nitrate, nitrite, phosphate, and sulfate often is desirable to characterize a water and/or to assess the need for specific treatment. Although conventional colorimetric, electrometric, or titrimetric methods are available for determining individual anions, only ion chromatography provides a single instrumental technique that may be used for their rapid, sequential measurement. Ion chromatography eliminates the need to use hazardous reagents and it effectively distinguishes among the halides (Br^-, Cl^-, and F^-) and the oxy-ions (SO_3^{2-}, SO_4^{2-} or NO_2^-, NO_3^-).

This method is applicable, after filtration to remove particles larger than 0.2 µm, to surface,

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ground, and wastewaters as well as drinking water. Some industrial process waters, such as boiler water and cooling water, also may be analyzed by this method.

4110 B. Ion Chromatography with Chemical Suppression of Eluent Conductivity

1. General Discussion

a. Principle: A water sample is injected into a stream of carbonate-bicarbonate eluent and passed through a series of ion exchangers. The anions of interest are separated on the basis of their relative affinities for a low capacity, strongly basic anion exchanger (guard and separator columns). The separated anions are directed through a hollow fiber cation exchanger membrane (fiber suppressor) or micromembrane suppressor bathed in continuously flowing strongly acid solution (regenerant solution). In the suppressor the separated anions are converted to their highly conductive acid forms and the carbonate-bicarbonate eluent is converted to weakly conductive carbonic acid. The separated anions in their acid forms are measured by conductivity. They are identified on the basis of retention time as compared to standards. Quantitation is by measurement of peak area or peak height.

b. Interferences: Any substance that has a retention time coinciding with that of any anion to be determined and produces a detector response will interfere. For example, relatively high concentrations of low-molecular-weight organic acids interfere with the determination of chloride and fluoride by isocratic analyses. A high concentration of any one ion also interferes with the resolution, and sometimes retention, of others. Sample dilution or gradient elution overcomes many interferences. To resolve uncertainties of identification or quantitation use the method of known additions. Spurious peaks may result from contaminants in reagent water, glassware, or sample processing apparatus. Because small sample volumes are used, scrupulously avoid contamination. Modifications such as preconcentration of samples, gradient elution, or reinjection of portions of the eluted sample may alleviate some interferences but require individual validation for precision and bias.

c. Minimum detectable concentration: The minimum detectable concentration of an anion is a function of sample size and conductivity scale used. Generally, minimum detectable concentrations are near 0.1 mg/L for Br^-, Cl^-, NO_3^-, NO_2^-, PO_4^{3-}, and SO_4^{2-} with a 100-µL sample loop and a 10-µS/cm full-scale setting on the conductivity detector. Lower values may be achieved by using a higher scale setting, an electronic integrator, or a larger sample size.

d. Limitations: This method is not recommended for the determination of F^- in unknown matrices. Equivalency studies have indicated positive or negative bias and poor precision in some samples. Recent interlaboratory studies show acceptable results. Two effects are common: first, F^- is difficult to quantitate at low concentrations because of the major negative contribution of the ‘‘water dip’’ (corresponding to the elution of water); second, the simple organic acids (formic, carbonic, etc.) elute close to fluoride and will interfere. Determine precision and bias

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before analyzing samples. F^- can be determined accurately by ion chromatography using special techniques such as dilute eluent or gradient elution using an NaOH eluent or alternative columns.

2. Apparatus

a. Ion chromatograph, including an injection valve, a sample loop, guard column, separator column, and fiber or membrane suppressors, a temperature-compensated small-volume conductivity cell and detector (6 µL or less), and a strip-chart recorder capable of full-scale response of 2 s or less. An electronic peak integrator is optional. Use an ion chromatograph capable of delivering 2 to 5 mL eluent/min at a pressure of 1400 to 6900 kPa.

b. Anion separator column, with styrene divinylbenzene-based low-capacity pellicular anion-exchange resin capable of resolving Br^-, Cl^-, NO_3^-, NO_2^-, PO_4^{3-}, and SO_4^{2-}.*#(2)

c. Guard column, identical to separator column†#(3) to protect separator column from fouling by particulates or organics.

d. Fiber suppressor or membrane suppressor:‡#(4) Cation-exchange membrane capable of continuously converting eluent and separated anions to their acid forms. Alternatively, use continuously regenerated suppression systems.

3. Reagents

a. Deionized or distilled water free from interferences at the minimum detection limit of each constituent, filtered through a 0.2-µm membrane filter to avoid plugging columns, and having a conductance of < 0.1 µS/cm.

b. Eluent solution, sodium bicarbonate-sodium carbonate, 0.0017*M* $NaHCO_3$-0.0018*M* Na_2CO_3: Dissolve 0.5712 g $NaHCO_3$ and 0.7632 g Na_2CO_3 in water and dilute to 4 L.

c. Regenerant solution, H_2SO_4, 0.025*N*: Dilute 2.8 mL conc H_2SO_4 to 4 L.

d. Standard anion solutions, 1000 mg/L: Prepare a series of standard anion solutions by weighing the indicated amount of salt, dried to a constant weight at 105°C, to 1000 mL. Store in plastic bottles in a refrigerator; these solutions are stable for at least 1 month. Verify stability.

Anion§	**Salt**	**Amount *g/L***
Cl^-	NaCl	1.6485
Br^-	NaBr	1.2876
NO_3^-	$NaNO_3$	1.3707 (226 mg NO_3^--N/L)
NO_2^-	$NaNO_2$	1.4998‖ (304 mg NO_2^--N/L)
PO_4^{3-}	KH_2PO_4	1.4330 (326 mg PO_4^{3-}-P/L

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Anion§	Salt	Amount g/L
SO_4^{2-}	K_2SO_4	1.8141

§ Expressed as compound.
‖ Do not oven-dry, but dry to constant weight in a desiccator.

e. Combined working standard solution, high range: Combine 12 mL of standard anion solutions, 1000 mg/L (¶ 3*d*) of NO_2^-, NO_3^-, HPO_4^{2-}, and Br^-, 20 mL of Cl^-, and 80 mL of SO_4^{2-}. Dilute to 1000 mL and store in a plastic bottle protected from light. Solution contains 12 mg/L each of NO_2^-, NO_3^-, HPO_4^{2-}, and Br^-, 20 mg/L of Cl^-, and 80 mg/L of SO_4^{2-}. Prepare fresh daily.

f. Combined working standard solution, low range: Dilute 25 mL of the high-range mixture (¶ 3*e*) to 100 mL and store in a plastic bottle protected from light. Solution contains 3 mg/L each of NO_2^-, NO_3^-, HPO_4^{2-}, and Br^-, 5 mg/L Cl^-, and 20 mg/L of SO_4^{2-}. Prepare fresh daily.

g. Alternative combined working standard solutions: Prepare appropriate combinations according to anion concentration to be determined. If NO_2^- and PO_4^{3-} are not included, the combined working standard is stable for 1 month. Dilute solutions containing NO_2^- and PO_4^{3-} must be made daily.

4. Procedure

a. System equilibration: Turn on ion chromatograph and adjust eluent flow rate to approximate the separation achieved in Figure 4110:1 (about 2 mL/min). Adjust detector to desired setting (usually 10 to 30 µS) and let system come to equilibrium (15 to 20 min). A stable base line indicates equilibrium conditions. Adjust detector offset to zero out eluent conductivity; with the fiber or membrane suppressor adjust the regeneration flow rate to maintain stability, usually 2.5 to 3 mL/min.

b. Calibration: Inject standards containing a single anion or a mixture and determine approximate retention times. Observed times vary with conditions but if standard eluent and anion separator column are used, retention always is in the order F^-, Cl^-, NO_2^-, Br^-, NO_3^-, HPO_4^{2-}, and SO_4^{2-}. Inject at least three different concentrations (one near the minimum reporting limit) for each anion to be measured and construct a calibration curve by plotting peak height or area against concentration on linear graph paper. Recalibrate whenever the detector setting, eluent, or regenerant is changed. To minimize the effect of the ‘‘water dip’’##(5) on F^- analysis, analyze standards that bracket the expected result or eliminate the water dip by diluting the sample with eluent or by adding concentrated eluent to the sample to give the same

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HCO_3^-/CO_3^{2-} concentration as in the eluent. If sample adjustments are made, adjust standards and blanks identically.

If linearity is established for a given detector setting, single standard calibration is acceptable. Record peak height or area and retention time for calculation of the calibration factor, *F*. However, a calibration curve will result in better precision and bias. HPO_4^{2-} is nonlinear below 1.0 mg/L.

c. Sample analysis: Remove sample particulates, if necessary, by filtering through a prewashed 0.2-µm-pore-diam membrane filter. Using a prewashed syringe of 1 to 10 mL capacity equipped with a male luer fitting inject sample or standard. Inject enough sample to flush sample loop several times: for 0.1 mL sample loop inject at least 1 mL. Switch ion chromatograph from load to inject mode and record peak heights and retention times on strip chart recorder. After the last peak (SO_4^{2-}) has appeared and the conductivity signal has returned to base line, another sample can be injected.

5. Calculations

Calculate concentration of each anion, in milligrams per liter, by referring to the appropriate calibration curve. Alternatively, when the response is shown to be linear, use the following equation:

$$C = H \times F \times D$$

where:

C = mg anion/L,
H = peak height or area,
F = response factor = concentration of standard/height (or area) of standard, and
D = dilution factor for those samples requiring dilution.

6. Quality Control

See Section 4020 for minimum QC guidelines.

7. Precision and Bias

The data in Table 4110:I, Table 4110:II, Table 4110:III, Table 4110:IV, Table 4110:V, Table 4110:VI, and Table 4110:VII were produced in a joint validation study with EPA and ASTM participation. Nineteen laboratories participated and used known additions of six prepared concentrates in three waters (reagent, waste, and drinking) of their choice.

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4110 C. Single-Column Ion Chromatography with Electronic Suppression of Eluent Conductivity and Conductimetric Detection

1. General Discussion

a. Principle: A small portion of a filtered, homogeneous, aqueous sample or a sample containing no particles larger than 0.45 µm is injected into an ion chromatograph. The sample merges with the eluent stream and is pumped through the ion chromatographic system. Anions are separated on the basis of their affinity for the active sites of the column packing material. Conductivity detector readings (either peak area or peak height) are used to compute concentrations.

b. Interferences: Any two species that have similar retention times can be considered to interfere with each other. This method has potential coelution interference between short-chain acids and fluoride and chloride. Solid-phase extraction cartridges can be used to retain organic acids and pass inorganic anions. The interference-free solution then can be introduced into the ion chromatograph for separation.

This method is usable but not recommended for fluoride. Acetate, formate, and carbonate interfere in determining fluoride under the conditions listed in Table 4110:VIII. Filtering devices may be used to remove organic materials for fluoride measurements; simultaneously, use a lower eluent flow rate.

Chlorate and bromide coelute under the specified conditions. Determine whether other anions in the sample coelute with the anions of interest.

Additional interference occurs when anions of high concentrations overlap neighboring anionic species. Minimize this by sample dilution with reagent water.

Best separation is achieved with sample pH between 5 and 9. When samples are injected the eluent pH will seldom change unless the sample pH is very low. Raise sample pH by adding a small amount of a hydroxide salt to enable the eluent to control pH.

Because method sensitivity is high, avoid contamination by reagent water and equipment. Determine any background or interference due to the matrix when adding the QC sample into any matrix other than reagent water.

c. Minimum detectable concentration: The minimum detectable concentration of an anion is a function of sample volume and the signal-to-noise ratio of the detector-recorder combination. Generally, minimum detectable concentrations are about 0.1 mg/L for the anions with an

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injection volume of 100 µL. Preconcentrators or using larger injection volumes can reduce detection limits to nanogram-per-liter levels for the common anions. However, coelution is a possible problem with large injection volumes. Determine method detection limit for each anion of interest.

d. Prefiltration: If particularly contaminated samples are run, prefilter before or during injection. If the guard column becomes contaminated, follow manufacturer's suggestions for cleanup.

2. Apparatus

a. Ion chromatograph, complete with all required accessories including syringes, analytical columns, gases, detector, and a data system. Required accessories are listed below.

b. Filter device, 0.45 µm, placed before separator column to protect it from fouling by particulates or organic constituents.*#(6)

c. Anion separator column, packed with low-capacity anion-exchange resin capable of resolving fluoride, chloride, nitrite, bromide, nitrate, orthophosphate, and sulfate.†#(7)

d. Conductivity detector, flow-through, with integral heat-exchange unit allowing automatic temperature control and with separate working and reference electrodes.

e. Pump, constant flow rate controlled, high-pressure liquid chromatographic type, to deliver 1.5 mL/min.

f. Data system, including one or more computer, integrator, or strip chart recorder compatible with detector output voltage.

g. Sample injector: Either an automatic sample processor or a manual injector. If manual injector is used, provide several glass syringes of > 200 µL capacity. The automatic device must be compatible and able to inject a minimum sample volume of 100 µL.

3. Reagents

a. Reagent water: Distilled or deionized water of 18 megohm-cm resistivity containing no particles larger than 0.20 µm.

b. Borate/gluconate concentrate: Combine 16.00 g sodium gluconate, 18.00 g boric acid, 25.00 g sodium tetraborate decahydrate, and 125 mL glycerin in 600 mL reagent water. Mix and dilute to 1 L with reagent water.

c. Eluent solution, 0.0110*M* borate, 0.0015*M* gluconate, 12% (v/v) acetonitrile: Combine 20 mL borate/gluconate concentrate, 120 mL HPLC-grade acetonitrile, and 20 mL HPLC-grade *n*-butanol, and dilute to 1 L with reagent water. Use an in-line filter before the separator column to assure freedom from particulates. If the base line drifts, degas eluent with an inert gas such as helium or argon.

d. Stock standard solutions: See Section 4110B.3*e*.

e. Combined working standard solutions, high-range: See Section 4110B.3*e*.

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f. Combined working standard solutions, low-range: See Section 4110B.3 *f*.

4. Procedure

a. System equilibration: Set up ion chromatograph in accordance with the manufacturer's directions. Install guard and separator columns and begin pumping eluent until a stable base line is achieved. The background conductivity of the eluent solution is 278 µS ± 10%.

b. Calibration: Determine retention time for each anion by injecting a standard solution containing only the anion of interest and noting the time required for a peak to appear. Retention times vary with operating conditions and with anion concentration. Late eluters show the greatest variation. The shift in retention time is inversely proportional to concentration. The order of elution is shown in Figure 4110:2.

Construct a calibration curve by injecting prepared standards including each anion of interest. Use at least three concentrations plus a blank. Cover the range of concentrations expected for samples. Use one concentration near but above the method detection limit established for each anion to be measured. Unless the detector's attenuation range settings have been proven to be linear, calibrate each setting individually. Construct calibration curve by plotting either peak height or peak area versus concentration. If a data system is being used, make a hard copy of the calibration curve available.

Verify that the working calibration curve is within ± 10% of the previous value on each working day; if not, reconstruct it. Also, verify when the eluent is changed and after every 20 samples. If response or retention time for any anion varies from the previous value by more than ± 10%, reconstruct the curve using fresh calibration standards.

c. Sample analysis: Inject enough sample (about two to three times the loop volume) to insure that sample loop is properly flushed. Inject sample into chromatograph and let all peaks elute before injecting another sample (usually this occurs in about 20 min). Compare response in peak height or peak area and retention time to values obtained in calibration.

5. Calculation

Determine the concentration of the anions of interest from the appropriate standard curve. If sample dilutions were made, calculate concentration:

$$C = A \times F$$

where:

C = anion concentration, mg/L,
A = mg/L from calibration curve, and
F = dilution factor.

6. Quality Control

a. If columns other than those listed in Section 4110C.2*c* are used, demonstrate that the resolution of all peaks is similar to that shown in Figure 4110:2.

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b. Generate accuracy and precision data with this method by using a reference standard of known concentration prepared independently of the laboratory making the analysis. Compare with data in Precision and Bias, below.

c. Analyze a quality control sample at least every 10 samples. Follow general guidelines from Section 4020.

7. Precision and Bias

Precision and bias data are given in Table 4110:IX.

8. Reference

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4120 SEGMENTED CONTINUOUS FLOW ANALYSIS*#(8)

4120 A. Introduction

1. Background and Applications

Air-segmented flow analysis (SFA) is a method that automates a large number of wet chemical analyses. An SFA analyzer can be thought of as a ‘‘conveyor belt’’ system for wet chemical analysis, in which reagents are added in a ‘‘production-line’’ manner. Applications have been developed to duplicate manual procedures precisely. SFA was first applied to analysis of sodium and potassium in human serum, with a flame photometer as the detection device, by removing protein interferences with a selectively porous membrane (dialyzer).

The advantages of segmented flow, compared to the manual method, include reduced sample and reagent consumption, improved repeatability, and minimal operator contact with hazardous materials. A typical SFA system can analyze 30 to 120 samples/ h. Reproducibility is enhanced by the precise timing and repeatability of the system. Because of this, the chemical reactions do not need to go to 100% completion. Decreasing the number of manual sample/solution manipulations reduces labor costs, improves workplace safety, and improves analytical precision. Complex chemistries using dangerous chemicals can be carried out in sealed systems. Unstable reagents can be made up in situ. An SFA analyzer uses smaller volumes of reagents and samples than manual methods, producing less chemical waste needing disposal.

SFA is not limited to single-phase colorimetric determinations. Segmented-flow techniques often include analytical procedures such as mixing, dilution, distillation, digestion, dialysis, solvent extractions, and/or catalytic conversion. In-line distillation methods are used for the determinations of ammonia, fluoride, cyanide, phenols, and other volatile compounds. In-line digestion can be used for the determination of total phosphorous, total cyanide, and total nitrogen

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(kjeldahl + NO_2 + NO_3). Dialysis membranes are used to eliminate interferences such as proteins and color, and other types of membranes are available for various analytical needs. SFA also is well-suited for automated liquid/liquid extractions, such as in the determination of MBAS. Packed-bed ion exchange columns can be used to remove interferences and enhance sensitivity and selectivity of the detection.

Specific automated SFA methods are described in the sections for the analytes of interest.

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4120 B. Segmented Flow Analysis Method

1. General Discussion

a. Principle: A rudimentary system (Figure 4120:1) contains four basic components: a sampling device, a liquid transport device such as a peristaltic pump, the analytical cartridge where the chemistry takes place, and the detector to quantify the analyte.

In a generalized system, samples are loaded onto an automatic sampler. The sampler arm moves the sample pickup needle between the sample cup and a wash reservoir containing a solution closely matching the sample matrix and free of the analyte. The wash solution is pumped continuously through the reservoir to eliminate cross-contamination. The sample is pumped to the analytical cartridge as a discrete portion separated from the wash by an air-bubble created during the sampler arm's travel from wash reservoir to sample cup and back.

In the analytical cartridge, the system adds the sample to the reagent(s) and introduces proportionately identical air-bubbles to reagent or sample stream. Alternatively, another gas or immiscible fluid can be substituted for air. The analyzer then proportions the analyte sample into a number of analytical segments depending on sample time, wash time, and segmentation frequency. Relative flow and initial reagent concentration determine the amount and concentration of each reagent added. The micro-circulation pattern enhances mixing, as do mixing coils, which swirl the analytical system to utilize gravitational forces. Chemical reactions, solvent separation, catalytic reaction, dilution, distillation, heating, and/or special applications take place in their appropriate sections of the analytical cartridge as the segmented stream flows toward the detector.

A typical SFA detector is a spectrophotometer that measures the color development at a

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specific wavelength. Other detectors, such as flame photometers and ion-selective electrodes, can be used. SFA detectors utilize flow-through cells, and typically send their output to a computerized data-collection system and/or a chart-recorder. The baseline is the reading when only the reagents and wash water are flowing through the system. Because gas bubbles are compressible, highly reflective, and electrically nonconductive, they severely distort the signal in the detector; therefore, many systems remove the bubbles before the optical light path. However, if the system removes the bubbles at any point within the system, the segregated liquids will be able to interact and pool. This interaction can cause cross-contamination or loss of wash, and decreases the rate at which samples can be processed. Real-time analog or digital data reconstruction techniques known as curve regeneration can remove the effect of pooling at the flow-cell debubbler and/or any other unsegmented zones of the system. ''Bubble-gating'' is a technique that does not remove the bubbles, but instead uses analog or digital processing to remove the distortion caused by the bubbles. Bubble-gating requires a sufficiently fast detector response time and requires that the volume of the measurement cell be smaller than the volume of the individual liquid segment.

b. Sample dispersion and interferences: Theoretically, the output of the detector is square-wave. Several carryover processes can deform the output exponentially. The first process, longitudinal dispersion, occurs as a result of laminar flow. Segmentation of the flow with air bubbles minimizes the dispersion and mixing between segments. The second process is axial or lag-phase dispersion. It arises from stagnant liquid film that wets the inner surfaces of the transmission tubing. Segmented streams depend on wet surfaces for hydraulic stability. The back-pressure within non-wet tubing increases in direct proportion to the number of bubbles it contains and causes surging and bubble breakup. Corrective measures include adding specific wetting agents (surfactants) to reagents and minimizing the length of transmission tubing.

Loose or leaking connections are another cause of carryover and can cause poor reproducibility. Wrap TFE tape around leaking screw fittings. When necessary, slightly flange the ends of types of tubing that require it for a tight connection. For other connections, sleeve one size of tubing over another size. Use a noninterfering lubricant for other tubing connections. Blockages in the tubing can cause back-pressure and leaks. Clean out or replace any blocked tubing or connection. A good indicator for problems is the bubble pattern; visually inspect the system for any abnormal bubble pattern that may indicate problems with flow.

For each analysis, check individual method for compounds that can interfere with color development and/or color reading. Other possible interferences include turbidity, color, and salinity. Turbid and/or colored samples may require filtration. In another interference-elimination technique, known as matrix correction, the solution is measured at two separate wavelengths, and the result at the interference wavelength is subtracted from that at the analytical wavelength.

2. Apparatus

a. Tubing and connections: Use mini- or micro-bore tubing on analytical cartridges. Replace flexible tubing that becomes discolored, develops a ''sticky'' texture, or loses ability to spring

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back into shape immediately after compression. Also see manufacturer's manual and specific methods.

b. Electrical equipment and connections: Make electrical connections with screw terminals or plug-and-socket connections. Use shielded electrical cables. Use conditioned power or a universal power supply if electrical current is subject to fluctuations. See manufacturer's manual for additional information.

c. Automated analytical equipment: Dedicate a chemistry manifold and tubing to each specific chemistry. See specific methods and manufacturer's manual for additional information.

d. Water baths: When necessary, use a thermostatically controlled heating/cooling bath to decrease analysis time and/or improve sensitivity. Several types of baths are available; the most common are coils heated or cooled by water or oil. Temperature-controlled laboratories reduce drift in temperature-sensitive chemistries if water baths are not used.

3. Reagents

Prepare reagents according to specific methods and manufacturer's instructions. If required, filter or degas a reagent. Use reagent water (see Section 1080) if available; if not, use a grade of water that is free of the analyte and interfering substances. Run blanks to demonstrate purity of the water used to prepare reagents and wash SFA system. Minimize exposure of reagents to air, and refrigerate if necessary. If reagents are made in large quantities, preferably decant a volume sufficient for one analytical run into a smaller container. If using a wetting agent, add it to the reagent just before the start of the run. Reagents and wetting agents have a limited shelf-life. Old reagents or wetting agents can produce poor reproducibility and distorted peaks. Do not change reagent solutions or add reagent to any reagent reservoirs during analysis. Always start with a sufficient quantity to last through the analytical run.

4. Procedure

For specific operating instructions, consult manufacturer's directions and methods for analytes of interest. At startup of a system, pump reagents and wash water through system until system has reached equilibrium (bubble pattern smooth and consistent) and base line is stable. Meanwhile, load samples and standards into sample cups or tubes and type corresponding tags into computer table. When ready, command computer to begin run. Most systems will run the highest standard to trigger the beginning of the run, followed by a blank to check return to base line, and then a set of standards covering the analytical range (sampling from lowest to highest concentration). Construct a curve plotting concentration against absorbance or detector reading and extrapolate results (many systems will do this automatically). Run a new curve daily immediately before use. Calculation and interpretation of results depend on individual chemistry and are analogous to the manual method. Insert blanks and standards periodically to check and correct for any drift of base line and/or sensitivity. Some systems will run a specific standard periodically as a ‘‘drift,’’ and automatically will adjust sample results. At end of a run, let system flush according to manufacturer's recommendations.

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5. Quality Control

See Section 4020 and individual methods for quality control methods and precision and bias data.

4130 INORGANIC NONMETALS BY FLOW INJECTION ANALYSIS*#(9)

4130 A. Introduction

1. Principle

Flow injection analysis (FIA) is an automated method of introducing a precisely measured portion of liquid sample into a continuously flowing carrier stream. The sample portion usually is injected into the carrier stream by either an injection valve with a fixed-volume sample loop or an injection valve in which a fixed time period determines injected sample volume. As the sample portion leaves the injection valve, it disperses into the carrier stream and forms an asymmetric Gaussian gradient in analyte concentration. This concentration gradient is detected continuously by either a color reaction or another analyte-specific detector through which the carrier and gradient flow.

When a color reaction is used as the detector, the color reaction reagents also flow continuously into the carrier stream. Each color reagent merges with the carrier stream and is added to the analyte gradient in the carrier in a proportion equal to the relative flow rates of the carrier stream and merging color reagent. The color reagent becomes part of the carrier after it is injected and has the effect of modifying or derivatizing the analyte in the gradient. Each subsequent color reagent has a similar effect, finally resulting in a color gradient proportional to the analyte gradient. When the color gradient passes through a flow cell placed in a flow-through absorbance detector, an absorbance peak is formed. The area of this peak is proportional to the analyte concentration in the injected sample. A series of calibration standards is injected to generate detector response data used to produce a calibration curve. It is important that the FIA flow rates, injected sample portion volume, temperature, and time the sample is flowing through the system (‘‘residence time’’) be the same for calibration standards and unknowns. Careful selection of flow rate, injected sample volume, frequency of sample injection, reagent flow rates, and residence time determines the precise dilution of the sample’s original analyte concentration into the useful concentration range of the color reaction. All of these parameters ultimately determine the sample throughput, dynamic range of the method, reaction time of the color reaction discrimination against slow interference reactions, signal-to-noise ratio, and method detection level (MDL).

2. Applications

FIA enjoys the advantages of all continuous-flow methods: There is a constantly measured

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reagent blank, the ‘‘base line’’ against which all samples are measured; high sample throughput encourages frequent use of quality control samples; large numbers of samples can be analyzed in batches; sample volume measurement, reagent addition, reaction time, and detection occur reproducibly without the need for discrete measurement and transfer vessels such as cuvettes, pipets, and volumetric flasks; and all samples share a single reaction manifold or vessel consisting of inert flow tubing.

Specific FIA methods are presented as Section 4500-Br^-.D, Section 4500-Cl^-.G, Section 4500-CN^-.N and Section 4500-CN^-.O, Section 4500-F^-.G, Section 4500-NH_3.H, Section 4500-NO_3^-.I, Section 4500-N.B, Section 4500-N_{org}.D, Section 4500-P.G, Section 4500-P.H, Section 4500-P.I, Section 4500-SiO_2.F, Section 4500-SO_4^{2-}.G, and Section 4500-S^{2-}.I.

4130 B. Quality Control

When FIA methods are used, follow a formal laboratory quality control program. The minimum requirements consist of an initial demonstration of laboratory capability and periodic analysis of laboratory reagent blanks, fortified blanks, and other laboratory solutions as a continuing check on performance. Maintain performance records that define the quality of the data generated.

See Section 1020, Quality Assurance, and Section 4020 for the elements of such a quality control program.

4140 INORGANIC ANIONS BY CAPILLARY ION ELECTROPHORESIS (PROPOSED)*#(10)

4140 A. Introduction

Determination of common inorganic anions such as fluoride, chloride, bromide, nitrite, nitrate, orthophosphate, and sulfate is a significant component of water quality analysis. Instrumental techniques that can determine multiple analytes in a single analysis, i.e., ion chromatography (Section 4110) and capillary ion electrophoresis, offer significant time and operating cost savings over traditional single-analyte wet chemical analysis.

Capillary ion electrophoresis is rapid (complete analysis in less than 5 min) and provides additional anion information, i.e., organic acids, not available with isocratic ion chromatography (IC). Operating costs are significantly less than those of ion chromatography. Capillary ion electrophoresis can detect all anions present in the sample matrix, providing an anionic ‘‘fingerprint.’’

Anion selectivity of capillary ion electrophoresis is different from that of IC and eliminates many of the difficulties present in the early portion of an IC chromatogram. For example, sample

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matrix neutral organics, water, and cations do not interfere with anion analysis, and fluoride is well resolved from monovalent organic acids. Sample preparation typically is dilution with reagent water and removal of suspended solids by filtration. If necessary, hydrophobic sample components such as oil and grease can be removed with the use of HPLC solid-phase extraction cartridges without biasing anion concentrations.

4140 B. Capillary Ion Electrophoresis with Indirect UV Detection

1. General Discussion

a. Principle: A buffered aqueous electrolyte solution containing a UV-absorbing anion salt (sodium chromate) and an electroosmotic flow modifier (OFM) is used to fill a 75-µm-ID silica capillary. An electric field is generated by applying 15 kV of applied voltage using a negative power supply; this defines the detector end of the capillary as the anode. Sample is introduced at the cathodic end of the capillary and anions are separated on the basis of their differences in mobility in the electric field as they migrate through the capillary. Cations migrate in the opposite direction and are not detected. Water and neutral organics are not attracted towards the anode; they migrate after the anions and thus do not interfere with anion analysis. Anions are detected as they displace charge-for-charge the UV-absorbing electrolyte anion (chromate), causing a net decrease in UV absorbance in the analyte anion zone compared to the background electrolyte. Detector polarity is reversed to provide positive mv response to the data system (Figure 4140:1). As in chromatography, the analytes are identified by their migration time and quantitated by using time-corrected peak area relative to standards. After the analytes of interest are detected, the capillary is purged with fresh electrolyte, eliminating the remainder of the sample matrix before the next analysis.

b. Interferences: Any anion that has a migration time similar to the analytes of interest can be considered an interference. This method has been designed to minimize potential interference typically found in environmental waters, groundwater, drinking water, and wastewater.

Formate is a common potential interference with fluoride; it is a common impurity in reagent water, has a migration time similar to that of fluoride, and is an indicator of loss of water purification system performance and TOC greater than 0.1 mg/L. The addition of 5 mg formate/L in the mixed working anion standard, and to sample where identification of fluoride is in question, aids in the correct identification of fluoride.

Generally, a high concentration of any one ion may interfere with resolution of analyte anions in close proximity. Dilution in reagent water usually is helpful. Modifications in the electrolyte formulation can overcome resolution problems but require individual validation for precision and bias. This method is capable of interference-free resolution of a 1:100 differential of Br^- to Cl^-, and NO_2^- and NO_3^- to SO_4^{2-}, and 1:1000 differential of Cl^- and SO_4^{2-}.

Dissolved ferric iron in the mg/L range gives a low bias for PO_4. However, transition metals do not precipitate with chromate because of the alkaline electrolyte pH.

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c. Minimum detectable concentrations: The minimum detectable concentration for an anion is a function of sample size. Generally, for a 30-s sampling time, the minimum detectable concentrations are 0.1 mg/L (Figure 4140:2). According to the method for calculating MDL given in Section 1030, the calculated detection limits are below 0.1 mg/L. These detection limits can be compromised by analyte impurities in the electrolyte.

d. Limitations: Samples with high ionic strength may show a decrease in analyte migration time. This variable is addressed by using normalized migration time with respect to a reference peak, chloride, for identification, and using time-corrected area for quantitation. With electrophoresis, published data indicate that analyte peak area is a function of migration time. At high analyte anion concentrations, peak shape becomes asymmetrical; this phenomenon is typical and is different from that observed in ion chromatography.

2. Apparatus

a. Capillary ion electrophoresis (CIE) system:*#(11) Various commercial instruments are available that integrate a negative high-voltage power supply, electrolyte reservoirs, covered sample carousel, hydrostatic sampling mechanism, capillary purge mechanism, self-aligning capillary holder, and UV detector capable of 254-nm detection in a single temperature-controlled compartment at 25°C. Optimal detection limits are attained with a fixed-wavelength UV detector with Hg lamp and 254-nm filter.

b. Capillary: 75-µm-ID × 375-µm-OD × 60-cm-long fused silica capillary with a portion of its outer coating removed to act as the UV detector window. Capillaries can be purchased premade* or on a spool and prepared as needed.

c. Data system:*#(12) HPLC-based integrator or computer. Optimum performance is attained with a computer data system and electrophoresis-specific data processing including data acquisition at 20 points/s, migration times determined at midpoint of peak width, identification based on normalized migration times with respect to a reference peak, and time-corrected peak area.

3. Reagents

a. Reagent water: See Section 1080. Ensure that water is analyte-free. The concentration of dissolved organic material will influence overall performance; preferably use reagent water with <50 µg TOC/L.

b. Chromate electrolyte solution: Prepare as directed from individual reagents, or purchase electrolyte preformulated.

1) *Sodium chromate concentrate,* 100 m*M:* In a 1-L volumetric flask dissolve 23.41 g sodium chromate tetrahydrate, $Na_2CrO_4 \cdot 4H_2O$, in 500 mL water and dilute to 1 L with water. Store in a capped glass or plastic container at ambient temperature; this reagent is stable for 1 year.

2) *Electroosmotic flow modifier concentrate,* 100 m*M:* In a 100-mL volumetric flask

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dissolve 3.365 g tetradecyltrimethyl ammonium bromide (TTAB), mol wt 336.4, in 50 mL water and dilute to 100 mL. Store in a capped glass or plastic container at ambient temperature; this reagent is stable for 1 year.

3) *Buffer concentrate,* 100 m*M:* In a 1-L volumetric flask dissolve 20.73 g 2-[*N*-cyclohexylamino]-ethane sulfonate (CHES), mol wt 207.29, in 500 mL water and dilute to 1 L. Store in a capped glass or plastic container at ambient temperature; this reagent is stable for 1 year.

4) *Calcium gluconate concentrate,* 1 m*M:* In a 1-L volumetric flask dissolve 0.43 g calcium gluconate, mol wt 430.38, in 500 mL water and dilute to 1 L. Store in a capped glass or plastic container at ambient temperature; this reagent is stable for 1 year.

5) *Sodium hydroxide solution,* NaOH, 100 m*M*: In a 1-L plastic volumetric flask dissolve 4 g sodium hydroxide, NaOH, in 500 mL water and dilute to 1 L. Store in a capped plastic container at ambient temperature; this reagent is stable for 1 month.

6) *Chromate electrolyte solution:* Prerinse an anion exchange cartridge in the hydroxide form with 10 mL 100-m*M* NaOH followed by 10 mL water; discard the washings. Slowly pass 4 mL 100-m*M* TTAB concentrate through the cartridge into a 100-mL volumetric flask. Rinse cartridge with 10 mL water and add to flask. (NOTE: This step is needed to convert the TTAB from the bromide form into the hydroxide form TTAOH. The step can be eliminated if commercially available 100 m*M* TTAOH is used.)

To the 100-mL volumetric flask containing the TTAOH add 4.7 mL sodium chromate concentrate, 10 mL CHES buffer concentrate, and 10 mL calcium gluconate concentrate. Mix and dilute to 100 mL with water. The pH should be 9 ± 0.1; final solution is 4.7 m*M* sodium chromate, 4 m*M* TTAOH, 10 m*M* CHES, and 0.1 m*M* calcium gluconate. Filter and degas through a 0.45-µm aqueous membrane, using a vacuum apparatus. Store any remaining electrolyte in a capped plastic container at ambient temperature for up to 1 month.

c. Standard anion solution, 1000mg/L*:* Prepare a series of individual standard anion solutions by adding the indicated amount of salt, dried to constant weight at 105°C, to 100mL with water. Store in plastic bottles; these solutions are stable for 3 months. (Alternatively, purchase individual certified 1000-mg/L anion standards and store following manufacturer's directions.)

Anion	**Salt**	**Amount *g/100mL***
Chloride	NaCl	0.1649
Bromide	NaBr	0.1288
Formate	$NaCO_2H$	0.1510
Fluoride	NaF	0.2210
Nitrite	$NaNO_2$	0.1499* (1000 mg NO_2^-/L = 304.3 mg NO_2^-–N/L)

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Anion	Salt	Amount g/100mL
Nitrate	$NaNO_3$	0.1371 (1000 mg NO_3^-/L = 225.8 mg NO_3^-–N/L)
Phosphate	Na_2HPO_4†	0.1500 (1000 mg PO_4^{3-}/L = 326.1 mg PO_4^{3-}–P/L)
Sulfate	Na_2SO_4†	0.1480 (1000 mg SO_4^{2-}/L = 676.3 mg SO_4^{2-}–S/L)

* Do not oven-dry, but dry to constant weight in a desiccator over phosphorous pentoxide.
† Potassium salts can be used, but with corresponding modification of salt amounts.

d. Mixed working anion standard solutions: Prepare at least three different working anion standard solutions that bracket the expected sample range, from 0.1 to 50 mg/L. Add 5 mg formate/ L to all standards. Use 0.1 mL standard anion solution/100 mL working anion solution (equal to 1 mg anion/L). (Above 50 mg/ L each anion, chloride, bromide, nitrite, sulfate, and nitrate are no longer baseline-resolved. Analytes that are not baseline-resolved may give a low bias. If the analytes are baseline-resolved, quantitation is linear to 100 mg/L.) Store in plastic containers in the refrigerator; prepare fresh standards weekly. Figure 4140:3 shows representative electropherograms of anion standards and Table 4140:I gives the composition of the standards.

e. Calibration verification sample: Use a certified performance evaluation standard, or equivalent, within the range of the mixed working anion standard solutions analyzed as an unknown. Refer to Section 4020.

f. Analyte known-addition sample: To each sample matrix add a known amount of analyte, and use to evaluate analyte recovery.

4. Procedure

a. Capillary conditioning: Set up CIE system according to manufacturer's instructions. Rinse capillary with 100 m*M* NaOH for 5 min. Place fresh degassed electrolyte into both reservoirs and purge capillary with electrolyte for 3 min to remove all previous solutions and air bubbles. Apply voltage of 15 kV and note the current; if the expected 14 ± 1 µA is observed, the CIE system is ready for use. Zero UV detector to 0.000 absorbance.

b. Analysis conditions: Program CE system to apply constant current of 14 µA for the run time. Use 30 s hydrostatic sampling time for all standard and sample introduction. Analysis time is 5 min.

c. Analyte migration time calibration: Determine migration time of each analyte daily using the midrange mixed working anion standard. Perform duplicate analysis to insure migration time stability. Use the midpoint of peak width, defined as midpoint between the start and stop integration marks, as the migration time for each analyte; this accounts for the observed non-symmetrical peak shapes. (Use of peak apex may result in analyte misidentification.) The

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migration order is always Cl^-, Br^-, NO_2^-, SO_4^{2-}, NO_3^-, F^-, and PO_4^{3-}. Dissolved HCO_3^- is the last peak in the standard (see Figure 4140:1). Set analyte migration time window as 2% of the migration time determined above, except for Cl^-, which is set at 10%. Chloride is always the first peak and is used as the reference peak for analyte qualitative identification; identify anions on the basis of normalized migration times with respect to the reference peak, or migration time ratio. (See Figure 4140:1 and Table 4140:II.)

d. Analyte response calibration: Analyze all three mixed working anion standards in duplicate. Plot time-corrected peak area for each analyte versus concentration using a linear regression through zero. (In capillary electrophoresis peak area is a function of analyte migration time, which may change during analyses. Time-corrected peak area is a well-documented CE normalization routine, i.e., peak area divided by migration time. (NOTE: *Do not use analyte peak height.*) Calibration is accepted as linear if regression coefficient of variation, R^2, is greater than 0.995. Linearity calibration curves for anions are shown in Figure 4140:4, Figure 4140:5 and Figure 4140:6.

e. Sample analysis: After initial calibration run samples in the following order: calibration verification sample, reagent blank, 10 unknown samples, calibration verification sample, reagent blank, etc. Filter samples containing high concentrations of suspended solids. If peaks are not baseline-resolved, dilute sample 1:5 with water and repeat analysis for unresolved analyte quantitation. Resolved analytes in the undiluted sample are considered correct quantitation. Electropherograms of typical samples are shown in Figure 4140:7, Figure 4140:8, and Figure 4140:9.

5. Calculation

Relate the time-corrected peak area for each sample analyte with the calibration curve to determine concentration of analyte. If the sample was diluted, multiply anion concentration by the dilution factor to obtain original sample concentration, as follows:

$$C = A \times F$$

where:

C = analyte concentration in original sample, mg/L,
A = analyte concentration from calibration curve, mg/L, and
F = scale factor or dilution factor. (For a 1:5 sample dilution, $F = 5$.)

6. Quality Control

a. Analytical performance check: Unless analyst has already demonstrated ability to generate data with acceptable precision and bias by this method, proceed as follows: Analyze seven replicates of a certified performance evaluation standard containing the analytes of interest. Calculate mean and standard deviation of these data. The mean must be within the performance evaluation standard's 95% confidence interval. Calculate percent relative standard deviation (RSD) for these data as (SD × 100) / mean; % RSD should conform to acceptance limit

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given in Section 1020B.

b. Calibration verification: Analyze an independent, certified performance evaluation standard at the beginning and end of the analyses, or if many samples are analyzed, after every 10 samples. The determined analyte concentration should be within ±10% of the true value, and the migration time of the Cl^- reference peak should be within 5% of the calibrated migration time. If the Cl^- reference peak differs by more than 5% of the calibrated migration time, repeat capillary conditioning and recalibrate before proceeding.

c. Water blank analysis: At the beginning of every set of analyses run a water blank to demonstrate that the water is free of analyte anions. Dissolved bicarbonate will always be observed as a positive or negative peak having a migration time greater than PO_4^{3-} and does not interfere with the analysis. Any negative peak indicates the presence of an anion impurity in the electrolyte; a positive peak indicates the presence of an impurity in the reagent water. If this is noted, discard electrolyte and prepare electrolyte and sample dilutions again with water from a different source.

d. Analyte recovery verification: For each sample matrix analyzed, e.g., drinking water, surface water, groundwater, or wastewater, analyze duplicate known-addition samples (¶ 3 *f*). Analyte recoveries should conform to acceptance limits given in Section 1020B.

e. Blind check sample: Analyze an unknown certified performance evaluation check sample at least once every 6 months to verify method accuracy.

f. Sample duplicates: Analyze one or more sample duplicates every 10 samples.

7. Precision and Bias

Table 4140:III compares results of capillary ion electrophoresis with those of other approved methods. Precision and bias data are given in Table 4140:IV and Table 4140:V. Comparison of other methods and capillary ion electrophoresis for wastewater effluent, drinking water, and landfill leachates are given in Table 4140:VI.

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4500-B BORON*#(13)

4500-B A. Introduction

1. Occurrence and Significance

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Boron (B) is the first element in Group IIIA of the periodic table; it has an atomic number of 5, an atomic weight of 10.81, and a valence of 3. The average abundance of B in the earth's crust is 9 ppm; in soils it is 18 to 63 ppm; in streams it is 10 µg/L; and in groundwaters it is 0.01 to 10 mg/L. The most important mineral is borax, which is used in the preparation of heat-resistant glasses, detergents, porcelain enamels, fertilizers, and fiberglass.

The most common form of boron in natural waters is H_3BO_3. Although boron is an element essential for plant growth, in excess of 2.0 mg/L in irrigation water, it is deleterious to certain plants and some plants may be affected adversely by concentrations as low as 1.0 mg/L (or even less in commercial greenhouses). Drinking waters rarely contain more than 1 mg B/L and generally less than 0.1 mg/L, concentrations considered innocuous for human consumption. Seawater contains approximately 5 mg B/L and this element is found in saline estuaries in association with other seawater salts.

The ingestion of large amounts of boron can affect the central nervous system. Protracted ingestion may result in a clinical syndrome known as borism.

2. Selection of Method

Preferably, perform analyses by the inductively coupled plasma method (Section 3120). The inductively coupled plasma/mass spectrometric method (Section 3125) also may be applied successfully in most cases (with lower detection limits), even though boron is not specifically listed as an analyte in the method.

The curcumin method (B) is applicable in the 0.10- to 1.0-mg/L range, while the carmine method (C) is suitable for the determination of boron concentration in the 1- to 10-mg/L range. The range of these methods can be extended by dilution or concentration of the sample.

3. Sampling and Storage

Store samples in polyethylene bottles or alkali-resistant, boron-free glassware.

4500-B B. Curcumin Method

1. General Discussion

a. Principle: When a sample of water containing boron is acidified and evaporated in the presence of curcumin, a red-colored product called rosocyanine is formed. The rosocyanine is taken up in a suitable solvent and the red color is compared with standards visually or photometrically.

b. Interference: NO_3^--N concentrations above 20 mg/L interfere. Significantly high results are possible when the total of calcium and magnesium hardness exceeds 100 mg/L as calcium carbonate ($CaCO_3$). Moderate hardness levels also can cause a considerable percentage error in the low boron range. This interference springs from the insolubility of the hardness salts in 95% ethanol and consequent turbidity in the final solution. Filter the final solution or pass the original

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sample through a column of strongly acidic cation-exchange resin in the hydrogen form to remove interfering cations. The latter procedure permits application of the method to samples of high hardness or solids content. Phosphate does not interfere.

c. Minimum detectable quantity: 0.2 μg B.

2. Apparatus

a. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer,* for use at 540 nm, with a minimum light path of 1 cm.

2) *Filter photometer,* equipped with a green filter having a maximum transmittance near 540 nm, with a minimum light path of 1 cm.

b. Evaporating dishes, 100- to 150-mL capacity, of high-silica glass,*#(14) platinum, or other suitable material.

c. Water bath, set at 55 ± 2°C.

d. Glass-stoppered volumetric flasks, 25- and 50-mL capacity.

e. Ion-exchange column, 50 cm long by 1.3 cm in diameter.

3. Reagents

Store all reagents in polyethylene or boron-free containers.

a. Stock boron solution: Dissolve 571.6 mg anhydrous boric acid, H_3BO_3, in distilled water and dilute to 1000 mL; 1.00 mL = 100 μg B. Because H_3BO_3 loses weight on drying at 105°C, use a reagent meeting ACS specifications and keep the bottle tightly stoppered to prevent entrance of atmospheric moisture.

b. Standard boron solution: Dilute 10.00 mL stock boron solution to 1000 mL with distilled water; 1.00 mL = 1.00 μg B.

c. Curcumin reagent: Dissolve 40 mg finely ground curcumin†#(15) and 5.0 g oxalic acid in 80 mL 95% ethyl alcohol. Add 4.2 mL conc HCl, make up to 100 mL with ethyl alcohol in a 100-mL volumetric flask, and filter if reagent is turbid (isopropyl alcohol, 95%, may be used in place of ethyl alcohol). This reagent is stable for several days if stored in a refrigerator.

d. Ethyl or isopropyl alcohol, 95%.

e. Reagents for removal of high hardness and cation interference:

1) *Strongly acidic cation-exchange resin.*

2) *Hydrochloric acid,* HCl, 1 + 5.

4. Procedure

a. Precautions: Closely control such variables as volumes and concentrations of reagents, as well as time and temperature of drying. Use evaporating dishes identical in shape, size, and composition to insure equal evaporation time because increasing the time increases intensity of the resulting color.

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b. Preparation of calibration curve: Pipet 0 (blank), 0.25, 0.50, 0.75, and 1.00 μg boron into evaporating dishes of the same type, shape, and size. Add distilled water to each standard to bring total volume to 1.0 mL. Add 4.0 mL curcumin reagent to each and swirl gently to mix contents thoroughly. Float dishes on a water bath set at 55 ± 2°C and let them remain for 80 min, which is usually sufficient for complete drying and removal of HCl. Keep drying time constant for standards and samples. After dishes cool to room temperature, add 10 mL 95% ethyl alcohol to each dish and stir gently with a polyethylene rod to insure complete dissolution of the red-colored product.

Wash contents of dish into a 25-mL volumetric flask, using 95% ethyl alcohol. Make up to mark with 95% ethyl alcohol and mix thoroughly by inverting. Read absorbance of standards and samples at a wavelength of 540 nm after setting reagent blank at zero absorbance. The calibration curve is linear from 0 to 1.00 μg boron. Make photometric readings within 1 h of drying samples.

c. Sample treatment: For waters containing 0.10 to 1.00 mg B/L, use 1.00 mL sample. For waters containing more than 1.00 mg B/L, make an appropriate dilution with boron-free distilled water, so that a 1.00-mL portion contains approximately 0.50 μg boron.

Pipet 1.00 mL sample or dilution into an evaporating dish. Unless the calibration curve is being determined at the same time, prepare a blank and a standard containing 0.50 μg boron and run in conjunction with the sample. Proceed as in ¶ 4*b*, beginning with ‘‘Add 4.0 mL curcumin reagent. . . .’’ If the final solution is turbid, filter through filter paper‡#(16) before reading absorbance. Calculate boron content from calibration curve.

d. Visual comparison: The photometric method may be adapted to visual estimation of low boron concentrations, from 50 to 200 μg/L, as follows: Dilute the standard boron solution 1 + 3 with distilled water; 1.00 mL = 0.20 μg B. Pipet 0, 0.05, 0.10, 0.15, and 0.20 μg boron into evaporating dishes as indicated in ¶ 4*b*. At the same time add an appropriate volume of sample (1.00 mL or portion diluted to 1.00 mL) to an identical evaporating dish. The total boron should be between 0.05 and 0.20 μg. Proceed as in ¶ 4*b*, beginning with ‘‘Add 4.0 mL curcumin reagent. . . .’’ Compare color of samples with standards within 1 h of drying samples.

e. Removal of high hardness and cation interference: Prepare an ion-exchange column of approximately 20 cm × 1.3 cm diam. Charge column with a strongly acidic cation-exchange resin. Backwash column with distilled water to remove entrained air bubbles. Keep the resin covered with liquid at all times. Pass 50 mL 1 + 5 HCl through column at a rate of 0.2 mL acid/mL resin in column/min and wash column free of acid with distilled water.

Pipet 25 mL sample, or a smaller sample of known high boron content diluted to 25 mL, onto the resin column. Adjust rate of flow to about 2 drops/s and collect effluent in a 50-mL volumetric flask. Wash column with small portions of distilled water until flask is filled to mark. Mix and transfer 2.00 mL into evaporating dish. Add 4.0 mL curcumin reagent and complete the analysis as described in ¶ 4*b* preceding.

5. Calculation

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Use the following equation to calculate boron concentration from absorbance readings:

$$\text{mg B/L} = \frac{A_2 \times C}{A_1 \times S}$$

where:

A_1 = absorbance of standard,
A_2 = absorbance of sample,
C = µg B in standard taken, and
S = mL sample.

6. Precision and Bias

A synthetic sample containing 240 µg B/L, 40 µg As/L, 250 µg Be/L, 20 µg Se/L, and 6 µg V/L in distilled water was analyzed in 30 laboratories by the curcumin method with a relative standard deviation of 22.8% and a relative error of 0%.

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4500-B C. Carmine Method

1. General Discussion

a. Principle: In the presence of boron, a solution of carmine or carminic acid in concentrated sulfuric acid changes from a bright red to a bluish red or blue, depending on the concentration of boron present.

b. Interference: The ions commonly found in water and wastewater do not interfere.

c. Minimum detectable quantity: 2 µg B.

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2. Apparatus

Colorimetric equipment: One of the following is required:

a. Spectrophotometer, for use at 585 nm, with a minimum light path of 1 cm.

b. Filter photometer, equipped with an orange filter having a maximum transmittance near 585 nm, with a minimum light path of 1 cm.

3. Reagents

Store all reagents in polyethylene or boron-free containers.

a. Standard boron solution: Prepare as directed in Method B, ¶ 3*b*.

b. Hydrochloric acid, HCl, conc and 1 + 11.

c. Sulfuric acid, H_2SO_4, conc.

d. Carmine reagent: Dissolve 920 mg carmine N.F. 40, or carminic acid, in 1 L conc H_2SO_4. (If unable to zero spectrophotometer, dilute carmine 1 + 1 with conc H_2SO_4 to replace above reagent.)

4. Procedure

a. Preliminary sample treatment: If sample contains less than 1 mg B/L, pipet a portion containing 2 to 20 µg B into a platinum dish, make alkaline with 1*N* NaOH plus a slight excess, and evaporate to dryness on a steam or hot water bath. If necessary, destroy any organic material by ignition at 500 to 550°C. Acidify cooled residue (ignited or not) with 2.5 mL 1 + 11 HCl and triturate with a rubber policeman to dissolve. Centrifuge if necessary to obtain a clear solution. Pipet 2.00 mL clear concentrate into a small flask or 30-mL test tube. Treat reagent blank identically.

b. Color development: Prepare a series of boron standard solutions (100, 250, 500, 750, and 1000 µg) in 100 mL with distilled water. Pipet 2.00 mL of each standard solution into a small flask or 30-mL test tube.

Treat blank and calibration standards exactly as the sample. Add 2 drops (0.1 mL) conc HCl, carefully introduce 10.0 mL conc H_2SO_4, mix, and let cool to room temperature. Add 10.0 mL carmine reagent, mix well, and after 45 to 60 min measure absorbance at 585 nm in a cell of 1-cm or longer light path, using the blank as reference.

To avoid error, make sure that no bubbles are present in the optical cell while photometric readings are being made. Bubbles may appear as a result of incomplete mixing of reagents. Because carmine reagent deteriorates, check calibration curve daily.

5. Calculation

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$$mg\ B/L = \frac{\mu g\ B\ (\text{in approx. 22 mL final volume})}{mL\ sample} \times 1.25$$

6. Precision and Bias

A synthetic sample containing 180 µg B/L, 50 µg As/L, 400 µg Be/L, and 50 µg Se/L in distilled water was analyzed in nine laboratories by the carmine method with a relative standard deviation of 35.5% and a relative error of 0.6%.

7. Bibliography

HATCHER, J.T. & L.V. WILCOX. 1950. Colorimetric determination of boron using carmine. *Anal. Chem.* 22:567.

4500-Br^- BROMIDE*#(17)

4500-Br^- A. Introduction

1. Occurrence

Bromide occurs in varying amounts in ground and surface waters in coastal areas as a result of seawater intrusion and sea-spray-affected precipitation. The bromide content of ground waters and stream baseflows also can be affected by connate water. Industrial and oil-field brine discharges can contribute to the bromide in water sources. Under normal circumstances, the bromide content of most drinking waters is small, seldom exceeding 1 mg/L. Even levels of <100 µg/L can lead to formation of bromate or brominated by-products in disinfected waters.

2. Selection of Method

Described here are a colorimetric procedure suitable for the determination of bromide in most drinking waters and a flow injection analysis method. Bromide preferably is determined by the ion chromatography method (Section 4110) or by capillary ion electrophoresis (Section 4140).

4500-Br^- B. Phenol Red Colorimetric Method

1. General Discussion

a. Principle: When a sample containing bromide ions (Br^-) is treated with a dilute solution of chloramine-T in the presence of phenol red, the oxidation of bromide and subsequent

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bromination of the phenol red occur readily. If the reaction is buffered to pH 4.5 to 4.7, the color of the brominated compound will range from reddish to violet, depending on the bromide concentration. Thus, a sharp differentiation can be made among various concentrations of bromide. The concentration of chloramine-T and timing of the reaction before dechlorination are critical.

b. Interference: Most materials present in ordinary tap water do not interfere, but oxidizing and reducing agents and higher concentrations of chloride and bicarbonate can interfere. Free chlorine in samples should be destroyed as directed in Section 5210B.4*e*2); analyze bromide in a portion of dechlorinated sample. Addition of substantial chloride to the pH buffer solution (see ¶ 3*a* below) can eliminate chloride interference for waters with very low bromide/chloride ratios, such as those affected by dissolved road salt. Small amounts of dissolved iodide do not interfere, but small concentrations of ammonium ion interfere substantially. Sample dilution may reduce interferences to acceptable levels for some saline and waste waters. However, if two dilutions differing by a factor of at least five do not give comparable values, the method is inapplicable. Bromide concentration in diluted samples must be within the range of the method (0.1 to 1 mg/L).

c. Minimum detectable concentration: 0.1 mg Br^-/L.

2. Apparatus

a. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer,* for use at 590 nm, providing a light path of at least 2 cm.

2) *Filter photometer,* providing a light path of at least 2 cm and equipped with an orange filter having a maximum transmittance near 590 nm.

3) *Nessler tubes,* matched, 100-mL, tall form.

b. Acid-washed glassware: Wash all glassware with 1 + 6 HNO_3 and rinse with distilled water to remove all trace of adsorbed bromide.

3. Reagents

a. Acetate buffer solution: Dissolve 90 g NaCl and 68 g sodium acetate trihydrate, $NaC_2H_3O_2 \cdot 3H_2O$, in distilled water. Add 30 mL conc (glacial) acetic acid and make up to 1 L. The pH should be 4.6 to 4.7.

b. Phenol red indicator solution: Dissolve 21 mg phenolsulfonephthalein sodium salt and dilute to 100 mL with distilled water.

c. Chloramine-T solution: Dissolve 500 mg chloramine-T, sodium p-toluenesulfonchloramide, and dilute to 100 mL with distilled water. Store in a dark bottle and refrigerate.

d. Sodium thiosulfate, 2*M:* Dissolve 49.6 g $Na_2S_2O_3 \cdot 5H_2O$ or 31.6 g $Na_2S_2O_3$ and dilute to 100 mL with distilled water.

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e. Stock bromide solution: Dissolve 744.6 mg anhydrous KBr in distilled water and make up to 1000 mL; 1.00 mL = 500 µg Br^-.

f. Standard bromide solution: Dilute 10.00 mL stock bromide solution to 1000 mL with distilled water; 1.00 mL = 5.00 µg Br^-.

4. Procedure

a. Preparation of bromide standards: Prepare at least six standards, 0, 0.20, 0.40, 0.60, 0.80 and 1.00 mg Br^-/L, by diluting 0.0, 2.00, 4.00, 6.00, 8.00, and 10.00 mL standard bromide solution to 50.00 mL with distilled water. Treat standards the same as samples in ¶ 4*b*.

b. Treatment of sample: Add 2 mL buffer solution, 2 mL phenol red solution, and 0.5 mL chloramine-T solution to 50.0 mL sample or two separate sample dilutions (see 1*b* above) such that the final bromide concentration is in the range of 0.1 to 1.0 mg Br^-/L. Mix thoroughly immediately after each addition. Exactly 20 min after adding chloramine-T, dechlorinate by adding, with mixing, 0.5 mL $Na_2S_2O_3$ solution. Compare visually in nessler tubes against bromide standards prepared simultaneously, or preferably read in a photometer at 590 nm against a reagent blank. Determine the bromide values from a calibration curve of mg Br^-/L (in 55 mL final volume) against absorbance. A 2.54-cm light path yields an absorbance value of approximately 0.36 for 1 mg Br^-/L.

5. Calculation

$$\text{mg Br}^-/\text{L} = \text{mg Br}^-/\text{L (from calibration curve)} \times \text{dilution factor (if any)}$$

Results are based on 55 mL final volume for samples and standards.

6. Bibliography

STENGER, V.A. & I.M. KOLTHOFF. 1935. Detection and colorimetric estimation of microquantities of bromide. *J. Amer. Chem. Soc.* 57:831.

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BASEL, C.L., J.D. DEFREESE & D.O. WHITTEMORE. 1982. Interferences in automated phenol red method for determination of bromide in water. *Anal. Chem.* 54:2090.

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4500-Br^- C. (Reserved)

4500-Br^- D. Flow Injection Analysis (PROPOSED)

1. General Discussion

a. Principle: Bromide is oxidized to bromine by chloramine-T, followed by substitution of bromine on phenol red to produce bromphenol blue. The absorbance measured at 590 nm is proportional to the concentration of bromide in the sample. Sodium thiosulfate is added to reduce interference from chloride.

This method is suitable for the determination of bromide in waters containing up to 20 000 mg Cl^-/L, including drinking, ground, and surface waters, and domestic and industrial wastes. The method determines total bromide, or, if the sample is filtered through a 0.45-µm-pore-size filter, the result is called ''dissolved bromide.'' The difference between total bromide and dissolved bromide is called ''insoluble bromide.''

Also see Section 4500-Br^-.A and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

Chloride interference is reduced by the addition of sodium thiosulfate. Chloramine-T dissociates in aqueous solution to form hypochlorous acid, which can then react with chloride, causing substitution of chloride at positions ortho to the hydroxy groups on phenol red, just as in bromination. Sodium thiosulfate reacts with chlorine to reduce this interferent to a selectivity (ratio of analyte to interferent concentration) of >28 000.

2. Apparatus

Flow injection analysis equipment consisting of:

a. FIA injection valve with sample loop or equivalent.

b. Multichannel proportioning pump.

c. FIA manifold with flow cell (Figure 4500-Br^-:1). Relative flow rates only are shown. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.*#(18)

d. Absorbance detector, 590 nm, 10-nm bandpass.

e. Valve control and data acquisition system.

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and all solutions. As an alternative to

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preparing reagents by weight/weight, use weight/volume.

a. Chloramine-T: To a tared 1-L container add 0.40 g chloramine-T hydrate (mol wt 227.65) and 999 g water. Cap and invert container to dissolve. Discard after 1 week.

b. Phenol red: To a tared 1-L container add 929 g water and 30.0 g glacial acetic acid. Swirl contents of container. Add 41.0 g sodium acetate and swirl container until it is dissolved. Add 0.040 g phenol red. Mix with a magnetic stirrer. Discard after 1 week.

c. Thiosulfate: To a tared 1-L container, add 724 g water and 500 g sodium thiosulfate pentahydrate, $Na_2S_2O_3 \cdot 5H_2O$. Dissolve by adding the solid slowly while stirring. The solid should be completely dissolved within 30 min. Gentle heating may be required. Discard after 1 week.

d. Stock bromide standard, 100.0 mg Br^-/L: To a 1-L volumetric flask add 0.129 g sodium bromide, NaBr. Dissolve in sufficient water, dilute to mark, and invert to mix.

e. Stock bromide standard, 10.0 mg Br^-/L: To a 500-mL volumetric flask add 50 mL stock standard (¶ 3*d*). Dilute to mark and invert to mix. Prepare fresh monthly.

f. Standard bromide solutions: Prepare bromide standards for the calibration curve in the desired concentration range, using the stock standard (¶ e), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-Br^-:1 and follow method supplied by manufacturer, or laboratory standard operating procedure for this method. Follow quality control guidelines outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold vs. bromide concentration. The calibration curve gives a good fit to a second-order polynomial.

6. Precision and Bias

a. Precision: With a 300-µL sample loop, ten replicates of a 5.0-mg Br^-/L standard gave a mean of 5.10 mg Br^-/L and a relative standard deviation of 0.73%.

b. Bias: With a 300-µ/L sample loop, solutions of sodium chloride were fortified in triplicate with bromide and mean blanks and recoveries were measured. From a 10 000-mg Cl^-/L solution, a blank gave 0.13 mg Br^-/L. Corrected for this blank, a 1.0-mg Br^-/L known addition gave 98% recovery and a 5.0-mg Br^-/L known addition gave 102 % recovery. From a 20 000 mg Cl^-/L solution, a blank gave 0.27 mg Br^-/L. Corrected for this blank, a 1.0-mg Br^-/L known addition gave 100% recovery and a 5.0-mg Br^-/L known addition gave 101% recovery.

c. MDL: Using a published MDL method[1] and a 300-µL sample loop, analysts ran 21 replicates of a 0.5-mg Br^-/L standard. These gave a mean of 0.468 mg Br^-/L, a standard deviation of 0.030 mg Br^-/L, and an MDL of 0.07 mg Br^-/L. A lower MDL may be obtained by

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increasing the sample loop volume and increasing the ratio of carrier flow rate to reagents flow rate.

7. Reference

1. U.S. Environmental Protection Agency. 1989. Definition and procedure for the determination of method detection limits. Appendix B to CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-CO_2 CARBON DIOXIDE*#(19)

4500-CO_2 A. Introduction

1. Occurrence and Significance

Surface waters normally contain less than 10 mg free carbon dioxide (CO_2) per liter while some groundwaters may easily exceed that concentration. The CO_2 content of a water may contribute significantly to corrosion. Recarbonation of a supply during the last stages of water softening is a recognized treatment process. The subject of saturation with respect to calcium carbonate is discussed in Section 2330.

2. Selection of Method

A nomographic and a titrimetric method are described for the estimation of free CO_2 in drinking water. The titration may be performed potentiometrically or with phenolphthalein indicator. Properly conducted, the more rapid, simple indicator method is satisfactory for field tests and for control and routine applications if it is understood that the method gives, at best, only an approximation.

The nomographic method (B) usually gives a closer estimation of the total free CO_2 when the pH and alkalinity determinations are made immediately and correctly at the time of sampling. The pH measurement preferably should be made with an electrometric pH meter, properly calibrated with standard buffer solutions in the pH range of 7 to 8. The error resulting from inaccurate pH measurements grows with an increase in total alkalinity. For example, an inaccuracy of 0.1 in the pH determination causes a CO_2 error of 2 to 4 mg/L in the pH range of 7.0 to 7.3 and a total alkalinity of 100 mg $CaCO_3$/L. In the same pH range, the error approaches 10 to 15 mg/L when the total alkalinity is 400 mg as $CaCO_3$/L.

Under favorable conditions, agreement between the titrimetric and nomographic methods is reasonably good. When agreement is not precise and the CO_2 determination is of particular importance, state the method used.

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The calculation of the total CO_2, free and combined, is given in Method D.

4500-CO_2 B. Nomographic Determination of Free Carbon Dioxide and the Three Forms of Alkalinity*#(20)

1. General Discussion

Diagrams and nomographs enable the rapid calculation of the CO_2, bicarbonate, carbonate, and hydroxide content of natural and treated waters. These graphical presentations are based on equations relating the ionization equilibria of the carbonates and water. If pH, total alkalinity, temperature, and total mineral content are known, any or all of the alkalinity forms and CO_2 can be determined nomographically.

A set of charts, Figure 4500-CO_2:1, Figure 4500-CO_2:2, Figure 4500-CO_2:3, and Figure 4500-CO_2:4 †#(21) is presented for use where their accuracy for the individual water supply is confirmed. The nomographs and the equations on which they are based are valid only when the salts of weak acids other than carbonic acid are absent or present in extremely small amounts.

Some treatment processes, such as superchlorination and coagulation, can affect significantly pH and total-alkalinity values of a poorly buffered water of low alkalinity and low total-dissolved-mineral content. In such instances the nomographs may not be applicable.

2. Precision and Bias

The precision possible with the nomographs depends on the size and range of the scales. With practice, the recommended nomographs can be read with a precision of 1%. However, the overall bias of the results depends on the bias of the analytical data applied to the nomographs and the validity of the theoretical equations and the numerical constants on which the nomographs are based. An approximate check of the bias of the calculations can be made by summing the three forms of alkalinity. Their sum should equal the total alkalinity.

3. Bibliography

MOORE, E.W. 1939. Graphic determination of carbon dioxide and the three forms of alkalinity. *J. Amer. Water Works Assoc.* 31:51.

4500-CO_2 C. Titrimetric Method for Free Carbon Dioxide

1. General Discussion

a. Principle: Free CO_2 reacts with sodium carbonate or sodium hydroxide to form sodium bicarbonate. Completion of the reaction is indicated potentiometrically or by the development of the pink color characteristic of phenolphthalein indicator at the equivalence pH of 8.3. A 0.01*N*

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sodium bicarbonate ($NaHCO_3$) solution containing the recommended volume of phenolphthalein indicator is a suitable color standard until familiarity is obtained with the color at the end point.

b. Interference: Cations and anions that quantitatively disturb the normal CO_2-carbonate equilibrium interfere with the determination. Metal ions that precipitate in alkaline solution, such as aluminum, chromium, copper, and iron, contribute to high results. Ferrous ion should not exceed 1.0 mg/L. Positive errors also are caused by weak bases, such as ammonia or amines, and by salts of weak acids and strong bases, such as borate, nitrite, phosphate, silicate, and sulfide. Such substances should not exceed 5% of the CO_2 concentration. The titrimetric method for CO_2 is inapplicable to samples containing acid mine wastes and effluent from acid-regenerated cation exchangers. Negative errors may be introduced by high total dissolved solids, such as those encountered in seawater, or by addition of excess indicator.

c. Sampling and storage: Even with a careful collection technique, some loss in free CO_2 can be expected in storage and transit. This occurs more frequently when the gas is present in large amounts. Occasionally a sample may show an increase in free CO_2 content on standing. Consequently, determine free CO_2 immediately at the point of sampling. Where a field determination is impractical, fill completely a bottle for laboratory examination. Keep the sample, until tested, at a temperature lower than that at which the water was collected. Make the laboratory examination as soon as possible to minimize the effect of CO_2 changes.

2. Apparatus

See Section 2310B.2.

3. Reagents

See Section 2310B.3.

4. Procedure

Follow the procedure given in Section 2310B.4*b*, phenolphthalein, or Section 2310B.4*d*, using end-point pH 8.3.

5. Calculation

$$\text{mg } CO_2/L = \frac{A \times N \times 44\,000}{\text{mL sample}}$$

where:

A = mL titrant and
N = normality of NaOH.

6. Precision and Bias

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Precision and bias of the titrimetric method are on the order of ±10% of the known CO_2 concentration.

4500-CO_2 D. Carbon Dioxide and Forms of Alkalinity by Calculation

1. General Discussion

When the total alkalinity of a water (Section 2320) is due almost entirely to hydroxides, carbonates, or bicarbonates, and the total dissolved solids (Section 2540) is not greater than 500 mg/ L, the alkalinity forms and free CO_2 can be calculated from the sample pH and total alkalinity. The calculation is subject to the same limitations as the nomographic procedure given above and the additional restriction of using a single temperature, 25°C. The calculations are based on the ionization constants:

$$K_1 = \frac{[H^+][HCO_3^-]}{[H_2CO_3^*]} \quad (K_1 = 10^{-6.36})$$

and

$$K_2 = \frac{[H^+][CO_3^{2-}]}{[HCO_3^-]} \quad (K_2 = 10^{-10.33})$$

where:

$$[H_2CO_3^*] = [H_2CO_3] + [CO_2(aq)]$$

Activity coefficients are assumed equal to unity.

2. Calculation

Compute the forms of alkalinity and sample pH and total alkalinity using the following equations:

a. Bicarbonate alkalinity:

$$HCO_3^- \text{ as mg } CaCO_3/L = \frac{T - 5.0 \times 10^{(pH - 10)}}{1 + 0.94 \times 10^{(pH - 10)}}$$

where:

T = total alkalinity, mg $CaCO_3$/L

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b. Carbonate alkalinity:

$$CO_3^{2-} \text{ as mg } CaCO_3/L = 0.94 \times B \times 10^{(pH-10)}$$

where:

B = bicarbonate alkalinity, from *a*.

c. Hydroxide alkalinity:

$$OH^- \text{ as mg } CaCO_3/L = 5.0 \times 10^{(pH-10)}$$

d. Free carbon dioxide:

$$\text{mg } CO_2/L = 2.0 \times B \times 10^{(6-pH)}$$

where:

B = bicarbonate alkalinity, from *a*.

e. Total carbon dioxide:

$$\text{mg total } CO_2/L = A + 0.44\,(2B + C)$$

where:

A = mg free CO_2/L,
B = bicarbonate alkalinity from *a*, and
C = carbonate alkalinity from *b*.

3. Bibliography

DYE, J.F. 1958. Correlation of the two principal methods of calculating the three kinds of alkalinity. *J. Amer. Water Works Assoc.* 50:812.

4500-CN⁻ CYANIDE*#(22)

4500-CN⁻ A. Introduction

1. General Discussion

‘‘Cyanide’’ refers to all of the CN groups in cyanide compounds that can be determined as the cyanide ion, CN^-, by the methods used. The cyanide compounds in which cyanide can be

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obtained as CN^- are classed as simple and complex cyanides.

Simple cyanides are represented by the formula $A(CN)_x$, where A is an alkali (sodium, potassium, ammonium) or a metal, and x, the valence of A, is the number of CN groups. In aqueous solutions of simple alkali cyanides, the CN group is present as CN^- and molecular HCN, the ratio depending on pH and the dissociation constant for molecular HCN ($pK_a \sim 9.2$). In most natural waters HCN greatly predominates.[1] In solutions of simple metal cyanides, the CN group may occur also in the form of complex metal-cyanide anions of varying stability. Many simple metal cyanides are sparingly soluble or almost insoluble [CuCN, AgCN, $Zn(CN)_2$], but they form a variety of highly soluble, complex metal cyanides in the presence of alkali cyanides.

Complex cyanides have a variety of formulae, but the alkali-metallic cyanides normally can be represented by $A_yM(CN)_x$. In this formula, A represents the alkali present y times, M the heavy metal (ferrous and ferric iron, cadmium, copper, nickel, silver, zinc, or others), and x the number of CN groups; x is equal to the valence of A taken y times plus that of the heavy metal. Initial dissociation of each of these soluble, alkali-metallic, complex cyanides yields an anion that is the radical $M(CN)_x^{y-}$. This may dissociate further, depending on several factors, with the liberation of CN^- and consequent formation of HCN.

The great toxicity to aquatic life of molecular HCN is well known;[2-5] it is formed in solutions of cyanide by hydrolytic reaction of CN^- with water. The toxicity of CN^- is less than that of HCN; it usually is unimportant because most of the free cyanide (CN group present as CN^- or as HCN) exists as HCN,[2-5] as the pH of most natural waters is substantially lower than the pK_a for molecular HCN. The toxicity to fish of most tested solutions of complex cyanides is attributable mainly to the HCN resulting from dissociation of the complexes.[2,4,5] Analytical distinction between HCN and other cyanide species in solutions of complex cyanides is possible.[2,5-9,10]

The degree of dissociation of the various metallocyanide complexes at equilibrium, which may not be attained for a long time, increases with decreased concentration and decreased pH, and is inversely related to the highly variable stability of the complexes.[2,4,5] The zinc- and cadmium-cyanide complexes are dissociated almost totally in very dilute solutions; thus these complexes can result in acute toxicity to fish at any ordinary pH. In equally dilute solutions there is much less dissociation for the nickel-cyanide complex and the more stable cyanide complexes formed with copper (I) and silver. Acute toxicity to fish from dilute solutions containing copper-cyanide or silver-cyanide complex anions can be due to the toxicity of the undissociated ions, although the complex ions are much less toxic than HCN.[2,5]

The iron-cyanide complex ions are very stable and not materially toxic; in the dark, acutely toxic levels of HCN are attained only in solutions that are not very dilute and have been aged for a long time. However, these complexes are subject to extensive and rapid photolysis, yielding

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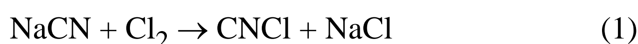
toxic HCN, on exposure of dilute solutions to direct sunlight.[2,11] The photodecomposition depends on exposure to ultraviolet radiation, and therefore is slow in deep, turbid, or shaded receiving waters. Loss of HCN to the atmosphere and its bacterial and chemical destruction concurrent with its production tend to prevent increases of HCN concentrations to harmful levels. Regulatory distinction between cyanide complexed with iron and that bound in less stable complexes, as well as between the complexed cyanide and free cyanide or HCN, can, therefore, be justified.

Historically, the generally accepted physicochemical technique for industrial waste treatment of cyanide compounds is alkaline chlorination:



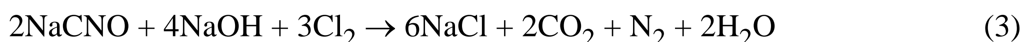
$$NaCN + Cl_2 \rightarrow CNCl + NaCl \qquad (1)$$

The first reaction product on chlorination is cyanogen chloride (CNCl), a highly toxic gas of limited solubility. The toxicity of CNCl may exceed that of equal concentrations of cyanide.[2,3,12] At an alkaline pH, CNCl hydrolyzes to the cyanate ion (CNO^-), which has only limited toxicity.

There is no known natural reduction reaction that may convert CNO^- to CN^-.[13] On the other hand, breakdown of toxic CNCl is pH- and time-dependent. At pH 9, with no excess chlorine present, CNCl may persist for 24 h.[14,15]

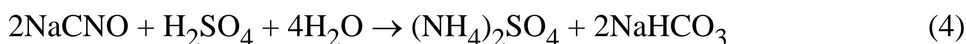
$$CNCl + 2NaOH \rightarrow NaCNO + NaCl + H_2O \qquad (2)$$

CNO^- can be oxidized further with chlorine at a nearly neutral pH to CO_2 and N_2:



$$2NaCNO + 4NaOH + 3Cl_2 \rightarrow 6NaCl + 2CO_2 + N_2 + 2H_2O \qquad (3)$$

CNO^- also will be converted on acidification to NH_4^+:



$$2NaCNO + H_2SO_4 + 4H_2O \rightarrow (NH_4)_2SO_4 + 2NaHCO_3 \qquad (4)$$

The alkaline chlorination of cyanide compounds is relatively fast, but depends equally on the dissociation constant, which also governs toxicity. Metal cyanide complexes, such as nickel, cobalt, silver, and gold, do not dissociate readily. The chlorination reaction therefore requires more time and a significant chlorine excess.[16] Iron cyanides, because they do not dissociate to any degree, are not oxidized by chlorination. There is correlation between the refractory properties of the noted complexes, in their resistance to chlorination and lack of toxicity.

Thus, it is advantageous to differentiate between *total cyanide* and *cyanides amenable to chlorination*. When total cyanide is determined, the almost nondissociable cyanides, as well as cyanide bound in complexes that are readily dissociable and complexes of intermediate stability, are measured. Cyanide compounds that are amenable to chlorination include free cyanide as well as those complex cyanides that are potentially dissociable, almost wholly or in large degree, and

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therefore, potentially toxic at low concentrations, even in the dark. The chlorination test procedure is carried out under rigorous conditions appropriate for measurement of the more dissociable forms of cyanide.

The free and potentially dissociable cyanides also may be estimated when using the *weak acid dissociable* procedure. These methods depend on a rigorous distillation, but the solution is only slightly acidified, and elimination of iron cyanides is insured by the earlier addition of precipitation chemicals to the distillation flask and by the avoidance of ultraviolet irradiation.

The *cyanogen chloride* procedure is common with the colorimetric test for cyanides amenable to chlorination. This test is based on the addition of chloramine-T and subsequent color complex formation with pyridine-barbituric acid solution. Without the addition of chloramine-T, only existing CNCl is measured. CNCl is a gas that hydrolyzes to CNO^-; sample preservation is not possible. Because of this, spot testing of CNCl levels may be best. This procedure can be adapted and used when the sample is collected.

There may be analytical requirements for the determination of CNO^-, even though the reported toxicity level is low. On acidification, CNO^- decomposes to ammonia (NH_3).[3] Molecular ammonia and metal-ammonia complexes are toxic to aquatic life.[17]

Thiocyanate (SCN^-) is not very toxic to aquatic life.[2,18] However, upon chlorination, toxic CNCl is formed, as discussed above.[2,3,12] At least where subsequent chlorination is anticipated, the determination of SCN^- is desirable. Thiocyanate is biodegradable; ammonium is released in this reaction. Although the typical detoxifying agents used in cyanide poisoning induce thiocyanate formation, biochemical cyclic reactions with cyanide are possible, resulting in detectable levels of cyanide from exposure to thiocyanate.[18] Thiocyanate may be analyzed in samples properly preserved for determination of cyanide; however, thiocyanate also can be preserved in samples by acidification with H_2SO_4 to pH ≤2.

2. Cyanide in Solid Waste

a. Soluble cyanide: Determination of soluble cyanide requires sample leaching with distilled water until solubility equilibrium is established. One hour of stirring in distilled water should be satisfactory. Cyanide analysis is then performed on the leachate. Low cyanide concentration in the leachate may indicate presence of sparingly soluble metal cyanides. The cyanide content of the leachate is indicative of residual solubility of insoluble metal cyanides in the waste.

High levels of cyanide in the leachate indicate soluble cyanide in the solid waste. When 500 mL distilled water are stirred into a 500-mg solid waste sample, the cyanide concentration (mg/L) of the leachate multiplied by 1000 will give the solubility level of the cyanide in the solid waste in milligrams per kilogram. The leachate may be analyzed for total cyanide and/or cyanide amenable to chlorination.

b. Insoluble cyanide: The insoluble cyanide of the solid waste can be determined with the total cyanide method by placing a 500-mg sample with 500 mL distilled water in the distillation

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flask and in general following the distillation procedure (Section 4500-CN^-.C). In calculating, multiply by 1000 to give the cyanide content of the solid sample in milligrams per kilogram. Insoluble iron cyanides in the solid can be leached out earlier by stirring a weighed sample for 12 to 16 h in a 10% NaOH solution. The leached and wash waters of the solid waste will give the iron cyanide content with the distillation procedure. Prechlorination will have eliminated all cyanide amenable to chlorination. Do not expose sample to sunlight.

3. Selection of Method

a. Total cyanide after distillation: After removal of interfering substances, the metal cyanide is converted to HCN gas, which is distilled and absorbed in sodium hydroxide (NaOH) solution.[19] Because of the catalytic decomposition of cyanide in the presence of cobalt at high temperature in a strong acid solution,[20,21] cobalticyanide is not recovered completely. Indications are that cyanide complexes of the noble metals, i.e., gold, platinum, and palladium, are not recovered fully by this procedure either. Distillation also separates cyanide from other color-producing and possibly interfering organic or inorganic contaminants. Subsequent analysis is for the simple salt, sodium cyanide (NaCN). Some organic cyanide compounds, such as cyanohydrins, are decomposed by the distillation. Aldehydes convert cyanide to cyanohydrins.

The absorption liquid is analyzed by a titrimetric, colorimetric, or cyanide-ion-selective electrode procedure:

1) The titration method (D) is suitable for cyanide concentrations above 1 mg/L.

2) The colorimetric methods (E, N, and O) are suitable for cyanide concentrations as low as 1 to 5 µg/L under ideal conditions. Method N uses flow injection analysis of the distillate. Method O uses flow injection analysis following transfer through a semipermeable membrane for separating gaseous cyanide, and colorimetric analysis. Method E uses conventional colorimetric analysis of the distillate from Method C.

3) The ion-selective electrode method (F) using the cyanide ion electrode is applicable in the concentration range of 0.05 to 10 mg/L.

b. Cyanide amenable to chlorination:

1) Distillation of two samples is required, one that has been chlorinated to destroy all amenable cyanide present and the other unchlorinated. Analyze absorption liquids from both tests for total cyanide. The observed difference equals cyanides amenable to chlorination.

2) The colorimetric methods, by conversion of amenable cyanide and SCN^- to CNCl and developing the color complex with pyridine-barbituric acid solution, are used for the determination of the total of these cyanides (H, N, and O). Repeating the test with the cyanide masked by the addition of formaldehyde provides a measure of the SCN^- content. When subtracted from the earlier results this provides an estimate of the amenable CN^- content. This method is useful for natural and ground waters, clean metal finishing, and heat treating effluents. Sanitary wastes may exhibit interference.

3) The *weak acid dissociable cyanides* procedure also measures the cyanide amenable to

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chlorination by freeing HCN from the dissociable cyanide. After being collected in a NaOH absorption solution, CN^- may be determined by one of the finishing procedures given for the total cyanide determination. An automated procedure (O) also is presented.

It should be noted that although cyanide amenable to chlorination and weak acid dissociable cyanide appear to be identical, certain industrial effluents (e.g., pulp and paper, petroleum refining industry effluents) contain some poorly understood substances that may produce interference. Application of the procedure for cyanide amenable to chlorination yields negative values. For natural waters and metal-finishing effluents, the direct colorimetric determination appears to be the simplest and most economical.

c. Cyanogen chloride: The colorimetric method for measuring cyanide amenable to chlorination may be used, but omit the chloramine-T addition. The spot test also may be used.

d. Spot test for sample screening: This procedure allows a quick sample screening to establish whether more than 50 µg/L cyanide amenable to chlorination is present. The test also may be used to estimate the CNCl content at the time of sampling.

e. Cyanate: CNO^- is converted to ammonium carbonate, $(NH_4)_2CO_3$, by acid hydrolysis at elevated temperature. Ammonia (NH_3) is determined before the conversion of the CNO^- and again afterwards. The CNO^- is estimated from the difference in NH_3 found in the two tests. [22-24] Measure NH_3 by either:

1) The selective electrode method, using the NH_3 gas electrode (Section 4500-NH_3.D); or

2) The colorimetric method, using the phenate method for NH_3 (Section 4500-NH_3.F or Section 4500-NH_3.G).

f. Thiocyanate: Use the colorimetric determination with ferric nitrate as a color-producing compound.

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4500-CN^- B. Preliminary Treatment of Samples

CAUTION—*Use care in manipulating cyanide-containing samples because of toxicity. Process in a hood or other well-ventilated area. Avoid contact, inhalation, or ingestion.*

1. General Discussion

The nature of the preliminary treatment will vary according to the interfering substance present. Sulfides, fatty acids, and oxidizing agents are removed by special procedures. Most other interfering substances are removed by distillation. The importance of the distillation procedure cannot be overemphasized.

2. Preservation of Samples

Oxidizing agents, such as chlorine, decompose most cyanides. Test by placing a drop of sample on a strip of potassium iodide (KI)-starch paper previously moistened with acetate buffer solution, pH 4 (Section 4500-Cl.C.3*e*). If a bluish discoloration is noted, add 0.1 g sodium arsenite ($NaAsO_2$)/L sample and retest. Repeat addition if necessary. Sodium thiosulfate or ascorbic acid also may be used, but avoid an excess greater than 0.1 g $Na_2S_2O_3$/ L. Manganese dioxide, nitrosyl chloride, etc., if present, also may cause discoloration of the test paper. If possible, carry out this procedure before preserving sample as described below. If the following test indicates presence of sulfide, oxidizing compounds would not be expected.

Oxidized products of sulfide convert CN^- to SCN^- rapidly, especially at high pH.[1] Test for S^{2-} by placing a drop of sample on lead acetate test paper previously moistened with acetic acid buffer solution, pH 4 (Section 4500-Cl.C.3*e*). Darkening of the paper indicates presence of S^{2-}. Add lead acetate, or if the S^{2-} concentration is too high, add powdered lead carbonate [$Pb(CO_3)_2$] to avoid significantly reducing pH. Repeat test until a drop of treated sample no longer darkens the acidified lead acetate test paper. Filter sample before raising pH for stabilization. When particulate, metal cyanide complexes are suspected, filter solution before removing S^{2-}. Reconstitute sample by returning filtered particulates to the sample bottle after S^{2-} removal. Homogenize particulates before analyses.

Aldehydes convert cyanide to cyanohydrin. Longer contact times between cyanide and the aldehyde and the higher ratios of aldehyde to cyanide both result in increasing losses of cyanide that are not reversible during analysis. If the presence of aldehydes is suspected, stabilize with NaOH at time of collection and add 2 mL 3.5% ethylenediamine solution per 100 mL of sample.

Because most cyanides are very reactive and unstable, analyze samples as soon as possible. If sample cannot be analyzed immediately, add NaOH pellets or a strong NaOH solution to raise sample pH to 12 to 12.5, add dechlorinating agent if sample is disinfected, and store in a closed, dark bottle in a cool place.

To analyze for CNCl collect a separate sample and omit NaOH addition because CNCl is

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converted rapidly to CNO^- at high pH. Make colorimetric estimation immediately after sampling.

3. Interferences

a. Oxidizing agents may destroy most of the cyanide during storage and manipulation. Add $NaAsO_2$ or $Na_2S_2O_3$ as directed above; avoid excess $Na_2S_2O_3$.

b. Sulfide will distill over with cyanide and, therefore, adversely affect colorimetric, titrimetric, and electrode procedures. Test for and remove S^{2-} as directed above. Treat 25 mL more than required for the distillation to provide sufficient filtrate volume.

c. Fatty acids that distill and form soaps under alkaline titration conditions make the end point almost impossible to detect. Remove fatty acids by extraction.[2] Acidify sample with acetic acid (1 + 9) to pH 6.0 to 7.0. (CAUTION—*Perform this operation in a hood as quickly as possible.*) Immediately extract with iso-octane, hexane, or $CHCl_3$ (preference in order named). Use a solvent volume equal to 20% of sample volume. One extraction usually is adequate to reduce fatty acid concentration below the interference level. Avoid multiple extractions or a long contact time at low pH to minimize loss of HCN. When extraction is completed, immediately raise pH to >12 with NaOH solution.

d. Carbonate in high concentration may affect the distillation procedure by causing the violent release of carbon dioxide with excessive foaming when acid is added before distillation and by reducing pH of the absorption solution. Use calcium hydroxide to preserve such samples.[3] Add calcium hydroxide slowly, with stirring, to pH 12 to 12.5. After precipitate settles, decant supernatant liquid for determining cyanide.

Insoluble complex cyanide compounds will not be determined. If such compounds are present, filter a measured amount of well-mixed treated sample through a glass fiber or membrane filter (47-mm diam or less). Rinse filter with dilute (1 to 9) acetic acid until effervescence ceases. Treat entire filter with insoluble material as insoluble cyanide (Section 4500-CN^-.A.2*b*) or add to filtrate before distillation.

e. Other possible interferences include substances that might contribute color or turbidity. In most cases, distillation will remove these.

Note, however, that the strong acid distillation procedure requires using sulfuric acid with various reagents. With certain wastes, these conditions may result in reactions that otherwise would not occur in the aqueous sample. As a quality control measure, periodically conduct addition and recovery tests with industrial waste samples.

f. Aldehydes convert cyanide to cyanohydrin, which forms nitrile under the distillation conditions. Only direct titration without distillation can be used, which reveals only non-complex cyanides. Formaldehyde interference is noticeable in concentrations exceeding 0.5 mg/L. Use the following spot test to establish absence or presence of aldehydes (detection limit 0.05 mg/L):[4-6]

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1) Reagents

a) *MBTH indicator solution:* Dissolve 0.05 g 3-methyl, 2-benzothiazolone hydrazone hydrochloride in 100 mL water. Filter if turbid.

b) *Ferric chloride oxidizing solution:* Dissolve 1.6 g sulfamic acid and 1 g $FeCl_3 \cdot 6H_2O$ in 100 mL water.

c) *Ethylenediamine solution,* 3.5%: Dilute 3.5 mL pharmaceutical-grade anhydrous $NH_2CH_2CH_2NH_2$ to 100 mL with water.

2) Procedure—If the sample is alkaline, add 1 + 1 H_2SO_4 to 10 mL sample to adjust pH to less than 8. Place 1 drop of sample and 1 drop distilled water for a blank in separate cavities of a white spot plate. Add 1 drop MBTH solution and then 1 drop $FeCl_3$ oxidizing solution to each spot. Allow 10 min for color development. The color change will be from a faint green-yellow to a deeper green with blue-green to blue at higher concentrations of aldehyde. The blank should remain yellow.

To minimize aldehyde interference, add 2 mL of 3.5% ethylenediamine solution/100 mL sample. This quantity overcomes the interference caused by up to 50 mg/L formaldehyde.

When using a known addition in testing, 100% recovery of the CN^- is not necessarily to be expected. Recovery depends on the aldehyde excess, time of contact, and sample temperature.

g. Glucose and other sugars, especially at the pH of preservation, lead to cyanohydrin formation by reaction of cyanide with aldose.[7] Reduce cyanohydrin to cyanide with ethylenediamine (see above). MBTH is not applicable.

h. Nitrite may form HCN during distillation in Methods C, G, and L, by reacting with organic compounds.[8,9] Also, NO_3^- may reduce to NO_2^-, which interferes. To avoid NO_2^- interference, add 2 g sulfamic acid to the sample before distillation. *Nitrate* also may interfere by reacting with SCN^- .[10]

i. Some sulfur compounds may decompose during distillation, releasing S, H_2S, or SO_2. Sulfur compounds may convert cyanide to thiocyanate and also may interfere with the analytical procedures for CN^-. To avoid this potential interference, add 50 mg $PbCO_3$ to the absorption solution before distillation. Filter sample before proceeding with the colorimetric or titrimetric determination.

Absorbed SO_2 forms Na_2SO_3 which consumes chloramine-T added in the colorimetric determination. The volume of chloramine-T added is sufficient to overcome 100 to 200 mg SO_3^{2-}/L. Test for presence of chloramine-T after adding it by placing a drop of sample on KI-starch test paper; add more chloramine-T if the test paper remains blank, or use Method F.

Some wastewaters, such as those from coal gasification or chemical extraction mining, contain high concentrations of sulfites. Pretreat sample to avoid overloading the absorption solution with SO_3^{2-}. Titrate a suitable sample iodometrically (Section 4500-O) with dropwise

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addition of 30% H_2O_2 solution to determine volume of H_2O_2 needed for the 500 mL distillation sample. Subsequently, add H_2O_2 dropwise while stirring, but in only such volume that not more than 300 to 400 mg SO_3^{2-}/L will remain. Adding a lesser quantity than calculated is required to avoid oxidizing any CN^- that may be present.

j. Alternate procedure: The strong acid distillation procedure uses concentrated acid with magnesium chloride to dissociate metal-cyanide complexes. In some instances, particularly with industrial wastes, it may be susceptible to interferences such as those from conversion of thiocyanate to cyanide in the presence of an oxidant, e.g., nitrate. If such interferences are present use a ligand displacement procedure with a mildly acidic medium with EDTA to dissociate metal-cyanide complexes.[10] Under such conditions thiocyanate is relatively stable and many oxidants, including nitrate, are weaker.

If any cyanide procedure is revised to meet specific requirements, obtain recovery data by the addition of known amounts of cyanide.

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4500-CN⁻ C. Total Cyanide after Distillation

1. General Discussion

Hydrogen cyanide (HCN) is liberated from an acidified sample by distillation and purging with air. The HCN gas is collected by passing it through an NaOH scrubbing solution. Cyanide concentration in the scrubbing solution is determined by titrimetric, colorimetric, or potentiometric procedures.

2. Apparatus

The apparatus is shown in Figure 4500-CN^-:1. It includes:

a. Boiling flask, 1 L, with inlet tube and provision for water-cooled condenser.

b. Gas absorber, with gas dispersion tube equipped with medium-porosity fritted outlet.

c. Heating element, adjustable.

d. Ground glass ST joints, TFE-sleeved or with an appropriate lubricant for the boiling flask and condenser. Neoprene stopper and plastic threaded joints also may be used.

3. Reagents

a. Sodium hydroxide solution: Dissolve 40 g NaOH in water and dilute to 1 L.

b. Magnesium chloride reagent: Dissolve 510 g $MgCl_2 \cdot 6H_2O$ in water and dilute to 1 L.

c. Sulfuric acid, H_2SO_4, 1 + 1.

d. Lead carbonate, $PbCO_3$, powdered.

e. Sulfamic acid, NH_2SO_3H.

4. Procedure

a. Add 500 mL sample, containing not more than 10 mg CN^-/ L (diluted if necessary with distilled water) to the boiling flask. If a higher CN^- content is anticipated, use the spot test (4500-CN^-.K) to approximate the required dilution. Add 10 mL NaOH solution to the gas scrubber and dilute, if necessary, with distilled water to obtain an adequate liquid depth in the absorber. Do not use more than 225 mL total volume of absorber solution. When S^{2-} generation from the distilling flask is anticipated add 50 or more mg powdered $PbCO_3$ to the absorber solution to precipitate S^{2-}. Connect the train, consisting of boiling flask air inlet, flask, condenser, gas washer, suction flask trap, and aspirator. Adjust suction so that approximately 1 air bubble/s enters the boiling flask. This air rate will carry HCN gas from flask to absorber and usually will prevent a reverse flow of HCN through the air inlet. If this air rate does not prevent

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sample backup in the delivery tube, increase air-flow rate to 2 air bubbles/s. Observe air purge rate in the absorber where the liquid level should be raised not more than 6.5 to 10 mm. Maintain air flow throughout the reaction.

b. Add 2 g sulfamic acid through the air inlet tube and wash down with distilled water.

c. Add 50 mL 1 1 H_2SO_4 through the air inlet tube. Rinse tube with distilled water and let air mix flask contents for 3 min. Add 20 mL $MgCl_2$ reagent through air inlet and wash down with stream of water. A precipitate that may form redissolves on heating.

d. Heat with rapid boiling, but do not flood condenser inlet or permit vapors to rise more than halfway into condenser. Adequate refluxing is indicated by a reflux rate of 40 to 50 drops/min from the condenser lip. Reflux for at least 1 h. Discontinue heating but continue air flow for 15 min. Cool and quantitatively transfer absorption solution to a 250-mL volumetric flask. Rinse absorber and its connecting tubing sparingly with distilled water and add to flask. Dilute to volume with distilled water and mix thoroughly.

e. Determine cyanide concentration in the absorption solution by procedure of Section 4500-CN^-.D, Section 4500-CN^-.E, or Section 4500-CN^-.F.

f. Distillation gives quantitative recovery of even refractory cyanides such as iron complexes. To obtain complete recovery of cobalticyanide use ultraviolet radiation pretreatment.[1,2] If incomplete recovery is suspected, distill again by refilling the gas washer with a fresh charge of NaOH solution and refluxing 1 h more. The cyanide from the second reflux, if any, will indicate completeness of recovery.

g. As a quality control measure, periodically test apparatus, reagents, and other potential variables in the concentration range of interest. As an example at least 100 ± 4% recovery from 1 mg CN^-/L standard should be obtained.

5. References

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4500-CN^- D. Titrimetric Method

1. General Discussion

a. Principle: CN^- in the alkaline distillate from the preliminary treatment procedure is titrated with standard silver nitrate ($AgNO_3$) to form the soluble cyanide complex, $Ag(CN)_2^-$. As soon as all CN^- has been complexed and a small excess of Ag^+ has been added, the excess Ag^+

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is detected by the silver-sensitive indicator, *p*-dimethylaminobenzalrhodanine, which immediately turns from a yellow to a salmon color.[1] The distillation has provided a 2:1 concentration. The indicator is sensitive to about 0.1 mg Ag/L. If titration shows that CN^- is below 1 mg/L, examine another portion colorimetrically or potentiometrically.

2. Apparatus

Koch microburet, 10-mL capacity.

3. Reagents

a. Indicator solution: Dissolve 20 mg *p*-dimethylaminobenzalrhodanine in 100 mL acetone.

b. Standard silver nitrate titrant: Dissolve 3.27 g $AgNO_3$ in 1 L distilled water. Standardize against standard NaCl solution, using the argentometric method with K_2CrO_4 indicator, as directed in Chloride, Section 4500-Cl^-.B.

Dilute 500 mL $AgNO_3$ solution according to the titer found so that 1.00 mL is equivalent to 1.00 mg CN^-.

c. Sodium hydroxide dilution solution: Dissolve 1.6 g NaOH in 1 L distilled water.

4. Procedure

a. From the absorption solution take a measured volume of sample so that the titration will require approximately 1 to 10 mL $AgNO_3$ titrant. Dilute to 100 mL using the NaOH dilution solution or to some other convenient volume to be used for all titrations. For samples with low cyanide concentration (≤5 mg/L) do not dilute. Add 0.5 mL indicator solution.

b. Titrate with standard $AgNO_3$ titrant to the first change in color from a canary yellow to a salmon hue. Titrate a blank containing the same amount of alkali and water, i.e., 100 mL NaOH dilution solution (or volume used for sample). As the analyst becomes accustomed to the end point, blank titrations decrease from the high values usually experienced in the first few trials to 1 drop or less, with a corresponding improvement in precision.

5. Calculation

$$\text{mg CN}^-/\text{L} = \frac{(A - B) \times 1000}{\text{mL original sample}} \times \frac{250}{\text{mL portion used}}$$

where:

A = mL standard $AgNO_3$ for sample and

B = mL standard $AgNO_3$ for blank.

6. Precision and Bias[2]

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Based on the results of six operators in three laboratories, the overall and single-operator precision of this method within its designated range may be expressed as follows:

Reagent water: $S_T = 0.04x + 0.038$
$S_o = 0.01x + 0.018$

Selected water matrices: $S_T = 0.06x + 0.711$
$S_o = 0.04x + 0.027$

where:

S_T = overall precision, mg/L,
S_o = single-operator precision, mg/L, and
x = cyanide concentration, mg/L.

Recoveries of known amounts of cyanide from reagent water and selected water matrices are:

Medium	Added *mg/L*	Recovered *mg/L*	*n*	S_T	Bias	% Bias
Reagent	2.00	2.10	18	0.1267	0.10	5
water	5.00	4.65	18	0.2199	–0.35	–7
	5.00	5.18	18	0.2612	0.18	4
Selected	2.00	2.80	18	0.8695	0.80	40
water	5.00	5.29	18	1.1160	0.29	6
matrices	5.00	5.75	18	0.9970	0.75	15

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4500-CN^- E. Colorimetric Method

1. General Discussion

a. Principle: CN^- in the alkaline distillate from preliminary treatment is converted to CNCl

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by reaction with chloramine-T at pH <8 without hydrolyzing to CNO^-.[1] (CAUTION—*CNCl is a toxic gas; avoid inhalation*.) After the reaction is complete, CNCl forms a red-blue color on addition of a pyridine-barbituric acid reagent. Maximum color absorbance in aqueous solution is between 575 and 582 nm. To obtain colors of comparable intensity, have the same salt content in sample and standards.

b. Interference: All known interferences are eliminated or reduced to a minimum by distillation.

2. Apparatus

Colorimetric equipment: One of the following is required:

a. Spectrophotometer, for use at 578 nm, providing a light path of 10 mm or longer.

b. Filter photometer, providing a light path of at least 10 mm and equipped with a red filter having maximum transmittance at 570 to 580 nm.

3. Reagents

a. Chloramine-T solution: Dissolve 1.0 g white, water-soluble powder in 100 mL water. Prepare weekly and store in refrigerator.

b. Stock cyanide solution: Dissolve approximately 1.6 g NaOH and 2.51 g KCN in 1 L distilled water. (CAUTION—*KCN is highly toxic; avoid contact or inhalation.*) Standardize against standard silver nitrate ($AgNO_3$) titrant as described in Section 4500-CN^-D.4, using 25 mL KCN solution. Check titer weekly because the solution gradually loses strength; 1 mL = 1 mg CN^-.

c. Standard cyanide solution: Based on the concentration determined for the KCN stock solution (¶ 3*b*) calculate volume required (approximately 10 mL) to prepare 1 L of a 10 µg CN^-/mL solution. Dilute with the NaOH dilution solution. Dilute 10 mL of the 10 µg CN^-/mL solution to 100 mL with the NaOH dilution solution; 1.0 mL = 1.0 µg CN^-. Prepare fresh daily and keep in a glass-stoppered bottle. (CAUTION—*Toxic; take care to avoid ingestion.*)

d. Pyridine-barbituric acid reagent: Place 15 g barbituric acid in a 250-mL volumetric flask and add just enough water to wash sides of flask and wet barbituric acid. Add 75 mL pyridine and mix. Add 15 mL conc hydrochloric acid (HCl), mix, and cool to room temperature. Dilute to volume and mix until barbituric acid is dissolved. The solution is stable for approximately 6 months if stored in an amber bottle under refrigeration; discard if precipitate develops.

e. Acetate buffer: Dissolve 410 g sodium acetate trihydrate, $NaC_2H_3O_2 \cdot 3H_2O$, in 500 mL of water. Add glacial acetic acid to adjust to pH 4.5, approximately 500 mL.

f. Sodium hydroxide dilution solution: Dissolve 1.6 g NaOH in 1 L distilled water.

4. Procedure

a. Preparation of standard curve: Pipet a series of standards containing 1 to 10 µg CN^- into

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50-mL volumetric flasks (0.02 to 0.2 µg CN^-/mL). Dilute to 40 mL with NaOH dilution solution. Use 40 mL of NaOH dilution solution as blank. Develop and measure absorbance in 10-mm cells as described in ¶ b for both standards and blank. For concentrations lower than 0.02 µg CN^-/mL use 100-mm cells.

Recheck calibration curve periodically and each time a new reagent is prepared.

b. Color development: Pipet a portion of absorption solution into a 50-mL volumetric flask and dilute to 40 mL with NaOH dilution solution. Add 1 mL acetate buffer and 2 mL chloramine-T solution, stopper, and mix by inversion twice. Let stand exactly 2 min.

Add 5 mL pyridine-barbituric acid reagent, dilute to volume with distilled water, mix thoroughly, and let stand exactly 8 min. Measure absorbance against distilled water at 578 nm.

Measure absorbance of blank (0.0 mg CN^-/L) using 40 mL NaOH dilution solution and procedures for color development.

5. Calculation

Use the linear regression feature available on most scientific calculators, or compute slope and intercept of standard curve as follows:

$$m = \frac{n \sum ca - \sum c \sum a}{n \sum a^2 - (\sum a)^2}$$

$$b = \frac{\sum a^2 \sum c - \sum a \sum ac}{n \sum a^2 - (\sum a)^2}$$

where:

a = absorbance of standard solution,
c = concentration of CN^- in standard, mg/L,
n = number of standard solutions,
m = slope of standard curve, and
b = intercept on c axis.

Include the blank concentration, 0.0 mg CN^-/L and blank absorbance in the calculations above.

$$CN^-, \text{mg/L} = (ma_1 + b) \times \frac{50}{X} \times \frac{250}{Y}$$

where:

X = absorption solution, mL,

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Y = original sample, mL, and
a_1 = absorbance of sample solution.

6. Precision and Bias[2]

Based on the results of nine operators in nine laboratories, the overall and single-operator precision of this method within its designated ranges may be expressed as follows:

$$\text{Reagent water: } S_T = 0.06x + 0.003$$
$$S_o = 0.11x + 0.010$$

$$\text{Selected water matrices: } S_T = 0.04x + 0.018$$
$$S_o = 0.04x + 0.008$$

where:

S_T = overall precision, mg/L,
S_o = single-operator precision, mg/L, and
x = cyanide concentration, mg/L.

Recoveries of known amounts of cyanide from reagent water and selected water matrices (coke plant and refinery wastes, sewage, and surface water) are:

Medium	Added *mg/L*	Recovered *mg/L*	*n*	S_T	Bias	% Bias
Reagent	0.060	0.060	26	0.0101	0.000	0
water	0.500	0.480	23	0.0258	–0.020	–4
	0.900	0.996	27	0.0669	0.096	11
Selected	0.060	0.060	25	0.0145	0.000	0
water	0.500	0.489	26	0.0501	–0.011	–3
matrices	0.900	0.959	24	0.0509	0.059	7

7. References

1. AMUS, E. & H. GARSCHAGEN. 1953. Über die Verwendung der Barbitsäure für die photometrische Bestimmund von Cyanid und Rhodanid. *Z. Anal. Chem.* 138:414.
2. AMERICAN SOCIETY FOR TESTING & MATERIALS. 1987. Research Rep. D2036:19-1131. American Soc. Testing & Materials, Philadelphia, Pa.

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4500-CN⁻ F. Cyanide-Selective Electrode Method

1. General Discussion

CN⁻ in the alkaline distillate from the preliminary treatment procedures can be determined potentiometrically by using a CN⁻-selective electrode in combination with a double-junction reference electrode and a pH meter having an expanded millivolt scale, or a specific ion meter. This method can be used to determine CN⁻ concentration in place of either the colorimetric or titrimetric procedures in the concentration range of 0.05 to 10 mg CN⁻/L.[1-3] If the CN⁻-selective electrode method is used, the previously described titration screening step can be omitted.

2. Apparatus

a. Expanded-scale pH meter or specific-ion meter.

*b. Cyanide-ion-selective electrode.**#(23)

c. Reference electrode, double-junction.

d. Magnetic mixer with TFE-coated stirring bar.

e. Koch microburet, 10-mL capacity.

3. Reagents

a. Stock standard cyanide solution: See Section 4500-CN⁻.E.3*b*.

b. Sodium hydroxide dilution solution: Dissolve 1.6 g NaOH in water and dilute to 1 L.

c. Standard cyanide solution: Dilute a calculated volume (approximately 25 mL) of stock KCN solution, based on the determined concentration, to 1000 mL with NaOH diluent. Mix thoroughly; 1 mL 25 µg CN⁻.

d. Dilute standard cyanide solution: Dilute 100.0 mL standard CN⁻ solution to 1000 mL with NaOH diluent; 1.00 mL = 2.5 µg CN⁻. Prepare daily and keep in a dark, glass-stoppered bottle.

e. Potassium nitrate solution: Dissolve 100 g KNO_3 in water and dilute to 1 L. Adjust to pH 12 with KOH. This is the outer filling solution for the double-junction reference electrode.

4. Procedure

a. Calibration: Using Koch microburet and standard CN⁻ solution, prepare four (or more) additional solutions containing 2.5, 0.25, 0.125, and 0.025 µg CN⁻/mL in NaOH dilution solution. Transfer approximately 100 mL of each of these standard solutions into a 250-mL beaker prerinsed with a small portion of standard being tested. Immerse CN⁻ and double-junction reference electrodes. Mix well on a magnetic stirrer at 25°C, maintaining as closely as possible the same stirring rate for all solutions.

Always progress from the lowest to the highest concentration of standard because otherwise

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equilibrium is reached only slowly. The electrode membrane dissolves in solutions of high CN^- concentration; do not use with a concentration above 25 μg CN^-/mL. After making measurements remove electrode and soak in water.

After equilibrium is reached (at least 5 min and not more than 10 min), record potential (millivolt) readings. Plot CN^- concentration on logarithmic axis of semilogarithmic paper versus potential developed in solution on linear axis. A straight line with a slope of approximately 59 mV per decade indicates that the instrument and electrodes are operating properly. Record slope of line obtained (millivolts/decade of concentration). The slope may vary somewhat from the theoretical value of 59.2 mV per decade because of manufacturing variation and reference electrode (liquid-junction) potentials. The slope should be a straight line and is the basis for calculating sample concentration. Follow manufacturer's instructions for direct-reading ion meters.

b. Measurement of sample: Place 100 mL of absorption liquid obtained in Section 4500-CN^-.C.4*d* (or an accurately measured portion diluted to 100 mL with NaOH dilution solution) into a 250-mL beaker. When measuring low CN^- concentrations, first rinse beaker and electrodes with a small volume of sample. Immerse CN^- and double-junction reference electrodes and mix on a magnetic stirrer at the same stirring rate used for calibration. After equilibrium is reached (at least 5 min and not more than 10 min), record values indicated on ion meter or found from graph prepared as above. Calculate concentration as directed below.

5. Calculations

$$\text{mg } CN^-/L = \mu g\ CN^-/mL \text{ from graph or meter} \times \frac{100}{x} \times \frac{250}{y}$$

where:

x = volume of absorption solution, mL, and
y = volume of original sample, mL.

6. Precision and Bias[4]

The precision of the CN^--ion-selective electrode method using the absorption solution from total cyanide distillation has been found in collaborative testing to be linear within its designated range.

Based on the results of six operators in five laboratories, the overall and single-operator precision of this method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.06x + 0.003$$

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$$S_o = 0.03x + 0.008$$

Selected water matrices: $S_T = 0.05x + 0.008$

$$S_o = 0.03x + 0.012$$

where:

S_T = overall precision, mg/L,

S_o = single-operator precision, mg/L, and

x = cyanide concentration, mg/L.

Recoveries of known amounts of cyanide from reagent water and selected water matrices are:

Medium	Added *mg/L*	Recovered *mg/L*	*n*	S_T	Bias	% Bias
Reagent	0.060	0.059	18	0.0086	–0.001	2
water	0.500	0.459	18	0.0281	–0.041	–8
	0.900	0.911	18	0.0552	0.011	1
	5.00	5.07	18	0.297	0.07	1
Selected	0.060	0.058	14	0.0071	–0.002	–3
water	0.500	0.468	21	0.0414	–0.032	–6
matrices	0.900	0.922	19	0.0532	0.022	2
	5.00	5.13	20	0.2839	0.13	3

7. References

1. ORION RESEARCH, INC. 1975. Cyanide Ion Electrode Instruction Manual. Cambridge, Mass.
2. FRANT, M.S., J.W. ROSS & J.H. RISEMAN. 1972. An electrode indicator technique for measuring low levels of cyanide. *Anal. Chem.* 44:2227.
3. SEKERKA, J. & J.F. LECHNER. 1976. Potentiometric determination of low levels of simple and total cyanides. *Water Res.* 10:479.
4. AMERICAN SOCIETY FOR TESTING & MATERIALS. 1987. Research Rep. D2036:19-1131. American Soc. Testing & Materials, Philadelphia, Pa.

4500-CN⁻ G. Cyanides Amenable to Chlorination after Distillation

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1. General Discussion

This method is applicable to the determination of cyanides amenable to chlorination.

After part of the sample is chlorinated to decompose the cyanides, both the chlorinated and the untreated sample are subjected to distillation as described in Section 4500-CN^-.C. The difference between the CN^- concentrations found in the two samples is expressed as cyanides amenable to chlorination.

Some unidentified organic chemicals may oxidize or form breakdown products during chlorination, giving higher results for cyanide after chlorination than before chlorination. This may lead to a negative value for cyanides amenable to chlorination after distillation for wastes from, for example, the steel industry, petroleum refining, and pulp and paper processing. Where such interferences are encountered use Method Section 4500-CN^-.I for determining dissociable cyanide.

Protect sample from exposure to ultraviolet radiation, and perform manipulations under incandescent light, to prevent photodecomposition of some metal-cyanide complexes by ultraviolet light.

2. Apparatus

a. Distillation apparatus: See Section 4500-CN^-.C.2.

b. Apparatus for determining cyanide by either the titrimetric method, Section 4500-CN^-.D.2, the colorimetric method, Section 4500-CN^-.E.2, or the electrode method, Section 4500-CN^-.F.2.

3. Reagents

a. All reagents listed in Section 4500-CN^-.C.3.

b. All reagents listed in Section 4500-CN^-.D.3, Section 4500-CN^-.E.3, or Section 4500-CN^-.F.3, depending on method of estimation.

c. Calcium hypochlorite solution: Dissolve 5 g $Ca(OCl)_2$ in 100 mL distilled water. Store in an amber-colored glass bottle in the dark. Prepare monthly.

d. Potassium iodide(KI)-starch test paper.

4. Procedure

a. Divide sample into two equal portions of 500 mL (or equal portions diluted to 500 mL) and chlorinate one as in ¶ b below. Analyze both portions for CN^-. The difference in determined concentrations is the cyanide amenable to chlorination.

b. Place one portion in a 1-L beaker covered with aluminum foil or black paper. Keep beaker covered with a wrapped watch glass during chlorination. Add $Ca(OCl)_2$ solution dropwise to sample while agitating and maintaining pH between 11 and 12 by adding NaOH solution. Test

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for chlorine by placing a drop of treated sample on a strip of KI-starch paper. A distinct blue color indicates sufficient chlorine (approximately 50 to 100 mg Cl_2/L). Maintain excess residual chlorine for 1 h while agitating. If necessary, add more $Ca(OCl)_2$ and/or NaOH.

c. After 1 h remove any residual chlorine by dropwise addition of $NaAsO_2$ solution (2 g/100 mL) or by addition of 8 drops H_2O_2 (3%) followed by 4 drops $Na_2S_2O_3$ solution (500 g/L). Test with KI-starch paper until there is no color change.

d. Distill both chlorinated and unchlorinated samples as in Section 4500-CN^-.C. Test according to Methods D, E, or F.

5. Calculation

$$\text{mg CN}^- \text{ amenable to chlorination/L} = G - H$$

where:

G = mg CN^-/L found in unchlorinated portion of sample and
H = mg CN^-/L found in chlorinated portion of sample.

For samples containing significant quantities of iron cyanides, it is possible that the second distillation will give a higher value for CN^- than the test for total cyanide, leading to a negative result. When the difference is within the precision limits of the method, report, ‘‘no determinable quantities of cyanide amenable to chlorination.’’ If the difference is greater than the precision limit, ascertain the cause such as presence of interferences, manipulation of the procedure, etc., or use Method I.

6. Precision and Bias[1]

The precision and bias information given in this section may not apply to waters of untested matrices.

a. Precision:

1) Colorimetric—Based on the results of eight operators in seven laboratories, the overall and single-operator precision of this test method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.18x + 0.005$$
$$S_o = 0.06x + 0.003$$

$$\text{Selected water matrices: } S_T = 0.20x + 0.009$$
$$S_o = 0.05x + 0.005$$

2) Titrimetric—Based on the results of six operators in three laboratories, the overall and

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single-operator precision of this test method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.01x + 0.439$$
$$S_o = 0.241 - 0.03x$$

$$\text{Selected water matrices: } S_T = 0.12x + 0.378$$
$$S_o = 0.209 - 0.01x$$

where:

S_T = overall precision, mg/L,
S_o = single-operator precision, mg/L, and
x = cyanide concentration, mg CN^-/L

b. Bias: Recoveries of known amount of cyanide amenable to chlorination from reagent water and selected water matrices are shown below:

Medium	Technique	Added *mg/L*	Recovered *mg/L*	*n*	S_T	Bias	% Bias
Reagent water	Colorimetric	0.008	0.009	21	0.0033	0.001	13
		0.019	0.023	20	0.0070	0.004	21
		0.080	0.103	20	0.0304	0.018	23
		0.191	0.228	21	0.0428	0.037	19
	Titrimetric	1.00	0.73	18	0.350	–0.27	–27
		1.00	0.81	18	0.551	–0.19	–19
		4.00	3.29	18	0.477	–0.71	–18
Selected water matrices	Colorimetric	0.008	0.013	17	0.0077	0.005	63
		0.019	0.025	18	0.0121	0.006	32
		0.080	0.100	18	0.0372	0.020	25
		0.191	0.229	18	0.0503	0.038	20
	Titrimetric	1.00	1.20	18	0.703	0.20	20
		1.00	1.10	18	0.328	0.10	10
		4.00	3.83	18	0.818	–0.17	–4

7. Reference

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1. AMERICAN SOCIETY FOR TESTING & MATERIALS. 1987. Research Rep. D2036:19-1131. American Soc. Testing & Materials, Philadelphia, Pa.

4500-CN^- H. Cyanides Amenable to Chlorination without Distillation (Short-Cut Method)

1. General Discussion

This method covers the determination of HCN and of CN complexes that are amenable to chlorination and also thiocyanates (SCN^-). The procedure does not measure cyanates (CNO^-) or iron cyanide complexes, but does determine cyanogen chloride (CNCl). It may be modified for use in presence of thiocyanates. The method requires neither lengthy distillation nor the chlorination of one sample before distillation. The recovery of CN^- from metal cyanide complexes will be comparable to that in Methods G and I.

The cyanides are converted to CNCl by chloramine-T after the sample has been heated. In the absence of nickel, copper, silver, and gold cyanide complexes or SCN^-, the CNCl may be developed at room temperature. The pyridine-barbituric acid reagent produces a red-blue color in the sample. The color can be estimated visually against standards or photometrically at 578 nm. The dissolved salt content in the standards used for the development of the calibration curve should be near the salt content of the sample, including the added NaOH and phosphate buffer.

The method's usefulness is limited by thiocyanate interference. Although the procedure allows the specific determination of CN^- amenable to chlorination (see Section 4500-CN^-.H.2 and Section 4500-CN^-.H.5) by masking the CN^- content and thereby establishing a correction for the thiocyanide content, the ratio of SCN^- to CN^- should not exceed 3 to be applicable. In working with unknown samples, screen the sample for SCN^- by the spot test (Section 4500-CN^-.K).

2. Interferences

a. Remove interfering agents as described in Section 4500-CN^-.B with the exception of NO_2^- and NO_3^- (Section 4500-CN^-.B.3*h*).

b. The SCN^- ion reacts with chloramine-T to give a positive error equivalent to its concentration. The procedure allows the separate determination of SCN^- and subtraction of this value from the results for the total. Use the spot test (Section 4500-CN^-.K) for SCN^- when its presence is suspected. If the SCN^- content is more than three times the CN^- content, use Method G or I.

c. Reducing chemical compounds, such as SO_3^{2-}, may interfere by consuming chlorine in the chloramine-T addition. A significant excess of chlorine is provided, but the procedure prescribes

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a test (Section 4500-CN^-.H.5*d*) to avoid this interference.

d. Color and turbidity may interfere with the colorimetric determination. Overcome this interference by extraction with chloroform (Section 4500-CN^-.B.3*c*) but omit reduction of the pH. Otherwise, use Method G or I.

Compensation for color and turbidity may be made by determining absorbance of a second sample solution to which all reagents except chloramine-T have been added.

e. Color intensity and absorption are affected by wide variations in total dissolved solids content of the sample.

For samples containing high concentrations of dissolved solids (3000 to 10 000 mg/L), add 6 g NaCl/L NaOH solution (1.6 g/L) used to prepare standards. For samples containing dissolved solids concentrations greater than 10 000 mg/L, add sufficient NaCl to the NaOH solution to approximate the dissolved solids content.

3. Apparatus

a. Apparatus listed in Section 4500-CN^-.E.2.

b. Hot water bath.

4. Reagents

a. Reagents listed in Section 4500-CN^-.B and Section 4500-CN^-.E.3.

b. Sodium chloride, NaCl, crystals.

c. Sodium carbonate, Na_2CO_3, crystals.

d. Sulfuric acid solution, H_2SO_4, 1*N*.

e. EDTA solution, 0.05*M:* Dissolve 18.5 g disodium salt of ethylenediamine tetraacetic acid in water and dilute to 1 L.

f. Formaldehyde solution, 10%: Dilute 27 mL formaldehyde (37% pharmaceutical grade) to 100 mL.

g. Phosphate buffer: Dissolve 138 g sodium dihydrogen phosphate monohydrate, $NaH_2PO_4 \cdot H_2O$, in water and dilute to 1 L. Refrigerate.

5. Procedure

a. Calibrate as directed in Section 4500-CN^-.E.1*a* and Section 4500-CN^-.E.4*a*. For samples with more than 3000 mg total dissolved solids/L, prepare a calibration curve from standards and blank NaOH solutions containing 6 g NaCl/L. Samples containing total dissolved solids exceeding 10 000 mg/L require appropriate standards and a new calibration curve.

b. Adjust pH of 50 mL sample to between 11.4 and 11.8. If acid is needed, add a small amount (0.2 to 0.4 g) of sodium carbonate and stir to dissolve. Then add HCl solution (1+9) dropwise while stirring. For raising the pH, use NaOH solution (40 g/L).

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c. Pipet 20.0 mL of adjusted sample into a 50-mL volumetric flask. If the cyanide concentration is greater than 0.3 mg/L, use a smaller portion and dilute to 20 mL with NaOH solution. Do not exceed the concentration limit of 0.3 mg/L.

d. To insure uniform color development, both in calibration and testing, maintain a uniform temperature. Immerse flasks in a water bath held at 27 ± 1°C for 10 min before adding reagents and keep samples in water bath until all reagents have been added.

Add 4 mL phosphate buffer and swirl to mix. Add one drop of EDTA solution, and mix.

e. Add 2 mL chloramine-T solution and swirl to mix. Place 1 drop of sample on potassium iodide-starch test paper that has been moistened previously with acetate buffer solution. Repeat the chloramine-T addition if required. After exactly 3 min, add 5 mL pyridine-barbituric acid reagent and swirl to mix.

f. Remove samples from water bath, dilute to volume, and mix. Allow 8 min from the addition of the pyridine-barbituric acid reagent for color development.

Determine absorbance at 578 nm in a 1.0-cm cell versus distilled water.

Calculate concentration of cyanide, mg/L in the original sample following instructions given in 4500-CN^- E.

g. If the presence of thiocyanate is suspected, pipet a second 20-mL portion of pH-adjusted sample into a 50-mL volumetric flask. Add 3 drops 10% formaldehyde solution. Mix and let stand 10 min. Place in a water bath at 27 ± 1°C for an additional 10 min before the addition of the reagent chemicals and hold in the bath until all reagents have been added.

Continue with *b* above.

Calculate the concentration of cyanide, as milligrams per liter, in the original sample following instructions given in Section 4500-CN^-.E.

h. In the presence of thiocyanate, cyanide amenable to chlorination is equal to the difference between the concentrations of cyanide obtained in and *g*.

6. Calculation

See Section 4500-CN^-.E.5.

Deduct SCN^- value from the results found when the CN^- has not been masked by formaldehyde addition (total) for cyanide content.

7. Precision and Bias[1]

This precision and bias information may not apply to waters of untested matrices.

a. Precision: Based on the results of 14 operators in nine laboratories, the overall and single-operator precision of this test method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.10x + 0.006$$

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$$S_o = 0.07x + 0.005$$

Selected water matrices: $S_T = 0.11x + 0.007$

$$S_o = 0.02x + 0.005$$

where:

S_T = overall precision, mg/L,

S_o = single-operator precision, mg/L, and

x = cyanide concentration, mg/L.

b. Bias: Recoveries of known amounts of cyanide from reagent water and selected water matrices including creek waters, diluted sewage (1 to 20), and industrial wastewater are shown below.

Medium	Added *mg/L* CN⁻	SCN⁻	Recovered *mg/L*	*n*	S_T	Bias	% Bias
Reagent water	0.005		0.007	42	0.0049	0.002	40
	0.027		0.036	41	0.0109	0.009	25
	0.090		0.100	42	0.0167	0.010	11
	0.090	0.080	0.080	39	0.0121	–0.010	11
	0.270		0.276	42	0.0320	0.006	2
Selected water matrices	0.005		0.003	40	0.0042	–0.002	40
	0.027		0.026	42	0.0093	–0.001	4
	0.090		0.087	42	0.0202	–0.003	3
	0.090	0.080	0.068	37	0.0146	–0.022	24
	0.270		0.245	41	0.0319	–0.025	9

8. Reference

1. AMERICAN SOCIETY FOR TESTING & MATERIALS. 1987. Research Rep. D2036:19-1074. American Soc. Testing & Materials, Philadelphia, Pa.

4500-CN⁻ I. Weak Acid Dissociable Cyanide

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1. General Discussion

Hydrogen cyanide (HCN) is liberated from a slightly acidified (pH 4.5 to 6.0) sample under the prescribed distillation conditions. The method does not recover CN^- from tight complexes that would not be amenable to oxidation by chlorine. The acetate buffer used contains zinc salts to precipitate iron cyanide as a further assurance of the selectivity of the method. In other respects the method is similar to 4500-CN^-.C.

2. Interferences

See Section 4500-CN^-.B.3.

Protect sample and apparatus from ultraviolet light to prevent photodecomposition of some metal-cyanide complexes and an increase in concentration of weak acid dissociable cyanide.

If procedure is used to determine low concentrations of cyanide in samples of ferri- and ferrocyanide, add more, e.g., fivefold excess, zinc acetate solution before adding acid and distilling.

3. Apparatus

See Section 4500-CN^-.C.2 and Figure 4500-CN^-:1, and also Section 4500-CN^-.D.2, Section 4500-CN^-.E.2, or Section 4500-CN^-.F.2, depending on method of estimation.

4. Reagents

a. Reagents listed in Section 4500-CN^-.C.3.

b. Reagents listed in Section 4500-CN^-.D.3, Section 4500-CN^-.E.3, or Section 4500-CN^-.F.3, depending on method of estimation.

c. Acetic acid, 1 + 9: Mix 1 volume of glacial acetic acid with 9 volumes of water.

d. Acetate buffer: Dissolve 410 g sodium acetate trihydrate ($NaC_2H_3O_2 \cdot 3H_2O$) in 500 mL water. Add glacial acetic acid to yield a solution pH of 4.5 (approximately 500 mL).

e. Zinc acetate solution, 100 g/L: Dissolve 120 g $Zn(C_2H_3O_2)_2 \cdot 2H_2O$ in 500 mL water. Dilute to 1 L.

f. Methyl red indicator.

5. Procedure

Follow procedure described in Section 4500-CN^-.C.4, but with the following modifications:

a. Do not add sulfamic acid, because NO_2^- and NO_3^- do not interfere.

b. Instead of H_2SO_4 and $MgCl_2$ reagents, add 20 mL each of the acetate buffer and zinc acetate solutions through air inlet tube. Also add 2 to 3 drops methyl red indicator. Rinse air inlet tube with water and let air mix contents. If the solution is not pink, add acetic acid (1 + 9) dropwise through air inlet tube until a pink color persists.

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c. Follow instructions beginning with Section 4500-CN^-.C.4*d*.

d. For determining CN^- in the absorption solution, use the preferred finish method (Section 4500-CN^-.D, Section 4500-CN^-.E, or Section 4500-CN^-.F).

6. Precision and Bias[1]

The precision and bias information given in this section may not apply to waters of untested matrices.

a. Precision:

1) Colorimetric—Based on the results of nine operators in nine laboratories, the overall and single-operator precision of this test method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.09x + 0.010$$
$$S_o = 0.08x + 0.005$$

$$\text{Selected water matrices: } S_T = 0.08x + 0.012$$
$$S_o = 0.05x + 0.008$$

2) Electrode—Based on the results of six operators in five laboratories, the overall and single-operator precision of this test method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.09x + 0.004$$
$$S_o = 0.02x - 0.009$$

$$\text{Selected water matrices: } S_T = 0.08x + 0.005$$
$$S_o = 0.02x + 0.004$$

3) Titrimetric—Based on the results of six operators in three laboratories, the overall and single-operator precision of this test method within its designated range may be expressed as follows:

$$\text{Reagent water: } S_T = 0.532 - 0.10x$$
$$S_o = 0.151 - 0.01x$$

$$\text{Selected water matrices: } S_T = 0.604 - 0.06x$$
$$S_o = 0.092 + 0.02x$$

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where:

S_T = overall precision,

S_o = single-operator precision, and

x = cyanide concentration, mg/L.

b. Bias: Recoveries of known amounts of cyanide from reagent water and selected water matrices are shown below.

Medium	Technique	Added *mg/L*	Recovered *mg/L*	*n*	S_T	Bias	% Bias
Reagent water	Colorimetric	0.030	0.030	25	0.0089	0.000	0
		0.100	0.117	27	0.0251	0.017	17
		0.400	0.361	27	0.0400	–0.039	–10
	Electrode	0.030	0.030	21	0.0059	0.000	0
		0.100	0.095	21	0.0163	–0.005	–5
		0.400	0.365	21	0.0316	–0.035	–9
		1.000	0.940	21	0.0903	–0.060	–6
	Titrimetric	1.00	1.35	18	0.4348	0.35	35
		1.00	1.38	18	0.3688	0.38	38
		4.00	3.67	18	0.1830	–0.33	–8
Selected water matrices	Colorimetric	0.030	0.029	15	0.0062	0.001	3
		0.100	0.118	24	0.0312	0.018	18
		0.400	0.381	23	0.0389	–0.019	–5
	Electrode	0.030	0.029	20	0.0048	–0.001	–3
		0.100	0.104	21	0.0125	0.004	4
		0.400	0.357	21	0.0372	–0.043	–11
		1.000	0.935	21	0.0739	–0.065	–7
	Titrimetric	1.00	1.55	18	0.5466	0.55	55
		1.00	1.53	18	0.4625	0.53	53
		4.00	3.90	18	0.3513	–0.10	–3

7. Reference

1. AMERICAN SOCIETY FOR TESTING & MATERIALS. 1987. Research Rep. D2036:19-1131. American Soc. Testing & Materials, Philadelphia, Pa.

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4500-CN⁻ J. Cyanogen Chloride

1. General Discussion

Cyanogen chloride (CNCl) is the first reaction product when cyanide compounds are chlorinated. It is a volatile gas, only slightly soluble in water, but highly toxic even in low concentrations. (CAUTION: *Avoid inhalation or contact*.) A mixed pyridine-barbituric acid reagent produces a red-blue color with CNCl.

Because CNCl hydrolyzes to cyanate (CNO^-) at a pH of 12 or more, collect a separate sample for CNCl analysis (See Section 4500-CN^-.B.2) in a closed container without sodium hydroxide (NaOH). A quick test with a spot plate or comparator as soon as the sample is collected may be the only procedure for avoiding hydrolysis of CNCl due to time lapse between sampling and analysis.

If starch-iodide (KI) test paper indicates presence of chlorine or other oxidizing agents, add sodium thiosulfate ($Na_2S_2O_3$) immediately as directed in Section 4500-CN^-.B.2.

2. Apparatus

See Section 4500-CN^-.E.2.

3. Reagents

a. Reagents listed in Section 4500-CN^-.E.3 and Section 4500-CN^-.H.4.

b. Phosphate buffer: Dissolve 138 g sodium dihydrogen phosphate monohydrate, $NaH_2PO_4 \cdot H_2O$, in water and dilute to 1 L. Refrigerate.

4. Procedure

a. Preparation of standard curve: Pipet a series of standards containing 1 to 10 μg CN^- into 50-mL volumetric flasks (0.02 to 0.2 μg CN^-/mL). Dilute to 20 mL with NaOH dilution solution. Use 20 mL of NaOH dilution solution for the blank. Add 2 mL chloramine-T solution and 4 mL phosphate buffer; stopper and mix by inversion two or three times. Add 5 mL pyridine-barbituric acid reagent, dilute to volume with water, mix thoroughly, and let stand exactly 8 min for color development. Measure absorbance at 578 nm in a 10-mm cell using distilled water as a reference. Calculate slope and intercept of the curve.

b. If sample pH is above 8, reduce it to 8.0 to 8.5 by careful addition of phosphate buffer. Measure 20 mL sample portion into 50-mL volumetric flask. If more than 0.20 mg CNCl-CN^-/L is present use a smaller portion diluted to 20 mL with water. Add 1 mL phosphate buffer, stopper and mix by inversion *one* time. Let stand 2 min. Add 5 mL pyridine-barbituric acid reagent, stopper and mix by inversion *one* time. Let color develop 3 min, dilute to volume with water,

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mix thoroughly, and let stand an additional 5 min. Measure absorbance at 578 nm in 10-mm cell using distilled water as a reference.

5. Calculation

Compute slope (m) and intercept (b) of standard curve as directed in 4500-CN⁻.E.5.

$$\text{Cyanogen chloride as } CN^-, \text{ mg/L} = (ma_1 + b) \times \frac{50}{\text{mL sample}}$$

where:

a_1 = absorbance of sample solution.

6. Precision[1]

Cyanogen chloride is unstable and round-robin testing is not possible. Single-operator precision is as follows:

Six operators made 70 duplicate analyses on samples of different concentrations within the applicable range of the method. The overall single-operator precision within its designated range may be expressed as follows:

$$\log S_o = (0.5308 \log c) - 1.9842$$
$$\log R = (0.5292 \log c) - 1.8436$$

where:

c = mg CNCl-CN⁻/L,
S_o = single-operator precision in the range of the method (precision is dependent on concentration), and
R = range between duplicate determinations.

The collaborative test data were obtained on reagent-grade water. For other matrices, these data may not apply.

7. Reference

1. AMERICAN SOCIETY FOR TESTING & MATERIALS. 1989. Research Rep. D4165:19-1100. American Soc. Testing & Materials, Philadelphia, Pa.

4500-CN⁻ K. Spot Test for Sample Screening

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1. General Discussion

The spot test procedure permits quick screening to establish whether more than 50 µg/L of cyanide amenable to chlorination is present. The test also establishes the presence or absence of cyanogen chloride (CNCl). With practice and dilution, the test reveals the approximate concentration range of these compounds by the color development compared with that of similarly treated standards.

When chloramine-T is added to cyanides amenable to chlorination, CNCl is formed. CNCl forms a red-blue color with the mixed reagent pyridine-barbituric acid. When testing for CNCl omit the chloramine-T addition. (CAUTION: *CNCl is a toxic gas; avoid inhalation.*)

The presence of formaldehyde in excess of 0.5 mg/L interferes with the test. A spot test for the presence of aldehydes and a method for removal of this interference are given in Section 4500-CN^-.B.3.

Thiocyanate (SCN^-) reacts with chloramine-T, thereby creating a positive interference. The CN^- can be masked with formaldehyde and the sample retested. This makes the spot test specific for SCN^-. In this manner it is possible to determine whether the spot discoloration is due to the presence of CN^-, SCN^-, or both.

2. Apparatus

a. Porcelain spot plate with 6 to 12 cavities.

b. Dropping pipets.

c. Glass stirring rods.

3. Reagents

a. Chloramine-T solution: See Section 4500-CN^-.E.3*a*.

b. Stock cyanide solution: See Section 4500-CN^-.E.3*b*.

c. Pyridine-barbituric acid reagent: See Section 4500-CN^-.E.3*d*.

d. Hydrochloric acid, HCl, 1 + 9.

e. Phenolphthalein indicator aqueous solution.

f. Sodium carbonate, Na_2CO_3, anhydrous.

g. Formaldehyde, 37%, pharmaceutical grade.

4. Procedure

If the solution to be tested has a pH greater than 10, neutralize a 20- to 25-mL portion. Add about 250 mg Na_2CO_3 and swirl to dissolve. Add 1 drop phenolphthalein indicator. Add 1 + 9 HCl dropwise with constant swirling until the solution becomes colorless. Place 3 drops sample and 3 drops distilled water (for blanks) in separate cavities of the spot plate. To each cavity, add 1 drop chloramine-T solution and mix with a clean stirring rod. Add 1 drop pyridine-barbituric

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acid solution to each cavity and again mix. After 1 min, the sample spot will turn pink to red if 50 μg/L or more of CN^- are present. The blank spot will be faint yellow because of the color of the reagents. Until familiarity with the spot test is gained, use, in place of the water blank, a standard solution containing 50 μg CN^-/ L for color comparison. This standard can be made by diluting the stock cyanide solution (¶ 3*b*).

If SCN^- is suspected, test a second sample pretreated as follows: Heat a 20- to 25-mL sample in a water bath at 50°C; add 0.1 mL formaldehyde and hold for 10 min. This treatment will mask up to 5 mg CN^-/L, if present. Repeat spot testing procedure. Color development indicates presence of SCN^-. Comparing color intensity in the two spot tests is useful in judging relative concentration of CN^- and SCN^-. If deep coloration is produced, serial dilution of sample and additional testing may allow closer approximation of the concentrations.

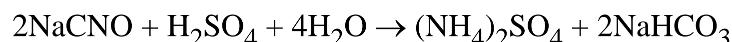
4500-CN⁻ L. Cyanates

1. General Discussion

Cyanate (CNO^-) may be of interest in analysis of industrial waste samples because the alkaline chlorination process used for the oxidation of cyanide yields cyanate in the second reaction.

Cyanate is unstable at neutral or low pH; therefore, stabilize the sample as soon as collected by adding sodium hydroxide (NaOH) to pH >12. Remove residual chlorine by adding sodium thiosulfate ($Na_2S_2O_3$) (see Section 4500-CN^-.B.2).

a. Principle: Cyanate hydrolyzes to ammonia when heated at low pH.



$$2NaCNO + H_2SO_4 + 4H_2O \rightarrow (NH_4)_2SO_4 + 2NaHCO_3$$

The ammonia concentration must be determined on one sample portion before acidification. The ammonia content before and after hydrolysis of cyanate may be measured by phenate (Section 4500-NH_3.F), or ammonia-selective electrode (Section 4500-NH_3.D) method.[1] The test is applicable to cyanate compounds in natural waters and industrial waste.

b. Interferences:

1) Organic nitrogenous compounds may hydrolyze to ammonia (NH_3) upon acidification. To minimize this interference, control acidification and heating closely.

2) Reduce oxidants that oxidize cyanate to carbon dioxide and nitrogen with $Na_2S_2O_3$ (see Section 4500-CN^-.G).

3) Industrial waste containing organic material may contain unknown interferences.

c. Detection limit: 1 to 2 mg CNO^-/L.

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2. Apparatus

a. Expanded-scale pH meter or selective-ion meter.

*b. Ammonia-selective electrode.**#(24)

c. Magnetic mixer, with TFE-coated stirring bar.

d. Heat barrier: Use a 3-mm-thick insulator under beaker to insulate against heat produced by stirrer motor.

3. Reagents

a. Stock ammonium chloride solution: Dissolve 3.819 g anhydrous NH_4Cl, dried at 100°C, in water, and dilute to 1 L; 1.00 mL=1.00 mg N=1.22 mg NH_3.

b. Standard ammonium chloride solution: From the stock NH_4Cl solution prepare standard solutions containing 1.0, 10.0, and 100.0 mg NH_3-N/L by diluting with ammonia-free water.

c. Sodium hydroxide, 10*N*: Dissolve 400 g NaOH in water and dilute to 1 L.

d. Sulfuric acid solution, H_2SO_4, 1 + 1.

e. Ammonium chloride solution: Dissolve 5.4 g NH_4Cl in distilled water and dilute to 1 L. (Use only for soaking electrodes.)

4. Procedure

a. Calibration: Daily, calibrate the ammonia electrode as in Section 4500-NH_3.F.4*b* and *c* using standard NH_4Cl solutions.

b. Treatment of sample: Dilute sample, if necessary, so that the CNO^- concentration is 1 to 200 mg/L or NH_3-N is 0.5 to 100 mg/L. Take or prepare at least 200 mL. From this 200 mL, take a 100-mL portion and, following the calibration procedure, establish the potential (millivolts) developed from the sample. Check electrode reading with prepared standards and adjust instrument calibration setting daily. Record NH_3-N content of untreated sample (*B*).

Acidify 100 mL of prepared sample by adding 0.5 mL 1 + 1 H_2SO_4 to a pH of 2.0 to 2.5. Heat sample to 90 to 95°C and maintain temperature for 30 min. Cool to room temperature and restore to original volume by adding ammonia-free water. Pour into a 150-mL beaker, immerse electrode, start magnetic stirrer, then add 1 mL 10*N* NaOH solution. With pH paper check that pH is greater than 11. If necessary, add more NaOH until pH 11 is reached.

After equilibrium has been reached (30 s) record the potential reading. Estimate NH_3-N content from calibration curve.

5. Calculations

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$$\text{mg } NH_3\text{-N derived from } CNO^-/L = A - B$$

where:

A = mg NH_3-N/L found in the acidified and heated sample portion and
B = mg NH_3-N/L found in untreated portion.

$$\text{mg } CNO^-/L = 3.0 \times (A - B)$$

6. Precision

No data on precision of this method are available. See Section 4500-NH_3.A.4 for precision of ammonia-selective electrode method.

7. Reference

1. THOMAS, R.F. & R.L. BOOTH. 1973. Selective electrode determination of ammonia in water and wastes. *Environ. Sci. Technol.* 7:523.

4500-CN^- M. Thiocyanate

1. General Discussion

When wastewater containing thiocyanate (SCN^-) is chlorinated, highly toxic cyanogen chloride (CNCl) is formed. At an acidic pH, ferric ion (Fe^{3+}) and SCN^- form an intense red color suitable for colorimetric determination.

a. Interference:

1) Hexavalent chromium (Cr^{6+}) interferes and is removed by adding ferrous sulfate ($FeSO_4$) after adjusting to pH 1 to 2 with nitric acid (HNO_3). Raising the pH to 9 with 1*N* sodium hydroxide (NaOH) precipitates Fe^{3+} and Cr^{3+}, which are then filtered out.

2) Reducing agents that reduce Fe^{3+} to Fe^{2+}, thus preventing formation of ferric thiocyanate complex, are destroyed by adding a few drops of hydrogen peroxide (H_2O_2). Avoid excess H_2O_2 to prevent reaction with SCN^-.

3) Industrial wastes may be highly colored or contain various interfering organic compounds. To eliminate these interferences,[1] use the pretreatment procedure given in ¶ 4*c* below. It is the analyst's responsibility to validate the method's applicability without pretreatment (¶ 4*b*). If in doubt, pretreat sample before proceeding with analysis (¶ 4*c*).

4) If sample contains cyanide amenable to chlorination and would be preserved for the cyanide determination at a high pH, sulfide could interfere by converting cyanide to SCN^-. To

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preserve SCN^- and CN^-, precipitate the sulfide by adding lead salts according to Section 4500-CN^-.B.2 before adding alkali; filter to remove precipitate.

5) Thiocyanate is biodegradable. Preserve samples at pH <2 by adding mineral acid and refrigerate.

6) If interferences from industrial wastes are not removed as directed in ¶ 4*c* below, consider adopting a solvent extraction technique with colorimetric or atomic absorption analysis of the extract.[2,3]

b. Application: 0.1 to 2.0 mg SCN^-/L in natural or wastewaters. For higher concentrations, use a portion of diluted sample.

2. Apparatus

a. Spectrophotometer or filter photometer, for use at 460 nm, providing a light path of 5 cm.

b. Glass adsorption column: Use a 50-mL buret with a glass-wool plug, and pack with macroreticular resin (¶ 3) approximately 40 cm high. For convenience, apply a powder funnel of the same diameter as the buret to the top with a short piece of plastic tubing.

3. Reagents

a. Ferric nitrate solution: Dissolve 404 g $Fe(NO_3)_3 \cdot 9H_2O$ in about 800 mL distilled water. Add 80 mL conc HNO_3 and dilute to 1 L.

b. Nitric acid solution, 0.1*N*: Mix 6.4 mL conc HNO_3 in about 800 mL distilled water and dilute to 1 L.

c. Stock thiocyanate solution: Dissolve 1.673 g potassium thiocyanate (KSCN) in distilled water and dilute to 1000 mL; 1.00 mL 1.00 mg SCN^-.

d. Standard thiocyanate solution: Dilute 10 mL stock solution to 1 L with distilled water; 1.00 mL = 0.01 mg SCN^-.

e. Sodium hydroxide solution, 4 g/L: Dissolve 4 g NaOH in about 800 mL distilled water and dilute to 1 L.

f. Macroreticular resin, 18 to 50 mesh:*#(25) The available resin may not be purified. Some samples have shown contamination with waxes and oil, giving poor permeability and adsorption. Purify as follows:

Place sufficient resin to fill the column or columns in a beaker and add 5 times the resin volume of acetone. Stir gently for 1 h. Pour off fines and acetone from settled resin and add 5 times the resin volume of hexane. Stir for 1 h. Pour off fines and hexane and add 5 times the resin volume of methanol. Stir for 15 min. Pour off methanol and add 3 times the resin volume of 0.1*N* NaOH. Stir for 15 min. Pour off NaOH solution and add 3 times the resin volume of 0.1*N* HNO_3 . Stir for 15 min. Pour off HNO_3 solution and add 3 times the resin volume of distilled water. Stir for 15 min. Drain excess water and use purified resin to fill the column. Store excess

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purified resin after covering it with distilled water. Keep in a closed jar.

g. Methyl alcohol.

4. Procedure

a. Preparation of calibration curve: Prepare a series of standards containing from 0.02 mg to 0.40 mg SCN^- by pipetting measured volumes of standard KSCN solution into 200-mL volumetric flasks and diluting with water. Mix well. Develop color according to ¶ b below. Plot absorbance against SCN^- concentration expressed as mg/50 mL sample. The absorbance plot should be linear.

b. Color development: Use a filtered sample or portion from a diluted solution so that the concentration of SCN^- is between 0.1 and 2 mg/L. Adjust pH to 2 with conc HNO_3 added dropwise. Pipet 50-mL portion to a beaker, add 2.5 mL ferric nitrate, and mix.

Fill a 5-cm absorption cell and measure absorbance against a reagent blank at 460 nm or close to the maximum absorbance found with the instrument being used. Measure absorbance of the developed color against a reagent blank within 5 min from adding the reagent. (The color develops within 30 s and fades on standing in light.)

c. Sample pretreatment:

1) Color and various organic compounds interfere with absorbance measurement. At pH 2, macroreticular resin removes these interfering materials by adsorption without affecting thiocyanate.

2) To prepare the adsorption column, fill it with resin, rinse with 100 mL methanol, and follow by rinses with 100 mL 0.1*N* NaOH, 100 mL 0.1*N* HNO_3, and finally with 100 mL distilled water. If previously purified resin is used, omit these preparatory steps.

3) When washing, regenerating, or passing a sample through the column, as solution level approaches resin bed, add and drain five separate 5-mL volumes of solution or water (depending on which is used in next step) to approximate bed height. After last 5-mL volume, fill column with remaining liquid. This procedure prevents undue mixing of solutions and helps void the column of the previous solution.

4) Acidify 150 mL sample (or a dilution) to pH 2 by adding conc HNO_3 dropwise while stirring. Pass it through the column at a flow rate not to exceed 20 mL/min. If the resin becomes packed and the flow rate falls to 4 to 5 mL/min, use gentle pressure through a manually operated hand pump or squeeze bulb on the column. In this case, use a separator funnel for the liquid reservoir instead of the powder funnel. Alternatively use a vacuum bottle as a receiver and apply gentle vacuum. Do not let liquid level drop below the adsorbent in the column.

5) When passing a sample through the column, measure 90 mL of sample in a graduated cylinder, and from this use the five 5-mL additions as directed in ¶ 3), then pour the remainder of the 90 mL into the column. Add rest of sample and collect 60 mL eluate to be tested after the first 60 mL has passed through the column.

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6) Prepare a new calibration curve using standards prepared according to ¶ 4*a*, but acidify standards according to ¶ 4*b*, and pass them through the adsorption column. Develop color and measure absorbance according to ¶ 4*b* against a reagent blank prepared by passing acidified, distilled water through the adsorption column.

7) Pipet 50 mL from the collected eluate to a beaker, add 2.5 mL ferric nitrate solution, and mix. Measure absorbance according to ¶ 4*b* against a reagent blank [see ¶ 6) above].

8) From the measured absorbance value, determine thiocyanate content of the sample or dilution using the absorbance plot.

9) Each day the column is in use, test a mid-range standard to check absorption curve.

10) Regenerate column between samples by rinsing with 100 mL 0.1*N* NaOH; 50 mL 0.1*N* HNO_3; and 100 mL water. Insure that the water has rinsed empty glass section of the buret. Occasionally rinse with 100 mL methanol for complete regeneration. Adsorbed weak organic acids and thiocyanate residuals from earlier tests are eluted by the NaOH rinse. Leave the column covered with the last rinse water for storage.

5. Calculation

Compute slope (m) and intercept (b) of standard curve as directed in Section 4500-CN^-.E.5.

Calculate thiocyanate concentration as follows:

$$\text{mg SCN}^-/\text{L} = (ma_1 + b) \times \text{dilution factor}$$

where:

a_1 = absorbance of sample solution.

6. Precision and Bias[4]

a. Precision: Based on the results of twelve operators in nine laboratories, at four levels of concentration, the precision of the test method within its designated range is linear with concentration and may be expressed as follows:

$$\text{Reagent water: } S_T = 0.093x + 0.0426$$
$$S_o = 0.045x + 0.010$$

$$\text{Water matrix: } S_T = 0.055x + 0.0679$$
$$S_o = 0.024x + 0.182$$

where:

S_T = overall precision, mg/L,

S_o = pooled single-operator precision, mg/L, and

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x = thiocyanate concentration, mg/L.

b. Bias: Recoveries of known amounts of thiocyanate from reagent water and selected water matrices including natural waters, laboratory effluent, steel mill effluent, and dechlorinated and treated sanitary effluents were as follows:

Medium	Added *mg/L*	Recovered *mg/L*	*n*	S_T	Bias	% Bias
Reagent	1.42	1.411	30	0.181	–0.009	–0.6
water	0.71	0.683	27	0.091	–0.027	–4
	0.35	0.329	30	0.084	–0.021	–6
	0.07	0.068	30	0.052	–0.002	–3
Selected	1.42	1.408	26	0.151	–0.012	–0.8
water	0.71	0.668	29	0.096	–0.042	–6
matrices	0.35	0.320	29	0.085	–0.030	–9
	0.07	0.050	29	0.079	–0.020	–29

For other matrices these data may not apply.

7. References

1. SPENCER, R.R., J. LEENHEER & V.C. MARTI. 1980. Automated colorimetric determination of thiocyanate, thiosulfate and tetrathionate in water. 94th Annu. Meeting. Assoc. Official Agricultural Chemists, Washington, D.C. 1981.
2. DANCHICK, R.S. & D.F. BOLTZ. 1968. Indirect spectrophotometric and atomic absorption spectrometric methods in determination of thiocyanate. *Anal. Chem.* 43:2215.
3. LUTHY, R.G. 1978. Manual of Methods: Preservation and Analysis of Coal Gasification Wastewaters. FE-2496-16, U.S. Dep. Energy, National Technical Information Serv., Springfield, Va.
4. AMERICAN SOCIETY FOR TESTING & MATERIALS. 1989. Research Rep. D4193:19-1099. American Soc. Testing & Materials, Philadelphia, Pa.

4500-CN^- N. Total Cyanide after Distillation, by Flow Injection Analysis (PROPOSED)

1. General Discussion

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a. Principle: Total cyanides are digested and steam-distilled from the sample as in Section 4500-CN⁻.C, cyanides amenable to chlorination are digested and steam-distilled from the sample as in Section 4500-CN⁻.G, or weak acid dissociable cyanides are digested and steam-distilled from the sample as in Section 4500-CN⁻.I, by using the apparatus described in Section 4500-CN⁻.C or an equivalent distillation apparatus. In any case, the distillate should consist of cyanide in 0.25*M* NaOH. The cyanide in this distillate is converted to cyanogen chloride, CNCl, by reaction with chloramine-T at pH less than 8. The CNCl then forms a red-blue dye by reacting with pyridine-barbituric acid reagent. The absorbance of this red dye is measured at 570 nm and is proportional to the total or weak acid dissociable cyanide in the sample.

Also see Section 4500-CN⁻.A and Section 4500-CN⁻.E, and Section 4130, Flow Injection Analysis (FIA).

b . Interferences: Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

Nonvolatile interferences are eliminated or minimized by the distillation procedure. Some of the known interferences are aldehydes, nitrate-nitrite, and oxidizing agents such as chlorine, thiocyanate, thiosulfate, and sulfide. Multiple interferences may require the analysis of a series of laboratory fortified sample matrices (LFM) to verify the suitability of the chosen treatment. See Section 4500-CN⁻.B for a discussion of preliminary treatment of samples to be distilled.

2. Apparatus

Flow injection analysis equipment consisting of:

a. FIA injection valve with sample loop or equivalent.

b. Multichannel proportioning pump.

c. FIA manifold (Figure 4500-CN⁻:2) with tubing heater and flow cell. Relative flow rates only are shown. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.*#(26)

d. Absorbance detector, 570 nm, 10-nm bandpass.

e. Injection valve control and data acquisition system.

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and all reagents with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. Carrier solution, 0.25*M*: In a 1-L plastic container dissolve 10.0 g NaOH in 1.00 L water.

b. Phosphate buffer, 0.71*M*: To a 1-L tared container add 97.0 g potassium phosphate, monobasic, anhydrous, KH_2PO_4, and 975 g water. Stir or shake until dissolved. Prepare fresh

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monthly.

c. Chloramine-T: Dissolve 2.0 g chloramine-T hydrate (mol wt 227.65) in 500 mL water. Prepare fresh daily.

d. Pyridine/barbituric acid: In fume hood, place 15.0 g barbituric acid in a tared 1-L container and add 100 g water, rinsing down sides of beaker to wet the barbituric acid. Add 73 g pyridine (C_5H_5N) with stirring and mix until barbituric acid dissolves. Add 18 g conc HCl, then an additional 825 g water, and mix. Prepare fresh weekly.

e. Stock cyanide standard, 100 mg CN^-/L: In a 1-L container, dissolve 2.0 g potassium hydroxide (KOH) in approximately 800 mL water. Add 0.250 g potassium cyanide (KCN). CAUTION: *KCN is highly toxic. Avoid inhalation of dust or contact with the solid or solutions.* Make to final weight of 1000 g with water and mix. Prepare fresh weekly or standardize weekly using procedure in Section 4500-CN^-.D.4.

f. Standard cyanide solution: Prepare cyanide standards in the desired concentration range, using the stock cyanide standard (¶ 3*e*) and diluting with the 0.25*M* NaOH carrier (¶ 3*a*).

4. Procedure

Set up a manifold equivalent to that in Figure 4500-CN^-:2 and follow method supplied by manufacturer or laboratory standard operating procedure for this method. Follow quality control guidelines outlined in Section 4020.

5. Calculation

Prepare standard curves by plotting absorbance of standards processed through manifold versus cyanide concentration. The calibration curve is linear.

6. Precision and Bias

a. Recovery and relative standard deviation: The results of single-laboratory studies with various matrices are given in Table 4500-CN^-:I.

b. MDL without distillation: Using a published MDL method,[1] analysts ran 21 replicates of an undistilled 0.010-mg CN^-/L standard with a 780-µL sample loop. These gave a mean of 0.010 mg CN^-/L, a standard deviation of 0.00012 mg CN^-/L, and an MDL of 0.0003 mg CN^-/L. A lower MDL may be obtained by increasing the sample loop volume and increasing the ratio of carrier flow rate to reagent flow rate.

c. MDL with distillation: Using a published MDL method,[1] analysts ran 21 replicates of a 0.0050-mg CN^-/L standard distilled using the distillation device†#(27) equivalent to the apparatus specified in 4500-CN^-.C. When the 0.25*M* NaOH distillates were determined with a 780-µL sample loop, they gave a mean of 0.0045 mg CN^-/L, a standard deviation of 0.0002 mg CN^-/L, and an MDL of 0.0006 mg CN^-/L.

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d. Precision study: Ten injections of an undistilled 0.050-mg CN^-/L standard gave a relative standard deviation of 0.21%.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1989. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 rev. 12.11 amended June 30, 1986. 49 CFR 43430.

4500-CN^- O. Total Cyanide and Weak Acid Dissociable Cyanide by Flow Injection Analysis (PROPOSED)

1. General Discussion

a. Principle: Total cyanide consists of various metal-cyanide complexes. To break down or digest these complexes to yield HCN, the sample is mixed with heated phosphoric acid and then irradiated with ultraviolet radiation. The resulting ‘‘donor stream’’ contains the product HCN (aq). This donor stream is passed over a silicone rubber gas permeation membrane. The HCN from the donor stream is extracted by the membrane as HCN (g) and is trapped in a parallel ‘‘acceptor stream’’ that consists of dilute sodium hydroxide, the equivalent of the distillate resulting from the digesting distillations in the sample preparation methods Section 4500-CN^-.C, Section 4500-CN^-.G, and Section 4500-CN^-.I.

As in Section 4500-CN^-.N, the cyanide in this acceptor stream or distillate is converted to cyanogen chloride, CNCl, by reaction with chloramine-T at pH less than 8. The CNCl then forms a red-blue dye by reacting with pyridine-barbituric acid reagent. The absorbance of this red dye is measured at 570 nm and is proportional to the total or weak acid dissociable cyanide in the sample.

The weak acid dissociable (WAD) cyanide method is similar except that ultraviolet radiation and phosphoric acid are not used in the donor stream. Instead, a solution of dihydrogen phosphate is used as the donor stream.

Also see Section 4500-CN^-.A, Section 4500-CN^-.E, and Section 4500-CN^-.N and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: Remove large or fibrous particulates by filtering the sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

Nonvolatile interferences are eliminated or minimized by the gas-permeable membrane.

Multiple interferences may require the analysis of a series of sample matrices with known additions to verify the suitability of the chosen treatment. See Section 4500-CN^-.B for a discussion of preliminary treatment of samples that will be distilled.

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1) Total cyanide interferences—Sulfide up to a concentration of 10 mg/L and thiocyanate up to a concentration of 20 mg/L do not interfere in the determination of 100 µg CN^-/L. When a sample containing nitrate at 100 mg NO_3^--N/L and 20 mg/L thiocyanate was treated with sulfamic acid, the determined value was 138.2 µg CN^-/L for a known concentration of 100 µg CN^-/L. When pretreated with ethylenediamine, a sample containing 50 mg formaldehyde/L did not interfere in the determination of cyanide.

2) WAD interferences—Sulfide up to 10 mg/L and thiocyanate up to 50 mg/L do not interfere in the determination of 0.1 mg/L cyanide.

2. Apparatus

Flow injection analysis equipment consisting of:

a. FIA injection valve with sample loop or equivalent.

b. Multichannel proportioning pump.

c. FIA manifold (Figure 4500-CN^-:3) with tubing heater, in-line ultraviolet digestion fluidics, a gas-permeable silicone rubber membrane and its holder, and flow cell. In Figure 4500-CN^-:3, relative flow rates only are shown. The tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE. The ultraviolet unit should consist of TFE tubing irradiated by a mercury discharge ultraviolet lamp emitting radiation at 254 nm.

d. Absorbance detector, 570 nm, 10-nm bandpass.

e. Injection valve control and data acquisition system.

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and all reagents with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L of solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. Phosphoric acid donor stream (total cyanide): To a 1-L volumetric flask, add approximately 700 mL water, then add 30 mL conc phosphoric acid, H_3PO_4. Mix and let solution cool. Dilute to mark. Prepare fresh monthly.

b. Dihydrogen phosphate donor stream (WAD cyanide): To a tared 1-L container add 97 g anhydrous potassium dihydrogen phosphate, KH_2PO_4, and 975 g water. Stir for 2 h or until the potassium phosphate has gone into solution. Degas with helium. Prepare fresh monthly.

c. NaOH acceptor stream, carrier, and diluent (total and WAD cyanide), 0.025*M* NaOH: To a 1-L container add 1.0 g sodium hydroxide (NaOH) and 999 g water. Mix with a magnetic stirrer for about 5 min. Cover with a laboratory film. Degas with helium. Prepare fresh daily.

d. Buffer (total and WAD cyanide), 0.71*M* phosphate: To a 1-L tared container add 97.0 g

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potassium phosphate, monobasic, anhydrous, KH_2PO_4, and 975 g water. Stir or shake until dissolved. Prepare fresh monthly.

e. Chloramine-T solution (total and WAD cyanide): Dissolve 3 g chloramine-T hydrate in 500 mL water. Degas with helium. Prepare fresh daily. NOTE: Chloramine-T is an air-sensitive solid. Preferably discard this chemical 6 months after opening.

f. Pyridine/barbituric acid solution (total and WAD cyanide): In the fume hood, place 15.0 g barbituric acid in a tared 1-L container and add 100 g water, rinsing down the sides of the beaker to wet the barbituric acid. Add 73 g pyridine (C_5H_5N) with stirring and mix until the barbituric acid dissolves. Add 18 g conc HCl, then add an additional 825 mL water and mix. Prepare fresh weekly.

g. Stock cyanide standard, 100 mg CN^-/L: In a 1-L container dissolve 2.0 g potassium hydroxide, KOH, in approximately 800 mL water. Add 0.250 g potassium cyanide, KCN. CAUTION: *KCN is highly toxic. Avoid inhalation of dust or contact with the solid or solutions.* Make to final weight of 1000 g with water and invert three times to mix. Prepare fresh weekly or standardize weekly using the procedure in Section 4500-CN^-D.4.

h. Standard cyanide solutions: Prepare cyanide standards in the desired concentration range, using the stock cyanide standard (¶ 3*g*) and diluting with the NaOH standards diluent (¶ 3*c*).

4. Procedure

Set up a manifold equivalent to that in Figure 4500-CN^-:3 and follow the method supplied by the manufacturer or laboratory standard operating procedure for this method. Follow quality control guidelines outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold vs. cyanide concentration. The calibration curve is linear.

6. Precision and Bias

a. MDL, total cyanide: A 420-µL sample loop was used in the total cyanide method. Using a published MDL method[1], analysts ran 21 replicates of a 10.0-µg CN^-/L standard. These gave a mean of 9.69 µg CN^-/L, a standard deviation of 0.86 µg CN^-/ L, and an MDL of 2.7 µg CN^-/L.

b. MDL, WAD cyanide: A 420-µL sample loop was used in the WAD cyanide method. Using a published MDL method[1], analysts ran 21 replicates of a 10.0-µg CN^-/L standard. These gave a mean of 11.5 µg CN^-/L, a standard deviation of 0.73 µg CN^-/ L, and an MDL of 2.3 µg CN^-/L.

c. Precision study, total cyanide: Seven injections of a 100.0-µg CN^-/L standard gave a relative standard deviation (RSD) of 1.0%.

d. Precision study, WAD cyanide: Ten injections of a 200.0-µg CN^-/L standard gave an

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RSD of 1.3%.

e. Recovery of total cyanide: Two injections each were made of solutions of potassium ferricyanide and potassium ferrocyanide, both at a concentration equivalent to 100 µg CN^-/L. Both gave an average recovery of 98%.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1989. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 rev. 12.11 amended June 30, 1986. 49 CFR 43430.

4500-Cl CHLORINE (RESIDUAL)*#(28)

4500-Cl A. Introduction

1. Effects of Chlorination

The chlorination of water supplies and polluted waters serves primarily to destroy or deactivate disease-producing microorganisms. A secondary benefit, particularly in treating drinking water, is the overall improvement in water quality resulting from the reaction of chlorine with ammonia, iron, manganese, sulfide, and some organic substances.

Chlorination may produce adverse effects. Taste and odor characteristics of phenols and other organic compounds present in a water supply may be intensified. Potentially carcinogenic chloroorganic compounds such as chloroform may be formed. Combined chlorine formed on chlorination of ammonia- or amine-bearing waters adversely affects some aquatic life. To fulfill the primary purpose of chlorination and to minimize any adverse effects, it is essential that proper testing procedures be used with a foreknowledge of the limitations of the analytical determination.

2. Chlorine Forms and Reactions

Chlorine applied to water in its molecular or hypochlorite form initially undergoes hydrolysis to form free chlorine consisting of aqueous molecular chlorine, hypochlorous acid, and hypochlorite ion. The relative proportion of these free chlorine forms is pH-and temperature-dependent. At the pH of most waters, hypochlorous acid and hypochlorite ion will predominate.

Free chlorine reacts readily with ammonia and certain nitrogenous compounds to form combined chlorine. With ammonia, chlorine reacts to form the chloramines: monochloramine, dichloramine, and nitrogen trichloride. The presence and concentrations of these combined forms depend chiefly on pH, temperature, initial chlorine-to-nitrogen ratio, absolute chlorine demand, and reaction time. Both free and combined chlorine may be present simultaneously. Combined

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chlorine in water supplies may be formed in the treatment of raw waters containing ammonia or by the addition of ammonia or ammonium salts. Chlorinated wastewater effluents, as well as certain chlorinated industrial effluents, normally contain only combined chlorine. Historically, the principal analytical problem has been to distinguish between free and combined forms of chlorine.

3. Selection of Method

In two separate but related studies, samples were prepared and distributed to participating laboratories to evaluate chlorine methods. Because of poor accuracy and precision and a high overall (average) total error in these studies, all orthotolidine procedures except one were dropped in the 14th edition of this work. The useful stabilized neutral orthotolidine method was deleted from the 15th edition because of the toxic nature of orthotolidine. The leuco crystal violet (LCV) procedure was dropped from the 17th edition because of its relative difficulty and the lack of comparative advantages.

a. Natural and treated waters: The iodometric methods (B and C) are suitable for measuring total chlorine concentrations greater than 1 mg/L, but the amperometric end point of Methods C and D gives greater sensitivity. All acidic iodometric methods suffer from interferences, generally in proportion to the quantity of potassium iodide (KI) and H^+ added.

The amperometric titration method (D) is a standard of comparison for the determination of free or combined chlorine. It is affected little by common oxidizing agents, temperature variations, turbidity, and color. The method is not as simple as the colorimetric methods and requires greater operator skill to obtain the best reliability. Loss of chlorine can occur because of rapid stirring in some commercial equipment. Clean and conditioned electrodes are necessary for sharp end points.

A low-level amperometric titration procedure (E) has been added to determine total chlorine at levels below 0.2 mg/L. This method is recommended only when quantification of such low residuals is necessary. The interferences are similar to those found with the standard amperometric procedure (D). The DPD methods (Methods F and G) are operationally simpler for determining free chlorine than the amperometric titration. Procedures are given for estimating the separate mono- and dichloramine and combined fractions. High concentrations of monochloramine interfere with the free chlorine determination unless the reaction is stopped with arsenite or thioacetamide. In addition, the DPD methods are subject to interference by oxidized forms of manganese unless compensated for by a blank.

The amperometric and DPD methods are unaffected by dichloramine concentrations in the range of 0 to 9 mg Cl as Cl_2/L in the determination of free chlorine. Nitrogen trichloride, if present, may react partially as free chlorine in the amperometric, DPD, and FACTS methods. The extent of this interference in the DPD methods does not appear to be significant.

The free chlorine test, syringaldazine (FACTS, Method H) was developed specifically for free chlorine. It is unaffected by significant concentrations of monochloramine, dichloramine, nitrate, nitrite, and oxidized forms of manganese.[1]

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Sample color and turbidity may interfere in all colorimetric procedures.

Organic contaminants may produce a false free chlorine reading in most colorimetric methods (see ¶ 3*b* below). Many strong oxidizing agents interfere in the measurement of free chlorine in all methods. Such interferences include bromine, chlorine dioxide, iodine, permanganate, hydrogen peroxide, and ozone. However, the reduced forms of these compounds—bromide, chloride, iodide, manganous ion, and oxygen, in the absence of other oxidants, do not interfere. Reducing agents such as ferrous compounds, hydrogen sulfide, and oxidizable organic matter generally do not interfere.

b. Wastewaters: The determination of total chlorine in samples containing organic matter presents special problems. Because of the presence of ammonia, amines, and organic compounds, particularly organic nitrogen, residual chlorine exists in a combined state. A considerable residual may exist in this form, but at the same time there may be appreciable unsatisfied chlorine demand. Addition of reagents in the determination may change these relationships so that residual chlorine is lost during the analysis. Only the DPD method for total chlorine is performed under neutral pH conditions. In wastewater, the differentiation between free chlorine and combined chlorine ordinarily is not made because wastewater chlorination seldom is carried far enough to produce free chlorine.

The determination of residual chlorine in industrial wastes is similar to that in domestic wastewater when the waste contains organic matter, but may be similar to the determination in water when the waste is low in organic matter.

None of these methods is applicable to estuarine or marine waters because the bromide is converted to bromine and bromamines, which are detected as free or total chlorine. A procedure for estimating this interference is available for the DPD method.

Although the methods given below are useful for the determination of residual chlorine in wastewaters and treated effluents, select the method in accordance with sample composition. Some industrial wastes, or mixtures of wastes with domestic wastewater, may require special precautions and modifications to obtain satisfactory results.

Determine free chlorine in wastewater by any of the methods provided that known interfering substances are absent or appropriate correction techniques are used. The amperometric method is the method of choice because it is not subject to interference from color, turbidity, iron, manganese, or nitrite nitrogen. The DPD method is subject to interference from high concentrations of monochloramine, which is avoided by adding thioacetamide immediately after reagent addition. Oxidized forms of manganese at all levels encountered in water will interfere in all methods except in the free chlorine measurement of amperometric titrations and FACTS, but a blank correction for manganese can be made in Methods F and G.

The FACTS method is unaffected by concentrations of monochloramine, dichloramine, nitrite, iron, manganese, and other interfering compounds normally found in domestic wastewaters.

For total chlorine in samples containing significant amounts of organic matter, use either the DPD methods (F and G), amperometric, or iodometric back titration method (C) to prevent

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contact between the full concentration of liberated iodine and the sample. With Method C, do not use the starch-iodide end point if the concentration is less than 1 mg/L. In the absence of interference, the amperometric and starch-iodide end points give concordant results. The amperometric end point is inherently more sensitive and is free of interference from color and turbidity, which can cause difficulty with the starch-iodide end point. On the other hand, certain metals, surface-active agents, and complex anions in some industrial wastes interfere in the amperometric titration and indicate the need for another method for such wastewaters. Silver in the form of soluble silver cyanide complex, in concentrations of 1.0 mg Ag/L, poisons the cell at pH 4.0 but not at 7.0. The silver ion, in the absence of the cyanide complex, gives extensive response in the current at pH 4.0 and gradually poisons the cell at all pH levels. Cuprous copper in the soluble copper cyanide ion, in concentrations of 5 mg Cu/L or less, poisons the cell at pH 4.0 and 7.0. Although iron and nitrite may interfere with this method, minimize the interference by buffering to pH 4.0 before adding KI. Oxidized forms of manganese interfere in all methods for total chlorine including amperometric titration. An unusually high content of organic matter may cause uncertainty in the end point.

Regardless of end-point detection, either phenylarsine oxide or thiosulfate may be used as the standard reducing reagent at pH 4. The former is more stable and is preferred.

The DPD titrimetric and colorimetric methods (F and G, respectively) are applicable to determining total chlorine in polluted waters. In addition, both DPD procedures and the amperometric titration method allow for estimating monochloramine and dichloramine fractions. Because all methods for total chlorine depend on the stoichiometric production of iodine, waters containing iodine-reducing substances may not be analyzed accurately by these methods, especially where iodine remains in the solution for a significant time. This problem occurs in Methods B and D. The back titration procedure (C) and Methods F and G cause immediate reaction of the iodine generated so that it has little chance to react with other iodine-reducing substances.

In all colorimetric procedures, compensate for color and turbidity by using color and turbidity blanks.

A method (I) for total residual chlorine using a potentiometric iodide electrode is proposed. This method is suitable for analysis of chlorine residuals in natural and treated waters and wastewater effluents. No differentiation of free and combined chlorine is possible. This procedure is an adaptation of other iodometric techniques and is subject to the same inferences.

4. Sampling and Storage

Chlorine in aqueous solution is not stable, and the chlorine content of samples or solutions, particularly weak solutions, will decrease rapidly. Exposure to sunlight or other strong light or agitation will accelerate the reduction of chlorine. Therefore, start chlorine determinations immediately after sampling, avoiding excessive light and agitation. Do not store samples to be analyzed for chlorine.

5. Reference

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4500-Cl B. Iodometric Method I

1. General Discussion

a. Principle: Chlorine will liberate free iodine from potassium iodide (KI) solutions at pH 8 or less. The liberated iodine is titrated with a standard solution of sodium thiosulfate ($Na_2S_2O_3$) with starch as the indicator. Titrate at pH 3 to 4 because the reaction is not stoichiometric at neutral pH due to partial oxidation of thiosulfate to sulfate.

b. Interference: Oxidized forms of manganese and other oxidizing agents interfere. Reducing agents such as organic sulfides also interfere. Although the neutral titration minimizes the interfering effect of ferric and nitrite ions, the acid titration is preferred because some forms of combined chlorine do not react at pH 7. Use only acetic acid for the acid titration; sulfuric acid (H_2SO_4) will increase interferences; *never use hydrochloric acid (HCl).* See ¶ A.3 for discussion of other interferences.

c. Minimum detectable concentration: The minimum detectable concentration approximates 40 µg Cl as Cl_2/L if 0.01*N* $Na_2S_2O_3$ is used with a 1000-mL sample. Concentrations below 1 mg/L cannot be determined accurately by the starch-iodide end point used in this method. Lower concentrations can be measured with the amperometric end point in Methods C and D.

2. Reagents

a. Acetic acid, conc (glacial).

b. Potassium iodide, KI, crystals.

c. Standard sodium thiosulfate, 0.1*N:* Dissolve 25 g $Na_2S_2O_3 \cdot 5H_2O$ in 1 L freshly boiled

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distilled water and standardize against potassium bi-iodate or potassium dichromate after at least 2 weeks storage. This initial storage is necessary to allow oxidation of any bisulfite ion present. Use boiled distilled water and add a few milliliters chloroform ($CHCl_3$) to minimize bacterial decomposition.

Standardize 0.1*N* $Na_2S_2O_3$ by one of the following:

1) Iodate method—Dissolve 3.249 g anhydrous potassium bi-iodate, $KH(IO_3)_2$, primary standard quality, or 3.567 g KIO_3 dried at 103 ± 2°C for 1 h, in distilled water and dilute to 1000 mL to yield a 0.1000*N* solution. Store in a glass-stoppered bottle.

To 80 mL distilled water, add, with constant stirring, 1 mL conc H_2SO_4, 10.00 mL 0.1000*N* $KH(IO_3)_2$, and 1 g KI. Titrate immediately with 0.1*N* $Na_2S_2O_3$ titrant until the yellow color of the liberated iodine almost is discharged. Add 1 mL starch indicator solution and continue titrating until the blue color disappears.

2) Dichromate method—Dissolve 4.904 g anhydrous potassium dichromate, $K_2Cr_2O_7$, of primary standard quality, in distilled water and dilute to 1000 mL to yield a 0.1000*N* solution. Store in a glass-stoppered bottle.

Proceed as in the iodate method, with the following exceptions: Substitute 10.00 mL 0.1000*N* $K_2Cr_2O_7$ for iodate and let reaction mixture stand 6 min in the dark before titrating with 0.1*N* $Na_2S_2O_3$ titrant.

$$\text{Normality } Na_2S_2O_3 = \frac{1}{\text{mL } Na_2S_2O_3 \text{ consumed}}$$

d. Standard sodium thiosulfate titrant, 0.01*N* or 0.025*N:* Improve the stability of 0.01*N* or 0.025*N* $Na_2S_2O_3$ by diluting an aged 0.1*N* solution, made as directed above, with freshly boiled distilled water. Add 4 g sodium borate and 10 mg mercuric iodide/L solution. For accurate work, standardize this solution daily in accordance with the directions given above, using 0.01*N* or 0.025*N* iodate or $K_2Cr_2O_7$. Use sufficient volumes of these standard solutions so that their final dilution is not greater than 1 + 4. To speed up operations where many samples must be titrated use an automatic buret of a type in which rubber does not come in contact with the solution. Standard titrants, 0.0100*N* and 0.0250*N*, are equivalent, respectively, to 354.5 µg and 886.3 µg Cl as Cl_2/1.00 mL.

e. Starch indicator solution: To 5 g starch (potato, arrowroot, or soluble), add a little cold water and grind in a mortar to a thin paste. Pour into 1 L of boiling distilled water, stir, and let settle overnight. Use clear supernate. Preserve with 1.25 g salicylic acid, 4 g zinc chloride, or a combination of 4 g sodium propionate and 2 g sodium azide/L starch solution. Some commercial starch substitutes are satisfactory.

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f. Standard iodine, 0.1*N:* See ¶ C.3*g*.

g. Dilute standard iodine, 0.0282*N:* See ¶ C.3*h*.

3. Procedure

a. Volume of sample: Select a sample volume that will require no more than 20 mL 0.01*N* $Na_2S_2O_3$ and no less than 0.2 mL for the starch-iodide end point. For a chlorine range of 1 to 10 mg/ L, use a 500-mL sample; above 10 mg/L, use proportionately less sample. Use smaller samples and volumes of titrant with the amperometric end point.

b. Preparation for titration: Place 5 mL acetic acid, or enough to reduce the pH to between 3.0 and 4.0, in a flask or white porcelain casserole. Add about 1 g KI estimated on a spatula. Pour sample in and mix with a stirring rod.

c. Titration: Titrate away from direct sunlight. Add 0.025*N* or 0.01*N* $Na_2S_2O_3$ from a buret until the yellow color of the liberated iodine almost is discharged. Add 1 mL starch solution and titrate until blue color is discharged.

If the titration is made with 0.025*N* $Na_2S_2O_3$ instead of 0.01*N*, then, with a 1-L sample, 1 drop is equivalent to about 50 µg/L. It is not possible to discern the end point with greater accuracy.

d. Blank titration: Correct result of sample titration by determining blank contributed by oxidizing or reducing reagent impurities. The blank also compensates for the concentration of iodine bound to starch at the end point.

Take a volume of distilled water corresponding to the sample used for titration in ¶s 3*a*–c, add 5 mL acetic acid, 1 g KI, and 1 mL starch solution. Perform blank titration as in 1) or 2) below, whichever applies.

1) If a blue color develops, titrate with 0.01*N* or 0.025*N* $Na_2S_2O_3$ to disappearance of blue color and record result. *B* (see ¶ 4, below) is negative.

2) If no blue color occurs, titrate with 0.0282*N* iodine solution until a blue color appears. Back-titrate with 0.01*N* or 0.025*N* $Na_2S_2O_3$ and record the difference. *B* is positive.

Before calculating the chlorine concentration, subtract the blank titration of ¶ 1) from the sample titration; or, if necessary, add the net equivalent value of the blank titration of ¶ 2).

4. Calculation

For standardizing chlorine solution for temporary standards:

$$\text{mg Cl as Cl}_2\text{/mL} = \frac{(A \pm B) \times N \times 35.45}{\text{mL sample}}$$

For determining total available residual chlorine in a water sample:

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$$\text{mg Cl as Cl}_2\text{/L} = \frac{(A \pm B) \times N \times 35\,450}{\text{mL sample}}$$

where:

A = mL titration for sample,
B = mL titration for blank (positive or negative), and
N = normality of $Na_2S_2O_3$.

5. Precision and Bias

Published studies[1,2] give the results of nine methods used to analyze synthetic water samples without interferences; variations of some of the methods appear in this edition. More current data are not now available.

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1. Water Chlorine (Residual) No. 1. 1969. Analytical Reference Service Rep. No. 35, U.S. Environmental Protection Agency, Cincinnati, Ohio.
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4500-Cl C. Iodometric Method II

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1. General Discussion

a. Principle: In this method, used for wastewater analysis, the end-point signal is reversed because the unreacted standard reducing agent (phenylarsine oxide or thiosulfate) remaining in the sample is titrated with standard iodine or standard iodate, rather than the iodine released being titrated directly. This indirect procedure is necessary regardless of the method of end-point detection, to avoid contact between the full concentration of liberated iodine and the wastewater.

When iodate is used as a back titrant, use only phosphoric acid. Do not use acetate buffer.

b. Interference: Oxidized forms of manganese and other oxidizing agents give positive interferences. Reducing agents such as organic sulfides do not interfere as much as in Method B. Minimize iron and nitrite interference by buffering to pH 4.0 before adding potassium iodide (KI). An unusually high content of organic matter may cause some uncertainty in the end point. Whenever manganese, iron, and other interferences definitely are absent, reduce this uncertainty and improve precision by acidifying to pH 1.0. Control interference from more than 0.2 mg nitrite/L with phosphoric acid-sulfamic acid reagent. A larger fraction of organic chloramines will react at lower pH along with interfering substances. See ¶ A.3 for a discussion of other interferences.

2. Apparatus

For a description of the amperometric end-point detection apparatus and a discussion of its use, see ¶ D.2*a*.

3. Reagents

a. Standard phenylarsine oxide solution, 0.005 64*N*: Dissolve approximately 0.8 g phenylarsine oxide powder in 150 mL 0.3*N* NaOH solution. After settling, decant 110 mL into 800 mL distilled water and mix thoroughly. Bring to pH 6 to 7 with 6*N* HCl and dilute to 950 mL with distilled water. CAUTION: *Severe poison, cancer suspect agent.*

Standardization—Accurately measure 5 to 10 mL freshly standardized 0.0282*N* iodine solution into a flask and add 1 mL KI solution. Titrate with phenylarsine oxide solution, using the amperometric end point (Method D) or starch solution (see ¶ B.2*e*) as an indicator. Adjust to 0.005 64*N* and recheck against the standard iodine solution; 1.00 mL = 200 µg available chlorine. (CAUTION: *Toxic—take care to avoid ingestion.*)

b. Standard sodium thiosulfate solution, 0.1*N*: See ¶ B.2*c*.

c. Standard sodium thiosulfate solution, 0.005 64*N*: Prepare by diluting 0.1*N* $Na_2S_2O_3$. For maximum stability of the dilute solution, prepare by diluting an aged 0.1*N* solution with freshly boiled distilled water (to minimize bacterial action) and add 4 g $Na_4B_4O_7$/L. To inhibit mold formation optionally add either 10 mg HgI_2 or 2 drops toluene per liter of solution. Standardize daily as directed in B.2*c* using 0.005 64*N* $K_2Cr_2O_7$ or iodate solution. Use sufficient volume of sample so that the final dilution does not exceed 1 + 2. Use an automatic buret of a type in which rubber does not come in contact with the solution. 1.00 mL = 200 µg available chlorine.

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d. Potassium iodide, KI, crystals.

e. Acetate buffer solution, pH 4.0: Dissolve 146 g anhydrous $NaC_2H_3O_2$, or 243 g $NaC_2H_3O_2 \cdot 3H_2O$, in 400 mL distilled water, add 480 g conc acetic acid, and dilute to 1 L with chlorine-demand-free water.

f. Standard arsenite solution, 0.1*N*: Accurately weigh a stoppered weighing bottle containing approximately 4.95 g arsenic trioxide, As_2O_3. Transfer without loss to a 1-L volumetric flask and again weigh bottle. Do not attempt to brush out adhering oxide. Moisten As_2O_3 with water and add 15 g NaOH and 100 mL distilled water. Swirl flask contents gently to dissolve. Dilute to 250 mL with distilled water and saturate with CO_2, thus converting all NaOH to $NaHCO_3$. Dilute to mark, stopper, and mix thoroughly. This solution will preserve its titer almost indefinitely. (CAUTION: *Severe poison. Cancer suspect agent.*)

$$\text{Normality} = \frac{\text{g } As_2O_3}{49.455}$$

g. Standard iodine solution, 0.1*N*: Dissolve 40 g KI in 25 mL chlorine-demand-free water, add 13 g resublimed iodine, and stir until dissolved. Transfer to a 1-L volumetric flask and dilute to mark.

Standardization—Accurately measure 40 to 50 mL 0.1*N* arsenite solution into a flask and titrate with 0.1*N* iodine solution, using starch solution as indicator. To obtain accurate results, insure that the solution is saturated with CO_2 at end of titration by passing current of CO_2 through solution for a few minutes just before end point is reached, or add a few drops of HCl to liberate sufficient CO_2 to saturate solution. Alternatively standardize against $Na_2S_2O_3$; see ¶ B.2*c*1).

Optionally, prepare 0.1000*N* iodine solution directly as a standard solution by weighing 12.69 g primary standard resublimed iodine. Because I_2 may be volatilized and lose from both solid and solution, transfer the solid immediately to KI as specified above. Never let solution stand in open containers for extended periods.

h. Standard iodine titrant, 0.0282*N*: Dissolve 25 g KI in a little distilled water in a 1-L volumetric flask, add correct amount of 0.1*N* iodine solution exactly standardized to yield a 0.0282*N* solution, and dilute to 1 L with chlorine-demand-free water. For accurate work, standardize daily according to directions in ¶ 3*g* above, using 5 to 10 mL of arsenite or $Na_2S_2O_3$ solution. Store in amber bottles or in the dark; protect solution from direct sunlight at all times and keep from all contact with rubber.

i. Starch indicator: See ¶ B.2*e*.

j. Standard iodate titrant, 0.005 64*N:* Dissolve 201.2 mg primary standard grade KIO_3, dried

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for 1 h at 103°C, or 183.3 mg primary standard anhydrous potassium bi-iodate in distilled water and dilute to 1 L.

k. Phosphoric acid solution, H_3PO_4, 1 + 9.

l. Phosphoric acid-sulfamic acid solution: Dissolve 20 g NH_2SO_3H in 1 L 1 + 9 phosphoric acid.

m. Chlorine-demand-free water: Prepare chlorine-demand-free water from good-quality distilled or deionized water by adding sufficient chlorine to give 5 mg/L free chlorine. After standing 2 d this solution should contain at least 2 mg/L free chlorine; if not, discard and obtain better-quality water. Remove remaining free chlorine by placing container in sunlight or irradiating with an ultraviolet lamp. After several hours take sample, add KI, and measure total chlorine with a colorimetric method using a nessler tube to increase sensitivity. Do not use before last trace of free and combined chlorine has been removed.

Distilled water commonly contains ammonia and also may contain reducing agents. Collect good-quality distilled or deionized water in a sealed container from which water can be drawn by gravity. To the air inlet of the container add an H_2SO_4 trap consisting of a large test tube half filled with 1 + 1 H_2SO_4 connected in series with a similar but empty test tube. Fit both test tubes with stoppers and inlet tubes terminating near the bottom of the tubes and outlet tubes terminating near the top of the tubes. Connect outlet tube of trap containing H_2SO_4 to the distilled water container, connect inlet tube to outlet of empty test tube. The empty test tube will prevent discharge to the atmosphere of H_2SO_4 due to temperature-induced pressure changes. Stored in such a container, chlorine-demand-free water is stable for several weeks unless bacterial growth occurs.

4. Procedure

a. Preparation for titration:

1) Volume of sample—For chlorine concentration of 10 mg/L or less, titrate 200 mL. For greater chlorine concentrations, use proportionately less sample and dilute to 200 mL with chlorine-demand-free water. Use a sample of such size that not more than 10 mL phenylarsine oxide solution is required.

2) Preparation for titration—Measure 5 mL 0.005 64*N* phenylarsine oxide or thiosulfate for chlorine concentrations from 2 to 5 mg/L, and 10 mL for concentrations of 5 to 10 mg/L, into a flask or casserole for titration with standard iodine or iodate. Start stirring. For titration by amperometry or standard iodine, also add excess KI (approximately 1 g) and 4 mL acetate buffer solution or enough to reduce the pH to between 3.5 and 4.2.

b. Titration: Use one of the following:

1) Amperometric titration—Add 0.0282*N* iodine titrant in small increments from a 1-mL buret or pipet. Observe meter needle response as iodine is added: the pointer remains practically stationary until the end point is approached, whereupon each iodine increment causes a

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temporary deflection of the microammeter, with the pointer dropping back to its original position. Stop titration at end point when a small increment of iodine titrant gives a definite pointer deflection upscale and the pointer does not return promptly to its original position. Record volume of iodine titrant used to reach end point.

2) Colorimetric (iodine) titration—Add 1 mL starch solution and titrate with 0.0282*N* iodine to the first appearance of blue color that persists after complete mixing.

3) Colorimetric (iodate) titration—To suitable flask or casserole add 200 mL chlorine-demand-free water and add, with agitation, the required volume of reductant, an excess of KI (approximately 0.5 g), 2 mL 10% H_3PO_4 solution, and 1 mL starch solution in the order given, and titrate immediately*#(29) with 0.005 64*N* iodate solution to the first appearance of a blue color that persists after complete mixing. Designate volume of iodate solution used as *A*. Repeat procedure, substituting 200 mL sample for the 200 mL chlorine-demand-free water. If sample is colored or turbid, titrate to the first change in color, using for comparison another portion of sample with H_3PO_4 added. Designate this volume of iodate solution as *B*.

5. Calculation

a. Titration with standard iodine:

$$\text{mg Cl as Cl}_2\text{/L} = \frac{(A - 5B) \times 200}{C}$$

where:

A = mL 0.005 64*N* reductant,
B = mL 0.0282 *N* I_2, and
C = mL sample.

b. Titration with standard iodate:

$$\text{mg Cl as Cl}_2\text{/L} = \frac{(A - B) \times 200}{C}$$

where:

A = mL $Na_2S_2O_3$,
B = mL iodate required to titrate $Na_2S_2O_3$, and
C = mL sample.

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6. Bibliography

See Section 4500-Cl.B.7.

4500-Cl D. Amperometric Titration Method

1. General Discussion

Amperometric titration requires a higher degree of skill and care than the colorimetric methods. Chlorine residuals over 2 mg/L are measured best by means of smaller samples or by dilution with water that has neither residual chlorine nor a chlorine demand. The method can be used to determine total chlorine and can differentiate between free and combined chlorine. A further differentiation into monochloramine and dichloramine fractions is possible by control of KI concentration and pH.

a. Principle: The amperometric method is a special adaptation of the polarographic principle. Free chlorine is titrated at a pH between 6.5 and 7.5, a range in which the combined chlorine reacts slowly. The combined chlorine, in turn, is titrated in the presence of the proper amount of KI in the pH range 3.5 to 4.5. When free chlorine is determined, the pH must not be greater than 7.5 because the reaction becomes sluggish at higher pH values, nor less than 6.5 because at lower pH values some combined chlorine may react even in the absence of iodide. When combined chlorine is determined, the pH must not be less than 3.5 because of increased interferences at lower pH values, nor greater than 4.5 because the iodide reaction is not quantitative at higher pH values. The tendency of monochloramine to react more readily with iodide than does dichloramine provides a means for further differentiation. The addition of a small amount of KI in the neutral pH range enables estimation of monochloramine content. Lowering the pH into the acid range and increasing the KI concentration allows the separation determination of dichloramine.

Organic chloramines can be measured as free chlorine, monochloramine, or dichloramine, depending on the activity of chlorine in the organic compound.

Phenylarsine oxide is stable even in dilute solution and each mole reacts with two equivalents of halogen. A special amperometric cell is used to detect the end point of the residual chlorine-phenylarsine oxide titration. The cell consists of a nonpolarizable reference electrode that is immersed in a salt solution and a readily polarizable noble-metal electrode that is in contact with both the salt solution and the sample being titrated. In some applications, end-point selectivity is improved by adding +200 mV to the platinum electrode versus silver, silver chloride. Another approach to end-point detection uses dual platinum electrodes, a mercury cell with voltage divider to impress a potential across the electrodes, and a microammeter. If there is no chlorine residual in the sample, the microammeter reading will be comparatively low because of cell polarization. The greater the residual, the greater the microammeter reading. The meter acts merely as a null-point indicator—that is, the actual meter reading is not important, but rather the relative readings as the titration proceeds. The gradual addition of phenylarsine oxide causes

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the cell to become more and more polarized because of the decrease in chlorine. The end point is recognized when no further decrease in meter reading can be obtained by adding more phenylarsine oxide.

b. Interference: Accurate determinations of free chlorine cannot be made in the presence of nitrogen trichloride, NCl_3, or chlorine dioxide, which titrate partly as free chlorine. When present, NCl_3 can titrate partly as free chlorine and partly as dichloramine, contributing a positive error in both fractions at a rate of approximately 0.1%/min. Some organic chloramines also can be titrated in each step. Monochloramine can intrude into the free chlorine fraction and dichloramine can interfere in the monochloramine fraction, especially at high temperatures and prolonged titration times. Free halogens other than chlorine also will titrate as free chlorine. Combined chlorine reacts with iodide ions to produce iodine. When titration for free chlorine follows a combined chlorine titration, which requires addition of KI, erroneous results may occur unless the measuring cell is rinsed thoroughly with distilled water between titrations.

Interference from copper has been noted in samples taken from copper pipe or after heavy copper sulfate treatment of reservoirs, with metallic copper plating out on the electrode. Silver ions also poison the electrode. Interference occurs in some highly colored waters and in waters containing surface-active agents. Very low temperatures slow response of measuring cell and longer time is required for the titration, but precision is not affected. A reduction in reaction rate is caused by pH values above 7.5; overcome this by buffering all samples to pH 7.0 or less. On the other hand, some substances, such as manganese, nitrite, and iron, do not interfere. The violent stirring of some commercial titrators can lower chlorine values by volatilization. When dilution is used for samples containing high chlorine content, take care that the dilution water is free of chlorine and ammonia and possesses no chlorine demand.

See ¶ A.3 for a discussion of other interferences.

2. Apparatus

a. End-point detection apparatus, consisting of a cell unit connected to a microammeter, with necessary electrical accessories. The cell unit includes a noble-metal electrode of sufficient surface area, a salt bridge to provide an electrical connection without diffusion of electrolyte, and a reference electrode of silver-silver chloride in a saturated sodium chloride solution connected into the circuit by means of the salt bridge. Numerous commercial systems are available.

Keep platinum electrode free of deposits and foreign matter. Vigorous chemical cleaning generally is unnecessary. Occasional mechanical cleaning with a suitable abrasive usually is sufficient. Keep salt bridge in good operating condition; do not allow it to become plugged nor permit appreciable flow of electrolyte through it. Keep solution surrounding reference electrode free of contamination and maintain it at constant composition by insuring an adequate supply of undissolved salt at all times. A cell with two metal electrodes polarized by a small DC potential also may be used. (See Bibliography.)

b. Agitator, designed to give adequate agitation at the noble-metal electrode surface to insure proper sensitivity. Thoroughly clean agitator and exposed electrode system to remove all

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chlorine-consuming contaminants by immersing them in water containing 1 to 2 mg/L free chlorine for a few minutes. Add KI to the same water and let agitator and electrodes remain immersed for 5 min. After thorough rinsing with chlorine-demand-free water or the sample to be tested, sensitized electrodes and agitator are ready for use. Remove iodide reagent completely from cell.

c. Buret: Commercial titrators usually are equipped with suitable burets (1 mL). Manual burets are available.*#(30)

d. Glassware, exposed to water containing at least 10 mg/L chlorine for 3 h or more before use and rinsed with chlorine-demand-free water.

3. Reagents

a. Standard phenylarsine oxide titrant: See ¶ C.3*a*.

b. Phosphate buffer solution, pH 7: Dissolve 25.4 g anhydrous KH_2PO_4 and 34.1 g anhydrous Na_2HPO_4 in 800 mL distilled water. Add 2 mL sodium hypochlorite solution containing 1% chlorine and mix thoroughly. Protect from sunlight for 2 d. Determine that free chlorine still remains in the solution. Then expose to sunlight until no chlorine remains. If necessary, carry out the final dechlorination with an ultraviolet lamp. Determine that no total chlorine remains by adding KI and measuring with one of the colorimetric tests. Dilute to 1 L with distilled water and filter if any precipitate is present.

c. Potassium iodide solution: Dissolve 50 g KI and dilute to 1 L with freshly boiled and cooled distilled water. Store in the dark in a brown glass-stoppered bottle, preferably in the refrigerator. Discard when solution becomes yellow.

d. Acetate buffer solution, pH 4: See ¶ C.3*e*.

4. Procedure

a. Sample volume: Select a sample volume requiring no more than 2 mL phenylarsine oxide titrant. Thus, for chlorine concentrations of 2 mg/L or less, take a 200-mL sample; for chlorine levels in excess of 2 mg/L, use 100 mL or proportionately less.

b. Free chlorine: Unless sample pH is known to be between 6.5 and 7.5, add 1 mL pH 7 phosphate buffer solution to produce a pH of 6.5 to 7.5. Titrate with standard phenylarsine oxide titrant, observing current changes on microammeter. Add titrant in progressively smaller increments until all needle movement ceases. Make successive buret readings when needle action becomes sluggish, signaling approach of end point. Subtract last very small increment that causes no needle response because of overtitration. Alternatively, use a system involving continuous current measurements and determine end point mathematically.

Continue titrating for combined chlorine as described in ¶ 4*c* below or for the separate monochloramine and dichloramine fractions as detailed in ¶s 4*e* and 4*f*.

c. Combined chlorine: To sample remaining from free-chlorine titration add 1.00 mL KI solution and 1 mL acetate buffer solution, in that order. Titrate with phenylarsine oxide titrant to

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the end point, as above. Do not refill buret but simply continue titration after recording figure for free chlorine. Again subtract last increment to give amount of titrant actually used in reaction with chlorine. (If titration was continued without refilling buret, this figure represents total chlorine. Subtracting free chlorine from total gives combined chlorine.) Wash apparatus and sample cell thoroughly to remove iodide ion to avoid inaccuracies when the titrator is used subsequently for a free chlorine determination.

d. Separate samples: If desired, determine total chlorine and free chlorine on separate samples. If sample pH is between 3.5 and 9.5 and total chlorine alone is required, treat sample immediately with 1 mL KI solution followed by 1 mL acetate buffer solution, and titrate with phenylarsine oxide titrant as described in ¶ 4*c* preceding.

e. Monochloramine: After titrating for free chlorine, add 0.2 mL KI solution to same sample and, without refilling buret, continue titration with phenylarsine oxide titrant to end point. Subtract last increment to obtain net volume of titrant consumed by monochloramine.

f. Dichloramine: Add 1 mL acetate buffer solution and 1 mL KI solution to same sample and titrate final dichloramine fraction as described above.

5. Calculation

Convert individual titrations for free chlorine, combined chlorine, total chlorine, monochloramine, and dichloramine by the following equation:

$$\text{mg Cl as } Cl_2/\text{L} = \frac{A \times 200}{\text{mL sample}}$$

where:

A = mL phenylarsine oxide titration.

6. Precision and Bias

See ¶ B.5.

7. Bibliography

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4500-Cl E. Low-Level Amperometric Titration Method

1. General Discussion

Detection and quantification of chlorine residuals below 0.2 mg/L require special modifications to the amperometric titration procedure. With these modifications chlorine concentrations at the 10-µg/L level can be measured. It is not possible to differentiate between free and combined chlorine forms. Oxidizing agents that interfere with the amperometric titration method (D) will interfere.

a. Principle: This method modifies D by using a more dilute titrant and a graphical procedure to determine the end point.

b. Interference: See ¶ D.1*b*.

2. Apparatus

See ¶ D.2.

3. Reagents

a. Potassium bi-iodate, 0.002 256*N*: Dissolve 0.7332 g anhydrous potassium bi-iodate, $KH(IO_3)_2$, in 500 mL chlorine-free distilled water and dilute to 1000 mL. Dilute 10.00 mL to 100.0 mL with chlorine-free distilled water. Use only freshly prepared solution for the standardization of phenylarsine oxide.

b. Potassium iodide, KI crystals.

c. Low-strength phenylarsine oxide titrant, 0.000 564*N*: Dilute 10.00 mL of 0.005 64*N* phenylarsine oxide (see C.3*a*) to 100.0 mL with chlorine-demand-free water (see C.3*m*).

Standardization—Dilute 5.00 mL 0.002 256*N* potassium bi-iodate to 200 mL with chlorine-free water. Add approximately 1.5 g KI and stir to dissolve. Add 1 mL acetate buffer and let stand in the dark for 6 min. Titrate using the amperometric titrator and determine the equivalence point as indicated below.

$$\text{Normality} = 0.002256 \times 5/A$$

where:

A = mL phenylarsine oxide titrant required to reach the equivalence point of standard bi-iodate.

d. Acetate buffer solution, pH 4: See ¶ C.3*e*.

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4. Procedure

Select a sample volume requiring no more than 2 mL phenylarsine oxide titrant. A 200-mL sample will be adequate for samples containing less than 0.2 mg total chlorine/L.

Before beginning titration, rinse buret with titrant several times. Rinse sample container with distilled water and then with sample. Add 200 mL sample to sample container and approximately 1.5 g KI. Dissolve, using a stirrer or mixer. Add 1 mL acetate buffer and place container in end-point detection apparatus. When the current signal stabilizes, record the reading. Initially adjust meter to a near full-scale deflection. Titrate by adding small, known, volumes of titrant. After each addition, record cumulative volume added and current reading when the signal stabilizes. If meter reading falls to near or below 10% of full-scale deflection, record low reading, readjust meter to near full-scale deflection, and record difference between low amount and readjusted high deflection. Add this value to all deflection readings for subsequent titrant additions. Continue adding titrant until no further meter deflection occurs. If fewer than three titrant additions were made before meter deflection ceased, discard sample and repeat analysis using smaller titrant increments.

Determine equivalence point by plotting total meter deflection against titrant volume added. Draw straight line through the first several points in the plot and a second, horizontal straight line corresponding to the final total deflection in the meter. Read equivalence point as the volume of titrant added at the intersection of these two lines.

5. Calculation

$$\text{mg Cl as } Cl_2/L = \frac{A \times 200 \times N}{B \times 0.00564}$$

where:

A = mL titrant at equivalence point,
B = sample volume, mL, and
N = phenylarsine oxide normality.

6. Bibliography

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4500-Cl F. DPD Ferrous Titrimetric Method

1. General Discussion

a. Principle: N,N-diethyl-*p*-phenylenediamine (DPD) is used as an indicator in the titrimetric procedure with ferrous ammonium sulfate (FAS). Where complete differentiation of chlorine

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species is not required, the procedure may be simplified to give only free and combined chlorine or total chlorine.

In the absence of iodide ion, free chlorine reacts instantly with DPD indicator to produce a red color. Subsequent addition of a small amount of iodide ion acts catalytically to cause monochloramine to produce color. Addition of iodide ion to excess evokes a rapid response from dichloramine. In the presence of iodide ion, part of the nitrogen trichloride (NCl_3) is included with dichloramine and part with free chlorine. A supplementary procedure based on adding iodide ion before DPD permits estimating proportion of NCl_3 appearing with free chlorine.

Chlorine dioxide (ClO_2) appears, to the extent of one-fifth of its total chlorine content, with free chlorine. A full response from ClO_2, corresponding to its total chlorine content, may be obtained if the sample first is acidified in the presence of iodide ion and subsequently is brought back to an approximately neutral pH by adding bicarbonate ion. Bromine, bromamine, and iodine react with DPD indicator and appear with free chlorine.

Addition of glycine before determination of free chlorine converts free chlorine to unreactive forms, with only bromine and iodine residuals remaining. Subtractions of these residuals from the residual measured without glycine permits differentiation of free chlorine from bromine and iodine.

b. pH control: For accurate results careful pH control is essential. At the proper pH of 6.2 to 6.5, the red colors produced may be titrated to sharp colorless end points. *Titrate as soon as the red color is formed in each step.* Too low a pH in the first step tends to make the monochloramine show in the free-chlorine step and the dichloramine in the monochloramine step. Too high a pH causes dissolved oxygen to give a color.

c. Temperature control: In all methods for differentiating free chlorine from chloramines, higher temperatures increase the tendency for chloramines to react and lead to increased apparent free-chlorine results. Higher temperatures also increase color fading. Complete measurements rapidly, especially at higher temperature.

d. Interference: The most significant interfering substance likely to be encountered in water is oxidized manganese. To correct for this, place 5 mL buffer solution and 0.5 mL sodium arsenite solution in the titration flask. Add 100 mL sample and mix. Add 5 mL DPD indicator solution, mix, and titrate with standard FAS titrant until red color is discharged. Subtract reading from Reading *A* obtained by the normal procedure as described in ¶ 3*a*1) of this method or from the total chlorine reading obtained in the simplified procedure given in ¶ 3*a*4). If the combined reagent in powder form (see below) is used, first add KI and arsenite to the sample and mix, then add combined buffer-indicator reagent.

As an alternative to sodium arsenite use a 0.25% solution of thioacetamide, adding 0.5 mL to 100 mL sample.

Interference by copper up to approximately 10 mg Cu/L is overcome by the EDTA incorporated in the reagents. EDTA enhances stability of DPD indicator solution by retarding deterioration due to oxidation, and in the test itself, provides suppression of dissolved oxygen

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errors by preventing trace metal catalysis.

Chromate in excess of 2 mg/L interferes with end-point determination. Add barium chloride to mask this interference by precipitation.

High concentrations of combined chlorine can break through into the free chlorine fraction. *If free chlorine is to be measured in the presence of more than 0.5 mg/L combined chlorine, use the thioacetamide modification.* If this modification is not used, a color-development time in excess of 1 min leads to progressively greater interference from monochloramine. Adding thioacetamide (0.5 mL 0.25% solution to 100 mL) immediately after mixing DPD reagent with sample completely stops further reaction with combined chlorine in the free chlorine measurement. Continue immediately with FAS titration to obtain free chlorine. Obtain total chlorine from the normal procedure, i.e., without thioacetamide.

Because high concentrations of iodide are used to measure combined chlorine and only traces of iodide greatly increase chloramine interference in free chlorine measurements, take care to avoid iodide contamination by rinsing between samples or using separate glassware.

See¶ A.3 for a discussion of other interferences.

e. Minimum detectable concentration: Approximately 18 µg Cl as Cl_2/L. This detection limit is achievable under ideal conditions; normal working detection limits typically are higher.

2. Reagents

a. Phosphate buffer solution: Dissolve 24 g anhydrous Na_2HPO_4 and 46 g anhydrous KH_2PO_4 in distilled water. Combine with 100 mL distilled water in which 800 mg disodium ethylenediamine tetraacetate dihydrate (EDTA) have been dissolved. Dilute to 1 L with distilled water and optionally add either 20 mg $HgCl_2$ or 2 drops toluene to prevent mold growth. Interference from trace amounts of iodide in the reagents can be negated by optional addition of 20 mg $HgCl_2$ to the solution. (CAUTION: *$HgCl_2$ is toxic—take care to avoid ingestion.*)

b. N,N-Diethyl-p-phenylenediamine (DPD) indicator solution: Dissolve 1 g DPD oxalate,*#(31) or 1.5 g DPD sulfate pentahydrate,†#(32) or 1.1 g anhydrous DPD sulfate in chlorine-free distilled water containing 8 mL 1 + 3 H_2SO_4 and 200 mg disodium EDTA. Make up to 1 L, store in a brown glass-stoppered bottle in the dark, and discard when discolored. Periodically check solution blank for absorbance and discard when absorbance at 515 nm exceeds 0.002/cm. (The buffer and indicator sulfate are available commercially as a combined reagent in stable powder form.) CAUTION: *The oxalate is toxic—take care to avoid ingestion.*

c. Standard ferrous ammonium sulfate (FAS) titrant: Dissolve 1.106 g $Fe(NH_4)_2(SO_4)_2\cdot 6H_2O$ in distilled water containing 1 mL 1 + 3 H_2SO_4 and make up to 1 L with freshly boiled and cooled distilled water. This standard may be used for 1 month, and the titer checked by potassium dichromate. For this purpose add 10 mL 1 + 5 H_2SO_4, 5 mL conc H_3PO_4, and 2 mL 0.1% barium diphenylamine sulfonate indicator to a 100-mL sample of FAS and titrate with potassium dichromate to a violet end point that persists for 30 s. FAS titrant equivalent to

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100 µg Cl as Cl_2/1.00 mL requires 20.00 mL dichromate for titration.

d. Potassium iodide, KI, crystals.

e. Potassium iodide solution: Dissolve 500 mg KI and dilute to 100 mL, using freshly boiled and cooled distilled water. Store in a brown glass-stoppered bottle, preferably in a refrigerator. Discard when solution becomes yellow.

f. Potassium dichromate solution, 0.691 g to 1000 mL.

g. Barium diphenylaminesulfonate, 0.1%: Dissolve 0.1 g $(C_6H_5NHC_6H_4\text{-}4\text{-}SO_3)_2Ba$ in 100 mL distilled water.

h. Sodium arsenite solution: Dissolve 5.0 g $NaAsO_2$ in distilled water and dilute to 1 L. (CAUTION: *Toxic—take care to avoid ingestion.*)

i. Thioacetamide solution: Dissolve 250 mg CH_3CSNH_2 in 100 mL distilled water. (CAUTION: *Cancer suspect agent. Take care to avoid skin contact or ingestion.*)

j. Chlorine-demand-free water: See ¶ C.3*m*.

k. Glycine solution: Dissolve 20 g glycine (aminoacetic acid) in sufficient chlorine-demand-free water to bring to 100 mL total volume. Store under refrigerated conditions and discard if cloudiness develops.

l. Barium chloride crystals, $BaCl_2 \cdot 2H_2O$.

3. Procedure

The quantities given below are suitable for concentrations of total chlorine up to 5 mg/L. If total chlorine exceeds 5 mg/L, use a smaller sample and dilute to a total volume of 100 mL. Mix usual volumes of buffer reagent and DPD indicator solution, or usual amount of DPD powder, with distilled water *before* adding sufficient sample to bring total volume to 100 mL. (If sample is added before buffer, test does not work.)

If chromate is present (>2 mg/L) add and mix 0.2 g $BaCl_2 \cdot 2H_2O$/100 mL sample before adding other reagents. If, in addition, sulfate is >500 mg/L, use 0.4 g $BaCl_2 \cdot 2H_2O$/100 mL sample.

a. Free chlorine or chloramine: Place 5 mL each of buffer reagent and DPD indicator solution in titration flask and mix (or use about 500 mg DPD powder). Add 100 mL sample, or diluted sample, and mix.

1) Free chlorine—Titrate rapidly with standard FAS titrant until red color is discharged (Reading *A*).

2) Monochloramine—Add one very small crystal of KI (about 0.5 mg) or 0.1 mL (2 drops) KI solution and mix. Continue titrating until red color is discharged again (Reading *B*).

3) Dichloramine—Add several crystals KI (about 1 g) and mix to dissolve. Let stand for 2 min and continue titrating until red color is discharged (Reading *C*). For dichloramine

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concentrations greater than 1 mg/L, let stand 2 min more if color driftback indicates slightly incomplete reaction. When dichloramine concentrations are not expected to be high, use half the specified amount of KI.

4) Simplified procedure for free and combined chlorine or total chlorine—Omit 2) above to obtain monochloramine and dichloramine together as combined chlorine. To obtain total chlorine in one reading, add full amount of KI at the start, with the specified amounts of buffer reagent and DPD indicator, and titrate after 2 min standing.

b. Nitrogen trichloride: Place one very small crystal of KI (about 0.5 mg) or 0.1 mL KI solution in a titration flask. Add 100 mL sample and mix. Add contents to a second flask containing 5 mL each of buffer reagent and DPD indicator solution (or add about 500 mg DPD powder direct to the first flask). Titrate rapidly with standard FAS titrant until red color is discharged (Reading *N*).

c. Free chlorine in presence of bromine or iodine: Determine free chlorine as in ¶ 3*a*1). To a second 100-mL sample, add 1 mL glycine solution before adding DPD and buffer. Titrate according to ¶ 3*a*1). Subtract the second reading from the first to obtain Reading A.

4. Calculation

For a 100-mL sample, 1.00 mL standard FAS titrant = 1.00 mg Cl as Cl_2/L.

Reading	NCl_3 Absent	NCl_3 Present
A	Free Cl	Free Cl
$B - A$	NH_2Cl	NH_2Cl
$C - B$	$NHCl_2$	$NHCl_2 + ½NCl_3$
N	—	Free Cl + $½NCl_3$
$2(N - A)$	—	NCl_3
$C - N$	—	$NHCl_2$

In the event that monochloramine is present with NCl_3, it will be included in *N*, in which case obtain NCl_3 from $2(N–B)$.

Chlorine dioxide, if present, is included in *A* to the extent of one-fifth of its total chlorine content.

In the simplified procedure for free and combined chlorine, only *A* (free Cl) and *C* (total Cl) are required. Obtain combined chlorine from $C–A$.

The result obtained in the simplified total chlorine procedure corresponds to *C*.

5. Precision and Bias

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See ¶ B.5.

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4500-Cl G. DPD Colorimetric Method

1. General Discussion

a. Principle: This is a colorimetric version of the DPD method and is based on the same principles. Instead of titration with standard ferrous ammonium sulfate (FAS) solution as in the titrimetric method, a colorimetric procedure is used.

b. Interference: See ¶ A.3 and ¶ F.1*d*. Compensate for color and turbidity by using sample to zero photometer. Minimize chromate interference by using the thioacetamide blank correction.

c. Minimum detectable concentration: Approximately 10 µg Cl as Cl_2/L. This detection limit is achievable under ideal conditions; normal working detection limits typically are higher.

2. Apparatus

a. Photometric equipment: One of the following is required:

1) *Spectrophotometer,* for use at a wavelength of 515 nm and providing a light path of 1 cm or longer.

2) *Filter photometer,* equipped with a filter having maximum transmission in the wavelength

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range of 490 to 530 nm and providing a light path of 1 cm or longer.

b. Glassware: Use separate glassware, including separate spectrophotometer cells, for free and combined (dichloramine) measurements, to avoid iodide contamination in free chlorine measurement.

3. Reagents

See ¶s F.2*a*, *b*, *c*, *d*, *e*, *h*, *i,* and *j*.

4. Procedure

a. Calibration of photometric equipment: Calibrate instrument with chlorine or potassium permanganate solutions.

1) Chlorine solutions—Prepare chlorine standards in the range of 0.05 to 4 mg/L from about 100 mg/L chlorine water standardized as follows: Place 2 mL acetic acid and 10 to 25 mL chlorine-demand-free water in a flask. Add about 1 g KI. Measure into the flask a suitable volume of chlorine solution. In choosing a convenient volume, note that 1 mL 0.025N $Na_2S_2O_3$ titrant (see ¶ B.2*d*) is equivalent to about 0.9 mg chlorine. Titrate with standardized 0.025N $Na_2S_2O_3$ titrant until the yellow iodine color almost disappears. Add 1 to 2 mL starch indicator solution and continue titrating to disappearance of blue color.

Determine the blank by adding identical quantities of acid, KI, and starch indicator to a volume of chlorine-demand-free water corresponding to the sample used for titration. Perform blank titration A or B, whichever applies, according to ¶ B.3*d*.

$$\text{mg Cl as Cl}_2\text{/mL} = \frac{(A + B) \times N \times 35.45}{\text{mL sample}}$$

where:

N = normality of $Na_2S_2O_3$,
A = mL titrant for sample,
B = mL titrant for blank (to be added or subtracted according to required blank titration. See B.3*d*).

Use chlorine-demand-free water and glassware to prepare these standards. Develop color by first placing 5 mL phosphate buffer solution and 5 mL DPD indicator reagent in flask and then adding 100 mL chlorine standard with thorough mixing as described in *b* and *c* below. Fill photometer or colorimeter cell from flask and read color at 515 nm. Return cell contents to flask and titrate with standard FAS titrant as a check on chlorine concentration.

2) Potassium permanganate solutions—Prepare a stock solution containing 891 mg $KMnO_4$/1000 mL. Dilute 10.00 mL stock solution to 100 mL with distilled water in a volumetric flask. When 1 mL of this solution is diluted to 100 mL with distilled water, a chlorine equivalent

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of 1.00 mg/L will be produced in the DPD reaction. Prepare a series of $KMnO_4$ standards covering the chlorine equivalent range of 0.05 to 4 mg/L. Develop color by first placing 5 mL phosphate buffer and 5 mL DPD indicator reagent in flask and adding 100 mL standard with thorough mixing as described in *b* and *c* below. Fill photometer or colorimeter cell from flask and read color at 515 nm. Return cell contents to flask and titrate with FAS titrant as a check on any absorption of permanganate by distilled water.

Obtain all readings by comparison to color standards or the standard curve before use in calculation.

b. Volume of sample: Use a sample volume appropriate to the photometer or colorimeter. The following procedure is based on using 10-mL volumes; adjust reagent quantities proportionately for other sample volumes. Dilute sample with chlorine-demand-free water when total chlorine exceeds 4 mg/L.

c. Free chlorine: Place 0.5 mL each of buffer reagent and DPD indicator reagent in a test tube or photometer cell. Add 10 mL sample and mix. Read color immediately (Reading *A*).

d. Monochloramine: Continue by adding one very small crystal of KI (about 0.1 mg) and mix. If dichloramine concentration is expected to be high, instead of small crystal add 0.1 mL (2 drops) freshly prepared KI solution (0.1 g/100 mL). Read color immediately (Reading *B*).

e. Dichloramine: Continue by adding several crystals of KI (about 0.1 g) and mix to dissolve. Let stand about 2 min and read color (Reading *C*).

f. Nitrogen trichloride: Place a very small crystal of KI (about 0.1 mg) in a clean test tube or photometer cell. Add 10 mL sample and mix. To a second tube or cell add 0.5 mL each of buffer and indicator reagents; mix. Add contents to first tube or cell and mix. Read color immediately (Reading *N*).

g. Chromate correction using thioacetamide: Add 0.5 mL thioacetamide solution (F.2*i*) to 100 mL sample. After mixing, add buffer and DPD reagent. Read color immediately. Add several crystals of KI (about 0.1 g) and mix to dissolve. Let stand about 2 min and read color. Subtract the first reading from Reading *A* and the second reading from Reading *C* and use in calculations.

h. Simplified procedure for total chlorine: Omit Step *d* above to obtain monochloramine and dichloramine together as combined chlorine. To obtain total chlorine in one reading, add the full amount of KI at the start, with the specified amounts of buffer reagent and DPD indicator. Read color after 2 min.

5. Calculation

Reading	NCl_3 Absent	NCl_3 Present
A	Free Cl	Free Cl
$B - A$	NH_2Cl	NH_2Cl

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Reading	NCl_3 Absent	NCl_3 Present
$C - B$	$NHCl_2$	$NHCl_2 + ½NCl_3$
N	—	Free Cl + $½NCl_3$
$2(N - A)$	—	NCl_3
$C - N$	—	$NHCl_2$

In the event that monochloramine is present with NCl_3, it will be included in Reading *N*, in which case obtain NCl_3 from $2(N–B)$.

6. Bibliography

See ¶ F.6.

4500-Cl H. Syringaldazine (FACTS) Method

1. General Discussion

a. Principle: The free (available) chlorine test, syringaldazine (FACTS) measures free chlorine over the range of 0.1 to 10 mg/L. A saturated solution of syringaldazine (3,5-dimethoxy-4-hydroxybenzaldazine) in 2-propanol is used. Syringaldazine is oxidized by free chlorine on a 1:1 molar basis to produce a colored product with an absorption maximum of 530 nm. The color product is only slightly soluble in water; therefore, at chlorine concentrations greater than 1 mg/L, the final reaction mixture must contain 2-propanol to prevent product precipitation and color fading.

The optimum color and solubility (minimum fading) are obtained in a solution having a pH between 6.5 and 6.8. At a pH less than 6, color development is slow and reproducibility is poor. At a pH greater than 7, the color develops rapidly but fades quickly. A buffer is required to maintain the reaction mixture pH at approximately 6.7. Take care with waters of high acidity or alkalinity to assure that the added buffer maintains the proper pH.

Temperature has a minimal effect on the color reaction. The maximum error observed at temperature extremes of 5 and 35°C is ± 10%.

b. Interferences: Interferences common to other methods for determining free chlorine do not affect the FACTS procedure. Monochloramine concentrations up to 18 mg/L, dichloramine concentrations up to 10 mg/L, and manganese concentrations (oxidized forms) up to 1 mg/L do not interfere. Trichloramine at levels above 0.6 mg/L produces an apparent free chlorine reaction. Very high concentrations of monochloramine (≥35 mg/L) and oxidized manganese (≥2.6 mg/L) produce a color with syringaldazine slowly. Ferric iron can react with syringaldazine; however, concentrations up to 10 mg/L do not interfere. Nitrite (≤250 mg/L), nitrate (≤100 mg/L), sulfate (≤1000 mg/L), and chloride (≤1000 mg/L) do not interfere. Waters

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with high hardness (≥500 mg/L) will produce a cloudy solution that can be compensated for by using a blank. Oxygen does not interfere.

Other strong oxidizing agents, such as iodine, bromine, and ozone, will produce a color.

c. Minimum detectable concentration: The FACTS procedure is sensitive to free chlorine concentrations of 0.1 mg/L or less.

2. Apparatus

Colorimetric equipment: One of the following is required:

a. Filter photometer, providing a light path of 1 cm for chlorine concentrations ≤1 mg/L or a light path from 1 to 10 mm for chlorine concentrations above 1 mg/L; also equipped with a filter having a band pass of 500 to 560 nm.

b. Spectrophotometer, for use at 530 nm, providing the light paths noted above.

3. Reagents

a. Chlorine-demand-free water: See C.3*m*. Use to prepare reagent solutions and sample dilutions.

b. Syringaldazine indicator: Dissolve 115 mg 3,5-dimethoxy-4-hydroxybenzaldazine*#(33) in 1 L 2-propanol.

c. 2-Propanol: To aid in dissolution use ultrasonic agitation or gentle heating and stirring. Redistill reagent-grade 2-propanol to remove chlorine demand. Use a 30.5-cm Vigreux column and take the middle 75% fraction. Alternatively, chlorinate good-quality 2-propanol to maintain a free residual overnight; then expose to UV light or sunlight to dechlorinate. CAUTION: *2-Propanol is extremely flammable.*

d. Buffer: Dissolve 17.01 g KH_2PO_4 in 250 mL water; pH should be 4.4. Dissolve 17.75 g Na_2HPO_4 in 250 mL water; the pH should be 9.9. Mix equal volumes of these solutions to obtain FACTS buffer, pH 6.6. Verify pH with pH meter. For waters containing considerable hardness or high alkalinity other pH 6.6 buffers can be used, for example, 23.21 g maleic acid and 16.5 mL 50% NaOH per liter of water.

e. Hypochlorite solution: Dilute household hypochlorite solution, which contains about 30 000 to 50 000 mg Cl equivalent/L, to a strength between 100 and 1000 mg/L. Standardize as directed in ¶ G.4*a*1).

4. Procedure

a. Calibration of photometer: Prepare a calibration curve by making dilutions of a standardized hypochlorite solution (¶ 3*e*). Develop and measure colors as described in ¶ 4*b*, below. Check calibration regularly, especially as reagent ages.

b. Free chlorine analysis: Add 3 mL sample and 0.1 mL buffer to a 5-mL-capacity test tube. Add 1 mL syringaldazine indicator, cap tube, and invert twice to mix. Transfer to a photometer tube or spectrophotometer cell and measure absorbance. Compare absorbance value obtained

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with calibration curve and report corresponding value as milligrams free chlorine per liter.

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4500-Cl I. Iodometric Electrode Technique

1. General Discussion

a. Principle: This method involves the direct potentiometric measurement of iodine released on the addition of potassium iodide to an acidified sample. A platinum-iodide electrode pair is used in combination with an expanded-scale pH meter.

b. Interference: All oxidizing agents that interfere with other iodometric procedures interfere. These include oxidized manganese and iodate, bromine, and cupric ions. Silver and mercuric ions above 10 and 20 mg/L interfere.

2. Apparatus

a. Electrodes: Use either a combination electrode consisting of a platinum electrode and an iodide ion-selective electrode or two individual electrodes. Both systems are available commercially.

b. pH/millivolt meter: Use an expanded-scale pH/millivolt meter with 0.1 mV readability or a direct-reading selective ion meter.

3. Reagents

a. pH 4 buffer solution: See ¶ C.3*e*.

b. Chlorine-demand-free water: See ¶ C.3*m*.

c. Potassium iodide solution: Dissolve 42 g KI and 0.2 g Na_2CO_3 in 500 mL chlorine-demand-free, distilled water. Store in a dark bottle.

d. Standard potassium iodate 0.002 81*N:* Dissolve 0.1002 g KIO_3 in chlorine-demand-free, distilled water and dilute to 1000 mL. Each 1.0 mL, when diluted to 100 mL, produces a solution equivalent to 1 mg/L as Cl_2.

4. Procedure

a. Standardization: Pipet into three 100-mL stoppered volumetric flasks 0.20, 1.00, and 5.00

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mL standard iodate solution. Add to each flask, and a fourth flask to be used as a reagent blank, 1 mL each of acetate buffer solution and KI solution. Stopper, swirl to mix, and let stand 2 min before dilution. Dilute each standard to 100 mL with chlorine-demand-free distilled water. Stopper, invert flask several times to mix, and pour into separate 150-mL beakers. Stir gently without turbulence, using a magnetic stirrer, and immerse electrode(s) in the 0.2-mg/L (0.2-mL) standard. Wait for the potential to stabilize and record potential in mV. Rinse electrodes with chlorine-demand-free water and repeat for each standard and for the reagent blank. Prepare a calibration curve by plotting, on semilogarithmic paper, potential (linear axis) against concentration. Determine apparent chlorine concentration in the reagent blank from this graph (Reading B).

b. Analysis: Select a volume of sample containing no more than 0.5 mg chlorine. Pipet 1 mL acetate buffer solution and 1 mL KI into a 100-mL glass-stoppered volumetric flask. Stopper, swirl and let stand for at least 2 min. Adjust sample pH to 4 to 5, if necessary (mid-range pH paper is adequate for pH measurement), by adding acetic acid. Add pH-adjusted sample to volumetric flask and dilute to mark. Stopper and mix by inversion several times. Let stand for 2 min. Pour into a 150-mL beaker, immerse the electrode(s), wait for the potential to stabilize, and record. If the mV reading is greater than that recorded for the 5-mg/L standard, repeat analysis with a smaller volume of sample.

5. Calculation

Determine chlorine concentration (mg/L) corresponding to the recorded mV reading from the standard curve. This is Reading A. Determine total residual chlorine from the following:

$$\text{Total residual chlorine} = A \times 100/V$$

where V = sample volume, mL. If total residual chlorine is below 0.2 mg/L, subtract apparent chlorine in reagent blank (Reading B) to obtain the true total residual chlorine value.

6. Bibliography

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4500-Cl⁻ CHLORIDE*#(34)

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4500-Cl^- A. Introduction

1. Occurrence

Chloride, in the form of chloride (Cl^-) ion, is one of the major inorganic anions in water and wastewater. The salty taste produced by chloride concentrations is variable and dependent on the chemical composition of water. Some waters containing 250 mg Cl^-/L may have a detectable salty taste if the cation is sodium. On the other hand, the typical salty taste may be absent in waters containing as much as 1000 mg/L when the predominant cations are calcium and magnesium.

The chloride concentration is higher in wastewater than in raw water because sodium chloride (NaCl) is a common article of diet and passes unchanged through the digestive system. Along the sea coast, chloride may be present in high concentrations because of leakage of salt water into the sewerage system. It also may be increased by industrial processes.

A high chloride content may harm metallic pipes and structures, as well as growing plants.

2. Selection of Method

Six methods are presented for the determination of chloride. Because the first two are similar in most respects, selection is largely a matter of personal preference. The argentometric method (B) is suitable for use in relatively clear waters when 0.15 to 10 mg Cl^- are present in the portion titrated. The end point of the mercuric nitrate method (C) is easier to detect. The potentiometric method (D) is suitable for colored or turbid samples in which color-indicated end points might be difficult to observe. The potentiometric method can be used without a pretreatment step for samples containing ferric ions (if not present in an amount greater than the chloride concentration), chromic, phosphate, and ferrous and other heavy-metal ions. The ferricyanide method (E) is an automated technique. Flow injection analysis (G), an automated colorimetric technique, is useful for analyzing large numbers of samples. Preferably determine chloride by ion chromatography (Section 4110). Chloride also can be determined by the capillary ion electrophoresis method (Section 4140). Methods (C and G) in which mercury, a highly toxic reagent, is used require special disposal practices to avoid improper sewage discharges. Follow appropriate regulatory procedures (see Section 1090).

3. Sampling and Storage

Collect representative samples in clean, chemically resistant glass or plastic bottles. The maximum sample portion required is 100 mL. No special preservative is necessary if the sample is to be stored.

4500-Cl^- B. Argentometric Method

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1. General Discussion

a. Principle: In a neutral or slightly alkaline solution, potassium chromate can indicate the end point of the silver nitrate titration of chloride. Silver chloride is precipitated quantitatively before red silver chromate is formed.

b. Interference: Substances in amounts normally found in potable waters will not interfere. Bromide, iodide, and cyanide register as equivalent chloride concentrations. Sulfide, thiosulfate, and sulfite ions interfere but can be removed by treatment with hydrogen peroxide. Orthophosphate in excess of 25 mg/L interferes by precipitating as silver phosphate. Iron in excess of 10 mg/L interferes by masking the end point.

2. Apparatus

a. Erlenmeyer flask, 250-mL.

b. Buret, 50-mL.

3. Reagents

a. Potassium chromate indicator solution: Dissolve 50 g K_2CrO_4 in a little distilled water. Add $AgNO_3$ solution until a definite red precipitate is formed. Let stand 12 h, filter, and dilute to 1 L with distilled water.

b. Standard silver nitrate titrant, 0.0141*M* (0.0141*N*): Dissolve 2.395 g $AgNO_3$ in distilled water and dilute to 1000 mL. Standardize against NaCl by the procedure described in ¶ 4*b* below; 1.00 mL = 500 µg Cl^-. Store in a brown bottle.

c. Standard sodium chloride, 0.0141*M* (0.0141*N*): Dissolve 824.0 mg NaCl (dried at 140°C) in distilled water and dilute to 1000 mL; 1.00 mL = 500 µg Cl^-.

d. Special reagents for removal of interference:

1) *Aluminum hydroxide suspension:* Dissolve 125 g aluminum potassium sulfate or aluminum ammonium sulfate, $AlK(SO_4)_2 \cdot 12H_2O$ or $AlNH_4(SO_4)_2 \cdot 12H_2O$, in 1 L distilled water. Warm to 60°C and add 55 mL conc ammonium hydroxide (NH_4OH) slowly with stirring. Let stand about 1 h, transfer to a large bottle, and wash precipitate by successive additions, with thorough mixing and decanting with distilled water, until free from chloride. When freshly prepared, the suspension occupies a volume of approximately 1 L.

2) *Phenolphthalein indicator solution.*

3) *Sodium hydroxide,* NaOH, 1*N*.

4) *Sulfuric acid,* H_2SO_4, 1*N*.

5) *Hydrogen peroxide,* H_2O_2, 30%.

4. Procedure

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a. Sample preparation: Use a 100-mL sample or a suitable portion diluted to 100 mL. If the sample is highly colored, add 3 mL $Al(OH)_3$ suspension, mix, let settle, and filter.

If sulfide, sulfite, or thiosulfate is present, add 1 mL H_2O_2 and stir for 1 min.

b. Titration: Directly titrate samples in the pH range 7 to 10. Adjust sample pH to 7 to 10 with H_2SO_4 or NaOH if it is not in this range. For adjustment, preferably use a pH meter with a non-chloride-type reference electrode. (If only a chloride-type electrode is available, determine amount of acid or alkali needed for adjustment and discard this sample portion. Treat a separate portion with required acid or alkali and continue analysis.) Add 1.0 mL K_2CrO_4 indicator solution. Titrate with standard $AgNO_3$ titrant to a pinkish yellow end point. Be consistent in end-point recognition.

Standardize $AgNO_3$ titrant and establish reagent blank value by the titration method outlined above. A blank of 0.2 to 0.3 mL is usual.

5. Calculation

$$\text{mg Cl}^-/\text{L} = \frac{(A - B) \times N \times 35\,450}{\text{mL sample}}$$

where:

A = mL titration for sample,
B = mL titration for blank, and
N = normality of $AgNO_3$.

$$\text{mg NaCl/L} = (\text{mg Cl}^-/\text{L}) \times 1.65$$

6. Precision and Bias

A synthetic sample containing 241 mg Cl^-/L, 108 mg Ca/L, 82 mg Mg/L; 3.1 mg K/L, 19.9 mg Na/L, 1.1 mg NO_3^--N/L, 0.25 mg NO_2^-- N/L, 259 mg SO_4^{2-}/L, and 42.5 mg total alkalinity/L (contributed by $NaHCO_3$) in distilled water was analyzed in 41 laboratories by the argentometric method, with a relative standard deviation of 4.2% and a relative error of 1.7%.

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November 16-20, 1986, Portland, Ore., p. 673. American Water Works Assoc., Denver, Colo.

4500-Cl^- C. Mercuric Nitrate Method

1. General Discussion

a. Principle: Chloride can be titrated with mercuric nitrate, $Hg(NO_3)_2$, because of the formation of soluble, slightly dissociated mercuric chloride. In the pH range 2.3 to 2.8, diphenylcarbazone indicates the titration end point by formation of a purple complex with the excess mercuric ions. Xylene cyanol FF serves as a pH indicator and end-point enhancer. Increasing the strength of the titrant and modifying the indicator mixtures extend the range of measurable chloride concentrations.

b. Interference: Bromide and iodide are titrated with $Hg(NO_3)_2$ in the same manner as chloride. Chromate, ferric, and sulfite ions interfere when present in excess of 10 mg/L.

2. Apparatus

a. Erlenmeyer flask, 250-mL.

b. Microburet, 5-mL with 0.01-mL graduation intervals.

3. Reagents

a. Standard sodium chloride, 0.0141*M* (0.0141*N*): See Method B, ¶ B.3*c* above.

b. Nitric acid, HNO_3, 0.1*N*.

c. Sodium hydroxide, NaOH, 0.1*N*.

d. Reagents for chloride concentrations below 100 mg/L:

1) *Indicator-acidifier reagent:* The HNO_3 concentration of this reagent is an important factor in the success of the determination and can be varied as indicated in a) or b) to suit the alkalinity range of the sample. Reagent a) contains sufficient HNO_3 to neutralize a total alkalinity of 150 mg as $CaCO_3$/L to the proper pH in a 100-mL sample. Adjust amount of HNO_3 to accommodate samples of alkalinity different from 150 mg/L.

a) Dissolve, in the order named, 250 mg s-diphenylcarbazone, 4.0 mL conc HNO_3, and 30 mg xylene cyanol FF in 100 mL 95% ethyl alcohol or isopropyl alcohol. Store in a dark bottle in a refrigerator. This reagent is not stable indefinitely. Deterioration causes a slow end point and high results.

b) Because pH control is critical, adjust pH of highly alkaline or acid samples to 2.5 ± 0.1 with 0.1*N* HNO_3 or NaOH, not with sodium carbonate (Na_2CO_3). Use a pH meter with a nonchloride type of reference electrode for pH adjustment. If only the usual chloride-type reference electrode is available for pH adjustment, determine amount of acid or alkali required to

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obtain a pH of 2.5 ± 0.1 and discard this sample portion. Treat a separate sample portion with the determined amount of acid or alkali and continue analysis. Under these circumstances, omit HNO_3 from indicator reagent.

2) *Standard mercuric nitrate titrant,* 0.007 05*M* (0.0141*N*): Dissolve 2.3 g $Hg(NO_3)_2$ or 2.5 g $Hg(NO_3)_2 \cdot H_2O$ in 100 mL distilled water containing 0.25 mL conc HNO_3. Dilute to just under 1 L. Make a preliminary standardization by following the procedure described in ¶ 4*a*. Use replicates containing 5.00 mL standard NaCl solution and 10 mg sodium bicarbonate ($NaHCO_3$) diluted to 100 mL with distilled water. Adjust titrant to 0.0141*N* and make a final standardization; 1.00 mL = 500 µg Cl^-. Store away from light in a dark bottle.

e. Reagent for chloride concentrations greater than 100 mg/L:

1) *Mixed indicator reagent:* Dissolve 0.50 g diphenylcarbazone powder and 0.05 g bromphenol blue powder in 75 mL 95% ethyl or isopropyl alcohol and dilute to 100 mL with the same alcohol.

2) *Strong standard mercuric nitrate titrant,* 0.0705*M* (0.141*N*) Dissolve 25 g $Hg(NO_3)_2 \cdot H_2O$ in 900 mL distilled water containing 5.0 mL conc HNO_3. Dilute to just under 1 L and standardize by following the procedure described in ¶ 4*b*. Use replicates containing 25.00 mL standard NaCl solution and 25 mL distilled water. Adjust titrant to 0.141*N* and make a final standardization; 1.00 mL = 5.00 mg Cl^-.

4. Procedure

a. Titration of chloride concentrations less than 100 mg/L: Use a 100-mL sample or smaller portion so that the chloride content is less than 10 mg.

Add 1.0 mL indicator-acidifier reagent. (The color of the solution should be green-blue at this point. A light green indicates pH less than 2.0; a pure blue indicates pH more than 3.8.) For most potable waters, the pH after this addition will be 2.5 ± 0.1. For highly alkaline or acid waters, adjust pH to about 8 before adding indicator-acidifier reagent.

Titrate with 0.0141*N* $Hg(NO_3)_2$ titrant to a definite purple end point. The solution turns from green-blue to blue a few drops before the end point.

Determine blank by titrating 100 mL distilled water containing 10 mg $NaHCO_3$.

b. Titration of chloride concentrations greater than 100 mg/L: Use a sample portion (5 to 50 mL) requiring less than 5 mL titrant to reach the end point. Measure into a 150-mL beaker. Add approximately 0.5 mL mixed indicator reagent and mix well. The color should be purple. Add 0.1*N* HNO_3 dropwise until the color just turns yellow. Titrate with strong $Hg(NO_3)_2$ titrant to first permanent dark purple. Titrate a distilled water blank using the same procedure.

5. Calculation

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$$\text{mg Cl}^-/\text{L} = \frac{(A - B) \times N \times 35\,450}{\text{mL sample}}$$

where:

A = mL titration for sample,
B = mL titration for blank, and
N = normality of $Hg(NO_3)_2$.

$$\text{mg NaCl/L} = (\text{mg Cl}^-/\text{L}) \times 1.65$$

6. Precision and Bias

A synthetic sample containing 241 mg Cl^-/L, 108 mg Ca/L, 82 mg Mg/L, 3.1 mg K/L, 19.9 mg Na/L, 1.1 mg NO_3^--N/L, 0.25 mg NO_2^--N/L, 259 mg SO_4^{2-}/L, and 42.5 mg total alkalinity/L (contributed by $NaHCO_3$) in distilled water was analyzed in 10 laboratories by the mercurimetric method, with a relative standard deviation of 3.3% and a relative error of 2.9%.

7. Bibliography

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4500-Cl^- D. Potentiometric Method

1. General Discussion

a. Principle: Chloride is determined by potentiometric titration with silver nitrate solution with a glass and silver-silver chloride electrode system. During titration an electronic voltmeter is used to detect the change in potential between the two electrodes. The end point of the titration is that instrument reading at which the greatest change in voltage has occurred for a small and constant increment of silver nitrate added.

b. Interference: Iodide and bromide also are titrated as chloride. Ferricyanide causes high results and must be removed. Chromate and dichromate interfere and should be reduced to the chromic state or removed. Ferric iron interferes if present in an amount substantially higher than the amount of chloride. Chromic ion, ferrous ion, and phosphate do not interfere.

Grossly contaminated samples usually require pretreatment. Where contamination is minor,

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some contaminants can be destroyed simply by adding nitric acid.

2. Apparatus

a. Glass and silver-silver chloride electrodes: Prepare in the laboratory or purchase a silver electrode coated with AgCl for use with specified instruments. Instructions on use and care of electrodes are supplied by the manufacturer.

b. Electronic voltmeter, to measure potential difference between electrodes: A pH meter may be converted to this use by substituting the appropriate electrode.

c. Mechanical stirrer, with plastic-coated or glass impeller.

3. Reagents

a. Standard sodium chloride solution, 0.0141*M* (0.0141*N*): See Section 4500-Cl^-.B.3*c*.

b. Nitric acid, HNO_3, conc.

c. Standard silver nitrate titrant, 0.0141*M* (0.0141*N*): See Section 4500-Cl^-.B.3*b*.

d. Pretreatment reagents:

1) *Sulfuric acid,* H_2SO_4, 1 + 1.

2) *Hydrogen peroxide,* H_2O_2, 30%.

3) *Sodium hydroxide,* NaOH, 1*N*.

4. Procedure

a. Standardization: The various instruments that can be used in this determination differ in operating details; follow the manufacturer's instructions. Make necessary mechanical adjustments. Then, after allowing sufficient time for warmup (10 min), balance internal electrical components to give an instrument setting of 0 mV or, if a pH meter is used, a pH reading of 7.0.

1) Place 10.0 mL standard NaCl solution in a 250-mL beaker, dilute to about 100 mL, and add 2.0 mL conc HNO_3. Immerse stirrer and electrodes.

2) Set instrument to desired range of millivolts or pH units. Start stirrer.

3) Add standard $AgNO_3$ titrant, recording scale reading after each addition. At the start, large increments of $AgNO_3$ may be added; then, as the end point is approached, add smaller and equal increments (0.1 or 0.2 mL) at longer intervals, so that the exact end point can be determined. Determine volume of $AgNO_3$ used at the point at which there is the greatest change in instrument reading per unit addition of $AgNO_3$.

4) Plot a differential titration curve if the exact end point cannot be determined by inspecting the data. Plot change in instrument reading for equal increments of $AgNO_3$ against volume of $AgNO_3$ added, using average of buret readings before and after each addition. The procedure is illustrated in Figure 4500-Cl^-:1.

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b. Sample analysis:

1) Pipet 100.0 mL sample, or a portion containing not more than 10 mg Cl^-, into a 250-mL beaker. In the absence of interfering substances, proceed with ¶ 3) below.

2) In the presence of organic compounds, sulfite, or other interferences (such as large amounts of ferric iron, cyanide, or sulfide) acidify sample with H_2SO_4, using litmus paper. Boil for 5 min to remove volatile compounds. Add more H_2SO_4, if necessary, to keep solution acidic. Add 3 mL H_2O_2 and boil for 15 min, adding chloride-free distilled water to keep the volume above 50 mL. Dilute to 100 mL, add NaOH solution dropwise until alkaline to litmus, then 10 drops in excess. Boil for 5 min, filter into a 250-mL beaker, and wash precipitate and paper several times with hot distilled water.

3) Add conc HNO_3 dropwise until acidic to litmus paper, then 2.0 mL in excess. Cool and dilute to 100 mL if necessary. Immerse stirrer and electrodes and start stirrer. Make any necessary adjustments according to the manufacturer's instructions and set selector switch to appropriate setting for measuring the difference of potential between electrodes.

4) Complete determination by titrating according to ¶ 4*a*4). If an end-point reading has been established from previous determinations for similar samples and conditions, use this predetermined end point. For the most accurate work, make a blank titration by carrying chloride-free distilled water through the procedure.

5. Calculation

$$\text{mg Cl}^-/\text{L} = \frac{(A - B) \times N \times 35\,450}{\text{mL sample}}$$

where:

A = mL $AgNO_3$,
B = mL blank, and
N = normality of titrant.

6. Precision and Bias

In the absence of interfering substances, the precision and bias are estimated to be about 0.12 mg for 5 mg Cl^-, or 2.5% of the amount present. When pretreatment is required to remove interfering substances, the precision and bias are reduced to about 0.25 mg for 5 mg Cl^-, or 5% of amount present.

7. Bibliography

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4500-Cl^- E. Automated Ferricyanide Method

1. General Discussion

a. Principle: Thiocyanate ion is liberated from mercuric thiocyanate by the formation of soluble mercuric chloride. In the presence of ferric ion, free thiocyanate ion forms a highly colored ferric thiocyanate, of which the intensity is proportional to the chloride concentration.

b. Interferences: Remove particulate matter by filtration or centrifugation before analysis. Guard against contamination from reagents, water, glassware, and sample preservation process. No chemical interferences are significant.

c. Application: The method is applicable to potable, surface, and saline waters, and domestic and industrial wastewaters. The concentration range is 1 to 200 mg Cl^-/L; it can be extended by dilution.

2. Apparatus

a. Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the interchangeable components shown in Figure 4500-Cl^-:2.

b. Filters, 480-nm.

3. Reagents

a. Stock mercuric thiocyanate solution: Dissolve 4.17 g $Hg(SCN)_2$ in about 500 mL methanol, dilute to 1000 mL with methanol, mix, and filter through filter paper.

b. Stock ferric nitrate solution: Dissolve 202 g $Fe(NO_3)_3 \cdot 9H_2O$ in about 500 mL distilled water, then carefully add 21 mL conc HNO_3. Dilute to 1000 mL with distilled water and mix. Filter through paper and store in an amber bottle.

c. Color reagent: Add 150 mL stock $Hg(SCN)_2$ solution to 150 mL stock $Fe(NO_3)_3$ solution.

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Mix and dilute to 1000 mL with distilled water. Add 0.5 mL polyoxyethylene 23 lauryl ether.*#(35)

d. Stock chloride solution: Dissolve 1.6482 g NaCl, dried at 140°C, in distilled water and dilute to 1000 mL; 1.00 mL = 1.00 mg Cl^-.

e. Standard chloride solutions: Prepare chloride standards in the desired concentration range, such as 1 to 200 mg/L, using stock chloride solution.

4. Procedure

Set up manifold as shown in Figure 4500-Cl^-:2 and follow general procedure described by the manufacturer.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against chloride concentrations in standards. Compute sample chloride concentration by comparing sample response with standard curve.

6. Precision and Bias

With an automated system in a single laboratory six samples were analyzed in septuplicate. At a concentration ranging from about 1 to 50 mg Cl^-/L the average standard deviation was 0.39 mg/L. The coefficient of variation was 2.2%. In two samples with added chloride, recoveries were 104% and 97%.

7. Bibliography

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O'BRIEN, J.E. 1962. Automatic analysis of chlorides in sewage. *Wastes Eng.* 33:670.

4500-Cl^- F. (RESERVED)

4500-Cl^- G. Mercuric Thiocyanate Flow Injection Analysis (PROPOSED)

1. General Discussion

a. Principle: A water sample containing chloride is injected into a carrier stream to which mercuric thiocyanate and ferric nitrate are added. The chloride complexes with the Hg(II), displacing the thiocyanate anion, which forms the highly colored ferric thiocyanate complex anion. The resulting peak's absorbance is measured at 480 nm. The peak area is proportional to the concentration of chloride in the original sample.

Also see Section 4500-Cl^-.A and Section 4130, Flow Injection Analysis (FIA).

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b. Interferences: Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

Substances such as sulfite and thiosulfate, which reduce iron(III) to iron(II) and mercury(II) to mercury(I), can interfere. Halides, which also form strong complexes with mercuric ion (e.g., Br^-, I^-), give a positive interference.

2. Apparatus

Flow injection analysis equipment consisting of:

a. FIA injection valve with sample loop.

b. Multichannel proportioning pump.

c. FIA manifold with flow cell (Figure 4500-Cl^-:3). Relative flow rates only are shown. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.*#(36)

d. Absorbance detector, 480 nm, 10-nm bandpass.

e. Valve control and data acquisition system.

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and all solutions.

a. Stock mercuric thiocyanate solution: In a 1-L volumetric flask, dissolve 4.17 g mercuric thiocyanate, $Hg(SCN)_2$, in about 500 mL methanol. Dilute to mark with methanol and mix. CAUTION: *Mercuric thiocyanate is toxic. Wear gloves!*

b. Stock ferric nitrate reagent, 0.5*M*: In a 1-L volumetric flask, dissolve 202 g ferric nitrate, $Fe(NO_3)_3 \cdot 9H_2O$, in approximately 800 mL water. Add 25 mL conc HNO_3 and dilute to mark. Invert to mix.

c. Color reagent: In a 500-mL volumetric flask, mix 75 mL stock mercuric thiocyanate solution with 75 mL stock ferric nitrate reagent and dilute to mark with water. Invert to mix. Vacuum filter through a 0.45-µm membrane filter. The color reagent also is available as a commercially prepared solution that is stable for several months.

d. Stock chloride standard, 1000 mg Cl^-/L: In a 105°C oven, dry 3 g primary standard grade sodium chloride, NaCl, overnight. In a 1-L volumetric flask, dissolve 1.648 g primary standard grade sodium chloride in about 500 mL water. Dilute to mark and invert to mix.

e. Standard chloride solutions: Prepare chloride standards for the calibration curve in the desired concentration range, using the stock standard (¶ 3*d*), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-Cl^-:3 and follow method supplied by

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manufacturer, or laboratory standard operating procedure for this method. Follow quality control procedures described in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold versus chloride concentration. The calibration curve gives a good fit to a second-order polynomial.

6. Precision and Bias

a. Recovery and relative standard deviation: The results of single-laboratory studies with various matrices are given in Table 4500-Cl^-:I .

b. MDL: A 100-µL sample loop was used in the method described above. Using a published MDL method[1] analysts ran 21 replicates of a 1.0-mg Cl^-/L standard. These gave a mean of 1.19 mg Cl^-/L, a standard deviation of 0.027 mg Cl^-/L, and an MDL of 0.07 mg Cl^-/L. This is only an estimate because the ratio of standard to the MDL is above guidelines (see Section 1030). A lower MDL may be obtained by increasing the sample loop volume and increasing the ratio of carrier flow rate to reagents flow rate. A higher MDL may be obtained by decreasing the sample loop volume and decreasing this ratio.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1989. Definition and Procedure for the Determination of Method Detection Limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-ClO_2 CHLORINE DIOXIDE*#(37)

4500-ClO_2 A. Introduction

Because the physical and chemical properties of chlorine dioxide resemble those of chlorine in many respects, read the entire discussion of Residual Chlorine (Section 4500-Cl) before attempting a chlorine dioxide determination.

1. Occurrence and Significance

Chlorine dioxide, ClO_2, has been used widely as a bleaching agent in the paper and pulp industry. It has been applied to water supplies to combat tastes and odors due to phenolic-type wastes, actinomycetes, and algae, as well as to oxidize soluble iron and manganese to a more easily removable form. It is a disinfectant, and some results suggest that it may be stronger than

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free chlorine or hypochlorite.

Chlorine dioxide is a deep yellow, volatile, unpleasant-smelling gas that is toxic and under certain conditions may react explosively. It should be handled with care in a vented area. The use of odor to warn of exposure to concentrations of health significance may not be adequate.

There are several methods of generating ClO_2; for laboratory purposes the acidification of a solution of sodium chlorite followed by suitable scrubbing and capture of the released gaseous ClO_2 is the most practical. CAUTION: *Sodium chlorite is a powerful oxidizer; keep out of direct contact with oxidizable material to avoid possibility of explosion.*

2. Selection of Method

The iodometric method (B) gives a very precise measure of total available strength of a solution in terms of its ability to liberate iodine from iodide. However, ClO_2, chlorine, chlorite, and hypochlorite are not distinguished easily by this technique. It is designed primarily, and best used, for standardizing ClO_2 solutions needed for preparation of temporary standards. It often is inapplicable to industrial wastes.

The amperometric methods (C and E) are useful when a knowledge of the various chlorine fractions in a water sample is desired. They distinguish various chlorine compounds of interest with good accuracy and precision, but require specialized equipment and considerable analytical skill.

The N,N-diethyl-*p*-phenylenediamine (DPD) method (D) has the advantages of a relatively easy-to-perform colorimetric test with the ability to distinguish between ClO_2 and some forms of chlorine. This technique is not as accurate as the amperometric method, but should yield results adequate for many common applications. NOTE: Reports in the literature indicate that the DPD method is subject to interference from monochloramine and chloraminoacetic acid, and the chlorite anion.[1]

3. Sampling and Storage

Determine ClO_2 promptly after collecting the sample. Do not expose sample to sunlight or strong artificial light and do not aerate to mix. Most of these methods can be performed on site, with prior calibration in the laboratory. Minimum ClO_2 losses occur when the determination is completed immediately at the site of sample collection.

4. Reference

1. CHISWELL, B. & K.R. O'HALLORAN. 1991. Use of Lissamine Green B as a spectrophotometric reagent for the determination of low residuals of chlorine dioxide. *Analyst* 116:657.

5. Bibliography

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4500-ClO_2 B. Iodometric Method

1. General Discussion

a. Principle: A pure solution of ClO_2 is prepared from gaseous ClO_2 by slowly adding dilute H_2SO_4 to a sodium chlorite ($NaClO_2$) solution. Contaminants such as chlorine are removed from the gas stream by a $NaClO_2$ scrubber; the gas is passed into distilled water in a steady stream of air. *See* CAUTION, ¶ A.1.

ClO_2 releases free iodine from a KI solution acidified with acetic acid or H_2SO_4. The liberated iodine is titrated with a standard solution of sodium thiosulfate ($Na_2S_2O_3$), with starch as the indicator.

b. Interference: There is little interference in this method, but temperature and strong light affect solution stability. Minimize ClO_2 losses by storing stock ClO_2 solution in a dark refrigerator and by preparing and titrating dilute ClO_2 solutions for standardization purposes at the lowest practicable temperature and in subdued light.

c. Minimum detectable concentration: One drop (0.05 mL) of 0.01*N* (0.01*M*) $Na_2S_2O_3$ is equivalent to 20 µg ClO_2/L (or 40 µg/ L in terms of available chlorine) when a 500-mL sample is titrated.

2. Reagents

All reagents listed for the determination of residual chlorine in Section 4500-Cl.B.2*a* - Section 4500-Cl.B.2*g* are required. Also needed are the following:

a. Stock chlorine dioxide solution: Prepare a gas generating and absorbing system as illustrated in Figure 4500-ClO_2:1. Connect aspirator flask, 500-mL capacity, with rubber tubing to a source of purified compressed air. Let air bubble through a layer of 300 mL distilled water in flask and then pass through a glass tube ending within 5 mm of the bottom of the 1-L

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gas-generating bottle. Conduct evolved gas via glass tubing through a scrubber bottle containing saturated $NaClO_2$ solution or a tower packed with flaked $NaClO_2$, and finally, via glass tubing, into a 2-L borosilicate glass collecting bottle where the gas is absorbed in 1500 mL distilled water. Provide an air outlet tube on collecting bottle for escape of air. Select for gas generation a bottle constructed of strong borosilicate glass and having a mouth wide enough to permit insertion of three separate glass tubes: the first leading almost to the bottom for admitting air, the second reaching below the liquid surface for gradual introduction of H_2SO_4, and the third near the top for exit of evolved gas and air. Fit to second tube a graduated cylindrical separatory funnel to contain H_2SO_4. Locate this system in a fume hood with an adequate shield.

Dissolve 10 g $NaClO_2$ in 750 mL distilled water and place in generating bottle. Carefully add 2 mL conc H_2SO_4 to 18 mL distilled water and mix. Transfer to funnel. Connect flask to generating bottle, generating bottle to scrubber, and the latter to collecting bottle. Pass a smooth current of air through the system, as evidenced by the bubbling rate in all bottles.

Introduce 5-mL increments of H_2SO_4 from funnel into generating bottle at 5-min intervals. Continue air flow for 30 min after last portion of acid has been added.

Store yellow stock solution in glass-stoppered dark-colored bottle in a dark refrigerator. The concentration of ClO_2 thus prepared varies between 250 and 600 mg/L, corresponding to approximately 500 to 1200 mg free chlorine/L.

b. Standard chlorine dioxide solution: Use this solution for preparing temporary ClO_2 standards. Dilute required volume of stock ClO_2 solution to desired strength with chlorine-demand-free water (see Section 4500-Cl.C.3*m*). Standardize solution by titrating with standard 0.01*N* (0.01*M*) or 0.025*N* (0.025*M*) $Na_2S_2O_3$ titrant in the presence of KI, acid, and starch indicator by following the procedure given in ¶ 3 below. A full or nearly full bottle of chlorine or ClO_2 solution retains its titer longer than a partially full one. When repeated withdrawals reduce volume to a critical level, standardize diluted solution at the beginning, midway in the series of withdrawals, and at the end of the series. Shake contents thoroughly before drawing off needed solution from middle of the glass-stoppered dark-colored bottle. Prepare this solution frequently.

3. Procedure

Select volume of sample, prepare for titration, and titrate sample and blank as described in Section 4500-Cl.B.3. The only exception is the following: *Let ClO_2 react in the dark with acid and KI for 5 min before starting titration.*

4. Calculations

Express ClO_2 concentrations in terms of ClO_2 or as free chlorine content. Free chlorine is defined as the total oxidizing power of ClO_2 measured by titrating iodine released by ClO_2 from an acidic solution of KI. Calculate result in terms of chlorine itself.

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For standardizing ClO_2 solution:

$$\text{mg } ClO_2/\text{mL} = \frac{(A \pm B) \times N \times 13.49}{\text{mL sample titrated}}$$

For determining ClO_2 temporary standards:

$$\text{mg } ClO_2 \text{ as } Cl_2/\text{mL} = \frac{(A \pm B) \times N \times 35.45}{\text{mL sample titrated}}$$

where:

A = mL titration for sample,
B = mL titration for blank (positive or negative, see Section 4500-Cl.B.3*d*), and
N = normality of $Na_2S_2O_3$ = molarity of $Na_2S_2O_3$.

5. Bibliography

POST, M.A. & W.A. MOORE. 1959. Determination of chlorine dioxide in treated surface waters. *Anal. Chem.* 31:1872.

4500-ClO_2 C. Amperometric Method I

1. General Discussion

a. Principle: The amperometric titration of ClO_2 is an extension of the amperometric method for chlorine. By performing four titrations with phenylarsine oxide, free chlorine (including hypochlorite and hypochlorous acid), chloramines, chlorite, and ClO_2 may be determined separately. The first titration step consists of conversion of ClO_2 to chlorite and chlorate through addition of sufficient NaOH to produce a pH of 12, followed by neutralization to a pH of 7 and titration of free chlorine. In the second titration KI is added to a sample that has been treated similarly with alkali and had the pH readjusted to 7; titration yields free chlorine and monochloramine. The third titration involves addition of KI and pH adjustment to 7, followed by titration of free chlorine, monochloramine, and one-fifth of the available ClO_2. In the fourth titration, addition of sufficient H_2SO_4 to lower the pH to 2 enables all available ClO_2 and chlorite, as well as the total free chlorine, to liberate an equivalent amount of iodine from the added KI and thus be titrated.

b. Interference: The interferences described in Section 4500-Cl.D.1*b* apply also to determination of ClO_2.

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2. Apparatus

The apparatus required is given in Section 4500-Cl.D.2*a* through *d*.

3. Reagents

All reagents listed for the determination of chlorine in Section 4500-Cl.D.3 are required. Also needed are the following:

a. Sodium hydroxide, NaOH, 6*N* (6*M*).

b. Sulfuric acid, H_2SO_4, 6*N* (3*M*), 1 + 5.

4. Procedure

Minimize effects of pH, time, and temperature of reaction by standardizing all conditions.

a. Titration of free available chlorine (hypochlorite and hypochlorous acid): Add sufficient 6*N* (6*M*) NaOH to raise sample pH to 12. After 10 min, add 6*N* (3*M*) H_2SO_4 to lower pH to 7. Titrate with standard phenylarsine oxide titrant to the amperometric end point as given in Section 4500-Cl.D. Record result as *A*.

b. Titration of free available chlorine and chloramine: Add 6*N* (6*M*) NaOH to raise sample pH to 12. After 10 min, add 6*N* (3*M*) H_2SO_4 to reduce pH to 7. Add 1 mL KI solution. Titrate with standard phenylarsine oxide titrant to the amperometric end point. Record result as *B*.

c. Titration of free available chlorine, chloramine, and one-fifth of available ClO_2: Adjust sample pH to 7 with pH 7 phosphate buffer solution. Add 1 mL KI solution. Titrate with standard phenylarsine oxide titrant to the amperometric end point. Record result as *C*.

d. Titration of free available chlorine, chloramines, ClO_2, and chlorite: Add 1 mL KI solution to sample. Add sufficient 6*N* (3*M*) H_2SO_4 to lower pH to 2. After 10 min, add sufficient 6*N* (6*M*) NaOH to raise pH to 7. Titrate with standard phenylarsine oxide titrant to the amperometric end point. Record result as *D*.

5. Calculation

Convert individual titrations (*A, B, C,* and *D*) into chlorine concentration by the following equation:

$$\text{mg Cl as Cl}_2\text{/L} = \frac{E \times 200}{\text{mL sample}}$$

where:

E = mL phenylarsine oxide titration for each individual sample *A, B, C,* or *D*.

Calculate ClO_2 and individual chlorine fractions as follows:

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$$\text{mg ClO}_2 \text{ as ClO}_2\text{/L} = 1.9\,(C - B)$$
$$\text{mg ClO}_2 \text{ as Cl}_2\text{/L} = 5\,(C - B)$$
$$\text{mg free available chlorine/L} = A$$
$$\text{mg chloramine/L as chlorine} = B - A$$
$$\text{mg chlorite/L as chlorine} = 4B - 5C + D$$

6. Bibliography

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4500-ClO_2 D. DPD Method

1. General Discussion

a. Principle: This method is an extension of the *N,N*-diethyl-*p*-phenylenediamine (DPD) method for determining free chlorine and chloramines in water. ClO_2 appears in the first step of this procedure but only to the extent of one-fifth of its total available chlorine content corresponding to reduction of ClO_2 to chlorite ion. If the sample is then acidified in the presence of iodide the chlorite also reacts. When neutralized by subsequent addition of bicarbonate, the color thus produced corresponds to the total available chlorine content of the ClO_2. If chlorite is present in the sample, this will be included in the step involving acidification and neutralization. Chlorite that did not result from ClO_2 reduction by the procedure will cause a positive error equal to twice this chlorite concentration. In evaluating mixtures of these various chloro-compounds, it is necessary to suppress free chlorine by adding glycine before reacting the sample with DPD reagent. Differentiation is based on the fact that glycine converts free chlorine instantaneously into chloroaminoacetic acid but has no effect on ClO_2.

b. Interference: The interference by oxidized manganese described in Section 4500-Cl.F.1*d* applies also to ClO_2 determination. Manganese interference appears as an increase in the first titrations after addition of DPD, with or without KI, and irrespective of whether there has been prior addition of glycine. Titration readings must be corrected suitably. Interference by chromate in wastewaters may be corrected similarly.

Iron contributed to the sample by adding ferrous ammonium sulfate (FAS) titrant may activate chlorite so as to interfere with the first end point of the titration. Suppress this effect with additional EDTA, disodium salt.

Exercise caution in the selection of this method, because of interferences from monochloramine and chloraminoacetic acid and the chlorite anion.

2. Reagents

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Reagents required in addition to those for the DPD free-combined chlorine method as listed in Section 4500-Cl.F.2 are as follows:

a. Glycine solution: Dissolve 10 g NH_2CH_2COOH in 100 mL distilled water.

b. Sulfuric acid solution: Dilute 5 mL conc H_2SO_4 to 100 mL with distilled water.

c. Sodium bicarbonate solution: Dissolve 27.5 g $NaHCO_3$ in 500 mL distilled water.

d. EDTA: Disodium salt of ethylenediamine tetraacetic acid, solid.

3. Procedure

For samples containing more than 5 mg/L total available chlorine follow the dilution procedure given in Section 4500-Cl.F.3.

a. Chlorine dioxide: Add 2 mL glycine solution to 100 mL sample and mix. Place 5 mL each of buffer reagent and DPD indicator solution in a separate titration flask and mix (or use about 500 mg DPD powder). Add about 200 mg EDTA, disodium salt. Then add glycine-treated sample and mix. Titrate rapidly with standard FAS titrant until red color is discharged (Reading *G*).

b. Free available chlorine and chloramine: Using a second 100-mL sample follow the procedures of Section 4500-Cl.F.3*a* adding about 200 mg EDTA, disodium salt, initially with the DPD reagents (Readings *A*, *B*, and *C*).

c. Total available chlorine including chlorite: After obtaining Reading *C* add 1 mL H_2SO_4 solution to the same sample in titration flask, mix, and let stand about 2 min. Add 5 mL $NaHCO_3$ solution, mix, and titrate (Reading *D*).

d. Colorimetric procedure: Instead of titration with standard FAS solution, colorimetric procedures may be used to obtain the readings at each stage. Calibrate colorimeters with standard permanganate solution as directed in Section 4500-Cl.G.4*a*. Use of additional EDTA, disodium salt, with the DPD reagents is not required in colorimetric procedures.

4. Calculations

For 100 mL sample, 1 mL FAS solution = 1 mg available chlorine/L.

In the absence of chlorite:

$$\text{Chlorine dioxide} = 5G \text{ (or } 1.9G \text{ expressed as } ClO_2)$$

$$\text{Free available chlorine} = A - G$$

$$\text{Monochloramine} = B - A$$

$$\text{Dichloramine} = C - B$$

$$\text{Total available chlorine} = C + 4G$$

If the step leading to Reading *B* is omitted, monochloramine and dichloramine are obtained together when:

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$$\text{Combined available chlorine} = C - A$$

If it is desired to check for presence of chlorite in sample, obtain Reading D. Chlorite is indicated if D is greater than $C + 4G$.

In the presence of chlorite:

$$\text{Chlorine dioxide} = 5G \text{ (or } 1.9G \text{ expressed as } ClO_2\text{)}$$
$$\text{Chlorite} = D - (C + 4G)$$
$$\text{Free available chlorine} = A - G$$
$$\text{Monochloramine} = B - A$$
$$\text{Dichloramine} = C - B$$
$$\text{Total available chlorine} = D$$

If B is omitted,

$$\text{Combined available chlorine} = C - A$$

5. Bibliography

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PALIN, A.T. 1975. Current DPD methods for residual halogen compounds and ozone in water. *J. Amer. Water Works Assoc.* 67:32.

4500-ClO_2 E. Amperometric Method II

1. General Discussion

a. Principle: Like Amperometric Method I (Section 4500-ClO_2.C), this procedure entails successive titrations of combinations of chlorine species. Subsequent calculations determine the concentration of each species. The equilibrium for reduction of the chlorine species of interest by iodide is pH-dependent.

The analysis of a sample for chlorine, chlorine dioxide, chlorite, and chlorate requires the following steps: determination of all of the chlorine (free plus combined) and one-fifth of the chlorine dioxide at pH 7; lowering sample pH to 2 and determination of the remaining four-fifths

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of the ClO_2 and all of the chlorite (the chlorite measured in this step comes from the chlorite originally present in the sample and that formed in the first titration); preparation of a second sample by purging with nitrogen to remove ClO_2 and by reacting with iodide at pH 7 to remove any chlorine remaining; lowering latter sample pH to 2 and determination of all chlorite present (this chlorite only comes from the chlorite originally present in the sample); and, in a third sample, determination of all of the relevant, oxidized chlorine species— chlorine, chlorine dioxide, chlorite, and chlorate—after reduction in hydrochloric acid.[1]

This procedure can be applied to concentrated solutions (10 to 100 mg/L) or dilute solutions (0.1 to 10 mg/L) by appropriate selection of titrant concentration and sample size.

b. Interferences: At pH values above 4, significant iodate formation is possible if iodine is formed in the absence of iodide;[2] this results in a negative bias in titrating the first and second samples. Acidification of these samples causes reduction of iodate to iodine and a positive bias. To prevent formation of iodate add 1 g KI granules to stirred sample.

A positive bias results from oxidation of iodide to iodine by dissolved oxygen in strongly acidic solutions.[1] To minimize this bias, use bromide as the reducing agent in titrating the third sample (bromide is not oxidized by oxygen under these conditions). After reaction is completed, add iodide, which will be oxidized to iodine by the bromine formed from the reduction of the original chlorine species. Add iodide carefully so that bromine gas is not lost. Rapid dilution of the sample with sodium phosphate decreases sample acidity and minimizes oxidation of iodide by oxygen. The pH of the solution to be titrated should be between 1.0 and 2.0. Carry a blank through the procedure as a check on iodide oxidation.

The potential for interferences from manganese, copper, and nitrate is minimized by buffering the sample to pH ≥ 4.[3,4] For the method presented here, the low pH required for the chlorite and chlorate analyses provides conditions favorable to manganese, copper, and nitrite interferences.

2. Apparatus

a. Titrators: See Section 4500-Cl.D.2*a* through *d*. Amperometric titrators with a platinum-platinum electrode system are more stable and require less maintenance. (NOTE: Chlorine dioxide may attack adhesives used to connect the platinum plate to the electrode, resulting in poor readings.)

If a potentiometric titrator is used, provide a platinum sensing electrode and a silver chloride reference electrode for end-point detection.

b. Glassware: Store glassware used in this method separately from other laboratory glassware and do not use for other purposes because ClO_2 reacts with glass to form a hydrophobic surface coating. To satisfy any ClO_2 demand, before first use immerse all glassware in a strong ClO_2 solution (200 to 500 mg/L) for 24 h and rinse only with water between uses.

c. Sampling: ClO_2 is volatile and will vaporize easily from aqueous solution. When

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sampling a liquid stream, minimize contact with air by placing a flexible sample line to reach the bottom of the sample container, letting several container volumes overflow, slowly removing sample line, and capping container with minimum headspace. Protect from sunlight. Remove sample portions with a volumetric pipet with pipet tip placed at bottom of container. Drain pipet by placing its tip below the surface of reagent or dilution water.

3. Reagents

a. Standard sodium thiosulfate, 0.100*N* (0.100*M*): See Section 4500-Cl.B.2*c*.

b. Standard phenylarsine oxide, 0.005 64*N* (0.005 64*M*): See Section 4500-Cl.C.3*a*. (Weigh out 1.25 g phenylarsine oxide and standardize to 0.005 64*M*.)

c. Phosphate buffer solution, pH 7: See Section 4500-Cl.D.3*b*.

d. Potassium iodide, KI, granules.

e. Saturated sodium phosphate solution: Prepare a saturated solution of $Na_2HPO_4 \cdot 12H_2O$ with cold deionized-distilled water.

f. Potassium bromide solution, 5%: Dissolve 5 g KBr and dilute to 100 mL. Store in a brown glass-stoppered bottle. Make fresh weekly.

g. Hydrochloric acid, HCl, conc.

h. Hydrochloric acid, HCl, 2.5*N* (2.5*M*): Cautiously add 200 mL conc HCl, with mixing, to distilled water, diluting to 1000 mL.

i. Purge gas: Use nitrogen gas for purging ClO_2 from samples. Assure that gas is free of contaminants and pass it through a 5% KI scrub solution. Discard solution at first sign of color.

4. Procedure

Use either sodium thiosulfate or phenylarsine oxide as titrant. Select concentration on basis of concentration range expected. The total mass of oxidant species should be no greater than about 15 mg. Make appropriate sample dilutions if necessary. A convenient volume for titration is 200 to 300 mL. Preferably analyze all samples and blanks in triplicate.

Minimize effects of pH, time, and temperature of reaction by standardizing all conditions.

a. Titration of residual chlorine and one-fifth of available ClO_2: Place 1 mL pH 7 phosphate buffer in beaker and add distilled-deionized dilution water if needed. Introduce sample with minimum aeration and add 1 g KI granules while stirring. Titrate to end point (see Section 4500-Cl.D). Record reading A = mL titrant/mL sample.

b. Titration of four-fifths of available ClO_2 and chlorite: Continuing with same sample, add 2 mL 2.5*N* (2.5*M*) HCl. Let stand in the dark for 5 min. Titrate to end point. Record reading B = mL titrant/mL sample.

c. Titration of nonvolatilized chlorine: Place 1 mL pH 7 phosphate buffer in purge vessel and add distilled-deionized dilution water if needed. Add sample and purge with nitrogen gas for 15 min. Use a gas-dispersion tube to give good gas-liquid contact. Add 1 g KI granules while

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stirring and titrate to end point. Record reading C = mL titrant/mL sample.

d. Titration of chlorite: Continuing with same sample, add 2 mL 2.5N (2.5M) HCl. Let stand in the dark for 5 min. Titrate to end point, and record reading D = mL titrant/mL sample.

e. Titration of chlorine, ClO_2, chlorate, and chlorite: Add 1 mL KBr and 10 mL conc HCl to 50-mL reaction flask and mix. Carefully add 15 mL sample, with minimum aeration. Mix and stopper immediately. Let stand in the dark for 20 min. Rapidly add 1 g KI granules and shake vigorously for 5 s. Rapidly transfer to titration flask containing 25 mL saturated Na_2HPO_4 solution. Rinse reaction flask thoroughly and add rinse water to titration flask. Final titration volume should be about 200 to 300 mL. Titrate to end point.

Repeat procedure of preceding paragraph using distilled-deionized water in place of sample to determine blank value.

Record reading E = (mL titrant sample − mL titrant blank)/ mL sample.

NOTE: The 15-mL sample volume can be adjusted to provide an appropriate dilution, but maintain the ratio of sample to HCl.

5. Calculations

Because the combining power of the titrants is pH-dependent, all calculations are based on the equivalents of reducing titrant required to react with equivalents of oxidant present. Use Table 4500-ClO2:I to obtain the equivalent weights to be used in the calculations.

In the following equations, N is the normality of the titrant used in equivalents per liter and A through E are as defined previously.

$$\text{Chlorite, mg ClO}_2^{-}\text{/L} = D \times N \times 16\,863$$

$$\text{Chlorate, mg ClO}_2^{2-}\text{/L} = [E - (A + B)] \times N \times 13\,909$$

$$\text{Chlorine dioxide, mg ClO}_2\text{/L} = (5/4) \times (B - D) \times N \times 13\,490$$

$$\text{Chlorine, mg Cl}_2\text{/L} = \text{A} - [(B - D)/4] \times N \times 35\,453$$

6. References

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4500-F^- FLUORIDE*#(38)

4500-F^- A. Introduction

A fluoride concentration of approximately 1.0 mg/L in drinking water effectively reduces dental caries without harmful effects on health. Fluoride may occur naturally in water or it may be added in controlled amounts. Some fluorosis may occur when the fluoride level exceeds the recommended limits. In rare instances the naturally occurring fluoride concentration may approach 10 mg/ L; such waters should be defluoridated.

Accurate determination of fluoride has increased in importance with the growth of the practice of fluoridation of water supplies as a public health measure. Maintenance of an optimal fluoride concentration is essential in maintaining effectiveness and safety of the fluoridation procedure.

1. Preliminary Treatment

Among the methods suggested for determining fluoride ion (F^-) in water, the electrode and colorimetric methods are the most satisfactory. Because both methods are subject to errors due to interfering ions (Table 4500-F^-:I), it may be necessary to distill the sample as directed in Section 4500-F-B before making the determination. When interfering ions are not present in excess of the tolerance of the method, the fluoride determination may be made directly without distillation.

2. Selection of Method

The electrode methods (C and G) are suitable for fluoride concentrations from 0.1 to more than 10 mg/L. Adding the prescribed buffer frees the electrode method from most interferences that adversely effect the SPADNS colorimetric method and necessitate preliminary distillation. Some substances in industrial wastes, such as fluoborates, may be sufficiently concentrated to present problems in electrode measurements and will not be measured without a preliminary distillation. Fluoride measurements can be made with an ion-selective electrode and either an

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expanded-scale pH meter or a specific ion meter, usually without distillation, in the time necessary for electrode equilibration.

The SPADNs method (D) has a linear analytical range of 0 to 1.40 mg F^-/L. Use of a nonlinear calibration can extend the range to 3.5 mg F^-/L. Color development is virtually instantaneous. Color determinations are made photometrically, using either a filter photometer or a spectrophotometer. A curve developed from standards is used for determining the fluoride concentration of a sample.

Fluoride also may be determined by the automated complexone method, Method E.

Ion chromatography (Section 4110) is an acceptable method if weaker eluents are used to separate fluoride from interfering peaks or fluoride can be determined by capillary ion electrophoresis (Section 4140).

The flow injection method (G) is a convenient automated technique for analyzing large numbers of samples.

3. Sampling and Storage

Preferably use polyethylene bottles for collecting and storing samples for fluoride analysis. Glass bottles are satisfactory if previously they have not contained high-fluoride solutions. Always rinse bottle with a portion of sample.

For the SPADNs method, never use an excess of dechlorinating agent. Dechlorinate with sodium arsenite rather than sodium thiosulfate when using the SPADNS method because the latter may produce turbidity that causes erroneous readings.

4500-F^- B. Preliminary Distillation Step

1. Discussion

Fluoride can be separated from other nonvolatile constituents in water by conversion to hydrofluoric or fluosilicic acid and subsequent distillation. The conversion is accomplished by using a strong, high-boiling acid. To protect against glassware etching, hydrofluoric acid is converted to fluosilicic acid by using soft glass beads. Quantitative fluoride recovery is approached by using a relatively large sample. Acid and sulfate carryover are minimized by distilling over a controlled temperature range.

Distillation will separate fluoride from most water samples. Some tightly bound fluoride, such as that in biological materials, may require digestion before distillation, but water samples seldom require such drastic treatment. Distillation produces a distillate volume equal to that of the original water sample so that usually it is not necessary to incorporate a dilution factor when expressing analytical results. The distillate will be essentially free of substances that might interfere with the fluoride determination if the apparatus used is adequate and distillation has been carried out properly. The only common volatile constituent likely to cause interference with colorimetric analysis of the distillate is chloride. When the concentration of chloride is high

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enough to interfere, add silver sulfate to the sulfuric acid distilling mixture to minimize the volatilization of hydrogen chloride.

Heating an acid-water mixture can be hazardous if precautions are not taken: *Mix acid and water thoroughly before heating.* Use of a quartz heating mantle and a magnetic stirrer in the distillation apparatus simplifies the mixing step.

2. Apparatus

a. Distillation apparatus consisting of a 1-L round-bottom long-neck borosilicate glass boiling flask, a connecting tube, an efficient condenser, a thermometer adapter, and a thermometer that can be read to 200°C. Use standard taper joints for all connections in the direct vapor path. Position the thermometer so that the bulb always is immersed in boiling mixture. The apparatus should be disassembled easily to permit adding sample. Substituting a thermoregulator and necessary circuitry for the thermometer is acceptable and provides some automation.

Alternative types of distillation apparatus may be used. Carefully evaluate any apparatus for fluoride recovery and sulfate carryover. The critical points are obstructions in the vapor path and trapping of liquid in the adapter and condenser. (The condenser should have a vapor path with minimum obstruction. A double-jacketed condenser, with cooling water in the outer jacket and the inner spiral tube, is ideal, but other condensers are acceptable if they have minimum obstructions. Avoid using Graham-type condensers.) Avoid using an open flame as a heat source if possible, because heat applied to the boiling flask above the liquid level causes superheating of vapor and subsequent sulfate carryover.

CAUTION: *Regardless of apparatus used, provide for thorough mixing of sample and acid; heating a non-homogenous acid-water mixture will result in bumping or possibly a violent explosion.*

The preferred apparatus is illustrated in Figure 4500-F^-:1.

b. Quartz hemispherical heating mantle, for full-voltage operation.

c. Magnetic stirrer, with TFE-coated stirring bar.

d. Soft glass beads.

3. Reagents

a. Sulfuric acid, H_2SO_4, conc, reagent grade.

b. Silver sulfate, Ag_2SO_4, crystals, reagent grade.

4. Procedure

a. Place 400 mL distilled water in the distilling flask and, with the magnetic stirrer operating, carefully add 200 mL conc H_2SO_4. Keep stirrer in operation throughout distillation. Add a few glass beads and connect the apparatus as shown in Figure 4500-F^-:1, making sure all joints are tight. Begin heating and continue until flask contents reach 180°C (because of heat retention by the mantle, it is necessary to discontinue heating when the temperature reaches 178°C to prevent

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overheating). Discard distillate. This process removes fluoride contamination and adjusts the acid-water ratio for subsequent distillations.

b. After the acid mixture remaining in the steps outlined in ¶ 4*a*, or previous distillations, has cooled to 80°C or below, add 300 mL sample, with stirrer operating, and distill until the temperature reaches 180°C. To prevent sulfate carryover, turn off heat before 178°C. Retain the distillate for analysis.

c. Add Ag_2SO_4 to the distilling flask at the rate of 5 mg/mg Cl^- when the chloride concentration is high enough to interfere (see Table 4500-F⁻:I).

d. Use H_2SO_4 solution in the flask repeatedly until contaminants from samples accumulate to such an extent that recovery is affected or interferences appear in the distillate. Check acid suitability periodically by distilling standard fluoride samples and analyzing for both fluoride and sulfate. After distilling samples containing more than 3 mg F^-/L, flush still by adding 300 mL distilled water, redistill, and combine the two fluoride distillates. If necessary, repeat flushing until the fluoride content of the last distillate is at a minimum. Include additional fluoride recovered with that of the first distillation. After periods of inactivity, similarly flush still and discard distillate.

5. Interpretation of Results

The recovery of fluoride is quantitative within the accuracy of the methods used for its measurement.

6. Bibliography

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4500-F^- C. Ion-Selective Electrode Method

1. General Discussion

a. Principle: The fluoride electrode is an ion-selective sensor. The key element in the fluoride electrode is the laser-type doped lanthanum fluoride crystal across which a potential is established by fluoride solutions of different concentrations. The crystal contacts the sample solution at one face and an internal reference solution at the other. The cell may be represented by:

$$Ag|AgCl, Cl^-(0.3M), F^-(0.001M) |LaF_3| \text{ test solution}|\text{reference electrode}$$

The fluoride electrode can be used with a standard calomel reference electrode and almost any

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modern pH meter having an expanded millivolt scale. Calomel electrodes contain both metallic and dissolved mercury; therefore, dispose of them only in approved sites or recycle. For this reason, the Ag/AgCl reference electrode is preferred.

The fluoride electrode measures the ion activity of fluoride in solution rather than concentration. Fluoride ion activity depends on the solution total ionic strength and pH, and on fluoride complexing species. Adding an appropriate buffer provides a nearly uniform ionic strength background, adjusts pH, and breaks up complexes so that, in effect, the electrode measures concentration.

b. Interference: Table 4500-F^-:I lists common interferences. Fluoride forms complexes with several polyvalent cations, notably aluminum and iron. The extent to which complexation takes place depends on solution pH, relative levels of fluoride, and complexing species. However, CDTA (cyclohexylenediaminetetraacetic acid), a component of the buffer, preferentially will complex interfering cations and release free fluoride ions. Concentrations of aluminum, the most common interference, up to 3.0 mg/L can be complexed preferentially. In acid solution, F^- forms a poorly ionized HF·HF complex but the buffer maintains a pH above 5 to minimize hydrogen fluoride complex formation. In alkaline solution hydroxide ion also can interfere with electrode response to fluoride ion whenever the hydroxide ion concentration is greater than one-tenth the concentration of fluoride ion. At the pH maintained by the buffer, no hydroxide interference occurs.

Fluoborates are widely used in industrial processes. Dilute solutions of fluoborate or fluoboric acid hydrolyze to liberate fluoride ion but in concentrated solutions, as in electroplating wastes, hydrolysis does not occur completely. Distill such samples or measure fluoborate with a fluoborate-selective electrode. Also distill the sample if the dissolved solids concentration exceeds 10 000 mg/L.

2. Apparatus

a. Expanded-scale or digital pH meter or ion-selective meter.

b. Sleeve-type reference electrode: Do not use fiber-tip reference electrodes because they exhibit erratic behavior in very dilute solutions.

c. Fluoride electrode.

d. Magnetic stirrer, with TFE-coated stirring bar.

e. Timer.

3. Reagents

a. Stock fluoride solution: Dissolve 221.0 mg anhydrous sodium fluoride, NaF, in distilled water and dilute to 1000 mL; 1.00 mL = 100 µg F^-.

b. Standard fluoride solution: Dilute 100 mL stock fluoride solution to 1000 mL with distilled water; 1.00 mL = 10.0 µg F^-.

c. Fluoride buffer: Place approximately 500 mL distilled water in a 1-L beaker and add 57

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mL glacial acetic acid, 58 g NaCl, and 4.0 g 1,2 cyclohexylenediaminetetraacetic acid (CDTA).*#(39) Stir to dissolve. Place beaker in a cool water bath and add slowly 6*N* NaOH (about 125 mL) with stirring, until pH is between 5.3 and 5.5. Transfer to a 1-L volumetric flask and add distilled water to the mark. This buffer, as well as a more concentrated version, is available commercially. In using the concentrated buffer follow the manufacturer's directions.

4. Procedure

a. Instrument calibration: No major adjustment of any instrument normally is required to use electrodes in the range of 0.2 to 2.0 mg F^-/L. For those instruments with zero at center scale adjust calibration control so that the 1.0 mg F^-/L standard reads at the center zero (100 mV) when the meter is in the expanded-scale position. This cannot be done on some meters that do not have a millivolt calibration control. To use a selective-ion meter follow the manufacturer's instructions.

b. Preparation of fluoride standards: Prepare a series of standards by diluting with distilled water 5.0, 10.0, and 20.0 mL of standard fluoride solution to 100 mL with distilled water. These standards are equivalent to 0.5, 1.0, and 2.0 mg F^-/L.

c. Treatment of standards and sample: In 100-mL beakers or other convenient containers add by volumetric pipet from 10 to 25 mL standard or sample. Bring standards and sample to same temperature, preferably room temperature. Add an equal volume of buffer. The total volume should be sufficient to immerse the electrodes and permit operation of the stirring bar.

d. Measurement with electrode: Immerse electrodes in each of the fluoride standard solutions and measure developed potential while stirring on a magnetic stirrer. Avoid stirring before immersing electrodes because entrapped air around the crystal can produce erroneous readings or needle fluctuations. Let electrodes remain in the solution 3 min (or until reading is constant) before taking a final millivolt reading. A layer of insulating material between stirrer and beaker minimizes solution heating. Withdraw electrodes, rinse with distilled water, and *blot dry* between readings. (CAUTION: Blotting may poison electrode if not done gently.) Repeat measurements with samples.

When using an expanded-scale pH meter or selective-ion meter, frequently recalibrate the electrode by checking potential reading of the 1.00-mg F^-/L standard and adjusting the calibration control, if necessary, until meter reads as before.

If a direct-reading instrument is not used, plot potential measurement of fluoride standards against concentration on two-cycle semilogarithmic graph paper. Plot milligrams F^- per liter on the logarithmic axis (ordinate), with the lowest concentration at the bottom of the graph. Plot millivolts on the abscissa. From the potential measurement for each sample, read the corresponding fluoride concentration from the standard curve.

The known-additions method may be substituted for the calibration method described. Follow the directions of the instrument manufacturer.

Selective-ion meters may necessitate using a slightly altered procedure, such as preparing

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1.00 and 10.0 mg F^-/L standards or some other concentration. Follow the manufacturer's directions. Commercial standards, often already diluted with buffer, frequently are supplied with the meter. Verify the stated fluoride concentration of these standards by comparing them with standards prepared by the analyst.

5. Calculation

$$\text{mg } F^-/L = \frac{\mu g\ F^-}{\text{mL sample}}$$

6. Precision and Bias

A synthetic sample containing 0.850 mg F^-/L in distilled water was analyzed in 111 laboratories by the electrode method, with a relative standard deviation of 3.6% and a relative error of 0.7%.

A second synthetic sample containing 0.750 mg F^-/L, 2.5 mg $(NaPO_3)_6$/L, and 300 mg alkalinity/L added as $NaHCO_3$, was analyzed in 111 laboratories by the electrode method, with a relative standard deviation of 4.8% and a relative error of 0.2%.

A third synthetic sample containing 0.900 mg F^-/L, 0.500 mg Al/L, and 200 mg SO_4^{2-}/L was analyzed in 13 laboratories by the electrode method, with a relative standard deviation of 2.9% and a relative error of 4.9%.

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4500-F^- D. SPADNS Method

1. General Discussion

a. Principle: The SPADNS colorimetric method is based on the reaction between fluoride and a zirconium-dye lake. Fluoride reacts with the dye lake, dissociating a portion of it into a colorless complex anion (ZrF_6^{2-}); and the dye. As the amount of fluoride increases, the color produced becomes progressively lighter.

The reaction rate between fluoride and zirconium ions is influenced greatly by the acidity of the reaction mixture. If the proportion of acid in the reagent is increased, the reaction can be made almost instantaneous. Under such conditions, however, the effect of various ions differs

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from that in the conventional alizarin methods. The selection of dye for this rapid fluoride method is governed largely by the resulting tolerance to these ions.

b. Interference: Table 4500-F^-:I lists common interferences. Because these are neither linear in effect nor algebraically additive, mathematical compensation is impossible. Whenever any one substance is present in sufficient quantity to produce an error of 0.1 mg/ L or whenever the total interfering effect is in doubt, distill the sample. Also distill colored or turbid samples. In some instances, sample dilution or adding appropriate amounts of interfering substances to the standards may be used to compensate for the interference effect. If alkalinity is the only significant interference, neutralize it with either hydrochloric or nitric acid. Chlorine interferes and provision for its removal is made.

Volumetric measurement of sample and reagent is extremely important to analytical accuracy. Use samples and standards at the same temperature or at least within 2°C. Maintain constant temperature throughout the color development period. Prepare different calibration curves for different temperature ranges.

2. Apparatus

Colorimetric equipment: One of the following is required:

a. Spectrophotometer, for use at 570 nm, providing a light path of at least 1 cm.

b. Filter photometer, providing a light path of at least 1 cm and equipped with a greenish yellow filter having maximum transmittance at 550 to 580 nm.

3. Reagents

a. Standard fluoride solution: Prepare as directed in the electrode method, Section 4500-F^-.C.3*b*.

b. SPADNS solution: Dissolve 958 mg SPADNS, sodium 2-(parasulfophenylazo)-1,8-dihydroxy-3,6-naphthalene disulfonate, also called 4,5-dihydroxy-3-(parasulfophenylazo)-2,7-naphthalenedisulfonic acid trisodium salt, in distilled water and dilute to 500 mL. This solution is stable for at least 1 year if protected from direct sunlight.

c. Zirconyl-acid reagent: Dissolve 133 mg zirconyl chloride octahydrate, $ZrOCl_2 \cdot 8H_2O$, in about 25 mL distilled water. Add 350 mL conc HCl and dilute to 500 mL with distilled water.

d. Acid zirconyl-SPADNS reagent: Mix equal volumes of SPADNS solution and zirconyl-acid reagent. The combined reagent is stable for at least 2 years.

e. Reference solution: Add 10 mL SPADNS solution to 100 mL distilled water. Dilute 7 mL conc HCl to 10 mL and add to the diluted SPADNS solution. The resulting solution, used for setting the instrument reference point (zero), is stable for at least 1 year. Alternatively, use a prepared standard of 0 mg F^-/L as a reference.

f. Sodium arsenite solution: Dissolve 5.0 g $NaAsO_2$ and dilute to 1 L with distilled water.

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(CAUTION: *Toxic—avoid ingestion.*)

4. Procedure

a. Preparation of standard curve: Prepare fluoride standards in the range of 0 to 1.40 mg F^-/L by diluting appropriate quantities of standard fluoride solution to 50 mL with distilled water. Pipet 5.00 mL each of SPADNS solution and zirconyl-acid reagent, or 10.00 mL mixed acid-zirconyl-SPADNS reagent, to each standard and mix well. Avoid contamination. Set photometer to zero absorbance with the reference solution and obtain absorbance readings of standards. Plot a curve of the milligrams fluoride-absorbance relationship. Prepare a new standard curve whenever a fresh reagent is made or a different standard temperature is desired. As an alternative to using a reference, set photometer at some convenient point (0.300 or 0.500 absorbance) with the prepared 0 mg F^-/L standard.

b. Sample pretreatment: If the sample contains residual chlorine, remove it by adding 1 drop (0.05 mL) $NaAsO_2$ solution/ 0.1 mg residual chlorine and mix. (Sodium arsenite concentrations of 1300 mg/L produce an error of 0.1 mg/L at 1.0 mg F^-/L.)

c. Color development: Use a 50.0-mL sample or a portion diluted to 50 mL with distilled water. Adjust sample temperature to that used for the standard curve. Add 5.00 mL each of SPADNS solution and zirconyl-acid reagent, or 10.00 mL acid-zirconyl-SPADNS reagent; mix well and read absorbance, first setting the reference point of the photometer as above. If the absorbance falls beyond the range of the standard curve, repeat using a diluted sample.

5. Calculation

$$\text{mg F}^-/\text{L} = \frac{A}{\text{mL sample}} \times \frac{B}{C}$$

where:

A = µg F^- determined from plotted curve,
B = final volume of diluted sample, mL, and
C = volume of diluted sample used for color development, mL.

When the prepared 0 mg F^-/L standard is used to set the photometer, alternatively calculate fluoride concentration as follows:

$$\text{mg F}^-/\text{L} = \frac{A_0 - A_x}{A_0 - A_1}$$

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where:

A_0 = absorbance of the prepared 0 mg F^-/L standard,

A_1 = absorbance of a prepared 1.0 mg F^-/L standard, and

A_x = absorbance of the prepared sample.

6. Precision and Bias

A synthetic sample containing 0.830 mg F^-/L and no interference in distilled water was analyzed in 53 laboratories by the SPADNS method, with a relative standard deviation of 8.0% and a relative error of 1.2%. After direct distillation of the sample, the relative standard deviation was 11.0% and the relative error 2.4%.

A synthetic sample containing 0.570 mg F^-/L, 10 mg Al/L, 200 mg SO_4^{2-}/L, and 300 mg total alkalinity/L was analyzed in 53 laboratories by the SPADNS method without distillation, with a relative standard deviation of 16.2% and a relative error of 7.0%. After direct distillation of the sample, the relative standard deviation was 17.2% and the relative error 5.3%.

A synthetic sample containing 0.680 mg F^-/L, 2 mg Al/L, 2.5 mg $(NaPO_3)_6$/L, 200 mg SO_4^{2-}/L, and 300 mg total alkalinity/L was analyzed in 53 laboratories by direct distillation and SPADNS methods with a relative standard deviation of 2.8% and a relative error of 5.9%.

7. Bibliography

BELLACK, E. & P.J. SCHOUBOE. 1968. Rapid photometric determination of fluoride with SPADNS-zirconium lake. *Anal. Chem.* 30:2032.

4500-F⁻ E. Complexone Method

1. General Discussion

a. Principle: The sample is distilled in the automated system, and the distillate is reacted with alizarin fluorine blue-lanthanum reagent to form a blue complex that is measured colorimetrically at 620 nm.

b. Interferences: Interferences normally associated with the determination of fluoride are removed by distillation.

c. Application: This method is applicable to potable, surface, and saline waters as well as domestic and industrial wastewaters. The range of the method, which can be modified by using the adjustable colorimeter, is 0.1 to 2.0 mg F^-/L.

2. Apparatus

An example of the required continuous-flow analytical instrument consists of the interchangeable components in the number and manner indicated in Figure 4500-F^-:2.

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3. Reagents

a. Standard fluoride solution: Prepare in appropriate concentrations from 0.10 to 2.0 mg F^-/L using the stock fluoride solution (see Section 4500-F⁻.C.3*a*).

b. Distillation reagent: Add 50 mL conc H_2SO_4 to about 600 mL distilled water. Add 10.00 mL stock fluoride solution (see Section 4500-F⁻.C.3*a*; 1.00 mL = 100 µg F^-) and dilute to 1000 mL.

c. Acetate buffer solution: Dissolve 60 g anhydrous sodium acetate, $NaC_2H_3O_2$, in about 600 mL distilled water. Add 100 mL conc (glacial) acetic acid and dilute to 1 L.

d. Alizarin fluorine blue stock solution: Add 960 mg alizarin fluorine,*#(40) $C_{14}H_7O_4 \cdot CH_2N(CH_2 \cdot COOH)_2$, to 100 mL distilled water. Add 2 mL conc NH_4OH and mix until dye is dissolved. Add 2 mL conc (glacial) acetic acid, dilute to 250 mL and store in an amber bottle in the refrigerator.

e. Lanthanum nitrate stock solution: Dissolve 1.08 g $La(NO_3)_3$ in about 100 mL distilled water, dilute to 250 mL, and store in refrigerator.

f. Working color reagent: Mix in the following order: 300 mL acetate buffer solution, 150 mL acetone, 50 mL tertiary butanol, 36 mL alizarin fluorine blue stock solution, 40 mL lanthanum nitrate stock solution, and 2 mL polyoxyethylene 23 lauryl ether.†#(41) Dilute to 1 L with distilled water. This reagent is stable for 2 to 4 d.

4. Procedure

No special handling or preparation of sample is required.

Set up manifold as shown in Figure 4500-F⁻:2 and follow the manufacturer's instructions.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against constituent concentrations in standards. Compute sample concentrations by comparing sample response with standard curve.

6. Precision and Bias

In a single laboratory four samples of natural water containing from 0.40 to 0.82 mg F^-/L were analyzed in septuplicate. Average precision was ± 0.03 mg F^-/L. To two of the samples, additions of 0.20 and 0.80 mg F^-/L were made. Average recovery of the additions was 98%.

7. Bibliography

WEINSTEIN, L.H., R.H. MANDL, D.C. MCCUNE, J.S. JACOBSON & A.E. HITCHCOCK. 1963. A semi-automated method for the determination of fluorine in air and plant tissues. *Boyce Thompson Inst.* 22:207.

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4500-F⁻ F. (Reserved)

4500-F⁻ G. Ion-Selective Electrode Flow Injection Analysis (PROPOSED)

1. General Discussion

a. Principle: Fluoride is determined potentiometrically by using a combination fluoride-selective electrode in a flow cell. The fluoride electrode consists of a lanthanum fluoride crystal across which a potential is developed by fluoride ions. The reference cell is a Ag/ AgCl/Cl^- cell. The reference junction is of the annular liquid-junction type and encloses the fluoride-sensitive crystal.

Also see Section 4500-F⁻.C and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

The polyvalent cations Si^{4+}, Al^{3+}, and Fe^{3+} interfere by forming complexes with fluoride. As part of the buffer reagent, 1,2-cyclohexyldiaminetetraacetic acid (CDTA) is added to preferentially complex these cations and eliminate this interference when these concentrations do not exceed 3.0 mg Al^{3+}/L and 20 mg Fe^{3+}/L.

Some interferents are removed by distillation; see Section 4500-F⁻.B. Drinking water samples generally do not require sample distillation.

2. Apparatus

Flow injection analysis equipment consisting of:

a. FIA injection valve with sample loop or equivalent.

b. Multichannel proportioning pump.

c. FIA manifold (Figure 4500-F⁻:3) with tubing heater and ion-selective electrode flow cell. In Figure 4500-F⁻:3, relative flow rates only are shown. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

d. Combination ion-selective electrode.

e. Injection valve control and data acquisition system.

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min.

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a. Carrier, 1.0 mg F^-/L: Add 10 mL or 10 g stock fluoride standard (¶ 3*d*) to 990 mL water and mix well.

b. Buffer: To a tared 1-L polyethylene container add 929.5 g water, 59.8 g glacial acetic acid, 30.0 g sodium hydroxide, NaOH, 58.0 g sodium chloride, NaCl, 0.5 g stock fluoride standard (¶ 3*d*), and 4.0 g 1,2-cyclohexyldiaminetetraacetic acid (CDTA) (also called trans-1,2-diaminocyclohexane). Stir on a magnetic stir plate until all material has dissolved.

c. Electrode conditioning solution: To a tared 1-L container, add 534 g buffer (¶ 3*b*) and 500 g carrier (¶ 3*a*). Shake or stir to mix thoroughly. Store fluoride electrode in this solution when it is not in use.

d. Stock fluoride standard, 100.0 mg F^-/L: In a 1-L volumetric flask, dissolve 0.2210 g sodium fluoride, NaF, in approximately 950 mL water. Dilute to mark with water and mix well. Store in a polyethylene bottle.

e. Standard fluoride solutions: Prepare fluoride standards in the desired concentration range, using the stock standard (¶ 3*d*), and diluting with water. A blank or zero concentration standard cannot be prepared for this method because it will give an undefined response from the fluoride electrode.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-F^-:3 and follow method supplied by manufacturer or laboratory standard operating procedure for this method. Follow quality control procedures outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting the electrode response to standards processed through the manifold vs. fluoride concentration. Standards greater than 1.0 mg F^-/L will give positive peaks, standards less than 1.0 mg F^-/L will give negative peaks, and the 1.0 mg F^-/L standard having the same concentration as the carrier will give no peak. The calibration curve gives a good fit to a second-order polynomial.

It is not necessary to plot the response versus log[F^-]; if this is done the calibration curve will still be a second-order polynomial because there is a concentration-dependent kinetic effect in the flowing stream electrode system.

6. Precision and Bias

The samples used in the studies described below were not distilled.

a. Recovery and relative standard deviation: The results of single-laboratory studies with various matrices are given in Table 4500-F^-:II.

b. MDL: A 390-µL sample loop was used in the method described above. Ten replicates of a 1.0-mg F^-/L standard were run to obtain an MDL of 0.02 mg F^-/L.

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c. Precision: Ten replicate standards of 2.0 mg F^-/L gave a % RSD of 0.5%.

4500-H⁺ PH VALUE*#(42)

4500-H⁺ A. Introduction

1. Principles

Measurement of pH is one of the most important and frequently used tests in water chemistry. Practically every phase of water supply and wastewater treatment, e.g., acid-base neutralization, water softening, precipitation, coagulation, disinfection, and corrosion control, is pH-dependent. pH is used in alkalinity and carbon dioxide measurements and many other acid-base equilibria. At a given temperature the *intensity* of the acidic or basic character of a solution is indicated by pH or hydrogen ion activity. Alkalinity and acidity are the acid- and base-neutralizing capacities of a water and usually are expressed as milligrams $CaCO_3$ per liter. Buffer capacity is the amount of strong acid or base, usually expressed in moles per liter, needed to change the pH value of a 1-L sample by 1 unit. pH as defined by Sorenson[1] is $-\log [H^+]$; it is the ''intensity'' factor of acidity. Pure water is very slightly ionized and at equilibrium the ion product is

$$[H^+][OH^-] = K_w = 1.01 \times 10^{-14} \text{ at } 25°C \quad (1)$$

and

$$[H^+] = [OH^-] = 1.005 \times 10^{-7}$$

where:

$[H^+]$ = activity of hydrogen ions, moles/L,
$[OH^-]$ = activity of hydroxyl ions, moles/L, and
K_w = ion product of water.

Because of ionic interactions in all but very dilute solutions, it is necessary to use the ''activity'' of an ion and not its molar concentration. Use of the term pH assumes that the activity of the hydrogen ion, a_{H^+}, is being considered. The *approximate* equivalence to molarity, $[H^+]$ can be presumed only in very dilute solutions (ionic strength <0.1).

A logarithmic scale is convenient for expressing a wide range of ionic activities. Equation 1

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in logarithmic form and corrected to reflect activity is:

$$(-\log_{10} a_{H^+}) + (-\log_{10} a_{OH^-}) = 14 \qquad (2)$$

or

$$\text{pH} + \text{pOH} = \text{p}K_w$$

where:

pH†#(43) = $\log_{10} a_{H^+}$ and
pOH = $\log_{10} a_{OH^-}$.

Equation 2 states that as pH increases pOH decreases correspondingly and vice versa because pK_w is constant for a given temperature. At 25°C, pH 7.0 is neutral, the activities of the hydrogen and hydroxyl ions are equal, and each corresponds to an approximate activity of 10^{-7} moles/L. The neutral point is temperature-dependent and is pH 7.5 at 0°C and pH 6.5 at 60°C.

The pH value of a highly dilute solution is approximately the same as the negative common logarithm of the hydrogen ion concentration. Natural waters usually have pH values in the range of 4 to 9, and most are slightly basic because of the presence of bicarbonates and carbonates of the alkali and alkaline earth metals.

2. Reference

1. SORENSON, S. 1909. Uber die Messung und die Bedeutung der Wasserstoff ionen Konzentration bei Enzymatischen Prozessen. *Biochem. Z.* 21:131.

4500-H^+ B. Electrometric Method

1. General Discussion

a. Principle: The basic principle of electrometric pH measurement is determination of the activ ity of the hydrogen ions by potentiometric measurement using a standard hydrogen electrode and a reference electrode. The hydrogen electrode consists of a platinum electrode across which hydrogen gas is bubbled at a pressure of 101 kPa. Because of difficulty in its use and the potential for poisoning the hydrogen electrode, the glass electrode commonly is used. The electromotive force (emf) produced in the glass electrode system varies linearly with pH. This linear relationship is described by plotting the measured emf against the pH of different buffers. Sample pH is determined by extrapolation.

Because single ion activities such as $a_H{}^+$ cannot be measured, pH is defined operationally on a potentiometric scale. The pH measuring instrument is calibrated potentiometrically with an

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indicating (glass) electrode and a reference electrode using National Institute of Standards and Technology (NIST) buffers having assigned values so that:

$$pH_B = -\log_{10} a_{H^+}$$

where:

pH_B = assigned pH of NIST buffer.

The operational pH scale is used to measure sample pH and is defined as:

$$pH_x = pH_B \pm \frac{F(E_x - E_s)}{2.303\ RT}$$

where:

pH_x = potentiometrically measured sample pH,
F = Faraday: 9.649×10^4 coulomb/mole,
E_x = sample emf, V,
E_s = buffer emf, V,
R = gas constant; 8.314 joule/(mole °K), and
T = absolute temperature, °K.

NOTE: Although the equation for pH_x appears in the literature with a plus sign, the sign of emf readings in millivolts for most pH meters manufactured in the U.S. is negative. The choice of negative sign is consistent with the IUPAC Stockholm convention concerning the sign of electrode potential.[1,2]

The activity scale gives values that are higher than those on Sorenson's scale by 0.04 units:

$$\text{pH (activity)} = \text{pH (Sorenson)} + 0.04$$

The equation for pH_x assumes that the emf of the cells containing the sample and buffer is due solely to hydrogen ion activity unaffected by sample composition. In practice, samples will have varying ionic species and ionic strengths, both affecting H^+ activity. This imposes an experimental limitation on pH measurement; thus, to obtain meaningful results, the differences between E_x and E_s should be minimal. Samples must be dilute aqueous solutions of simple solutes ($<0.2M$). (Choose buffers to bracket the sample.) Determination of pH cannot be made accurately in nonaqueous media, suspensions, colloids, or high-ionic-strength solutions.

b. Interferences: The glass electrode is relatively free from interference from color, turbidity, colloidal matter, oxidants, reductants, or high salinity, except for a sodium error at pH > 10.

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Reduce this error by using special ‘‘low sodium error’’ electrodes.

pH measurements are affected by temperature in two ways: mechanical effects that are caused by changes in the properties of the electrodes and chemical effects caused by equilibrium changes. In the first instance, the Nernstian slope increases with increasing temperature and electrodes take time to achieve thermal equilibrium. This can cause long-term drift in pH. Because chemical equilibrium affects pH, standard pH buffers have a specified pH at indicated temperatures.

Always report temperature at which pH is measured.

2. Apparatus

a. pH meter consisting of potentiometer, a glass electrode, a reference electrode, and a temperature-compensating device. A circuit is completed through the potentiometer when the electrodes are immersed in the test solution. Many pH meters are capable of reading pH or millivolts and some have scale expansion that permits reading to 0. 001 pH unit, but most instruments are not that precise.

For routine work use a pH meter accurate and reproducible to 0.1 pH unit with a range of 0 to 14 and equipped with a temperature-compensation adjustment.

Although manufacturers provide operating instructions, the use of different descriptive terms may be confusing. For most instruments, there are two controls: intercept (set buffer, asymmetry, standardize) and slope (temperature, offset); their functions are shown diagramatically in Figure 4500-H^+:1 and Figure 4500-H^+:2. The intercept control shifts the response curve laterally to pass through the isopotential point with no change in slope. This permits bringing the instrument on scale (0 mV) with a pH 7 buffer that has no change in potential with temperature.

The slope control rotates the emf/pH slope about the isopotential point (0 mV/pH 7). To adjust slope for temperature without disturbing the intercept, select a buffer that brackets the sample with pH 7 buffer and adjust slope control to pH of this buffer. The instrument will indicate correct millivolt change per unit pH at the test temperature.

b. Reference electrode consisting of a half cell that provides a constant electrode potential. Commonly used are calomel and silver: silver-chloride electrodes. Either is available with several types of liquid junctions.

The liquid junction of the reference electrode is critical because at this point the electrode forms a salt bridge with the sample or buffer and a liquid junction potential is generated that in turn affects the potential produced by the reference electrode. Reference electrode junctions may be annular ceramic, quartz, or asbestos fiber, or the sleeve type. The quartz type is most widely used. The asbestos fiber type is not recommended for strongly basic solutions. Follow the manufacturer’s recommendation on use and care of the reference electrode.

Refill nonsealed electrodes with the correct electrolyte to proper level and make sure junction is properly wetted.

c. Glass electrode: The sensor electrode is a bulb of special glass containing a fixed concentration of HCl or a buffered chloride solution in contact with an internal reference

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electrode. Upon immersion of a new electrode in a solution the outer bulb surface becomes hydrated and exchanges sodium ions for hydrogen ions to build up a surface layer of hydrogen ions. This, together with the repulsion of anions by fixed, negatively charged silicate sites, produces at the glass-solution interface a potential that is a function of hydrogen ion activity in solution.

Several types of glass electrodes are available. Combination electrodes incorporate the glass and reference electrodes into a single probe. Use a ‘‘low sodium error’’ electrode that can operate at high temperatures for measuring pH over 10 because standard glass electrodes yield erroneously low values. For measuring pH below 1 standard glass electrodes yield erroneously high values; use liquid membrane electrodes instead.

d. Beakers: Preferably use polyethylene or TFE*#(44) beakers.

e. Stirrer: Use either a magnetic, TFE-coated stirring bar or a mechanical stirrer with inert plastic-coated impeller.

f. Flow chamber: Use for continuous flow measurements or for poorly buffered solutions.

3. Reagents

a. General preparation: Calibrate the electrode system against standard buffer solutions of known pH. Because buffer solutions may deteriorate as a result of mold growth or contamination, prepare fresh as needed for accurate work by weighing the amounts of chemicals specified in Table 4500-H+:I, dissolving in distilled water at 25°C, and diluting to 1000 mL. This is particularly important for borate and carbonate buffers.

Boil and cool distilled water having a conductivity of less than 2 µmhos/cm. To 50 mL add 1 drop of saturated KCl solution suitable for reference electrode use. If the pH of this test solution is between 6.0 and 7.0, use it to prepare all standard solutions.

Dry KH_2PO_4 at 110 to 130°C for 2 h before weighing but do not heat unstable hydrated potassium tetroxalate above 60°C nor dry the other specified buffer salts.

Although ACS-grade chemicals generally are satisfactory for preparing buffer solutions, use certified materials available from the National Institute of Standards and Technology when the greatest accuracy is required. For routine analysis, use commercially available buffer tablets, powders, or solutions of tested quality. In preparing buffer solutions from solid salts, insure complete solution.

As a rule, select and prepare buffer solutions classed as primary standards in Table 4500-H+:I; reserve secondary standards for extreme situations encountered in wastewater measurements. Consult Table 4500-H+:II for accepted pH of standard buffer solutions at temperatures other than 25°C. In routine use, store buffer solutions and samples in polyethylene bottles. Replace buffer solutions every 4 weeks.

b. Saturated potassium hydrogen tartrate solution: Shake vigorously an excess (5 to 10 g) of finely crystalline $KHC_4H_4O_6$ with 100 to 300 mL distilled water at 25°C in a glass-stoppered bottle. Separate clear solution from undissolved material by decantation or filtration. Preserve for

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2 months or more by adding one thymol crystal (8 mm diam) per 200 mL solution.

c. Saturated calcium hydroxide solution: Calcine a well-washed, low-alkali grade $CaCO_3$ in a platinum dish by igniting for 1 h at 1000°C. Cool, hydrate by slowly adding distilled water with stirring, and heat to boiling. Cool, filter, and collect solid $Ca(OH)_2$ on a fritted glass filter of medium porosity. Dry at 110°C, cool, and pulverize to uniformly fine granules. Vigorously shake an excess of fine granules with distilled water in a stoppered polyethylene bottle. Let temperature come to 25°C after mixing. Filter supernatant under suction through a sintered glass filter of medium porosity and use filtrate as the buffer solution. Discard buffer solution when atmospheric CO_2 causes turbidity to appear.

d. Auxiliary solutions: 0.1*N* NaOH, 0.1*N* HCl, 5*N* HCl (dilute five volumes 6*N* HCl with one volume distilled water), and acid potassium fluoride solution (dissolve 2 g KF in 2 mL conc H_2SO_4 and dilute to 100 mL with distilled water).

4. Procedure

a. Instrument calibration: In each case follow manufacturer's instructions for pH meter and for storage and preparation of electrodes for use. Recommended solutions for short-term storage of electrodes vary with type of electrode and manufacturer, but generally have a conductivity greater than 4000 µmhos/cm. Tap water is a better substitute than distilled water, but pH 4 buffer is best for the single glass electrode and saturated KCl is preferred for a calomel and Ag/AgCl reference electrode. Saturated KCl is the preferred solution for a combination electrode. Keep electrodes wet by returning them to storage solution whenever pH meter is not in use.

Before use, remove electrodes from storage solution, rinse, blot dry with a soft tissue, place in initial buffer solution, and set the isopotential point (¶ 2*a* above). Select a second buffer within 2 pH units of sample pH and bring sample and buffer to same temperature, which may be the room temperature, a fixed temperature such as 25°C, or the temperature of a fresh sample. Remove electrodes from first buffer, rinse thoroughly with distilled water, blot dry, and immerse in second buffer. Record temperature of measurement and adjust temperature dial on meter so that meter indicates pH value of buffer at test temperature (this is a slope adjustment).

Use the pH value listed in the tables for the buffer used at the test temperature. Remove electrodes from second buffer, rinse thoroughly with distilled water and dry electrodes as indicated above. Immerse in a third buffer below pH 10, approximately 3 pH units different from the second; the reading should be within 0.1 unit for the pH of the third buffer. If the meter response shows a difference greater than 0.1 pH unit from expected value, look for trouble with the electrodes or potentiometer (see ¶ 5*a* and ¶ 5*b* below).

The purpose of standardization is to adjust the response of the glass electrode to the instrument. When only occasional pH measurements are made standardize instrument before each measurement. When frequent measurements are made and the instrument is stable, standardize less frequently. If sample pH values vary widely, standardize for each sample with a buffer having a pH within 1 to 2 pH units of the sample.

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b. Sample analysis: Establish equilibrium between electrodes and sample by stirring sample to insure homogeneity; stir gently to minimize carbon dioxide entrainment. For buffered samples or those of high ionic strength, condition electrodes after cleaning by dipping them into sample for 1 min. Blot dry, immerse in a fresh portion of the same sample, and read pH.

With dilute, poorly buffered solutions, equilibrate electrodes by immersing in three or four successive portions of sample. Take a fresh sample to measure pH.

5. Trouble Shooting

a. Potentiometer: To locate trouble source disconnect electrodes and, using a short-circuit strap, connect reference electrode terminal to glass electrode terminal. Observe change in pH when instrument calibration knob is adjusted. If potentiometer is operating properly, it will respond rapidly and evenly to changes in calibration over a wide scale range. A faulty potentiometer will fail to respond, will react erratically, or will show a drift upon adjustment. Switch to the millivolt scale on which the meter should read zero. If inexperienced, do not attempt potentiometer repair other than maintenance as described in instrument manual.

b. Electrodes: If potentiometer is functioning properly, look for the instrument fault in the electrode pair. Substitute one electrode at a time and cross-check with two buffers that are about 4 pH units apart. A deviation greater than 0.1 pH unit indicates a faulty electrode. Glass electrodes fail because of scratches, deterioration, or accumulation of debris on the glass surface. Rejuvenate electrode by alternately immersing it three times each in 0.1*N* HCl and 0.1*N* NaOH. If this fails, immerse tip in KF solution for 30 s. After rejuvenation, soak in pH 7.0 buffer overnight. Rinse and store in pH 7.0 buffer. Rinse again with distilled water before use. Protein coatings can be removed by soaking glass electrodes in a 10% pepsin solution adjusted to pH 1 to 2.

To check reference electrode, oppose the emf of a questionable reference electrode against another one of the same type that is known to be good. Using an adapter, plug good reference electrode into glass electrode jack of potentiometer; then plug questioned electrode into reference electrode jack. Set meter to read millivolts and take readings with both electrodes immersed in the same electrolyte (KCl) solution and then in the same buffer solution. The millivolt readings should be 0 ± 5 mV for both solutions. If different electrodes are used, i.e., silver: silver-chloride against calomel or vice versa, the reading will be 44 ± 5 mV for a good reference electrode.

Reference electrode troubles generally are traceable to a clogged junction. Interruption of the continuous trickle of electrolyte through the junction causes increase in response time and drift in reading. Clear a clogged junction by applying suction to the tip or by boiling tip in distilled water until the electrolyte flows freely when suction is applied to tip or pressure is applied to the fill hole. Replaceable junctions are available commercially.

6. Precision and Bias

By careful use of a laboratory pH meter with good electrodes, a precision of ±0.02 pH unit and an accuracy of ±0.05 pH unit can be achieved. However, ±0.1 pH unit represents the limit of

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accuracy under normal conditions, especially for measurement of water and poorly buffered solutions. For this reason, report pH values to the nearest 0.1 pH unit. A synthetic sample of a Clark and Lubs buffer solution of pH 7.3 was analyzed electrometrically by 30 laboratories with a standard deviation of ±0.13 pH unit.

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4500-I IODINE*#(45)

4500-I A. Introduction

1. Uses and Forms

Elemental iodine is not a natural constituent of natural waters. Iodine may be added to potable and swimming pool waters as a disinfectant. For wastewaters, iodine has had limited application. Use of iodine generally is restricted to personal or remote water supplies where ease of application, storage stability, and an inertness toward organic matter are important considerations. Some swimming pool waters are treated with iodine to lessen eye burn among swimmers and to provide a stable disinfectant residual less affected by adverse environmental conditions.

Iodine is applied in the elemental form or produced in situ by the simultaneous addition of an iodide salt and a suitable oxidant. In the latter case, an excess of iodide may be maintained to serve as a reservoir for iodine production; the determination of iodide is desirable for disinfectant control (see Iodide, Section 4500-I).

Elemental I_2 can undergo hydrolysis to form hypoiodous acid (HOI), which can dissociate to form hypoiodite (OI^-) under strongly basic conditions. Hypoiodous acid/hypoiodite ion may further disproportionate to form iodate. In the presence of excess iodide, iodine may react with iodide to form tri-iodide ion (I_3^-). The rate and the extent to which these reactions may occur depend on pH and the concentration of iodide in the solution. Basic conditions favor formation of hypoiodite and iodate. Acidic conditions and the presence of iodide favor formation of iodine and tri-iodide ion. Thus, the relative concentrations of these iodine species in the resulting solution can be quite variable. Hypoiodous acid/hypoiodite also can act as an iodinating agent, reacting with organic compounds to form iodinated organic compounds. Elemental I_2, hypoiodous acid, hypoiodite ion, and tri-iodide ion are considered active iodine. There is no generally accepted method for the determination of each of these species individually. Most analytical methods use the oxidizing power of all forms of active iodine for its determination and the results usually are expressed as an equivalent concentration of elemental iodine. The effects of iodate or dissolved organic iodine on these methods have not been thoroughly investigated.

2. Selection of Method

For potable and swimming pool waters treated with elemental iodine, both the amperometric titration and leuco crystal violet colorimetric methods give acceptable results. However, oxidized forms of manganese interfere with the leuco crystal violet method. Where the iodide and chloride ion concentrations are above 50 mg/L and 200 mg/L, respectively, interference in color production may occur in the leuco crystal violet method and the amperometric method is

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preferred. However, because of the extreme sensitivity of the leuco crystal violet method, this interference may be eliminated by sample dilution to obtain halogen ion concentrations less than 50 mg/L.

For wastewaters or highly polluted waters, organic constituents normally do not interfere with either the amperometric or leuco crystal violet procedures. Determine which of the methods yields the more acceptable results, because specific substances present in these waters may interfere in one method but not in the other. Certain metallic cations such as copper and silver interfere in the amperometric titration procedure. Iodate, which is a naturally occurring species of iodine in marine waters, will also interfere in the amperometric titration by reacting with excess iodide under acidic conditions to form I_2 and/or I_3^-. The rate of the reaction is most pH-dependent between pH 3 and 5. Thus, the magnitude of this interference may depend on the concentration of iodate present and the analytical conditions. The leuco crystal violet method is relatively free of interference from these and other cations and anions with the exceptions noted previously.

For waters containing iodine coexisting with free chlorine, combined chlorine, or other excess oxidants, of the methods described only the leuco crystal violet method can determine iodine specifically. This condition occurs in the in-situ production of iodine by the reaction of iodide and excess oxidant. Under these conditions, the amperometric method would continue to titrate the iodine produced in a cyclic reaction until exhaustion of the oxidant.

4500-I B. Leuco Crystal Violet Method

1. General Discussion

The leuco crystal violet method determines aqueous iodine present as elemental iodine and hypoiodous acid. Excess common oxidants do not interfere. While the method utilizes the sum of the oxidative power of all forms of active iodine residuals, the results are expressed as the equivalent concentration of iodine. The method also is capable of determining the sum of iodine and free iodide concentrations; the free iodide concentration can be determined by difference (see Iodide, Section 4500-I^-).

a. Principle: Mercuric chloride added to aqueous elemental iodine solutions causes essentially complete hydrolysis of iodine and the stoichiometric production of hypoiodous acid. The compound 4,4′,4″-methylidynetris (*N,N*-dimethylaniline), also known by the common name of leuco crystal violet, reacts instantaneously with the hypoiodous acid to form crystal violet dye. The absorbance of this dye is highly pH-dependent. The maximum absorbance is produced in the pH range of 3.5 to 4.0 and is measured at a wavelength of 592 nm. Below a pH of 3.5, the absorbance drops precipitously. Above a pH of about 4.7, the excess leuco crystal violet in the sample precipitates and masks the absorbance of the crystal violet dye. Accurate pH control is essential to maximize precision. The absorbance follows Beer's law over a wide range of iodine concentrations and the developed color is stable for several hours.

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In the presence of certain excess oxidants such as free chlorine or chloramines, the iodine residual will exist exclusively in the form of hypoiodous acid. The leuco crystal violet is relatively insensitive to the combined forms of chlorine while any free chlorine is converted to chloramine by reaction with an ammonium salt incorporated in the test reagents. All the hypoiodous acid is determined. As hypoiodous acid, the weight concentration value found, expressed as an equivalent elemental I_2 concentration, is equal to twice that of an elemental I_2 solution of the same weight concentration.

b. Interference: Oxidized forms of manganese interfere by oxidizing the indicator to crystal violet dye and yield apparent high iodine concentrations.

Iodide and chloride ion concentrations above 50 mg/L and 200 mg/L, respectively, interfere by inhibiting full color production. Dilute the sample to eliminate this interference.

Combined chlorine residuals normally do not interfere provided that the test is completed within 5 min after adding the indicator solution. Eliminate interference from free chlorine by adding an ammonium salt buffer to form combined chlorine.

c. Minimum detectable concentration: 10 μg I as I_2/L.

2. Apparatus

a. Colorimetric equipment: One of the following is required:

1) *Filter photometer,* with a light path of 1 cm or longer, equipped with an orange filter having maximum transmittance near 592 nm.

2) *Spectrophotometer,* for use at 592 nm, with a light path of 1 cm or longer.

b. Volumetric flasks, 100-mL, with plastic caps or ground-glass stoppers.

c. Glassware: Completely remove reducing substances from glassware or plastic containers, including containers for storage of reagent solutions (see Section 4500-Cl.D.2*d*).

3. Reagents

a. Iodine-demand-free water: See Section 4500-I^-.B.3*a*. Prepare all stock iodine and reagent solutions with iodine-demand-free water.

b. Stock iodine solution: Prepare a saturated iodine solution by dissolving 20 g elemental iodine in 300 mL water. Let stand several hours. Decant iodine solution and dilute 170 mL to 2000 mL. Standardize solution by titrating with standard sodium thiosulfate ($Na_2S_2O_3$) titrant as described in Section 4500-Cl.B.3*b* and *c* or amperometrically as in Section 4500-I.C.

Calculate iodine concentration:

$$\text{mg I as } I_2\text{/mL} = \text{normality of iodine solution} \times 126.9$$

Prepare a working solution of 10 μg I as I_2/mL by appropriate dilution of the standardized

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stock solution.

c. Citric buffer solution, pH 3.8: See Section 4500-I^-.B.3*c*.

d. Leuco crystal violet indicator: See Section 4500-I^-.B.3*d*.

e. Sodium thiosulfate solution: See Section 4500-I^-.B.3*f*.

4. Procedure

a. Preparation of temporary iodine standards: For greater accuracy, standardize working solution immediately before use by the amperometric titration method (Method C). Prepare standards in the range of 0.1 to 6.0 mg I as I_2/L by adding 1 to 60 mL working solution to 100 mL glass-stoppered volumetric flasks, in increments of 1 mL or larger. Adjust these volumes if the measured iodine concentration of working solution varies by 5% or more from 10 µg I as I_2/mL.

Measure 50.0 mL of each diluted iodine working solution into a 100-mL glass-stoppered volumetric flask. Add 1.0 mL citric buffer solution, gently swirl to mix, and let stand for at least 30 s. Add 1.0 mL leuco crystal violet indicator and swirl to develop color. Dilute to 100 mL and mix.

b. Photometric calibration: Transfer colored temporary standards of known iodine concentrations to cells of 1-cm light path and read absorbance in a photometer or spectrophotometer at a wavelength of 592 nm against a distilled water reference. Plot absorbance values against iodine concentrations to construct a curve that follows Beer's law.

c. Color development of iodine sample: Measure 50.0 mL sample into a 100-mL volumetric flask and treat as described for preparation of temporary iodine standards, ¶ 4*a*. Match test sample visually with temporary standards or read absorbance photometrically and refer to standard calibration curve for the iodine equivalent.

d. Samples containing >6.0mg I as I_2/L: Place approximately 25mL water in a 100-mL volumetric flask. Add 1.0mL citric buffer solution and a measured volume of 25mL or less of sample. Mix and let stand for at least 30s. Add 1.0mL leuco crystal violet indicator, mix, and dilute to mark. Match visually with standards or read absorbance photometrically and compare with calibration curve from which the initial iodine is obtained by applying the dilution factor. Select one of the following sample volumes to remain within optimum iodine range:

Iodine *mg/L*	**Sample Volume Required** *mL*
6.0–12.0	25.0
12.0–30	10.0
30–60	5.0

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e. Samples containing both chlorine and iodine: For samples containing free or combined chlorine and iodine, follow procedure given in ¶ 4*c* or *d* above but read absorbance within 5 min after adding leuco crystal violet indicator.

f. Compensation for turbidity and color: Compensate for natural color or turbidity by adding 5 mL $Na_2S_2O_3$ solution to a 50-mL sample. Add reagents to sample as described previously and use as blank to set zero absorbance on the photometer. Measure all samples in relation to this blank and, from calibration curve, determine concentrations of iodine.

5. Bibliography

BLACK, A.P. & G.P. WHITTLE. 1967. New methods for the colorimetric determination of halogen residuals. Part I. Iodine, iodide, and iodate. *J. Amer. Water Works Assoc*. 59:471.

4500-I C. Amperometric Titration Method

1. General Discussion

The amperometric titration method for iodine is a modification of the amperometric method for residual chlorine (see Section 4500-Cl.D). Iodine residuals over 7 mg/L are best measured with smaller samples or by dilution. In most cases the titration results represent free iodine because combined iodine rarely is encountered.

a. Principle: The principle of the amperometric method as described for the determination of total residual chlorine is applicable to the determination of residual iodine. Iodine is determined using buffer solution, pH 4.0, and potassium iodide (KI) solution. Maintain pH at 4.0 because at pH values less than 3.5 substances such as oxidized forms of manganese interfere, while at pH values greater than 4.5, the reaction is not quantitative. Adding KI improves the sharpness of the end point.

b. Interference: Free chlorine and the interferences described in Section 4500-Cl.D.1*b* also interfere in the iodine determination.

2. Apparatus

See Section 4500-Cl.D.2*a* through *d*.

3. Reagents

With the exception of phosphate buffer solution, pH 7.0, all reagents listed for the determination of residual chlorine in Section 4500-Cl.D.3 are required. Standardized phenylarsine oxide solution (1 mL = 1 mg chlorine/L for a 200-mL sample) is equivalent to 3.58 mg I as I_2/mL for a 200-mL sample.

4. Procedure

a. Sample volume: Select a sample volume that will require no more than 2 mL phenylarsine

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oxide titrant. For iodine concentrations of 7 mg/L or less, take a 200-mL volume; for iodine levels above 7 mg/L, use 100 mL or proportionately less diluted to 200 mL with water.

b. Free iodine: To the sample add 1 mL KI solution and 1 mL acetate buffer, pH 4.0 solution. Titrate with phenylarsine oxide titrant to the end point described in Section 4500-Cl.D.4.

5. Calculation

Calculate the iodine concentration by the following equation:

$$\text{mg I as } I_2\text{/L} = \frac{A \times 3.58 \times 200}{\text{mL sample}}$$

where:

A = mL phenylarsine oxide titration to the end point.

6. Bibliography

MARKS, H.C. & J.R. GLASS. 1942. A new method of determining residual chlorine. *J. Amer. Water Works Assoc*. 34:1227.

4500-I⁻ IODIDE*#(46)

4500-I⁻ A. Introduction

1. Occurrence

Iodide is found in natural waters at concentrations ranging from 40 µg I^-/L in coastal surface seawater to <1 µg I^-/L in deep ocean water and fresh water. Higher concentrations may be found in brines, certain industrial wastes, and waters treated with iodine. Iodide is thermodynamically unstable relative to iodate in oxygenated waters.

2. Selection of Method

The leuco crystal violet method (B) is applicable to iodide concentrations of 50 to 6000 µg/L. The catalytic reduction method (C) is applicable to iodide concentrations of 80 µg I^-/L or less. The voltammetric method (D) is the most sensitive method. It can be used for samples with iodide concentrations of 0.13 to 10.2 µg I^-/L. It is also species-specific. It is insensitive to iodate, iodine, and most organic iodine compounds. It requires minimal sample manipulation, aside from an occasional dilution for samples with high concentrations of iodide. Thus, the concentrations of iodide in many types of water samples may be determined directly with the voltammetric method.

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The choice of method depends on the sample and concentration to be determined. The high chloride concentrations of brines, seawater, and many estuarine waters will interfere with color development in the leuco crystal violet method. In the presence of iodine, the leuco crystal violet method gives the sum of iodine and iodide. Iodide may be determined by the difference after concentration of iodine has been estimated independently (see Section 4500-I). In the catalytic reduction method, As(III), under acidic conditions, is a strong reducing agent and will reduce the oxidized forms of iodine to iodide. Thus, this method measures not only iodide, but also the sum of all the inorganic iodine species including iodide, iodate, hypoiodous acid, hypoiodite ion, and elemental iodine. Because iodate is the thermodynamically stable form of dissolved iodine in oxygenated natural waters and is frequently the dominant species of dissolved iodine, the catalytic reduction method is likely to overestimate the concentration of iodide. This method works well only under exactly reproducible conditions.

4500-I⁻ B. Leuco Crystal Violet Method

1. General Discussion

a. Principle: Iodide is selectively oxidized to iodine by the addition of potassium peroxymonosulfate, $KHSO_5$. The iodine produced reacts instantaneously with the colorless indicator reagent containing 4,4′,4″-methylidynetris (*N,N*-dimethylaniline), also known as leuco crystal violet, to produce the highly colored crystal violet dye. The developed color is sufficiently stable for the determination of an absorbance value and adheres to Beer's law over a wide range of iodine concentrations. Absorbance is highly pH-dependent, and must be measured within the pH range of 3.5 to 4.0 at a wavelength of 592 nm. Accurate control of pH is essential for maximum precision. (See Section 4500-I.B.1*a*.) Follow the general principles for quality control (Section 4020).

b. Interference: Chloride concentrations greater than 200 mg/ L may interfere with color development. Reduce these interferences by diluting sample to contain less than 200 mg Cl^-/L.

2. Apparatus

a. Colorimetric equipment: One of the following is required:

1) *Filter photometer,* providing a light path of 1 cm or longer, equipped with an orange filter having maximum transmittance near 592 nm.

2) *Spectrophotometer*, for use at 592 nm, providing a light path of 1 cm or longer.

b. Volumetric flasks: 100-mL with plastic caps or ground-glass stoppers.

c. Glassware: Completely remove any reducing substances from all glassware or plastic containers, including containers for storing reagent solutions (see Section 4500-Cl.D.2*d*).

3. Reagents

a. Iodine-demand-free water: Prepare a 1-m ion-exchange column of 2.5 to 5 cm diam,

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containing strongly acid cation and strong basic anion exchange resins. If a commercial analytical-grade mixed-bed resin is used, verify that compounds that react with iodine are removed. Pass distilled water at a slow rate through the resin bed and collect in clean container that will protect the treated water from undue exposure to the atmosphere.

Prepare all stock iodide and reagent solutions with iodine-demand-free water.

b. Stock iodide solution: Dissolve 1.3081 g KI in water and dilute to 1000 mL; 1 mL = 1 mg I^-.

c. Citric buffer solution, pH 3.8:

1) *Citric acid:* Dissolve 192.2 g $C_6H_8O_7$ or 210.2 g $C_6H_8O_7 \cdot H_2O$ and dilute to 1 L with water.

2) *Ammonium hydroxide,* 2*N*: Add 131 mL conc NH_4OH to about 700 mL water and dilute to 1 L. Store in a polyethylene bottle.

3) *Final buffer solution*: Slowly add, with mixing, 350 mL 2*N* NH_4OH solution to 670 mL citric acid. Add 80 g ammonium dihydrogen phosphate ($NH_4H_2PO_4$) and stir to dissolve.

d. Leuco crystal violet indicator: Measure 200 mL water and 3.2 mL conc sulfuric acid (H_2SO_4) into a brown glass container of at least 1-L capacity. Introduce a magnetic stirring bar and mix at moderate speed. Add 1.5 g 4,4′,4″-methylidynetris (*N,N*-dimethylaniline)*#(47) and with a small amount of water wash down any reagent adhering to neck or sides of container. Mix until dissolved.

To 800 mL water, add 2.5 g mercuric chloride ($HgCl_2$) and stir to dissolve. With mixing, add $HgCl_2$ solution to leuco crystal violet solution. For maximum stability, adjust pH of final solution to 1.5 or less, adding, if necessary, conc H_2SO_4 dropwise. Store in a brown glass bottle away from direct sunlight. Discard after 6 months. Do not use a rubber stopper.

e. Potassium peroxymonosulfate solution: Obtain $KHSO_5$ as a commercial product,†#(48) which is a stable powdered mixture containing 42.8% $KHSO_5$ by weight and a mixture of $KHSO_4$ and K_2SO_4. Dissolve 1.5 g powder in water and dilute to 1 L.

f. Sodium thiosulfate solution: Dissolve 5.0 g $Na_2S_2O_3 \cdot H_2O$ in water and dilute to 1 L.

4. Procedure

a. Preparation of temporary iodine standards: Add suitable portions of stock iodide solution, or of dilutions of stock iodide solution, to water to prepare a series of 0.1 to 6.0 mg I^-/L in increments of 0.1 mg/L or larger.

Measure 50.0 mL dilute KI standard solution into a 100-mL glass-stoppered volumetric flask. Add 1.0 mL citric buffer and 0.5 mL $KHSO_5$ solution. Swirl to mix and let stand approximately 1 min. Add 1.0 mL leuco crystal violet indicator, mix, and dilute to 100 mL. For best results, read absorbance as described below within 5 min after adding indicator solution.

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b. Photometric calibration: Transfer colored temporary standards of known iodide concentrations to cells of 1-cm light path and read absorbance in a photometer or spectrophotometer at a wavelength of 592 nm against a water reference. Plot absorbance values against iodide concentrations to construct a curve that follows Beer's law.

c. Color development of sample: Measure a 50.0-mL sample into a 100-mL volumetric flask and treat as described for preparation of temporary iodine standards, ¶ 4*a*. Read absorbance photometrically and refer to standard calibration curve for iodide equivalent.

d. Samples containing >6.0mg I^-*/L*: Place approximately 25mL water in a 100-mL volumetric flask. Add 1.0mL citric buffer and a measured volume of 25mL or less of sample. Add 0.5mL $KHSO_5$ solution. Swirl to mix and let stand approximately 1min. Add 1.0mL leuco crystal violet indicator, mix, and dilute to 100mL.

Read absorbance photometrically and compare with calibration curve from which the initial iodide concentration is obtained by applying the dilution factor. Select one of the following sample volumes to remain within the optimum iodide range.

Iodide *mg/L*	Sample Volume Required *mL*
6.0–12.0	25.0
12.0–30	10.0
30–60	5.0

e. Determination of iodide in the presence of iodine: On separate samples determine (1) total iodide and iodine, and (2) iodine. The iodide concentration is the difference between the iodine determined and the total iodine-iodide obtained. Determine iodine by not adding $KHSO_5$ solution in the iodide method and by comparing the absorbance value to the calibration curve developed for iodide.

f. Compensation for turbidity and color: Compensate for natural color or turbidity by adding 5 mL $Na_2S_2O_3$ solution to a 50-mL sample. Add reagents to sample as described previously and use as the blank to set zero absorbance on photometer. Measure all samples in relation to this blank and, from the calibration curve, determine concentrations of iodide or total iodine-iodide.

5. Bibliography

BLACK, A.P. & G.P. WHITTLE. 1967. New methods for the colorimetric determination of halogen residuals. Part I. Iodine, iodide, and iodate. *J. Amer. Water Works Assoc*. 59:471.

4500-I^- C. Catalytic Reduction Method

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1. General Discussion

a. Principle: Iodide can be determined by using its ability to catalyze the reduction of ceric ions by arsenious acid. The effect is nonlinearly proportional to the amount of iodide present. The reaction is stopped after a specific time interval by the addition of ferrous ammonium sulfate. The resulting ferric ions are directly proportional to the remaining ceric ions and develop a relatively stable color complex with potassium thiocyanate.

Pretreatment by digestion with chromic acid and distillation is necessary to estimate the nonsusceptible bound forms of iodine.

b. Interferences: The formation of noncatalytic forms of iodine and the inhibitory effects of silver and mercury are reduced by adding an excess of sodium chloride (NaCl) that sensitizes the reaction. Iodate, hypoiodous acid/hypoiodite ion, and elemental iodine interfere. Under acidic conditions, As(III) may reduce these forms of inorganic iodine to iodide and include them as iodide in the subsequent detection of iodide.

2. Apparatus

a. Water bath, capable of temperature control to 30 ± 0.5°C.

b. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer,* for use at wavelengths of 510 or 525 nm and providing a light path of 1 cm.

2) *Filter photometer,* providing a light path of 1 cm and equipped with a green filter having maximum transmittance near 525 nm.

c. Test tubes, 2 × 15 cm.

d. Stopwatch.

3. Reagents

Store all stock solutions in tightly stoppered containers in the dark. Prepare all reagent solutions in distilled water.

a. Distilled water, containing less than 0.3 µg total I/L.

b. Sodium chloride solution: Dissolve 200.0 g NaCl in water and dilute to 1 L. Recrystallize the NaCl if an interfering amount of iodine is present, using a water-ethanol mixture.

c. Arsenious acid: Dissolve 4.946 g As_2O_3 in water, add 0.20 mL conc H_2SO_4, and dilute to 1000 mL.

d. Sulfuric acid, H_2SO_4, conc.

e. Ceric ammonium sulfate: Dissolve 13.38 g $Ce(NH_4)_4(SO_4)_4 \cdot 4H_2O$ in water, add 44 mL conc H_2SO_4, and make up to 1 L.

f. Ferrous ammonium sulfate reagent: Dissolve 1.50 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in 100 mL

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distilled water containing 0.6 mL conc H_2SO_4. Prepare daily.

g. Potassium thiocyanate solution: Dissolve 4.00 g KSCN in 100 mL water.

h. Stock iodide solution: Dissolve 261.6 mg anhydrous KI in water and dilute to 1000 mL; 1.00 mL = 200 µg I^-.

i. Intermediate iodide solution: Dilute 20.00 mL stock iodide solution to 1000 mL with water; 1.00 mL = 4.00 µg I^-.

j. Standard iodide solution: Dilute 25.00 mL intermediate iodide solution to 1000 mL with water; 1.00 mL = 0.100 µg I^-.

4. Procedure

a. Sample size: Add 10.00 mL sample, or a portion made up to 10.00 mL with water, to a 2- × 15-cm test tube. If possible, keep iodide content in the range 0.2 to 0.6 µg. Use thoroughly clean glassware and apparatus.

b. Color measurement: Add reagents in the following order: 1.00 mL NaCl solution, 0.50 mL As_2O_3 solution, and 0.50 mL conc H_2SO_4.

Place reaction mixture and ceric ammonium sulfate solution in 30°C water bath and let come to temperature equilibrium. Add 1.0 mL ceric ammonium sulfate solution, mix by inversion, and start stopwatch to time reaction. Use an inert clean test tube stopper when mixing. After 15 ± 0.1 min remove sample from water bath and add immediately 1.00 mL ferrous ammonium sulfate reagent with mixing, whereupon the yellow ceric ion color should disappear. Then add, with mixing, 1.00 mL KSCN solution. Replace sample in water bath. Within 1 h after adding thiocyanate read absorbance in a photometric instrument. Maintain temperature of solution and cell compartment at 30 ± 0.5°C until absorbance is determined. If several samples are run, start reactions at 1-min intervals to allow time for additions of ferrous ammonium sulfate and thiocyanate. (If temperature control of cell compartment is not possible, let final solution come to room temperature and measure absorbance with cell compartment at room temperature.)

c. Calibration standards: Treat standards containing 0, 0.2, 0.4, 0.6, and 0.8 µg I^-/10.00 mL of solution as in ¶ 4*b* above. Run with each set of samples to establish a calibration curve.

5. Calculation

$$\text{mg } I^-/L = \frac{\mu g \text{ I (in 15 mL final volume)}}{\text{mL sample}}$$

6. Precision and Bias

Results obtained by this method are reproducible on samples of Los Angeles source waters, and have been reported to be accurate to ±0.3 µg I^-/L on samples of Yugoslavian water

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containing from 0 to 14.0 µg I^-/L. Follow general principles for quality control (Section 4020).

7. Bibliography

ROGINA, B. & M. DUBRAVCIC. 1953. Microdetermination of iodides by arresting the catalytic reduction of ceric ions. *Analyst* 78:594.

DUBRAVCIC, M. 1955. Determination of iodine in natural waters (sodium chloride as a reagent in the catalytic reduction of ceric ions). *Analyst* 80:295.

4500-I^- D. Voltammetric Method

1. General Discussion

a. Principle: Iodide is deposited onto the surface of a static mercury drop electrode (SMDE) as mercurous iodide under an applied potential for a specified period of time. The deposited mercurous iodide is reduced by a cathodic potential scan. This reaction gives rise to a current peak at about −0.33 V relative to the saturated calomel electrode. The height of the current peak is directly proportional to the concentration of iodide in solution, which is quantified by the method of internal standard additions.

b. Interferences: Sulfide can interfere. Remove it as hydrogen sulfide by acidifying the sample and then purging it with air. Adjust pH of sample back to about pH 8 before analysis.

2. Apparatus

a. Voltammetric analyzer system, consisting of a potentiostat, static mercury drop electrode (SMDE), stirrer, and plotter, that can be operated in the cathodic stripping square wave voltammetry-SMDE mode with adjustable deposition potential, deposition time, equilibration time, scan rate, scan range, scan increment, pulse height, frequency, and drop size.

A saturated calomel electrode is used as the reference electrode through a salt bridge.

b. Glassware: Wash glassware and other surfaces contacting the sample or reagents with 10% (v/v) HCl (low in iodide); thoroughly rinse with reagent water (see Section 1080) before use.

3. Reagents

Use chemicals low in iodide whenever available.

a. Oxygen-free water: Remove oxygen in reagent water (see Section 1080) by bubbling it with argon gas while boiling it for 20 min in an erlenmeyer flask. Let water cool while argon bubbling continues. Tightly stopper flask and store water under nitrogen. Prepare water immediately before use.

b. Alkaline pyrogallol solution: Dissolve 30 g pyrogallol in 200 mL oxygen-free water. Dissolve 120 g potassium hydroxide (KOH) in 400 mL oxygen-free water. Mix 300 mL KOH solution with 100 mL pyrogallol solution.

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c. Sodium sulfite solution, 1*M:* Dissolve 1.26 g sodium sulfite, Na_2SO_3, in oxygen-free water and dilute to 10 mL.

d. Sodium sulfite solution, 0.1*M:* Dilute 5.0 mL 1*M* sodium sulfite solution to 50 mL. Prepare fresh daily.

e. Oxygen-free argon gas: Bubble argon gas (at least 99.99% pure) through a series of three traps containing, respectively, alkaline pyrogallol solution, 0.1*M* sodium sulfite solution, and oxygen-free water.

f. Standard iodide solution: Dry several grams potassium iodide, KI, in an oven at 80°C overnight. Dissolve 1.660 g dried KI in reagent water (see Section 1080) and dilute to 500 mL. Dilute 5 mL solution to 500 mL, and dilute 5 mL of the latter solution to 500 mL.

g. Polyethylene glycol p-*isooctylphenyl ether (PEG-IOPE) solution,* 0.2%: Dilute 0.2 mL commercially available reagent*#(49) to 100 mL in reagent water (see Section 1080).

4. Procedure

a. Sample measurement: Transfer 10 mL sample, 0.05 mL PEG-IOPE solution, and 0.2 mL 1*M* Na_2SO_3 solution (which also acts as the supporting electrolyte in fresh-water samples) to polarographic cell containing a magnetic stirrer. Purge solution with oxygen-free argon gas for 1 min. Set electrode at SMDE mode. Record a voltammogram in the cathodic stripping square wave voltammetry mode under the following conditions: deposition potential, –0.15 V; deposition time, 60 s; equilibration time, 5 s; scan rate, 200 mV/s; scan range, 0.15 to –0.6 V; scan increment, 2 mV; pulse height, 20 mV; frequency, 100 Hz; and the largest drop size. Measure magnitude of current peak above baseline at center of peak at an applied potential of about –0.33 V relative to saturated calomel electrode in the voltammogram.

b. Internal standard additions: Add 0.1 mL 2 µ*M* standard KI solution to the cell. Purge solution with oxygen-free argon gas for 0.5 min. Record a voltammogram under conditions described in ¶ 4*a* and again determine magnitude of current peak. Repeat procedure twice, for a total of three additions.

c. Blank determination: Determine method reagent blank by treating reagent water as a sample.

5. Calculation

For the *j*th addition of the standard KI (j = 0, 1, 2, 3), compute the following variables:

$$Y_j = I_j (V_x + jV_s + V_c)$$

$$X_j = jV_s C_s$$

where:

I_j = height of *j*th peak, nA,

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V_x = sample volume, mL,

V_s = volume of standard KI added during each internal addition, mL,

V_c = total volume of PEG-IOPE solution and sodium sulfite added during analysis, mL, and

C_s = concentration of iodide in standard KI solution, n*M*.

Determine slope, *B*, and intercept, *A*, of line relating Y_j to X_j by linear least squares method. Calculate concentration of iodide in sample as:

$$C_x = \frac{A}{B \times V_x}$$

where:

C_x = concentration of iodide, n*M*, and other terms are as defined above.

If there is a reagent blank, subtract the reagent blank from C_x to get true concentration in sample.

Multiply C_x (or blank-corrected C_x) by 0.1269 to obtain concentration in µg/L.

6. Precision

In one laboratory, using seawater samples with a concentration of iodide of about 6 µg I/L, the precision was about ±5%. Follow general principles of quality control as in Section 4020.

7. Bibliography

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4500-IO_3^- IODATE*#(50)

4500-IO_3^- A. Introduction

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1. Occurrence

Iodate is found in natural waters at concentrations ranging from 60 µg I/L in deep ocean water to undetectable (3 µg I/L) in estuarine water and fresh water. Iodate is the thermodynamically stable form of dissolved inorganic iodine in waters containing dissolved oxygen; it is absent in anoxic waters.

2. Selection of Method

The differential pulse polarographic method is species-specific and highly sensitive. It is applicable to iodate concentrations of 3 to at least 130 µg I/L and can determine iodate in the presence of other iodine species such as iodide and organic iodine. It can be used for the direct determination of iodate in many types of water samples.

3. Sampling and Storage

Collect representative samples in clean glass or plastic bottles. Clean sample bottles with 10% (v/v) hydrochloric acid (low in iodate) and thoroughly rinse them with reagent water (see Section 1080) before use. Most samples can be analyzed directly without further treatment. Highly turbid samples may be filtered through glass fiber filters before analysis. For storage of up to 2 d, refrigerate sample at 4°C. For longer storage, freeze sample and store at or below −5°C. Frozen samples can be stored for at least 1 month.

4500-IO_3^- B. Polarographic Method

1. General Discussion

a. Principle: Under mildly basic conditions, iodate is reduced to iodide at a dropping mercury electrode by a cathodic potential scan. This reaction gives rise to a current peak centered around −1.1 V relative to the saturated calomel electrode. The height of the current peak is directly proportional to the concentration of iodate, which is quantified by the method of standard additions.

b. Interferences: Dissolved oxygen and zinc interfere. Remove dissolved oxygen by bubbling oxygen-free argon gas through sample and by reacting oxygen with added sodium sulfite. Remove interference from zinc by complexing with EDTA (ethylene diaminetetraacetate).

2. Apparatus

a. Polarographic analyzer system: A polarographic analyzer system, consisting of a potentiostat, a static mercury drop electrode (SMDE), a stirrer, and a plotter, that can be operated in the difference pulse polarography-SMDE mode with adjustable drop time, scan increment, pulse height, scan range, and drop size.

Use a saturated calomel electrode as the reference electrode.

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b. Glassware: Acid-wash glassware and other surfaces contacting sample or reagents with 10% (v/v) HCl (low in iodate); thoroughly rinse with reagent water (see Section 1080) before use.

3. Reagents

Use chemicals low in iodate whenever available.

a. Oxygen-free water: See Section 4500-I^-.D.3*a*.

b. Alkaline pyrogallol solution: See Section 4500-I^-.D.3*b*.

c. Oxygen-free argon gas: See Section 4500-I^-.D.3*e*.

d. Sodium sulfite solution: See Section 4500-I^-.D.3*c*.

e. Standard iodate solution, 25 *µM:* Dry several grams potassium iodate, KIO_3, in an oven at 80°C overnight. Dissolve 1.070 g dried KIO_3 in reagent water (see Section 1080) and dilute to 1000 mL. Dilute 5 mL of this solution to 1000 mL.

*f. Na_2*EDTA solution, 0.1*M:* Dissolve 3.722 g $Na_2EDTA \cdot 2H_2O$ (disodium ethylenediaminetetraacetate) in reagent water (see Section 1080) and dilute to 100 mL.

g. Supporting electrolyte: Dissolve 54.8 g sodium chloride, 0.30 g potassium bromide, and 1.05 g sodium bicarbonate in reagent water (see Section 1080) to form a final volume of 250 mL.

4. Procedure

a. Sample measurement: Transfer 5 mL sample and 0.5 mL supporting electrolyte to polarographic cell containing a magnetic stirrer. Check pH of solution to make sure it is about 8. (For marine waters with salinities above 15, the supporting electrolyte is not needed.) Remove dissolved oxygen by bubbling sample rigorously with oxygen-free argon gas for 0.5 min with stirring. Add 0.1 mL 1*M* sodium sulfite solution to sample and purge, with stirring, with oxygen-free argon gas for one additional minute. Add 0.01 mL 0.1*M* disodium EDTA and purge, with stirring, with oxygen-free argon for another 0.5 min. Set electrode in the SMDE mode. Record a polarogram in the differential pulse polarography mode under the following conditions: drop time, 1 s; scan increment, 6 mV; pulse height, 0.06 V; and scan range, −0.65 to −1.35 V. Use a medium drop size that allows mercury droplets to be formed and dislodged from the dropping mercury electrode at a steady and consistent rate. Measure height of current peak above base line at an applied potential of about −1.1 V relative to the saturated calomel electrode.

b. Internal standard additions: Add 0.05 mL 25 µ*M* standard iodate solution to cell. Purge solution, with stirring, with oxygen-free argon gas for 0.5 min.

Record a polarogram under conditions described in ¶ 4*a* and determine height of current peak again. Repeat this procedure two additional times.

c. Blank determination: Determine method reagent blank by treating reagent water as a sample.

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5. Calculation

Follow calculations given in Section 4500-I^-.D.5, with substitution of appropriate compounds in the definitions of terms.

6. Precision

In one laboratory, analyzing seawater samples with a concentration of iodate of 60 µg I/L, the precision was about ±3%. Follow general principles for quality control (see Section 4020).

7. Bibliography

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4500-N NITROGEN*#(51)

4500-N A. Introduction

In waters and wastewaters the forms of nitrogen of greatest interest are, in order of decreasing oxidation state, nitrate, nitrite, ammonia, and organic nitrogen. All these forms of nitrogen, as well as nitrogen gas (N_2), are biochemically interconvertible and are components of the nitrogen cycle. They are of interest for many reasons.

Organic nitrogen is defined functionally as organically bound nitrogen in the trinegative oxidation state. It does not include all organic nitrogen compounds. Analytically, organic nitrogen and ammonia can be determined together and have been referred to as ‘‘kjeldahl nitrogen,’’ a term that reflects the technique used in their determination. Organic nitrogen includes such natural materials as proteins and peptides, nucleic acids and urea, and numerous synthetic organic materials. Typical organic nitrogen concentrations vary from a few hundred micrograms per liter in some lakes to more than 20 mg/L in raw sewage.

Total oxidized nitrogen is the sum of nitrate and nitrite nitrogen. Nitrate generally occurs in trace quantities in surface water but may attain high levels in some groundwater. In excessive amounts, it contributes to the illness known as methemoglobinemia in infants. A limit of 10 mg nitrate as nitrogen/L has been imposed on drinking water to prevent this disorder. Nitrate is found only in small amounts in fresh domestic wastewater but in the effluent of nitrifying biological treatment plants nitrate may be found in concentrations of up to 30 mg nitrate as

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nitrogen/ L. It is an essential nutrient for many photosynthetic autotrophs and in some cases has been identified as the growth-limiting nutrient.

Nitrite is an intermediate oxidation state of nitrogen, both in the oxidation of ammonia to nitrate and in the reduction of nitrate. Such oxidation and reduction may occur in wastewater treatment plants, water distribution systems, and natural waters. Nitrite can enter a water supply system through its use as a corrosion inhibitor in industrial process water. Nitrite is the actual etiologic agent of methemoglobinemia. Nitrous acid, which is formed from nitrite in acidic solution, can react with secondary amines (RR′NH) to form nitrosamines (RR′N-NO), many of which are known to be carcinogens. The toxicologic significance of nitrosation reactions in vivo and in the natural environment is the subject of much current concern and research.

Ammonia is present naturally in surface and wastewaters. Its concentration generally is low in groundwaters because it adsorbs to soil particles and clays and is not leached readily from soils. It is produced largely by deamination of organic nitrogen-containing compounds and by hydrolysis of urea. At some water treatment plants ammonia is added to react with chlorine to form a combined chlorine residual. Ammonia concentrations encountered in water vary from less than 10 μg ammonia nitrogen/L in some natural surface and groundwaters to more than 30 mg/L in some wastewaters.

In this manual, organic nitrogen is referred to and reported as organic N, nitrate nitrogen as NO_3^--N, nitrite nitrogen as NO_2^--N, and ammonia nitrogen as NH_3-N.

Total nitrogen can be determined through oxidative digestion of all digestible nitrogen forms to nitrate, followed by quantitation of the nitrate. Two procedures, one using a persulfate/UV digestion (Section 4500-N.B), and the other using persulfate digestion (Section 4500-N.C) are presented. The procedures give good results for total nitrogen, composed of organic nitrogen (including some aromatic nitrogen-containing compounds), ammonia, nitrite, and nitrate. Molecular nitrogen is not determined and recovery of some industrial nitrogen-containing compounds is low.

Chloride ions do not interfere with persulfate oxidation, but the rate of reduction of nitrate to nitrite (during subsequent nitrate analysis by cadmium reduction) is significantly decreased by chlorides. Ammonium and nitrate ions adsorbed on suspended pure clay or silt particles should give a quantitative yield from persulfate digestion. If suspended matter remains after digestion, remove it before the reduction step.

If suspended organic matter is dissolved by the persulfate digestion reagent, yields comparable to those from true solutions are obtained; if it is not dissolved, the results are unreliable and probably reflect a negative interference. The persulfate method is not effective in wastes with high organic loadings. Dilute such samples and re-analyze until results from two dilutions agree.

4500-N B. In-Line UV/Persulfate Digestion and Oxidation with Flow Injection Analysis (PROPOSED)

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1. General Discussion

a. Principle: Nitrogen compounds are digested and oxidized in-line to nitrate by use of heated alkaline persulfate and ultraviolet radiation. The digested sample is injected onto the manifold where its nitrate is reduced to nitrite by a cadmium granule column. The nitrite then is determined by diazotization with sulfanilamide under acidic conditions to form a diazonium ion. The diazonium ion is coupled with *N*-(1-naphthyl)ethylenediamine dihydrochloride. The resulting pink dye absorbs at 540 nm and is proportional to total nitrogen.

This method recovers nearly all forms of organic and inorganic nitrogen, reduced and oxidized, including ammonia, nitrate, and nitrite. It differs from the total kjeldahl nitrogen method described in Section 4500-N_{org}.D, which does not recover the oxidized forms of nitrogen. This method recovers nitrogen components of biological origin such as amino acids, proteins, and peptides as ammonia, but may not recover the nitrogenous compounds of some industrial wastes such as amines, nitro-compounds, hydrazones, oximes, semicarbazones, and some refractory tertiary amines.

See Section 4500-N.A for a discussion of the various forms of nitrogen found in waters and wastewaters, Section 4500-N_{org}.A and Section 4500-N_{org}.B for a discussion of total nitrogen methods, and Section 4130, Flow Injection Analysis (FIA). Also see Section 4500-N.C for a similar, batch total nitrogen method that uses only persulfate.

b. Interferences: Remove large or fibrous particulates by filtering sample though glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

2. Apparatus

Flow injection analysis equipment consisting of:

a. FIA injection valve with sample loop or equivalent.

b. Multichannel proportioning pump.

c. FIA manifold (Figure 4500-N:1) with tubing heater, in-line ultraviolet digestion fluidics including a debubbler consisting of a gas-permeable TFE membrane and its holder, and flow cell. In Figure 4500-N:1, relative flow rates only are shown. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE. The block marked ‘‘UV’’ should consist of TFE tubing irradiated by a mercury discharge ultraviolet lamp emitting radiation at 254 nm.

d. Absorbance detector, 540 nm, 10-nm bandpass.

e. Injection valve control and data acquisition system.

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and for all solutions. To prevent bubble formation, degas carrier and all reagents with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing

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reagents by weight/weight, use weight/volume.

a. Borate solution, $Na_2B_4O_7 \cdot 10H_2O$: In a 1-L volumetric flask dissolve 38.0 g $Na_2B_4O_7 \cdot 10H_2O$ and 3.0 g sodium hydroxide, NaOH, in approximately 900 mL water, using a magnetic stirring bar. Gentle heating will speed dissolution. Adjust to pH 9.0 with NaOH or conc hydrochloric acid (HCl). Dilute to mark and invert to mix.

b. Persulfate solution, $K_2S_2O_8$: Potassium persulfate solid reagent usually contains nitrogen contamination. Higher contamination levels result in larger blank peaks.

To a tared 1-L container, add 975 g water and 49 g $K_2S_2O_8$. Add a magnetic stirring bar, dissolve persulfate, and dilute to mark. Invert to mix.

c. Ammonium chloride buffer: CAUTION: *Fumes. Use a hood.* To a 1-L volumetric flask add 500 mL water, 105 mL conc HCl, and 95 mL conc ammonium hydroxide, NH_4OH. Dissolve, dilute to mark, and invert to mix. Adjust to pH 8.5 with 1*N* HCl or 1*N* NaOH solution.

d. Sulfanilamide color reagent: To a tared, dark, 1-L container add 876 g water, 170 g 85% phosphoric acid, H_3PO_4, 40.0 g sulfanilamide, and 1.0 g *N*-(1-naphthyl)ethylenediamine dihydrochloride (NED). Shake to wet solids and stir for 30 min to dissolve. Store in a dark bottle and discard when solution turns dark pink.

e. Cadmium column: See Section 4500-NO_3^-.I.3*c*, d, and *e*.

f. Stock nitrate standard, 1000 mg N/L: In a 1-L volumetric flask dissolve 7.221 g potassium nitrate, KNO_3 (dried at 60°C for 1 h), or 4.93 g sodium nitrite, $NaNO_2$, in about 800 mL water. Dilute to mark and invert to mix. When refrigerated the standard may be stored for up to 3 months.

g. Standard solutions: Prepare nitrate standards in the desired concentration range, using stock nitrate standards (¶ 3*f*), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-N:1 and follow method supplied by manufacturer, or laboratory standard operating procedure for this method.

Carry both standards and samples through this procedure. If samples have been preserved with sulfuric acid, preserve standards similarly. Samples may be homogenized. Turbid samples may be filtered, since digestion effectiveness on nitrogen-containing particles is unknown; however, organic nitrogen may be lost in the filtration.

5. Calculation

Prepare standard curves by plotting absorbance of standards processed through the manifold versus nitrogen concentration. The calibration curve is linear.

Verify digestion efficiency by determining urea, glutamic acid, or nicotinic acid standards (Section 4500-N.C.3*d*) at regular intervals. In the concentration range of the method, the

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recovery of these compounds should be >95%.

6. Quality Control

See Section 4130B.

7. Precision and Bias

a. MDL: Using a 70-µL sample loop and a published MDL method,[1] analysts ran 21 replicates of a 0.20-mg N/L standard. These gave a mean of 0.18 mg N/L, a standard deviation of 0.008 mg N/L, and MDL of 0.020 mg N/L.

b. Precision study: Ten injections each of a 4.00-mg N/L standard and of a 10.0-mg N/L standard both gave a relative standard deviation of 0.6%.

c. Recovery of total nitrogen: Table 4500-N:I shows recoveries for various nitrogen compounds determined at 10 mg N/L and 4.0 mg N/L. All compounds were determined in triplicate.

d. Ammonia recoveries from wastewater treatment plant effluent with known additions: To a sample of wastewater treatment plant effluent, ammonium chloride was added at two concentrations, 2.50 and 5.00 mg N/L, and analyses were made in triplicate to give mean recoveries of 96% and 95%, respectively. A sample with no additions also was diluted twofold in triplicate to give a mean recovery of 99%.

8. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 Rev. 1.11 amended June 30, 1986. 49 CFR 43430, October 26, 1984.

4500-N C. Persulfate Method

1. General Discussion

The persulfate method determines total nitrogen by oxidation of all nitrogenous compounds to nitrate. Should ammonia, nitrate, and nitrite be determined individually, ‘‘organic nitrogen’’ can be obtained by difference.

a. Principle: Alkaline oxidation at 100 to 110°C converts organic and inorganic nitrogen to nitrate. Total nitrogen is determined by analyzing the nitrate in the digestate.

b. Selection of nitrate measurement method: Automated or manual cadmium reduction may be used to determine total nitrogen levels below 2.9 mg N/L. Results summarized in Table 4500-N:II were obtained using automated cadmium reduction.

2. Apparatus

a. Autoclave, or hotplate and pressure cooker capable of developing 100 to 110°C for 30

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min.

*b. Glass culture tubes:**#(52) 30-mL screw-capped (polypropylene linerless caps), 20 mm OD × 150 mm long. Clean before initial use by autoclaving with digestion reagent.

c. Apparatus for nitrate determination: See Section 4500-NO_3^-.E or Section 4500-NO_3^-.F.

d. Automated analytical equipment: An example of the continuous-flow analytical instrument consists of components shown in Figure 4500-NO_3^-:2.

3. Reagents

a. Ammonia-free and nitrate-free water: Prepare by ion exchange or distillation methods as directed in Section 4500-NH_3.B.3*a* and Section 4500-NO_3^-.B.3*a*.

b. Stock nitrate solution: Prepare as directed in Section 4500-NO_3^-.B.3*b*.

c. Intermediate nitrate solution: Prepare as directed in Section 4500-NO_3^-.B.3*c*.

d. Stock glutamic acid solution: Dry glutamic acid, $C_3H_5NH_2(COOH)_2$, in an oven at 105°C for 24 h. Dissolve 1.051 g in water and dilute to 1000 mL; 1.00 mL = 100 µg N. Preserve with 2 mL $CHCl_3$/L.

e. Intermediate glutamic acid solution: Dilute 100 mL stock glutamic acid solution to 1000 mL with water; 1.00 mL = 10.0 µg N. Preserve with 2 mL $CHCl_3$/L.

gc

f. Digestion reagent: Dissolve 20.1 g low nitrogen (<0.001% N) potassium persulfate, $K_2S_2O_8$, and 3.0 g NaOH in water and dilute to 1000 mL just before use.

g. Borate buffer solution: Dissolve 61.8 g boric acid, H_3BO_3, and 8.0 g NaOH in water and dilute to 1000 mL.

h. Copper sulfate solution: Dissolve 2.0 g $CuSO_4 \cdot 5H_2O$ in 90 mL water and dilute to 100 mL.

i. Ammonium chloride solution: Dissolve 10.0 g NH_4Cl in 1 L water. Adjust to pH 8.5 by adding three or four NaOH pellets as necessary or NaOH solution before bringing to volume. This reagent is stable for 2 weeks when refrigerated.

j. Color reagent: Combine 1500 mL water, 200.0 mL conc phosphoric acid, H_3PO_4, 20.0 g sulfanilamide, and 1.0 g *N*-(1-naphthyl)-ethylenediamine dihydrochloride. Dilute to 2000 mL. Add 2.0 mL polyoxyethylene 23 lauryl ether.†#(53) Store at 4°C in the dark. Prepare fresh reagent every 6 weeks. Alternatively, prepare proportionally smaller volumes to minimize waste.

4. Procedure

a. Calibration curve: Prepare NO_3^- calibration standards in the range 0 to 2.9 mg NO_3^--N/L by diluting to 100 mL the following volumes of intermediate nitrate solution: 0, 1.00, 2.00, 4.00 .

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. . 29.0 mL. Treat standards in the same manner as samples.

b. Digestion check standard: Prepare glutamic acid digestion check standard of 2.9 mg N/L by diluting, to 100 mL, a 29.0-mL volume of intermediate glutamic acid solution. Treat digestion check standard in the same manner as samples.

c. Digestion: Samples preserved with acid cannot be analyzed by this method. To a culture tube, add 10.0 mL sample or standard or a portion diluted to 10.0 mL. Add 5.0 mL digestion reagent. Cap tightly. Mix by inverting twice. Heat for 30 min in an autoclave or pressure cooker at 100 to 110°C. Slowly cool to room temperature. Add 1.0 mL borate buffer solution. Mix by inverting at least twice.

d. Blank: Carry a reagent blank through all steps of the procedure and apply necessary corrections to the results.

e. Nitrate measurement: Determine nitrate by cadmium reduction. Set up manifold as shown in Figure 4500-NO_3^-:2, but use reagents specified in Section 4500-N_{org}.C.3.

5. Calculation

Prepare the standard curve by plotting the absorbances or peak heights of the nitrate calibration standards carried through the digestion procedure against their nitrogen concentrations. Compute organic N sample concentration by comparing sample absorbance or peak height with the standard curve.

6. Precision and Bias

See Table 4500-N:II.

7. Bibliography

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4500-NH_3 NITROGEN (AMMONIA)*#(54)

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4500-NH_3 A. Introduction

1. Selection of Method

The two major factors that influence selection of the method to determine ammonia are concentration and presence of interferences. In general, direct manual determination of low concentrations of ammonia is confined to drinking waters, clean surface or groundwater, and good-quality nitrified wastewater effluent. In other instances, and where interferences are present or greater precision is necessary, a preliminary distillation step (B) is required.

A titrimetric method (C), an ammonia-selective electrode method (D), an ammonia-selective electrode method using known addition (E), a phenate method (F), and two automated versions of the phenate method (G and H) are presented. Methods D, E, F, G, and H may be used either with or without sample distillation. The data presented in Table 4500-NH_3:I and Table 4500-NH_3:III should be helpful in selecting the appropriate method of analysis.

Nesslerization has been dropped as a standard method, although it has been considered a classic water quality measurement for more than a century. The use of mercury in this test warrants its deletion because of the disposal problems.

The distillation and titration procedure is used especially for NH_3-N concentrations greater than 5 mg/L. Use boric acid as the absorbent following distillation if the distillate is to be titrated.

The ammonia-selective electrode method is applicable over the range from 0.03 to 1400 mg NH_3-N/L.

The manual phenate method is applicable to both fresh water and seawater and is linear to 0.6 mg NH_3-N/L. Distill into sulfuric acid (H_2SO_4) absorbent for the phentate method when interferences are present.

The automated phenate method is applicable over the range of 0.02 to 2.0 mg NH_3-N/L.

2. Interferences

Glycine, urea, glutamic acid, cyanates, and acetamide hydrolyze very slowly in solution on standing but, of these, only urea and cyanates will hydrolyze on distillation at pH of 9.5. Hydrolysis amounts to about 7% at this pH for urea and about 5% for cyanates. Volatile alkaline compounds such as hydrazine and amines will influence titrimetric results. Residual chlorine reacts with ammonia; remove by sample pretreatment. If a sample is likely to contain residual chlorine, immediately upon collection, treat with dechlorinating agent as in Section 4500-NH_3.B.3*d*.

3. Storage of Samples

Most reliable results are obtained on fresh samples. If samples are to be analyzed within 24 h

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of collection, refrigerate unacidified at 4°C. For preservation for up to 28 d, freeze at − 20°C unacidified, or preserve samples by acidifying to pH <2 and storing at 4°C. If acid preservation is used, neutralize samples with NaOH or KOH immediately before making the determination. CAUTION: Although acidification is suitable for certain types of samples, it produces interferences when exchangeable ammonium is present in unfiltered solids.

4. Bibliography

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4500-NH_3 B. Preliminary Distillation Step

1. General Discussion

The sample is buffered at pH 9.5 with a borate buffer to decrease hydrolysis of cyanates and organic nitrogen compounds. It is distilled into a solution of boric acid when titration is to be used or into H_2SO_4 when the phenate method is used. The ammonia in the distillate can be determined either colorimetrically by the phenate method or titrimetrically with standard H_2SO_4 and a mixed indicator or a pH meter. The choice between the colorimetric and the acidimetric methods depends on the concentration of ammonia. Ammonia in the distillate also can be determined by the ammonia-selective electrode method, using 0.04*N* H_2SO_4 to trap the ammonia.

2. Apparatus

a. Distillation apparatus: Arrange a borosilicate glass flask of 800- to 2000-mL capacity attached to a vertical condenser so that the outlet tip may be submerged below the surface of the receiving acid solution. Use an all-borosilicate-glass apparatus or one with condensing units constructed of block tin or aluminum tubes.

b. pH meter.

3. Reagents

a. Ammonia-free water: Prepare by ion-exchange or distillation methods:

1) Ion exchange—Prepare ammonia-free water by passing distilled water through an ion-exchange column containing a strongly acidic cation-exchange resin mixed with a strongly basic anion-exchange resin. Select resins that will remove organic compounds that interfere with the ammonia determination. Some anion-exchange resins tend to release ammonia. If this occurs, prepare ammonia-free water with a strongly acidic cation-exchange resin. Regenerate the column

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according to the manufacturer's instructions. Check ammonia-free water for the possibility of a high blank value.

2) Distillation—Eliminate traces of ammonia in distilled water by adding 0.1 mL conc H_2SO_4 to 1 L distilled water and redistilling. Alternatively, treat distilled water with sufficient bromine or chlorine water to produce a free halogen residual of 2 to 5 mg/ L and redistill after standing at least 1 h. Discard the first 100 mL distillate. Check redistilled water for the possibility of a high blank.

It is very difficult to store ammonia-free water in the laboratory without contamination from gaseous ammonia. However, if storage is necessary, store in a tightly stoppered glass container to which is added about 10 g ion-exchange resin (preferably a strongly acidic cation-exchange resin)/L ammonia-free water. For use, let resin settle and decant ammonia-free water. If a high blank value is produced, replace the resin or prepare fresh ammonia-free water.

Use ammonia-free distilled water for preparing all reagents, rinsing, and sample dilution.

b. Borate buffer solution: Add 88 mL 0.1*N* NaOH solution to 500 mL approximately 0.025*M* sodium tetraborate ($Na_2B_4O_7$) solution (9.5 g $Na_2B_4O_7 \cdot 10\ H_2O$/L) and dilute to 1 L.

c. Sodium hydroxide, 6*N*.

d. Dechlorinating reagent: Dissolve 3.5 g sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) in water and dilute to 1 L. Prepare fresh weekly. Use 1 mL reagent to remove 1 mg/L residual chlorine in 500-mL sample.

e. Neutralization agent.

1) *Sodium hydroxide,* NaOH, 1*N*.

2) *Sulfuric acid*, H_2SO_4, 1*N*.

f. Absorbent solution, plain boric acid: Dissolve 20 g H_3BO_3 in water and dilute to 1 L.

g. Indicating boric acid solution: See Section 4500-NH_3.C.3*a* and *b*.

h. Sulfuric acid, 0.04*N:* Dilute 1.0 mL conc H_2SO_4 to 1 L.

4. Procedure

a. Preparation of equipment: Add 500 mL water and 20 mL borate buffer, adjust pH to 9.5 with 6*N* NaOH solution, and add to a distillation flask. Add a few glass beads or boiling chips and use this mixture to steam out the distillation apparatus until distillate shows no traces of ammonia.

b. Sample preparation: Use 500 mL dechlorinated sample or a known portion diluted to 500 mL with water. When NH_3-N concentration is less than 100 μg/L, use a sample volume of 1000 mL. Remove residual chlorine by adding, at the time of collection, dechlorinating agent equivalent to the chlorine residual. If necessary, neutralize to approximately pH 7 with dilute acid or base, using a pH meter.

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Add 25 mL borate buffer solution and adjust to pH 9.5 with 6*N* NaOH using a pH meter.

c. Distillation: To minimize contamination, leave distillation apparatus assembled after steaming out and until just before starting sample distillation. Disconnect steaming-out flask and immediately transfer sample flask to distillation apparatus. Distill at a rate of 6 to 10 mL/min with the tip of the delivery tube below the surface of acid receiving solution. Collect distillate in a 500-mL erlenmeyer flask containing 50 mL indicating boric acid solution for titrimetric method. Distill ammonia into 50 mL 0.04*N* H_2SO_4 for the ammonia-selective electrode method and for the phenate method. Collect at least 200 mL distillate. Lower distillation receiver so that the end of the delivery tube is free of contact with the liquid and continue distillation during the last minute or two to cleanse condenser and delivery tube. Dilute to 500 mL with water.

When the phenate method is used for determining NH_3-N, neutralize distillate with 1*N* NaOH solution.

d. Ammonia determination: Determine ammonia by the titrimetric method (C), the ammonia-selective electrode methods (D and E), or the phenate methods (F and G).

5. Bibliography

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4500-NH_3 C. Titrimetric Method

1. General Discussion

The titrimetric method is used only on samples that have been carried through preliminary distillation (see Section 4500-NH_3.B). The following table is useful in selecting sample volume for the distillation and titration method.

Ammonia Nitrogen in Sample *mg/L*	Sample Volume *mL*

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Ammonia Nitrogen in Sample *mg/L*	Sample Volume *mL*
5–10	250
10–20	100
20–50	50.0
50–100	25.0

2. Apparatus

Distillation apparatus: See Section 4500-NH_3.B.2*a* and Section 4500-NH_3.B.2*b*.

3. Reagents

Use ammonia-free water in making all reagents and dilutions.

a. Mixed indicator solution: Dissolve 200 mg methyl red indicator in 100 mL 95% ethyl or isopropyl alcohol. Dissolve 100 mg methylene blue in 50 mL 95% ethyl or isopropyl alcohol. Combine solutions. Prepare monthly.

b. Indicating boric acid solution: Dissolve 20 g H_3BO_3 in water, add 10 mL mixed indicator solution, and dilute to 1 L. Prepare monthly.

c. Standard sulfuric acid titrant, 0.02*N:* Prepare and standardize as directed in Alkalinity, Section 2320B.3*c*. For greatest accuracy, standardize titrant against an amount of Na_2CO_3 that has been incorporated in the indicating boric acid solution to reproduce the actual conditions of sample titration; 1.00 mL = 14 × normality × 1000 µg N. (For 0.02*N*, 1.00 mL = 280 µg N.)

4. Procedure

a. Proceed as described in Section 4500-NH_3.B using indicating boric acid solution as absorbent for the distillate.

b. Sludge or sediment samples: Rapidly weigh to within ±1% an amount of wet sample, equivalent to approximately 1 g dry weight, in a weighing bottle or crucible. Wash sample into a 500-mL kjeldahl flask with water and dilute to 250 mL. Proceed as in ¶ 4*a* but add a piece of paraffin wax to distillation flask and collect only 100 mL distillate.

c. Titrate ammonia in distillate with standard 0.02*N* H_2SO_4 titrant until indicator turns a pale lavender.

d. Blank: Carry a blank through all steps of the procedure and apply the necessary correction to the results.

5. Calculation

a. Liquid samples:

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$$\text{mg NH}_3\text{-N/L} = \frac{(A - B) \times 280}{\text{mL sample}}$$

b. Sludge or sediment samples:

$$\text{mg NH}_3\text{-N/kg} = \frac{(A - B) \times 280}{\text{g dry wt sample}}$$

where:

A = volume of H_2SO_4 titrated for sample, mL, and
B = volume of H_2SO_4 titrated for blank, mL.

6. Precision and Bias

Three synthetic samples containing ammonia and other constituents dissolved in distilled water were distilled and analyzed by titration.

Sample 1 contained 200 µg NH_3-N/L, 10 mg Cl^-/L, 1.0 mg NO_3^--N/L, 1.5 mg organic N/L, 10.0 mg PO_4^{3-}/L, and 5.0 mg silica/L. The relative standard deviation and relative error for the 21 participating laboratories were 69.8% and 20%, respectively.

Sample 2 contained 800 µg NH_3-N/L, 200 mg Cl^-/L, 1.0 mg NO_3^--N/L, 0.8 mg organic N/L, 5.0 mg PO_4^{3-}/L, and 15.0 mg silica/L. The relative standard deviation and relative error for the 20 participating laboratories were 28.6% and 5%, respectively.

Sample 3 contained 1500 µg NH_3-N/L, 400 mg Cl^-/L, 1.0 mg NO_3^--N/L, 0.2 mg organic N/L, 0.5 mg PO_4^{3-}/L, and 30.0 mg silica/L. The relative standard deviation and relative error for the 21 participating laboratories were 21.6%, and 2.6%, respectively.

7. Bibliography

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4500-NH_3 D. Ammonia-Selective Electrode Method

1. General Discussion

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a. Principle: The ammonia-selective electrode uses a hydrophobic gas-permeable membrane to separate the sample solution from an electrode internal solution of ammonium chloride. Dissolved ammonia ($NH_{3(aq)}$ and NH_4^+) is converted to $NH_{3(aq)}$ by raising pH to above 11 with a strong base. $NH_{3(aq)}$ diffuses through the membrane and changes the internal solution pH that is sensed by a pH electrode. The fixed level of chloride in the internal solution is sensed by a chloride ion-selective electrode that serves as the reference electrode. Potentiometric measurements are made with a pH meter having an expanded millivolt scale or with a specific ion meter.

b. Scope and application: This method is applicable to the measurement of 0.03 to 1400 mg NH_3-N/L in potable and surface waters and domestic and industrial wastes. High concentrations of dissolved ions affect the measurement, but color and turbidity do not. Sample distillation is unnecessary. Use standard solutions and samples that have the same temperature and contain about the same total level of dissolved species. The ammonia-selective electrode responds slowly below 1 mg NH_3-N/L; hence, use longer times of electrode immersion (2 to 3 min) to obtain stable readings.

c. Interference: Amines are a positive interference. This may be enhanced by acidification. Mercury and silver interfere by complexing with ammonia, unless the NaOH/EDTA solution (3*c*) is used.

d. Sample preservation: Refrigerate at 4°C for samples to be analyzed within 24 h. Preserve samples high in organic and nitrogenous matter, and any other samples for longer storage, by lowering pH to 2 or less with conc H_2SO_4.

2. Apparatus

a. Electrometer: A pH meter with expanded millivolt scale capable of 0.1 mV resolution between −700 mV and +700 mV or a specific ion meter.

*b. Ammonia-selective electrode.**#(55)

c. Magnetic stirrer, thermally insulated, with TFE-coated stirring bar.

3. Reagents

a. Ammonia-free water: See Section 4500-NH_3.B.3*a*. Use for making all reagents.

b. Sodium hydroxide, 10*N*.

c. NaOH/EDTA solution, 10*N*: Dissolve 400 g NaOH in 800 mL water. Add 45.2 g ethylenediaminetetraacetic acid, tetrasodium salt, tetrahydrate ($Na_4EDTA \cdot 4\ H_2O$) and stir to dissolve. Cool and dilute to 1000 mL.

d. Stock ammonium chloride solution: Dissolve 3.819 g anhydrous NH_4Cl (dried at 100°C) in water, and dilute to 1000 mL; 1.00 mL = 1.00 mg N = 1.22 mg NH_3.

e. Standard ammonium chloride solutions: See ¶ 4*a* below.

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4. Procedure

a. Preparation of standards: Prepare a series of standard solutions covering the concentrations of 1000, 100, 10, 1, and 0.1 mg NH_3-N/L by making decimal dilutions of stock NH_4Cl solution with water.

b. Electrometer calibration: Place 100 mL of each standard solution in a 150-mL beaker. Immerse electrode in standard of lowest concentration and mix with a magnetic stirrer. Limit stirring speed to minimize possible loss of ammonia from the solution. Maintain the same stirring rate and a temperature of about 25°C throughout calibration and testing procedures. Add a sufficient volume of 10*N* NaOH solution (1 mL usually is sufficient) to raise pH above 11. If the presence of silver or mercury is possible, use NaOH/EDTA solution in place of NaOH solution. If it is necessary to add more than 1 mL of either NaOH or NaOH/ EDTA solution, note volume used, because it is required for subsequent calculations. Keep electrode in solution until a stable millivolt reading is obtained. Do not add NaOH solution before immersing electrode, because ammonia may be lost from a basic solution. Repeat procedure with remaining standards, proceeding from lowest to highest concentration. Wait until the reading has stablized (at least 2 to 3 min) before recording millivolts for standards and samples containing ≤ 1 mg NH_3-N/L.

c. Preparation of standard curve: Using semilogarithmic graph paper, plot ammonia concentration in milligrams NH_3-N per liter on the log axis vs. potential in millivolts on the linear axis starting with the lowest concentration at the bottom of the scale. If the electrode is functioning properly a tenfold change of NH_3-N concentration produces a potential change of about 59 mV.

d. Calibration of specific ion meter: Refer to manufacturer's instructions and proceed as in ¶s 4*a* and b.

e. Measurement of samples: Dilute if necessary to bring NH_3-N concentration to within calibration curve range. Place 100 mL sample in 150-mL beaker and follow procedure in ¶ 4*b* above. Record volume of 10*N* NaOH added. Read NH_3-N concentration from standard curve.

5. Calculation

$$\text{mg } NH_3\text{-N/L} = A \times B \times \left[\frac{100 + D}{100 + C}\right]$$

where:

A = dilution factor,
B = concentration of NH_3-N/L, mg/L, from calibration curve,
C = volume of 10*N* NaOH added to calibration standards, mL, and

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D = volume of 10N NaOH added to sample, mL.

6. Precision and Bias

For the ammonia-selective electrode in a single laboratory using surface water samples at concentrations of 1.00, 0.77, 0.19, and 0.13 mg NH_3-N/L, standard deviations were ±0.038, ±0.017, ±0.007, and ±0.003, respectively. In a single laboratory using surface water samples at concentrations of 0.10 and 0.13 mg NH_3-N/L, recoveries were 96% and 91%, respectively. The results of an interlaboratory study involving 12 laboratories using the ammonia-selective electrode on distilled water and effluents are summarized in Table 4500-NH3:I.

7. Bibliography

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4500-NH_3 E. Ammonia-Selective Electrode Method Using Known Addition

1. General Discussion

a. Principle: When a linear relationship exists between concentration and response, known addition is convenient for measuring occasional samples because no calibration is needed. Because an accurate measurement requires that the concentration at least double as a result of the addition, sample concentration must be known within a factor of three. Total concentration of ammonia can be measured in the absence of complexing agents down to 0.8 mg NH_3-N/L or in the presence of a large excess (50 to 100 times) of complexing agent. Known addition is a convenient check on the results of direct measurement.

b. See Section 4500-NH_3.D.1 for further discussion.

2. Apparatus

Use apparatus specified in Section 4500-NH_3.D.2.

3. Reagents

Use reagents specified in Section 4500-NH_3.D.3.

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Add standard ammonium chloride solution approximately 10 times as concentrated as samples being measured.

4. Procedure

a. Dilute 1000 mg/L stock solution to make a standard solution about 10 times as concentrated as the sample concentrate.

b. Add 1 mL 10*N* NaOH to each 100 mL sample and immediately immerse electrode. When checking a direct measurement, leave electrode in 100 mL of sample solution. Use magnetic stirring throughout. Measure mV reading and record as E_1.

c. Pipet 10 mL of standard solution into sample. Thoroughly stir and immediately record new mV reading as E_2.

5. Calculation

a. $\Delta E = E_1 - E_2$.

b. From Table 4500-NH_3:II find the concentration ratio, Q, corresponding to change in potential, ΔE. To determine original total sample concentration, multiply Q by the concentration of the added standard:

$$C_o = Q\,C_s$$

where:

C_o = total sample concentration, mg/L,
Q = reading from known-addition table, and
C_s = concentration of added standard, mg/L.

c. To check a direct measurement, compare results of the two methods. If they agree within ±4%, the measurements probably are good. If the known-addition result is much larger than the direct measurement, the sample may contain complexing agents.

6. Precision and Bias

In 38 water samples analyzed by both the phenate and the known-addition ammonia-selective electrode method, the electrode method yielded a mean recovery of 102% of the values obtained by the phenate method when the NH_3-N concentrations varied between 0.30 and 0.78 mg/L. In 57 wastewater samples similarly compared, the electrode method yielded a mean recovery of 108% of the values obtained by the phenate method using distillation when the NH_3-N concentrations varied between 10.2 and 34.7 mg N/L. In 20 instances in which two to four replicates of these samples were analyzed, the mean standard deviation was 1.32 mg N/L. In three measurements at a sewer outfall, distillation did not change statistically the value obtained by the electrode method. In 12 studies using standards in the 2.5- to 30-mg N/L range, average

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recovery by the phenate method was 97% and by the electrode method 101%.

4500-NH_3 F. Phenate Method

1. General Discussion

a. Principle: An intensely blue compound, indophenol, is formed by the reaction of ammonia, hypochlorite, and phenol catalyzed by sodium nitroprusside.

b. Interferences: Complexing magnesium and calcium with citrate eliminates interference produced by precipitation of these ions at high pH. There is no interference from other trivalent forms of nitrogen. Remove interfering turbidity by distillation orfiltration. If hydrogen sulfide is present, remove by acidifying samples to pH 3 with dilute HCl and aerating vigorously until sulfide odor no longer can be detected.

2. Apparatus

Spectrophotometer for use at 640 nm with a light path of 1 cm or greater.

3. Reagents

a. Phenol solution: Mix 11.1 mL liquified phenol (≥89%) with 95% v/v ethyl alcohol to a final volume of 100 mL. Prepare weekly. CAUTION: *Wear gloves and eye protection when handling phenol; use good ventilation to minimize all personnel exposure to this toxic volatile substance.*

b. Sodium nitroprusside, 0.5% w/v: Dissolve 0.5 g sodium nitroprusside in 100 mL deionized water. Store in amber bottle for up to 1 month.

c. Alkaline citrate: Dissolve 200 g trisodium citrate and 10 g sodium hydroxide in deionized water. Dilute to 1000 mL.

d. Sodium hypochlorite, commercial solution, about 5%. This solution slowly decomposes once the seal on the bottle cap is broken. Replace about every 2 months.

e. Oxidizing solution: Mix 100 mL alkaline citrate solution with 25 mL sodium hypochlorite. Prepare fresh daily.

f. Stock ammonium solution: See Section 4500-NH_3.D.3*d*.

g. Standard ammonium solution: Use stock ammonium solution and water to prepare a calibration curve in a range appropriate for the concentrations of the samples.

4. Procedure

To a 25-mL sample in a 50-mL erlenmeyer flask, add, with thorough mixing after each addition, 1 mL phenol solution, 1 mL sodium nitroprusside solution, and 2.5 mL oxidizing solution. Cover samples with plastic wrap or paraffin wrapper film. Let color develop at room temperature (22 to 27°C) in subdued light for at least 1 h. Color is stable for 24 h. Measure absorbance at 640 nm. Prepare a blank and at least two other standards by diluting stock

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ammonia solution into the sample concentration range. Treat standards the same as samples.

5. Calculations

Prepare a standard curve by plotting absorbance readings of standards against ammonia concentrations of standards. Compute sample concentration by comparing sample absorbance with the standard curve.

6. Precision and Bias

For the manual phenate method, reagent water solutions of ammonium sulfate were prepared and analyzed by two analysts in each of three laboratories. Results are summarized in Table 4500-NH_3:III.

7. Bibliography

SOLORZANO, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnol. Oceanogr.* 14:799.

PARSONS, T.R., Y. MAITA & C.M. LALLI. 1984. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Elmsford, N.Y.

4500-NH_3 G. Automated Phenate Method

1. General Discussion

a. Principle: Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside.

b. Interferences: Seawater contains calcium and magnesium ions in sufficient concentrations to cause precipitation during analysis. Adding EDTA and sodium potassium tartrate reduces the problem. Eliminate any marked variation in acidity or alkalinity among samples because intensity of measured color is pH-dependent. Likewise, insure that pH of wash water and standard ammonia solutions approximates that of sample. For example, if sample has been preserved with 0.8 mL conc H_2SO_4/L, include 0.8 mL conc H_2SO_4/L in wash water and standards. Remove interfering turbidity by filtration. Color in the samples that absorbs in the photometric range used for analysis interferes.

c. Application: Ammonia nitrogen can be determined in potable, surface, and saline waters as well as domestic and industrial wastewaters over a range of 0.02 to 2.0 mg/L when photometric measurement is made at 630 to 660 nm in a 10- to 50-mm tubular flow cell at rates of up to 60 samples/h. Determine higher concentrations by diluting the sample.

2. Apparatus

Automated analytical equipment. An example of the required continuous-flow analytical

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instrument consists of the interchangeable components shown in Figure 4500-NH_3:1.

3. Reagents

a. Ammonia-free distilled water: See Section 4500-NH_3.B.3*a*. Use for preparing all reagents and dilutions.

b. Sulfuric acid, H_2SO_4, 5*N*, air scrubber solution: Carefully add 139 mL conc H_2SO_4 to approximately 500 mL water, cool to room temperature, and dilute to 1 L.

c. Sodium phenate solution: In a 1-L erlenmeyer flask, dissolve 93 mL liquid (≥89%) phenol in 500 mL water. In small increments and with agitation, cautiously add 32 g NaOH. Cool flask under running water and dilute to 1 L. CAUTION: *Minimize exposure of personnel to this compound by wearing gloves and eye protection, and using proper ventilation.*

d. Sodium hypochlorite solution: Dilute 250 mL bleach solution containing 5.25% NaOCl to 500 mL with water.

e. EDTA reagent: Dissolve 50 g disodium ethylenediamine tetraacetate and approximately six pellets NaOH in 1 L water. For salt-water samples where EDTA reagent does not prevent precipitation of cations, use sodium potassium tartrate solution prepared as follows:

Sodium potassium tartrate solution: To 900 mL water add 100 g $NaKC_4H_4O_6\cdot 4H_2O$, two pellets NaOH, and a few boiling chips, and boil gently for 45 min. Cover, cool, and dilute to 1 L. Adjust pH to 5.2 ± 0.05 with H_2SO_4. Let settle overnight in a cool place and filter to remove precipitate. Add 0.5 mL polyoxyethylene 23 lauryl ether*#(56) solution and store in stoppered bottle.

f. Sodium nitroprusside solution: Dissolve 0.5 g $Na_2(NO)Fe(CN)_5\cdot 2H_2O$ in 1 L water.

g. Ammonia standard solutions: See Section 4500-NH_3.D.3*c* and *d*. Use standard ammonia solution and water to prepare the calibration curve in the appropriate ammonia concentration range. To analyze saline waters use substitute ocean water of the following composition to prepare calibration standards:

Constituent	Concentration *g/L*
NaCl	24.53
$MgCl_2$	5.20
$CaCl_2$	1.16
KCl	0.70
$SrCl_2$	0.03
Na_2SO_4	4.09

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Constituent	Concentration *g/L*
$NaHCO_3$	0.20
KBr	0.10
H_3BO_3	0.03
NaF	0.003

Subtract blank background response of substitute seawater from standards before preparing standard curve.

4. Procedure

a. Eliminate marked variation in acidity or alkalinity among samples. Adjust pH of wash water and standard ammonia solutions to approximately that of sample.

b. Set up manifold and complete system as shown in Figure 4500-NH_3:1.

c. Obtain a stable base line with all reagents, feeding wash water through sample line.

d. Typically, use a 60/h, 6:1 cam with a common wash.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against NH_3-N concentrations in standards. Compute sample NH_3-N concentration by comparing sample response with standard curve.

6. Precision and Bias

For an automated phenate system in a single laboratory using surface water samples at concentrations of 1.41, 0.77, 0.59, and 0.43 mg NH_3-N/L, the standard deviation was ±0.005 mg/L, and at concentrations of 0.16 and 1.44 mg NH_3-N/L, recoveries were 107 and 99%, respectively.

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4500-NH_3 H. Flow Injection Analysis (PROPOSED)

1. General Discussion

a. Principle: A water sample containing ammonia or ammonium cation is injected into an FIA carrier stream to which a complexing buffer, alkaline phenol, and hypochlorite are added. This reaction, the Berthelot reaction, produces the blue indophenol dye. The blue color is intensified by the addition of nitroferricyanide. The resulting peak's absorbance is measured at 630 nm. The peak area is proportional to the concentration of ammonia in the original sample.

Also see Section 4500-NH_3.F and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

Also see Section 4500-NH_3.A. Some interferents are removed by distillation; see Section 4500-NH_3.B.

2. Apparatus

Flow injection analysis equipment consisting of:

a. FIA injection valve with sample loop or equivalent.

b. Multichannel proportioning pump.

c. FIA manifold (Figure 4500-NH_3:2) with tubing heater and flow cell. In Figure 4500-NH_3:2, relative flow rates only are shown. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.*#(57)

d. Absorbance detector, 630 nm, 10-nm bandpass.

e. Injection valve control and data acquisition system.

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and all solutions. To prevent bubble formation, degas carrier and buffer with helium. Use He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. Buffer: To a 1-L tared container add 50.0 g disodium ethylenediamine tetraacetate and 5.5 g sodium hydroxide, NaOH. Add 968 mL water. Mix with a magnetic stirrer until dissolved.

b. Phenolate: CAUTION: *Wear gloves. Phenol causes severe burns and is rapidly absorbed into the body through the skin.* To a tared 1-L container, add 888 g water. Add 94.2 g 88% liquefied phenol or 83 g crystalline phenol, C_6H_5OH. While stirring, slowly add 32 g NaOH.

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Cool and invert to mix thoroughly. Do not degas.

c. Hypochlorite: To a tared 500-mL container add 250 g 5.25% sodium hypochlorite, NaOCl bleach solution†#(58) and 250 g water. Stir or shake to mix.

d. Nitroprusside: To a tared 1-L container add 3.50 g sodium nitroprusside (sodium nitroferricyanide), $Na_2Fe(CN)_5NO \cdot 2H_2O$, and 1000 g water. Invert to mix.

e. Stock ammonia standard, 1000 mg N/L: In a 1-L volumetric flask dissolve 3.819 g ammonium chloride, NH_4Cl, that has been dried for 2 h at 110°C, in about 800 mL water. Dilute to mark and invert to mix.

f. Standard ammonia solutions: Prepare ammonia standards in desired concentration range, using the stock standard (¶ 3*e*), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-NH_3:2 and follow method supplied by manufacturer or laboratory standard operating procedure for this method. Follow quality control procedures described in Section 4020.

5. Calculations

Prepare standard curves by plotting the absorbance of standards processed through the manifold versus ammonia concentration. The calibration curve is linear.

6. Precision and Bias

a. Recovery and relative standard deviation: The results of single-laboratory studies with various matrices are given in Table 4500-NH_3:IV.

b. MDL: A 650-µL sample loop was used in the method described above. Using a published MDL method,[1] analysts ran 21 replicates of a 0.020-mg N/L standard. These gave a mean of 0.0204 mg N/L, a standard deviation of 0.0007 mg N/L, and an MDL of 0.002 mg N/L.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1989. Definition and Procedure for the Determination of Method Detection Limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-NO_2^- NITROGEN (NITRITE)*#(59)

4500-NO_2^- A. Introduction

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1. Occurrence and Significance

For a discussion of the chemical characteristics, sources, and effects of nitrite nitrogen, see Section 4500-N.

2. Selection of Method

The colorimetric method (B) is suitable for concentrations of 5 to 1000 µg NO_2^--N/L (See ¶ B.1*a*). Nitrite values can be obtained by the automated method given in Section 4500-NO_3^-.E with the Cu-Cd reduction step omitted. Additionally, nitrite nitrogen can be determined by ion chromatography (Section 4110), and by flow injection analysis (see Section 4130 and Section 4500-NO_3^-.I).

4500-NO_2^- B. Colorimetric Method

1. General Discussion

a. Principle: Nitrite (NO_2^-) is determined through formation of a reddish purple azo dye produced at pH 2.0 to 2.5 by coupling diazotized sulfanilamide with *N*-(1-naphthyl)-ethylenediamine dihydrochloride (NED dihydrochloride). The applicable range of the method for spectrophotometric measurements is 10 to 1000 µg NO_2^--N/L. Photometric measurements can be made in the range 5 to 50 µg N/L if a 5-cm light path and a green color filter are used. The color system obeys Beer's law up to 180 µg N/ L with a 1-cm light path at 543 nm. Higher NO_2^- concentrations can be determined by diluting a sample.

b. Interferences: Chemical incompatibility makes it unlikely that NO_2^-, free chlorine, and nitrogen trichloride (NCl_3) will coexist. NCl_3 imparts a false red color when color reagent is added. The following ions interfere because of precipitation under test conditions and should be absent: Sb^{3+}, Au^{3+}, Bi^{3+}, Fe^{3+}, Pb^{2+}, Hg^{2+}, Ag^{+}, chloroplatinate ($PtCl_6^{2-}$), and metavanadate (VO_3^{2-}). Cupric ion may cause low results by catalyzing decomposition of the diazonium salt. Colored ions that alter the color system also should be absent. Remove suspended solids by filtration.

c. Storage of sample: Never use acid preservation for samples to be analyzed for NO_2^-. Make the determination promptly on fresh samples to prevent bacterial conversion of NO_2^- to NO_3^- or NH_3. For short-term preservation for 1 to 2 d, freeze at –20°C or store at 4°C.

2. Apparatus

Colorimetric equipment: One of the following is required:

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a. Spectrophotometer, for use at 543 nm, providing a light path of 1 cm or longer.

b. Filter photometer, providing a light path of 1 cm or longer and equipped with a green filter having maximum transmittance near 540 nm.

3. Reagents

a. Nitrite-free water: If it is not known that the distilled or demineralized water is free from NO_2^-, use either of the following procedures to prepare nitrite-free water:

1) Add to 1 L distilled water one small crystal each of $KMnO_4$ and either $Ba(OH)_2$ or $Ca(OH)_2$. Redistill in an all-borosilicate-glass apparatus and discard the initial 50 mL of distillate. Collect the distillate fraction that is free of permanganate; a red color with DPD reagent (Section 4500-Cl.F.2*b*) indicates the presence of permanganate.

2) Add 1 mL conc H_2SO_4 and 0.2 mL $MnSO_4$ solution (36.4 g $MnSO_4 \cdot H_2O$/100 mL distilled water) to each 1 L distilled water, and make pink with 1 to 3 mL KMnO4 solution (400 mg $KMnO_4$/ L distilled water). Redistill as described in the preceding paragraph.

Use nitrite-free water in making all reagents and dilutions.

b. Color reagent: To 800 mL water add 100 mL 85% phosphoric acid and 10 g sulfanilamide. After dissolving sulfanilamide completely, add 1 g *N*-(1-naphthyl)-ethylenediamine dihydrochloride. Mix to dissolve, then dilute to 1 L with water. Solution is stable for about a month when stored in a dark bottle in refrigerator.

c. Sodium oxalate, 0.025*M* (0.05*N*): Dissolve 3.350 g $Na_2C_2O_4$, primary standard grade, in water and dilute to 1000 mL.

d. Ferrous ammonium sulfate, 0.05*M* (0.05*N*): Dissolve 19.607 g $Fe(NH_4)_2\,(SO_4)_2 \cdot 6H_2O$ plus 20 mL conc H_2SO_4 in water and dilute to 1000 mL. Standardize as in Section 5220B.3*d*.

e. Stock nitrite solution: Commercial reagent-grade $NaNO_2$ assays at less than 99%. Because NO_2^- is oxidized readily in the presence of moisture, use a fresh bottle of reagent for preparing the stock solution and keep bottles tightly stoppered against the free access of air when not in use. To determine $NaNO_2$ content, add a known excess of standard 0.01*M* (0.05*N*) $KMnO_4$ solution (see ¶ h below), discharge permanganate color with a known quantity of standard reductant such as 0.025*M* $Na_2C_2O_4$ or 0.05*M* $Fe(NH_4)_2(SO_4)_2$, and back-titrate with standard permanganate solution.

1) Preparation of stock solution—Dissolve 1.232 g $NaNO_2$ in water and dilute to 1000 mL; 1.00 mL = 250 µg N. Preserve with 1 mL $CHCl_3$.

2) Standardization of stock nitrite solution—Pipet, in order, 50.00 mL standard 0.01*M* (0.05*N*) $KMnO_4$, 5 mL conc H_2SO_4, and 50.00 mL stock NO_2^- solution into a glass-stoppered flask or bottle. Submerge pipet tip well below surface of permanganate-acid solution while

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adding stock NO_2^- solution. Shake gently and warm to 70 to 80°C on a hot plate. Discharge permanganate color by adding sufficient 10-mL portions of standard 0.025*M* $Na_2C_2O_4$. Titrate excess $Na_2C_2O_4$ with 0.01*M* (0.05*N*) $KMnO_4$ to the faint pink end point. Carry a water blank through the entire procedure and make the necessary corrections in the final calculation as shown in the equation below.

If standard 0.05*M* ferrous ammonium sulfate solution is substituted for $Na_2C_2O_4$, omit heating and extend reaction period between $KMnO_4$ and Fe^{2+} to 5 min before making final $KMnO_4$ titration.

Calculate NO_2^--N content of stock solution by the following equation:

$$A = \frac{[(B \times C) - (D \times E)] \times 7}{F}$$

where:

A = mg NO_2^--N/mL in stock $NaNO_2$ solution,
B = total mL standard $KMnO_4$ used,
C = normality of standard $KMnO_4$,
D = total mL standard reductant added,
E = normality of standard reductant, and
F = mL stock $NaNO_2$ solution taken for titration.

Each 1.00 mL 0.01*M* (0.05*N*) $KMnO_4$ consumed by the $NaNO_2$ solution corresponds to 1750 µg NO_2^--N.

f. Intermediate nitrite solution: Calculate the volume, *G*, of stock NO_2^- solution required for the intermediate NO_2^- solution from $G = 12.5/A$. Dilute the volume *G* (approximately 50 mL) to 250 mL with water; 1.00 mL = 50.0 µg N. Prepare daily.

g. Standard nitrite solution: Dilute 10.00 mL intermediate NO_2^- solution to 1000 mL with water; 1.00 mL = 0.500 µg N. Prepare daily.

h. Standard potassium permanganate titrant, 0.01*M* (0.05*N*): Dissolve 1.6 g $KMnO_4$ in 1 L distilled water. Keep in a brown glass-stoppered bottle and age for at least 1 week. Carefully decant or pipet supernate without stirring up any sediment. Standardize this solution frequently by the following procedure:

Weigh to the nearest 0.1 mg several 100- to 200-mg samples of anhydrous $Na_2C_2O_4$ into 400-mL beakers. To each beaker, in turn, add 100 mL distilled water and stir to dissolve. Add 10

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mL 1 + 1 H_2SO_4 and heat rapidly to 90 to 95°C. Titrate rapidly with permanganate solution to be standardized, while stirring, to a slight pink end-point color that persists for at least 1 min. Do not let temperature fall below 85°C. If necessary, warm beaker contents during titration; 100 mg will consume about 6 mL solution. Run a blank on distilled water and H_2SO_4.

$$\text{Normality of } KMnO_4 = \frac{\text{g } Na_2C_2O_4}{(A - B) \times 0.335\ 05}$$

where:

A = mL titrant for sample and
B = mL titrant for blank.

Average the results of several titrations.

4. Procedure

a. Removal of suspended solids: If sample contains suspended solids, filter through a 0.45-µm-pore-diam membrane filter.

b. Color development: If sample pH is not between 5 and 9, adjust to that range with 1*N* HCl or NH_4OH as required. To 50.0 mL sample, or to a portion diluted to 50.0 mL, add 2 mL color reagent and mix.

c. Photometric measurement: Between 10 min and 2 h after adding color reagent to samples and standards, measure absorbance at 543 nm. As a guide use the following light paths for the indicated NO_2^--N concentrations:

Light Path Length *cm*	NO_2^--N *µg/L*
1	2–25
5	2–6
10	<2

5. Calculation

Prepare a standard curve by plotting absorbance of standards against NO_2^--N concentration. Compute sample concentration directly from curve.

6. Precision and Bias

In a single laboratory using wastewater samples at concentrations of 0.04, 0.24, 0.55, and

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1.04 mg NO^{-}_{3} + NO_2^{-}-N/L, the standard deviations were ±0.005, ± 0.004, ± 0.005, and ± 0.01, respectively. In a single laboratory using wastewater samples at concentrations of 0.24, 0.55, and 1.05 mg NO_3^{-} + NO_2^{-}-N/L, the recoveries were 100%, 102%, and 100%, respectively.[1]

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1979. Methods for Chemical Analysis of Water and Wastes. Method 353. 3. U.S. Environmental Protection Agency, Washington, D.C.

8. Bibliography

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4500-NO_2^{-} C. (Reserved)

4500-NO_3^{-} NITROGEN (NITRATE)*#(60)

4500-NO_3^{-} A. Introduction

1. Selection of Method

Determination of nitrate (NO_3^{-}) is difficult because of the relatively complex procedures required, the high probability that interfering constituents will be present, and the limited concentration ranges of the various techniques.

An ultraviolet (UV) technique (Method B) that measures the absorbance of NO_3^{-} at 220 nm is suitable for screening uncontaminated water (low in organic matter).

Screen a sample; if necessary, then select a method suitable for its concentration range and probable interferences. Nitrate may be determined by ion chromatography (Section 4110) or capillary ion electrophoresis (Section 4140). Applicable ranges for other methods are: nitrate electrode method (D), 0.14 to 1400 mg NO_3^{-}-N/L; cadmium reduction method (E), 0.01 to 1.0 mg NO_3^{-}-N/L; automated cadmium reduction methods (F and I), 0.001 to 10 mg NO_3^{-}-N/L. For higher NO_3^{-}-N concentrations, dilute into the range of the selected method.

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Colorimetric methods (B, E) require an optically clear sample. Filter turbid sample through 0.45-µm-pore-diam membrane filter. Test filters for nitrate contamination.

2. Storage of Samples

Start NO_3^- determinations promptly after sampling. If storage is necessary, store for up to 2 d at 4°C; disinfected samples are stable much longer without acid preservation. For longer storage of unchlorinated samples, preserve with 2 mL conc H_2SO_4/L and store at 4°C. NOTE: When sample is preserved with acid, NO_3^- and NO_2^- cannot be determined as individual species.

4500-NO_3^- B. Ultraviolet Spectrophotometric Screening Method

1. General Discussion

a. Principle: Use this technique only for screening samples that have low organic matter contents, i.e., uncontaminated natural waters and potable water supplies. The NO_3^- calibration curve follows Beer's law up to 11 mg N/L.

Measurement of UV absorption at 220 nm enables rapid determination of NO_3^-. Because dissolved organic matter also may absorb at 220 nm and NO_3^- does not absorb at 275 nm, a second measurement made at 275 nm may be used to correct the NO_3^- value. The extent of this empirical correction is related to the nature and concentration of organic matter and may vary from one water to another. Consequently, this method is not recommended if a significant correction for organic matter absorbance is required, although it may be useful in monitoring NO_3^- levels within a water body with a constant type of organic matter. Correction factors for organic matter absorbance can be established by the method of additions in combination with analysis of the original NO_3^- content by another method. Sample filtration is intended to remove possible interference from suspended particles. Acidification with 1*N* HCl is designed to prevent interference from hydroxide or carbonate concentrations up to 1000 mg $CaCO_3$/L. Chloride has no effect on the determination.

b. Interference: Dissolved organic matter, surfactants, NO_2^-, and Cr^{6+} interfere. Various inorganic ions not normally found in natural water, such as chlorite and chlorate, may interfere. Inorganic substances can be compensated for by independent analysis of their concentrations and preparation of individual correction curves. For turbid samples, see ¶ A.1.

2. Apparatus

Spectrophotometer, for use at 220 nm and 275 nm with matched silica cells of 1-cm or longer light path.

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3. Reagents

a. Nitrate-free water: Use redistilled or distilled, deionized water of highest purity to prepare all solutions and dilutions.

b. Stock nitrate solution: Dry potassium nitrate (KNO_3) in an oven at 105°C for 24 h. Dissolve 0.7218 g in water and dilute to 1000 mL; 1.00 mL = 100 µg NO_3^--N. Preserve with 2 mL $CHCl_3$/L. This solution is stable for at least 6 months.

c. Intermediate nitrate solution: Dilute 100 mL stock nitrate solution to 1000 mL with water; 1.00 mL = 10.0 µg NO_3^--N. Preserve with 2 mL $CHCl_3$/L. This solution is stable for 6 months.

d. Hydrochloric acid solution, HCl, 1*N*.

4. Procedure

a. Treatment of sample: To 50 mL clear sample, filtered if necessary, add 1 mL HCl solution and mix thoroughly.

b. Preparation of standard curve: Prepare NO_3^- calibration standards in the range 0 to 7 mg NO_3^--N/L by diluting to 50 mL the following volumes of intermediate nitrate solution: 0, 1.00, 2.00, 4.00, 7.00 . . . 35.0 mL. Treat NO_3^- standards in same manner as samples.

c. Spectrophotometric measurement: Read absorbance or transmittance against redistilled water set at zero absorbance or 100% transmittance. Use a wavelength of 220 nm to obtain NO_3^- reading and a wavelength of 275 nm to determine interference due to dissolved organic matter.

5. Calculation

For samples and standards, subtract two times the absorbance reading at 275 nm from the reading at 220 nm to obtain absorbance due to NO_3^-. Construct a standard curve by plotting absorbance due to NO_3^- against NO_3^--N concentration of standard. Using corrected sample absorbances, obtain sample concentrations directly from standard curve. NOTE: If correction value is more than 10% of the reading at 220 nm, do not use this method.

6. Bibliography

HOATHER, R.C. & R.F. RACKMAN. 1959. Oxidized nitrogen and sewage effluents observed by ultraviolet spectrophotometry. *Analyst* 84:549. GOLDMAN, E. & R. JACOBS. 1961. Determination of nitrates by ultraviolet absorption. *J. Amer. Water Works Assoc.* 53:187.

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4500-NO_3^- C. (Reserved)

4500-NO_3^- D. Nitrate Electrode Method

1. General Discussion

a. Principle: The NO_3^- ion electrode is a selective sensor that develops a potential across a thin, porous, inert membrane that holds in place a water-immiscible liquid ion exchanger. The electrode responds to NO_3^- ion activity between about 10^{-5} and 10^{-1} *M* (0.14 to 1400 mg NO_3^--N/L). The lower limit of detection is determined by the small but finite solubility of the liquid ion exchanger.

b. Interferences: Chloride and bicarbonate ions interfere when their weight ratios to NO_3^--N are >10 or >5, respectively. Ions that are potential interferences but do not normally occur at significant levels in potable waters are NO_2^-, CN^-, S^{2-}, Br^-, I^-, ClO_3^-, and ClO_4^-. Although the electrodes function satisfactorily in buffers over the range pH 3 to 9, erratic responses have been noted where pH is not held constant. Because the electrode responds to NO_3^- activity rather than concentration, ionic strength must be constant in all samples and standards. Minimize these problems by using a buffer solution containing Ag_2SO_4 to remove Cl^-, Br^-, I^-, S^{2-}, and CN^-, sulfamic acid to remove NO_2^-, a buffer at pH 3 to eliminate HCO_3^- and to maintain a constant pH and ionic strength, and $Al_2(SO_4)_3$ to complex organic acids.

2. Apparatus

a. pH meter, expanded-scale or digital, capable of 0.1 mV resolution.

*b. Double-junction reference electrode.**#(61) Fill outer chamber with $(NH_4)_2SO_4$ solution.

c. Nitrate ion electrode:†#(62) Carefully follow manufacturer's instructions regarding care and storage.

d. Magnetic stirrer: TFE-coated stirring bar.

3. Reagents

a. Nitrate-free water: Prepare as described in ¶ B.3*a*. Use for all solutions and dilutions.

b. Stock nitrate solution: Prepare as described in ¶ B.3*b*.

c. Standard nitrate solutions: Dilute 1.0, 10, and 50 mL stock nitrate solution to 100 mL with water to obtain standard solutions of 1.0, 10, and 50 mg NO_3^--N/L, respectively.

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d. Buffer solution: Dissolve 17.32 g $Al_2(SO_4)_3 18H_2O$, 3.43 g Ag_2SO_4, 1.28 g H_3BO_3, and 2.52 g sulfamic acid (H_2NSO_3H), in about 800 mL water. Adjust to pH 3.0 by slowly adding 0.10*N* NaOH. Dilute to 1000 mL and store in a dark glass bottle.

e. Sodium hydroxide, NaOH, 0.1*N*.

f. Reference electrode filling solution: Dissolve 0.53 g $(NH_4)_2SO_4$ in water and dilute to 100 mL.

4. Procedure

a. Preparation of calibration curve: Transfer 10 mL of 1 mg NO_3^--N/L standard to a 50-mL beaker, add 10 mL buffer, and stir with a magnetic stirrer. Immerse tips of electrodes and record millivolt reading when stable (after about 1 min). Remove electrodes, rinse, and blot dry. Repeat for 10-mg NO_3^--N/L and 50-mg NO_3^--N/L standards. Plot potential measurements against NO_3^--N concentration on semilogarithmic graph paper, with NO_3^--N concentration on the logarithmic axis (abscissa) and potential (in millivolts) on the linear axis (ordinate). A straight line with a slope of +57 ±3 mV/decade at 25°C should result. Recalibrate electrodes several times daily by checking potential reading of the 10 mg NO_3^--N standard and adjusting the calibration control until the reading plotted on the calibration curve is displayed again.

b. Measurement of sample: Transfer 10 mL sample to a 50-mL beaker, add 10 mL buffer solution, and stir (for about 1 min) with a magnetic stirrer. Measure standards and samples at about the same temperature. Immerse electrode tips in sample and record potential reading when stable (after about 1 min). Read concentration from calibration curve.

5. Precision

Over the range of the method, precision of ±0.4 mV, corresponding to 2.5% in concentration, is expected.

6. Bibliography

LANGMUIR, D. & R.I. JACOBSON. 1970. Specific ion electrode determination of nitrate in some fresh waters and sewage effluents. *Environ. Sci. Technol*. 4:835.

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4500-NO_3^- E. Cadmium Reduction Method

1. General Discussion

a. Principle: NO_3^- is reduced almost quantitatively to nitrite (NO_2^-) in the presence of cadmium (Cd). This method uses commercially available Cd granules treated with copper sulfate ($CuSO_4$) and packed in a glass column.

The NO_2^- produced thus is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye that is measured colorimetrically. A correction may be made for any NO_2^- present in the sample by analyzing without the reduction step. The applicable range of this method is 0.01 to 1.0 mg NO_3^--N/L. The method is recommended especially for NO_3^- levels below 0.1 mg N/L where other methods lack adequate sensitivity.

b. Interferences: Suspended matter in the column will restrict sample flow. For turbid samples, see ¶ A.1. Concentrations of iron, copper, or other metals above several milligrams per liter lower reduction efficiency. Add EDTA to samples to eliminate this interference. Oil and grease will coat the Cd surface. Remove by pre-extraction with an organic solvent (see Section 5520). Residual chlorine can interfere by oxidizing the Cd column, reducing its efficiency. Check samples for residual chlorine (see DPD methods in Section 4500-Cl). Remove residual chlorine by adding sodium thiosulfate ($Na_2S_2O_3$) solution (Section 4500-NH_3.B.3*d*). Sample color that absorbs at about 540 nm interferes.

2. Apparatus

a. Reduction column: Purchase or construct the column*#(63) (Figure 4500-NO_3^-:1) from a 100-mL volumetric pipet by removing the top portion. The column also can be constructed from two pieces of tubing joined end to end: join a 10-cm length of 3-cm-ID tubing to a 25-cm length of 3.5-mm-ID tubing. Add a TFE stopcock with metering valve[1] to control flow rate.

b. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer,* for use at 543 nm, providing a light path of 1 cm or longer.

2) *Filter photometer*, with light path of 1 cm or longer and equipped with a filter having maximum transmittance near 540 nm.

3. Reagents

a. Nitrate-free water: See ¶ B.3*a*. The absorbance of a reagent blank prepared with this water should not exceed 0.01. Use for all solutions and dilutions.

b. Copper-cadmium granules: Wash 25 g new or used 20- to 100-mesh Cd granules†#(64)

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with 6*N* HCl and rinse with water. Swirl Cd with 100 mL 2% $CuSO_4$ solution for 5 min or until blue color partially fades. Decant and repeat with fresh $CuSO_4$ until a brown colloidal precipitate begins to develop. Gently flush with water to remove all precipitated Cu.

c. Color reagent: Prepare as directed in Section 4500-NO_2^-B.3*b*.

d. Ammonium chloride-EDTA solution: Dissolve 13 g NH_4Cl and 1.7 g disodium ethylenediamine tetraacetate in 900 mL water. Adjust to pH 8.5 with conc NH_4OH and dilute to 1 L.

e. Dilute ammonium chloride-EDTA solution: Dilute 300 mL NH_4Cl-EDTA solution to 500 mL with water.

f. Hydrochloric acid, HCl, 6*N*.

g. Copper sulfate solution, 2%: Dissolve 20 g $CuSO_4.5H_2O$ in 500 mL water and dilute to 1 L.

h. Stock nitrate solution: Prepare as directed in ¶ B.3*b*.

i. Intermediate nitrate solution: Prepare as directed in ¶ B.3*c*.

j. Stock nitrite solution: See Section 4500-NO_2^-.B.3*e*.

k. Intermediate nitrite solution: See Section 4500-NO_2^-.B.3*f*.

l. Working nitrite solution: Dilute 50.0 mL intermediate nitrite solution to 500 mL with nitrite-free water; 1.00 mL = 5 µg NO_2^--N.

4. Procedure

a. Preparation of reduction column: Insert a glass wool plug into bottom of reduction column and fill with water. Add sufficient Cu-Cd granules to produce a column 18.5 cm long. Maintain water level above Cu-Cd granules to prevent entrapment of air. Wash column with 200 mL dilute NH_4Cl-EDTA solution. Activate column by passing through it, at 7 to 10 mL/min, at least 100 mL of a solution composed of 25% 1.0 mg NO_3^--N/L standard and 75% NH_4Cl-EDTA solution.

b. Treatment of sample:

1) Turbidity removal—For turbid samples, see ¶ A.1.

2) pH adjustment—Adjust pH to between 7 and 9, as necessary, using a pH meter and dilute HCl or NaOH. This insures a pH of 8.5 after adding NH_4Cl- EDTA solution.

3) Sample reduction—To 25.0 mL sample or a portion diluted to 25.0 mL, add 75 mL NH_4Cl-EDTA solution and mix. Pour mixed sample into column and collect at a rate of 7 to 10 mL/min. Discard first 25 mL. Collect the rest in original sample flask. There is no need to wash columns between samples, but if columns are not to be reused for several hours or longer, pour

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50 mL dilute NH_4Cl-EDTA solution on to the top and let it pass through the system. Store Cu-Cd column in this solution and never let it dry.

4) Color development and measurement—As soon as possible, and not more than 15 min after reduction, add 2.0 mL color reagent to 50 mL sample and mix. Between 10 min and 2 h afterward, measure absorbance at 543 nm against a distilled water-reagent blank. NOTE: If NO_3^- concentration exceeds the standard curve range (about 1 mg N/L), use remainder of reduced sample to make an appropriate dilution and analyze again.

c. Standards: Using the intermediate NO_3^-- N solution, prepare standards in the range 0.05 to 1.0 mg NO_3^--N/L by diluting the following volumes to 100 mL in volumetric flasks: 0.5, 1.0, 2.0, 5.0, and 10.0 mL. Carry out reduction of standards exactly as described for samples. Compare at least one NO_2^- standard to a reduced NO_3^- standard at the same concentration to verify reduction column efficiency. Reactivate Cu-Cd granules as described in ¶ 3*b* above when efficiency of reduction falls below about 75%.

5. Calculation

Obtain a standard curve by plotting absorbance of standards against NO_3^--N concentration. Compute sample concentrations directly from standard curve. Report as milligrams oxidized N per liter (the sum of NO_3^--N plus NO_2^--N) unless the concentration of NO_2^--N is separately determined and subtracted.

6. Precision and Bias

In a single laboratory using wastewater samples at concentrations of 0.04, 0.24, 0.55, and 1.04 mg $NO_3^- + NO_2^-$-N/L, the standard deviations were ±0.005, ±0.004, ±0.005, and ±0.01, respectively. In a single laboratory using wastewater with additions of 0.24, 0.55, and 1.05 mg $NO_3^- + NO_2^-$-N/L, the recoveries were 100%, 102%, and 100%, respectively.[2]

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4500-NO_3^- F. Automated Cadmium Reduction Method

1. General Discussion

a. Principle: See ¶ E.1*a*.

b. Interferences: Sample turbidity may interfere. Remove by filtration before analysis. Sample color that absorbs in the photometric range used for analysis also will interfere.

c. Application: Nitrate and nitrite, singly or together in potable, surface, and saline waters and domestic and industrial wastewaters, can be determined over a range of 0.5 to 10 mg N/L.

2. Apparatus

Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the components shown in Figure 4500-NO_3^-:2.

3. Reagents

a. Deionized distilled water: See ¶ B.3*a*.

b. Copper sulfate solution: Dissolve 20 g $CuSO_4 \cdot 5H_2O$ in 500 mL water and dilute to 1 L.

c. Wash solution: Use water for unpreserved samples. For samples preserved with H_2SO_4, add 2 mL conc H_2SO_4/L wash water.

d. Copper-cadmium granules: See ¶ E.3*b*.

e. Hydrochloric acid, HCl, conc.

f. Ammonium hydroxide, NH_4OH, conc.

g. Color reagent: To approximately 800 mL water, add, while stirring, 100 mL conc H_3PO_4, 40 g sulfanilamide, and 2 g *N*-(1-naphthyl)-ethylenediamine dihydrochloride. Stir until dissolved and dilute to 1 L. Store in brown bottle and keep in the dark when not in use. This solution is stable for several months.

h. Ammonium chloride solution: Dissolve 85 g NH_4Cl in water and dilute to 1 L. Add 0.5 mL polyoxyethylene 23 lauryl ether.*#(65)

i. Stock nitrate solution: See ¶ B.3*b*.

j. Intermediate nitrate solution: See ¶ B.3*c*.

k. Standard nitrate solutions: Using intermediate NO_3^--N solution and water, prepare standards for calibration curve in appropriate nitrate range. Compare at least one NO_2^- standard

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to a NO_3^- standard at the same concentration to verify column reduction efficiency. To examine saline waters prepare standard solutions with the substitute ocean water described in Section 4500-NH_3.G.3*g*.

l. Standard nitrite solution: See Section 4500-NO_2^-.B.3*g*.

4. Procedure

Set up manifold as shown in Figure 4500-NO_3^-:2 and follow general procedure described by the manufacturer.

If sample pH is below 5 or above 9, adjust to between 5 and 9 with either conc HCl or conc NH_4OH.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against NO_3^--N concentration in standards. Compute sample NO_3^--N concentration by comparing sample response with standard curve.

6. Precision and Bias

Data obtained in three laboratories with an automated system based on identical chemical principles but having slightly different configurations are given in the table below. Analyses were conducted on four natural water samples containing exact increments of inorganic nitrate:

Increment as NO_3^--N µg/L	Standard Deviation µg N/L	Bias %	Bias µg N/L
290	12	+5.75	+17
350	92	+18.10	+63
2310	318	+4.47	+103
2480	176	–2.69	–67

In a single laboratory using surface water samples at concentrations of 100, 200, 800, and 2100 µg N/L, the standard deviations were 0, ±40, ±50, and ±50 µg/L, respectively, and at concentrations of 200 and 2200 µg N/L, recoveries were 100 and 96%, respectively.

Precision and bias for the system described herein are believed to be comparable.

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4500-NO_3^- G. (Reserved)

4500-NO_3^- H. Automated Hydrazine Reduction Method

1. General Discussion

a. Principle: NO_3^- is reduced to NO_2^- with hydrazine sulfate. The NO_2^-(originally present) plus reduced NO_3^- is determined by diazotization with sulfanilamide and coupling with *N*-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye that is measured colorimetrically.

b. Interferences: Sample color that absorbs in the photometric range used will interfere. Concentrations of sulfide ion of less than 10 mg/L cause variations of NO_3^- and NO_2^- concentrations of ±10%.

c. Application: $NO_3^- + NO_2^-$ in potable and surface water and in domestic and industrial wastes can be determined over a range of 0.01 to 10 mg N/L.

2. Apparatus

Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the components shown in Figure 4500-NO_3^-:3.

3. Reagents

a. Color developing reagent: To approximately 500 mL water add 200 mL conc phosphoric acid and 10 g sulfanilamide. After sulfanilamide is dissolved completely, add 0.8 g *N*-(1-naphthyl)-ethylenediamine dihydrochloride. Dilute to 1 L with water, store in a dark bottle, and refrigerate. Solution is stable for approximately 1 month.

b. Copper sulfate stock solution: Dissolve 2.5 g $CuSO_4 \cdot 5H_2O$ in water and dilute to 1 L.

c. Copper sulfate dilute solution: Dilute 20 mL stock solution to 2 L.

d. Sodium hydroxide stock solution, 10*N:* Dissolve 400 g NaOH in 750 mL water, cool, and

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dilute to 1 L.

e. Sodium hydroxide, 1.0*N:* Dilute 100 mL stock NaOH solution to 1 L.

f. Hydrazine sulfate stock solution: Dissolve 27.5 g $N_2H_4 \cdot H_2SO_4$ in 900 mL water and dilute to 1 L. This solution is stable for approximately 6 months. CAUTION: *Toxic if ingested. Mark container with appropriate warning.*

g. Hydrazine sulfate dilute solution: Dilute 22 mL stock solution to 1 L.

h. Stock nitrate solution: See ¶ B.3*b*.

i. Intermediate nitrate solution: See ¶ B.3*c*.

j. Standard nitrate solutions: Prepare NO_3^- calibration standards in the range 0 to 10 mg/L by diluting to 100 mL the following volumes of stock nitrate solution: 0, 0.5, 1.0, 2.0 . . . 10.0 mL. For standards in the range of 0.01 mg/L use intermediate nitrate solution. Compare at least one nitrite standard to a nitrate standard at the same concentration to verify the efficiency of the reduction.

k. Standard nitrite solution: See Section 4500-NO_2^-.B.3*e*, Section 4500-NO_2^-.B.3*f*, and Section 4500-NO_2^-.B.3*g*.

4. Procedure

Set up manifold as shown in Figure 4500-NO_3^-:3 and follow general procedure described by manufacturer. Run a 2.0-mg NO_3^--N/L and a 2.0-mg NO_2^--N/L standard through the system to check for 100% reduction of nitrate to nitrite. The two peaks should be of equal height; if not, adjust concentration of the hydrazine sulfate solution: If the NO_3^- peak is lower than the NO_2^- peak, increase concentration of hydrazine sulfate until they are equal; if the NO_3^- peak is higher than the NO_2^- reduce concentration of hydrazine sulfate. When correct concentration has been determined, no further adjustment should be necessary.

5. Calculation

Prepare a standard curve by plotting response of processed standards against known concentrations. Compute concentrations of samples by comparing response with standard curve.

6. Precision and Bias

In a single laboratory using drinking water, surface water, and industrial waste at concentrations of 0.39, 1.15, 1.76, and 4.75 mg NO_3^--N/L, the standard deviations were ±0.02, ±0.01, ±0.02, and ±0.03, respectively. In a single laboratory using drinking water at concentrations of 0.75 and 2.97 mg NO_3^--N/ L, the recoveries were 99% and 101%.[1]

7. Reference

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1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1979. Methods for Chemical Analysis of Water and Wastes. U.S. Environmental Protection Agency, Washington, D.C.

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4500-NO_3^- I. Cadmium Reduction Flow Injection Method (PROPOSED)

1. General Discussion

a. Principle: The nitrate in the sample is reduced quantitatively to nitrite by passage of the sample through a copperized cadmium column. The resulting nitrite plus any nitrite originally in the sample is determined as a sum by diazotizing the nitrite with sulfanilamide followed by coupling with *N*-(1-naphthyl)ethylenediamine dihydrochloride. The resulting water-soluble dye has a magenta color; absorbance of the color at 540 nm is proportional to the nitrate + nitrite in the sample. This sum also is known as total oxidized nitrogen (TON).

Nitrite alone can be determined by removing the cadmium column, recalibrating the method, and repeating the sample analyses. A TON and a nitrite FIA method also can be run in parallel for a set of samples. In this arrangement, the concentrations determined in the nitrite method can be subtracted from the corresponding concentrations determined in the TON method to give the resulting nitrate concentrations of the samples.

Also see Section 4500-NO_2^- and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: Remove large or fibrous particulates by filtering sample through glass wool. Guard against nitrate and nitrite contamination from reagents, water, glassware, and the sample preservation process.

Residual chlorine can interfere by oxidizing the cadmium reduction column. Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. Eliminate this interference by pre-extracting sample with an organic solvent.

Low results would be obtained for samples that contain high concentrations of iron, copper, or other metals. In this method, EDTA is added to the buffer to reduce this interference.

Also see Section 4500-NO_2^-.B.1*b* and *c* and Section 4500-NO_3^-.A.2 and Section 4500-NO_3^-.B.1*b*.

2. Apparatus

Flow injection analysis equipment consisting of:

a. FIA injection valve with sample loop or equivalent.

b. Multichannel proportioning pump.

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c. FIA manifold (Figure 4500-NO_3^-:4) with flow cell. Relative flow rates only are shown in Figure 4500-NO_3^-:4. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

d. Absorbance detector, 540 nm, 10-nm bandpass.

e. Injection valve control and data acquisition system.

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. Ammonium chloride buffer: CAUTION: *Fumes. Use a hood.* To a tared 1-L container add 800.0 g water, 126 g conc hydrochloric acid, HCl, 55.6 g ammonium hydroxide, NH_4OH, and 1.0 g disodium EDTA. Shake until dissolved. The pH of this buffer should be 8.5.

b. Sulfanilamide color reagent: To a tared, dark 1-L container add 876 g water, 170 g 85% phosphoric acid, H_3PO_4, 40.0 g sulfanilamide, and 1.0 g *N*-(1-naphthyl)ethylenediamine dihydrochloride (NED). Shake until wetted and stir with stir bar for 30 min until dissolved. This solution is stable for 1 month.

c. Hydrochloric acid, HCl, 1*M*: To a 100-mL container, add 92 g water, then add 9.6 g conc HCl. Stir or shake to mix.

d. Copper sulfate solution, 2%*:* To a 1-L container, add 20 g copper sulfate pentahydrate, $CuSO_4 \cdot 5H_2O$, to 991 g water. Stir or shake to dissolve.

e. Copperized cadmium granules: Place 10 to 20 g coarse cadmium granules (0.3- to 1.5-mm-diam) in a 250-mL beaker. Wash with 50 mL acetone, then water, then two 50-mL portions 1*M* HCl (¶ 3*c*). Rinse several times with water. CAUTION: *Cadmium is toxic and carcinogenic. Collect and store all waste cadmium. When handling cadmium, wear gloves and follow the precautions described on the cadmium's Material Safety Data Sheet.*

Add 100 mL 2% copper sulfate solution (¶ 3*d*) to cadmium prepared above. Swirl for about 5 min, then decant the liquid and repeat with a fresh 100-mL portion of the 2% copper sulfate solution. Continue this process until the blue aqueous copper color persists. Decant and wash with at least five portions of ammonium chloride buffer (¶ 3*a*) to remove colloidal copper. The cadmium should be black or dark gray. The copperized cadmium granules may be stored in a bottle under ammonium chloride buffer.

f. Stock nitrate standard, 200 mg N/L: In a 1-L volumetric flask dissolve 1.444 g potassium nitrate, KNO_3, in about 600 mL water. Add 2 mL chloroform. Dilute to mark and invert to mix. This solution is stable for 6 months.

g. Stock nitrite standard, 200.0 mg N/L: In a 1-L volumetric flask dissolve 0.986 g sodium

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nitrite, $NaNO_2$, or 1.214 g potassium nitrite, KNO_2, in approximately 800 mL water. Add 2 mL chloroform. Dilute to mark and invert to mix. Refrigerate.

h. Standard solution: Prepare nitrate or nitrite standards in the desired concentration range, using the stock standards (¶ 3 *f* or *g*), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-NO_3^-:4 and pack column with copperized cadmium granules. Follow methods supplied by column and instrument manufacturer or laboratory's standard operating procedure for this method. Follow quality control procedures outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold versus TON or nitrite concentration. The calibration curve is linear.

If TON includes measurable nitrite concentrations, it is important that the cadmium column be 100% efficient. If the efficiency is less, the nitrite in the sample will give a positive percent error equal to the difference from 100%, causing an error in TON and nitrate determinations. To measure efficiency of the cadmium column, prepare two calibration curves, one using nitrate standards and one using equimolar nitrite standards. The column efficiency is:

$$\text{Column efficiency} = 100\% \times (\text{slope of nitrate curve}/\text{slope of nitrite curve})$$

Determine column efficiency at least weekly.

6. Precision and Bias

In the studies described below, nitrate was measured. There was no significant concentration of nitrite in the samples.

a. Recovery and relative standard deviation: Table 4500-NO_3^-:I gives results of single-laboratory studies.

b. MDL: A 800-µL sample loop was used in the method described above. Using a published MDL method,[1] analysts ran 21 replicates of a 2.00-µg N/L standard. These gave a mean of 1.82 µg N/L, a standard deviation of 0.098 µg N/L, and MDL of 0.25 µg N/L. A lower MDL may be obtained by increasing the sample loop volume and increasing the ratio of carrier flow rate to reagent flow rate.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

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4500-N_{org} NITROGEN (ORGANIC)*#(66)

4500-N_{org} A. Introduction

1. Selection of Method

The kjeldahl methods (B and C) determine nitrogen in the trinegative state. They fail to account for nitrogen in the form of azide, azine, azo, hydrazone, nitrate, nitrite, nitrile, nitro, nitroso, oxime, and semi-carbazone. ‘‘Kjeldahl nitrogen’’ is the sum of organic nitrogen and ammonia nitrogen.

The major factor that influences the selection of a macro- or semi-micro-kjeldahl method to determine organic nitrogen is its concentration. The macro-kjeldahl method is applicable for samples containing either low or high concentrations of organic nitrogen but requires a relatively large sample volume for low concentrations. In the semi-micro-kjeldahl method, which is applicable to samples containing high concentrations of organic nitrogen, the sample volume should be chosen to contain organic plus ammonia nitrogen in the range of 0.2 to 2 mg.

The block digestion method (D) is a micro method with an automated analysis step capable of measuring organic nitrogen as low as 0.1 mg/L when blanks are carefully controlled.

2. Storage of Samples

The most reliable results are obtained on fresh samples. If an immediate analysis is not possible, preserve samples for kjeldahl digestion by acidifying to pH 1.5 to 2.0 with concentrated H_2SO_4 and storing at 4°C. Do not use $HgCl_2$ because it will interfere with ammonia removal.

3. Interferences

a. Nitrate: During kjeldahl digestion, nitrate in excess of 10 mg/L can oxidize a portion of the ammonia released from the digested organic nitrogen, producing N_2O and resulting in a negative interference. When sufficient organic matter in a low state of oxidation is present, nitrate can be reduced to ammonia, resulting in a positive interference. The conditions under which significant interferences occur are not well defined and there is no proven way to eliminate the interference in conjunction with the kjeldahl methods described herein.

b. Inorganic salts and solids: The acid and salt content of the kjeldahl digestion reagent is intended to produce a digestion temperature of about 380°C. If the sample contains a very large quantity of salt or inorganic solids that dissolve during digestion, the temperature may rise above 400°C, at which point pyrolytic loss of nitrogen begins to occur. To prevent an excessive digestion temperature, add more H_2SO_4 to maintain the acid-salt balance. Not all salts cause precisely the same temperature rise, but adding 1 mL H_2SO_4/g salt in the sample gives reasonable results. Add the extra acid and the digestion reagent to both sample and reagent

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blank. Too much acid will lower the digestion temperature below 380°C and result in incomplete digestion and recovery. If necessary, add sodium hydroxide-sodium thiosulfate before the final distillation step to neutralize the excess acid.

Large amounts of salt or solids also may cause bumping during distillation. If this occurs, add more dilution water after digestion.

c. Organic matter: During kjeldahl digestion, H_2SO_4 oxidizes organic matter to CO_2 and H_2O. If a large amount of organic matter is present, a large amount of acid will be consumed, the ratio of salt to acid will increase, and the digestion temperature will increase. If enough organic matter is present, the temperature will rise above 400°C, resulting in pyrolytic loss of nitrogen. To prevent this, add to the digestion flask 10 mL conc H_2SO_4/3 g COD. Alternately, add 50 mL more digestion reagent/g COD. Additional sodium hydroxide-sodium thiosulfate reagent may be necessary to keep the distillation pH high. Because reagents may contain traces of ammonia, treat the reagent blank identically with the samples.

4. Use of a Catalyst

Mercury has been the catalyst of choice for kjeldahl digestion. Because of its toxicity and problems associated with legal disposal of mercury residues, a less toxic catalyst is recommended. Digestion of some samples may be complete or nearly complete without the use of a catalyst. Effective digestion results from the use of a reagent having a salt/acid ratio of 1 g/mL with copper as catalyst (¶ B.3*a*), and specified temperature (¶ B.2*a*) and time (¶ B.4*c*)). If a change is made in the reagent formula, report the change and indicate percentage recovery relative to the results for similar samples analyzed using the previous formula.

Before results are considered acceptable, determine nitrogen recovery from samples with known additions of nicotinic acid, to test completeness of digestion; and with ammonium chloride to test for loss of nitrogen.

4500-N_{org} B. Macro-Kjeldahl Method

1. General Discussion

a. Principle: In the presence of H_2SO_4, potassium sulfate (K_2SO_4), and cupric sulfate ($CuSO_4$) catalyst, amino nitrogen of many organic materials is converted to ammonium. Free ammonia also is converted to ammonium. After addition of base, the ammonia is distilled from an alkaline medium and absorbed in boric or sulfuric acid. The ammonia may be determined colorimetrically, by ammonia-selective electrode, or by titration with a standard mineral acid.

b. Selection of ammonia measurement method: The sensitivity of colorimetric methods makes them particularly useful for determining organic nitrogen levels below 5 mg/L. The titrimetric and selective electrode methods of measuring ammonia in the distillate are suitable for determining a wide range of organic nitrogen concentrations. Selective electrode methods and

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automated colorimetric methods may be used for measurement of ammonia in digestate without distillation. Follow equipment manufacturer's instructions.

2. Apparatus

a. Digestion apparatus: Kjeldahl flasks with a total capacity of 800 mL yield the best results. Digest over a heating device adjusted so that 250 mL water at an initial temperature of 25°C can be heated to a rolling boil in approximately 5 min. For testing, preheat heaters for 10 min if gas-operated or 30 min if electric. A heating device meeting this specification should provide the temperature range of 375 to 385°C for effective digestion.

b. Distillation apparatus: See Section 4500-NH_3.B.2*a*.

c. Apparatus for ammonia determination: See Section 4500-NH_3.C.2, 4500-NH_3.D.2, Section 4500-NH_3.F.2, or Section 4500-NH_3.G.2.

3. Reagents

Prepare all reagents and dilutions in ammonia-free water.

All of the reagents listed for the determination of Nitrogen (Ammonia), Section 4500-NH_3.C.3, Section 4500-NH_3.D.3, Section 4500-NH_3.F.3, or Section 4500-NH_3.G.3, are required, plus the following:

a. Digestion reagent: Dissolve 134 g K_2SO_4 and 7.3 g $CuSO_4$ in about 800 mL water. Carefully add 134 mL conc H_2SO_4. When it has cooled to room temperature, dilute the solution to 1 L with water. Mix well. Keep at a temperature close to 20°C to prevent crystallization.

b. Sodium hydroxide-sodium thiosulfate reagent: Dissolve 500 g NaOH and 25 g $Na_2S_2O_3 \cdot 5H_2O$ in water and dilute to 1 L.

c. Borate buffer solution: See Section 4500-NH_3.B.3*b*.

d. Sodium hydroxide, NaOH, 6*N*.

4. Procedure

a. Selection of sample volume and sample preparation: Place a measured volume of sample in an 800-mL kjeldahl flask. Select sample size from the following tabulation:

Organic Nitrogen in Sample *mg/L*	Sample Size *mL*
0–1	500
1–10	250
10–20	100
20–50	50.0
50–100	25.0

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If necessary, dilute sample to 300 mL, neutralize to pH 7, and dechlorinate as described in Section 4500-NH_3.B.4*b*.

b. Ammonia removal: Add 25 mL borate buffer and then 6*N* NaOH until pH 9.5 is reached. Add a few glass beads or boiling chips such as Hengar Granules #12 and boil off 300 mL. If desired, distill this fraction and determine ammonia nitrogen. Alternately, if ammonia has been determined by the distillation method, use residue in distilling flask for organic nitrogen determination.

For sludge and sediment samples, weigh wet sample in a crucible or weighing bottle, transfer contents to a kjeldahl flask, and determine kjeldahl nitrogen. Follow a similar procedure for ammonia nitrogen and organic nitrogen determined by difference. Determinations of organic and kjeldahl nitrogen on dried sludge and sediment samples are not accurate because drying results in loss of ammonium salts. Measure dry weight of sample on a separate portion.

c. Digestion: Cool and add carefully 50 mL digestion reagent (or substitute 6.7 mL conc H_2SO_4, 6.7 g K_2SO_4, and 0.365 g $CuSO_4$) to distillation flask. Add a few glass beads and, after mixing, heat under a hood or with suitable ejection equipment to remove acid fumes. Boil briskly until the volume is greatly reduced (to about 25 to 50 mL) and copious white fumes are observed (fumes may be dark for samples high in organic matter). Then continue to digest for an additional 30 min. As digestion continues, colored or turbid samples will become transparent and pale green. After digestion, let cool, dilute to 300 mL with water, and mix. Tilt flask away from personnel and carefully add 50 mL sodium hydroxide-thiosulfate reagent to form an alkaline layer at flask bottom. Connect flask to a steamed-out distillation apparatus and swirl flask to insure complete mixing. The pH of the solution should exceed 11.0.

d. Distillation: Distill and collect 200 mL distillate. Use 50 mL indicating boric acid as absorbent solution when ammonia is to be determined by titration. Use 50 mL 0.04*N* H_2SO_4 solution as absorbent for manual phenate or electrode methods. Extend tip of condenser well below level of absorbent solution and do not let temperature in condenser rise above 29°C. Lower collected distillate free of contact with condenser tip and continue distillation during last 1 or 2 min to cleanse condenser.

e. Final ammonia measurement: Use the titration, ammonia-selective electrode, manual phenate, or automated phenate method, Section 4500-NH_3.C, Section 4500-NH_3.D, Section 4500-NH_3.F, and Section 4500-NH_3.G, respectively.

f. Standards: Carry a reagent blank and standards through all steps of the procedure.

5. Calculation

See Section 4500-NH_3.C.5, Section 4500-NH_3.D.5, Section 4500-NH_3.F.5, or Section 4500-NH_3.G.5.

6. Precision and Bias

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Two analysts in one laboratory prepared reagent water solutions of nicotinic acid and digested them by the macro-kjeldahl method. Ammonia in the distillate was determined by titration. Results are summarized in Table 4500-N_{org}:I.

7. Bibliography

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4500-N_{org} C. Semi-Micro-Kjeldahl Method

1. General Discussion

See Section 4500-N_{org}.B.1.

2. Apparatus

a. Digestion apparatus: Use kjeldahl flasks with a capacity of 100 mL in a semi-micro-kjeldahl digestion apparatus*#(67) equipped with heating elements to accommodate kjeldahl flasks and a suction outlet to vent fumes. The heating elements should provide the temperature range of 375 to 385°C for effective digestion.

b. Distillation apparatus: Use an all-glass unit equipped with a steam-generating vessel containing an immersion heater†#(68) (Figure 4500-N_{org}:1).

c. pH meter.

d. Apparatus for ammonia determination: See Section 4500-NH_3.C.2, Section 4500-NH_3.D.2, Section 4500-NH_3.F.2, or Section 4500-NH_3.G.2.

3. Reagents

All of the reagents listed for the determination of Nitrogen (Ammonia) (Section 4500-NH_3.B.3) and Nitrogen (Organic) macro-kjeldahl (Section 4500-N_{org}.B.3) are required.

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Prepare all reagents and dilutions with ammonia-free water.

4. Procedure

a. Selection of sample volume: Determine the sample size from the following tabulation:

Organic Nitrogen in Sample *mg/L*	Sample Size *mL*
4–40	50
8–80	25
20–200	10
40–400	5

For sludge and sediment samples weigh a portion of wet sample containing between 0.2 and 2 mg organic nitrogen in a crucible or weighing bottle. Transfer sample quantitatively to a 100-mL beaker by diluting it and rinsing the weighing dish several times with small quantities of water. Make the transfer using as small a quantity of water as possible and do not exceed a total volume of 50 mL. Measure dry weight of sample on a separate portion.

b. Ammonia removal: Pipet 50 mL sample or an appropriate volume diluted to 50 mL with water into a 100-mL beaker. Add 3 mL borate buffer and adjust to pH 9.5 with 6*N* NaOH, using a pH meter. Quantitatively transfer sample to a 100-mL kjeldahl flask and boil off 30 mL. Alternatively, if ammonia removal is not required, digest samples directly as described in ¶ c below. Distillation following this direct digestion yields kjeldahl nitrogen concentration rather than organic nitrogen.

c. Digestion: Carefully add 10 mL digestion reagent to kjeldahl flask containing sample. Add five or six glass beads (3- to 4-mm size) to prevent bumping during digestion. Set each heating unit on the micro-kjeldahl digestion apparatus to its medium setting and heat flasks under a hood or with suitable ejection equipment to remove fumes of SO_3. Continue to boil briskly until solution becomes transparent and pale green and copious fumes are observed. Then turn each heating unit up to its maximum setting and digest for an additional 30 min. Cool. Quantitatively transfer digested sample by diluting and rinsing several times into micro-kjeldahl distillation apparatus so that total volume in distillation apparatus does not exceed 30 mL. Add 10 mL sodium hydroxide-thiosulfate reagent and turn on steam.

d. Distillation: Control rate of steam generation to boil contents in distillation unit so that neither escape of steam from tip of condenser nor bubbling of contents in receiving flask occurs. Distill and collect 30 to 40 mL distillate below surface of 10 mL absorbent solution contained in a 125-mL erlenmeyer flask. Use indicating boric acid for a titrimetric finish. Use 10 mL 0.04*N* H_2SO_4 solution for collecting distillate for the phenate or electrode methods. Extend tip of condenser well below level of absorbent solution and do not let temperature in condenser rise

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above 29°C. Lower collected distillate free of contact with condenser tip and continue distillation during last 1 or 2 min to cleanse condenser.

e. Standards: Carry a reagent blank and standards through all steps of procedure and apply necessary correction to results.

f. Final ammonia measurement: Use the titration, ammonia-selective electrode, manual phenate, or automated phenate method, Section 4500-NH_3.C, Section 4500-NH_3.D, Section 4500-NH_3.F, and Section 4500-NH_3.G, respectively.

5. Calculation

See Section 4500-NH_3.C.5, Section 4500-NH_3.D.5, Section 4500-NH_3.F.5, or Section 4500-NH_3.G.5.

6. Precision and Bias

No data on the precision and bias of the semi-micro-kjeldahl method are available.

7. Bibliography

See Section 4500-N_{org}.B.7.

4500-N_{org} D. Block Digestion and Flow Injection Analysis (PROPOSED)

1. General Discussion

a. Principle: Samples of drinking, ground, and surface waters and of domestic and industrial wastes are digested in a block digestor with sulfuric acid and copper sulfate as a catalyst. The digestion recovers nitrogen components of biological origin, such as amino acids, proteins, and peptides, as ammonia, but may not recover the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones, and some refractory tertiary amines. Nitrate is not recovered. See Section 4500-N for a discussion of the various forms of nitrogen found in waters and wastewaters, Section 4500-N_{org}.A and Section 4500-N_{org}.B for a discussion of kjeldahl nitrogen methods, and Section 4130, Flow Injection Analysis (FIA).

The digested sample is injected onto the FIA manifold where its pH is controlled by raising it to a known, basic pH by neutralization with a concentrated buffer. This in-line neutralization converts the ammonium cation to ammonia, and also prevents undue influence of the sulfuric acid matrix on the pH-sensitive color reaction that follows. The ammonia thus produced is heated with salicylate and hypochlorite to produce a blue color that is proportional to the ammonia concentration. The color is intensified by adding sodium nitroprusside. The presence of EDTA in the buffer prevents precipitation of calcium and magnesium. The resulting peak's absorbance is measured at 660 nm. The peak area is proportional to the concentration of total

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kjeldahl nitrogen in the original sample.

b. Interferences: Remove large or fibrous particulates by filtering the sample through glass wool.

The main source of interference is ammonia. Ammonia is an airborne contaminant that is removed rapidly from ambient air by the digestion solution. Guard against ammonia contamination in reagents, water, glassware, and the sample preservation process. It is particularly important to prevent ammonia contamination in the sulfuric acid used for the digestion. Open sulfuric acid bottles away from laboratories in which ammonia or ammonium chloride have been used as reagents and store sulfuric acid away from such reagents. Ensure that the open ends of the block digestor's tubes can be covered to prevent ammonia from being scrubbed from the fume hood make-up air during the digestion.

If a sample consumes more than 10% of the sulfuric acid during digestion, the pH-dependent color reaction will show a matrix effect. The color reaction buffer will accommodate a range of 5.4%±0.4% H_2SO_4 (v/v) in the diluted digested sample. Sample matrices with a high concentration of carbohydrates or other organic material may consume more than 10% of the acid during digestion. If this effect is suspected, titrate digested sample with standardized sodium hydroxide to determine whether more than 10% of the sulfuric acid has been consumed during digestion. The block digestor also should have a means to prevent loss of sulfuric acid from the digestion tubes during the digestion period.

Also see Section 4500-N_{org}.A and Section 4500-N_{org}.B.

2. Apparatus

Digestion and flow injection analysis equipment consisting of:

a. Block digestor capable of maintaining a temperature of 380°C for 2 h.

b. Digestion tubes capable of being heated to 380°C for 2 h and having a cover to prevent ammonia contamination and loss of sulfuric acid.

c. FIA injection valve with sample loop or equivalent.

d. Multichannel proportioning pump.

e. FIA manifold (Figure 4500-N_{org}:2) with tubing heater and flow cell. Relative flow rates only are shown in Figure 4500-N_{org}:2. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

f. Absorbance detector, 660 nm, 10-nm bandpass.

g. Injection valve control and data acquisition system.

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by

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weight/weight, use weight/volume.

a. Digestion solution: In a 1-L volumetric flask, dissolve 134.0 g potassium sulfate, K_2SO_4, and 7.3 g copper sulfate, $CuSO_4$, in 800 mL water. Then add slowly while swirling 134 mL conc sulfuric acid, H_2SO_4. Let cool, dilute to mark, and invert to mix.

b. Carrier and diluent: To a tared 1-L container, add 496 g digestion solution (¶ 3*a*) and 600 g water. Shake until dissolved.

c. Sodium hydroxide, NaOH, 0.8*M*: To a tared 1-L plastic container, add 32.0 g NaOH and 985.0 g water. Stir or shake until dissolved.

d. Buffer: To a tared 1-L container add 941 g water. Add and completely dissolve 35.0 g sodium phosphate dibasic heptahydrate, $Na_2HPO_4 \cdot 7H_2O$. Add 20.0 g disodium EDTA (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add 50 g NaOH. Stir or shake until dissolved.

e. Salicylate/nitroprusside: To a tared 1-L dark container, add 150.0 g sodium salicylate (salicylic acid sodium salt), $C_6H_4(OH)(COO)Na$, 1.00 g sodium nitroprusside (sodium nitroferricyanide dihydrate), $Na_2Fe(CN)_5NO \cdot 2H_2O$, and 908 g water. Stir or shake until dissolved. Prepare fresh monthly.

f. Hypochlorite: To a tared 250-mL container, add 16 g commercial 5.25% sodium hypochlorite bleach solution*#(69) and 234 g deionized water. Shake to mix.

g. Stock standard, 250 mg N/L: In a 1-L volumetric flask dissolve 0.9540 g ammonium chloride, NH_4Cl (dried for 2 h at 110°C), in about 800 mL water. Dilute to mark and invert to mix.

h. Standard ammonia solutions: Prepare ammonia standards in desired concentration range, using the stock standard (¶ 3*g*) and diluting with water.

i. Simulated digested standards: To prepare calibration standards without having to digest the standards prepared in ¶ 3*h*, proceed as follows:

Stock standard, 5.00 mg N/L: To a tared 250-mL container add about 5.0 g stock standard (250 mg N/L). Divide actual weight of solution added by 0.02 and make up to this resulting total weight with diluent (¶ 3*b*). Shake to mix. Prepare working standards from this stock standard, diluting with the diluent (¶ 3*b*), not water.

4. Procedure

a. Digestion procedure: Carry both standards and samples through this procedure.

To a 75-mL block digestor tube add 25.0 mL sample or standard and then add 10 mL digestion solution (¶ 3*a*) and mix. Add four alundum granules to each tube for smooth boiling. Place tubes in preheated block digestor for 1 h at 200°C. After 1 h, increase block temperature to 380°C and continue to digest for 1 h at 380°C. Remove tubes from block and let cool for about 10 min. Dilute each to 25.0 mL with water and mix with vortex mixer. Cover tubes to prevent

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ammonia contamination.

b. FIA analysis: Set up a manifold equivalent to that in Figure 4500-N_{org}:2 and analyze digested standards and samples by method supplied by manufacturer or laboratory standard operating procedure. Follow quality control protocols described in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold versus ammonia concentration. The calibration curve is linear.

6. Precision and Bias

a. Recovery and relative standard deviation: Table 4500-N_{org}:II gives results of single-laboratory studies.

b. MDL: A 130-µL sample loop was used in the method described above. Using a published MDL method,[1] analysts ran 21 replicates of a 0.1-mg N/L standard. These gave a mean of 0.103 mg N/L, a standard deviation of 0.014 mg N/L, and MDL of 0.034 mg N/L. A lower MDL may be obtained by increasing the sample loop volume and increasing the ratio of carrier flow rate to reagents flow rate.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-O OXYGEN (DISSOLVED)*#(70)

4500-O A. Introduction

1. Significance

Dissolved oxygen (DO) levels in natural and wastewaters depend on the physical, chemical, and biochemical activities in the water body. The analysis for DO is a key test in water pollution and waste treatment process control.

2. Selection of Method

Two methods for DO analysis are described: the Winkler or iodometric method and its modifications and the electrometric method using membrane electrodes. The iodometric method[1] is a titrimetric procedure based on the oxidizing property of DO while the membrane electrode procedure is based on the rate of diffusion of molecular oxygen across a membrane.[2] The choice

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of procedure depends on the interferences present, the accuracy desired, and, in some cases, convenience or expedience.

3. References

1. WINKLER, L.W. 1888. The determination of dissolved oxygen in water. *Berlin. Deut. Chem. Ges.* 21:2843.
2. MANCY, K.H. & T. JAFFE. 1966. Analysis of Dissolved Oxygen in Natural and Waste Waters. Publ. No. 999-WP-37, U.S. Public Health Serv., Washington, D.C.

4500-O B. Iodometric Methods

1. Principle

The iodometric test is the most precise and reliable titrimetric procedure for DO analysis. It is based on the addition of divalent manganese solution, followed by strong alkali, to the sample in a glass-stoppered bottle. DO rapidly oxidizes an equivalent amount of the dispersed divalent manganous hydroxide precipitate to hydroxides of higher valency states. In the presence of iodide ions in an acidic solution, the oxidized manganese reverts to the divalent state, with the liberation of iodine equivalent to the original DO content. The iodine is then titrated with a standard solution of thiosulfate.

The titration end point can be detected visually, with a starch indicator, or electrometrically, with potentiometric or dead-stop techniques.[1] Experienced analysts can maintain a precision of ±50 μg/L with visual end-point detection and a precision of ±5 μg/L with electrometric end-point detection.[1,2]

The liberated iodine also can be determined directly by simple absorption spectrophotometers.[3] This method can be used on a routine basis to provide very accurate estimates for DO in the microgram-per-liter range provided that interfering particulate matter, color, and chemical interferences are absent.

2. Selection of Method

Before selecting a method consider the effect of interferences, particularly oxidizing or reducing materials that may be present in the sample. Certain oxidizing agents liberate iodine from iodides (positive interference) and some reducing agents reduce iodine to iodide (negative interference). Most organic matter is oxidized partially when the oxidized manganese precipitate is acidified, thus causing negative errors.

Several modifications of the iodometric method are given to minimize the effect of interfering materials.[2] Among the more commonly used procedures are the azide modification,[4] the permanganate modification,[5] the alum flocculation modification,[6] and the copper sulfate-sulfamic acid flocculation modification.[7,8] The azide modification (C) effectively

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removes interference caused by nitrite, which is the most common interference in biologically treated effluents and incubated BOD samples. Use the permanganate modification (D) in the presence of ferrous iron. When the sample contains 5 or more mg ferric iron salts/L, add potassium fluoride (KF) as the first reagent in the azide modification or after the permanganate treatment for ferrous iron. Alternately, eliminate Fe(III) interference by using 85 to 87% phosphoric acid (H_3PO_4) instead of sulfuric acid (H_2SO_4) for acidification. This procedure has not been tested for Fe(III) concentrations above 20 mg/L.

Use the alum flocculation modification (E) in the presence of suspended solids that cause interference and the copper sulfate-sulfamic acid flocculation modification (F) on activated-sludge mixed liquor.

3. Collection of Samples

Collect samples very carefully. Methods of sampling are highly dependent on source to be sampled and, to a certain extent, on method of analysis. Do not let sample remain in contact with air or be agitated, because either condition causes a change in its gaseous content. Samples from any depth in streams, lakes, or reservoirs, and samples of boiler water, need special precautions to eliminate changes in pressure and temperature. Procedures and equipment have been developed for sampling waters under pressure and unconfined waters (e.g., streams, rivers, and reservoirs). Sampling procedures and equipment needed are described in American Society for Testing and Materials Special Technical Publication No. 148-1 and in U.S. Geological Survey Water Supply Paper No. 1454.

Collect surface water samples in narrow-mouth glass-stoppered BOD bottles of 300-mL capacity with tapered and pointed ground-glass stoppers and flared mouths. Avoid entraining or dissolving atmospheric oxygen. In sampling from a line under pressure, attach a glass or rubber tube to the tap and extend to bottom of bottle. Let bottle overflow two or three times its volume and replace stopper so that no air bubbles are entrained.

Suitable samplers for streams, ponds, or tanks of moderate depth are of the APHA type shown in Figure 4500-O:1. Use a Kemmerer-type sampler for samples collected from depths greater than 2 m. Bleed sample from bottom of sampler through a tube extending to bottom of a 250- to 300-mL BOD bottle. Fill bottle to overflowing (overflow for approximately 10 s), and prevent turbulence and formation of bubbles while filling. Record sample temperature to nearest degree Celsius or more precisely.

4. Preservation of Samples

Determine DO immediately on all samples containing an appreciable oxygen or iodine demand. Samples with no iodine demand may be stored for a few hours without change after adding manganous sulfate ($MnSO_4$) solution, alkali-iodide solution, and H_2SO_4, followed by shaking in the usual way. Protect stored samples from strong sunlight and titrate as soon as possible.

For samples with an iodine demand, preserve for 4 to 8 h by adding 0.7 mL conc H_2SO_4 and

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1 mL sodium azide solution (2 g NaN_3/100 mL distilled water) to the BOD bottle. This will arrest biological activity and maintain DO if the bottle is stored at the temperature of collection or water-sealed and kept at 10 to 20°C. As soon as possible, complete the procedure, using 2 mL $MnSO_4$ solution, 3 mL alkali-iodide solution, and 2 mL conc H_2SO_4.

5. References

1. POTTER, E.C. & G.E. EVERITT. 1957. Advances in dissolved oxygen microanalysis. *J. Appl. Chem.* 9:642.
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6. RUCHHOFT, C.C. & W.A. MOORE. 1940. The determination of biochemical oxygen demand and dissolved oxygen of river mud suspensions. *Ind. Eng. Chem.*, Anal. Ed. 12:711.
7. PLACAK, O.R. & C.C. RUCHHOFT. 1941. Comparative study of the azide and Rideal-Stewart modifications of the Winkler method in the determination of biochemical oxygen demand. *Ind. Eng. Chem.,* Anal. Ed. 13:12.
8. RUCHHOFT, C.C. & O.R. PLACAK. 1942. Determination of dissolved oxygen in activated-sludge sewage mixtures. *Sewage Works J.* 14:638.

4500-O C. Azide Modification

1. General Discussion

Use the azide modification for most wastewater, effluent, and stream samples, especially if samples contain more than 50 µg NO_2^--N/L and not more than 1 mg ferrous iron/L. Other reducing or oxidizing materials should be absent. If 1 mL KF solution is added before the sample is acidified and there is no delay in titration, the method is applicable in the presence of 100 to 200 mg ferric iron/L.

2. Reagents

a. Manganous sulfate solution: Dissolve 480 g $MnSO_4 \cdot 4H_2O$, 400 g $MnSO_4 \cdot 2H_2O$, or 364 g $MnSO_4 \cdot H_2O$ in distilled water, filter, and dilute to 1 L. The $MnSO_4$ solution should not give a

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color with starch when added to an acidified potassium iodide (KI) solution.

b. Alkali-iodide-azide reagent:

1) For saturated or less-than-saturated samples—Dissolve 500 g NaOH (or 700 g KOH) and 135 g NaI (or 150 g KI) in distilled water and dilute to 1 L. Add 10 g NaN_3 dissolved in 40 mL distilled water. Potassium and sodium salts may be used interchangeably. This reagent should not give a color with starch solution when diluted and acidified.

2) For supersaturated samples—Dissolve 10 g NaN_3 in 500 mL distilled water. Add 480 g sodium hydroxide (NaOH) and 750 g sodium iodide (NaI), and stir until dissolved. There will be a white turbidity due to sodium carbonate (Na_2CO_3), but this will do no harm. CAUTION—*Do not acidify this solution because toxic hydrazoic acid fumes may be produced.*

c. Sulfuric acid, H_2SO_4, conc: One milliliter is equivalent to about 3 mL alkali-iodide-azide reagent.

d. Starch: Use either an aqueous solution or soluble starch powder mixtures.

To prepare an aqueous solution, dissolve 2 g laboratory-grade soluble starch and 0.2 g salicylic acid, as a preservative, in 100 mL hot distilled water.

e. Standard sodium thiosulfate titrant: Dissolve 6.205 g $Na_2S_2O_3 \cdot 5H_2O$ in distilled water. Add 1.5 mL 6*N* NaOH or 0.4 g solid NaOH and dilute to 1000 mL. Standardize with bi-iodate solution.

f. Standard potassium bi-iodate solution, 0.0021*M:* Dissolve 812.4 mg $KH(IO_3)_2$ in distilled water and dilute to 1000 mL.

Standardization—Dissolve approximately 2 g KI, free from iodate, in an erlenmeyer flask with 100 to 150 mL distilled water. Add 1 mL 6*N* H_2SO_4 or a few drops of conc H_2SO_4 and 20.00 mL standard bi-iodate solution. Dilute to 200 mL and titrate liberated iodine with thiosulfate titrant, adding starch toward end of titration, when a pale straw color is reached. When the solutions are of equal strength, 20.00 mL 0.025*M* $Na_2S_2O_3$ should be required. If not, adjust the $Na_2S_2O_3$ solution to 0.025*M*.

3. Procedure

a. To the sample collected in a 250- to 300-mL bottle, add 1 mL $MnSO_4$ solution, followed by 1 mL alkali-iodide-azide reagent. If pipets are dipped into sample, rinse them before returning them to reagent bottles. Alternatively, hold pipet tips just above liquid surface when adding reagents. Stopper carefully to exclude air bubbles and mix by inverting bottle a few times. When precipitate has settled sufficiently (to approximately half the bottle volume) to leave clear supernate above the manganese hydroxide floc, add 1.0 mL conc H_2SO_4. Restopper and mix by inverting several times until dissolution is complete. Titrate a volume corresponding to 200 mL original sample after correction for sample loss by displacement with reagents. Thus, for a total of 2 mL (1 mL each) of $MnSO_4$ and alkali-iodide-azide reagents in a 300-mL bottle, titrate 200 ×

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300/(300 − 2) = 201 mL.

b. Titrate with 0.025*M* $Na_2S_2O_3$ solution to a pale straw color. Add a few drops of starch solution and continue titration to first disappearance of blue color. If end point is overrun, back-titrate with 0.0021*M* bi-iodate solution added dropwise, or by adding a measured volume of treated sample. Correct for amount of bi-iodate solution or sample. Disregard subsequent recolorations due to the catalytic effect of nitrite or to traces of ferric salts that have not been complexed with fluoride.

4. Calculation

a. For titration of 200 mL sample, 1 mL 0.025*M* $Na_2S_2O_3$ = 1 mg DO/L.

b. To express results as percent saturation at 101.3 kPa, use the solubility data in Table 4500-O:I. Equations for correcting solubilities to barometric pressures other than mean sea level and for various chlorinities are given below the table.

5. Precision and Bias

DO can be determined with a precision, expressed as a standard deviation, of about 20 µg/L in distilled water and about 60 µg/L in wastewater and secondary effluents. In the presence of appreciable interference, even with proper modifications, the standard deviation may be as high as 100 µg/L. Still greater errors may occur in testing waters having organic suspended solids or heavy pollution. Avoid errors due to carelessness in collecting samples, prolonging the completion of test, or selecting an unsuitable modification.

6. References

1. BENSON, B.B. & D. KRAUSE, JR. 1984. The concentration and isotopic fractionation of oxygen dissolved in freshwater and seawater in equilibrium with the atmosphere. *Limnol. Oceanogr.* 29:620.
2. BENSON, B.B. & D. KRAUSE, JR. 1980. The concentration and isotopic fractionation of gases dissolved in fresh water in equilibrium with the atmosphere: I. Oxygen. *Limnol. Oceanogr.* 25:662.
3. MORTIMER, C.H. 1981. The oxygen content of air-saturated fresh waters over ranges of temperature and atmospheric pressure of limnological interest. *Int. Assoc. Theoret. Appl. Limnol.,* Communication No. 22, Stuttgart, West Germany.
4. SULZER, F. & W.M. WESTGARTH. 1962. Continuous D. O. recording in activated sludge. *Water Sewage Works* 109: 376.
5. UNITED NATIONS EDUCATIONAL, SCIENTIFIC & CULTURAL ORGANIZATION. 1981. Background Papers and Supporting Data on the Practical Salinity Scale 1978. Tech. Paper Mar. Sci. No. 37.

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4500-O D. Permanganate Modification

1. General Discussion

Use the permanganate modification only on samples containing ferrous iron. Interference from high concentrations of ferric iron (up to several hundred milligrams per liter), as in acid mine water, may be overcome by the addition of 1 mL potassium fluoride (KF) and azide, provided that the final titration is made immediately after acidification.

This procedure is ineffective for oxidation of sulfite, thiosulfate, polythionate, or the organic matter in wastewater. The error with samples containing 0.25% by volume of digester waste from the manufacture of sulfite pulp may amount to 7 to 8 mg DO/L. With such samples, use the alkali-hypochlorite modification.[1] At best, however, the latter procedure gives low results, the deviation amounting to 1 mg/L for samples containing 0.25% digester wastes.

2. Reagents

All the reagents required for Method C, and in addition:

a. Potassium permanganate solution: Dissolve 6.3 g $KMnO_4$ in distilled water and dilute to 1 L.

b. Potassium oxalate solution: Dissolve 2 g $K_2C_2O_4 \cdot H_2O$ in 100 mL distilled water; 1 mL will reduce about 1.1 mL permanganate solution.

c. Potassium fluoride solution: Dissolve 40 g $KF \cdot 2H_2O$ in distilled water and dilute to 100 mL.

3. Procedure

a. To a sample collected in a 250- to 300-mL bottle add, below the surface, 0.70 mL conc H_2SO_4, 1 mL $KMnO_4$ solution, and 1 mL KF solution. Stopper and mix by inverting. Never add more than 0.7 mL conc H_2SO_4 as the first step of pretreatment. Add acid with a 1-mL pipet graduated to 0.1 mL. Add sufficient $KMnO_4$ solution to obtain a violet tinge that persists for 5 min. If the permanganate color is destroyed in a shorter time, add additional $KMnO_4$ solution, but avoid large excesses.

b. Remove permanganate color completely by adding 0.5 to 1.0 mL $K_2C_2O_4$ solution. Mix well and let stand in the dark to facilitate the reaction. Excess oxalate causes low results; add only enough $K_2C_2O_4$ to decolorize the $KMnO_4$ completely without an excess of more than 0.5 mL. Complete decolorization in 2 to 10 min. If it is impossible to decolorize the sample without adding a large excess of oxalate, the DO result will be inaccurate.

c. From this point the procedure closely parallels that in Section 4500-O.C.3. Add 1 mL $MnSO_4$ solution and 3 mL alkali-iodide-azide reagent. Stopper, mix, and let precipitate settle a

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short time; acidify with 2 mL conc H_2SO_4. When 0.7 mL acid, 1 mL KF solution, 1 mL $KMnO_4$ solution, 1 mL $K_2C_2O_4$ solution, 1 mL $MnSO_4$ solution, and 3 mL alkali-iodide-azide (or a total of 7.7 mL reagents) are used in a 300-mL bottle, take $200 \times 300/(300 - 7.7) = 205$ mL for titration.

This correction is slightly in error because the $KMnO_4$ solution is nearly saturated with DO and 1 mL would add about 0.008 mg oxygen to the DO bottle. However, because precision of the method (standard deviation, 0.06 mL thiosulfate titration, or 0.012 mg DO) is 50% greater than this error, a correction is unnecessary. When substantially more $KMnO_4$ solution is used routinely, use a solution several times more concentrated so that 1 mL will satisfy the permanganate demand.

4. Reference

1. THERIAULT, E.J. & P.D. MCNAMEE. 1932. Dissolved oxygen in the presence of organic matter, hypochlorites, and sulfite wastes. *Ind. Eng. Chem.,* Anal. Ed. 4:59.

4500-O E. Alum Flocculation Modification

1. General Discussion

Samples high in suspended solids may consume appreciable quantities of iodine in acid solution. The interference due to solids may be removed by alum flocculation.

2. Reagents

All the reagents required for the azide modification (Section 4500-O.C.2) and in addition:

a. Alum solution: Dissolve 10 g aluminum potassium sulfate, $AlK(SO_4)_2 \cdot 12H_2O$, in distilled water and dilute to 100 mL.

b. Ammonium hydroxide, NH_4OH, conc.

3. Procedure

Collect sample in a glass-stoppered bottle of 500 to 1000 mL capacity, using the same precautions as for regular DO samples. Add 10 mL alum solution and 1 to 2 mL conc NH_4OH. Stopper and invert gently for about 1 min. Let sample settle for about 10 min and siphon clear supernate into a 250- to 300-mL DO bottle until it overflows. Avoid sample aeration and keep siphon submerged at all times. Continue sample treatment as in Section 4500-O.C.3 or an appropriate modification.

4500-O F. Copper Sulfate-Sulfamic Acid Flocculation Modification

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1. General Discussion

This modification is used for biological flocs such as activated sludge mixtures, which have high oxygen utilization rates.

2. Reagents

All the reagents required for the azide modification (Section 4500-O.C.2) and,, in addition:

Copper sulfate-sulfamic acid inhibitor solution: Dissolve 32 g technical-grade NH_2SO_2OH without heat in 475 mL distilled water. Dissolve 50 g $CuSO_4 \cdot 5H_2O$ in 500 mL distilled water. Mix the two solutions and add 25 mL conc acetic acid.

3. Procedure

Add 10 mL $CuSO_4$-NH_2SO_2OH inhibitor to a 1-L glass-stoppered bottle. Insert bottle in a special sampler designed so that bottle fills from a tube near bottom and overflows only 25 to 50% of bottle capacity. Collect sample, stopper, and mix by inverting. Let suspended solids settle and siphon relatively clear supernatant liquor into a 250- to 300-mL DO bottle. Continue sample treatment as rapidly as possible by the azide (Section 4500-O.C.3) or other appropriate modification.

4500-O G. Membrane Electrode Method

1. General Discussion

Various modifications of the iodometric method have been developed to eliminate or minimize effects of interferences; nevertheless, the method still is inapplicable to a variety of industrial and domestic wastewaters.[1] Moreover, the iodometric method is not suited for field testing and cannot be adapted easily for continuous monitoring or for DO determinations in situ.

Polarographic methods using the dropping mercury electrode or the rotating platinum electrode have not been reliable always for the DO analysis in domestic and industrial wastewaters because impurities in the test solution can cause electrode poisoning or other interferences.[2,3] With membrane-covered electrode systems these problems are minimized, because the sensing element is protected by an oxygen-permeable plastic membrane that serves as a diffusion barrier against impurities.[4–6] Under steady-state conditions the current is directly proportional to the DO concentration.*#(71)

Membrane electrodes of the polarographic[4] as well as the galvanic[5] type have been used for DO measurements in lakes and reservoirs,[8] for stream survey and control of industrial effluents,[9,10] for continuous monitoring of DO in activated sludge units,[11] and for estuarine and oceanographic studies.[12] Being completely submersible, membrane electrodes are suited for analysis in situ. Their portability and ease of operation and maintenance make them particularly convenient for field applications. In laboratory investigations, membrane electrodes have been

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used for continuous DO analysis in bacterial cultures, including the BOD test.[5,13]

Membrane electrodes provide an excellent method for DO analysis in polluted waters, highly colored waters, and strong waste effluents. They are recommended for use especially under conditions that are unfavorable for use of the iodometric method, or when that test and its modifications are subject to serious errors caused by interferences.

a. Principle: Oxygen-sensitive membrane electrodes of the polarographic or galvanic type are composed of two solid metal electrodes in contact with supporting electrolyte separated from the test solution by a selective membrane. The basic difference between the galvanic and the polarographic systems is that in the former the electrode reaction is spontaneous (similar to that in a fuel cell), while in the latter an external source of applied voltage is needed to polarize the indicator electrode. Polyethylene and fluorocarbon membranes are used commonly because they are permeable to molecular oxygen and are relatively rugged.

Membrane electrodes are commercially available in some variety. In all these instruments the ''diffusion current'' is linearly proportional to the concentration of molecular oxygen. The current can be converted easily to concentration units (e.g., milligrams per liter) by a number of calibration procedures.

Membrane electrodes exhibit a relatively high temperature coefficient largely due to changes in the membrane permeability.[6] The effect of temperature on the electrode sensitivity, ϕ (microamperes per milligram per liter), can be expressed by the following simplified relationship:[6]

$$\log \phi = 0.43\, mt + b$$

where:

t = temperature, °C,
m = constant that depends on the membrane material, and
b = constant that largely depends on membrane thickness.

If values of ϕ and m are determined for one temperature (ϕ_0 and t_0), it is possible to calculate the sensitivity at any desired temperature (ϕ and t) as follows:

$$\log \phi = \log \phi_0 + 0.43\, m\, (t - t_0)$$

Nomographic charts for temperature correction can be constructed easily[7] and are available from some manufacturers. An example is shown in Figure 4500-O:2, in which, for simplicity, sensitivity is plotted versus temperature on semilogarithmic coordinates. Check one or two points frequently to confirm original calibration. If calibration changes, the new calibration should be parallel to the original, provided that the same membrane material is used.

Temperature compensation also can be made automatically by using thermistors in the electrode circuit.[4] However, thermistors may not compensate fully over a wide temperature

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range. For certain applications where high accuracy is required, use calibrated nomographic charts to correct for temperature effect.

To use the DO membrane electrode in estuarine waters or in wastewaters with varying ionic strength, correct for effect of salting-out on electrode sensitivity.[6,7] This effect is particularly significant for large changes in salt content. Electrode sensitivity varies with salt concentration according to the following relationship:

$$\log \phi_S = 0.43\, m_S C_S + \log \phi_0$$

where:

ϕ_S, ϕ_0 = sensitivities in salt solution and distilled water, respectively,
C_S = salt concentration (preferably ionic strength), and
m_S = constant (salting-out coefficient).

If ϕ_0 and m_S are determined, it is possible to calculate sensitivity for any value of C_S. Conductivity measurements can be used to approximate salt concentration (C_S). This is particularly applicable to estuarine waters. Figure 4500-O:3 shows calibration curves for sensitivity of varying salt solutions at different temperatures.

b. Interference: Plastic films used with membrane electrode systems are permeable to a variety of gases besides oxygen, although none is depolarized easily at the indicator electrode. Prolonged use of membrane electrodes in waters containing such gases as hydrogen sulfide (H_2S) tends to lower cell sensitivity. Eliminate this interference by frequently changing and calibrating the membrane electrode.

c. Sampling: Because membrane electrodes offer the advantage of analysis in situ they eliminate errors caused by sample handling and storage. If sampling is required, use the same precautions suggested for the iodometric method.

2. Apparatus

Oxygen-sensitive membrane electrode, polarographic or galvanic, with appropriate meter.

3. Procedure

a. Calibration: Follow manufacturer's calibration procedure exactly to obtain guaranteed precision and accuracy. Generally, calibrate membrane electrodes by reading against air or a sample of known DO concentration (determined by iodometric method) as well as in a sample with zero DO. (Add excess sodium sulfite, Na_2SO_3, and a trace of cobalt chloride, $CoCl_2$, to bring DO to zero.) Preferably calibrate with samples of water under test. Avoid an iodometric calibration where interfering substances are suspected. The following illustrate the recommended procedures:

1) Fresh water—For unpolluted samples where interfering substances are absent, calibrate in

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the test solution or distilled water, whichever is more convenient.

2) Salt water—Calibrate directly with samples of seawater or waters having a constant salt concentration in excess of 1000 mg/L.

3) Fresh water containing pollutants or interfering substances— Calibrate with distilled water because erroneous results occur with the sample.

4) Salt water containing pollutants or interfering substances— Calibrate with a sample of clean water containing the same salt content as the sample. Add a concentrated potassium chloride (KCl) solution (see Conductivity, Section 2510 and Table 2510:I) to distilled water to produce the same specific conductance as that in the sample. For polluted ocean waters, calibrate with a sample of unpolluted seawater.

5) Estuary water containing varying quantities of salt—Calibrate with a sample of uncontaminated seawater or distilled or tap water. Determine sample chloride or salt concentration and revise calibration to account for change of oxygen solubility in the estuary water.[7]

b. Sample measurement: Follow all precautions recommended by manufacturer to insure acceptable results. Take care in changing membrane to avoid contamination of sensing element and also trapping of minute air bubbles under the membrane, which can lead to lowered response and high residual current. Provide sufficient sample flow across membrane surface to overcome erratic response (see Figure 4500-O:4 for a typical example of the effect of stirring).

c. Validation of temperature effect: Check frequently one or two points to verify temperature correction data.

4. Precision and Bias

With most commercially available membrane electrode systems an accuracy of ±0.1 mg DO/L and a precision of ±0.05 mg DO/ L can be obtained.

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4500-O_3 OZONE (RESIDUAL)*#(72)

4500-O_3 A. Introduction

1. Sources

Ozone, a potent germicide, is used also as an oxidizing agent for the oxidation of organic compounds that produce taste and odor in drinking water, for the destruction of organic coloring matter, and for the oxidation of reduced iron or manganese salts to insoluble oxides.

2. Selection of Method

Ozone residual in water is determined by the indigo method. Residual ozone decays rapidly. Depending on water quality, the ozone residual half-life may be several seconds to a few minutes. Methods also are available for determining ozone in process gases.[1,2]

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4500-O_3 B. Indigo Colorimetric Method

1. General Discussion

The indigo colorimetric method is quantitative, selective, and simple; it replaces methods based on the measurement of total oxidant. The method is applicable to lake water, river infiltrate, manganese-containing groundwaters, extremely hard groundwaters, and even biologically treated domestic wastewaters.

a. Principle: In acidic solution, ozone rapidly decolorizes indigo. The decrease in absorbance is linear with increasing concentration. The proportionality constant at 600 nm is 0.42 ± 0.01/cm/mg/L (Δ = 20 000/*M*·cm) compared to the ultraviolet absorption of pure ozone of = 2950/*M*·cm at 258 nm).[1]

b. Interferences: Hydrogen peroxide (H_2O_2) and organic peroxides decolorize the indigo reagent very slowly. H_2O_2 does not interfere if ozone is measured in less than 6 h after adding reagents. Organic peroxides may react more rapidly. Fe(III) does not interfere. Mn(II) does not interfere but it is oxidized by ozone to forms that decolorize the reagent. Correct for this decolorization by making the measurement relative to a blank in which the ozone has been destroyed selectively. Without the corrective procedure, 0.1 mg/L ozonated manganese gives a response of about 0.08 mg/L apparent ozone. Chlorine also interferes. Low concentrations of chlorine (<0.1 mg/L) can be masked by malonic acid. Bromine, which can be formed by oxidation of Br^-, interferes (1 mole HOBr corresponds to 0.4 mole ozone). In the presence of HOBr or chlorine in excess of 0.1 mg/L, an accurate measurement of ozone cannot be made with this method.

c. Minimum detectable concentration: For the spectrophotometric procedure using thermostated cells and a high-quality photometer, the low-range procedure will measure down to 2 µg O_3/L. The practical lower limit for residual measurement is 10 to 20 µg/L.

d. Sampling: React sample with indigo as quickly as possible, because the residual may decay rapidly. Avoid loss of ozone residual due to off-gassing during sample collection. Do not run sample down side of flask. Add sample so that completely decolorized zones are eliminated quickly by swirling or stirring.

2. Apparatus

Photometer: Spectrophotometer or filter colorimeter for use at 600 ± 10 nm.

3. Reagents

a. Indigo stock solution: Add about 500 mL distilled water and 1 mL conc phosphoric acid to a 1-L volumetric flask. With stirring, add 770 mg potassium indigo trisulfonate, $C_{16}H_7N_2O_{11}S_3K_3$ (use only high-grade reagent, commercially available at about 80 to 85%

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purity). Fill to mark with distilled water. A 1:100 dilution exhibits an absorbance of 0.20 ± 0.010 cm at 600 nm. The stock solution is stable for about 4 months when stored in the dark. Discard when absorbance of a 1:100 dilution falls below 0.16/ cm. Do not change concentration of dye for higher ranges of ozone residual. Volume of dye used may be adjusted.

b. Indigo reagent I: To a 1-L volumetric flask add 20 mL indigo stock solution, 10 g sodium dihydrogen phosphate (NaH_2PO_4), and 7 mL conc phosphoric acid. Dilute to mark. Prepare solution fresh when its absorbance decreases to less than 80% of its initial value, typically within a week.

c. Indigo reagent II: Proceed as with indigo reagent I, but add 100 mL indigo stock solution instead of 20 mL.

d. Malonic acid reagent: Dissolve 5 g malonic acid in water and dilute to 100 mL.

e. Glycine reagent: Dissolve 7 g glycine in water and dilute to 100 mL.

4. Procedure

a. Spectrophotometric, volumetric procedure:

1) Concentration range 0.01 to 0.1 mg O_3/L—Add 10.0 mL indigo reagent I to each of two 100-mL volumetric flasks. Fill one flask (blank) to mark with distilled water. Fill other flask to mark with sample. Measure absorbance of both solutions at ± 10 nm as soon as possible but at least within 4 h. Preferably use 10-cm cells. Calculate the ozone concentration from the difference between the absorbances found in sample and blank (¶ 5*a* below). (NOTE: A maximum delay of 4 h before spectrophotometric reading can be tolerated only for drinking water samples. For other sample types that cannot be read immediately, determine the relationship between time and absorbance.)

2) Range 0.05 to 0.5 mg O_3/L—Proceed as above using 10.0 mL indigo reagent II instead of reagent I. Preferably measure absorbance in 4- or 5-cm cells.

3) Concentrations greater than 0.3 mg O_3/L—Proceed using indigo reagent II, but for these higher ozone concentrations use a correspondingly smaller sample volume. Dilute resulting mixture to 100 mL with distilled water.

4) Control of interferences—In presence of low chlorine concentration (<0.1 mg/L), place 1 mL malonic acid reagent in both flasks before adding sample and/or filling to mark. Measure absorbance as soon as possible, within 60 min (Br^-, Br_2, and HOBr are only partially masked by malonic acid).

In presence of manganese prepare a blank solution using sample, in which ozone is selectively destroyed by addition of glycine. Place 0.1 mL glycine reagent in 100-mL volumetric flask (blank) and 10.0 mL indigo reagent II in second flask (sample). Pipet exactly the same volume of sample into each flask. Adjust dose so that decolorization in second flask is easily visible but complete bleaching does not result (maximum 80 mL).

Insure that pH of glycine/sample mixture in blank flask (before adding indigo) is not below 6

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because reaction between ozone and glycine becomes very slow at low pH. Stopper flasks and mix by carefully inverting. Add 10.0 mL indigo reagent II to blank flask only 30 to 60 s after sample addition. Fill both flasks to the mark with ozone-free water and mix thoroughly. Measure absorbance of both solutions at comparable contact times of approximately 30 to 60 min (after this time, residual manganese oxides further discolor indigo only slowly and the drift of absorbance in blank and sample become comparable). Reduced absorbance in blank flask results from manganese oxides while that in sample flask is due to ozone plus manganese oxide.

5) Calibration—Because ozone is unstable, base measurements on known and constant loss of absorbance of the indigo reagent (f = 0.42 + 0.01/cm/mg O_3/L). For maximum accuracy analyze the lot of potassium indigo trisulfonate (no commercial lot has been found to deviate from f = 0.42) using the iodometric procedure.

When using a filter photometer, readjust the conversion factor, f, by comparing photometer sensitivity with absorbance at 600 nm by an accurate spectrophotometer.

b. Spectrophotometric, gravimetric procedure:

1) Add 10.0 mL indigo reagent II to 100-mL volumetric flask and fill flask (blank) to mark with distilled water. Obtain tare weight of a second flask (volumetric or erlenmeyer). Add 10.0 mL indigo reagent II to second flask. Fill directly with sample (do not run water down side), and swirl second flask until blue solution has turned to a light blue color. Weigh flask containing indigo and sample.

2) Preferably using 10-cm cells, measure absorbance of both solutions at 600 ± 10 nm as soon as possible, but at least within 4 h. NOTE: A maximum delay of 4 h before spectrophotometric reading is suitable only for drinking water samples. For other sample types, test the time drift.

5. Calculations

a. Spectrophotometric, volumetric method:

$$\text{mg } O_3/\text{L} = \frac{100 \times \Delta A}{f \times b \times V}$$

where:

ΔA = difference in absorbance between sample and blank,
b = path length of cell, cm,
V = volume of sample, mL (normally 90 mL), and
f = 0.42.

The factor f is based on a sensitivity factor of 20 000/cm for the change of absorbance (600

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nm) per mole of added ozone per liter. It was calibrated by iodometric titration. The UV absorbance of ozone in pure water may serve as a secondary standard: the factor f = 0.42 corresponds to an absorption coefficient for aqueous ozone, = 2950/M·cm at 258 nm.

b. Spectrophotometric, gravimetric method:

$$\text{mg } O_3/L = \frac{(A_B \times 100) - (A_S \times V_T)}{f \times V_S \times b}$$

where:

A_B, A_S = absorbance of blank and sample, respectively,

V_S = volume of sample, mL = [(final weight − tare weight) g × 1.0 mL/g] − 10 mL,

V_T = total volume of sample plus indigo, mL = (final weight − tare weight) g × 1.0 mL/g,

b = path length of cell, cm, and

f = 0.42 (see ¶ a above).

6. Precision and Bias

For the spectrophotometric volumetric procedure in the absence of interferences, the relative error is less than 5% without special sampling setups. In laboratory testing this may be reduced to 1%. No data are available for the spectrophotometric gravimetric procedure.

Because this method is based on the differences in absorbance between the sample and blank (ΔA) the method is not applicable in the presence of chlorine. If the manganese content exceeds the ozone, precision is reduced. If the ratio of manganese to ozone is less than 10:1, ozone concentrations above 0.02 mg/L may be determined with a relative error of less than 20%.

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4500-P PHOSPHORUS*#(73)

4500-P A. Introduction

1. Occurrence

Phosphorus occurs in natural waters and in wastewaters almost solely as phosphates. These are classified as orthophosphates, condensed phosphates (pyro-, meta-, and other polyphosphates), and organically bound phosphates. They occur in solution, in particles or detritus, or in the bodies of aquatic organisms.

These forms of phosphate arise from a variety of sources. Small amounts of orthophosphate or certain condensed phosphates are added to some water supplies during treatment. Larger quantities of the same compounds may be added when the water is used for laundering or other cleaning, because these materials are major constituents of many commercial cleaning preparations. Phosphates are used extensively in the treatment of boiler waters. Orthophosphates applied to agricultural or residential cultivated land as fertilizers are carried into surface waters with storm runoff and to a lesser extent with melting snow. Organic phosphates are formed primarily by biological processes. They are contributed to sewage by body wastes and food residues, and also may be formed from orthophosphates in biological treatment processes or by receiving water biota.

Phosphorus is essential to the growth of organisms and can be the nutrient that limits the primary productivity of a body of water. In instances where phosphate is a growth-limiting nutrient, the discharge of raw or treated wastewater, agricultural drainage, or certain industrial wastes to that water may stimulate the growth of photosynthetic aquatic micro- and macroorganisms in nuisance quantities.

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Phosphates also occur in bottom sediments and in biological sludges, both as precipitated inorganic forms and incorporated into organic compounds.

2. Definition of Terms

Phosphorus analyses embody two general procedural steps: (*a*+) conversion of the phosphorus form of interest to dissolved orthophosphate, and (*b*) colorimetric determination of dissolved orthophosphate. The separation of phosphorus into its various forms is defined analytically but the analytical differentiations have been selected so that they may be used for interpretive purposes.

Filtration through a 0.45-µm-pore-diam membrane filter separates dissolved from suspended forms of phosphorus. No claim is made that filtration through 0.45-µm filters is a true separation of suspended and dissolved forms of phosphorus; it is merely a convenient and replicable analytical technique designed to make a gross separation.

Membrane filtration is selected over depth filtration because of the greater likelihood of obtaining a consistent separation of particle sizes. Prefiltration through a glass fiber filter may be used to increase the filtration rate.

Phosphates that respond to colorimetric tests without preliminary hydrolysis or oxidative digestion of the sample are termed ‘‘reactive phosphorus.’’ While reactive phosphorus is largely a measure of orthophosphate, a small fraction of any condensed phosphate present usually is hydrolyzed unavoidably in the procedure. Reactive phosphorus occurs in both dissolved and suspended forms.

Acid hydrolysis at boiling-water temperature converts dissolved and particulate condensed phosphates to dissolved orthophosphate. The hydrolysis unavoidably releases some phosphate from organic compounds, but this may be reduced to a minimum by judicious selection of acid strength and hydrolysis time and temperature. The term ‘‘acid-hydrolyzable phosphorus’’ is preferred over ‘‘condensed phosphate’’ for this fraction.

The phosphate fractions that are converted to orthophosphate only by oxidation destruction of the organic matter present are considered ‘‘organic’’ or ‘‘organically bound’’ phosphorus. The severity of the oxidation required for this conversion depends on the form—and to some extent on the amount—of the organic phosphorus present. Like reactive phosphorus and acid-hydrolyzable phosphorus, organic phosphorus occurs both in the dissolved and suspended fractions.

The total phosphorus as well as the dissolved and suspended phosphorus fractions each may be divided analytically into the three chemical types that have been described: reactive, acid-hydrolyzable, and organic phosphorus. Figure 4500-P:1 shows the steps for analysis of individual phosphorus fractions. As indicated, determinations usually are conducted only on the unfiltered and filtered samples. Suspended fractions generally are determined by difference; however, they may be determined directly by digestion of the material retained on a glass-fiber filter.

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3. Selection of Method

a. Digestion methods: Because phosphorus may occur in combination with organic matter, a digestion method to determine total phosphorus must be able to oxidize organic matter effectively to release phosphorus as orthophosphate. Three digestion methods are given in Section 4500-P.B.3, Section 4500-P.B.4, and Section 4500-P.B.5. The perchloric acid method, the most drastic and time-consuming method, is recommended only for particularly difficult samples such as sediments. The nitric acid-sulfuric acid method is recommended for most samples. By far the simplest method is the persulfate oxidation technique. Persulfate oxidation is coupled with ultraviolet light for a more efficient digestion in an automated in-line digestion/determination by flow injection analysis (4500-P.I). It is recommended that persulfate oxidation methods be checked against one or more of the more drastic digestion techniques and be adopted if identical recoveries are obtained.

After digestion, determine liberated orthophosphate by Method C, D, E, F, G, or H. The colorimetric method used, rather than the digestion procedure, governs in matters of interference and minimum detectable concentration.

b. Colorimetric method: Three methods of orthophosphate determination are described. Selection depends largely on the concentration range of orthophosphate. The vanadomolybdophosphoric acid method (C) is most useful for routine analysis in the range of 1 to 20 mg P/L. The stannous chloride method (D) or the ascorbic acid method (E) is more suited for the range of 0.01 to 6 mg P/L. An extraction step is recommended for the lower levels of this range and when interferences must be overcome. Automated versions of the ascorbic acid method (F, G, and H) also are presented. Careful attention to procedure may allow application of these methods to very low levels of phosphorus, such as those found in unimpaired fresh water.

Ion chromatography (Section 4110) and capillary ion electrophoresis (Section 4140) are useful for determination of orthophosphate in undigested samples.

4. Precision and Bias

To aid in method selection, Table 4500-P:I presents the results of various combinations of digestions, hydrolysis, and colorimetric techniques for three synthetic samples of the following compositions:

Sample 1: 100 µg orthosphosphate phosphorus (PO_4^{3-}-P/L), 80 µg condensed phosphate phosphorus/L (sodium hexametaphosphate), 30 µg organic phosphorus/L (adenylic acid), 1.5 mg NH_3-N/L, 0.5 mg NO_3-N/L, and 400 mg Cl^-/L.

Sample 2: 600 µg PO_4^{3-}-P/L, 300 µg condensed phosphate phosphorus/L (sodium hexametaphosphate), 90 µg organic phosphorus/L (adenylic acid), 0.8 mg NH_3-N/L, 5.0 mg NO_3^--N/L, and 400 mg Cl^-/L.

Sample 3: 7.00 mg PO_4^{3-}-P/L, 3.00 mg condensed phosphate phosphorus/L (sodium

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hexametaphosphate), 0.230 mg organic phosphorus/L (adenylic acid), 0.20 mg NH_3-N/L, 0.05 mg NO_3^-- N/L, and 400 mg Cl^-/L.

5. Sampling and Storage

If dissolved phosphorus forms are to be differentiated, filter sample immediately after collection. Preserve by freezing at or below −10°C. In some cases 40 mg $HgCl_2$/L may be added to the samples, especially when they are to be stored for long periods before analysis. CAUTION: *$HgCl_2$ is a hazardous substance; take appropriate precautions in disposal; use of $HgCl_2$ is not encouraged.* Do not add either acid or $CHCl_3$ as a preservative when phosphorus forms are to be determined. If total phosphorus alone is to be determined, add H_2SO_4 or HCl to pH<2 and cool to 4°C, or freeze without any additions.

Do not store samples containing low concentrations of phosphorus in plastic bottles unless kept in a frozen state because phosphates may be adsorbed onto the walls of plastic bottles.

Rinse all glass containers with hot dilute HCl, then rinse several times in reagent water. Never use commercial detergents containing phosphate for cleaning glassware used in phosphate analysis.

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4500-P B. Sample Preparation

For information on selection of digestion method (¶s 3 through 5 below), see 4500-P.A.3*a*.

1. Preliminary Filtration

Filter samples for determination of dissolved reactive phosphorus, dissolved acid-hydrolyzable phosphorus, and total dissolved phosphorus through 0.45-µm membrane filters. A glass fiber filter may be used to prefilter hard-to-filter samples.

Wash membrane filters by soaking in distilled water before use because they may contribute significant amounts of phosphorus to samples containing low concentrations of phosphate. Use

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one of two washing techniques: (*a*) soak 50 filters in 2 L distilled water for 24 h; (*b*) soak 50 filters in 2 L distilled water for 1 h, change distilled water, and soak filters an additional 3 h. Membrane filters also may be washed by running several 100-mL portions of distilled water through them. This procedure requires more frequent determination of blank values to ensure consistency in washing and to evaluate different lots of filters.

2. Preliminary Acid Hydrolysis

The acid-hydrolyzable phosphorus content of the sample is defined operationally as the difference between reactive phosphorus as measured in the untreated sample and phosphate found after mild acid hydrolysis. Generally, it includes condensed phosphates such as pyro-, tripoly-, and higher-molecular-weight species such as hexametaphosphate. In addition, some natural waters contain organic phosphate compounds that are hydrolyzed to orthophosphate under the test conditions. Polyphosphates generally do not respond to reactive phosphorus tests but can be hydrolyzed to orthophosphate by boiling with acid.

After hydrolysis, determine reactive phosphorus by a colorimetric method (C, D, or E). Interferences, precision, bias, and sensitivity will depend on the colorimetric method used.

a. Apparatus:

Autoclave or pressure cooker, capable of operating at 98 to 137 kPa.

b. Reagents:

1) *Phenolphthalein indicator aqueous solution.*

2) *Strong acid solution:* Slowly add 300 mL conc H_2SO_4 to about 600 mL distilled water. When cool, add 4.0 mL conc HNO_3 and dilute to 1 L.

3) Sodium hydroxide, NaOH, 6*N*.

c. Procedure: To 100-mL sample or a portion diluted to 100 mL, add 0.05 mL (1 drop) phenolphthalein indicator solution. If a red color develops, add strong acid solution dropwise, to just discharge the color. Then add 1 mL more.

Boil gently for at least 90 min, adding distilled water to keep the volume between 25 and 50 mL. Alternatively, heat for 30 min in an autoclave or pressure cooker at 98 to 137 kPa. Cool, neutralize to a faint pink color with NaOH solution, and restore to the original 100-mL volume with distilled water.

Prepare a calibration curve by carrying a series of standards containing orthophosphate (see colorimetric method C, D, or E) through the hydrolysis step. Do not use orthophosphate standards without hydrolysis, because the salts added in hydrolysis cause an increase in the color intensity in some methods.

Determine reactive phosphorus content of treated portions, using Method C, D, or E. This gives the sum of polyphosphate and orthophosphate in the sample. To calculate its content of acid-hydrolyzable phosphorus, determine reactive phosphorus in a sample portion that has not been hydrolyzed, using the same colorimetric method as for treated sample, and subtract.

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3. Perchloric Acid Digestion

a. Apparatus:

1) *Hot plate:* A 30- × 50-cm heating surface is adequate.

2) *Safety shield.*

3) *Safety goggles.*

4) *Erlenmeyer flasks,* 125-mL, acid-washed and rinsed with distilled water.

b. Reagents:

1) *Nitric acid,* HNO_3, conc.

2) *Perchloric acid,* $HClO_4 \cdot 2H_2O$, purchased as 70 to 72% $HClO_4$, reagent-grade.

3) *Sodium hydroxide,* NaOH, 6*N*.

4) *Methyl orange indicator solution.*

5) *Phenolphthalein indicator aqueous solution.*

c. Procedure: CAUTION—*Heated mixtures of $HClO_4$ and organic matter may explode violently. Avoid this hazard by taking the following precautions: (a) Do not add $HClO_4$ to a hot solution that may contain organic matter. (b) Always initiate digestion of samples containing organic matter with HNO_3. Complete digestion using the mixture of HNO_3 and $HClO_4$. (c) Do not fume with $HClO_4$ in ordinary hoods. Use hoods especially constructed for $HClO_4$ fuming or a glass fume eradicator*#(74) connected to a water pump. (d) Never let samples being digested with $HClO_4$ evaporate to dryness.*

Measure sample containing the desired amount of phosphorus (this will be determined by whether Method C, D, or E is to be used) into a 125-mL erlenmeyer flask. Acidify to methyl orange with conc HNO_3, add another 5 mL conc HNO_3, and evaporate on a steam bath or hot plate to 15 to 20 mL.

Add 10 mL each of conc HNO_3 and $HClO_4$ to the 125-mL conical flask, cooling the flask between additions. Add a few boiling chips, heat on a hot plate, and evaporate gently until dense white fumes of $HClO_4$ just appear. If solution is not clear, cover neck of flask with a watch glass and keep solution barely boiling until it clears. If necessary, add 10 mL more HNO_3 to aid oxidation.

Cool digested solution and add 1 drop aqueous phenolphthalein solution. Add 6*N* NaOH solution until the solution just turns pink. If necessary, filter neutralized solution and wash filter liberally with distilled water. Make up to 100 mL with distilled water.

Determine the PO_4^{3-}-P content of the treated sample by Method C, D, or E.

Prepare a calibration curve by carrying a series of standards containing orthophosphate (see Method C, D, or E) through digestion step. Do not use orthophosphate standards without

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treatment.

4. Sulfuric Acid-Nitric Acid Digestion

a. Apparatus:

1) *Digestion rack:* An electrically or gas-heated digestion rack with provision for withdrawal of fumes is recommended. Digestion racks typical of those used for micro-kjeldahl digestions are suitable.

2) *Micro-kjeldahl flasks.*

b. Reagents:

1) *Sulfuric acid,* H_2SO_4, conc.

2) *Nitric acid,* HNO_3, conc.

3) *Phenolphthalein indicator aqueous solution.*

4) *Sodium hydroxide,* NaOH, 1*N*.

c. Procedure: Into a micro-kjeldahl flask, measure a sample containing the desired amount of phosphorus (this is determined by the colorimetric method used). Add 1 mL conc H_2SO_4 and 5 mL conc HNO_3.

Digest to a volume of 1 mL and then continue until solution becomes colorless to remove HNO_3.

Cool and add approximately 20 mL distilled water, 0.05 mL (1 drop) phenolphthalein indicator, and as much 1*N* NaOH solution as required to produce a faint pink tinge. Transfer neutralized solution, filtering if necessary to remove particulate material or turbidity, into a 100-mL volumetric flask. Add filter washings to flask and adjust sample volume to 100 mL with distilled water.

Determine phosphorus by Method C, D, or E, for which a separate calibration curve has been constructed by carrying standards through the acid digestion procedure.

5. Persulfate Digestion Method

a. Apparatus:

1) *Hot plate:* A 30- × 50-cm heating surface is adequate.

2) *Autoclave:* An autoclave or pressure cooker capable of developing 98 to 137 kPa may be used in place of a hot plate.

3) *Glass scoop,* to hold required amounts of persulfate crystals.

b. Reagents:

1) *Phenolphthalein indicator aqueous solution.*

2) *Sulfuric acid solution:* Carefully add 300 mL conc H_2SO_4 to approximately 600 mL distilled water and dilute to 1 L with distilled water.

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3) *Ammonium persulfate,* $(NH_4)_2S_2O_8$, solid, or potassium persulfate, $K_2S_2O_8$, solid.

4) *Sodium hydroxide,* NaOH, 1*N*.

c. Procedure: Use 50 mL or a suitable portion of thoroughly mixed sample. Add 0.05 mL (1 drop) phenolphthalein indicator solution. If a red color develops, add H_2SO_4 solution dropwise to just discharge the color. Then add 1 mL H_2SO_4 solution and either 0.4 g solid $(NH_4)_2S_2O_8$ or 0.5 g solid $K_2S_2O_8$.

Boil gently on a preheated hot plate for 30 to 40 min or until a final volume of 10 mL is reached. Organophosphorus compounds such as AMP may require as much as 1.5 to 2 h for complete digestion. Cool, dilute to 30 mL with distilled water, add 0.05 mL (1 drop) phenolphthalein indicator solution, and neutralize to a faint pink color with NaOH. Alternatively, heat for 30 min in an autoclave or pressure cooker at 98 to 137 kPa. Cool, add 0.05 mL (1 drop) phenolphthalein indicator solution, and neutralize to a faint pink color with NaOH. Make up to 100 mL with distilled water. In some samples a precipitate may form at this stage, but do not filter. For any subsequent subdividing of the sample, shake well. The precipitate (which is possibly a calcium phosphate) redissolves under the acid conditions of the colorimetric reactive phosphorus test. Determine phosphorus by Method C, D, or E, for which a separate calibration curve has been constructed by carrying standards through the persulfate digestion procedure.

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4500-P C. Vanadomolybdophosphoric Acid Colorimetric Method

1. General Discussion

a. Principle: In a dilute orthophosphate solution, ammonium molybdate reacts under acid conditions to form a heteropoly acid, molybdophosphoric acid. In the presence of vanadium, yellow vanadomolybdophosphoric acid is formed. The intensity of the yellow color is proportional to phosphate concentration.

b. Interference: Positive interference is caused by silica and arsenate only if the sample is heated. Negative interferences are caused by arsenate, fluoride, thorium, bismuth, sulfide, thiosulfate, thiocyanate, or excess molybdate. Blue color is caused by ferrous iron but this does not affect results if ferrous iron concentration is less than 100 mg/L. Sulfide interference may be removed by oxidation with bromine water. Ions that do not interfere in concentrations up to 1000

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mg/L are Al^{3+}, Fe^{3+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Li^{+}, Na^{+}, K^{+}, NH_4^{+}, Cd^{2+}, Mn^{2+}, Pb^{2+}, Hg^{+}, Hg^{2+}, Sn^{2+}, Cu^{2+}, Ni^{2+}, Ag^{+}, U^{4+}, Zr^{4+}, AsO_3^{-}, Br^{-}, CO_3^{2-}, ClO_4^{-}, CN^{-}, IO_3^{-}, SiO_4^{4-}, NO_3^{-}, NO_2^{-}, SO_4^{2-}, SO_3^{2-}, pyrophosphate, molybdate, tetraborate, selenate, benzoate, citrate, oxalate, lactate, tartrate, formate, and salicylate. If HNO_3 is used in the test, Cl^{-} interferes at 75 mg/L.

c. Minimum detectable concentration: The minimum detectable concentration is 200 µg P/L in 1-cm spectrophotometer cells.

2. Apparatus

a. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer,* for use at 400 to 490 nm.

2) *Filter photometer,* provided with a blue or violet filter exhibiting maximum transmittance between 400 and 470 nm.

The wavelength at which color intensity is measured depends on sensitivity desired, because sensitivity varies tenfold with wavelengths 400 to 490 nm. Ferric iron causes interference at low wavelengths, particularly at 400 nm. A wavelength of 470 nm usually is used. Concentration ranges for different wavelengths are:

P Range *mg/L*	Wavelength *nm*
1.0– 5.0	400
2.0–10	420
4.0–18	470

b. Acid-washed glassware: Use acid-washed glassware for determining low concentrations of phosphorus. Phosphate contamination is common because of its absorption on glass surfaces. Avoid using commercial detergents containing phosphate. Clean all glassware with hot dilute HCl and rinse well with distilled water. Preferably, reserve the glassware only for phosphate determination, and after use, wash and keep filled with water until needed. If this is done, acid treatment is required only occasionally.

*c. Filtration apparatus and filter paper.**#(75)

3. Reagents

a. Phenolphthalein indicator aqueous solution.

b. Hydrochloric acid, HCl, 1 + 1. H_2SO_4, $HClO_4$, or HNO_3 may be substituted for HCl. The acid concentration in the determination is not critical but a final sample concentration of 0.5*N* is recommended.

c. Activated carbon.†#(76) Remove fine particles by rinsing with distilled water.

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d. Vanadate-molybdate reagent:

1) *Solution A:* Dissolve 25 g ammonium molybdate, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, in 300 mL distilled water.

2) *Solution B:* Dissolve 1.25 g ammonium metavanadate, NH_4VO_3, by heating to boiling in 300 mL distilled water. Cool and add 330 mL conc HCl. Cool Solution B to room temperature, pour Solution A into Solution B, mix, and dilute to 1 L.

e. Standard phosphate solution: Dissolve in distilled water 219.5 mg anhydrous KH_2PO_4 and dilute to 1000 mL; 1.00 mL = 50.0 µg PO_4^{3-}-P.

4. Procedure

a. Sample pH adjustment: If sample pH is greater than 10, add 0.05 mL (1 drop) phenolphthalein indicator to 50.0 mL sample and discharge the red color with 1 + 1 HCl before diluting to 100 mL.

b. Color removal from sample: Remove excessive color in sample by shaking about 50 mL with 200 mg activated carbon in an erlenmeyer flask for 5 min and filter to remove carbon. Check each batch of carbon for phosphate because some batches produce high reagent blanks.

c. Color development in sample: Place 35 mL or less of sample, containing 0.05 to 1.0 mg P, in a 50-mL volumetric flask. Add 10 mL vanadate-molybdate reagent and dilute to the mark with distilled water. Prepare a blank in which 35 mL distilled water is substituted for the sample. After 10 min or more, measure absorbance of sample versus a blank at a wavelength of 400 to 490 nm, depending on sensitivity desired (see ¶ 2*a* above). The color is stable for days and its intensity is unaffected by variation in room temperature.

d. Preparation of calibration curve: Prepare a calibration curve by using suitable volumes of standard phosphate solution and proceeding as in ¶ 4*c*. When ferric ion is low enough not to interfere, plot a family of calibration curves of one series of standard solutions for various wavelengths. This permits a wide latitude of concentrations in one series of determinations. Analyze at least one standard with each set of samples.

5. Calculation

$$\text{mg P/L} = \frac{\text{mg P(in 50 mL final volume)} \times 1000}{\text{mL sample}}$$

6. Precision and Bias

See Table 4500-P:I.

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4500-P D. Stannous Chloride Method

1. General Discussion

a. Principle: Molybdophosphoric acid is formed and reduced by stannous chloride to intensely colored molybdenum blue. This method is more sensitive than Method C and makes feasible measurements down to 7 µg P/L by use of increased light path length. Below 100 µg P/L an extraction step may increase reliability and lessen interference.

b. Interference: See Section 4500-P.C.1*b*.

c. Minimum detectable concentration: The minimum detectable concentration is about 3 µg P/L. The sensitivity at 0.3010 absorbance is about 10 µg P/L for an absorbance change of 0.009.

2. Apparatus

The same apparatus is required as for Method C, except that a pipetting bulb is required for the extraction step. Set spectrophotometer at 625 nm in the measurement of benzene-isobutanol extracts and at 690 nm for aqueous solutions. If the instrument is not equipped to read at 690 nm, use a wavelength of 650 nm for aqueous solutions, with somewhat reduced sensitivity and precision.

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3. Reagents

a. Phenolphthalein indicator aqueous solution.

b. Strong-acid solution: Prepare as directed in Section 4500-P.B.2*b*2).

c. Ammonium molybdate reagent I: Dissolve 25 g $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in 175 mL distilled water. Cautiously add 280 mL conc H_2SO_4 to 400 mL distilled water. Cool, add molybdate solution, and dilute to 1 L.

d. Stannous chloride reagent I: Dissolve 2.5 g fresh $SnCl_2\cdot 2H_2O$ in 100 mL glycerol. Heat in a water bath and stir with a glass rod to hasten dissolution. This reagent is stable and requires neither preservatives nor special storage.

e. Standard phosphate solution: Prepare as directed in Section 4500-P.C.3*e*.

f. Reagents for extraction:

1) *Benzene-isobutanol solvent:* Mix equal volumes of benzene and isobutyl alcohol. (CAUTION—*This solvent is highly flammable.*)

2) *Ammonium molybdate reagent II:* Dissolve 40.1 g $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in approximately 500 mL distilled water. Slowly add 396 mL ammonium molybdate reagent I. Cool and dilute to 1 L.

3) *Alcoholic sulfuric acid solution:* Cautiously add 20 mL conc H_2SO_4 to 980 mL methyl alcohol with continuous mixing.

4) *Dilute stannous chloride reagent II:* Mix 8 mL stannous chloride reagent I with 50 mL glycerol. This reagent is stable for at least 6 months.

4. Procedure

a. Preliminary sample treatment: To 100 mL sample containing not more than 200 µg P and free from color and turbidity, add 0.05 mL (1 drop) phenolphthalein indicator. If sample turns pink, add strong acid solution dropwise to discharge the color. If more than 0.25 mL (5 drops) is required, take a smaller sample and dilute to 100 mL with distilled water after first discharging the pink color with acid.

b. Color development: Add, with thorough mixing after each addition, 4.0 mL molybdate reagent I and 0.5 mL (10 drops) stannous chloride reagent I. Rate of color development and intensity of color depend on temperature of the final solution, each 1°C increase producing about 1% increase in color. Hence, hold samples, standards, and reagents within 2°C of one another and in the temperature range between 20 and 30°C.

c. Color measurement: After 10 min, but before 12 min, using the same specific interval for all determinations, measure color photometrically at 690 nm and compare with a calibration curve, using a distilled water blank. Light path lengths suitable for various concentration ranges are as follows:

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Approximate P Range mg/L	Light Path cm
0.3–2	0.5
0.1–1	2
0.007–0.2	10

Always run a blank on reagents and distilled water. Because the color at first develops progressively and later fades, maintain equal timing conditions for samples and standards. Prepare at least one standard with each set of samples or once each day that tests are made. The calibration curve may deviate from a straight line at the upper concentrations of the 0.3 to 2.0-mg/L range.

d. Extraction: When increased sensitivity is desired or interferences must be overcome, extract phosphate as follows: Pipet a 40-mL sample, or one diluted to that volume, into a 125-mL separatory funnel. Add 50.0 mL benzene-isobutanol solvent and 15.0 mL molybdate reagent II. Close funnel at once and shake vigorously for exactly 15 s. If condensed phosphate is present, any delay will increase its conversion to orthophosphate. Remove stopper and withdraw 25.0 mL of separated organic layer, using a pipet with safety bulb. Transfer to a 50-mL volumetric flask, add 15 to 16 mL alcoholic H_2SO_4 solution, swirl, add 0.50 mL (10 drops) dilute stannous chloride reagent II, swirl, and dilute to the mark with alcoholic H_2SO_4. Mix thoroughly. After 10 min, but before 30 min, read against the blank at 625 nm. Prepare blank by carrying 40 mL distilled water through the same procedure used for the sample. Read phosphate concentration from a calibration curve prepared by taking known phosphate standards through the same procedure used for samples.

5. Calculation

Calculate as follows:

a. Direct procedure:

$$\text{mg P/L} = \frac{\text{mg P (in approximately 104.5 mL final volume)} \times 1000}{\text{mL sample}}$$

b. Extraction procedure:

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$$\text{mg P/L} = \frac{\text{mg P (in 50 mL final volume)} \times 1000}{\text{mL sample}}$$

6. Precision and Bias

See Table 4500-P:I.

4500-P E. Ascorbic Acid Method

1. General Discussion

a. Principle: Ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid—phosphomolybdic acid—that is reduced to intensely colored molybdenum blue by ascorbic acid.

b. Interference: Arsenates react with the molybdate reagent to produce a blue color similar to that formed with phosphate. Concentrations as low as 0.1 mg As/L interfere with the phosphate determination. Hexavalent chromium and NO_2^- interfere to give results about 3% low at concentrations of 1 mg/L and 10 to 15% low at 10 mg/L. Sulfide (Na_2S) and silicate do not interfere at concentrations of 1.0 and 10 mg/L.

c. Minimum detectable concentration: Approximately 10 µg P/L. P ranges are as follows:

Approximate P Range *mg/L*	Light Path *cm*
0.30–2.0	0.5
0.15–1.30	1.0
0.01–0.25	5.0

2. Apparatus

a. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer,* with infrared phototube for use at 880 nm, providing a light path of 2.5 cm or longer.

2) *Filter photometer,* equipped with a red color filter and a light path of 0.5 cm or longer.

b. Acid-washed glassware: See Section 4500-P.C.2*b*.

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3. Reagents

a. Sulfuric acid, H_2SO_4, 5*N:* Dilute 70 mL conc H_2SO_4 to 500 mL with distilled water.

b. Potassium antimonyl tartrate solution: Dissolve 1.3715 g $K(SbO)C_4H_4O_6 \cdot {}^1/_2H_2O$ in 400 mL distilled water in a 500-mL volumetric flask and dilute to volume. Store in a glass-stoppered bottle.

c. Ammonium molybdate solution: Dissolve 20 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 500 mL distilled water. Store in a glass-stoppered bottle.

d. Ascorbic acid, 0.1*M:* Dissolve 1.76 g ascorbic acid in 100 mL distilled water. The solution is stable for about 1 week at 4°C.

e. Combined reagent: Mix the above reagents in the following proportions for 100 mL of the combined reagent: 50 mL 5*N* H_2SO_4, 5 mL potassium antimonyl tartrate solution, 15 mL ammonium molybdate solution, and 30 mL ascorbic acid solution. *Mix after addition of each reagent.* Let all reagents reach room temperature before they are mixed and mix in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. The reagent is stable for 4 h.

f. Stock phosphate solution: See Section 4500-P.C.3*e*.

g. Standard phosphate solution: Dilute 50.0 mL stock phosphate solution to 1000 mL with distilled water; 1.00 mL = 2.50 µg P.

4. Procedure

a. Treatment of sample: Pipet 50.0 mL sample into a clean, dry test tube or 125-mL erlenmeyer flask. Add 0.05 mL (1 drop) phenolphthalein indicator. If a red color develops add 5*N* H_2SO_4 solution dropwise to just discharge the color. Add 8.0 mL combined reagent and mix thoroughly. After at least 10 min but no more than 30 min, measure absorbance of each sample at 880 nm, using reagent blank as the reference solution.

b. Correction for turbidity or interfering color: Natural color of water generally does not interfere at the high wavelength used. For highly colored or turbid waters, prepare a blank by adding all reagents except ascorbic acid and potassium antimonyl tartrate to the sample. Subtract blank absorbance from absorbance of each sample.

c. Preparation of calibration curve: Prepare individual calibration curves from a series of six standards within the phosphate ranges indicated in ¶ 1*c* above. Use a distilled water blank with the combined reagent to make photometric readings for the calibration curve. Plot absorbance vs. phosphate concentration to give a straight line passing through the origin. Test at least one phosphate standard with each set of samples.

5. Calculation

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$$\text{mg P/L} = \frac{\text{mg P (in approximately 58 mL final volume)} \times 1000}{\text{mL sample}}$$

6. Precision and Bias

The precision and bias values given in Table 4500-P:I are for a single-solution procedure given in the 13th edition. The present procedure differs in reagent-to-sample ratios, no addition of solvent, and acidity conditions. It is superior in precision and bias to the previous technique in the analysis of both distilled water and river water at the 228-µg P/L level (Table 4500-P:II).

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4500-P F. Automated Ascorbic Acid Reduction Method

1. General Discussion

a. Principle: Ammonium molybdate and potassium antimonyl tartrate react with orthophosphate in an acid medium to form an antimony-phosphomolybdate complex, which, on reduction with ascorbic acid, yields an intense blue color suitable for photometric measurement.

b. Interferences: As much as 50 mg Fe^{3+}/L, 10 mg Cu/L, and 10 mg SiO_2/L can be tolerated. High silica concentrations cause positive interference.

In terms of phosphorus, the results are high by 0.005, 0.015, and 0.025 mg/L for silica concentrations of 20, 50, and 100 mg/ L, respectively. Salt concentrations up to 20% (w/v) cause an error of less than 1%. Arsenate (AsO_4^{3-}) is a positive interference.

Eliminate interference from NO_2^- and S^{2-} by adding an excess of bromine water or a saturated potassium permanganate ($KMnO_4$) solution. Remove interfering turbidity by filtration

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before analysis. Filter samples for total or total hydrolyzable phosphorus only after digestion. Sample color that absorbs in the photometric range used for analysis also will interfere. See also Section 4500-P.E.1*b*.

c. Application: Orthophosphate can be determined in potable, surface, and saline waters as well as domestic and industrial wastewaters over a range of 0.001 to 10.0 mg P/L when photometric measurements are made at 650 to 660 or 880 nm in a 15-mm or 50-mm tubular flow cell. Determine higher concentrations by diluting sample. Although the automated test is designed for orthophosphate only, other phosphorus compounds can be converted to this reactive form by various sample pretreatments described in Section 4500-P.B.1, Section 4500-P.B.2, and Section 4500-P.B.5.

2. Apparatus

a. Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the interchangeable components shown in Figure 4500-P:2. A flow cell of 15 or 50 mm and a filter of 650 to 660 or 880 nm may be used.

b. Hot plate or autoclave.

c. Acid-washed glassware: See Section 4500-P.C.2*b*.

3. Reagents

a. Potassium antimonyl tartrate solution: Dissolve 0.3 g $K(SbO)C_4H_4O_6 \cdot {}^1/_2H_2O$ in approximately 50 mL distilled water and dilute to 100 mL. Store at 4°C in a dark, glass-stoppered bottle.

b. Ammonium molybdate solution: Dissolve 4 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 100 mL distilled water. Store in a plastic bottle at 4°C.

c. Ascorbic acid solution: See Section 4500-P.E.3*d*.

d. Combined reagent: See Section 4500-P.E.3*e*.

e. Dilute sulfuric acid solution: Slowly add 140 mL conc H_2SO_4 to 600 mL distilled water. When cool, dilute to 1 L.

f. Ammonium persulfate, $(NH_4)_2S_2O_8$, crystalline.

g. Phenolphthalein indicator aqueous solution.

h. Stock phosphate solution: Dissolve 439.3 mg anhydrous KH_2PO_4, dried for 1 h at 105°C, in distilled water and dilute to 1000 mL; 1.00 mL = 100 µg P.

i. Intermediate phosphate solution: Dilute 100.0 mL stock phosphate solution to 1000 mL with distilled water; 1.00 mL = 10.0 µg P.

j. Standard phosphate solutions: Prepare a suitable series of standards by diluting appropriate volumes of intermediate phosphate solution.

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4. Procedure

Set up manifold as shown in Figure 4500-P:2 and follow the general procedure described by the manufacturer.

Add 0.05 mL (1 drop) phenolphthalein indicator solution to approximately 50 mL sample. If a red color develops, add H_2SO_4 (¶ 3*e*) dropwise to just discharge the color.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against P concentration in standards. Compute sample P concentration by comparing sample response with standard curve.

6. Precision and Bias

Six samples were analyzed in a single laboratory in septuplicate. At an average PO_4^{3-} concentration of 0.340 mg/L, the average deviation was 0.015 mg/L. The coefficient of variation was 6.2%. In two samples with added PO_4^{3-}, recoveries were 89 and 96%.

7. Bibliography

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4500-P G. Flow Injection Analysis for Orthophosphate (PROPOSED)

1. General Discussion

a. Principle: The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex that absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

Also see Section 4500-P.A, Section 4500-P.B, and Section 4500-P.F, and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation

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process.

Silica forms a pale blue complex that also absorbs at 880 nm. This interference is generally insignificant because a silica concentration of approximately 30 mg/L would be required to produce a 0.005 mg P/L positive error in orthophosphate.

Concentrations of ferric iron greater than 50 mg/L cause a negative error due to competition with the complex for the reducing agent ascorbic acid. Treat samples high in iron with sodium bisulfite to eliminate this interference, as well as the interference due to arsenates.

Glassware contamination is a problem in low-level phosphorus determinations. Wash glassware with hot dilute HCl and rinse with reagent water. Commercial detergents are rarely needed but, if they are used, use special phosphate-free preparations.

Also see Section 4500-P.F.

2. Apparatus

Flow injection analysis equipment consisting of:

a. FIA injection valve with sample loop or equivalent.

b. Multichannel proportioning pump.

c. FIA manifold (Figure 4500-P:3) with tubing heater and flow cell. Relative flow rates only are shown in Figure 4500-P:3. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

d. Absorbance detector, 880 nm, 10-nm bandpass.

e. Injection valve control and data acquisition system.

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. Stock ammonium molybdate solution: To a tared 1-L container add 40.0 g ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$] and 983 g water. Mix with a magnetic stirrer for at least 4 h. Store in plastic and refrigerate.

b. Stock antimony potassium tartrate solution: To a 1-L dark, tared container add 3.0 g antimony potassium tartrate (potassium antimonyl tartrate hemihydrate), $K(SbO)C_4H_4O_6\cdot {}^1/_2H_2O$, and 995 g water. Mix with a magnetic stirrer until dissolved. Store in a dark bottle and refrigerate.

c. Working molybdate color reagent: To a tared 1-L container add 680 g water, then add 64.4 g conc sulfuric acid. (CAUTION: *This solution becomes very hot!*) Swirl to mix. When mixture can be handled comfortably, add 213 g stock ammonium molybdate solution (¶ 3*a*) and 72.0 g stock antimony potassium tartrate solution (¶ 3*b*). Shake and degas with helium.

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d. Ascorbic acid solution: To a tared 1-L container, add 60.0 g granular ascorbic acid and 975 g water. Stir or shake until dissolved. Degas this reagent with helium, then add 1.0 g dodecyl sulfate, $CH_3(CH_2)_{11}OSO_3Na$, stirring gently to mix. Prepare fresh weekly.

e. Stock orthophosphate standard, 25.00 mg P/L: In a 1-L volumetric flask dissolve 0.1099 g primary standard grade anhydrous potassium phosphate monobasic (KH_2PO_4) that has been dried for 1 h at 105°C in about 800 mL water. Dilute to mark with water and invert to mix.

f. Standard orthophosphate solutions: Prepare orthophosphate standards in desired concentration range, using stock standard (¶ 3*e*) and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-P:3 and follow method supplied by manufacturer or laboratory standard operating procedure. Use quality control protocols outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold versus orthophosphate concentration. The calibration curve is linear.

6. Precision and Bias

a. Recovery and relative standard deviation: Table 4500-P:III gives results of single-laboratory studies.

b. MDL: A 700-µL sample loop was used in the method described above. Using a published MDL method,[1] analysts ran 21 replicates of a 5.0-µg P/L standard. These gave a mean of 5.26 µg P/L, a standard deviation of 0.264 µg P/L, and MDL of 0.67 µg P/L.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 Rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-P H. Manual Digestion and Flow Injection Analysis for Total Phosphorus (PROPOSED)

1. General Discussion

a. Principle: Polyphosphates are converted to the orthophosphate form by a sulfuric acid digestion and organic phosphorus is converted to orthophosphate by a persulfate digestion. When the resulting solution is injected onto the manifold, the orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a

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complex. This complex is reduced with ascorbic acid to form a blue complex that absorbs light at 880 nm. The absorbance is proportional to the concentration of total phosphorus in the sample.

See Section 4500-P.A for a discussion of the various forms of phosphorus found in waters and wastewaters, Section 4500-P.B for a discussion of sample preparation and digestion, and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: See Section 4500-P.G.1*b*.

2. Apparatus

Digestion and flow injection analysis equipment consisting of:

a. Hotplate or autoclave.

b. FIA injection valve with sample loop or equivalent.

c. Multichannel proportioning pump.

d. FIA manifold (Figure 4500-P:4) with tubing heater and flow cell. Relative flow rates only are shown in Figure 4500-P:4. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

Prepare reagents listed in Section 4500-P.G.3*a*, *b*, *d*, *e*, and *f*, and in addition:

a. Sulfuric acid carrier, H_2SO_4, 0.13*M*: To a tared 1-L container add 993 g water, then add 13.3 g conc H_2SO_4. Shake carefully to mix. Degas daily. Prepare fresh weekly.

b. Molybdate color reagent: To a tared 1-L container add 694 g water, then add 38.4 g conc H_2SO_4. (CAUTION: *The solution becomes very hot!*) Swirl to mix. When mixture can be handled comfortably, add 72.0 g stock antimony potassium tartrate (¶ G.3*b*) and 213 g stock ammonium molybdate (¶ G.3*a*). Shake to mix, and degas.

4. Procedure

See Section 4500-P.B.4 or 5 for digestion procedures. Carry both standards and samples through the digestion. The resulting solutions should be about 0.13*M* in sulfuric acid to match the concentration of the carrier. If the solutions differ more than 10% from this concentration, adjust concentration of carrier's sulfuric acid to match that of digested samples.

Set up a manifold equivalent to that in Figure 4500-P:4 and analyze digested samples and standards by following method supplied by manufacturer or laboratory's standard operating procedure. Use quality control protocols outlined in Section 4020.

5. Calculations

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Prepare standard curves by plotting absorbance of standards processed through the manifold versus phosphorus concentration. The calibration curve is linear.

6. Precision and Bias

a. MDL: A 780-µL sample loop was used in the method described above. Using a published MDL method,[1] analysts ran 21 replicates of a 3.5-µg P/L standard. These gave a mean of 3.53 µg P/L, a standard deviation of 0.82 µg P/L, and MDL of 2.0 µg P/L. The MDL is limited mainly by the precision of the digestion.

b. Precision study: Ten injections of a 100.0-µg P/L standard gave a percent relative standard deviation of 0.3%.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 Rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-P I. In-line UV/Persulfate Digestion and Flow Injection Analysis for Total Phosphorus (PROPOSED)

1. General Discussion

a. Principle: Organic phosphorus is converted in-line to orthophosphate by heat, ultraviolet radiation, and persulfate digestion. At the same time, inorganic polyphosphates are converted to orthophosphate by in-line sulfuric acid digestion. The digestion processes occur before sample injection. A portion of the digested sample is then injected and its orthophosphate concentration determined by the flow injection method described in Section 4500-P.H.1.

See Section 4500-P.A for a discussion of the various forms of phosphorus found in waters and wastewaters, Section 4500-P.B for a discussion of sample preparation and digestion, and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: See Section 4500-P.G.1*b*.

2. Apparatus

Flow injection analysis equipment consisting of:

a. FIA injection valve with sample loop or equivalent.

b. Multichannel proportioning pump.

c. FIA manifold (Figure 4500-P:5) with tubing heater, in-line ultraviolet digestion fluidics including a debubbler consisting of a gas-permeable TFE membrane and its holder, and flow cell. Relative flow rates only are shown in Figure 4500-P:5. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material

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such as TFE. The block marked ''UV'' should consist of TFE tubing irradiated by a mercury discharge ultraviolet lamp emitting radiation at 254 nm.

d. Absorbance detector, 880 nm, 10-nm bandpass.

e. Injection valve control and data acquisition system.

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and all reagents with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. Digestion reagent 1: To a tared 1-L container, add 893.5 g water, then slowly add 196.0 g sulfuric acid, H_2SO_4. CAUTION: *This solution becomes very hot!* Prepare weekly. Degas before using.

b. Digestion reagent 2: To a tared 1-L container, add 1000 g water, then add 26 g potassium persulfate, $K_2S_4O_8$. Mix with a magnetic stirrer until dissolved. Prepare weekly. Degas before using.

c. Sulfuric acid carrier, 0.71*M*: To a tared 1-L container, slowly add 70 g H_2SO_4 to 962 g water. Add 5 g sodium chloride, NaCl. Let cool, then degas with helium. Add 1.0 g sodium dodecyl sulfate. Invert to mix. Prepare weekly.

d. Stock ammonium molybdate: To a tared 1-L container add 40.0 g ammonium molybdate tetrahydrate, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, and 983 g water. Mix with a magnetic stirrer for at least 4 h. The solution can be stored in plastic for up to 2 months if refrigerated.

e. Stock antimony potassium tartrate: To a 1-L dark, plastic, tared container add 3.0 g antimony potassium tartrate (potassium antimonyl tartrate trihydrate), $C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$, and 995 g water. Mix with a magnetic stirrer until dissolved. The solution can be stored in a dark plastic container for up to 2 months if refrigerated.

f. Molybdate color reagent: To a tared 1-L container add 715 g water, then 213 g stock ammonium molybdate (¶ 3*e*) and 72.0 g stock antimony potassium tartrate (¶ 3 *f*). Add and dissolve 22.8 g sodium hydroxide, NaOH. Shake and degas with helium. Prepare weekly.

g. Ascorbic acid: To a tared 1-L container add 70.0 g ascorbic acid and 975 g water. Mix with a magnetic stirrer until dissolved. Degas with helium. Add 1.0 g sodium dodecyl sulfate. Mix with a magnetic stirrer. Prepare fresh every 2 d.

h. Stock orthophosphate standard, 1000 mg P/L: In a 1-L volumetric flask dissolve 4.396 g primary standard grade anhydrous potassium phosphate monobasic, KH_2PO_4 (dried for 1 h at 105°C), in about 800 mL water. Dilute to mark with water and invert to mix. Prepare monthly.

i. Standard solutions: Prepare orthophosphate standards in desired concentration range, using stock orthophosphate standards (¶ 3*i*), and diluting with water. If the samples are preserved

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with sulfuric acid, ensure that stock standard and diluted standards solutions are of the same concentration.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-P:5 and follow method supplied by manufacturer or laboratory's standard operating procedure. Use quality control procedures described in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through manifold versus phosphorus concentration. The calibration curve is linear.

Verify digestion efficiency by determining tripolyphosphate and trimethylphosphate standards at regular intervals. In the concentration range of the method, the recovery of either of these compounds should be >95%.

6. Precision and Bias

a. MDL: A 390-µL sample loop was used in the method described above. Using a published MDL method,[1] analysts ran 21 replicates of a 0.10-mg P/L orthophosphate standard. These gave a mean of 0.10 mg P/L, a standard deviation of 0.003 mg P/L, and MDL of 0.007 mg P/L.

b. Precision of recovery study: Ten injections of a 10.0-mg P/L trimethylphosphate standard gave a mean percent recovery of 98% and a percent relative standard deviation of 0.8%.

c. Recovery of total phosphorus: Two organic and two inorganic complex phosphorus compounds were determined in triplicate at three concentrations. The results are shown in Table 4500-P:IV.

d. Comparison of in-line digestion with manual digestion method: Samples from a wastewater treatment plant influent and effluent and total phosphorus samples at 2.0 mg P/L were determined in duplicate with both manual persulfate digestion followed by the method in Section 4500-PH and in-line digestion method. Table 4500-P:V gives the results of this comparison, and Figure 4500-P:6 shows the correlation between manual and in-line total phosphorus methods.

4500-$KMnO_4$ POTASSIUM PERMANGANATE*#(77)

4500-$KMnO_4$ A. Introduction

1. Occurrence and Significance

Potassium permanganate, $KMnO_4$, has been widely used in both potable and nonpotable water sources. It has been applied to water supplies to remove taste, odor, color, iron,

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manganese, and sulfides and to control trihalomethanes (THMs) and zebra mussels. Municipal and industrial waste treatment facilities use potassium permanganate for odor control, toxic pollutant destruction, bio-augmentation, and grease removal.

Potassium permanganate is produced as a dark black-purple crystalline material. It has a solubility in water of 60 g/L at 20°C. The color of potassium permanganate solutions ranges from faint pink (dilute) to deep purple (concentrated). Under normal conditions the solid material is stable. However, as with all oxidizing agents, avoid contact with acids, peroxides, and all combustible organic or readily oxidizable materials.

2. Sampling and Storage

If kept dry, solid potassium permanganate may be stored indefinitely. Potassium permanganate solutions, made in oxidant-demand-free water, are stable for long periods of time if kept in an amber bottle out of direct sunlight.[1] For samples obtained from other water sources (those having an oxidant demand), analyze potassium permanganate on site, as soon as possible after sample collection.

3. Reference

1. DAY, R.A. & A.L. UNDERWOOD. 1986. Quantitative Analysis, 5th ed. Prentice-Hall, Englewood Cliffs, N.J.

4500-$KMnO_4$ B. Spectrophotometric Method

1. General Discussion

a. Principle: This method is a direct determination of aqueous potassium permanganate solutions. The concentration (pink to violet color) is directly proportional to the absorbance as measured at 525 nm. The concentrations are best determined from the light-absorbing characteristics of a filtered sample by means of a spectrophotometer.

b. Application: This method is applicable to ground and surface waters.

c. Interference: Turbidity and manganese dioxide interfere. Remove by the filtration methods described below. Other color-producing compounds also interfere. Compensate for color by using an untreated sample to zero the spectrophotometer.

d. Minimum detectable concentration: As a guide, use the following light paths for the indicated $KMnO_4$ concentrations measured at 525nm. Sample dilution may be required, depending on the initial concentration.

Cell Path Length *cm*	Range *mg $KMnO_4$/L*	Expected Absorbance for 1-mg/L $KMnO_4$ Solution

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Cell Path Length cm	Range mg $KMnO_4$/L	Expected Absorbance for 1-mg/L $KMnO_4$ Solution
1	0.5–100	0.016
2.5	0.2–25	0.039
5	0.1–20	0.078

2. Apparatus

a. Photometric equipment: Use one of the following:

1) *Spectrophotometer,* for use at a wavelength of 525 nm and providing a light path of 1 cm or longer.

2) *Filter photometer,* equipped with a filter having a maximum transmittance at or near 525 nm and providing a light path of 1 cm or longer.

b. Filtration apparatus.#(78)*

c. Filters: Use 0.22-µm filters that do not react with $KMnO_4$ (or smallest glass fiber filters available).

3. Reagents

Use potassium-permanganate-demand-free water (¶ e below) for all reagent preparation and dilutions.

a. Calcium chloride solution, $CaCl_2$, 1*M:* Dissolve 111 g $CaCl_2$ in water and dilute to 1 L.

b. Sulfuric acid, H_2SO_4, 20%: Add 20 g conc H_2SO_4 slowly, with stirring, to 80 mL water. After cooling, adjust final volume to 100 mL.

c. Sodium oxalate, $Na_2C_2O_4$, primary standard.

d. Sodium thiosulfate solution, $Na_2S_2O_3 \cdot 5H_2O$, 0.019*M:* Dissolve 0.471 g $Na_2S_2O_3 \cdot 5H_2O$ in water and dilute to 100 mL.

e. Potassium-permanganate-demand-free water: Add one small crystal $KMnO_4$ to 1 L distilled or deionized water; let stand. After 1 to 2 d, a residual pink color should be present; if not, discard and obtain better-quality water or increase permanganate added. Redistill in an all-borosilicate-glass apparatus and discard initial 50 mL distillate. Collect distillate fraction that is free of permanganate: a red color with DPD reagent (see Section 4500-Cl.F.2*b*) indicates presence of permanganate.

f. Potassium permanganate standard, $KMnO_4$, 0.006*M:* Dissolve 1.000 g $KMnO_4$ in water and dilute to 1000 mL. Standardize as follows: Accurately weigh about 0.1 g primary standard grade sodium oxalate and dissolve it in 150 mL water in a 250-mL erlenmeyer flask. Add 20 mL

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20% H_2SO_4 and heat to 70 to 80°C. Titrate the warm oxalate solution with the potassium permanganate standard until a pink coloration persists (60 s). Calculate the potassium permanganate concentration:

$$\text{mg KMnO}_4\text{/L} = \frac{W \times 1000}{2.1197 \times V}$$

where:

W = weight of sodium oxalate, mg, and
V = mL $KMnO_4$ titrant.

4. Procedure

a. Calibration of photometric equipment: Prepare calibration curve by diluting standardized potassium permanganate solution. Make dilutions appropriate for the cell path length and range desired. Plot absorbance (y axis) versus $KMnO_4$ concentration (x axis). Calculate a best-fit line through the points. Preferably perform a calibration check with a known $KMnO_4$ standard before any analysis to ensure that equipment is in proper working order.

b. Potassium permanganate analysis: Check zero on spectrophotometer at 525 nm with deionized water. If the water is soft (i.e.,<40 mg/L hardness as $CaCO_3$), add 1 mL $CaCl_2$ solution/L sample (111 mg/L as $CaCl_2$) to aid in removal of any colloidal manganese dioxide and suspended solids. Pass 50 mL sample through a 0.22-µm filter. Rinse spectrophotometer cell with two or three portions of filtrate. Fill cell and check that no air bubbles are present in the solution or on the sides of the cell. Measure absorbance at 525 nm (Reading A). For best results, minimize time between filtration and reading absorbance. To 100 mL sample, add 0.1 mL $CaCl_2$ solution. Add 0.1 mL sodium thiosulfate solution per 1 mg/L $KMnO_4$ (based on Reading A). Pass through a 0.22-µm filter and measure absorbance (Reading B).

5. Calculation

$$\text{Correct absorbance} = A - B$$

where:

A = absorbance of sample, and
B = absorbance of blank.

Compare corrected absorbance value obtained with the calibration curve and report the corresponding value as milligrams potassium permanganate per liter.

6. Precision and Bias

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Based on the results obtained by eight analysts in a single laboratory, the overall precision (pooled standard deviation) was determined to be 0.035 mg/L for a 1-cm cell. The mean recovery for 12 measurements at 4 initial concentrations was 98%. The method detection level (MDL), as determined using Method 1030, was 0.083 mg $KMnO_4$/L.

4500-SiO_2 SILICA*#(79)

4500-SiO_2 A. Introduction

1. Occurrence and Significance

Silicon does not occur free in nature, but rather as free silica (SiO_2) in coarsely crystalline (quartz, rock crystal, amethyst, etc.) and microcrystalline (flint, chert, jasper, etc.) varieties of quartz, the major component of sand and sandstone. Silicon is found in combination with other elements in silicates, represented by feldspar, hornblende, mica, asbestos, and other clay minerals. Silicates also occur in rocks such as granite, basalt, and shale. Silicon therefore is usually reported as silica (SiO_2) when rocks, sediments, soils, and water are analyzed. The average abundance of silica in different rock types is 7 to 80%, in typical soils 50 to 80%, and in surface and groundwater 14 mg/L.

The common aqueous forms of silica are H_4SiO_4 and $H_3SiO_4^-$. In the presence of magnesium, it can form scale deposits in boilers and in steam turbines. It is considered a nonessential trace element for most plants, but essential for most animals. Chronic exposure to silica dust can be toxic. There is no U.S. EPA drinking water standard MCL for silica.

A more complete discussion of the occurrence and chemistry of silica in natural waters is available.[1]

2. Selection of Method

Perform analyses by the electrothermal atomic absorption method (3113B) or one of the colorimetric methods (C, D, E, or F), depending on the fraction to be measured. The inductively coupled plasma mass spectrometric method (3125) or the inductively coupled plasma method (3120) also may be applied successfully in most cases (with lower detection limits), even though silica is not specifically listed as an analyte in the method.

Methods 3120 and 3125 determine total silica. Methods C, D, E, and F determine molybdate-reactive silica. As noted in Section 4500-SiO_2.C.4, it is possible to convert other forms of silica to the molybdate-reactive form for determination by these methods. Method 3111D determines more than one form of silica. It will determine all dissolved silica and some colloidally dispersed silica. The determination of silica present in micrometer and submicrometer particles will depend on the size distribution, composition, and structure of the particles; thus

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Method 3111D cannot be said to determine total silica.

Method C is recommended for relatively pure waters containing from 0.4 to 25 mg SiO_2/L. As with most colorimetric methods, the range can be extended, if necessary, by diluting, by concentrating, or by varying the light path. Interferences due to tannin, color, and turbidity are more severe with this method than with Method D. Moreover, the yellow color produced by Method C has a limited stability and attention to timing is necessary. When applicable, however, it offers greater speed and simplicity than Method D because one reagent fewer is used; one timing step is eliminated; and many natural waters can be analyzed without dilution, which is not often the case with Method D. Method D is recommended for the low range, from 0.04 to 2 mg SiO_2/L. This range also can be extended if necessary. Such extension may be desirable if interference is expected from tannin, color, or turbidity. A combination of factors renders Methods D, E, and F less susceptible than Method C to those interferences; also, the blue color in Methods D, E, and F is more stable than the yellow color in Method C. However, many samples will require dilution because of the high sensitivity of the method. Permanent artificial color standards are not available for the blue color developed in Method D.

The yellow color produced by Method C and the blue color produced by Methods D, E, and F are affected by high concentrations of salts. With seawater the yellow color intensity is decreased by 20 to 35% and the blue color intensity is increased by 10 to 15%. When waters of high ionic strength are analyzed by these methods, use silica standards of approximately the same ionic strengths.[2]

Method E or F may be used where large numbers of samples are analyzed regularly. Method 3111D is recommended for broad-range use. Although Method 3111D is usable from 1 to 300 mg SiO_2/L, optimal results are obtained from about 20 to 300 mg/L. The range can be extended upward by dilution if necessary. This method is rapid and does not require any timing step.

The inductively coupled plasma method (3120) also may be used in analyses for silica.

3. Sampling and Storage

Collect samples in bottles of polyethylene, other plastic, or hard rubber, especially if there will be a delay between collection and analysis. Borosilicate glass is less desirable, particularly with waters of pH above 8 or with seawater, in which cases a significant amount of silica in the glass can dissolve. Freezing to preserve samples for analysis of other constituents can lower soluble silica values by as much as 20 to 40% in waters that have a pH below 6. Do not acidify samples for preservation because silica precipitates in acidic solutions.

4. References

1. HEM, J.D. 1985. Study and interpretation of the Chemical Characteristics of Natural Water, 3rd ed. U.S. Geol. Surv. Water Supply Pap. No. 2254.
2. FANNING, K.A. & M.E.Q. PILSON. 1973. On the spectrophotometric determination of dissolved silica in natural waters. *Anal. Chem.* 45:136.

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4500-SiO_2 B. (Reserved)

4500-SiO_2 C. Molybdosilicate Method

1. General Discussion

a. Principle: Ammonium molybdate at pH approximately 1.2 reacts with silica and any phosphate present to produce heteropoly acids. Oxalic acid is added to destroy the molybdophosphoric acid but not the molybdosilicic acid. Even if phosphate is known to be absent, the addition of oxalic acid is highly desirable and is a mandatory step in both this method and Method D. The intensity of the yellow color is proportional to the concentration of ‘‘molybdate-reactive’’ silica. In at least one of its forms, silica does not react with molybdate even though it is capable of passing through filter paper and is not noticeably turbid. It is not known to what extent such ‘‘unreactive’’ silica occurs in waters. Terms such as ‘‘colloidal,’’ ‘‘crystalloidal,’’ and ‘‘ionic’’ have been used to distinguish among various forms of silica but such terminology cannot be substantiated. ‘‘Molybdate-unreactive’’ silica can be converted to the ‘‘molybdate-reactive’’ form by heating or fusing with alkali. Molybdate-reactive or unreactive does not imply reactivity, or lack of it, toward *other* reagents or processes.

b. Interference: Because both apparatus and reagents may contribute silica, avoid using glassware as much as possible and use reagents low in silica. Also, make a blank determination to correct for silica so introduced. In both this method and Method D, tannin, large amounts of iron, color, turbidity, sulfide, and phosphate interfere. Treatment with oxalic acid eliminates interference from phosphate and decreases interference from tannin. If necessary, use photometric compensation to cancel interference from color or turbidity.

c. Minimum detectable concentration: Approximately 1 mg SiO_2/L can be detected in 50-mL nessler tubes.

2. Apparatus

a. Platinum dishes, 100-mL.

b. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer,* for use at 410 nm, providing a light path of 1 cm or longer. See Table 4500-SiO2:I for light path selection.

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2) *Filter photometer,* providing a light path of 1 cm or longer and equipped with a violet filter having maximum transmittance near 410 nm.

3) *Nessler tubes,* matched, 50-mL, tall form.

3. Reagents

For best results, set aside and use batches of chemicals low in silica. Use distilled reagent water in making reagents and dilutions. Store all reagents in plastic containers to guard against high blanks.

a. Sodium bicarbonate, $NaHCO_3$, powder.

b. Sulfuric acid, H_2SO_4, 1*N*.

c. Hydrochloric acid, HCl, 1 + 1.

d. Ammonium molybdate reagent: Dissolve 10 g $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in water, with stirring and gentle warming, and dilute to 100 mL. Filter if necessary. Adjust to pH 7 to 8 with silica-free NH_4OH or NaOH and store in a polyethylene bottle to stabilize. (If the pH is not adjusted, a precipitate gradually forms. If the solution is stored in glass, silica may leach out and cause high blanks.) If necessary, prepare silica-free NH_4OH by passing gaseous NH_3 into distilled water contained in a plastic bottle.

e. Oxalic acid solution: Dissolve 7.5 g $H_2C_2O_4\cdot H_2O$ in water and dilute.

f. Stock silica solution: Dissolve 4.73 g sodium metasilicate nonahydrate, $Na_2SiO_3\cdot 9H_2O$, in water and dilute to 1000 mL. For work of highest accuracy, analyze 100.0-mL portions by the gravimetric method.[1] Store in a tightly stoppered plastic bottle.

g. Standard silica solution: Dilute 10.00 mL stock solution to 1000 mL with water; 1.00 mL = 10.0 µg SiO_2. Calculate exact concentration from concentration of stock silica solution. Store in a tightly stoppered plastic bottle.

h. Permanent color solutions:

1) *Potassium chromate solution:* Dissolve 630 mg K_2CrO_4 in water and dilute to 1 L.

2) *Borax solution:* Dissolve 10 g sodium borate decahydrate, $Na_2B_4O_7\cdot 10H_2O$, in water and dilute to 1 L.

4. Procedure

a. Color development: To 50.0 mL sample add in rapid succession 1.0 mL 1 + 1 HCl and 2.0 mL ammonium molybdate reagent. Mix by inverting at least six times and let stand for 5 to 10 min. Add 2.0 mL oxalic acid solution and mix thoroughly. Read color after 2 min but before 15 min, measuring time from addition of oxalic acid. Because the yellow color obeys Beer’s law, measure photometrically or visually.

b. To detect the presence of molybdate-unreactive silica, digest sample with $NaHCO_3$ before

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color development. This digestion is not necessarily sufficient to convert all molybdate-unreactive silica to the molybdate-reactive form. Complex silicates and higher silica polymers may require extended fusion with alkali at high temperatures or digestion under pressure for complete conversion. Omit digestion if all the silica is known to react with molybdate.

Prepare a clear sample by filtration if necessary. Place 50.0 mL, or a smaller portion diluted to 50 mL, in a 100-mL platinum dish. Add 200 mg silica-free $NaHCO_3$ and digest on a steam bath for 1 h. Cool and add slowly, with stirring, 2.4 mL 1*N* H_2SO_4. Do not interrupt analysis but proceed *at once* with remaining steps. Transfer quantitatively to a 50-mL nessler tube and make up to mark with water. (Tall-form 50-mL nessler tubes are convenient for mixing even if the solution subsequently is transferred to an absorption cell for photometric measurement.)

c. Preparation of standards: If $NaHCO_3$ pretreatment is used, add to the standards (approximately 45 mL total volume) 200 mg $NaHCO_3$ and 2.4 mL 1*N* H_2SO_4, to compensate both for the slight amount of silica introduced by the reagents and for the effect of the salt on color intensity. Dilute to 50.0 mL.

d. Correction for color or turbidity: Prepare a special blank for every sample that needs such correction. Carry two identical portions of each such sample through the procedure, including $NaHCO_3$ treatment if this is used. To one portion add all reagents as directed in ¶ 4*a* preceding. To the other portion add HCl and oxalic acid but no molybdate. Adjust photometer to zero absorbance with the blank containing no molybdate before reading absorbance of molybdate-treated sample.

e. Photometric measurement: Prepare a calibration curve from a series of approximately six standards to cover the optimum ranges cited in Table 4500-SiO2:I. Follow direction of ¶ 4*a* above on suitable portions of standard silica solution diluted to 50.0 mL in nessler tubes. Set photometer at zero absorbance with water and read all standards, including a reagent blank, against water. Plot micrograms silica in the final (55 mL) developed solution against photometer readings. Run a reagent blank and at least one standard with each group of samples to confirm that the calibration curve previously established has not shifted.

f. Visual comparison: Make a set of permanent artificial color standards, using K_2CrO_4 and borax solutions. Mix liquid volumes specified in Table 4500-SiO2:II and place them in well-stoppered, appropriately labeled 50-mL nessler tubes. Verify correctness of these permanent artificial standards by comparing them visually against standards prepared by analyzing portions of the standard silica solution. Use permanent artificial color standards only for visual comparison.

5. Calculation

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$$\text{mg SiO}_2\text{/L} = \frac{\mu\text{g SiO}_2\text{ (in 55 mL final volume)}}{\text{mL sample}}$$

Report whether $NaHCO_3$ digestion was used.

6. Precision and Bias

A synthetic sample containing 5.0 mg SiO_2/L, 10 mg Cl^-/L, 0.20 mg NH_3-N/L, 1.0 mg NO_3^--N/L, 1.5 mg organic N/L, and 10.0 mg PO_4^{3-}/L in distilled water was analyzed in 19 laboratories by the molybdosilicate method with a relative standard deviation of 14.3% and a relative error of 7.8%.

Another synthetic sample containing 15.0 mg SiO_2/L, 200 mg Cl^-/L, 0.800 mg NH_3-N/L, 1.0 mg NO_3^--N/L, 0.800 mg organic N/L, and 5.0 mg PO_4^{3-}/L in distilled water was analyzed in 19 laboratories by the molybdosilicate method, with a relative standard deviation of 8.4% and a relative error of 4.2%.

A third synthetic sample containing 30.0 mg SiO_2/L, 400 mg Cl^-/L, 1.50 mg NH_3-N/L, 1.0 mg NO_3^--N/L, 0.200 mg organic N/L, and 0.500 mg PO_4^{3-}/L, in distilled water was analyzed in 20 laboratories by the molybdosilicate method, with a relative standard deviation of 7.7% and a relative error of 9.8%.

All results were obtained after sample digestion with $NaHCO_3$.

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4500-SiO_2 D. Heteropoly Blue Method

1. General Discussion

a. Principle: The principles outlined under Method C, ¶ 1*a*, also apply to this method. The yellow molybdosilicic acid is reduced by means of aminonaphtholsulfonic acid to heteropoly blue. The blue color is more intense than the yellow color of Method C and provides increased sensitivity.

b. Interference: See Section 4500-SiO_2.C.1*b*.

c. Minimum detectable concentration: Approximately 20 µg SiO_2/L can be detected in 50-mL nessler tubes and 50 µg SiO_2/L spectrophotometrically with a 1-cm light path at 815 nm.

2. Apparatus

a. Platinum dishes, 100-mL.

b. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer,* for use at approximately 815 nm. The color system also obeys Beer's law at 650 nm, with appreciably reduced sensitivity. Use light path of 1 cm or longer. See Table 4500-SiO2:I for light path selection.

2) *Filter photometer,* provided with a red filter exhibiting maximum transmittance in the wavelength range of 600 to 815 nm. Sensitivity improves with increasing wavelength. Use light path of 1 cm or longer.

3) *Nessler tubes,* matched, 50-mL, tall form.

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3. Reagents

For best results, set aside and use batches of chemicals low in silica. Store all reagents in plastic containers to guard against high blanks. Use distilled water that does not contain detectable silica after storage in glass.

All of the reagents listed in Section 4500-SiO_2.C.3 are required, and in addition:

Reducing agent: Dissolve 500 mg 1-amino-2-naphthol-4-sulfonic acid and 1 g Na_2SO_3 in 50 mL distilled water, with gentle warming if necessary; add this to a solution of 30 g $NaHSO_3$ in 150 mL distilled water. Filter into a plastic bottle. Discard when solution becomes dark. Prolong reagent life by storing in a refrigerator and away from light. Do not use aminonaphtholsulfonic acid that is incompletely soluble or that produces reagents that are dark even when freshly prepared.*#(80)

4. Procedure

a. Color development: Proceed as in 4500-SiO_2.C.4*a* up to and including the words, ‘‘Add 2.0 mL oxalic acid solution and mix thoroughly.’’ Measuring time from the moment of adding oxalic acid, wait at least 2 min but not more than 15 min, add 2.0 mL reducing agent, and mix thoroughly. After 5 min, measure blue color photometrically or visually. If $NaHCO_3$ pretreatment is used, follow 4500-SiO_2.C.4*b*.

b. Photometric measurement: Prepare a calibration curve from a series of approximately six standards to cover the optimum range indicated in Table 4500-SiO2:I. Carry out the steps described above on suitable portions of standard silica solution diluted to 50.0 mL in nessler tubes; pretreat standards if $NaHCO_3$ digestion is used (see Section 4500-SiO_2.C.4*b*). Adjust photometer to zero absorbance with distilled water and read all standards, including a reagent blank, against distilled water. If necessary to correct for color or turbidity in a sample, see Section 4500-SiO_2.C.4*d*. To the special blank add HCl and oxalic acid, but no molybdate or reducing agent. Plot micrograms silica in the final 55 mL developed solution against absorbance. Run a reagent blank and at least one standard with each group of samples to check the calibration curve.

c. Visual comparison: Prepare a series of not less than 12 standards, covering the range 0 to 120 µg SiO_2, by placing the calculated volumes of standard silica solution in 50-mL nessler tubes, diluting to mark with distilled water, and developing color as described in ¶ a preceding.

5. Calculation

$$\text{mg } SiO_2/L = \frac{\mu g\ SiO_2 \text{ (in 55 mL final volume)}}{\text{mL sample}}$$

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Report whether $NaHCO_3$ digestion was used.

6. Precision and Bias

A synthetic sample containing 5.0 mg SiO_2/L, 10 mg Cl^-/L, 0.200 mg NH_3-N/L, 1.0 mg NO_3^--N/L, 1.5 mg organic N/L, and 10.0 mg PO_4^{3-}/L in distilled water was analyzed in 11 laboratories by the heteropoly blue method, with a relative standard deviation of 27.2% and a relative error of 3.0%.

A second synthetic sample containing 15 mg SiO_2/L, 200 mg Cl^-/L, 0.800 mg NH_3-N/L, 1.0 mg NO_3^--N/L, 0.800 mg organic N/L, and 5.0 mg PO_4^{3-}/L in distilled water was analyzed in 11 laboratories by the heteropoly blue method, with a relative standard deviation of 18.0% and a relative error of 2.9%.

A third synthetic sample containing 30.0 mg SiO_2/L, 400 mg Cl^-/L, 1.50 mg NH_3-N/L, 1.0 mg NO_3^--N/L, 0.200 mg organic N/L, and 0.500 mg PO_4^{3-}/L in distilled water was analyzed in 10 laboratories by the heteropoly blue method with a relative standard deviation of 4.9% and a relative error of 5.1%.

All results were obtained after sample digestion with $NaHCO_3$.

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4500-SiO_2 E. Automated Method for Molybdate-Reactive Silica

1. General Discussion

a. Principle: This method is an adaptation of the heteropoly blue method (Method D) utilizing a continuous-flow analytical instrument.

b. Interferences: See Section 4500-SiO_2.C.1*b*. If particulate matter is present, filter sample or use a continuous filter as an integral part of the system.

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c. Application: This method is applicable to potable, surface, domestic, and other waters containing 0 to 20 mg SiO_2/L. The range of concentration can be broadened to 0 to 80 mg/L by substituting a 15-mm flow cell for the 50-mm flow cell shown in Figure 4500-SiO_2:1.

2. Apparatus

Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the interchangeable components shown in Figure 4500-SiO_2:1.

3. Reagents

a. Sulfuric acid, H_2SO_4, 0.05*M* (0.1*N*).

b. Ammonium molybdate reagent: Dissolve 10 g $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in 1 L 0.05*M* H_2SO_4. Filter and store in an amber plastic bottle.

c. Oxalic acid solution: Dissolve 50 g oxalic acid in 900 mL distilled water and dilute to 1 L.

d. Reducing agent: Dissolve 120 g $NaHSO_3$ and 4 g Na_2SO_3 in 800 mL warm distilled water. Add 2 g 1-amino-2-naphthol-4-sulfonic acid, mix well, and dilute to 1 L. Filter into amber plastic bottle for storage.

To prepare working reagent, dilute 100 mL to 1 L with distilled water. Make working reagent daily.

e. Standard silica solution: See 4500-SiO_2.C.3*g*.

4. Procedure

Set up manifold as shown in Figure 4500-SiO_2:1 and follow the general procedure described by the manufacturer. Determine absorbance at 660 nm. Use quality control procedures given in Section 4020.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against SiO_2 concentration in standards. Compute sample SiO_2 concentration by comparing sample response with standard curve.

6. Precision and Bias

For 0 to 20 mg SiO_2/L, when a 50-mm flow cell was used at 40 samples/h, the detection limit was 0.1 mg/L, sensitivity (concentration giving 0.398 absorbance) was 7.1 mg/L, and the coefficient of variation (95% confidence level at 7.1 mg/L) was 1.6%. For 0 to 80 mg SiO_2/L, when a 15-mm flow cell was used at 50 samples/h, detection limit was 0.5 mg/L, sensitivity was 31 mg/ L, and coefficient of variation at 31 mg/L was 1.5%.

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4500-SiO_2 F. Flow Injection Analysis for Molybdate-Reactive Silicate (PROPOSED)

1. General Discussion

Silicate reacts with molybdate under acidic conditions to form yellow beta-molybdosilicic acid. This acid is subsequently reduced with stannous chloride to form a heteropoly blue complex that has an absorbance maximum at 820 nm. Oxalic acid is added to reduce the interference from phosphate.

Collect samples in polyethylene or other plastic bottles and refrigerate at 4°C. Chemical preservation for silica is not recommended. Adding acid may cause polymerization of reactive silicate species. Freezing decreases silicate concentrations, especially at concentrations greater than 100 µg SiO_2/L. If filtration is required, preferably use a 0.45-µm TFE filter. Samples may be held for 28 d.

Also see Section 4500-SiO_2.A, Section 4500-SiO_2.D, and Section 4500-SiO_2.E, and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: Remove large or fibrous particulates by filtering sample through inert filter.

The interference due to phosphates is reduced by the addition of oxalic acid as a reagent on the flow injection manifold. By the following method, a solution of 1000 µg P/L was determined as 20 µg SiO_2/L. Verify extent of phosphate interference by determining a solution of phosphate at the highest concentration that is expected to occur.

Tannin and large amounts of iron or sulfides are interferences. Remove sulfides by boiling an acidified sample. Add disodium EDTA to eliminate interference due to iron. Treat with oxalic acid to decrease interference from tannin.

Sample color and turbidity can interfere. Determine presence of these interferences by analyzing samples without the presence of the molybdate.

Avoid silica contamination by storing samples, standards, and reagents in plastic. Do not use glass-distilled water for reagents or standards.

2. Apparatus

Flow injection analysis equipment consisting of:

a. FIA injection valve with sample loop or equivalent.

b. Multichannel proportioning pump.

c. FIA manifold (Figure 4500-SiO_2:2) with tubing heater and flow cell. Relative flow rates only are shown in Figure 4500-SiO_2:2. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.*#(81)

d. Absorbance detector, 820 nm, 10-nm bandpass.

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e. Injection valve control and data acquisition system.

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. All reagents can also be prepared on a weight/volume basis if desired.

a. Molybdate: To a tared 500-mL container add 20.0 g ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$]. Add 486 g warm water and 14.8 g conc sulfuric acid (H_2SO_4). Stir or shake until dissolved. Store in plastic and refrigerate. Prepare fresh monthly and discard if precipitate or blue color is observed.

b. Oxalic acid: To a tared 500-mL container add 50.0 g oxalic acid ($HO_2CCO_2H\cdot 2H_2O$) and 490 g water. Stir or shake until dissolved. Store in plastic.

c. Stannous chloride: To a tared 1-L container, add 978 g water. Add 40.0 g conc H_2SO_4. Dissolve 2.0 g hydroxylamine hydrochloride in this solution. Then dissolve 0.30 g stannous chloride. Prepare fresh weekly.

d. Stock silicate standard, 100 mg SiO_2/L: In a 1-L volumetric flask dissolve 0.473 g sodium metasilicate nonahydrate ($Na_2SiO_3\cdot 9H_2O$) in approximately 800 mL water. Dilute to mark and invert three times. Alternatively, use a commercially prepared standard solution, especially if nonstoichiometry of the solid metasilicate is suspected; the original degree of polymerization of the sodium metasilicate, which depends on storage time, can affect free silica concentration of the resulting solution.

e. Standard silicate solutions: Prepare silicate standards in the desired concentration range, using the stock standard (¶ 3*d*), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-SiO_2:2 and follow method supplied by manufacturer or laboratory standard operating procedure.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold versus silicate concentration. The calibration curve is linear.

6. Precision and Bias

Twenty-one replicates of a 5.0-µg SiO_2/L standard were analyzed with a 780-µL sample loop by a published MDL method.[1] These gave a mean of 4.86 µg SiO_2/L, a standard deviation of 0.31 µg SiO_2/L, and an MDL of 0.78 µg SiO_2/L.

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7. Quality Control

Follow procedures outlined in Section 4020.

8. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1989. Definition and Procedure for the Determination of Method Detection Limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-S^{2-} SULFIDE*#(82)

4500-S^{2-} A. Introduction

1. Occurrence and Significance

Sulfide often is present in groundwater, especially in hot springs. Its common presence in wastewaters comes partly from the decomposition of organic matter, sometimes from industrial wastes, but mostly from the bacterial reduction of sulfate. Hydrogen sulfide escaping into the air from sulfide-containing wastewater causes odor nuisances. The threshold odor concentration of H_2S in clean water is between 0.025 and 0.25 µg/L. Gaseous H_2S is very toxic and has claimed the lives of numerous workers in sewers. At levels toxic to humans it interferes with the olfactory system, giving a false sense of the safe absence of H_2S. It attacks metals directly and indirectly has caused serious corrosion of concrete sewers because it is oxidized biologically to H_2SO_4 on the pipe wall. Dissolved H_2S is toxic to fish and other aquatic organisms.

2. Categories of Sulfides

From an analytical standpoint, three categories of sulfide in water and wastewater are distinguished.

a. Total sulfide includes dissolved H_2S and HS^-, as well as acid-soluble metallic sulfides present in suspended matter. The S^{2-} is negligible, amounting to less than 0.5% of the dissolved sulfide at pH 12, less than 0.05% at pH 11, etc. Copper and silver sulfides are so insoluble that they do not respond in ordinary sulfide determinations; they can be ignored for practical purposes.

b. Dissolved sulfide is that remaining after suspended solids have been removed by flocculation and settling.

c. Un-ionized hydrogen sulfide may be calculated from the concentration of dissolved sulfide, the sample pH, and the practical ionization constant of H_2S.

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Figure 4500-S^{2-}:1 shows analytical flow paths for sulfide determinations under various conditions and options.

3. Sampling and Storage

Take samples with minimum aeration. Either analyze samples immediately after collection or preserve for later analysis with zinc acetate solution. To preserve a sample for a total sulfide determination put zinc acetate and sodium hydroxide solutions into bottle before filling it with sample. Use 4 drops of 2*N* zinc acetate soution per 100 mL sample. Increase volume of zinc acetate solution if the sulfide concentration is expected to be greater than 64 mg/L. The final pH should be at least 9. Add more NaOH if necessary. Fill bottle completely and stopper.

4. Qualitative Tests

A qualitative test for sulfide often is useful. It is advisable in the examination of industrial wastes containing interfering substances that may give a false negative result in the methylene blue method (D).

a. Antimony test: To about 200 mL sample, add 0.5 mL saturated solution of potassium antimony tartrate and 0.5 mL 6*N* HCl in excess of phenolphthalein alkalinity.

Yellow antimony sulfide (Sb_2S_3) is discernible at a sulfide concentration of 0.5 mg/L. Comparisons with samples of known sulfide concentration make the technique roughly quantitative. The only known interferences are metallic ions such as lead, which hold the sulfide so firmly that it does not produce Sb_2S_3, and dithionite, which decomposes in acid solution to produce sulfide.

b. Silver-silver sulfide electrode test: Dilute sample 1:1 with alkaline antioxidant reagent (see ¶ G.3*a* below). Measure electrode potential relative to a double-junction reference electrode and estimate the sulfide concentration from an old calibration curve or the example calibration curve in the electrode manual. This gives a reasonable estimate of sulfide concentration if the electrode is in good condition.

c. Lead acetate paper and silver foil tests: Confirm odors attributed to H_2S with lead acetate paper. On exposure to the vapor of a slightly acidified sample, the paper becomes blackened by formation of PbS. A strip of silver foil is more sensitive than lead acetate paper. Clean the silver by dipping in NaCN solution and rinse. CAUTION: *NaCN is toxic, handle with care.* Silver is suitable particularly for long-time exposure in the vicinity of possible H_2S sources because black Ag_2S is permanent whereas PbS slowly oxidizes.

5. Selection of Quantitative Methods

Iodine oxidizes sulfide in acid solution. A titration based on this reaction is an accurate method for determining sulfide at concentrations above 1 mg/L if interferences are absent and if loss of H_2S is avoided. The iodometric method (F) is useful for standardizing the methylene blue colorimetric methods (D, E, and I) and is suitable for analyzing samples freshly taken from wells

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or springs. The method can be used for wastewater and partly oxidized water from sulfur springs if interfering substances are removed first. The automated methylene blue method with distillation (I) is useful for a variety of samples containing more than 1 mg S^{-}/L.

The methylene blue method (D) is based on the reaction of sulfide, ferric chloride, and dimethyl-*p*-phenylenediamine to produce methylene blue. Ammonium phosphate is added after color development to remove ferric chloride color. The procedure is applicable at sulfide concentrations between 0.1 and 20.0 mg/L. The automated methlylene blue method (E) is similar to Method D. A gas dialysis technique separates the sulfide from the sample matrix. Gas dialysis eliminates most interferences, including turbidity and color. The addition of the antioxidant ascorbic acid improves sulfide recoveries. The method is applicable at sulfide concentrations between 0.002 and 0.100 mg/L.

Potentiometric methods utilizing a silver electrode (G) may be suitable. From the potential of the electrode relative to a reference electrode an estimate can be made of the sulfide concentration, but careful attention to details of procedures and frequent standardizations are needed to secure good results. The electrode is useful particularly as an end-point indicator for titration of dissolved sulfide with silver nitrate. The ion-selective electrode method is unaffected by sample color or turbidity and is applicable for concentrations greater than 0.03 mg/L.

6. Preparation of Sulfide Standards

Take care in preparing reliable stock solutions of sulfide for calibration and quality control. Prepare sulfide standards from sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$) crystals. These crystals usually have excess water present on the surface, in addition to a layer of contamination from oxidation products (polysulfides, polythionates, and sulfate) of sulfide reacting with atmospheric oxygen. Further, solutions of sulfide are prone to ready oxidation by dissolved and atmospheric oxygen. Use reagent water to prepare sulfide standards and sample dilutions. Boil and degas with either argon or nitrogen while cooling. Purchase the smallest amount of solid standards possible and keep no longer than 1 year. Preferably handle and store solid sulfide standards and stock solutions in an inert atmosphere glove bag or glove box to reduce contamination due to oxidation.

Preferably remove single crystals of $Na_2S \cdot 9H_2O$ from reagent bottle with nonmetallic tweezers; quickly rinse in degassed reagent water to remove surface contamination. Blot crystal dry with a tissue, then rapidly transfer to a tared, stoppered weighing bottle containing 5 to 10 mL degassed reagent water. Repeat procedure until desired amount of sodium sulfide is in weighing bottle. Determine amount of $Na_2S \cdot 9H_2O$ in weighing bottle by difference, then multiply the weight by 0.133 to determine the amount of S^{2-}. Avoid excess agitation and mixing of the solution with atmospheric oxygen. Quantitatively transfer and dilute entire contents of weighing bottle to an appropriate size volumetric flask with degassed reagent water to prepare a known concentration sulfide stock solution (3.750 g $Na_2S \cdot 9H_2O$ diluted to a final volume of 500 mL will give a stock solution of which 1.00 mL = 1.00 mg S^{2-}). Alternatively, purchase

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precertified stock solutions of sulfide. Verify concentration of stock solution daily using the iodometric method (F). Store stock solution with minimum headspace for no more than 1 week.

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4500-S^{2-} B. Separation of Soluble and Insoluble Sulfides

Unless the sample is entirely free from suspended solids (dissolved sulfide equals total sulfide), to measure dissolved sulfide first remove insoluble matter. This can be done by producing an aluminum hydroxide floc that is settled, leaving a clear supernatant for analysis.

1. Apparatus

Glass bottles with stoppers: Use 100 mL if sulfide will be determined by the methylene blue method and 500 to 1000 mL if by the iodometric method.

2. Reagents

a. Sodium hydroxide solution, NaOH, 6*N*.

b. Aluminum chloride solution: Because of the hygroscopic and caking tendencies of this chemical, purchase 100-g bottles of $AlCl_3 \cdot 6H_2O$. Dissolve contents of a previously unopened 100-g bottle in 144 mL distilled water.

3. Procedure

a. To a 100-mL glass bottle add 0.2 mL (nominally 4 drops) 6*N* NaOH. Fill bottle with sample and immediately add 0.2 mL (4 drops) $AlCl_3$ solution. Stopper bottle with no air under stopper. Rotate back and forth about a transverse axis vigorously for 1 min or longer to flocculate contents. Vary volumes of these added chemicals to get good clarification without using excessively large amounts and to produce a pH of 6 to 9. If a 500- or 1000-mL bottle is used, add proportionally larger amounts of reagents.

b. Let settle until reasonably clear supernatant can be drawn off. With proper flocculation, this may take 5 to 15 min. Do not wait longer than necessary.

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c. Either analyze the supernatant immediately or preserve with 2*N* zinc acetate (see Section 4500-S^{2-}.C).

4500-S^{2-} C. Sample Pretreatment to Remove Interfering Substances or to Concentrate the Sulfide

The iodometric method suffers interference from reducing substances that react with iodine, including thiosulfate, sulfite, and various organic compounds, both solid and dissolved.

Strong reducing agents also interfere in the methylene blue method (D) by preventing formation of the blue color. Thiosulfate at concentrations about 10 mg/L may retard color formation or completely prevent it. Ferrocyanide produces a blue color. Sulfide itself prevents the reaction if its concentration is very high, in the range of several hundred milligrams per liter. To avoid the possibility of false negative results, use the antimony method to obtain a qualitative result in industrial wastes likely to contain sulfide but showing no color by the methylene blue method. Iodide, which is likely to be present in oil-field wastewaters, may diminish color formation if its concentration exceeds 2 mg/L. Many metals (e.g., Hg, Cd, Cu) form insoluble sulfides and give low recoveries.

Eliminate interferences due to sulfite, thiosulfate, iodide, and many other soluble substances, but not ferrocyanide, by first precipitating ZnS, removing the supernatant, and replacing it with distilled water. Use the same procedure, even when not needed for removal of interferences, to concentrate sulfide. The automated methylene blue method (E) is relatively free from interferences because gas dialysis separates the sulfide from the sample matrix.

1. Apparatus

Glass bottles with stoppers: See Section 4500-S^{2-}.B.1.

2. Reagents

a. Zinc acetate solution: Dissolve 220 g $Zn(C_2H_3O_2)_2 \cdot 2H_2O$ in 870 mL water; this makes 1 L solution.

b. Sodium hydroxide solution, NaOH, 6*N*.

3. Procedure

a. Put 0.20 mL (4 drops) zinc acetate solution and 0.10 mL (2 drops) 6*N* NaOH into a 100-mL glass bottle, fill with sample, and add 0.10 mL (2 drops) 6*N* NaOH solution. Stopper with no air bubbles under stopper and mix by rotating back and forth vigorously about a transverse axis. For the iodometric procedure, use a 500-mL bottle or other convenient size, with proportionally larger volumes of reagents. Vary volume of reagents added according to sample so that the resulting precipitate is not excessively bulky and settles readily. Add enough NaOH to raise the pH above 9. Let precipitate settle for 30 min. The treated sample is relatively stable and can be held for several hours. However, if much iron is present, oxidation may be fairly

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rapid.

b. If the iodometric method is to be used, collect precipitate on a glass fiber filter and continue at once with titration according to the procedure of Method F. If the methylene blue method (D) is used, let precipitate settle for 30 min and decant as much supernatant as possible without loss of precipitate. Refill bottle with distilled water, shake to resuspend precipitate, and quickly withdraw a sample. If interfering substances are present in high concentration, settle, decant, and refill a second time. If sulfide concentration is known to be low, add only enough water to bring volume to one-half or one-fifth of original volume. Use this technique for analyzing samples of very low sulfide concentrations. After determining the sulfide concentration colorimetrically, multiply the result by the ratio of final to initial volume. No concentration or pretreatment steps to remove interferences are necessary for Method E.

4500-S^{2-} D. Methylene Blue Method

1. Apparatus

a. Matched test tubes, approximately 125 mm long and 15 mm OD.

b. Droppers, delivering 20 drops/mL methylene blue solution. To obtain uniform drops hold dropper in a vertical position and let drops form slowly.

c. If photometric rather than visual color determination will be used, either:

1) *Spectrophotometer,* for use at a wavelength of 664 nm with cells providing light paths of 1 cm and 1 mm, or other path lengths, or

2) *Filter photometer,* with a filter providing maximum transmittance near 660 nm.

2. Reagents

a. Amine-sulfuric acid stock solution: Dissolve 27 g *N*,*N*-dimethyl-*p*-phenylenediamine oxalate*#(83) in an iced mixture of 50 mL conc H_2SO_4 and 20 mL distilled water. Cool and dilute to 100 mL with distilled water. Use fresh oxalate because an old supply may be oxidized and discolored to a degree that results in interfering colors in the test. Store in a dark glass bottle. When this stock solution is diluted and used in the procedure with a sulfide-free sample, it first will be pink but then should become colorless within 3 min.

b. Amine-sulfuric acid reagent: Dilute 25 mL amine-sulfuric acid stock solution with 975 mL 1 + 1 H_2SO_4. Store in a dark glass bottle.

c. Ferric chloride solution: Dissolve 100 g $FeCl_3 \cdot 6H_2O$ in 40 mL water.

d. Sulfuric acid solution, H_2SO_4, 1 + 1.

e. Diammonium hydrogen phosphate solution: Dissolve 400 g $(NH_4)_2HPO_4$ in 800 mL distilled water.

f. Methylene blue solution I: Use USP grade dye or one certified by the Biological Stain

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Commission. The dye content should be reported on the label and should be 84% or more. Dissolve 1.0 g in distilled water and make up to 1 L. This solution will be approximately the correct strength, but because of variation between different lots of dye, standardize against sulfide solutions of known strength and adjust its concentration so that 0.05 mL (1 drop) = 1.0 mg sulfide/L.

Standardization—Prepare five known-concentration sulfide standards ranging from 1 to 8 mg/L as described in 4500-S^{2-}.A.6, or proceed as follows: Put several grams of clean, washed crystals of $Na_2S \cdot 9H_2O$ into a small beaker. Add somewhat less than enough water to cover crystals. Stir occasionally for a few minutes, then pour solution into another vessel. This solution reacts slowly with oxygen but the change is insignificant if analysis is performed within a few hours. Prepare solution daily. To 1 L distilled water add 1 drop of Na_2S solution and mix. Immediately determine sulfide concentration by the methylene blue procedure and by the iodometric procedure. Repeat, using more than 1 drop Na_2S solution or smaller volumes of water, until at least five tests have been made, with a range of sulfide concentrations between 1 and 8 mg/L. Calculate average percent error of the methylene blue result as compared to the iodometric result. If the average error is negative, that is, methylene blue results are lower than iodometric results, dilute methylene blue solution by the same percentage, so that a greater volume will be used in matching colors. If methylene blue results are high, increase solution strength by adding more dye.

g. Methylene blue solution II: Dilute 10.00 mL of adjusted methylene blue solution I to 100 mL with reagent water.

3. Procedure

a. Color development: Transfer 7.5 mL sample to each of two matched test tubes, using a special wide-tip pipet or filling to marks on test tubes. If sample has been preserved with zinc acetate, shake vigorously before taking subsample. Add to Tube A 0.5 mL amine-sulfuric acid reagent and 0.15 mL (3 drops) $FeCl_3$ solution. Mix immediately by inverting slowly, only once. (Excessive mixing causes low results by loss of H_2S as a gas before it has had time to react). To Tube B add 0.5 mL 1 + 1 H_2SO_4 and 0.15 mL (3 drops) $FeCl_3$ solution and mix. The presence of S^{2-} will be indicated by the appearance of blue color in Tube A. Color development usually is complete in about 1 min, but a longer time often is required for fading out of the initial pink color. Wait 3 to 5 min and add 1.6 mL $(NH_4)_2HPO_4$ solution to each tube. Wait 3 to 15 min and make color comparisons. If zinc acetate was used, wait at least 10 min before making a visual color comparison.

b. Color determination:

1) Visual color estimation—Add methylene blue solution I or II, depending on sulfide concentration and desired accuracy, dropwise, to the second tube, until color matches that developed in first tube. If the concentration exceeds 20 mg/L, repeat test with a portion of

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sample diluted tenfold.

With methylene blue solution I, adjusted so that 0.05 mL (1 drop) = 1.0 mg S^{2-}/L when 7.5 mL of sample are used:

$$\text{mg } S^{2-}/L = \text{no. drops solution I} + 0.1 \text{ (no. drops solution II)}$$

2) Photometric color measurement—A cell with a light path of 1 cm is suitable for measuring sulfide concentrations from 0.1 to 2.0 mg/L. Use shorter or longer light paths for higher or lower concentrations. This method is suitable for sample concentrations up to 20 mg/L. Zero instrument with a portion of treated sample from Tube B. Prepare calibration curves on basis of colorimetric tests made on Na_2S solutions simultaneously analyzed by the iodometric method, plotting concentration vs. absorbance. A linear relationship between concentration and absorbance can be assumed from 0 to 1.0 mg/L.

Read sulfide concentration from calibration curve.

4. Precision and Bias

In a study by two chemists working in the same laboratory, the standard deviation estimated from 34 sets of duplicate sulfide measurements was 0.04 mg/L for concentrations between 0.2 and 1.5 mg/L. The average recoveries of known additions were 92% for 40 samples containing 0.5 to 1.5 mg/L and 89% for samples containing less than 0.1 mg/L.

5. Bibliography

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4500-S^{2-} E. Gas Dialysis, Automated Methylene Blue Method

1. Apparatus

a. Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the interchangeable components shown in Figure 4500-S^{2-}:2.

The sampler is equipped with a mixer to stir samples before analysis and the gas dialysis membrane, which is maintained at room temperature, separates H_2S from the sample matrix.

2. Reagents

a. N,N-*dimethyl*-p-*phenylenediamine stock solution:* Dissolve 1 g *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride in 500 mL 6*N* HCl. Prepare fresh monthly. Store in an amber bottle.

b. N,N-*dimethyl*-p-*phenylenediamine working solution:* Dilute 190 mL

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N,N-dimethyl-*p*-phenylenediamine stock solution to 1 L. Store in an amber bottle. Prepare weekly.

c. Ferric chloride stock solution: Dissolve 13.5 g $FeCl_3 \cdot 6H_2O$ in 500 mL 5*N* HCl. Store in an amber bottle. Prepare fresh monthly.

d. Working ferric chloride solution: Dilute 190 mL ferric chloride stock solution to 1 L. Store in an amber bottle. Prepare fresh weekly.

e. Hydrochloric acid, HCl, 6*N*:

f. Sodium hydroxide stock solution, NaOH, 1*N*.

g. Sodium hydroxide, NaOH, 0.01*N*: Dilute 10 mL NaOH stock solution to 1 L.

h. Sulfide stock solution, 1.00 mg S^{2-}/1.00 mL: See Section 4500-S^{2-}.A.6.

i. Sulfide intermediate standard solution: Dilute 10 mL sulfide stock solution to 1 L with water. Prepare fresh daily. Standardize by iodometric titration method, Section 4500-S^{2-}.F. 1 mL ≈ 0.01 mg S^{2-}.

j. Sulfide tertiary standard solution: Dilute 50 mL sulfide intermediate solution to 500 mL with 0.01*N* NaOH. Prepare fresh daily. Use standardization value from ¶ 2*i* to determine exact concentration. 1.00 mL ≈ 0.001 mg S^{2-}.

k. Working sulfide standard solutions: Prepare a suitable series of standards by diluting appropriate volumes of sulfide tertiary standing solutions with 0.01*N* NaOH. Prepare fresh daily.

l. Zinc acetate preservative solution: Dissolve 220 g $Zn(C_2H_3O_2)_2 \cdot 2H_2O$ in 870 mL water (this makes 1 L solution).

3. Procedure

For unpreserved, freshly collected samples and sulfide working standards, add, in order, 4 drops 2*N* zinc acetate, 0.5 mL 6*N* NaOH, and 400 mg ascorbic acid/100 mL. For preserved samples, add 0.5 mL 6*N* NaOH and 400 mg ascorbic acid/100 mL. Shake well.

Let precipitate settle for at least 30 min. Pour a portion of well-mixed sample or working standard into a sample cup. Set up manifold as shown in Figure 4500-S^{2-}:2 and follow the general procedure described by the manufacturer. Determine absorbance at 660 nm.

4. Calculation

Prepare standard curves by plotting peak heights of standards processed through the manifold against S^{2-} concentration in the standards. Compute S^{2-} sample concentration by comparing sample response with standard curve.

5. Precision and Bias

In a single laboratory, samples with S^{2-} concentrations of 0.012, 0.015, 0.034, and 0.085 mg/L had standard deviations of 0.001, 0.001, 0.001, and 0.001 mg/L, respectively, with coefficients of variation of 8.3%, 6.3%, 2.9%, and 1.2%, respectively. In two environmental

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samples with added S^{2-}, recoveries were 104.2% and 97.6%.

6. Bibliography

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4500-S^{2-} F. Iodometric Method

1. Reagents

a. Hydrochloric acid, HCl, 6*N*.

b. Standard iodine solution, 0.0250*N*: Dissolve 20 to 25 g KI in a little water and add 3.2 g iodine. After iodine has dissolved, dilute to 1000 mL and standardize against 0.0250*N* $Na_2S_2O_3$, using starch solution as indicator.

c. Standard sodium thiosulfate solution, 0.0250*N*: See Section 4500-O.C.2*e*.

d. Starch solution: See Section 4500-O.C.2*d*.

2. Procedure

a. Measure from a buret into a 500-mL flask an amount of iodine solution estimated to be an excess over the amount of sulfide present. Add distilled water, if necessary, to bring volume to about 20 mL. Add 2 mL 6*N* HCl. Pipet 200 mL sample into flask, discharging sample under solution surface. If iodine color disappears, add more iodine until color remains. Back-titrate with $Na_2S_2O_3$ solution, adding a few drops of starch solution as end point is approached, and continuing until blue color disappears.

b. If sulfide was precipitated with zinc and ZnS filtered out, return filter with precipitate to original bottle and add about 100 mL water. Add iodine solution and HCl and titrate as in ¶ 2*a* above.

3. Calculation

One milliliter 0.0250*N* iodine solution reacts with 0.4 mg S^{2-}:

$$\text{mg } S^{2-}/\text{L} = \frac{[(A \times B) - (C \times D)] \times 16\,000}{\text{mL sample}}$$

where:

A = mL iodine solution,
B = normality of iodine solution,

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C = mL $Na_2S_2O_3$ solution, and
D = normality of $Na_2S_2O_3$ solution.

4. Precision

The precision of the end point varies with the sample. In clean waters it should be determinable within 1 drop, which is equivalent to 0.1 mg/L in a 200-mL sample.

4500-S^{2-} G. Ion-Selective Electrode Method

1. General Discussion

a. Principle: The potential of a silver/sulfide ion-selective electrode (ISE) is related to the sulfide ion activity. An alkaline antioxidant reagent (AAR) is added to samples and standards to inhibit oxidation of sulfide by oxygen and to provide a constant ionic strength and pH. Use of the AAR allows calibration in terms of total dissolved sulfide concentration. All samples and standards must be at the same temperature. Sulfide concentrations between 0.032 mg/L ($1 \times 10^{-6}M$) and 100 mg/L can be measured without preconcentration. For lower concentrations, preconcentration is necessary.

b. Interferences: Humic substances may interfere with Ag/S-ISE measurements. For highly colored water (high concentration of humic substances), use the method of standard additions to check results. Sulfide is oxidized by dissolved oxygen. Sulfide oxidation may cause potential readings to drift in the direction of decreasing concentration, i.e., to more positive values. Flush surface of samples and standards with nitrogen to minimize contact with atmospheric oxygen for low-level measurements. Temperature changes may cause potentials to drift either upward or downward. Therefore, let standards and samples come to the same temperature. If samples cannot be analyzed immediately, preserve dissolved sulfide by precipitating with zinc acetate (Section 4500-S^{2-}.C).

2. Apparatus

*a. Silver/sulfide electrode:**#(84)

b. Double-junction reference electrode.

c. Electrode polishing strips.†#(85)

d. pH meter with millivolt scale, capable of 0.1-mV resolution. Meters that can be calibrated in concentration and that perform standard-additions calculations are available.

e. Electrochemical cell: Make suitable cell from a 150-mL beaker and a sheet of rigid plastic (PVC or acrylic) with holes drilled to allow insertion of the electrodes and a tube for flushing the headspace with nitrogen. Alternatively, purchase a polarographic cell with gas transfer tube.‡#(86)

f. Gas dispersion tube: Use to deaerate water for preparing reagents and standards.

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g. Magnetic stirrer and stirring bar: Use a piece of styrofoam or cardboard to insulate the cell from the magnetic stirrer.

3. Reagents

a. Alkaline antioxidant reagent (AAR): To approximately 600 mL deaerated reagent water (DRW) in a 1-L volumetric flask, add 80 g NaOH, 35 g ascorbic acid, and 67 g Na_2H_2EDTA. Swirl to dissolve and dilute to 1 L. The color of freshly prepared AAR will range from colorless to yellow. Store in a tightly capped brown glass bottle. Discard when solution becomes brown.

b. Lead perchlorate, 0.1*M:* Dissolve 4.60 g $Pb(ClO_4)_2 \cdot 3H_2O$ in 100 mL reagent water. Standardize by titrating with Na_2H_2EDTA. Alternatively, use commercially available 0.1*M* $Pb(ClO_4)_2$ solutions.

c. Sulfide stock solution, 130 mg/L: See 4500-S^{2-}.A.6, and dilute 13.0 mL of 1.00 mg S^{2-}/mL stock to 100.0 mL with AAR. Alternatively, add 500 mL AAR and 10 g $Na_2S \cdot 9H_2O$ to a 1-L volumetric flask; dissolve. Dilute to 1 L with DRW. Use deaerated artificial seawater (DASW), Table 8010:III, or 0.7*M* NaCl if sulfide concentrations are to be determined in seawater. Standardize stock solution by titrating with 0.1*M* $Pb(ClO_4)_2$. Pipet 50 mL sulfide stock solution into the electrochemical cell. (Use 10 mL with a small-volume polarographic cell.) Insert Ag/S electrode and reference electrode and read initial potential. Titrate with 0.1*M* $Pb(ClO_4)_2$. Let electrode potential stabilize and record potential after each addition. Locate equivalence point as in Section 4500-Cl^-.D.4*a*. Alternatively, linearize the titration curve.[1] Calculate the function F_1 for points before the equivalence point.

$$F_1 = (V_o + V)10^{\frac{E}{m}}$$

where:

V_o = volume of stock solution, mL,
V = titrant volume, mL,
E = potential, mV, and
m = slope of calibration curve, mV/log unit.

Plot F_1 as a function of titrant volume. Extrapolate to find the intersection with the x-axis; that is, the equivalence point. Calculate sulfide concentration in the stock solution from:

$$C = \frac{V_{eq}[Pb]}{V_o}$$

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where:

C = sulfide concentration, mg/L,
V_{eq} = equivalence volume, mL,
$[Pb]$ = concentration of Pb in titrant, mg/L, and
V_o = volume of stock solution, mL.

Store stock solution in a tightly capped bottle for 1 week or less. The stock solution also can be standardized iodometrically (see Section 4500-S^{2-}.E). CAUTION: *Store in a fume hood.*

d. Sulfide standards: Prepare sulfide standards daily by serial dilution of stock. Add AAR and $Zn(C_2H_3O_2)_2$ solutions to 100-mL volumetric flasks. Add sulfide solutions and dilute to volume with DRW (or DASW). Refer to Table 4500-S^{2-}:I for volumes. Prepare at least one standard with a concentration less than the lowest sample concentration.

4. Procedure

Check electrode performance and calibrate daily. Check electrode potential in a sulfide standard every 2 h. The procedure depends on the sulfide concentration and the time between sample collection and sulfide determination. If the total sulfide concentration is greater than 0.03 mg/L ($1 \times 10^{-6}M$) and the time delay is only a few minutes, sulfide can be determined directly. Otherwise, precipitate ZnS and filter as described in Section 4500-S^{2-}.C.

a. Check electrode performance: Pipet 50 mL AAR, 50 mL DWR, and 1 mL sulfide stock solution into the measurement cell. Place Ag/S and reference electrodes in the solution and read potential. Add 10 mL stock solution and read potential. The change in potential should be -28 ± 2 mV. If it is not, follow the troubleshooting procedure in the electrode manual.

b. Calibration: Place electrodes in the most dilute standard but use calibration standards that bracket the sulfide concentrations in the samples. Record potential when the rate of change is less than 0.3 mV/min. (This may take up to 30 min for very low sulfide concentrations, i.e., less than 0.03 mg/L.) Rinse electrodes, blot dry with a tissue, and read potential of the next highest standard. For a meter that can be calibrated directly in concentration, follow manufacturer's directions. For other meters, plot potential as a function of the logarithm (base 10) of the sulfide concentration. For potentials in the linear range, calculate the slope and intercept of the linear portion of the calibration plot.

c. Sulfide determination by comparison with calibration curve, no ZnS precipitation: Add 40 mL AAR, 0.15 mL (3 drops) zinc acetate, and 50 mL sample to a 100-mL volumetric flask. Dilute to 100 mL with AAR. Pour into the electrochemical cell and insert the electrodes. Record potential when the rate of change is less than 0.3 mV/min. Read sulfide concentration from the calibration curve. Alternatively, for potentials in the linear range, calculate the sulfide concentration from:

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$$S_{Tot} = 10^{\frac{E-b}{m}}$$

where:

E = electrode potential and

b and m are the intercept and slope of the calibration curve. For a meter that can be calibrated directly in concentration, follow the manufacturer's directions.

d. Sulfide determination by comparison with calibration curve, with ZnS precipitation: Place filter with ZnS precipitate in a 150-mL beaker containing a stir bar. Wash sample bottle with 50 mL AAR and 20 mL DRW and pour the washings into the beaker. Stir to dissolve precipitate. Remove filter with forceps while rinsing it into the beaker with a minimum amount of DRW. Quantitatively transfer to a 100-mL volumetric flask and dilute to mark with DRW. Pour into the electrochemical cell and place the electrodes in the solution. Measure potential as in ¶ 4*c* above. Calculate sulfide concentration (¶ 4*c*).

e. Sulfide determination by standard addition with or without ZnS precipitation: Measure the Ag/S-ISE electrode potential as in ¶ c or *d* above. Add sulfide stock solution and measure potential again. Calculate sulfide concentration as follows:

$$C_o = \frac{fC_s}{(1+f)10^{\frac{E_s-E_o}{m}} - 1}$$

where:

C_o and C_s = sulfide concentrations in sample and known addition,

E_o and E_s = potentials measured for sample and known addition,

m = slope of calibration curve (approximately 28 mV/log S^{2-}, and

f = ratio of known-addition volume to sample volume.

f. Sulfide determination by titration: Use the same procedure as for standardizing the sulfide stock solution (¶ 3*c*). The minumum sulfide concentration for determination by titration is 0.3 mg/L ($10^{-5}M$).

5. Precision

For sulfide determination by comparison with the calibration curve, the relative standard deviation varies with the sulfide concentration. RSD values of 23% for 0.0091 mg/L and 5% for 0.182 mg/L have been reported.[2] (0.0091 µg/L was below the range for which the potential varied linearly with the logarithm of the sulfide concentration, i.e., the Nernstian range.) For

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sulfide determination by standard addition, the precision is greatest if the amount of sulfide added is as large as possible while staying within the linear range.[3]

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4500-S^{2-} H. Calculation of Un-ionized Hydrogen Sulfide

Hydrogen sulfide (H_2S) and bisulfide ion (HS^-), which together constitute dissolved sulfide, are in equilibrium with hydrogen ions:

$$H_2S \; H^+ + HS^-$$

The conditional ionization constant, which is valid for the temperature and ionic strength of the water of interest, relates the concentrations of H_2S and HS^-:

$$K'_1 = \frac{[H^+]\,[HS^-]}{[H_2S]}$$

The conditional constant is used to calculate the distribution of dissolved sulfide between the two species. The conditional ionization constant of H_2S is approximately 7.0. It differs from 7.0 by less than 0.2 log units for the ionic strengths and temperatures likely to be encountered in water-quality monitoring. The fraction of sulfide present as H_2S can be estimated with an error of less than 40% from Figure 4500-S^{2-}:3. If more accuracy is needed, use the methods given below.

1. Calculation for Fresh Water and Brackish Water ($I < 0.1M$)

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Calculate the dissociation constant for zero ionic strength (pK_1) and the temperature of interest.[1] If the temperature is 25°C, then pK_1 is 6.98. Otherwise:

$$pK_1\ (T) = 32.55 + 1519.44/T - 15.672 \log_{10} T + 0.02722T$$

where T is temperature (°K, i.e., T °C + 273.15). Next, calculate the ionic strength I as in Table 2330:I, the Debye-Huckel A parameter, and the negative logarithm of the monovalent ion activity coefficient (pf_m):

$$A = 0.7083 - 2.277 \times 10^{-3}T + 5.399 \times 10^{-6}T^2$$

$$A = 0.7083 - 2.277 \times 10^{-3}T + 5.399 \times 10^{-6}T^2$$

$$pf_m = A\left(\frac{\sqrt{I}}{1 + \sqrt{I}} - 0.3I\right)$$

Calculate the conditional ionization constant, K'_1, and the hydrogen ion concentration, $[H^+]$:

$$K'_1 = 10^{-pK_1 + 2\,pf_m}$$

$$[H^+] = 10^{-pH + pf_m}$$

Finally, calculate the un-ionized hydrogen sulfide concentration, $[H_2S]$, from the total sulfide concentration, S_T:

$$[H_2S] = \frac{S_T}{1 + \dfrac{K'_1}{[H^+]}}$$

Sample calculation: Total sulfide concentration 0.32 mg/L ($1.0 \times 10^{-5}M$), pH 6.75, ionic strength 0.02M, temperature 15.5°C.

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$$pK_1 = 32.55 \times \frac{1519.44}{288.65} - 15.672 \times \log_{10}(288.65) + 0.02722 \times 288.65 = 7.11$$

$$A = 0.7083 - 2.277 \times 10^{-3} \times 288.65 + 5.399 \times 10^{-6} \times (288.65)^2 = 0.501$$

$$pf_m = 0.501 \times \left(\frac{\sqrt{0.02}}{1 + \sqrt{0.02}} - 0.3 \times 0.02\right) = 0.059$$

$$K'_1 = 10^{-7.11 + 2 \times 0.059} = 1.014 \times 10^{-7}$$

$$[H^+] = 10^{-6.75 + 0.059} = 2.037 \times 10^{-7}$$

$$[H_2S] = \frac{1 \times 10^{-5}}{1 + \frac{1.014 \times 10^{-7}}{2.037 \times 10^{-7}}} = 6.68 \times 10^{-6} M = 0.21 \text{ mg/L (as S)}$$

2. Calculation for Seawater and Estuarine Water

This procedure differs only in calculating the conditional ionization constant, which can be calculated accurately.[1] The (potentially) largest source of error in calculating un-ionized hydrogen sulfide in seawater is the hydrogen ion concentration. Calibrate the pH electrode in artificial seawater at the temperature of the water of interest.[2] Alternatively, if the pH electrode is calibrated using NIST buffers (as in Section 4500-H), measure pH of dilute acid (10^{-4}–$10^{-3}N$ HNO_3, HCl, or $HClO_4$) in artificial seawater diluted to the salinity of the water of interest and at the temperature of interest and calculate a correction factor.[3] (Prepare artificial seawater as in Table 8010:III, substituting NaCl for NaF, $NaHCO_3$, and $Na_2SiO_3 \cdot 9H_2O$ on an equimolar basis.)

Calculate pK'_1 as outlined in Section 4500-S^{2-}.H.1. Calculate the coefficients A and B[1] (A and B are not Debye-Huckel parameters):

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$$A = -0.2391 + \frac{35.685}{T}$$

$$B = 0.0109 - \frac{0.3776}{T}$$

Calculate pK'_1:

$$pK'_1 = pK_1 + A\sqrt{S} + BS$$

where:

S = salinity, g/kg.

Calculate K'_1:

$$K'_1 = 10^{-pK'_1}$$

Sample calculation: Total sulfide concentration 0.32 mg/L ($1 \times 10^{-5} M$), pH 6.75, salinity 35 g/kg ($I = 0.7M$), temperature 15.5°C.

$$A = -0.2391 + \frac{35.685}{288.65}$$
$$= -0.115$$

$$B = 0.0109 - \frac{0.3776}{288.65}$$
$$= 0.009\ 59$$

From 4500-S^{2-}.H.1, $pK_1 = 7.11$.

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$$pK_1' = 7.11 - 0.115\sqrt{35} + 0.00959 \times 35$$
$$= 6.77$$

$$K_1' = 10^{-6.77}$$
$$= 1.70 \times 10^{-7}$$

$$pf_m = 0.501 \times \left(\frac{\sqrt{0.7}}{1 + \sqrt{0.7}} - 0.3 \times 0.7\right)$$
$$= 0.12$$

$$[H^+] = 10^{-6.75+0.12}$$
$$= 2.34 \times 10^{-7}$$

$$[H_2S] = \frac{1 \times 10^{-5}}{1 + \dfrac{1.7 \times 10^{-7}}{2.3 \times 10^{-7}}}$$
$$= 5.8 \times 10^{-6} M$$
$$= 0.19 \text{ mg/L (as S)}$$

3. References

1. MILLERO, F.J. 1986. The thermodynamics and kinetics of the hydrogen sulfide system in natural waters. *Mar. Chem.* 18:121.
2. MILLERO, F.J. 1986. The pH of estuarine waters. *Limnol. Oceanogr.* 31 :839.
3. SIGEL, H., A.D. ZUBERBUHLER & O. YAMAUCHI. 1991. Comments on potentiometric pH titrations and the relationship between pH-meter reading and hydrogen ion concentration. *Anal. Chim. Acta.* 255:63.

4. Bibliography

ARCHER, D.G. & P. WANG. 1990. The dielectric constant of water and Debye-Huckel Limiting Law Slopes. *J. Phys. Chem. Ref. Data* 12: 817.

4500-S^{2-} I. Distillation, Methylene Blue Flow Injection Analysis (PROPOSED)

1. General Discussion

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a. Principle: Water and wastewater samples are distilled into a sodium hydroxide trapping solution and the distillate is analyzed. Hydrogen sulfide (H_2S) reacts in acid media and in the presence of ferric chloride with two molecules of *N*, *N*-dimethyl-*p*-phenylenediamine to form methylene blue. The resulting color is read at 660 nm.

b. Sample preservation: Because H_2S oxidizes rapidly, analyze samples and standards without delay. To preserve samples, add 4 drops 2*M* zinc acetate to 100 mL sample and adjust pH to >9 with 6*M* NaOH, then cool to 4°C. Samples are distilled into a trapping solution resulting in 0.25*M* NaOH matrix.

Also see Section 4500-S^{2-}.A, Section 4500-S^{2-}.B, and Section 4500-S^{2-}.E, and Section 4130, Flow Injection Analysis (FIA).

c. Interferences: This method measures total sulfide, which is defined as the acid-soluble sulfide fraction of a sample. Total sulfide includes both acid-soluble sulfides such as H_2S, and acid-soluble metal sulfides present in suspended matter. This method does not measure acid-insoluble sulfides such as CuS.

Most nonvolatile interferences are eliminated by distillation. Strong reducing agents inhibit color formation at concentrations of several hundred milligrams per liter. Iodide interferes at concentrations greater than 2 mg I/L.

Also see Section 4500-S^{2-}.A and Section 4500-S^{2-}.B.

2. Apparatus

a. Distillation apparatus consisting of a glass or polypropylene micro-distillation device*#(87) capable of distilling 6 mL or more of sample into a 0.25*M* NaOH final concentration trapping solution.

b. Flow injection analysis equipment consisting of:

1) *FIA injection valve* with sample loop or equivalent.

2) *Multichannel proportioning pump.*

3) *FIA manifold* (Figure 4500-S^{2-}:4) with cation exchange column and flow cell. Relative flow rates only are shown in Figure 4500-S^{2-}:4. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

4) *Absorbance detector,* 660 nm, 10-nm bandpass.

5) *Injection valve control and data acquisition system.*

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min.

a. Sodium hydroxide carrier and diluent, NaOH, 0.25*M*: In a 2-L volumetric flask, dissolve 20 g NaOH in approximately 1800 mL water. Dilute to mark and mix with a magnetic stirrer

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until dissolved. Store in a plastic container.

b. Hydrochloric acid, HCl, 3*M*: To a tared 1-L container, add 752 g water and then slowly add 295 g conc HCl. Invert to mix.

c. Hydrochloric acid, HCl, 0.20*M*: To a tared 1-L container, add 983.5 g water. Then add 19.7 g conc HCl. Invert to mix.

d. N,N-*dimethyl*-p-*phenylenediamine:* In a 1-L volumetric flask dissolve 1.0 g *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride, $(CH_3)_2NC_6H_4NH_2\cdot 2HCl$, in about 800 mL 3*M* HCl (¶ 3*b*). Dilute to mark and invert to mix. If solution appears dark, it is likely that the *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride is decomposed; discard, and use fresh reagent.

e. Ferric chloride: In a 500-mL volumetric flask dissolve 6.65 g ferric chloride hexahydrate, $FeCl_3\cdot 6H_2O$, in about 450 mL 0.20*M* HCl (¶ 3*c*). Dilute to mark with water and invert to mix.

f. Stock sulfide standard, 100 mg S^{2-}/L: In a 1-L volumetric flask dissolve 0.7491 g sodium sulfide nonahydrate, $Na_2S\cdot 9H_2O$, in approximately 900 mL NaOH diluent (¶ 3*a*). Dilute to mark and invert to mix.

g. Standard solutions: Prepare sulfide standards in desired concentration range, using stock standard (¶ 3 *f*), and diluting with NaOH diluent (¶ 3*a*).

h. Sulfuric acid distillation releasing solution, H_2SO_4, 9*M*: To a tared 500-mL container, add 150.0 g water, then add slowly while swirling, in increments of 40 g, 276 g conc H_2SO_4. CAUTION: *Solution will become very hot.* Allow to cool before using.

4. Procedure

a. Distillation: This procedure is designed for the determination of sulfides in aqueous solutions, solid waste materials, or effluents. To preserve and remove sulfide from interfering substances, distill samples immediately after collection.

Follow manufacturer's instructions for use of distillation apparatus. Add sufficient 9*M* H_2SO_4 (¶ 3*h*) to sample to dissolve ZnS (s), digest total sulfides, and release the sulfide as hydrogen sulfide gas. Immediately place sample on-line with the receiving vessel or collector tube and distill hydrogen sulfide and water in the sample into a 0.25*M* trapping solution.

b. Flow injection analysis: Set up a manifold equivalent to that in Figure 4500-S^{2-}:4 and follow method supplied by the manufacturer or laboratory standard operating procedure. The carrier concentration should be identical to the final concentration of NaOH in the trapping solution from the distillation procedure (¶ 4*a*). Follow quality control protocols outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold versus sulfide concentration.

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6. Precision and Bias

a. MDL: A 200-µL sample loop was used in the method described above. Using a published method,[1] analysts ran 21 replicates of a 10.0-mg S^{2-}/L standard. These gave a mean of 9.0 mg S^{2-}/L, a standard deviation of 0.23 mg S^{2-}/L, and MDL of 0.58 mg S^{2-}/L. A higher MDL may be obtained by decreasing sample loop volume.

b. Precision: Ten injections of a distilled 50-mg S^{2-}/L standard gave a mean of 49.4 mg S^{2-}/L, a standard deviation of 0.27 mg S^{2-}/L, and percent relative standard deviation of 0.54.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 Rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-SO_3^{2-} SULFITE*#(88)

4500-SO_3^{2-} A. Introduction

1. Occurrence

Sulfite ions (SO_3^{2-}) may occur in boilers and boiler feedwaters treated with sulfite for dissolved oxygen control, in natural waters or wastewaters as a result of industrial pollution, and in treatment plant effluents dechlorinated with sulfur dioxide (SO_2). Excess sulfite ion in boiler waters is deleterious because it lowers the pH and promotes corrosion. Control of sulfite ion in wastewater treatment and discharge may be important environmentally, principally because of its toxicity to fish and other aquatic life and its rapid oxygen demand.

2. Selection of Method

The iodometric titration method is suitable for relatively clean waters with concentrations above 2 mg SO_3^{2-}/L. The phenanthroline colorimetric determination, following evolution of sulfite from the sample matrix as SO_2, is preferred for low levels of sulfite.

4500-SO_3^{2-} B. Iodometric Method

1. General Discussion

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a. Principle: An acidified sample containing sulfite (SO_3^{2-}) is titrated with a standardized potassium iodide-iodate titrant. Free iodine, liberated by the iodide-iodate reagent, reacts with SO_3^{2-}. The titration endpoint is signalled by the blue color resulting from the first excess of iodine reacting with a starch indicator.

b. Interferences: The presence of other oxidizable materials, such as sulfide, thiosulfate, and Fe^{2+} ions, can cause apparently high results for sulfite. Some metal ions, such as Cu^{2+}, may catalyze the oxidation of SO_3^{2-} to SO_4^{2-} when the sample is exposed to air, thus leading to low results. NO_2^- will react with SO_3^{2-} in the acidic reaction medium and lead to low sulfite results unless sulfamic acid is added to destroy nitrite. Addition of EDTA as a complexing agent at the time of sample collection inhibits Cu^{2+} catalysis and promotes oxidation of ferrous to ferric iron before analysis. Sulfide and thiosulfate ions normally would be expected only in samples containing certain industrial discharges, but must be accounted for if present. Sulfide may be removed by adding about 0.5 g zinc acetate and analyzing the supernatant of the settled sample. However, thiosulfate may have to be determined by an independent method (e.g., the formaldehyde/iodometric method[1]), and then the sulfite determined by difference.

c. Minimum detectable concentration: 2 mg SO_3^{2-}/L.

2. Reagents

a. Sulfuric acid: H_2SO_4, 1 + 1.

b. Standard potassium iodide-iodate titrant, 0.002083*M:* Dissolve 0.4458 g primary-grade anhydrous KIO_3 (dried for 4 h at 120°C), 4.35 g KI, and 310 mg sodium bicarbonate ($NaHCO_3$) in distilled water and dilute to 1000 mL; 1.00 mL = 500 μg SO_3^{2-}.

c. Sulfamic acid, NH_2SO_3H, crystalline.

d. EDTA reagent: Dissolve 2.5 g disodium EDTA in 100 mL distilled water.

e. Starch indicator: To 5 g starch (potato, arrowroot, or soluble) in a mortar, add a little cold distilled water and grind to a paste. Add mixture to 1 L boiling distilled water, stir, and let settle overnight. Use clear supernatant. Preserve by adding either 1.3 g salicylic acid, 4 g $ZnCl_2$, or a combination of 4 g sodium propionate and 2 g sodium azide to 1 L starch solution.

3. Procedure

a. Sample collection: Collect a fresh sample, taking care to minimize contact with air. Fix cooled samples (<50°C) immediately by adding 1 mL EDTA solution/100 mL sample. Cool hot samples to 50°C or below. Do not filter.

b. Titration: Add 1 mL H_2SO_4 and 0.1 g NH_2SO_3H crystals to a 250-mL erlenmeyer flask or other suitable titration vessel. Accurately measure 50 to 100 mL EDTA-stabilized sample into flask, keeping pipet tip below liquid surface. Add 1 mL starch indicator solution. Titrate

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immediately with standard KI-KIO_3 titrant, while swirling flask, until a faint permanent blue color develops. Analyze a reagent blank using distilled water instead of sample.

4. Calculation

$$\text{mg } SO_3^{2-}/\text{L} = \frac{(A - B) \times M \times 6 \times 40\,000}{\text{mL sample}}$$

where:

A = mL titrant for sample,
B = mL titrant for blank, and
M = molarity of KI-KIO_3 titrant.

5. Precision and Bias

Three laboratories analyzed five replicate portions of a standard sulfite solution and of secondary treated wastewater effluent to which sulfite was added. The data are summarized below. Individual analyst's precision ranged from 0.7 to 3.6% standard deviation (N = 45).

Sample	$\overline{X}$ *mg/L*	Standard Deviation, σ *mg/L*	Relative Standard Deviation %
Standard, 6.3 mg SO_3^{2-}/L	4.5	0. 25	5.5
Secondary effluent with 2.0 mg SO_3^{2-}/L	2.1	0.28	13.4
Secondary effluent with 4.0 mg SO_3^{2-}/L	3.6	0.17	4.8

6. Reference

1. KURTENACKER, A. 1924. The aldehyde-bisulfite reaction in mass analysis. *Z. Anal. Chem.* 64:56.

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4500-SO_3^{2-} C. Phenanthroline Method

1. General Discussion

a. Principle: An acidified sample is purged with nitrogen gas and the liberated SO_2 is trapped in an absorbing solution containing ferric ion and 1,10-phenanthroline. Ferric iron is reduced to the ferrous state by SO_2, producing the orange tris(1,10-phenanthroline) iron(II) complex. After excess ferric iron is removed with ammonium bifluoride, the phenanthroline complex is measured colorimetrically at 510 nm.[1]

b. Interferences: See Section 4500-SO_3^{2-}.B..

c. Minimum detectable concentration: 0.01 mg SO_3^{2-}/L.

2. Apparatus

a. Apparatus for evolution of SO_2: Figure 4500-SO_3^{2-}:1 shows the following components:

1) *Gas flow meter,* with a capacity to measure 2 L/min of pure nitrogen gas.

2) *Gas washing bottle,* 250-mL, with coarse-porosity, 12- mm-diam fritted cylinder gas dispersion tube.

3) *Tubing connectors,* quick-disconnect, polypropylene.

4) *Tubing,* flexible PVC, for use in all connections.

5) *Nessler tube,* 100-mL.

b. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer,* for use at 510 nm, providing a light path of 1 cm or longer.

2) *Filter photometer,* providing a light path of 1 cm or longer and equipped with a green filter having maximum transmittance near 510 nm.

3. Reagents

a. 1,10-phenanthroline solution, 0.03*M:* Dissolve 5.95 g 1,10-phenanthroline in 100 mL 95% ethanol. Dilute to 1 L with distilled water. Discard if solution becomes colored.

b. Ferric ammonium sulfate solution, 0.01*M:* Dissolve 4.82 g $NH_4Fe(SO_4)_2 \cdot 12H_2O$ in 1 L distilled water to which has been added 1 mL conc H_2SO_4 to suppress ferric hydrolysis. Filter through a glass fiber filter if insoluble matter is visible. If necessary, adjust volume of acid so that a mixture of 10 parts of phenanthroline solution and one part of ferric ammonium sulfate solution will have a pH between 5 and 6.

c. Ammonium bifluoride, 5%: Dissolve 25 g NH_4HF_2 in 500 mL distilled water. Store in a polyethylene bottle and dispense with a plastic pipet.

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d. Potassium tetrachloromercurate, (TCM), K_2HgCl_4, 0.04*M:* Dissolve 10.86 g $HgCl_2$, 5.96 g KCl, and 0.066 g disodium EDTA in distilled water and dilute to 1 L. Adjust pH to 5.2. This reagent normally is stable for 6 months, but discard if a precipitate forms.[2]

e. Dilute TCM-stabilized sulfite standard: Dissolve 0.5 g Na_2SO_3 in 500 mL distilled water. Standardize on the day of preparation, but wait at least 30 min to allow the rate of oxidation to slow. Determine molarity by titrating with standard 0.0125*M* potassium iodide-iodate titrant using starch indicator (see Section 4500-SO_3^{2-}.B). Calculate molarity of working standard as follows:

$$\text{Molarity of } SO_3^{2-} \text{ standard} = \frac{(A - B) \times M}{\text{mL sample}}$$

where:

A = titrant for sample, mL,
B = titrant for blank, mL,
M = molarity of potassium iodide-iodate titrant.

Because stock Na_2SO_3 solution is unstable, immediately after standardization, pipet 10 mL into a 500-mL volumetric flask partially filled with TCM and dilute to mark with TCM. Calculate the concentration of this dilute sulfite solution by multiplying the stock solution concentration by 0.02. This TCM-stabilized standard is stable for 30 d if stored at 5°C. Discard as soon as any precipitate is noticed at the bottom.

f. Standard potassium iodide-iodate titrant, 0.0125*M:* See Section 4500-SO_3^{2-}.B..

g. Hydrochloric acid, 1 + 1.

h. Octyl alcohol, reagent-grade.

i. Sulfamic acid, 10%: Dissolve 10 g NH_2SO_3H in 100 mL distilled water. This reagent can be kept for a few days if protected from air.

j. EDTA reagent: See Section 4500-SO_3^{2-}.B..

4. Procedure

a. Sample collection: Collect a fresh sample taking care to minimize contact with air. Fix cooled samples (<50°C) immediately by adding 1 mL EDTA solution for each 100 mL sample.

b. SO_2 evolution: Prepare the absorbing solution by adding 5 mL 1,10-phenanthroline solution, 0.5 mL ferric ammonium sulfate solution, 25 mL distilled water, and 5 drops octyl alcohol (to act as defoamer) to a 100-mL nessler tube; insert a gas dispersion tube. Add 1 mL

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sulfamic acid solution to the gas washing bottle and 100 mL of sample or a portion containing less than 100 µg SO_3^{2-} diluted to 100 mL. Add 10 mL 1 + 1 HCl and immediately connect the gas washing bottle to the gas train as shown in Figure 4500-SO_3^{2-}:1. Place a spring or rubber band on the gas washing bottle to keep the top securely closed during gas flow. Adjust nitrogen flow to 2.0 L/min and purge for 60 min.

c. Colorimetric measurement: After exactly 60 min, turn off nitrogen flow, disconnect nessler tube, and immediately add 1 mL ammonium bifluoride solution. Remove gas dispersion tube after rinsing it with distilled water into the tube and forcing the rinse water into the nessler tube with a rubber bulb. Dilute to 50 mL in the nessler tube and mix by rapidly moving the tube in a circular motion. Do not let rubber stoppers or PVC tubing come in contact with the absorbing solution. After at least 5 min from the time of adding ammonium bifluoride, read the absorbance versus distilled water at 510 nm using either a 5-cm cell for a range of 0 to 30 µg SO_3^{2-} per portion or a 1-cm cell for a range of 0 to 100 µg SO_3^{2-}. Avoid transferring octyl alcohol into the cell by letting it rise to the surface of the absorbing solution and transferring the clear lower solution to the cell with a pipet. Make a calibration curve by analyzing a procedure blank and at least three standards. Run at least one standard with each set of samples. For maximum accuracy hold samples and standards at the same temperature and keep the time interval from start of purging to the addition of ammonium bifluoride constant. This is easier to do if several gas trains are used simultaneously in parallel. If ambient temperatures are subject to frequent fluctuation, a water bath may be used to control color development at a fixed temperature.

5. Calculation

$$\text{mg } SO_3^{2-}/\text{L} = \frac{\mu\text{g } SO_3^{2-} \text{ from calibration curve}}{\text{mL sample}}$$

6. Precision and Bias

Three laboratories analyzed five replicate portions of a standard sulfite solution and of secondary treated wastewater effluent to which sulfite was added. The data are summarized below. Individual analyst's precision ranged from 4.1 to 10.5% standard deviation (*N* = 45).

Sample	$\bar{X}$ *mg/L*	Standard Deviation, σ *mg/L*	Relative Standard Deviation %
Standard,			
4.7 mg SO_3^{2-}/L	3.7	0.78	21

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Sample	$\bar{X}$ mg/L	Standard Deviation, σ mg/L	Relative Standard Deviation %
Secondary effluent with 0.12 mg SO_3^{2-}/L	0.12	0.03	25
Secondary effluent with 4.0 mg SO_3^{2-}/L	3.7	0.30	8.0

7. References

1. STEPHENS, B.G. & F. LINDSTROM. 1964. Spectrophotometric determination of sulfur dioxide suitable for atmospheric analysis. *Anal. Chem.* 36:1308.
2. WEST, P. W. & G.C. GAEKE. 1956. Fixation of sulfur dioxide as sulfitomercurate and subsequent colorimetric determination. *Anal. Chem.* 28:1816.

4500-SO_4^{2-} SULFATE*#(89)

4500-SO_4^{2-} A. Introduction

1. Occurrence

Sulfate (SO_4^{2-}) is widely distributed in nature and may be present in natural waters in concentrations ranging from a few to several thousand milligrams per liter. Mine drainage wastes may contribute large amounts of SO_4^{2-} through pyrite oxidation. Sodium and magnesium sulfate exert a cathartic action.

2. Selection of Method

The ion chromatographic method (4110) and capillary ion electrophoresis (CIE—see Section 4140) are suitable for sulfate concentrations above 0.1 mg/L. The gravimetric methods (C and D) are suitable for SO_4^{2-} concentrations above 10 mg/L. The turbidimetric method (E) is applicable in the range of 1 to 40 mg SO_4^{2-} /L. The automated methylthymol blue methods (F and G) are the procedures for analyzing large numbers of samples for sulfate alone when the equipment is available; over 30 samples can be analyzed per hour. Methods C, D, F, G, 4110, or

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CIE (4140) are preferred for accurate results.

3. Sampling and Storage

In the presence of organic matter certain bacteria may reduce SO_4^{2-} to S^{2-}. To avoid this, store samples at 4°C.

4500-SO_4^{2-} B. (Reserved)

4500-SO_4^{2-} C. Gravimetric Method with Ignition of Residue

1. General Discussion

a. Principle: Sulfate is precipitated in a hydrochloric acid (HCl) solution as barium sulfate ($BaSO_4$) by the addition of barium chloride ($BaCl_2$).

The precipitation is carried out near the boiling temperature, and after a period of digestion the precipitate is filtered, washed with water until free of Cl^-, ignited or dried, and weighed as $BaSO_4$.

b. Interference: The gravimetric determination of SO_4^{2-} is subject to many errors, both positive and negative. In potable waters where the mineral concentration is low, these may be of minor importance.

1) Interferences leading to high results—Suspended matter, silica, $BaCl_2$ precipitant, NO_3^-, SO_3^{2-} and occluded mother liquor in the precipitate are the principal factors in positive errors. Suspended matter may be present in both the sample and the precipitating solution; soluble silicate may be rendered insoluble and SO_3^{2-} may be oxidized to SO_4^{2-} during analysis. Barium nitrate [$Ba(NO_3)_2$], $BaCl_2$, and water are occluded to some extent with the $BaSO_4$ although water is driven off if the temperature of ignition is sufficiently high.

2) Interferences leading to low results—Alkali metal sulfates frequently yield low results. This is true especially of alkali hydrogen sulfates. Occlusion of alkali sulfate with $BaSO_4$ causes substitution of an element of lower atomic weight than barium in the precipitate. Hydrogen sulfates of alkali metals act similarly and, in addition, decompose on being heated. Heavy metals, such as chromium and iron, cause low results by interfering with the complete precipitation of SO_4^{2-} and by formation of heavy metal sulfates. $BaSO_4$ has small but significant solubility, which is increased in the presence of acid. Although an acid medium is necessary to prevent precipitation of barium carbonate and phosphate, it is important to limit its concentration to minimize the solution effect.

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2. Apparatus

a. Steam bath.

b. Drying oven, equipped with thermostatic control.

c. Muffle furnace, with temperature indicator.

d. Desiccator.

e. Analytical balance, capable of weighing to 0.1 mg.

f. Filter: Use one of the following:

1) *Filter paper,* acid-washed, ashless hard-finish, sufficiently retentive for fine precipitates.

2) *Membrane filter,* with a pore size of about 0.45 µm.

g. Filtering apparatus, appropriate to the type of filter selected. (Coat membrane filter holder with silicone fluid to prevent precipitate from adhering.)

3. Reagents

a. Methyl red indicator solution: Dissolve 100 mg methyl red sodium salt in distilled water and dilute to 100 mL.

b. Hydrochloric acid, HCl, 1 + 1.

c. Barium chloride solution: Dissolve 100 g $BaCl_2 \cdot 2H_2O$ in 1 L distilled water. Filter through a membrane filter or hard- finish filter paper before use; 1 mL is capable of precipitating approximately 40 mg SO_4^{2-}.

d. Silver nitrate-nitric acid reagent: Dissolve 8.5 g $AgNO_3$ and 0.5 mL conc HNO_3 in 500 mL distilled water.

*e. Silicone fluid.**#(90)

4. Procedure

a. Removal of silica: If the silica concentration exceeds 25 mg/L, evaporate sample nearly to dryness in a platinum dish on a steam bath. Add 1 mL HCl, tilt, and rotate dish until the acid comes in complete contact with the residue. Continue evaporation to dryness. Complete drying in an oven at 180°C and if organic matter is present, char over flame of a burner. Moisten residue with 2 mL distilled water and 1 mL HCl, and evaporate to dryness on a steam bath. Add 2 mL HCl, take up soluble residue in hot water, and filter. Wash insoluble silica with several small portions of hot distilled water. Combine filtrate and washings. Discard residue.

b. Precipitation of barium sulfate: Adjust volume of clarified sample to contain approximately 50 mg SO_4^{2-} in a 250-mL volume. Lower concentrations of SO_4^{2-} may be tolerated if it is impracticable to concentrate sample to the optimum level, but in such cases limit total volume to 150 mL. Adjust pH with HCl to pH 4.5 to 5.0, using a pH meter or the orange color of methyl red indicator. Add 1 to 2 mL HCl. Heat to boiling and, while stirring gently,

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slowly add warm $BaCl_2$ solution until precipitation appears to be complete; then add about 2 mL in excess. If amount of precipitate is small, add a total of 5 mL $BaCl_2$ solution. Digest precipitate at 80 to 90°C, preferably overnight but for not less than 2 h.

c. Filtration and weighing: Mix a small amount of ashless filter paper pulp with the $BaSO_4$, quantitatively transfer to a filter, and filter at room temperature. The pulp aids filtration and reduces the tendency of the precipitate to creep. Wash precipitate with small portions of warm distilled water until washings are free of Cl^- as indicated by testing with $AgNO_3$-HNO_3 reagent. Place filter and precipitate in a weighed platinum crucible and ignite at 800°C for 1 h. Do not let filter paper flame. Cool in desiccator and weigh.

5. Calculation

$$\text{mg } SO_4^{2-}/L = \frac{\text{mg } BaSO_4 \times 411.6}{\text{mL sample}}$$

6. Precision and Bias

A synthetic sample containing 259 mg SO_4^{2-}/L, 108 mg Ca^{2+}/L, 82 mg Mg^{2+}/L, 3.1 mg K^+/L, 19.9 mg Na^+/L, 241 mg Cl^-/L, 0.250 mg NO_2^--N/L, 1.1 mg NO_3^--N/L, and 42.5 mg total alkalinity/L (contributed by $NaHCO_3$) was analyzed in 32 laboratories by the gravimetric method, with a relative standard deviation of 4.7% and a relative error of 1.9%.

7. Bibliography

HILLEBRAND, W.F. et al. 1953. Applied Inorganic Analysis, 2nd ed. John Wiley & Sons, New York, N.Y.

KOLTHOFF, I.M., E.J. MEEHAN, E.B. SANDELL & S. BRUCKENSTEIN. 1969. Quantitative Chemical Analysis, 4th ed. Macmillan Co., New York, N.Y.

4500-SO_4^{2-} D. Gravimetric Method with Drying of Residue

1. General Discussion

See Method C, preceding.

2. Apparatus

With the exception of the filter paper, all of the apparatus cited in Section 4500-SO_4^{2-}.C. is required, plus the following:

a. Filters: Use one of the following:

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1) *Fritted-glass filter,* fine (''F'') porosity, with a maximum pore size of 5 µm.

2) *Membrane filter,* with a pore size of about 0.45 µm.

b. Vacuum oven.

3. Reagents

All the reagents listed in Section 4500-SO_4^{2-}.C. are required.

4. Procedure

a. Removal of interference: See Section 4500-SO_4^{2-}.C..

b. Precipitation of barium sulfate: See Section 4500-SO_4^{2-}.C..

c. Preparation of filters:

1) Fritted glass filter—Dry to constant weight in an oven maintained at 105°C or higher, cool in desiccator, and weigh.

2) Membrane filter—Place filter on a piece of filter paper or a watch glass and dry to constant weight*#(91) in a vacuum oven at 80°C, while maintaining a vacuum of at least 85 kPa or in a conventional oven at a temperature of 103 to 105°C. Cool in desiccator and weigh membrane only.

d. Filtration and weighing: Filter $BaSO_4$ at room temperature. Wash precipitate with several small portions of warm distilled water until washings are free of Cl^-, as indicated by testing with $AgNO_3$-HNO_3 reagent. If a membrane filter is used add a few drops of silicone fluid to the suspension before filtering, to prevent adherence of precipitate to holder. Dry filter and precipitate by the same procedure used in preparing filter. Cool in a desiccator and weigh.

5. Calculation

$$\text{mg } SO_4^{2-}/\text{L} = \frac{\text{mg } BaSO_4 \times 411.6}{\text{mL sample}}$$

6. Bibliography

See Section 4500-SO_4^{2-}.C.

4500-SO_4^{2-} E. Turbidimetric Method

1. General Discussion

a. Principle: Sulfate ion (SO_4^{2-}) is precipitated in an acetic acid medium with barium

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chloride ($BaCl_2$) so as to form barium sulfate ($BaSO_4$) crystals of uniform size. Light absorbance of the $BaSO_4$ suspension is measured by a photometer and the SO_4^{2-} concentration is determined by comparison of the reading with a standard curve.

b. Interference: Color or suspended matter in large amounts will interfere. Some suspended matter may be removed by filtration. If both are small in comparison with the SO_4^{2-} concentration, correct for interference as indicated in ¶ 4*d* below. Silica in excess of 500 mg/L will interfere, and in waters containing large quantities of organic material it may not be possible to precipitate $BaSO_4$ satisfactorily.

In potable waters there are no ions other than SO_4^{2-} that will form insoluble compounds with barium under strongly acid conditions. Make determination at room temperature; variation over a range of 10°C will not cause appreciable error.

c. Minimum detectable concentration: Approximately 1 mg SO_4^{2-}/L.

2. Apparatus

a. Magnetic stirrer: Use a constant stirring speed. It is convenient to incorporate a fixed resistance in series with the motor operating the magnetic stirrer to regulate stirring speed. Use magnets of identical shape and size. The exact speed of stirring is not critical, but keep it constant for each run of samples and standards and adjust it to prevent splashing.

b. Photometer: One of the following is required, with preference in the order given:

1) *Nephelometer.*

2) *Spectrophotometer,* for use at 420 nm, providing a light path of 2.5 to 10 cm.

3) *Filter photometer,* equipped with a violet filter having maximum transmittance near 420 nm and providing a light path of 2.5 to 10 cm.

c. Stopwatch or electric timer.

d. Measuring spoon, capacity 0.2 to 0.3 mL.

3. Reagents

a. Buffer solution A: Dissolve 30 g magnesium chloride, $MgCl_2 \cdot 6H_2O$, 5 g sodium acetate, $CH_3COONa \cdot 3H_2O$, 1.0 g potassium nitrate, KNO_3, and 20 mL acetic acid, CH_3COOH (99%), in 500 mL distilled water and make up to 1000 mL.

b. Buffer solution B (required when the sample SO_4^{2-} concentration is less than 10 mg/L): Dissolve 30 g $MgCl_2 \cdot 6H_2O$, 5 g $CH_3COONa \cdot 3H_2O$, 1.0 g KNO_3, 0.111 g sodium sulfate, Na_2SO_4, and 20 mL acetic acid (99%) in 500 mL distilled water and make up to 1000 mL.

c. Barium chloride, $BaCl_2$, crystals, 20 to 30 mesh. In standardization, uniform turbidity is produced with this mesh range and the appropriate buffer.

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d. Standard sulfate solution: Prepare a standard sulfate solution as described in 1) or 2) below; 1.00 mL = 100 µg SO_4^{2-}.

1) Dilute 10.4 mL standard 0.0200*N* H_2SO_4 titrant specified in Alkalinity, Section 2320B.3*c*, to 100 mL with distilled water.

2) Dissolve 0.1479 g anhydrous Na_2SO_4 in distilled water and dilute to 1000 mL.

4. Procedure

a. Formation of barium sulfate turbidity: Measure 100 mL sample, or a suitable portion made up to 100 mL, into a 250-mL erlenmeyer flask. Add 20 mL buffer solution and mix in stirring apparatus. While stirring, add a spoonful of $BaCl_2$ crystals and begin timing immediately. Stir for 60 ± 2 s at constant speed.

b. Measurement of barium sulfate turbidity: After stirring period has ended, pour solution into absorption cell of photometer and measure turbidity at 5 ± 0.5 min.

c. Preparation of calibration curve: Estimate SO_4^{2-} concentration in sample by comparing turbidity reading with a calibration curve prepared by carrying SO_4^{2-} standards through the entire procedure. Space standards at 5-mg/L increments in the 0- to 40-mg/L SO_4^{2-} range. Above 40 mg/L accuracy decreases and $BaSO_4$ suspensions lose stability. Check reliability of calibration curve by running a standard with every three or four samples.

d. Correction for sample color and turbidity: Correct for sample color and turbidity by running blanks to which $BaCl_2$ is not added.

5. Calculation

$$\text{mg } SO_4^{2-}/\text{L} = \frac{\text{mg } SO_4^{2-} \times 1000}{\text{mL sample}}$$

If buffer solution A was used, determine SO_4^{2-} concentration directly from the calibration curve after subtracting sample absorbance before adding $BaCl_2$. If buffer solution B was used subtract SO_4^{2-} concentration of blank from apparent SO_4^{2-} concentration as determined above; because the calibration curve is not a straight line, this is not equivalent to subtracting blank absorbance from sample absorbance.

6. Precision and Bias

With a turbidimeter,*#(92) in a single laboratory with a sample having a mean of 7.45 mg SO_4^{2-}/L, a standard deviation of 0.13 mg/L and a coefficient of variation of 1.7% were obtained.

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Two samples dosed with sulfate gave recoveries of 85 and 91%.

7. Bibliography

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THOMAS, J.F. & J.E. COTTON. 1954. A turbidimetric sulfate determination. *Water Sewage Works* 101:462.

ROSSUM, J.R. & P. VILLARRUZ. 1961. Suggested methods for turbidimetric determination of sulfate in water. *J. Amer. Water Works Assoc.* 53: 873.

4500-SO_4^{2-} F. Automated Methylthymol Blue Method

1. General Discussion

a. Principle: Barium sulfate is formed by the reaction of the SO_4^{2-} with barium chloride ($BaCl_2$) at a low pH. At high pH excess barium reacts with methylthymol blue to produce a blue chelate. The uncomplexed methylthymol blue is gray. The amount of gray uncomplexed methylthymol blue indicates the concentration of SO_4^{2-}.

b. Interferences: Because many cations interfere, use an ion-exchange column to remove interferences.

Molybdenum, often used to treat cooling waters, has been shown to cause a strong positive bias with this method, even with as little as 1 mg Mo/L.

c. Application: This method is applicable to potable, ground, surface, and saline waters as well as domestic and industrial wastewaters over a range from about 10 to 300 mg SO_4^{2-}/L.

2. Apparatus

a. Automated analytical equipment: An example of the required continuous-flow analytical instrument consists of the interchangeable components shown in Figure 4500-SO_4^{2-}:1.

b. Ion-exchange column: Fill a piece of 2-mm-ID glass tubing about 20 cm long with the ion-exchange resin.*#(93) To simplify filling column put resin in distilled water and aspirate it into the tubing, which contains a glass-wool plug. After filling, plug other end of tube with glass wool. Avoid trapped air in the column.

3. Reagents

a. Barium chloride solution: Dissolve 1.526 g $BaCl_2 \cdot 2H_2O$ in 500 mL distilled water and dilute to 1 L. Store in a polyethylene bottle.

b. Methylthymol blue reagent: Dissolve 118.2 mg methylthymol blue†#(94) in 25 mL $BaCl_2$ solution. Add 4 mL 1*N* HCl and 71 mL distilled water and dilute to 500 mL with 95% ethanol.

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Store in a brown glass bottle. Prepare fresh daily.

c. Buffer solution, pH 10.1: Dissolve 6.75 g NH_4Cl in 500 mL distilled water. Add 57 mL conc NH_4OH and dilute to 1 L with distilled water. Adjust pH to 10.1 and store in a polyethylene bottle. Prepare fresh monthly.

d. EDTA reagent: Dissolve 40 g tetrasodium ethylenediaminetetraacetate in 500 mL pH 10.1 buffer solution. Dilute to 1 L with pH 10.1 buffer solution and store in a polyethylene bottle.

e. Sodium hydroxide solution, 0.36*N:* Dissolve 7.2 g NaOH in 250 mL distilled water. Cool and make up to 500 mL with distilled water.

f. Stock sulfate solution: Dissolve 1.479 g anhydrous Na_2SO_4 in 500 mL distilled water and dilute to 1000 mL; 1.00 mL = 1.00 mg SO_4^{2-}.

g. Standard sulfate solutions: Prepare in appropriate concentrations from 10 to 300 mg SO_4^{2-}/L, using the stock sulfate solution.

4. Procedure

Set up the manifold as shown in Figure 4500-SO_4^{2-}:1 and follow the general procedure described by the manufacturer.

After use, rinse methylthymol blue and NaOH reagent lines in water for a few minutes, rinse them in the EDTA solution for 10 min, and then rinse in distilled water.

5. Calculation

Prepare standard curves by plotting peak heights of standards processed through the manifold against SO_4^{2-} concentrations in standards. Compute sample SO_4^{2-} concentration by comparing sample peak height with standard curve.

6. Precision and Bias

In a single laboratory a sample with an average concentration of about 28 mg SO_4^{2-}/L had a standard deviation of 0.68 mg/L and a coefficient of variation of 2.4%. In two samples with added SO_4^{2-}, recoveries were 91% and 100%.

7. Bibliography

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COLOROS, E., M. R. PANESAR & F.P. PERRY. 1976. Linearizing the calibration curve in determination of sulfate by the methylthymol blue method. *Anal. Chem.* 48:1693.

4500-SO_4^{2-} G. Methylthymol Blue Flow Injection Analysis (PROPOSED)

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1. General Discussion

a. Principle: At pH 13.0 barium forms a blue complex with methylthymol blue (MTB). This gives a dark blue base line. The sample is injected into a low, but known, concentration of sulfate. The sulfate from the sample then reacts with the ethanolic barium-MTB solution and displaces the MTB from the barium to give barium sulfate and uncomplexed MTB. Uncomplexed MTB has a grayish color. The pH is raised with NaOH and the color of the gray uncomplexed MTB is measured at 460 nm. The intensity of gray color is proportional to the sulfate concentration.

Also see Section 4500-SO_4^{2-}.A and Section 4500-SO_4^{2-}.F, and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: Remove large or fibrous particulates by filtering sample through glass wool. Guard against nitrate and nitrite contamination from reagents, water, glassware, and the sample preservation process.

A cation-exchange column removes multivalent cations such as Ca^{2+} and Mg^{2+}. A midrange sulfate standard containing a typical level of hardness as $CaCO_3$ can be run periodically to check the performance of the column. Any decrease in peak height from that of a sulfate standard without added $CaCO_3$ indicates the need to regenerate or replace the resin.

Neutralize samples that have pH less than 2. High acid concentrations can displace multivalent cations from the column.

Orthophosphate forms a precipitate with barium at high pH. If samples are known to be high in orthophosphate, make a recovery study using added amounts of sulfate, or run a sample blank containing only the orthophosphate matrix.

Also see Section 4500-SO_4^{2-}.F..

2. Apparatus

Flow injection analysis equipment consisting of:

a. FIA injection valve with sample loop or equivalent.

b. Multichannel proportioning pump.

c. FIA manifold (Figure 4500-SO_4^{2-}:2) with cation-exchange column and flow cell. Relative flow rates only are shown in Figure 4500-SO_4^{2-}:2. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

d. Absorbance detector, 460 nm, 10-nm bandpass.

e. Injection valve control and data acquisition system.

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas

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carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. Carrier solution, 0.30 mg SO_4^{2-}/L*:* To a tared 1-L container, add 0.30 g 1000 mg/L stock sulfate standard (¶ 3*h*) and 999.7 g water. Shake or stir to mix. Degas with helium.

b. Barium chloride solution, 6.24m*M*: To a tared 1-L container, add 1.526 g barium chloride dihydrate, $BaCl_2 \cdot 2H_2O$, and 995 g water. Shake or stir until dissolved. Degas with helium.

c. Hydrochloric acid, HCl, 1.0*M:* To a tared 1-L container, add 913 g water and 99.6 g conc HCl (specific gravity 1.20, 37%). CAUTION: *Fumes.* Shake or stir to mix well. Degas with helium.

d. Barium—MTB color reagent: NOTE: The purity of the methylthymol blue and the denaturants in the alcohol are critical. Use the sources specified below, or test the material from alternative sources for suitability before using.

To a tared 500-mL dry brown plastic bottle, place 0.236 g methylthymol blue, 3,3′-bis[*N,N*-di(carboxymethyl)amino-methyl]-thymol-sulfonephthalein, pentasodium salt. Add 50 g barium chloride solution (¶ 3*b*), which may be used to aid in transfer of the dye. Swirl to dissolve. Add 4.0 g of 1.0*M* HCl (¶ 3*c*) and mix. The solution should turn orange. Add 71 g water and 321 g ethanol (ethyl alcohol, specially denatured anhydrous alcohol*#(95)). Stir or shake to mix well. The pH should be 2.5. Prepare solution the day before use and store, refrigerated, in a brown plastic bottle. Let warm to room temperature before using, then degas with helium.

e. Stock sodium hydroxide solution, NaOH, 50% (w/v) solution*:* To a glass 1-L container add 500 g water and 500.0 g NaOH. Dilute to 1 L. CAUTION: *The solution becomes very hot.* Shake or stir until dissolved. Cool to ambient. Store in a plastic bottle.

f. Working sodium hydroxide solution, NaOH, 0.18*M:* To a tared plastic 1-L container add 982 g water and 19.8 g stock NaOH solution (¶ 3*e*). Shake or stir to mix. Degas with helium.

g. Cation exchange column preparation: Prepare approximately 0.5 g ion exchange resin,†#(96) 50 to 100 mesh, by mixing with sufficient water to make a slurry. Remove one end fitting from the threaded glass column. Fill column with water and aspirate slurry into column or let it settle into column by gravity. Take care to avoid trapping air bubbles in column or fittings at this point and during all subsequent operations. When resin has settled, replace end fitting. To ensure a good seal, remove any resin particles from the threads of glass, column end, and end fitting. To store column, join ends of the TFE tubing.

To test column effectiveness, make up two midrange standards, one of only sodium sulfate and the other with an identical concentration of sodium sulfate but with hardness typical of the samples. If the column is depleted, the standard with hardness will give a lower response because the divalent Mg^{2+} and Ca^{2+} cations are complexing with the free MTB. If depletion has occurred, repack column with fresh resin.

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h. Stock sulfate standard, 1000 mg SO_4^{2-}/L*:* Dry approximately 2 g sodium sulfate, Na_2SO_4, at 105° overnight. Cool in a desiccator. In a 1-L volumetric flask, add 1.479 g of dried sodium sulfate to about 800 mL water. Dissolve by swirling, dilute to mark, and mix by inversion.

i. Standard solutions: Prepare sulfate standards in desired concentration range, using the stock standard (¶ 3*h*), and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-SO_4^{2-}:2 and follow method supplied by manufacturer or laboratory standard operating procedure. Follow quality control protocols outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting adsorbance of standards processed through the manifold versus sulfate concentration.

6. Precision and Bias

a. Recovery and relative standard deviation: Table 4500-SO_4^{2-}:I gives results of single-laboratory studies.

b. MDL: A 180-µL sample loop was used in the method described above. Using a published MDL method,[1] analysts ran 21 replicates of a 5.00-mg SO_4^{2-}/L standard. These gave a mean of 4.80 mg SO_4^{2-}/L, a standard deviation of 0.69 mg SO_4^{2-}/L, and MDL of 1.8 mg SO_4^{2-}/L.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

Figures

Figure 4110:1. Typical inorganic anion separation. Eluent: 1.7 m*M* $NaHCO_3$, 1.8 m*M* Na_2CO_3; sample loop: 50 µL; flow: 2.0 mL/min; column: Dionex AG4A plus AS4A.

Anion	Time	Conc *mg/L*
Fluoride	1.15	2
Chloride	1.72	20

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Anion	Time	Conc mg/L
Nitrite	2.03	10
Bromide	2.93	5
Nitrate	3.20	10
Orthophosphate	5.10	10
Sulfate	6.82	20

Figure 4110:2. Typical inorganic anion separation. Eluent: borate/gluconate; flow rate: 1.5 mL/min; injection volume: 100 µL.

Anion	Time *min*	Conc *mg/L*
1. System—peak	—	—
2. Cl^-	2.3	40
3. NO_2^-	3.1	10
4. Br^-	4.2	40
5. NO_3^-	5.3	20
6. PO_4^{3-}	5.7	40
7. SO_4^{2-}	8.3	80

Figure 4120:1 Schematic of a segmented flow analyzer.

Figure 4140:1. Electropherogram of the inorganic anions and typically found organic acids using capillary ion electrophoresis and chromate electrolyte. Electrolyte: 4.7 m*N* Na_2CrO_4/4.0 m*M* TTAOH/10 m*M* CHES/0.1 m*M* calcium gluconate; capillary: 75-µm-ID × 375-µm-OD × 60-cm length, uncoated silica; voltage: 15 kV using a negative power supply; current: 14 ± 1 µA; sampling: hydrostatic at 10 cm for 30 s; detection: indirect UV with Hg lamp and 254-nm filter.

Anion	Conc *mg/L*	Migration Time *min*	Migration Time Ratio to Cl	Peak Area	Time-Cor Peak Are
Chloride	2.0	3.200	1.000	1204	376.04
Bromide	4.0	3.296	1.030	1147	348.05

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Anion	Conc *mg/L*	Migration Time *min*	Migration Time Ratio to Cl	Peak Area	Time-Cor Peak Are
Nitrite	4.0	3.343	1.045	2012	601.72
Sulfate	4.0	3.465	1.083	1948	562.05
Nitrate	4.0	3.583	1.120	1805	503.69
Oxalate	5.0	3.684	1.151	3102	842.14
Fluoride	1.0	2.823	1.195	1708	446.65
Formate	5.0	3.873	1.210	1420	366.61
o-Phosphate	4.0	4.004	1.251	2924	730.25
Carbonate & bicarbonate		4.281	1.338		
Acetate	5.0	4.560	1.425	3958	868.01

Figure 4140:2. Electropherogram of 0.1 mg/L inorganic anions at minimum detection level. Seven replicates of 0.1 mg/L inorganic anion standard used to calculate minimum detection levels, as mg/L, using analytical protocol described in Section 1030E.

Chloride = 0.046
Nitrate = 0.084
Bromide = 0.090
Fluoride = 0.020
Nitrite = 0.072
Phosphate = 0.041
Sulfate = 0.032

Figure 4140:3. Representative electropherograms of Youden anion standards. For composition of standards, see Table 4140:I.

Figure 4140:4. Linearity calibration curve for chloride, bromide, and sulfate. Three data points were used per concentration; based on Youden pair design.

Figure 4140:5. Linearity calibration curve for fluoride and o-phosphate. Three data points were used per concentration; based on Youden pair design.

Figure 4140:6. Linearity calibration curve for nitrite and nitrate. Three data points were used per concentration; based on Youden pair design.

Figure 4140:7. Electropherogram of typical drinking water.

Chloride = 24.72 mg/L
Sulfate = 7.99 mg/L
Nitrate = 0.36 mg/L
Fluoride < 0.10 mg/L
Carbonate & bicarbonate = natural

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Figure 4140:8. Electropherogram of typical municipal wastewater discharge, undiluted.

Chloride = 93.3 mg/L
Nitrate = 0.46 mg/L
Sulfate = 60.3 mg/L
Nitrate < 40.8 mg/L
Carbonate & bicarbonate = natural

Figure 4140:9. Electropherogram of typical industrial wastewater discharge, undiluted.

Chloride = 2.0 mg/L
Nitrite = 1.6 mg/L
Sulfate = 34.7 mg/L
Nitrate = 16.5 mg/L
Formate < 0.05 mg/L
Phosphate = 12.3 mg/L
Carbonate & bicarbonate = natural

Figure 4500-Br^-:1. FIA bromide manifold.

Figure 4500-CO_2:1. Nomograph for evaluation of hydroxide ion concentration. To use: Align temperature (Scale 1) and total dissolved solids (Scale 5); pivot on Line 2 to proper pH (Scale 3); read hydroxide ion concentration, as mg $CaCO_3$/L, on Scale 4. (Example: For 13°C temperature, 240 mg total dissolved solids/L, pH 9.8, the hydroxide ion concentration is found to be 1.4 mg as $CaCO_3$/L.)

Figure 4500-CO_2:2. Nomograph for evaluation of bicarbonate alkalinity. To use: Align temperature (Scale 1) and total dissolved solids (Scale 3); pivot on Line 2 to proper pH (Scale 4) and read constant on Scale 5; locate constant on Scale 6 and align with nonhydroxide alkalinity (found with aid of Figure 4500-CO_2:1) on Scale 7; read bicarbonate alkalinity on Scale 8. (Example: For 13°C temperature, 240 mg total dissolved solids/L, pH 9.8, and 140 mg alkalinity/L, the bicarbonate content is found to be 90 mg as $CaCO_3$/L.)

Figure 4500-CO_2:3. Nomograph for evaluation of carbonate alkalinity. To use: Align temperature (Scale 1) and total dissolved solids (Scale 3); pivot on Line 2 to proper pH (Scale 4) and read constant on Scale 5; locate constant on Scale 6 and align with nonhydroxide alkalinity (found with aid of Figure 4500-CO_2:1) on Scale 7; read carbonate alkalinity on Scale 8. (Example: For 13°C temperature, 240 mg total dissolved solids/ L, pH 9.8, and 140 mg alkalinity/L, the carbonate content is found to be 50 mg as $CaCO_3$/L.)

Figure 4500-CO_2:4. Nomograph for evaluation of free carbon dioxide content. To use: Align temperature (Scale 1) and total dissolved solids (Scale 3), which determines Point P_1 on Line 2; align pH (Scale 4) and bicarbonate alkalinity (Scale 7), which determines Point P_2 on Line 6; align P_1 and P_2 and read free carbon dioxide on Scale 5. (Example: For 13°C temperature, 560 mg total dissolved solids/L, pH 7.4, and 320 mg alkalinity/ L, the free

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carbon dioxide content is found to be 28 mg/L.)

Figure 4500-CN^-:1. Cyanide distillation apparatus.

Figure 4500-CN^-:2. FIA cyanide manifold.

Figure 4500-CN^-:3. FIA in-line total and WAD cyanide manifold.

Figure 4500-Cl^-:1. Example of differential titration curve (end point is 25.5 mL).

Figure 4500-Cl^-:2. Flow scheme for automated chloride analysis.

Figure 4500-Cl^-:3. FIA chloride manifold.

Figure 4500-ClO_2:1. Chlorine dioxide generation and absorption system.

Figure 4500-F^-:1. Direct distillation apparatus for fluoride.

Figure 4500-F^-:2. Fluoride manifold.

Figure 4500-F^-:3. FIA fluoride manifold.

Figure 4500-H^+:1. Electrode potential vs. pH. Intercept control shifts response curve laterally.

Figure 4500-H^+:2. Typical pH electrode response as a function of temperature.

Figure 4500-N:1. FIA in-line total nitrogen manifold.

Figure 4500-NH_3:1. Ammonia manifold.

Figure 4500-NH_3:2. FIA ammonia manifold.

Figure 4500-NO_3^-:1. Reduction column.

Figure 4500-NO_3^-:2. Nitrate-nitrite manifold.

Figure 4500-NO_3^-:3. Nitrate-nitrite manifold.

Figure 4500-NO_3^-:4. FIA nitrate + nitrite manifold.

Figure 4500-N_{org}:1. Micro-kjeldahl distillation apparatus.

Figure 4500-N_{org}:2. FIA total kjeldahl nitrogen manifold.

Figure 4500-O:1. DO and BOD sampler assembly.

Figure 4500-O:2. Effect of temperature on electrode sensitivity.

Figure 4500-O:3. The salting-out effect at different temperatures.

Figure 4500-O:4. Typical trend of effect of stirring on electrode response.

Figure 4500-P:1. Steps for analysis of phosphate fractions.

* Direct determination of phosphorus on the membrane filter containing suspended matter will be required where greater precision than that obtained by difference is desired. Digest filter with HNO_3 and follow by perchloric acid. Then perform colorimetry.

† Total phosphorus measurements on highly saline samples may be difficult because of

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precipitation of large quantities of salt as a result of digestion techniques that drastically reduce sample volume. For total phosphorus analyses on such samples, directly determine total dissolved phosphorus and total suspended phosphorus and add the results.
‡ In determination of total dissolved or total suspended reactive phosphorus, anomalous results may be obtained on samples containing large amounts of suspended sediments. Very often results depend largely on the degree of agitation and mixing to which samples are subjected during analysis because of a time-dependent desorption of orthophosphate from the suspended particles.

Figure 4500-P:2. Phosphate manifold for automated analytical system.

Figure 4500-P:3. FIA orthophosphate manifold.

Figure 4500-P:4. FIA total phosphorus manifold.

Figure 4500-P:5. FIA in-line total phosphorus manifold.

Figure 4500-P:6. Correlation between manual and in-line total phosphorus methods.

Figure 4500-SiO_2:1. Silica manifold.

Figure 4500-SiO_2:2. FIA manifold.

Figure 4500-S^{2-}:1. Analytical flow paths for sulfide determinations.

Figure 4500-S^{2-}:2. Sulfide manifold.

Figure 4500-S^{2-}:3. Proportions of H_2S and HS^- in dissolved sulfide.

Figure 4500-S^{2-}:4. FIA sulfide manifold.

Figure 4500-SO_3^{2-}:1. Apparatus for evolution of SO_2 from samples for colorimetric analysis.

Figure 4500-SO_4^{2-}:1. Sulfate manifold.

Figure 4500-SO_4^{2-}:2. FIA manifold.

Tables

TABLE 4110:I. DETERMINATION OF BIAS FOR FLUORIDE

Water	Amount Added *mg/L*	Amount Found *mg/L*	S_t	S_0	Bias %

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Water	Amount Added *mg/L*	Amount Found *mg/L*	S_t	S_0	Bias %
Reagent	0.26	0.25	0.08	0.11	–3.8
	0.34	0.29	0.11		–14.7
	2.12	2.12	0.07	0.12	0.0
	2.55	2.48	0.14		–2.7
	6.79	6.76	0.20	0.19	–0.4
	8.49	8.46	0.30		–0.4
Drinking	0.26	0.24	0.08	0.05	–7.7
	0.34	0.34	0.11		0.0
	2.12	2.09	0.18	0.06	–1.4
	2.55	2.55	0.16		0.0
	6.79	6.84	0.54	0.25	+0.7
	8.49	8.37	0.75		–1.4
Waste	0.26	0.25	0.15	0.06	–3.8
	0.34	0.32	0.08		–5.9
	2.12	2.13	0.22	0.15	+0.5
	2.55	2.48	0.16		–2.7
	6.79	6.65	0.41	0.20	–2.1
	8.49	8.27	0.36		–2.6

Source: American Society for Testing and Materials. 1992. Method D4327. Annual Book of ASTM Standards, Vol. 11.01 Water. American Soc. Testing Materials, Philadelphia, Pa.

TABLE 4110:II. DETERMINATION OF BIAS FOR CHLORIDE

Water	Amount Added *mg/L*	Amount Found *mg/L*	S_t	S_0	Bias %
Reagent	0.78	0.79	0.17	0.29	+1.3
	1.04	1.12	0.46		+7.7
	6.50	6.31	0.27	0.14	–2.9
	7.80	7.76	0.39		–0.5
	20.8	20.7	0.54	0.62	–0.5
	26.0	25.9	0.58		–0.4

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Water	Amount Added mg/L	Amount Found mg/L	S_t	S_0	Bias %
Drinking	0.78	0.54	0.35	0.20	–30.8
	1.04	0.51	0.38		–51.0
	6.50	5.24	1.35	1.48	–19.4
	7.80	6.02	1.90		–22.8
	20.8	20.0	2.26	1.14	–3.8
	26.0	24.0	2.65		–7.7
Waste	0.78	0.43	0.32	0.39	–44.9
	1.04	0.65	0.48		–37.5
	6.50	4.59	1.82	0.83	–29.4
	7.80	5.45	2.02		–30.1
	20.8	18.3	2.41	1.57	–11.8
	26.0	23.0	2.50		–11.5

Source: American Society for Testing and Materials. 1992. Method D4327. Annual Book of ASTM Standards, Vol. 11.01 Water. American Soc. Testing Materials, Philadelphia, Pa.

TABLE 4110:III. DETERMINATION OF BIAS FOR NITRITE NITROGEN

Water	Amount Added mg/L	Amount Found mg/L	S_t	S_0	Bias %
Reagent	0.36	0.37	0.04	0.04	+2.8
	0.48	0.48	0.06		0.0
	3.00	3.18	0.12	0.06	+6.0
	3.60	3.83	0.12		+6.4
	9.60	9.84	0.36	0.26	+2.5
	12.0	12.1	0.27		+0.6
Drinking	0.36	0.30	0.13	0.03	–16.7
	0.48	0.40	0.14		–16.7
	3.00	3.02	0.23	0.12	+0.7
	3.60	3.62	0.22		+0.6
	9.60	9.59	0.44	0.28	–0.1
	12.0	11.6	0.59		–3.1

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Water	Amount Added mg/L	Amount Found mg/L	S_t	S_0	Bias %
Waste	0.36	0.34	0.06	0.04	–5.6
	0.48	0.46	0.07		–4.2
	3.00	3.18	0.13	0.10	+6.0
	3.60	3.76	0.18		+4.4
	9.60	9.74	0.49	0.26	+1.5
	12.0	12.0	0.56		+0.3

Source: American Society for Testing and Materials. 1992. Method D4327. Annual Book of ASTM Standards, Vol. 11.01 Water. American Soc. Testing Materials, Philadelphia, Pa.

TABLE 4110:IV. DETERMINATION OF BIAS FOR BROMIDE

Water	Amount Added mg/L	Amount Found mg/L	S_t	S_0	Bias %
Water	0.63	0.69	0.11	0.05	+9.5
	0.84	0.85	0.12		+1.2
	5.24	5.21	0.22	0.21	–0.6
	6.29	6.17	0.35		–1.9
	16.8	17.1	0.70	0.36	+1.6
	21.0	21.3	0.93		+1.5
Drinking	0.63	0.63	0.13	0.04	0.0
	0.84	0.81	0.13		–3.6
	5.24	5.11	0.23	0.13	–2.5
	6.29	6.18	0.30		–1.7
	16.8	17.0	0.55	0.57	+0.9
	21.0	20.9	0.65		–0.4
Waste	0.63	0.63	0.15	0.09	0.0
	0.84	0.85	0.15		+1.2
	5.24	5.23	0.36	0.11	–0.2
	6.29	6.27	0.46		–0.3
	16.8	16.6	0.69	0.43	–1.0
	21.0	21.1	0.63		+0.3

Source: American Society for Testing and Materials. 1992. Method D4327. Annual Book of ASTM Standards, Vol. 11.01 Water. American Soc. Testing Materials, Philadelphia, Pa.

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TABLE 4110:V. DETERMINATION OF BIAS FOR NITRATE NITROGEN

Water	Amount Added *mg/L*	Amount Found *mg/L*	S_t	S_0	Bias %
Reagent	0.42	0.42	0.04	0.02	0.0
	0.56	0.56	0.06		0.0
	3.51	3.34	0.15	0.08	–4.8
	4.21	4.05	0.28		–3.8
	11.2	11.1	0.47	0.34	–1.1
	14.0	14.4	0.61		+2.6
Drinking	0.42	0.46	0.08	0.03	+9.5
	0.56	0.58	0.09		+3.6
	3.51	3.45	0.27	0.10	–1.7
	4.21	4.21	0.38		0.0
	11.2	11.5	0.50	0.48	+2.3
	14.0	14.2	0.70		+1.6
Waste	0.42	0.36	0.07	0.06	–14.6
	0.56	0.40	0.16		–28.6
	3.51	3.19	0.31	0.07	–9.1
	4.21	3.84	0.28		–8.8
	11.2	10.9	0.35	0.51	–3.0
	14.0	14.1	0.74		+0.4

Source: American Society for Testing and Materials. 1992. Method D4327. Annual Book of ASTM Standards, Vol. 11.01 Water. American Soc. Testing Materials, Philadelphia, Pa.

TABLE 4110:VI. DETERMINATION OF BIAS FOR ORTHOPHOSPHATE

Water	Amount Added *mg/L*	Amount Found *mg/L*	S_t	S_0	Bias %
Reagent	0.69	0.69	0.06	0.06	0.0
	0.92	0.98	0.15		+6.5
	5.77	5.72	0.36	0.18	–0.9
	6.92	6.78	0.42		–2.0
	18.4	18.8	1.04	0.63	+2.1
	23.1	23.2	0.35		+0.4

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Water	Amount Added *mg/L*	Amount Found *mg/L*	S_t	S_0	Bias %
Drinking	0.69	0.70	0.17	0.17	+1.4
	0.92	0.96	0.20		+4.3
	5.77	5.43	0.52	0.40	–5.9
	6.92	6.29	0.72		–9.1
	18.4	18.0	0.68	0.59	–2.2
	23.1	22.6	1.07		–2.0
Waste	0.68	0.64	0.26	0.09	–7.2
	0.92	0.82	0.28		–10.9
	5.77	5.18	0.66	0.34	–10.2
	6.92	6.24	0.74		–9.8
	18.4	17.6	2.08	1.27	–4.1
	23.1	22.4	0.87		–3.0

Source: American Society for Testing and Materials. 1992. Method D4327. Annual Book of ASTM Standards, Vol. 11.01 Water. American Soc. Testing Materials, Philadelphia, Pa.

TABLE 4110:VII. DETERMINATION OF BIAS FOR SULFATE

Water	Amount Added *mg/L*	Amount Found *mg/L*	S_t	S_0	Bias %
Reagent	2.85	2.83	0.32	0.52	–0.7
	3.80	3.83	0.92		+0.8
	23.8	24.0	1.67	0.68	+0.8
	28.5	28.5	1.56		–0.1
	76.0	76.8	3.42	2.33	+1.1
	95.0	95.7	3.59		+0.7
Drinking	2.85	1.12	0.37	0.41	–60.7
	3.80	2.26	0.97		–40.3
	23.8	21.8	1.26	0.51	–8.4
	28.5	25.9	2.48		–9.1
	76.0	74.5	4.63	2.70	–2.0
	95.0	92.3	5.19		–2.8

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Water	Amount Added *mg/L*	Amount Found *mg/L*	S_t	S_0	Bias %
Waste	2.85	1.89	0.37	0.24	–33.7
	3.80	2.10	1.25		–44.7
	23.8	20.3	3.19	0.58	–14.7
	28.5	24.5	3.24		–14.0
	76.0	71.4	5.65	3.39	–6.1
	95.0	90.3	6.80		–5.0

Source: American Society for Testing and Materials. 1992. Method D4327. Annual Book of ASTM Standards, Vol. 11.01 Water. American Soc. Testing Materials, Philadelphia, Pa.

TABLE 4110:VIII. DETECTION LIMITS FOR ANIONS IN REAGENT WATER*

Anion	Retention Time *min*	MDL†[1] *mg/L*
Cl^-	2.3	0.035
NO_2^-	3.1	0.022
Br^-	4.2	0.110
NO_3^-	5.3	0.035
PO_4^{3-}	5.7	0.110
SO_4^{2-}	8.3	0.350

* Standard conditions as defined in text.

† MDL calculated from the peak height in mm taken from chart recorder.

TABLE 4110:IX. SINGLE-COLUMN CHROMATOGRAPHY SINGLE-OPERATOR PRECISION AND BIAS*

Anion	Sample Type†	Amount Added *mg/L*	Mean Recovery %	SD *mg/L*	Anion	Sample Type†	Amount Added *mg/L*	Mean Recovery %
Cl^-	RW	16	105	1.6	NO3+–	RW	8	103
	DW	16	98	1.9		DW	8	87
NO2+–	RW	4	101	0.10	PO4+3–	RW	16	113
	DW	4	101	0.43		DW	16	110
Br^-	RW	16	104	0.75	SO4+2–	RW	32	101
	DW	16	98	2.3		DW	32	94

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*Data provided by EPA/EMSL, Cincinnati, Ohio 95268. Seven replicates were analyzed for each anion and sample type.
†RW = reagent water; DW = drinking water.

TABLE 4140:I. COLLABORATIVE DESIGN AS FOUR YOUDEN PAIR SETS

Anion	Anion Concentration in Individual Youden Pair Standards *mg/L*							
	1	2	3	4	5	6	7	8
Cl^-	0.7	2.0	3.0	15.0	40.0	20.0	50.0	0.5
Br^-	2.0	3.0	15.0	40.0	20.0	50.0	0.7	0.5
NO_2^-	3.0	40.0	20.0	15.0	50.0	0.5	2.0	0.7
SO_4^{2-}	40.0	50.0	0.5	0.7	2.0	3.0	15.0	20.0
NO_3^-	15.0	20.0	40.0	50.0	0.5	0.7	2.0	3.0
F^-	2.0	0.7	0.5	3.0	10.0	7.0	20.0	25.0
PO_4^{3-}	50.0	40.0	20.0	0.5	3.0	2.0	0.7	15.0

* The collaborative design is intended to demonstrate performance between 0.1 and 50 mg anion/L, except for fluoride between 0.1 and 25 mg/L. The concentrations among anions are varied so as not to have any one standard at all low or all high anion concentrations.

TABLE 4140:II. ANION MIGRATION TIME REPRODUCIBILITY FROM YOUDEN PAIR STANDARDS

Youden Standard	Anion Midpoint Migration Time, Average of Triplicate Samplings *min*						
	Cl^-	Br^-	NO_2^-	SO_4^{2-}	NO_3^-	F^-	PO_4^{3-}
1	3.132	3.226	3.275	3.405	3.502	3.761	3.906
2	3.147	3.239	3.298	3.431	3.517	3.779	3.931
3	3.138	3.231	3.283	3.411	3.497	3.771	3.925
4	3.158	3.244	3.307	3.434	3.510	3.781	3.963
5	3.184	3.271	3.331	3.435	3.551	3.787	3.981
6	3.171	3.260	3.312	3.418	3.537	3.776	3.964
7	3.191	3.272	3.315	3.437	3.544	3.773	3.978
8	3.152	3.248	3.294	3.418	3.526	3.739	3.954
SD	0.021	0.015	0.018	0.012	0.20	0.015	0.027
%RSD	0.67%	0.46%	0.55%	0.36%	0.56%	0.40%	0.68%

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Youden Standard	Anion Midpoint Migration Time, Average of Triplicate Samplings *min*						
	Cl^-	Br^-	NO_2^-	SO_4^{2-}	NO_3^-	F^-	PO_4^{3-}

* Average SD = 0.018 min = 1.1 s; average %RSD = 0.53%.

TABLE 4140:III. COMPARISON OF CAPILLARY ION ELECTROPHORESIS AND OTHER METHODS

Source	Statistic	Value for Given Anion *mg/L*					
		Cl^-	NO_2^-	SO_4^{2-}	NO_3^-	F^-	PO_4^{3-}
Performance evaluation standard	True value	43.00	1.77	37.20	15.37	2.69	6.29
Wet chemical and ion chromatography methods	Measured mean	43.30	1.77	37.00	15.42	2.75	6.38
	Measured SD	3.09	0.07	2.24	1.15	0.26	0.21
CIE using chromate electrolyte‡	Average (n = 18)	43.34	1.64	37.11	14.41	2.64	6.34
	CIE/mean	1.003	0.927	1.003	0.935	0.959	0.993
	CIE/true value	1.008	0.927	0.996	0.938	0.981	1.008

* Purchased from APG Laboratories, June 1996; diluted 1:100 with deionized water.
† Measured result is the average from numerous laboratories using approved *Standard Methods* and EPA wet chemistry and ion chromatography methods.
‡ CIE results determined in July 1996 with proposed EPA and ASTM method, operationally identical to 4140; they are the average from four laboratories using the Youdenpair standards for quantitation. These data can be considered known addition of the performance evaluation standard in reagent water; they conform to quality control acceptance limits given in Section 1020.

TABLE 4140:IV. CAPILLARY ION ELECTROPHORESIS REPRODUCIBILITY AND PRECISION*

Laboratory No.	Anion Concentration *mg/L*				
	Cl^-	NO_2^-	SO_4^{2-}	NO_3^-	F^-
1 (n = 5)	43.22 ± 0.22	1.58 ± 0.09	36.39 ± 0.33	14.57 ± 0.12	2.54 ±
2 (n = 5)	43.68 ± 0.61	1.58 ± 0.08	37.01 ± 0.37	13.94 ± 0.09	2.69 ±
3 (n = 5)	43.93 ± 0.39	1.60 ± 0.06	37.68 ± 0.24	15.05 ± 0.11	2.69 ±

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	Anion Concentration *mg/L*				
Laboratory No.	Cl^-	NO_2^-	SO_4^{2-}	NO_3^-	F^-
4 (*n* = 3)	42.51 ± 0.22	1.78 ± 0.06	37.34 ± 0.19	14.06 ± 0.07	2.69 ±
Average (mean ± SD)	43.34 ± 0.36	1.64 ± 0.07	37.11 ± 0.28	14.41 ± 0.10	2.64 ±
%RSD	0.83%	4.5%	0.77%	0.67%	1.6

* Results from four laboratories analyzing the performance evaluation standard using the Youden pair standards for quantitation. Only one laboratory reported results for PO_4^{3-} as 6.34 ± 0.02 mg/L on triplicate samplings yielding %RSD of 0.07%. Calculated replicate reproducibility and precision conform to the quality control acceptance limits given in Section 1020.

TABLE 4140:V. CAPILLARY ION ELECTROPHORESIS KNOWN-ADDITION RECOVERY AND PRECISION OF PERFORMANCE EVALUATION STANDARD WITH DRINKING WATER

	Value for Given Anion				
Variable	Cl^-	NO_2^-	SO_4^{2-}	NO_3^-	F^-
Milford drinking water (MDW) *n* = 3, concentration as mg/L	24.72 ± 0.18	Not detected	7.99 ± 0.07	0.36 ± 0.05	Not detected
%RSD	0.73	—	0.91	13.3	—
Performance evaluation standard (PES), concentration as mg/L	43.00	1.77	37.20	15.37	2.69
MDW + PES,* *n* = 3, concentration as mg/L	66.57 ± 0.34	1.74 ± 0.03	45.19 ± 0.17	15.42 ± 0.12	2.62 ± 0.07
%RSD	0.51%	1.85%	0.38%	0.79%	2.69%
% Recovery	97.9%	98.3%	100.2%	98.1%	97.4%

* Performance evaluation standard diluted 1:100 with Milford drinking water. Calculated analyte recovery and precision conform to the quality control acceptance limits given in Section 1020.

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TABLE 4140:VI. COMPARISON OF CAPILLARY ION ELECTROPHORESIS WITH CHROMATE ELECTROLYTE WITH OTHER METHODS FOR THE DETERMINATION OF ANIONS

			Concentration by Given Method* *mg/L*		
Anion	**Matrix**	**Sample No.**	**Wet Chemical or Other Method†**	**IC‡**	**CIE**
Chloride	Effluent	1	—	149	147
		2	—	162	161
		3	—	152	151
		4	—	139	139
		5	—	111	110
		6	—	109	107
		7	—	3.6	3.5
	Drinking water	1	5.5	5.1	5.0
		2	5.5	5.0	5.0
		3	5.3	5.2	5.2
		4	5.5	5.1	5.1
		5	5.3	5.0	5.1
		6	5.3	4.9	4.9
		7	5.5	4.9	4.9
	Landfill leachate	1	0.1	<0.1	ND
		2	230	245	240
Fluoride	Effluent	1	1.7	1.2	1.5
		2	0.9	0.6	0.6
		3	0.8	0.5	0.6
		4	0.8	0.4	0.7
		5	0.9	0.5	0.8
		6	0.9	0.5	0.7
		7	<0.1	ND	<0.1

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Anion	Matrix	Sample No.	Concentration by Given Method* *mg/L*		
			Wet Chemical or Other Method†	IC‡	CIE
	Drinking water	1	1.2	0.9	0.9
		2	1.3	0.9	0.9
		3	1.3	0.9	0.9
		4	1.3	0.9	0.9
		5	1.3	0.9	0.9
		6	0.9	0.6	0.6
		7	1.3	0.9	0.9
	Landfill leachate	1	<0.2	ND	ND
		2	16	10.6	10.9
Sulfate	Effluent	1	98	87.5	86.4
		2	110	95.3	95.9
		3	130	118	115
		4	130	139	136
		5	110	113	110
		6	100	107	106
		7	6	5.6	5.8
	Drinking water	1	6	5.8	6.0
		2	6	5.8	6.0
		3	6	5.9	6.1
		4	6	5.9	6.1
		5	5	5.8	6.2
		6	4	3.0	3.4
		7	5	5.8	6.1
	Landfill leachate	1	<1	ND	ND

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Anion	Matrix	Sample No.	Concentration by Given Method* *mg/L* Wet Chemical or Other Method†	IC‡	CIE
		2	190	211	201
Nitrite nitrate§ (as N)	Effluent	1	0.3	ND	ND
		2	—	ND	ND
		3	—	ND	ND
		4	—	ND	0.5
		5	—	2.1	2.4
		6	2.4	1.9	2.2
		7	0.7	0.3	0.4
	Drinking water	1	0.6	0.3	0.4
		2	0.6	0.3	0.4
		3	0.4	0.3	0.4
		4	0.6	0.3	0.3
		5	0.6	0.3	0.4
		6	0.3	0.1	0.1
		7	0.5	0.3	0.4
	Landfill leachate	1	—	ND	ND
		2	—	ND	ND
Orthophosphate (as P)	Effluent	1	3.4	ND	2.8
		2	4.9	ND	4.4
		3	4.7	ND	4.5
		4	5.3	ND	4.2
		5	3.0	ND	3.0
		6	2.9	ND	2.3
		7	<0.1	ND	0.04

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Anion	Matrix	Sample No.	Concentration by Given Method* *mg/L*		
			Wet Chemical or Other Method†	IC‡	CIE
	Drinking water	1	<0.1	ND	ND
		2	<0.1	ND	ND
		3	—	ND	ND
		4	<0.1	ND	ND
		5	<0.1	ND	ND
		6	—	ND	ND
		7	—	ND	ND
	Landfill leachate	1	<0.1	ND	0.1
		2	2.2	1.6	1.4

* — = test not performed; ND = not detected.
† Methods used were: chloride—iodometric method (4500-Cl.C); fluoride—ion selective electrode method (4500-F.C); sulfate—turbidimetric method (4500-SO_4.E); nitrite+ nitrate (total)—cadmium reduction method (4500-NO_3.E); orthophosphate—ascorbic acid method (4500-P.E).
‡ Single-column ion chromatography with direct conductivity detection (4110C).
§ Each technique gave separate nitrite and nitrate values; because of their interconvertability, results were added for comparison purposes.

TABLE 4500-CN^-:I. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition *mg CN^-/L*	Recovery %	Relative Standard Deviation %
Wastewater treatment	Reference sample*	—	94	—
plant	Blank†	0.050	96	—
influent		0.10	96	—
	Site A‡	0	—	<0.5
		0.010	104	—
		0.020	104	—
	Site B‡	0	—	<0.5
		0.010	101	—

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Matrix	Sample/Blank Designation	Known Addition *mg CN¯/L*	Recovery %	Relative Standard Deviation %
		0.020	106	—
	Site C‡	0	—	<0.5
		0.010	103	—
		0.020	108	—
Wastewater treatment plant effluent	Reference sample*	—	95	—
	Blank†	0.050	88	—
		0.10	95	—
	Site A‡	0	—	<0.5
		0.010	112	—
		0.020	106	—
	Site B‡	0	—	<0.5
		0.010	110	—
		0.020	105	—
	Site C‡	0	—	<0.5
		0.010	101	—
		0.020	106	—
Landfill leachate	Reference sample*	—	98	—
	Blank†	0.050	96	—
		0.10	98	—
	Site A‡	0	—	<0.5
		0.050	114	—
		0.10	106	—
	Site B‡	0	—	<0.5
		0.050	104	—
		0.10	104	—
	Site C‡	0	—	<0.5
		0.050	103	—

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Matrix	Sample/Blank Designation	Known Addition *mg CN⁻/L*	Recovery %	Relative Standard Deviation %
		0.10	107	—

* U.S. EPA QC sample, 0.498 mg CN^-/L, diluted five-fold.
† Determined in duplicate.
‡ Samples diluted five-fold. Samples without known additions determined four times; sample with known additions determined in duplicate; typical relative differences between duplicates <0.5%.

TABLE 4500-Cl⁻:I. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition *mg Cl⁻/L*	Recovery %	Relative Standard Deviation %
Wastewater treatment	Reference sample*	—	101	—
plant influent	Blank†	10	104	—
		20	102	—
	Site A‡	0	—	0.4
		10	92	—
		20	101	—
	Site B‡	0	—	0.2
		10	97	—
		20	106	—
	Site C‡	0	—	0.4
		10	102	—
		20	102	—
Wastewater treatment	Reference sample*	—	101	—
plant effluent	Blank†	10	104	—
		20	102	—
	Site A‡	0	—	0.3
		10	98	—
		20	101	—
	Site B‡	0	—	0.2
		10	99	—
		20	103	—

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Matrix	Sample/Blank Designation	Known Addition mg Cl^-/L	Recovery %	Relative Standard Deviation %
	Site C‡	0	—	0.4
		10	91	—
		20	97	—
Landfill leachate‖	Reference sample*	—	100	—
	Blank†	10	101	—
		20	100	—
	Site A§	0	—	0.3
		10	97	—
		20	103	—
	Site B§	0	—	0.2
		10	89	—
		20	103	—
	Site C§	0	—	0.5
		10	89	—
		20	103	—

* U.S. EPA nutrient QC sample, 51.7 mg Cl^-/L.
† Determined in duplicate.
‡ Samples diluted 5-fold. Samples without known additions determined four times; samples with known additions determined in duplicate. Typical relative difference between duplicates 0.2%.
§ Sample from Site A diluted 50-fold, those from B and C 100-fold. Samples without known additions determined four times; samples with known additions determined in duplicate; typical relative difference between duplicates 0.5%.

TABLE 4500-ClO_2:I. EQUIVALENT WEIGHTS FOR CALCULATING CONCENTRATIONS ON THE BASIS OF MASS

pH	Species	Molecular Weight mg/mol	Electrons Transferred	Equivalent Weight mg/eq
7	Chlorine dioxide	67 452	1	67 452
2, 0.1	Chlorine dioxide	67 452	5	13 490
7, 2, 0.1	Chlorine	70 906	2	35 453
2, 0.1	Chlorite	67 452	4	16 863
0.1	Chlorate	83 451	6	13 909

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TABLE 4500-F^-:I. CONCENTRATION OF SOME SUBSTANCES CAUSING 0.1-MG/L ERROR AT 1.0 MG F/L IN FLUORIDE METHODS

Substance	Method C (Electrode) Conc *mg/L*	Method C (Electrode) Type of Error*	Method D (SPADNS) Conc *mg/L*	Method D (SPADNS) Type of Error*
Alkalinity ($CaCO_3$)	7 000	+	5 000	–
Aluminum (Al^{3+})	3.0	–	0.1†	–
Chloride (Cl^-)	20 000		7 000	+
Chlorine	5 000			Remove completely with arsenite
Color & turbidity				Remove or compensate for
Iron	200	–	10	–
Hexametaphosphate ($[NaPO_3]$)	50 000		1.0	+
Phosphate (PO_4^{3-})	50 000		16	+
Sulfate (SO_4^{2-})	50 000	–	200	–

* + denotes positive error
– denotes negative error
Blank denotes no measurable error.
† On immediate reading. Tolerance increases with time: after 2 h, 3.0; after 4 h, 30.

TABLE 4500-F^-:II. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition *mg F^-/L*	Recovery %	Relative Standard Deviation %
Wastewater treatment	Reference sample*	—	101	—

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Matrix	Sample/Blank Designation	Known Addition *mg F⁻/L*	Recovery %	Relative Standard Deviation %
plant	Blank†	1.0	91	—
influent		2.0	97	—
	Site A‡	0	—	4.8
		1.0	93	—
		2.0	82	—
	Site B‡	0	—	6.4
		1.0	96	—
		2.0	86	—
	Site C‡§	0	—	15
		1.0	99	—
		2.0	86	—
Wastewater treatment	Reference sample*	—	103	—
plant	Blank†	1.0	97	—
effluent		2.0	97	—
	Site A‡	0	—	ND
		1.0	ND	—
		2.0	ND	—
	Site B‡	0	—	<0.1
		1.0	80	—
		2.0	78	—
	Site C‡	0	—	<0.1
		1.0	93	—
		2.0	91	—
Landfill leachate‖	Reference sample*	—	99	—
	Blank†	1.0	87	—
		2.0	88	—
	Site A‡	0	—	13

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Matrix	Sample/Blank Designation	Known Addition *mg F⁻/L*	Recovery %	Relative Standard Deviation %
		1.0	74	—
		2.0	68	—
	Site B‡	0	—	10
		1.0	68	—
		2.0	73	—
	Site C‡	0	—	32
		1.0	66	—
		2.0	79	—

ND = not detectable.

* U.S. EPA QC sample, 1.81 mg F^-/L.
† Determined in duplicate.
‡ Samples without known additions determined four times; samples with knownadditions determined in duplicate. Typical difference between duplicates for influent 5%, for effluent 6%.
§ Mean concentration 0.18 mg F^-/L.
‖ All sites had mean concentration of <0.2 mg F^-/L.

TABLE 4500-H^+:I. PREPARATION OF PH STANDARD SOLUTIONS[3]

Standard Solution (molality)	pH at 25°C	Weight of Chemicals Needed/1000 mL Aqueous Solution at 25°C
Primary standards:		
Potassium hydrogen tartrate (saturated at 25°C)	3.557	> 7 g $KHC_4H_4O_6$*
0.05 potassium dihydrogen citrate	3.776	11.41 g $KH_2C_6H_5O_7$
0.05 potassium hydrogen phthalate	4.004	10.12 g $KHC_8H_4O_4$
0.025 potassium dihydrogen phosphate + 0.025 disodium hydrogen phosphate	6.863	3.387 g KH_2PO_4+ 3.533 g Na_2HPO_4†
0.008 695 potassium dihydrogen phosphate + 0.030 43 disodium hydrogen phosphate	7.415	1.179 g KH_2PO_4 + 4.303 g Na_2HPO_4†
0.01 sodium borate decahydrate (borax)	9.183	3.80 g $Na_2B_4O_7 \cdot 10H_2O$†

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Standard Solution (molality)	pH at 25°C	Weight of Chemicals Needed/1000 mL Aqueous Solution at 25°C
0.025 sodium bicarbonate + 0.025 sodium carbonate	10.014	2.092 g $NaHCO_3$ + 2.640 g Na_2CO_3
Secondary standards:		
0.05 potassium tetroxalate dihydrate	1.679	12.61 g $KH_3C_4O_8 \cdot 2H_2O$
Calcium hydroxide (saturated at 25°C)	12.454	> 2 g $Ca(OH)_2$*

* Approximate solubility.
† Prepare with freshly boiled and cooled distilled water (carbon-dioxide-free).

TABLE 4500-H^+:II. STANDARD PH VALUES[3]

	Primary Standards						
Temperature °*C*	Tartrate (Saturated)	Citrate (0.05*M*)	Phthalate (0.05*M*)	Phosphate (1:1)	Phosphate (1:3.5)	Borax (0.01*M*)	Bicarbonate-Carbonate (0.025*M*)
0			4.003	6.982	7.534	9.460	10.321
5			3.998	6.949	7.501	9.392	10.248
10			3.996	6.921	7.472	9.331	10.181
15			3.996	6.898	7.449	9.276	10.120
20			3.999	6.878	7.430	9.227	10.064
25	3.557	3.776	4.004	6.863	7.415	9.183	10.014
30	3.552		4.011	6.851	7.403	9.143	9.968
35	3.549		4.020	6.842	7.394	9.107	9.928
37			4.024	6.839	7.392	9.093	
40	3.547		4.030	6.836	7.388	9.074	9.891
45	3.547		4.042	6.832	7.385	9.044	9.859
50	3.549		4.055	6.831	7.384	9.017	9.831
55	3.554		4.070				
60	3.560		4.085				
70	3.580		4.12				
80	3.609		4.16				
90	3.650		4.19				
85	3.674		4.21				

TABLE 4500-N:I. RECOVERIES OF TOTAL NITROGEN

	Mean Recovery%	
Compound	10 mg N/L	4 mg N/L
Ammonium chloride	98.1	99.7

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Compound	Mean Recovery%	
	10 mg N/L	4 mg N/L
Sodium nitrite	100.5	101.8
Glycine	101.0	100.8
Glutamic acid	99.7	99.2
Ammonium *p*-toluenesulfonate	99.6	97.4
Glycine *p*-toluenesulfonate	101.4	102.3
Nicotinic acid	98.6	102.0
Urea	94.9	98.0
EDTA	89.4	89.4

TABLE 4500-N:II. PRECISION DATA FOR TOTAL NITROGEN, PERSULFATE METHOD, BASED ON TRIPLICATE ANALYSES OF NICOTINIC ACID

Lab/ Analyst	Nicotinic Acid *mg N/L*	Recovery of N *%*	Standard Deviation *mg/L*	Relative Standard Deviation *%*
1/1	0.5	104	0.019	3.82
1/2	0.5	99.7	0.012	2.44
2/1	0.5	97.7	0.035	7.02
2/2	0.5	87.8	0.024	4.89
3/1	0.5	105	0.072	13.7
3/2	0.5	95.3	0.015	3.20
1/1	1.0	98.5	0.023	2.32
1/2	1.0	99.3	0.022	2.21
2/1	1.0	113	0.053	5.31
2/2	1.0	97.1	0.031	3.10
3/1	1.0	96.2	0.019	1.97
3/2	1.0	102	0.025	2.46
1/1	2.0	100	0.030	1.50
1/2	2.0	97.8	0.014	0.7
2/1	2.0	104	0.069	3.45
2/2	2.0	100	0.080	3.98
3/1	2.0	95.3	0.078	4.12
3/2	2.0	98.3	0.015	0.75

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TABLE 4500-NH_3:I. PRECISION AND BIAS OF AMMONIA-SELECTIVE ELECTRODE

Level *mg/L*	Matrix	Mean Recovery %	Precision	
			Overall % *RSD*	Single Operator % *RSD*
0.04	Distilled water	200	125	25
	Effluent water	100	75	0
0.10	Distilled water	180	50	10
	Effluent water	470	610	10
0.80	Distilled water	105	14	5
	Effluent water	105	38	7.5
20	Distilled water	95	10	5
	Effluent water	95	15	10
100	Distilled water	98	5	2
	Effluent water	97	—	—
750	Distilled water	97	10.4	1.6
	Effluent water	99	14.1	1.3

Source: AMERICAN SOCIETY FOR TESTING AND MATERIALS. Method 1426-79. American Soc. Testing Materials, Philadelphia, Pa.

TABLE 4500-NH_3:II. * VALUES OF Q VS. ΔE (59 MV SLOPE) FOR 10% VOLUME CHANGE

ΔE	Q	ΔE	Q	ΔE	Q	ΔE	Q	ΔE	Q
5.0	0.297	9.0	0.178	16.0	0.0952	24.0	0.0556	32.0	0.0354
5.1	0.293	9.1	0.176	16.2	0.0938	24.2	0.0549	32.2	0.0351
5.2	0.288	9.2	0.174	16.4	0.0924	24.4	0.0543	32.4	0.0347
5.3	0.284	9.3	0.173	16.6	0.0910	24.6	0.0536	32.6	0.0343
5.4	0.280	9.4	0.171	16.8	0.0897	24.8	0.0530	32.8	0.0340
5.5	0.276	9.5	0.169	17.0	0.0884	25.0	0.0523	33.0	0.0335
5.6	0.272	9.6	0.167	17.2	0.0871	25.2	0.0517	33.2	0.0333
5.7	0.268	9.7	0.165	17.4	0.0858	25.4	0.0511	33.4	0.0329
5.8	0.264	9.8	0.164	17.6	0.0846	25.6	0.0505	33.6	0.0326
5.9	0.260	9.9	0.162	17.8	0.0834	25.8	0.0499	33.8	0.0323

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ΔE	Q	ΔE	Q	ΔE	Q	ΔE	Q	ΔE	Q
6.0	0.257	10.0	0.160	18.0	0.0822	26.0	0.0494	34.0	0.0319
6.1	0.253	10.2	0.157	18.2	0.0811	26.2	0.0488	34.2	0.0316
6.2	0.250	10.4	0.154	18.4	0.0799	26.4	0.0482	34.4	0.0313
6.3	0.247	10.6	0.151	18.6	0.0788	26.6	0.0477	34.6	0.0310
6.4	0.243	10.8	0.148	18.8	0.0777	26.8	0.0471	34.8	0.0307
6.5	0.240	11.0	0.145	19.0	0.0767	27.0	0.0466	35.0	0.0304
6.6	0.237	11.2	0.143	19.2	0.0756	27.2	0.0461	36.0	0.0289
6.7	0.234	11.4	0.140	19.4	0.0746	27.4	0.0456	37.0	0.0275
6.8	0.231	11.6	0.137	19.6	0.0736	27.6	0.0450	38.0	0.0261
6.9	0.228	11.8	0.135	19.8	0.0726	27.8	0.0445	39.0	0.0249
7.0	0.225	12.0	0.133	20.0	0.0716	28.0	0.0440	40.0	0.0237
7.1	0.222	12.2	0.130	20.2	0.0707	28.2	0.0435	41.0	0.0226
7.2	0.219	12.4	0.128	20.4	0.0698	28.4	0.0431	42.0	0.0216
7.3	0.217	12.6	0.126	20.6	0.0689	28.6	0.0426	43.0	0.0206
7.4	0.214	12.8	0.123	20.8	0.0680	28.8	0.0421	44.0	0.0196
7.5	0.212	13.0	0.121	21.0	0.0671	29.0	0.0417	45.0	0.0187
7.6	0.209	13.2	0.119	21.2	0.0662	29.2	0.0412	46.0	0.0179
7.7	0.207	13.4	0.117	21.4	0.0654	29.4	0.0408	47.0	0.0171
7.8	0.204	13.6	0.115	21.6	0.0645	29.6	0.0403	48.0	0.0163
7.9	0.202	13.8	0.113	21.8	0.0637	29.8	0.0399	49.0	0.0156
8.0	0.199	14.0	0.112	22.0	0.0629	30.0	0.0394	50.0	0.0149
8.1	0.197	14.2	0.110	22.2	0.0621	30.2	0.0390	51.0	0.0143
8.2	0.195	14.4	0.108	22.4	0.0613	30.4	0.0386	52.0	0.0137
8.3	0.193	14.6	0.106	22.6	0.0606	30.6	0.0382	53.0	0.0131
8.4	0.190	14.8	0.105	22.8	0.0598	30.8	0.0378	54.0	0.0125
8.5	0.188	15.0	0.103	23.0	0.0591	31.0	0.0374	55.0	0.0120
8.6	0.186	15.2	0.1013	23.2	0.0584	31.2	0.0370	56.0	0.0115
8.7	0.184	15.4	0.0997	23.4	0.0576	31.4	0.0366	57.0	0.0110

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ΔE	Q	ΔE	Q	ΔE	Q	ΔE	Q	ΔE	Q
8.8	0.182	15.6	0.0982	23.6	0.0569	31.6	0.0362	58.0	0.0105
8.9	0.180	15.8	0.0967	23.8	0.0563	31.8	0.0358	59.0	0.0101

* Orion Research Inc. Instruction Manual, Ammonia Electrode, Model 95-12, Boston, MA. 02129.

TABLE 4500-NH_3:III. PRECISION DATA FOR MANUAL PHENATE METHOD BASED ON TRIPLICATE ANALYSES OF AMMONIUM SULFATE

Lab/ Analyst	NH_3-N Concentration *mg/L*	Optical Density	Relative Standard Deviation %
1/1	0.1	0.129	1.55
1/2	0.1	0.114	9.66
2/1	0.1	0.100	10.2
2/2	0.1	0.122	2.36
3/1	0.1	0.112	3.61
3/2	0.1	0.107	1.94
1/1	0.3	0.393	0.39
1/2	0.3	0.364	0.32
2/1	0.3	0.372	2.64
2/2	0.3	0.339	0.90
3/1	0.3	0.370	0.31
3/2	0.3	0.373	0.46
1/1	0.5	0.637	0.77
1/2	0.5	0.630	0.56
2/1	0.5	0.624	1.65
2/2	0.5	0.618	0.86
3/1	0.5	0.561	0.27
3/2	0.5	0.569	0.91

TABLE 4500-NH_3:IV. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition *mg NH_3-N/L*	Recovery %	Relative Standard Deviation %
Wastewater treatment	Reference sample*	—	104	—

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Matrix	Sample/Blank Designation	Known Addition *mg NH_3-N/L*	Recovery %	Relative Standard Deviation %
plant	Blank†	0.4	100	—
influent		0.8	102	—
	Site A‡§	0	—	0.5
		0.4	108	—
		0.8	105	—
	Site B‡§	0	—	0.5
		0.4	105	—
		0.8	105	—
	Site C‡§	0	—	1.1
		0.4	109	—
		0.8	107	—
Wastewater treatment	Reference sample*	—	106	—
plant	Blank†	0.4	105	—
effluent		0.8	105	—
	Site A‡‖	0	—	ND
		0.4	90	—
		0.8	88	—
	Site B‡‖	0	—	<0.1
		0.4	93	—
		0.8	94	—
	Site C‡‖	0	—	<0.1
		0.4	89	—
		0.8	91	—
Landfill leachate‖	Reference sample*	—	106	—
	Blank†	0.4	105	—
		0.8	106	—
	Site A‡#	0	—	1.9

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Matrix	Sample/Blank Designation	Known Addition *mg NH_3-N/L*	Recovery %	Relative Standard Deviation %
		0.4	125	—
		0.8	114	—
	Site B‡#	0	—	0.4
		0.4	96	—
		0.8	106	—
	Site C‡#	0	—	0.2
		0.4	102	—
		0.8	107	—

ND = not detectable.
* U.S. EPA QC sample, 1.98 mg N/L.
† Determined in duplicate.
‡ Samples without known additions determined four times; samples with known additions determined in duplicate.
§ Site A and C samples diluted 20-fold, Site B sample diluted 100-fold. Typical relative difference between duplicates 0.2%.
|| Samples not diluted. Typical relative difference between duplicates <1%.
Site A and C samples diluted 50-fold; Site B sample diluted 150-fold. Typical relative difference between duplicates 0.3%.

TABLE 4500-NO_3^-:I. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition *mg NO_3^--N/L*	Recovery %	Relative Standard Deviation %
Wastewater treatment	Reference sample*	—	102	—
plant	Blank†	0.2	100	—
influent		0.4	100	—
	Site A‡	0	—	<1
		0.2	101	—
		0.4	96	—
	Site B‡	0	—	<1
		0.2	90	—
		0.4	88	—
	Site C‡	0	—	<1

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Matrix	Sample/Blank Designation	Known Addition *mg NO_3^--N/L*	Recovery %	Relative Standard Deviation %
		0.2	95	—
		0.4	95	—
Wastewater treatment plant effluent	Reference sample*	—	102	—
	Blank†	0.2	100	—
		0.4	95	—
	Site A‡	0	—	0.9
		0.2	95	—
		0.4	102	—
	Site B‡	0	—	0.7
		0.2	91	—
		0.4	101	—
	Site C‡	0	—	0.5
		0.2	91	—
		0.4	96	—
Landfill leachate	Reference sample*	—	98	—
	Blank†	0.2	100	—
		0.4	98	—
	Site A‡	0	—	<1
		0.2	104	—
		0.4	96	—
	Site B‡	0	—	<1
		0.2	95	—
		0.4	94	—
	Site C‡	0	—	<1
		0.2	91	—
		0.4	93	—

* U.S. EPA QC sample, 1.98 mg N/L.
† Determined in duplicate.
‡ Samples without known additions determined four times; samples with known additions determined in duplicate. Typical difference

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between duplicates: influent, 2%; effluent, 1%; leachate, <1%.

TABLE 4500-N_{org}:I. PRECISION DATA FOR KJELDAHL NITROGEN METHOD BASED ON MEAN OF TRIPLICATE ANALYSES OF NICOTINIC ACID

Lab/ Analyst	Nicotinic Acid *mg N/L*	Recovery of N *%*	Standard Deviation *mg/L*	Relative Standard Deviation *%*
1/1	5	93.3	0.16	3.46
1/2	5	101	0.16	3.17
1/1	10	87.7	0.16	1.84
1/2	10	91.5	0.28	3.06
1/1	20	95.7	0.16	0.84
1/2	20	95.7	0.58	3.03
2/1	0.5	97.4	0.005	1.04
2/2	0.5	95.3	0.027	5.46
3/1	0.5	87.3	0.130	29.9
4/1	0.5	113	0.235	41.7
2/1	1.0	103	0.012	1.15
2/2	1.0	101	0.046	4.63
3/1	1.0	84.3	0.081	9.66
4/1	1.0	99.3	0.396	39.9
2/1	2.0	104	0	0
2/2	2.0	99.2	0.029	1.44
3/1	2.0	89.2	0.071	3.98
4/1	2.0	112	0.139	6.18

TABLE 4500-N_{ORG}:II. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition *mg N/L*	Recovery %	Relative Standard Deviation %
Wastewater treatment	Reference sample*	—	97	—
plant	Blank†	3.0	97	—
influent		6.0	99	—
	Site A‡§	0	—	3.3

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Matrix	Sample/Blank Designation	Known Addition *mg N/L*	Recovery %	Relative Standard Deviation %
		3.0	91	—
		6.0	95	—
	Site B‡§	0	—	3.6
		3.0	115	—
		6.0	93	—
	Site C‡§	0	—	5.1
		3.0	97	—
		6.0	107	—
Wastewater treatment plant effluent	Reference sample*	—	92	—
	Blank†	3.0	97	—
		6.0	100	—
	Site A‡‖	0	—	5.4
		3.0	94	—
		6.0	100	—
	Site B‡‖	0	—	4.1
		3.0	119	—
		6.0	81	—
	Site C‡‖	0	—	7.3
		3.0	93	—
		6.0	105	—
Landfill leachate	Reference sample*	—	96	—
	Blank†	3.0	101	—
		6.0	99	—
	Site A‡#	0	—	3.3
		3.0	95	—
		6.0	98	—
	Site B‡#	0	—	4.4
		3.0	134	—
		6.0	85	—

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Matrix	Sample/Blank Designation	Known Addition *mg N/L*	Recovery %	Relative Standard Deviation %
	Site C‡#	0	—	3.8
		3.0	98	—
		6.0	105	—

* U.S. EPA nutrient QC sample, 1.52 mg N/L.
† Determined in duplicate.
‡ Samples without known additions determined four times; samples with known additions determined in duplicate.
§ Sample dilutions: A - 5-fold; B - 10-fold; C - 5-fold. Typical relative difference between duplicates 3%.
‖ Sample dilutions: A - none; B - 2-fold; C - none. Typical relative difference between duplicates 1%.
Sample dilutions: A, B, and C - 25-fold. Typical relative difference between duplicates 4%.

TABLE 4500-O:I. SOLUBILITY OF OXYGEN IN WATER EXPOSED TO WATER-SATURATED AIR AT ATMOSPHERIC PRESSURE (101.3 KPA)[1]

	Oxygen Solubility *mg/L*					
Temperature °*C*	Chlorinity: 0	5.0	10.0	15.0	20.0	25.0
0.0	14.621	13.728	12.888	12.097	11.355	10.657
1.0	14.216	13.356	12.545	11.783	11.066	10.392
2.0	13.829	13.000	12.218	11.483	10.790	10.139
3.0	13.460	12.660	11.906	11.195	10.526	9.897
4.0	13.107	12.335	11.607	10.920	10.273	9.664
5.0	12.770	12.024	11.320	10.656	10.031	9.441
6.0	12.447	11.727	11.046	10.404	9.799	9.228
7.0	12.139	11.442	10.783	10.162	9.576	9.023
8.0	11.843	11.169	10.531	9.930	9.362	8.826
9.0	11.559	10.907	10.290	9.707	9.156	8.636
10.0	11.288	10.656	10.058	9.493	8.959	8.454
11.0	11.027	10.415	9.835	9.287	8.769	8.279
12.0	10.777	10.183	9.621	9.089	8.586	8.111
13.0	10.537	9.961	9.416	8.899	8.411	7.949
14.0	10.306	9.747	9.218	8.716	8.242	7.792
15.0	10.084	9.541	9.027	8.540	8.079	7.642
16.0	9.870	9.344	8.844	8.370	7.922	7.496
17.0	9.665	9.153	8.667	8.207	7.770	7.356

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	Oxygen Solubility mg/L					
Temperature °C	Chlorinity: 0	5.0	10.0	15.0	20.0	25.0
18.0	9.467	8.969	8.497	8.049	7.624	7.221
19.0	9.276	8.792	8.333	7.896	7.483	7.090
20.0	9.092	8.621	8.174	7.749	7.346	6.964
21.0	8.915	8.456	8.021	7.607	7.214	6.842
22.0	8.743	8.297	7.873	7.470	7.087	6.723
23.0	8.578	8.143	7.730	7.337	6.963	6.609
24.0	8.418	7.994	7.591	7.208	6.844	6.498
25.0	8.263	7.850	7.457	7.083	6.728	6.390
26.0	8.113	7.711	7.327	6.962	6.615	6.285
27.0	7.968	7.575	7.201	6.845	6.506	6.184
28.0	7.827	7.444	7.079	6.731	6.400	6.085
29.0	7.691	7.317	6.961	6.621	6.297	5.990
30.0	7.559	7.194	6.845	6.513	6.197	5.896
31.0	7.430	7.073	6.733	6.409	6.100	5.806
32.0	7.305	6.957	6.624	6.307	6.005	5.717
33.0	7.183	6.843	6.518	6.208	5.912	5.631
34.0	7.065	6.732	6.415	6.111	5.822	5.546
35.0	6.950	6.624	6.314	6.017	5.734	5.464
36.0	6.837	6.519	6.215	5.925	5.648	5.384
37.0	6.727	6.416	6.119	5.835	5.564	5.305
38.0	6.620	6.316	6.025	5.747	5.481	5.228
39.0	6.515	6.217	5.932	5.660	5.400	5.152
40.0	6.412	6.121	5.842	5.576	5.321	5.078
41.0	6.312	6.026	5.753	5.493	5.243	5.005
42.0	6.213	5.934	5.667	5.411	5.167	4.933
43.0	6.116	5.843	5.581	5.331	5.091	4.862
44.0	6.021	5.753	5.497	5.252	5.017	4.793
45.0	5.927	5.665	5.414	5.174	4.944	4.724
46.0	5.835	5.578	5.333	5.097	4.872	4.656
47.0	5.744	5.493	5.252	5.021	4.801	4.589
48.0	5.654	5.408	5.172	4.947	4.730	4.523

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	Oxygen Solubility *mg/L*					
Temperature °C	**Chlorinity: 0**	**5.0**	**10.0**	**15.0**	**20.0**	**25.0**
49.0	5.565	5.324	5.094	4.872	4.660	4.457
50.0	5.477	5.242	5.016	4.799	4.591	4.392

NOTE:

1. The table provides three decimal places to aid interpolation. When computing saturation values to be used with measured values, such as in computing DO deficit in a receiving water, precision of measured values will control choice of decimal places to be used.
2. Equations are available to compute DO concentration in fresh water[1-3] and in seawater[1] at equilibrium with water-saturated air. Figures and tables also are available.[3]

   Calculate the equilibrium oxygen concentration, C^*, from equation:

   $$\ln C^* = -139.344\,11 + (1.575\,701 \times 10^5/T) - (6.642\,308 \times 10^7/T^2) + (1.243\,800 \times 10^{10}/T^3) - (8.621\,949 \times 10^{11}/T^4) - \text{Chl}\,[(3.1929) \times 10^{-2}) - (1.9428 \times 10^1/T) + (3.8673 \times 10^3/T^2)]$$

   where:

   C^* = equilibrium oxygen concentration at 101.325 kPa, mg/L,
   T = temperature (°K) = °C + 273.150, (°C is between 0.0 and 40.0 in the equation; the table is accurate up to 50.0), and
   Chl = Chlorinity (see definition in Note 4, below).

   Example 1: At 20°C and 0.000 Chl, ln C^* = 2.207 442 and C^* = 9.092 mg/L;
   Example 2: At 20°C and 15.000 ChL,
   ln C^* = (2.207 442) − 15.000 (0.010 657)
   = 2.0476 and C^* = 7.749 mg/L.

   When salinity is used, replace the chlorinity term (−Chl[. . .]) by:
   $- S(1.7674 \times 10^{-2}) - (1.0754 \times 10^1/T) + (2.1407 \times 10^3/T^2)$

   where:

   S = salinity (see definition in Note 4, below).

$$C_p = C^*P\left[\frac{(1 - P_{wv}/P)(1 - \theta P)}{(1 - P_{wv})(1 - \theta)}\right]$$

3. For nonstandard conditions of pressure:

   where:

   C_p = equilibrium oxygen concentration at nonstandard pressure, mg/L,
   C^* = equilibrium oxygen concentration at standard pressure of 1 atm, mg/L.
   P = nonstandard pressure, atm,

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P_{wv} = partial pressure of water vapor, atm, computed from:

$\ln P_{wv} = 11.8571 - (3840.70/T) - (216\,961/T^2)$,

T = temperature, °K,

$\theta = 0.000\,975 - (1.426 \times 10^{-5} t) + (6.436 \times 10^{-8} t^2)$, and

t = temperature, °C.

N.B.: Although not explicit in the above, the quantity in brackets in the equation for C_p has dimensions of atm^{-1} per Reference 4, so that P multiplied by this quantity is dimensionless.

Also, the equation for ln P_{wv} is strictly valid for fresh water only, but for practical purposes no error is made by neglecting the effect of salinity. An equation for P_{wv} that includes the salinity factor may be found in Reference 1.

Example 3: At 20°C, 0.000 Chl, and 0.700 atm,
$C_p = C^* P\,(0.990\,092) = 6.30$ mg/L.

4. Definitions:
Salinity: Although salinity has been defined traditionally as the total solids in water after all carbonates have been converted to oxides, all bromide and iodide have been replaced by chloride, and all organic matter has been oxidized (see Section 2520), the new scale used to define salinity is based on the electrical conductivity of seawater relative to a specified solution of KCl in water.[5] The scale is dimensionless and the traditional dimension of parts per thousand (i.e., g/kg of solution) no longer applies.

Chlorinity: Chlorinity is defined in relation to salinity as follows:

$$\text{Salinity} = 1.806\,55 \times \text{chlorinity}$$

Although chlorinity is not equivalent to chloride concentration, the factor for converting a chloride concentration in seawater to include bromide, for example, is only 1.0045 (based on the relative molecular weights and amounts of the two ions). Therefore, for practical purposes, chloride concentration (in g/kg of solution) is nearly equal to chlorinity in seawater. For wastewater, it is necessary to know the ions responsible for the solution's electrical conductivity to correct for their effect on oxygen solubility and use of the tabular value. If this is not done, the equation is inappropriate unless the relative composition of the wastewater is similar to that of seawater.

TABLE 4500-P:I. PRECISION AND BIAS DATA FOR MANUAL PHOSPHORUS METHODS

	Phosphorus Concentration					
Method	**Ortho-phosphate μg/L**	**Poly-phosphate μg/L**	**Total μg/L**	**No. of Laboratories**	**Relative Standard Deviation %**	**Relative Error %**
Vanadomolybdophosphoric acid	100			45	75.2	21.6
	600			43	19.6	10.8
	7000			44	8.6	5.4
Stannous chloride	100			45	25.5	28.7
	600			44	14.2	8.0
	7000			45	7.6	4.3

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Method	Phosphorus Concentration Ortho-phosphate μg/L	Poly-phosphate μg/L	Total μg/L	No. of Laboratories	Relative Standard Deviation %	Relative Error %
Ascorbic acid	100			3	9.1	10.0
	600			3	4.0	4.4
	7000			3	5.2	4.9
Acid hydrolysis +		80		37	106.8	7.4
vanadomolybdophosphoric		300		38	66.5	14.0
acid		3000		37	36.1	23.5
Acid hydrolysis + stannous		80		39	60.1	12.5
chloride		300		36	47.6	21.7
		3000		38	37.4	22.8
Persulfate +			210	32	55.8	1.6
vanadomolybdophosphoric			990	32	23.9	2.3
acid			10 230	31	6.5	0.3
Sulfuric-nitric acids +			210	23	65.6	20.9
vanadomolybdophosphoric			990	22	47.3	0.6
acid			10 230	20	7.0	0.4
Perchloric acid +			210	4	33.5	45.2
vanadomolybdophosphoric			990	5	20.3	2.6
acid			10 230	6	11.7	2.2
Persulfate + stannous			210	29	28.1	9.2
chloride			990	30	14.9	12.3
			10 230	29	11.5	4.3
Sulfuric-nitric acids +			210	20	20.8	1.2
stannous chloride			990	17	8.8	3.2
			10 230	19	7.5	0.4

TABLE 4500-P:II. COMPARISON OF PRECISION AND BIAS OF ASCORBIC ACID METHODS

	Relative Standard Deviation %	Relative Error %

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Method	phosphate µg/L	phosphate µg/L	Total µg/L	No. of Laboratories	Deviation %	Error %

TABLE 4500-P:II. COMPARISON OF PRECISION AND BIAS OF ASCORBIC ACID METHODS

Ascorbic Acid Method	Phosphorus Concentration, Dissolved Orthophosphate µg/L	No. of Labora-tories	Relative Standard Deviation %		Relative Error %	
			Distilled Water	River Water	Distilled Water	River Water
13th Edition[1]	228	8	3.87	2.17	4.01	2.08
Current method[2]	228	8	3.03	1.75	2.38	1.39

TABLE 4500-P:III. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition *mg P/L*	Recovery %	Relative Standard Deviation %
Wastewater treatment	Reference sample*	—	101	—
plant	Blank†	0.05	96	—
influent		0.1	95	—
	Site A‡§	0	—	0.7
		0.05	98	—
		0.1	101	—
	Site B‡§	0	—	5
		0.05	75	—
		0.1	91	—
	Site C‡§	0	—	0.6
		0.05	88	—
		0.1	97	—

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Matrix	Sample/Blank Designation	Known Addition *mg P/L*	Recovery %	Relative Standard Deviation %
Wastewater treatment	Reference sample*	—	100	—
plant	Blank†	0.05	96	—
effluent		0.1	96	—
	Site A‡‖	0	—	0.7
		0.05	94	—
		0.1	96	—
	Site B‡‖	0	—	0.3
		0.05	94	—
		0.1	99	—
	Site C‡‖	0	—	0.5
		0.05	109	—
		0.1	107	—
Landfill leachate	Reference sample*	—	98	—
	Blank†	0.05	94	—
		0.1	95	—
	Site A‡#	0	—	0.9
		0.05	105	—
		0.1	106	—
	Site B‡#	0	—	6.7
		0.05	89	—
		0.1	94	—
	Site C‡#	0	—	0.9
		0.05	110	—
		0.1	109	—

* U.S. EPA QC sample, 0.109 mg P/L.
† Determined in duplicate.
‡ Samples without known additions determined four times; samples with known additions determined in duplicate.
§ Sample dilutions: A - 5-fold; B - 100-fold; C - 10-fold. Typical relative difference between duplicates 0.5%.
‖ Sample dilutions: A - 5-fold; B - 20-fold; C - 10-fold. Typical relative difference between duplicates 0.3%.
Sample dilutions: A - 20-fold; B - 10-fold; C - 20-fold. Typical relative difference between duplicates 1%.

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TABLE 4500-P:IV. RECOVERIES OF TOTAL PHOSPHORUS

Compound	Known Concentration *mg P/L*	Mean Concentration Recovered *mg P/L*	Recovery %	Relative Standard Deviation %
Sodium	10	8.99	90.3	0.5
pyrophosphate	2	1.81	90.2	0.6
	0.2	0.19	93.4	1.0
Phenylphosphate	10	10.10	101.5	0.2
	2	2.12	105.0	5.6
	0.2	0.20	101.3	1.0
Trimethylphosphate	10	8.99	90.3	0.2
	2	1.86	92.7	0.3
	0.2	0.18	95.3	1.1
Sodium	10	10.61	106.7	1.0
tripolyphosphate	2	2.14	106.6	0.2
	0.2	0.22	108.9	0.9

TABLE 4500-P:V. COMPARISON OF MANUAL AND IN-LINE TOTAL PHOSPHORUS METHODS

Samples	Concentration by Manual Persulfate Digestion Method *mg P/L*	Concentration by In-Line Digestion Method *mg P/L*	Relative Difference %
Influent (I2)	5.93	5.52	–6.9
Influent (I3)	5.03	4.50	–10.5
Influent (I5)	2.14	2.11	–1.4
Influent (I6)	1.88	1.71	–9.0
Effluent (E1)	3.42	2.87	–16.1
Effluent (E2)	3.62	3.55	–1.9
Effluent (E3)	3.26	3.34	+2.4
Effluent (E4)	8.36	8.16	–2.4

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Samples	Concentration by Manual Persulfate Digestion Method *mg P/L*	Concentration by In-Line Digestion Method *mg P/L*	Relative Difference %
Effluent (E5)	0.65	0.71	+9.2
Effluent (E6)	0.74	0.81	+9.5
Phenylphosphate	1.95	1.91	–2.1
Trimethylphosphate	1.87	1.80	–3.7
Sodium pyrophosphate	1.90	1.73	–8.9
Sodium tripolyphosphate	1.84	1.73	–6.0

TABLE 4500-SiO_2:I. SELECTION OF LIGHT PATH LENGTH FOR VARIOUS SILICA CONCENTRATIONS

	Method C	Method D	
		Silica in 55 mL Final Volume *µg*	
Light Path *cm*	Silica in 55 mL Final Volume *µg*	650 nm Wavelength	815 nm Wavelength
1	200–1300	40–300	20–100
2	100–700	20–150	10–50
5	4–250	7–50	4–20
10	20–130	4–30	2–10

TABLE 4500-SIO_2:II. PREPARATION OF PERMANENT COLOR STANDARDS FOR VISUAL DETERMINATION OF SILICA

Values in Silica µg	Potassium Chromate Solution *mL*	Borax Solution *mL*	Water *mL*

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Values in Silica µg	Potassium Chromate Solution mL	Borax Solution mL	Water mL
0	0.0	25	30
100	1.0	25	29
200	2.0	25	28
400	4.0	25	26
500	5.0	25	25
750	7.5	25	22
1000	10.0	25	20

TABLE 4500-S^{2-}:I. DILUTION OF SULFIDE STOCK SOLUTION FOR PREPARATION OF STANDARDS (100 mL TOTAL VOLUME)

Dilution	Alkaline Antioxidant Reagent mL	Sulfide Solution	Sulfide Solution mL	1 *M* Zinc Acetate mL
1:10	45	Stock	10	0.15
1:100	50	Stock	1	0.15
1:1 000	45	1:100	10	0.14
1:10 000	50	1:100	1	0.15

TABLE 4500-SO_4^{2-}:I. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition mg SO_4^{2-}/L	Recovery %	Relative Standard Deviation %
Wastewater treatment	Reference sample*	—	99	—
plant	Blank†	10.0	99	—
influent		20.0	99	—
	Site A‡	0	—	0.7
		10.0	109	—

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Matrix	Sample/Blank Designation	Known Addition *mg* SO_4^{2-}/*L*	Recovery %	Relative Standard Deviation %
		20.0	110	—
	Site B‡	0	—	0.7
		10.0	106	—
		20.0	112	—
	Site C‡	0	—	1.9
		10.0	104	—
		20.0	107	—
Wastewater treatment plant effluent	Reference sample*	—	99	—
	Blank†	10.0	95	—
		20.0	99	—
	Site A‡	0	—	0.9
		10.0	108	—
		20.0	108	—
	Site B‡	0	—	2.4
		10.0	107	—
		20.0	107	—
	Site C‡	0	—	0.6
		10.0	97	—
		20.0	104	—
Landfill leachate	Reference sample*	—	100	—
	Blank†	10.0	100	—
		20.0	99	—
	Site A‡	0	—	0.7
		10.0	106	—
		20.0	110	—
	Site B‡	0	—	0.5
		10.0	106	—
		20.0	107	—

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Matrix	Sample/Blank Designation	Known Addition *mg* SO_4^{2-}/*L*	Recovery %	Relative Standard Deviation %
	Site C‡	0	—	0.9
		10.0	101	—
		20.0	103	—

* U.S. EPA QC sample, 20.0 mg SO_4^{2-}/L.

† Determined in duplicate.

‡ Samples without known additions determined four times; samples with known additions determined in duplicate. Typical relative difference between duplicates1%. Sample dilutions: Influent and effluent, all sites - 5-fold; leachate A - 100-fold; B - 50-fold; C - 10-fold.

Part 5000 AGGREGATE ORGANIC CONSTITUENTS

5010 INTRODUCTION

5010 A. General Discussion

Analyses for organic matter in water and wastewater can be classified into two general types of measurements: those that quantify an aggregate amount of organic matter comprising organic constituents with a common characteristic and those that quantify individual organic compounds. The latter can be found in Part 6000. The former, described here in Part 5000, have been grouped into four categories: oxygen-demanding substances, organically bound elements, classes of compounds, and formation potentials.

Methods for total organic carbon and chemical oxygen demand are used to assess the total amount of organics present. Gross fractions of the organic matter can be identified analytically, as in the measurement of BOD, which is an index of the biodegradable organics present, oil and grease, which represents material extractable from a sample by a nonpolar solvent, or dissolved organic halide (DOX), which measures organically bound halogens. Trihalomethane formation potential is an aggregate measure of the total concentration of trihalomethanes formed upon chlorination of a water sample.

Analyses of organics are made to assess the concentration and general composition of organic matter in raw water supplies, wastewaters, treated effluents, and receiving waters; and to

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determine the efficiency of treatment processes.

5010 B. Sample Collection and Preservation

The sampling, field treatment, preservation, and storage of samples taken for organic matter analysis are covered in detail in the individual introductions to the methods. If possible, analyze samples immediately because preservatives often interfere with the tests. Otherwise, store at a low temperature (4°C) immediately after collection to preserve most samples. Use chemical preservatives only when they are shown not to interfere with the examinations to be made (see Section 1060). Never use preservatives for samples to be analyzed for BOD. When preservatives are used, add them to the sample bottle initially so that all portions are preserved as soon as collected. No single method of preservation is entirely satisfactory; choose the preservative with due regard to the determinations that are to be made. All methods of preservation may be inadequate when applied to samples containing significant amounts of suspended matter.

5020 QUALITY ASSURANCE/QUALITY CONTROL

Part 1000 contains important information relevant to analyses included in Part 5000. Give particular attention to Section 1020B (Quality Control), Section 1060 (Collection and Preservation of Samples), Section 1080 (Reagent Water), and Section 1090 (Laboratory Occupational Health and Safety), all of which are critical for many of the Part 5000 methods.

Take special precautions when analyses are performed by independent laboratories. Reliable use of independent laboratories deserves the same quality assurance procedures observed for in-house analyses: replicate samples, samples with known additions, and blanks.

Preparation of samples with known additions may not be feasible for certain analyses. In such cases, consider using a mixture, in varying ratios, of several samples. Use the reported concentrations in the samples and the proportions in which they were mixed to calculate the expected concentration in the mixture. Examine laboratory performance using externally prepared standards and check samples (see Section 1020B).

Reagent water (Section 1080) should give satisfactory results for most of the analyses in Part 5000, but additional purification steps may be needed for certain methods, such as dissolved organic halogen (DOX) and disinfection by-product formation potential (DBPFP).

5210 BIOCHEMICAL OXYGEN DEMAND (BOD)*#(97)

5210 A. Introduction

1. General Discussion

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The biochemical oxygen demand (BOD) determination is an empirical test in which standardized laboratory procedures are used to determine the relative oxygen requirements of wastewaters, effluents, and polluted waters. The test has its widest application in measuring waste loadings to treatment plants and in evaluating the BOD-removal efficiency of such treatment systems. The test measures the molecular oxygen utilized during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulfides and ferrous iron. It also may measure the amount of oxygen used to oxidize reduced forms of nitrogen (nitrogenous demand) unless their oxidation is prevented by an inhibitor. The seeding and dilution procedures provide an estimate of the BOD at pH 6.5 to 7.5.

Measurements of oxygen consumed in a 5-d test period (5-d BOD or BOD_5, Section 5210B), oxygen consumed after 60 to 90 d of incubation (ultimate BOD or UBOD, Section 5210C), and continuous oxygen uptake (respirometric method, Section 5210D) are described here. Many other variations of oxygen demand measurements exist, including using shorter and longer incubation periods and tests to determine rates of oxygen uptake. Alternative seeding, dilution, and incubation conditions can be chosen to mimic receiving-water conditions, thereby providing an estimate of the environmental effects of wastewaters and effluents.

The UBOD measures the oxygen required for the total degradation of organic material (ultimate carbonaceous demand) and/or the oxygen to oxidize reduced nitrogen compounds (ultimate nitrogenous demand). UBOD values and appropriate kinetic descriptions are needed in water quality modeling studies such as UBOD: BOD_5 ratios for relating stream assimilative capacity to regulatory requirements; definition of river, estuary, or lake deoxygenation kinetics; and instream ultimate carbonaceous BOD (UCBOD) values for model calibration.

2. Carbonaceous Versus Nitrogenous BOD

A number of factors, for example, soluble versus particulate organics, settleable and floatable solids, oxidation of reduced iron and sulfur compounds, or lack of mixing may affect the accuracy and precision of BOD measurements. Presently, there is no way to include adjustments or corrections to account for the effect of these factors.

Oxidation of reduced forms of nitrogen, such as ammonia and organic nitrogen, can be mediated by microorganisms and exert nitrogenous demand. Nitrogenous demand historically has been considered an interference in the determination of BOD, as clearly evidenced by the inclusion of ammonia in the dilution water. The interference from nitrogenous demand can now be prevented by an inhibitory chemical.[1] If an inhibiting chemical is not used, the oxygen demand measured is the sum of carbonaceous and nitrogenous demands.

Measurements that include nitrogenous demand generally are not useful for assessing the oxygen demand associated with organic material. Nitrogenous demand can be estimated directly from ammonia nitrogen (Section 4500-NH_3); and carbonaceous demand can be estimated by subtracting the theoretical equivalent of the reduced nitrogen oxidation from uninhibited test results. However, this method is cumbersome and is subject to considerable error. Chemical

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inhibition of nitrogenous demand provides a more direct and more reliable measure of carbonaceous demand.

The extent of oxidation of nitrogenous compounds during the 5-d incubation period depends on the concentration and type of microorganisms capable of carrying out this oxidation. Such organisms usually are not present in raw or settled primary sewage in sufficient numbers to oxidize sufficient quantities of reduced nitrogen forms in the 5-d BOD test. Many biological treatment plant effluents contain sufficient numbers of nitrifying organisms to cause nitrification in BOD tests. Because oxidation of nitrogenous compounds can occur in such samples, inhibition of nitrification as directed in Section 5210B.4*e*6) is recommended for samples of secondary effluent, for samples seeded with secondary effluent, and for samples of polluted waters.

Report results as carbonaceous biochemical oxygen demand ($CBOD_5$) when inhibiting the nitrogenous oxygen demand. When nitrification is not inhibited, report results as BOD_5.

3. Dilution Requirements

The BOD concentration in most wastewaters exceeds the concentration of dissolved oxygen (DO) available in an air-saturated sample. Therefore, it is necessary to dilute the sample before incubation to bring the oxygen demand and supply into appropriate balance. Because bacterial growth requires nutrients such as nitrogen, phosphorus, and trace metals, these are added to the dilution water, which is buffered to ensure that the pH of the incubated sample remains in a range suitable for bacterial growth. Complete stabilization of a sample may require a period of incubation too long for practical purposes; therefore, 5 d has been accepted as the standard incubation period.

If the dilution water is of poor quality, the BOD of the dilution water will appear as sample BOD. This effect will be amplified by the dilution factor. A positive bias will result. The methods included below (Section 5210B and Section 5210C) contain both a dilution-water check and a dilution-water blank. Seeded dilution waters are checked further for acceptable quality by measuring their consumption of oxygen from a known organic mixture, usually glucose and glutamic acid.

The source of dilution water is not restricted and may be distilled, tap, or receiving-stream water free of biodegradable organics and bioinhibitory substances such as chlorine or heavy metals. Distilled water may contain ammonia or volatile organics; deionized waters often are contaminated with soluble organics leached from the resin bed. Use of copper-lined stills or copper fittings attached to distilled water lines may produce water containing excessive amounts of copper (see Section 3500-Cu).

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5210 B. 5-Day BOD Test

1. General Discussion

a. Principle: The method consists of filling with sample, to overflowing, an airtight bottle of the specified size and incubating it at the specified temperature for 5 d. Dissolved oxygen is measured initially and after incubation, and the BOD is computed from the difference between initial and final DO. Because the initial DO is determined shortly after the dilution is made, all oxygen uptake occurring after this measurement is included in the BOD measurement.

b. Sampling and storage: Samples for BOD analysis may degrade significantly during storage between collection and analysis, resulting in low BOD values. Minimize reduction of BOD by analyzing sample promptly or by cooling it to near-freezing temperature during storage. However, even at low temperature, keep holding time to a minimum. Warm chilled samples to 20 ± 3°C before analysis.

1) Grab samples—If analysis is begun within 2 h of collection, cold storage is unnecessary. If analysis is not started within 2 h of sample collection, keep sample at or below 4°C from the time of collection. Begin analysis within 6 h of collection; when this is not possible because the sampling site is distant from the laboratory, store at or below 4°C and report length and temperature of storage with the results. In no case start analysis more than 24 h after grab sample collection. When samples are to be used for regulatory purposes make every effort to deliver samples for analysis within 6 h of collection.

2) Composite samples—Keep samples at or below 4°C during compositing. Limit compositing period to 24 h. Use the same criteria as for storage of grab samples, starting the measurement of holding time from end of compositing period. State storage time and conditions as part of the results.

2. Apparatus

a. Incubation bottles: Use glass bottles having 60 mL or greater capacity (300-mL bottles

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having a ground-glass stopper and a flared mouth are preferred). Clean bottles with a detergent, rinse thoroughly, and drain before use. As a precaution against drawing air into the dilution bottle during incubation, use a water seal. Obtain satisfactory water seals by inverting bottles in a water bath or by adding water to the flared mouth of special BOD bottles. Place a paper or plastic cup or foil cap over flared mouth of bottle to reduce evaporation of the water seal during incubation.

b. Air incubator or water bath, thermostatically controlled at 20 ±1°C. Exclude all light to prevent possibility of photosynthetic production of DO.

3. Reagents

Prepare reagents in advance but discard if there is any sign of precipitation or biological growth in the stock bottles. Commercial equivalents of these reagents are acceptable and different stock concentrations may be used if doses are adjusted proportionally.

a. Phosphate buffer solution: Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 \cdot 7H_2O$, and 1.7 g NH_4Cl in about 500 mL distilled water and dilute to 1 L. The pH should be 7.2 without further adjustment. Alternatively, dissolve 42.5 g KH_2PO_4 or 54.3 g K_2HPO_4 in about 700 mL distilled water. Adjust pH to 7.2 with 30% NaOH and dilute to 1 L.

b. Magnesium sulfate solution: Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in distilled water and dilute to 1 L.

c. Calcium chloride solution: Dissolve 27.5 g $CaCl_2$ in distilled water and dilute to 1 L.

d. Ferric chloride solution: Dissolve 0.25 g $FeCl_3 \cdot 6H_2O$ in distilled water and dilute to 1 L.

e. Acid and alkali solutions, 1*N*, for neutralization of caustic or acidic waste samples.

1) Acid—Slowly and while stirring, add 28 mL conc sulfuric acid to distilled water. Dilute to 1 L.

2) Alkali—Dissolve 40 g sodium hydroxide in distilled water. Dilute to 1 L.

f. Sodium sulfite solution: Dissolve 1.575 g Na_2SO_3 in 1000 mL distilled water. This solution is not stable; prepare daily.

g. Nitrification inhibitor, 2-chloro-6-(trichloromethyl) pyridine.*#(98)

h. Glucose-glutamic acid solution: Dry reagent-grade glucose and reagent-grade glutamic acid at 103°C for 1 h. Add 150 mg glucose and 150 mg glutamic acid to distilled water and dilute to 1 L. Prepare fresh immediately before use.

i. Ammonium chloride solution: Dissolve 1.15 g NH_4Cl in about 500 mL distilled water, adjust pH to 7.2 with NaOH solution, and dilute to 1 L. Solution contains 0.3 mg N/mL.

j. Dilution water: Use demineralized, distilled, tap, or natural water for making sample dilutions.

4. Procedure

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a. Preparation of dilution water: Place desired volume of water (¶ 3 *j*) in a suitable bottle and add 1 mL each of phosphate buffer, $MgSO_4$, $CaCl_2$, and $FeCl_3$ solutions/L of water. Seed dilution water, if desired, as described in ¶ 4*d*. Test dilution water as described in ¶ 4*h* so that water of assured quality always is on hand.

Before use bring dilution water temperature to 20 ± 3°C. Saturate with DO by shaking in a partially filled bottle or by aerating with organic-free filtered air. Alternatively, store in cotton-plugged bottles long enough for water to become saturated with DO. Protect water quality by using clean glassware, tubing, and bottles.

b. Dilution water storage: Source water (¶ 3 *j*) may be stored before use as long as the prepared dilution water meets quality control criteria in the dilution water blank (¶ 4*h*). Such storage may improve the quality of some source waters but may allow biological growth to cause deterioration in others. Preferably do not store prepared dilution water for more than 24 h after adding nutrients, minerals, and buffer unless dilution water blanks consistently meet quality control limits. Discard stored source water if dilution water blank shows more than 0.2 mg/L DO depletion in 5 d.

c. Glucose-glutamic acid check: Because the BOD test is a bioassay its results can be influenced greatly by the presence of toxicants or by use of a poor seeding material. Distilled waters frequently are contaminated with copper; some sewage seeds are relatively inactive. Low results always are obtained with such seeds and waters. Periodically check dilution water quality, seed effectiveness, and analytical technique by making BOD measurements on a mixture of 150 mg glucose/L and 150 mg glutamic acid/L as a ‘‘standard’’ check solution. Glucose has an exceptionally high and variable oxidation rate but when it is used with glutamic acid, the oxidation rate is stabilized and is similar to that obtained with many municipal wastes. Alternatively, if a particular wastewater contains an identifiable major constituent that contributes to the BOD, use this compound in place of the glucose-glutamic acid.

Determine the 5-d 20°C BOD of a 2% dilution of the glucose-glutamic acid standard check solution using the techniques outlined in ¶s 4*d-j*. Adjust concentrations of commercial mixtures to give 3 mg/L glucose and 3 mg/L glutamic acid in each GGA test bottle. Evaluate data as described in ¶ 6, Precision and Bias.

d. Seeding:

1) Seed source—It is necessary to have present a population of microorganisms capable of oxidizing the biodegradable organic matter in the sample. Domestic wastewater, unchlorinated or otherwise-undisinfected effluents from biological waste treatment plants, and surface waters receiving wastewater discharges contain satisfactory microbial populations. Some samples do not contain a sufficient microbial population (for example, some untreated industrial wastes, disinfected wastes, high-temperature wastes, or wastes with extreme pH values). For such wastes seed the dilution water or sample by adding a population of microorganisms. The preferred seed is effluent or mixed liquor from a biological treatment system processing the waste. Where such seed is not available, use supernatant from domestic wastewater after settling at room

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temperature for at least 1 h but no longer than 36 h. When effluent or mixed liquor from a biological treatment process is used, inhibition of nitrification is recommended.

Some samples may contain materials not degraded at normal rates by the microorganisms in settled domestic wastewater. Seed such samples with an adapted microbial population obtained from the undisinfected effluent or mixed liquor of a biological process treating the waste. In the absence of such a facility, obtain seed from the receiving water below (preferably 3 to 8 km) the point of discharge. When such seed sources also are not available, develop an adapted seed in the laboratory by continuously aerating a sample of settled domestic wastewater and adding small daily increments of waste. Optionally use a soil suspension or activated sludge, or a commercial seed preparation to obtain the initial microbial population. Determine the existence of a satisfactory population by testing the performance of the seed in BOD tests on the sample. BOD values that increase with time of adaptation to a steady high value indicate successful seed adaptation.

2) Seed control—Determine BOD of the seeding material as for any other sample. This is the *seed control*. From the value of the seed control and a knowledge of the seeding material dilution (in the dilution water) determine seed DO uptake. Ideally, make dilutions of seed such that the largest quantity results in at least 50% DO depletion. A plot of DO depletion, in milligrams per liter, versus milliters of seed for all bottles having a 2-mg/L depletion and a 1.0-mg/L minimum residual DO should present a straight line for which the slope indicates DO depletion per milliliter of seed. The DO-axis intercept is oxygen depletion caused by the dilution water and should be less than 0.1 mg/L (¶ 4*h*). Alternatively, divide DO depletion by volume of seed in milliliters for each seed control bottle having a 2-mg/L depletion and a 1.0-mg/L residual DO. Average the results for all bottles meeting minimum depletion and residual DO criteria. The DO uptake attributable to the seed added to each bottle should be between 0.6 and 1.0 mg/L, but the amount of seed added should be adjusted from this range to that required to provide glucose-glutamic acid check results in the range of 198 ± 30.5 mg/L. To determine DO uptake for a test bottle, subtract DO uptake attributable to the seed from total DO uptake (see ¶ 5).

Techniques for adding seeding material to dilution water are described for two sample dilution methods (¶ 4*f*).

e. Sample pretreatment: Check pH of all samples before testing unless previous experience indicates that pH is within the acceptable range.

1) Samples containing caustic alkalinity (pH >8.5) or acidity (pH <6.0)—Neutralize samples to pH 6.5 to 7.5 with a solution of sulfuric acid (H_2SO_4) or sodium hydroxide (NaOH) of such strength that the quantity of reagent does not dilute the sample by more than 0.5%. The pH of dilution water should not be affected by the lowest sample dilution. Always seed samples that have been pH-adjusted.

2) Samples containing residual chlorine compounds—If possible, avoid samples containing residual chlorine by sampling ahead of chlorination processes. If the sample has been chlorinated but no detectable chlorine residual is present, seed the dilution water. If residual chlorine is present, dechlorinate sample and seed the dilution water (¶ 4*f*). Do not test

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chlorinated/dechlorinated samples without seeding the dilution water. In some samples chlorine will dissipate within 1 to 2 h of standing in the light. This often occurs during sample transport and handling. For samples in which chlorine residual does not dissipate in a reasonably short time, destroy chlorine residual by adding Na_2SO_3 solution. Determine required volume of Na_2SO_3 solution on a 100- to 1000-mL portion of neutralized sample by adding 10 mL of 1 + 1 acetic acid or 1 + 50 H_2SO_4, 10 mL potassium iodide (KI) solution (10 g/100 mL) per 1000 mL portion, and titrating with Na_2SO_3 solution to the starch-iodine end point for residual. Add to neutralized sample the relative volume of Na_2SO_3 solution determined by the above test, mix, and after 10 to 20 min check sample for residual chlorine. (NOTE: Excess Na_2SO_3 exerts an oxygen demand and reacts slowly with certain organic chloramine compounds that may be present in chlorinated samples.)

3) Samples containing other toxic substances—Certain industrial wastes, for example, plating wastes, contain toxic metals. Such samples often require special study and treatment.

4) Samples supersaturated with DO—Samples containing more than 9 mg DO/L at 20°C may be encountered in cold waters or in water where photosynthesis occurs. To prevent loss of oxygen during incubation of such samples, reduce DO to saturation at 20°C by bringing sample to about 20°C in partially filled bottle while agitating by vigorous shaking or by aerating with clean, filtered compressed air.

5) Sample temperature adjustment—Bring samples to 20 ± 1°C before making dilutions.

6) Nitrification inhibition—If nitrification inhibition is desired add 3 mg 2-chloro-6-(trichloro methyl) pyridine (TCMP) to each 300-mL bottle before capping or add sufficient amounts to the dilution water to make a final concentration of 10 mg/L. (NOTE: Pure TCMP may dissolve slowly and can float on top of the sample. Some commercial formulations dissolve more readily but are not 100% TCMP; adjust dosage accordingly.) Samples that may require nitrification inhibition include, but are not limited to, biologically treated effluents, samples seeded with biologically treated effluents, and river waters. Note the use of nitrogen inhibition in reporting results.

f. Dilution technique: Make several dilutions of sample that will result in a residual DO of at least 1 mg/L and a DO uptake of at least 2 mg/L after a 5-d incubation. Five dilutions are recommended unless experience with a particular sample shows that use of a smaller number of dilutions produces at least two bottles giving acceptable minimum DO depletion and residual limits. A more rapid analysis, such as COD, may be correlated approximately with BOD and serve as a guide in selecting dilutions. In the absence of prior knowledge, use the following dilutions: 0.0 to 1.0% for strong industrial wastes, 1 to 5% for raw and settled wastewater, 5 to 25% for biologically treated effluent, and 25 to 100% for polluted river waters.

Prepare dilutions either in graduated cylinders or volumetric glassware, and then transfer to BOD bottles or prepare directly in BOD bottles. Either dilution method can be combined with any DO measurement technique. The number of bottles to be prepared for each dilution depends

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on the DO technique and the number of replicates desired.

When using graduated cylinders or volumetric flasks to prepare dilutions, and when seeding is necessary, add seed either directly to dilution water or to individual cylinders or flasks before dilution. Seeding of individual cylinders or flasks avoids a declining ratio of seed to sample as increasing dilutions are made. When dilutions are prepared directly in BOD bottles and when seeding is necessary, add seed directly to dilution water or directly to the BOD bottles. When a bottle contains more than 67% of the sample after dilution, nutrients may be limited in the diluted sample and subsequently reduce biological activity. In such samples, add the nutrient, mineral, and buffer solutions (¶ 3*a* through *e*) directly to individual BOD bottles at a rate of 1 mL/L (0.33 mL/300-mL bottle) or use commercially prepared solutions designed to dose the appropriate bottle size.

1) Dilutions prepared in graduated cylinders or volumetric flasks—If the azide modification of the titrimetric iodometric method (Section 4500-O.C) is used, carefully siphon dilution water, seeded if necessary, into a 1- to 2-L-capacity flask or cylinder. Fill half full without entraining air. Add desired quantity of carefully mixed sample and dilute to appropriate level with dilution water. Mix well with a plunger-type mixing rod; avoid entraining air. Siphon mixed dilution into two BOD bottles. Determine initial DO on one of these bottles. Stopper the second bottle tightly, water-seal, and incubate for 5 d at 20°C. If the membrane electrode method is used for DO measurement, siphon dilution mixture into one BOD bottle. Determine initial DO on this bottle and replace any displaced contents with sample dilution to fill the bottle. Stopper tightly, water-seal, and incubate for 5 d at 20°C.

2) Dilutions prepared directly in BOD bottles—Using a wide-tip volumetric pipet, add the desired sample volume to individual BOD bottles of known capacity. Add appropriate amounts of seed material either to the individual BOD bottles or to the dilution water. Fill bottles with enough dilution water, seeded if necessary, so that insertion of stopper will displace all air, leaving no bubbles. For dilutions greater than 1:100 make a primary dilution in a graduated cylinder before making final dilution in the bottle. When using titrimetric iodometric methods for DO measurement, prepare two bottles at each dilution. Determine initial DO on one bottle. Stopper second bottle tightly, water-seal, and incubate for 5 d at 20°C. If the membrane electrode method is used for DO measurement, prepare only one BOD bottle for each dilution. Determine initial DO on this bottle and replace any displaced contents with dilution water to fill the bottle. Stopper tightly, water-seal, and incubate for 5 d at 20°C. Rinse DO electrode between determinations to prevent cross-contamination of samples.

Use the azide modification of the iodometric method (Section 4500-O.C) or the membrane electrode method (Section 4500-O.G) to determine initial DO on all sample dilutions, dilution water blanks, and where appropriate, seed controls.

If the membrane electrode method is used, the azide modification of the iodometric method (Method 4500-O.C) is recommended for calibrating the DO probe.

g. Determination of initial DO: If the sample contains materials that react rapidly with DO, determine initial DO immediately after filling BOD bottle with diluted sample. If rapid initial

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DO uptake is insignificant, the time period between preparing dilution and measuring initial DO is not critical but should not exceed 30 min.

h. Dilution water blank: Use a dilution water blank as a rough check on quality of unseeded dilution water and cleanliness of incubation bottles. Together with each batch of samples incubate a bottle of unseeded dilution water. Determine initial and final DO as in ¶s 4*g* and j. The DO uptake should not be more than 0.2 mg/L and preferably not more than 0.1 mg/L Discard all dilution water having a DO uptake greater than 0.2 mg/L and either eliminate source of contamination or select an alternate dilution water source..

i. Incubation: Incubate at 20°C ± 1°C BOD bottles containing desired dilutions, seed controls, dilution water blanks, and glucose-glutamic acid checks. Water-seal bottles as described in ¶ 4 *f*.

j. Determination of final DO: After 5 d incubation determine DO in sample dilutions, blanks, and checks as in ¶ 4*g*.

5. Calculation

For each test bottle meeting the 2.0-mg/L minimum DO depletion and the 1.0-mg/L residual DO, calculate BOD_5 as follows:

When dilution water is not seeded:

$$BOD_5,\ mg/L = \frac{D_1 - D_2}{P}$$

When dilution water is seeded:

$$BOD_5,\ mg/L = \frac{D_1 - D_2}{P}$$

where:

D_1 = DO of diluted sample immediately after preparation, mg/L,
D_2 = DO of diluted sample after 5 d incubation at 20°C, mg/L,
P = decimal volumetric fraction of sample used,
B_1 = DO of seed control before incubation, mg/L (¶ 4*d*),
B_2 = DO of seed control after incubation mg/L (¶ 4*d*), and
f = ratio of seed in diluted sample to seed in seed control = (% seed in diluted sample)/(% seed in seed control).

If seed material is added directly to sample or to seed control bottles:

f = (volume of seed in diluted sample)/(volume of seed in seed control)

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Report results as $CBOD_5$ if nitrification is inhibited.

If more than one sample dilution meets the criteria of a residual DO of at least 1 mg/L and a DO depletion of at least 2 mg/L and there is no evidence of toxicity at higher sample concentrations or the existence of an obvious anomaly, average results in the acceptable range.

In these calculations, do not make corrections for DO uptake by the dilution water blank during incubation. This correction is unnecessary if dilution water meets the blank criteria stipulated above. If the dilution water does not meet these criteria, proper corrections are difficult ; do not record results or, as a minimum, mark them as not meeting quality control criteria.

6. Precision and Bias

There is no measurement for establishing bias of the BOD procedure. The glucose-glutamic acid check prescribed in ¶ 4*c* is intended to be a reference point for evaluation of dilution water quality, seed effectiveness, and analytical technique. Single-laboratory tests using a 300-mg/L mixed glucose-glutamic acid solution provided the following results:

Number of months: 14
Number of triplicates: 421
Average monthly recovery: 204 mg/L
Average monthly standard deviation: 10.4 mg/L

In a series of interlaboratory studies,[1] each involving 2 to 112 laboratories (and as many analysts and seed sources), 5-d BOD measurements were made on synthetic water samples containing a 1:1 mixture of glucose and glutamic acid in the total concentration range of 3.3 to 231 mg/L. The regression equations for mean value, $\bar{X}$, and standard deviation, S, from these studies were:

$$\bar{X} = 0.658 \text{ (added level, mg/L)} + 0.280 \text{ mg/L}$$
$$S = 0.100 \text{ (added level, mg/L)} + 0.547 \text{ mg/L}$$

For the 300-mg/L mixed primary standard, the average 5-d BOD would be 198 mg/L with a standard deviation of 30.5 mg/L. When nitrification inhibitors are used, GGA test results falling outside the 198 ± 30.5 control limit quite often indicate use of incorrect amounts of seed. Adjust amount of seed added to the GGA test to achieve results falling within this range.

a. *Control limits:* Because of many factors affecting BOD tests in multilaboratory studies and the resulting extreme variability in test results, one standard deviation, as determined by interlaboratory tests, is recommended as a control limit for individual laboratories. Alternatively, for each laboratory, establish its control limits by performing a minimum of 25 glucose-glutamic acid checks (¶ 4*c*) over a period of several weeks or months and calculating the mean and standard deviation. Use the mean ±3 standard deviations as the control limit for future glucose-glutamic acid checks. Compare calculated control limits to the single-laboratory tests

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presented above and to interlaboratory results. If control limits are outside the range of 198 ± 30.5, re-evaluate the control limits and investigate source of the problem. If measured BOD for a glucose-glutamic acid check is outside the accepted control limit range, reject tests made with that seed and dilution water.

b. *Working range and detection limit:* The working range is equal to the difference between the maximum initial DO (7 to 9 mg/L) and minimum DO residual of 1 mg/L multiplied by the dilution factor. A lower detection limit of 2 mg/L is established by the requirement for a minimum DO depletion of 2 mg/L.

7. Reference

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5210 C. Ultimate BOD Test (PROPOSED)

1. General Discussion

The ultimate BOD test is an extension of the 5-d dilution BOD test as described in 5210B but with a number of specific test requirements and differences in application. The user should be familar with the 5210B procedure before conducting tests for UBOD.

a. Principle: The method consists of placing a single sample dilution in full, airtight bottles and incubating under specified conditions for an extended period depending on wastewater, effluent, river, or estuary quality.[1] Dissolved oxygen (DO) is measured (with probes) initially and intermittently during the test. From the DO versus time series, UBOD is calculated by an appropriate statistical technique. For improved accuracy, run tests in triplicate.

Bottle size and incubation time are flexible to accommodate individual sample characteristics and laboratory limitations. Incubation temperature, however, is 20°C. Most effluents and some naturally occurring surface waters contain materials with oxygen demands exceeding the DO available in air-saturated water. Therefore, it is necessary either to dilute the sample or to monitor DO frequently to ensure that low DO or anaerobic conditions do not occur. When DO concentrations approach 2 mg/L, the sample should be reaerated.

Because bacterial growth requires nutrients such as nitrogen, phosphorus, and trace metals, the necessary amounts may be added to the dilution water together with buffer to ensure that pH

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remains in a range suitable for bacterial growth and seed to provide an adequate bacterial population. However, if the result is being used to estimate the rate of oxidation of naturally occurring surface waters, addition of nutrients and seed probably accelerates the decay rate and produces misleading results. If only UBOD is desired, it may be advantageous to add supplemental nutrients that accelerate decay and reduce the test duration. When nutrients are used, they also should be used in the dilution water blank. Because of the wide range of water and wastewater characteristics and varied applications of UBOD data, no specific nutrient or buffer formulations are included.

The extent of oxidation of nitrogenous compounds during the prescribed incubation period depends on the presence of microorganisms capable of carrying out this oxidation. Such organisms may not be present in wastewaters in sufficient numbers to oxidize significant quantities of reduced nitrogen. This situation may be reversed in naturally occurring surface waters. Erratic results may be obtained when a nitrification inhibitor is used;[2] therefore, the specified method precludes use of a nitrogen inhibitor unless prior experimental evidence on the particular sample suggests that it is acceptable.*#(99) Monitor NO_2^--N and NO_3^--N to compute the oxygen equivalency of the nitrification reaction. When these values are subtracted from the DO vs. time series, the carbonaceous BOD time series can be constructed.[3] *b. Sampling and storage:* See Section 5210B.1*b*.

2. Apparatus

a. Incubation bottles: Glass bottles with ground-glass stoppers,†#(100) 2-L (or larger) capacity. Glass serum bottles of 4- to 10-L capacity are available. Alternatively use nonground-glass bottles with nonbiodegradable plastic caps as a plug insert. Do not reuse the plugs because discoloration occurs with continued use. Replace plugs every 7 to 14 d. Do not use rubber stoppers that may exert an oxygen demand. Clean bottles with a detergent and wash with dilute HCl (3*N*) to remove surface films and precipitated inorganic salts; rinse thoroughly with DI water before use. Cover top of bottles with paper after rinsing to prevent dust from collecting. To prevent drawing air into the sample bottle during incubation, use a water seal. If the bottle does not have a flared mouth, construct a water seal by making a watertight dam around the stopper (or plug) and fill with water from the reservoir as necessary. Cover dam with clean aluminum foil to retard evaporation. If a 2-L BOD bottle is used, fill reservoir with sample and cover with a polyethylene cap before incubation.

Place a clean magnetic stirring bar in each bottle to mix contents before making DO measurement or taking a subsample. Do not remove the magnets until the test is complete.

Alternatively use a series of 300-mL BOD bottles as described in 5210B, if larger bottles are not available or incubation space is limited.

b. Reservoir bottle: 4-L or larger glass bottle. Close with screw plastic cap or non-rubber plug.

c. Incubator or water bath, thermostatically controlled at 20 ± 1°C. Exclude all light to prevent the possibility of photosynthetic production of DO.

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d. Oxygen-sensitive membrane electrode: See Section 4500-O.G.2.

3. Procedure

a. River water samples: Preferably fill large BOD bottle (>2 L, or alternatively 6 or more 300-mL BOD bottles) with sample at 20°C. Add no nutrients, seed, or nitrification inhibitor if in-bottle decay rates will be used to estimate in-stream rates. Do not dilute sample unless it is known by pretesting or by experience to have a high ultimate BOD (>20 mg/L).

Measure DO in each bottle, stopper, and make an airtight seal. Incubate at 20°C in the dark.

Measure DO in each bottle at intervals of at least 2 to 5 d over a period of 30 to 60 d (minimum of 6 to 8 readings) or longer under special circumstances. To avoid oxygen depletion in samples containing NH_3-N, measure DO more frequently until nitrification has taken place. If DO falls to about 2 mg/L, reaerate as directed below. Replace sample lost by the cap and DO probe displacement by adding 1 to 2 mL sample from the reservoir bottle.

When DO approaches 2 mg/L, reaerate. Pour a small amount of sample into a clean vessel and reaerate the remainder directly in the bottle by vigorous shaking or bubbling with purified air (medical grade). Refill bottle from the storage reservoir and measure DO. This concentration becomes the initial DO for the next measurement. If using 300-mL BOD bottles, pour all of the sample from the several bottles used into a clean vessel, reaerate, and refill the small bottles.

Analyze for nitrate plus nitrite nitrogen (NO_3^--N + NO_2^--N) (see Section 4500-NO_2^- and Section 4500-NO_3^-) on Days 0, 5, 10, 15, 20, and 30. Alternatively, determine NO_2^--N and NO_3^--N each time DO is determined, thereby producing corresponding BOD and nitrogen determinations. If the ultimate demand occurs at a time greater than 30 d, make additional analyses at 30-d intervals. Remove 10 to 20 mL from the bottle for these analyses. Refill bottle as necessary from the reservoir bottle. Preserve NO_2^--N + NO_3^--N subsample with H_2SO_4 to pH <2 and refrigerate. If the purpose of the UBOD test is to assess the UBOD and not to provide data for rate calculations, measure nitrate nitrogen concentration only at Day 0 and on the last day of the test (kinetic rate estimates are not useful when the nitrification reaction is not followed).

Calculate oxygen consumption during each time interval and make appropriate corrections for nitrogenous oxygen demand. Correct by using 3.43 × the NH_3-N to NO_2^--N conversion plus 1.14 × the NO_2^--N to NO_3^--N conversion to reflect the stoichiometry of the oxidation of NH_4^+ to NO_2^- or NO_3^-.

When using a dilution water blank, subtract DO uptake of the blank from the total DO consumed. High-quality reagent water without nutrients typically will consume a maximum of 1 mg DO/L in a 30- to 90-d period. If DO uptake of the dilution water is greater than 0.5 mg/L for a 20-d period, or 1 mg/L for a 90-d period, report the magnitude of the correction and try to obtain higher-quality dilution water for use with subsequent UBOD tests.

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When the weekly DO consumption drops below 1 to 2% of the total accumulative consumption, calculate the ultimate BOD using a nonlinear regression method.

b. Wastewater treatment plant samples: Use high-quality reagent water (see Section 1080) for dilution water. Add no nitrification inhibitors if decay rates are desired. If seed and nutrients are necessary, add the same amounts of each to the dilution water blank. Use minimal sample dilution. As a rule of thumb, the ultimate BOD of the diluted sample should be in the range of 20 to 30 mg/L. Dilution to this level probably will require two or three sample reaerations during the incubation period to avoid having dissolved oxygen concentrations fall below 2 mg/L.

Use 2-L or larger BOD bottles (alternatively, multiple 300-mL BOD bottles) for each dilution. Add desired volume of sample to each bottle and fill with dilution water.

Fill a BOD bottle with dilution water to serve as a dilution water blank. Treat blank the same as all samples. Follow procedure given in ¶ 3*a*) and incubate for at least as long as UBOD test.

4. Calculations

An example of results obtained for a wastewater sample, undiluted, without seed and nutrients, is given in Table 5210:I.

UBOD can be estimated by using a first-order model described as follows:

$$BOD_t = UBOD\,(1 - e^{-kt})$$

where:

BOD_t = oxygen uptake measured at time t, mg/L, and
k = first-order oxygen uptake rate.

The data in Table 5210:I were analyzed with a nonlinear regression technique applied to the above first-order model.[4] However, a first-order kinetic model may not always be the best choice. Significantly better statistical fits usually are obtained with alternative kinetic models including sum of two first-order and logistic function models.[1,3-8]

5. Precision and Bias

The precision of the ultimate BOD test was assessed with a series of replicate tests in a single laboratory. Interlaboratory studies have not been conducted.

Reference	Replicate No.	UBOD *mg/L*	Precision Summary*
2	1	154	μ = 151 mg/L
	2	154	
	3	145	CV = 3.5%

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Reference	Replicate No.	UBOD *mg/L*	Precision Summary*
5	1	10.3	
	2	11.1	
	3	9.6	μ = 10.0 mg/L
	4	9.9	
	5	9.8	CV = 5.8%
	6	9.6	
6	1	12.8	μ = 12.4 mg/L
	2	12.6	
	3	12.6	CV = 4.4%
	4	11.6	

*μ = mean, CV = coefficient of variation.

Bias was assessed by determining the BOD of a known concentration of glucose (150 mg/L) and glutamic acid (150 mg/L). This solution has a UBOD of 321 mg/L to 308 mg/L, depending on extent of nitrification. The results of the study conducted in triplicate were:[1]

Estimated* UBOD *mg/L*	Theoretical BOD *mg/L*	Percent Difference
276	308/321	–10/–14
310	308/321	+1/–3
303	308/321	–2/–6

*By statistical model.

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5210 D. Respirometric Method (PROPOSED)

1. General Discussion

a. Principle: Respirometric methods provide direct measurement of the oxygen consumed by microorganisms from an air or oxygen-enriched environment in a closed vessel under conditions of constant temperature and agitation.

b. Uses: Respirometry measures oxygen uptake more or less continuously over time. Respirometric methods are useful for assessing: biodegradation of specific chemicals; treatability of organic industrial wastes; the effect of known amounts of toxic compounds on the oxygen-uptake reaction of a test wastewater or organic chemical; the concentration at which a pollutant or a wastewater measurably inhibits biological degradation; the effect of various treatments such as disinfection, nutrient addition, and pH adjustment on oxidation rates; the oxygen requirement for essentially complete oxidation of biologically oxidizable matter; the need for using adapted seed in other biochemical oxygen-uptake measurements, such as the dilution BOD test; and stability of sludges.

Respirometric data typically will be used comparatively, that is, in a direct comparison between oxygen uptakes from two test samples or from a test sample and a control. Because of inherent differences among uses, among seed cultures, among applications of results, and among instruments, a single procedure for respirometric tests applicable to all cases cannot be defined. Therefore, only basic recommendations and guidelines for overall test setup and procedure are given. Follow manufacturer's instructions for operating details for specific commercial instruments.

c. Types of respirometers: Four principal types of commercial respirometers are available. Manometric respirometers relate oxygen uptake to the change in pressure caused by oxygen

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consumption while maintaining a constant volume. Volumetric respirometers measure oxygen uptake in incremental changes in gas volume while maintaining a constant pressure at the time of reading. Electrolytic respirometers monitor the amount of oxygen produced by electrolysis of water to maintain a constant oxygen pressure within the reaction vessel. Direct-input respirometers deliver oxygen to the sample from a pure oxygen supply through metering on demand as detected by minute pressure differences. Most respirometers have been instrumented to permit data collection and processing by computer. Reaction-vessel contents are mixed by using a magnetic or mechanical stirring device or by bubbling the gaseous phase within the reaction vessel through the liquid phase. All respirometers remove carbon dioxide produced during biological growth by suspending a concentrated adsorbent (granular or solution) within the closed reaction chamber or by recirculating the gas phase through an external scrubber.

d. Interferences: Evolution of gases other than CO_2 may introduce errors in pressure or volume measurements; this is uncommon in the presence of dissolved oxygen. Incomplete CO_2 absorption will introduce errors if appropriate amounts and concentrations of alkaline absorbent are not used. Temperature fluctuations or inadequate mixing will introduce error. Fluctuations in barometric pressure can cause errors with some respirometers. Become familiar with the limits of the instrument to be used.

e. Minimum detectable concentration: Most commercial respirometers can detect oxygen demand in increments as small as 0.1 mg but test precision depends on the total amount of oxygen consumed at the time of reading, the precision of pressure or volume measurement, and the effect of temperature and barometric pressure changes. Upper limits of oxygen uptake rate are determined by the ability to transfer oxygen into the solution from the gas phase, which typically is related to mixing intensity. Transfer limits typically range from less than 10 mg O_2/L/h for low-intensity mixing to above 100 mg O_2/L/h for high-intensity mixing.

f. Relationship to dilution BOD: Variations in waste composition, substrate concentration, mixing, and oxygen concentrations from one wastewater source to another generally preclude use of a general relationship between oxygen uptake by respirometers and the 5-d, 20°C, BOD (see 5210B, above). Reasonably accurate correlations may be possible for a specific wastewater. The incubation period for respirometric measurements need not be 5 d because equally valid correlations can be made between the 5-d dilution BOD and respirometric oxygen uptake at any time after 2 d.[1,2] The point of common dilution and respirometric BOD seems to occur at about 2 to 3 d incubation for municipal wastewaters. Correlations between respirometric measurements and 5-d BOD for industrial wastes and specific chemicals are less certain. Respirometric measurements also can provide an indication of the ultimate biochemical oxygen demand (UBOD) (see Section 5210C). In many cases, it is reasonable to consider that the 28-to 30-d oxygen uptake is essentially equal to the UBOD.[3]

More commonly, respirometers are used as a diagnostic tool. The continuous readout of oxygen consumption in respirometric measurements indicates lag, toxicity, or any abnormalities in the biodegradation reaction. The change in the normal shape of an oxygen-uptake curve in the

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first few hours may help to identify the effect of toxic or unusual wastes entering a treatment plant in time to make operating corrections.

g. Relationship to other test methods and protocols: This method supports most of the protocols and guidelines established by the European Organization for Economic Co-operation and Development[3] (OECD) that require measurement of oxygen uptake.

h. Sampling and storage:

1) Grab samples—If analysis is begun within 2 h of sample collection, cold storage is unnecessary. Otherwise, keep sample at or below 4°C from the time of collection. Begin analysis within 6 h of collection; when this is not possible, store at or below 4°C and report length and temperature of storage. Never start analysis more than 24 h after grab sample collection.

2) Composite samples—Keep samples at or below 4°C during compositing. Limit compositing period to 24 h. Use the same criteria as for storage of grab samples, starting the measurement of holding time from the end of the compositing period. State storage time and conditions with results.

2. Apparatus

a. Respirometer system: Use commercial apparatus and check manufacturer's instructions for specific system requirements, reaction vessel type and volume, and instrument operating characteristics.

b. Incubator or water bath: Use a constant-temperature room, incubator chamber, or water bath to control temperature to ±1°C. Exclude all light to prevent oxygen formation by algae in the sample. Use red, actinic-coated bottles for analysis outside of a darkened incubator.

3. Reagents

Formulations of reagent solutions are given for 1-L volumes, but smaller or larger volumes may be prepared according to need. Discard any reagent showing signs of biological growth or chemical precipitation. Stock solutions can be sterilized by autoclaving to provide longer shelf life.

a. Distilled water: Use only high-quality water distilled from a block tin or all-glass still (see Section 1080). Deionized water may be used but often contains high bacterial counts. The water must contain less than 0.01 mg heavy metals/L and be free of chlorine, chloramines, caustic alkalinity, organic material, or acids. Make all reagents with this water. When other waters are required for special-purpose testing, state clearly their source and quality characteristics.

b. Phosphate buffer solution, 1.5*N:* Dissolve 207 g sodium dihydrogen phosphate, $NaH_2PO_4 \cdot H_2O$, in water. Neutralize to pH 7.2 with 6*N* KOH (¶ 3*g* below) and dilute to 1 L.

c. Ammonium chloride solution, 0.71*N:* Dissolve 38.2 g ammonium chloride, NH_4Cl, in water. Neutralize to pH 7.0 with KOH. Dilute to 1.0 L; 1 mL = 10 mg N.

d. Calcium chloride solution, 0.25*N:* Dissolve 27.7 g $CaCl_2$ in water and dilute to 1 L; 1 mL = 10 mg Ca.

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e. Magnesium sulfate solution, 0.41*N:* Dissolve 101 g $MgSO_4 \cdot 7H_2O$ in water and dilute to 1 L; 1 mL = 10 mg Mg.

f. Ferric chloride solution, 0.018*N:* Dissolve 4.84 g $FeCl_3 \cdot 6H_2O$ in water and dilute to 1 L; 1 mL = 1.0 mg Fe.

g. Potassium hydroxide solution, 6*N:* Dissolve 336 g KOH in about 700 mL water and dilute to 1 L. CAUTION: *Add KOH to water slowly and use constant mixing to prevent excessive heat buildup.* Alternately, use commercial solutions containing 30 to 50% KOH by weight.

h. Acid solutions, 1*N:* Add 28 mL conc H_2SO_4 or 83 mL conc HCl to about 700 mL water. Dilute to 1 L.

i. Alkali solution, 1*N:* Add 40 g NaOH to 700 mL water. Dilute to 1 L.

j. Nitrification inhibitor: Reagent-grade 2-chloro-6-(trichloromethyl) pyridine (TCMP) or equivalent.[3]*#(101)

k. Glucose-glutamic acid solution: Dry reagent-grade glucose and reagent-grade glutamic acid at 103°C for 1 h. Add 15.0 g glucose and 15.0 g glutamic acid to distilled water and dilute to 1 L. Neutralize to pH 7.0 using 6*N* potassium hydroxide (¶ 3*g*). This solution may be stored for up to 1 week at 4°C.

l. Electrolyte solution (for electrolytic respirometers): Use manufacturer's recommended solution.

m. Sodium sulfite solution, 0.025*N:* Dissolve 1.575 g Na_2SO_3 in about 800 mL water. Dilute to 1 L. This solution is not stable; prepare daily or as needed.

n. Trace element solution: Dissolve 40 mg $MnSO_4 \cdot 4H_2O$, 57 mg H_3BO_3, 43 mg $ZnSO_4 \cdot 7H_2O$, 35 mg $(NH_4)_6\ Mo_7O_{24}$, and 100 mg Fe-chelate ($FeCl_3$-EDTA) in about 800 mL water. Dilute to 1 L. Sterilize at 120°C and 200 kPa (2 atm) pressure for 20 min.

o. Yeast extract solution:[3] Add 15 mg laboratory- or pharmaceutical-grade brewer's yeast extract to 100 mL water. Make this solution fresh immediately before each test in which it is used.

p. Nutrient solution:[3] Add 2.5 mL phosphate buffer solution (3*b*), 0.65 mL ammonium chloride solution (3*c*), 1.0 mL calcium chloride solution (3*d*), 0.22 mL magnesium sulfate solution (3*a*), 0.1 mL ferric chloride solution (3 *f*), 1 mL trace element solution (3*n*), and 1 mL yeast extract solution (3*o*) to about 900 mL water. Dilute to 1 L. This nutrient solution and those of ¶s n and o above are specifically formulated for use with the OECD method.[3] (NOTE: A 10:1 concentrated nutrient solution can be made and diluted accordingly.)

4. Procedure

a. Instrument operation: Follow respirometer manufacturer's instructions for assembly, testing, calibration, and operation of the instrument. NOTE: The manufacturer's stated maximum

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and minimum limits of measurement are not always the same as the instrument output limits. Make sure that test conditions are within the limits of measurement.

b. Sample volume: Sample volume or concentration of organic chemicals to be added to test vessels is a function of expected oxygen uptake characteristics and oxygen transfer capability of the instrument. Small volumes or low concentrations may be required for high-strength wastes. Large volumes may be required for low-strength wastes to improve accuracy.

c. Data recording interval: Set instrument to give data readings at suitable intervals. Intervals of 15 min to 6 h typically are used.

d. Sample preparation:

1) Homogenization—If sample contains large settleable or floatable solids, homogenize it with a blender and transfer representative test portions while all solids are in suspension. If there is a concern for changing sample characteristics, skip this step.

2) pH adjustment—Neutralize samples to pH 7.0 with H_2SO_4 or NaOH of such strength (¶s 3*h* and ¶ 3*i*) that reagent quantity does not dilute the sample more than 0.5%.

3) Dechlorination—Avoid analyzing samples containing residual chlorine by collecting the samples ahead of chlorination processes. If residual chlorine is present, aerate as described in ¶ 5) below or let stand in light for 1 to 2 h. If a chlorine residual persists, add Na_2SO_3 solution. Determine required volume of Na_2SO_3 solution by adding 10 mL 1 + 1 acetic acid or 1 + 50 H_2SO_4 and 10 mL potassium iodide solution (10 g/100 mL) to a portion of the sample. Titrate with 0.025*N* Na_2SO_3 solution to the starch-iodine end point (see Section 4500-Cl.B). Add to the neutralized sample a proportional volume of Na_2SO_3 solution determined above, mix, and after 10 to 20 min check for residual chlorine. Re-seed the sample (see ¶ 4*h* below).

4) Samples containing toxic substances—Certain industrial wastes contain toxic metals or organic compounds. These often require special study and treatment.[3]

5) Initial oxygen concentration—If samples contain dissolved oxygen concentrations above or below the desired concentration, agitate or aerate with clean and filtered compressed air for about 1 h immediately before testing. Minimum and maximum actual DO concentrations will vary with test objectives. In some cases, pure oxygen may be added to respirometer vessels to increase oxygen levels above ambient.

6) Temperature adjustment—Bring samples and dilution water to desired test temperature (±1°C) before making dilutions or transferring to test vessels.

e. Sample dilution: Use distilled water or water from other appropriate sources free of organic matter. In some cases, receiving stream water may be used for dilution. Add desired sample volume to test vessels using a wide-tip volumetric pipet or other suitable volumetric glassware. Add dilution water to bring sample to about 80% of desired final volume. Add appropriate amounts of nutrients, minerals, buffer, nitrification inhibitor if desired, and seed culture as described in ¶s 4 *f* and h below. Dilute sample to desired final volume. The number of

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test vessels to prepare for each dilution depends on test objectives and number of replicates desired.

f. Nutrients, minerals, and buffer: Add sufficient ammonia nitrogen to provide a COD:N:P ratio of 100:5:1 or a TOC:N:P ratio of 30:5:1. Add 2 mL each of calcium, magnesium, ferric chloride, and trace mineral solutions to each liter of diluted sample unless sufficient amounts of these minerals are present in the original sample. Phosphorus requirements will be met by the phosphate buffer if it is used (1 mL/50 mg/L COD or ultimate BOD of diluted sample usually is sufficient to maintain pH between 6.8 and 7.2). Be cautious in adding phosphate buffer to samples containing metal salts because metal phosphates may precipitate and show less toxic or beneficial effect than when phosphate is not present. For OECD-compatible tests, substitute the nutrient, mineral, and buffer amounts listed in ¶ 3 *p* for the above nutrient/ mineral/buffer quantities.

g. Nitrification inhibition: If nitrification inhibition is desired, add 10 mg 2-chloro-6-(trichloromethyl) pyridine (TCMP)/L sample in the test vessel. Samples that may nitrify readily include biologically treated effluents, samples seeded with biologically treated effluents, and river waters.[4]

h. Seeding: See 5210B.4*d*1) for seed preparation. Use sufficient amounts of seed culture to prevent major lags in the oxygen uptake reaction but not so much that the oxygen uptake of the seed exceeds about 10% of the oxygen uptake of the seeded sample.

Determine the oxygen uptake of the seeding material as for any other sample. This is the seed control. Typically, the seed volume in the seed control should be 10 times the volume used in seeded samples.

i. Incubation: Incubate samples at 20°C or other suitable temperature ±1.0°C. Take care that the stirring device does not increase the temperature of the sample.

5. Calculations

To convert instrument readings to oxygen uptake, refer to manufacturer's procedures.

Correct oxygen uptake for seed and dilution by the following equation:

$$C = [A - B(S_A/S_B)](1000/N_A)$$

where:

C = corrected oxygen uptake of sample, mg/L,
A = measured oxygen uptake in seeded sample, mg,
B = measured oxygen uptake in seed control, mg,
S_A = volume of seed in Sample A, mL,
S_B = volume of seed in Sample B, mL, and
N_A = volume of undiluted sample in Sample A, mL.

6. Quality Control

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Periodically use the following procedure to check distilled water quality, instrument quality, instrument function, and analytical technique by making oxygen uptake measurements using a mixture of glucose and glutamic acid as a standard check solution.

Adjust water for sample formulation to test temperature and saturate with DO by aerating with clean, organic-free filtered air. Protect water quality by using clean glassware, tubing, and bottles.

Prepare a *test solution* by adding 10 mL glucose-glutamic acid solution (¶ 3*k*); 6 mL phosphate buffer (¶ 3*b*); 2 mL each of ammonium chloride (¶ 3*c*), magnesium sulfate (¶ 3*e*), calcium chloride (¶ 3*d*), ferric chloride (¶ 3 *f*), and trace element solution (¶ 3*n*) to approximately 800 mL water. Add 10 mg nitrification inhibitor (TCMP)/L. Add sufficient seed from a suitable source as described in ¶ 4*h* to give a lag time less than 6 h (usually 25 mL supernatant from settled primary effluent/L test solution is sufficient). Dilute to 1 L. Adjust temperature to 20 ± 1°C.

Prepare a *seed blank* by diluting 500 mL or more of the seed solution to 800 mL with distilled water. Add the same amount of buffer, nutrients, and TCMP as in the test solution, and dilute to 1 L. Adjust temperature to 20 ± 1°C.

Place test solution and seed blank solution in separate reaction vessels of respirometer and incubate for 5 d at 20°C. Run at least three replicates of each. The seed-corrected oxygen uptake after 5 d incubation should be 260 ± 30 mg/L. If the value of the check is outside this range, repeat the test using a fresh seed culture and seek the cause of the problem.

7. Precision and Bias

a. Precision: No standard is available to check the accuracy of respirometric oxygen uptake measurements. To obtain laboratory precision data, use a glucose-glutamic acid mixture (¶ 6 above) having a known theoretical maximum oxygen uptake value. Tests with this and similar organic compound mixtures have shown that the standard deviation, expressed as the coefficient of variation, C_v, is approximately 5% for samples having total oxygen uptakes of 50 to 100 mg/L and 3% for more concentrated samples.[1,2] Individual instruments have different readability limits that can affect precision. The minimum response or sensitivity of most commercial respirometers ranges from 0.05 to 1 mg oxygen. Check manufacturer's specifications for sensitivity of the instrument at hand.

b. Control limits: To establish laboratory control limits, perform a minimum of 25 glucose-glutamic acid checks over a period of several weeks or months and calculate mean and standard deviation. If measured oxygen uptake in 5 d at 20°C is outside the 260 ± 30 mg/L range, re-evaluate procedure to identify source of error. For other samples, use the mean ±3 standard deviations as the control limit.

c. Working range and detection limits: The working range and detection limits are established by the limits of each commercial instrument. Refer to manufacturer's specifications.

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5220 CHEMICAL OXYGEN DEMAND (COD)*#(102)

5220 A. Introduction

Chemical oxygen demand (COD) is defined as the amount of a specified oxidant that reacts with the sample under controlled conditions. The quantity of oxidant consumed is expressed in terms of its oxygen equivalence. Because of its unique chemical properties, the dichromate ion ($Cr_2O_7^{2-}$) is the specified oxidant in Methods Section 5220B, Section 5220C, and Section 5220D; it is reduced to the chromic ion (Cr^{3+}) in these tests. Both organic and inorganic components of a sample are subject to oxidation, but in most cases the organic component predominates and is of the greater interest. If it is desired to measure either organic or inorganic COD alone, additional steps not described here must be taken to distinguish one from the other.

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COD is a defined test; the extent of sample oxidation can be affected by digestion time, reagent strength, and sample COD concentration.

COD often is used as a measurement of pollutants in wastewater and natural waters. Other related analytical values are biochemical oxygen demand (BOD), total organic carbon (TOC), and total oxygen demand (TOD). In many cases it is possible to correlate two or more of these values for a given sample. BOD is a measure of oxygen consumed by microorganisms under specific conditions; TOC is a measure of organic carbon in a sample; TOD is a measure of the amount of oxygen consumed by all elements in a sample when complete (total) oxidation is achieved.

In a COD analysis, hazardous wastes of mercury, hexavalent chromium, sulfuric acid, silver, and acids are generated. Methods Section 5220C and Section 5220D reduce these waste problems but may be less accurate and less representative. (See ¶ 2 below.)

1. Selection of Method

The open reflux method (B) is suitable for a wide range of wastes where a large sample size is preferred. The closed reflux methods (C and D) are more economical in the use of metallic salt reagents and generate smaller quantities of hazardous waste, but require homogenization of samples containing suspended solids to obtain reproducible results. Ampules and culture tubes with premeasured reagents are available commercially. Measurements of sample volumes as well as reagent volumes and concentrations are critical. Consequently, obtain specifications as to limits of error for premixed reagents from manufacturer before use.

Determine COD values of >50 mg O_2/L by using procedures Section 5220B.4*a*, Section 5220C.4, or Section 5220D.4. Use procedure Section 5220B.4*b* to determine, with lesser accuracy, COD values from 5 to 50 mg O_2/L.

2. Interferences and Limitations

Oxidation of most organic compounds is 95 to 100% of the theoretical value. Pyridine and related compounds resist oxidation and volatile organic compounds will react in proportion to their contact with the oxidant. Straight-chain aliphatic compounds are oxidized more effectively in the presence of a silver sulfate catalyst.

The most common interferent is the chloride ion. Chloride reacts with silver ion to precipitate silver chloride, and thus inhibits the catalytic activity of silver. Bromide, iodide, and any other reagent that inactivates the silver ion can interfere similarly. Such interferences are negative in that they tend to restrict the oxidizing action of the dichromate ion itself. However, under the rigorous digestion procedures for COD analyses, chloride, bromide, or iodide can react with dichromate to produce the elemental form of the halogen and the chromic ion. Results then are in error on the high side. The difficulties caused by the presence of the chloride can be overcome largely, though not completely, by complexing with mercuric sulfate ($HgSO_4$) before the refluxing procedure. Although 1 g $HgSO_4$ is specified for 50 mL sample, a lesser amount may be used where sample chloride concentration is known to be less than 2000 mg/L, as long as

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a 10:1 weight ratio of $HgSO_4$:Cl^- is maintained. Do not use the test for samples containing more than 2000 mg Cl^-/L. Techniques designed to measure COD in saline waters are available.[1,2]

Halide interferences may be removed by precipitation with silver ion and filtration before digestion. This approach may introduce substantial errors due to the occlusion and carrydown of COD matter from heterogenous samples.

Ammonia and its derivatives, in the waste or generated from nitrogen-containing organic matter, are not oxidized. However, elemental chlorine reacts with these compounds. Hence, corrections for chloride interferences are difficult.

Nitrite (NO_2^-) exerts a COD of 1.1 mg O_2/mg NO_2^--N. Because concentrations of NO_2^- in waters rarely exceed 1 or 2 mg NO_2^--N/L, the interference is considered insignificant and usually is ignored. To eliminate a significant interference due to NO_2^-, add 10 mg sulfamic acid for each mg NO_2^--N present in the sample volume used; add the same amount of sulfamic acid to the reflux vessel containing the distilled water blank.

Reduced inorganic species such as ferrous iron, sulfide, manganous manganese, etc., are oxidized quantitatively under the test conditions. For samples containing significant levels of these species, stoichiometric oxidation can be assumed from known initial concentration of the interfering species and corrections can be made to the COD value obtained.

The silver, hexavalent chromium, and mercury salts used in the COD determinations create hazardous wastes. The greatest problem is in the use of mercury. If the chloride contribution to COD is negligible, $HgSO_4$ can be omitted. Smaller sample sizes (see Section 5220C and Section 5220D) reduce the waste. Recovery of the waste material may be feasible if allowed by regulatory authority.[3]

3. Sampling and Storage

Preferably collect samples in glass bottles. Test unstable samples without delay. If delay before analysis is unavoidable, preserve sample by acidification to pH ≤ 2 using conc H_2SO_4. Blend (homogenize) all samples containing suspended solids before analysis. If COD is to be related to BOD, TOC, etc., ensure that all tests receive identical pretreatment. Make preliminary dilutions for wastes containing a high COD to reduce the error inherent in measuring small sample volumes.

4. References

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5220 B. Open Reflux Method

1. General Discussion

a. Principle: Most types of organic matter are oxidized by a boiling mixture of chromic and sulfuric acids. A sample is refluxed in strongly acid solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). After digestion, the remaining unreduced $K_2Cr_2O_7$ is titrated with ferrous ammonium sulfate to determine the amount of $K_2Cr_2O_7$ consumed and the oxidizable matter is calculated in terms of oxygen equivalent. Keep ratios of reagent weights, volumes, and strengths constant when sample volumes other than 50 mL are used. The standard 2-h reflux time may be reduced if it has been shown that a shorter period yields the same results. Some samples with very low COD or with highly heterogeneous solids content may need to be analyzed in replicate to yield the most reliable data. Results are further enhanced by reacting a maximum quantity of dichromate, provided that some residual dichromate remains.

2. Apparatus

a. Reflux apparatus, consisting of 500- or 250-mL erlenmeyer flasks with ground-glass 24/40 neck and 300-mm jacket Liebig, West, or equivalent condenser with 24/40 ground-glass joint, and a hot plate having sufficient power to produce at least 1.4 W/cm^2 of heating surface, or equivalent.

b. Blender.

c. Pipets, Class A and wide-bore.

3. Reagents

a. Standard potassium dichromate solution, 0.04167*M*: Dissolve 12.259 g $K_2Cr_2O_7$, primary standard grade, previously dried at 150°C for 2 h, in distilled water and dilute to 1000 mL. This reagent undergoes a six-electron reduction reaction; the equivalent concentration is 6 × 0.04167*M* or 0.2500*N*.

b. Sulfuric acid reagent: Add Ag_2SO_4, reagent or technical grade, crystals or powder, to conc H_2SO_4 at the rate of 5.5 g Ag_2SO_4/kg H_2SO_4. Let stand 1 to 2 d to dissolve. Mix.

c. Ferroin indicator solution: Dissolve 1.485 g 1,10-phenanthroline monohydrate and 695 mg $FeSO_4 \cdot 7H_2O$ in distilled water and dilute to 100 mL. This indicator solution may be purchased already prepared.*#(103)

d. Standard ferrous ammonium sulfate (FAS) titrant, approximately 0.25*M*: Dissolve 98 g

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$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water. Add 20 mL conc H_2SO_4, cool, and dilute to 1000 mL. Standardize this solution daily against standard $K_2Cr_2O_7$ solution as follows:

Dilute 25.00 mL standard $K_2Cr_2O_7$ to about 100 mL. Add 30 mL conc H_2SO_4 and cool. Titrate with FAS titrant using 0.10 to 0.15 mL (2 to 3 drops) ferroin indicator.

Molarity of FAS solution

$$= \frac{\text{Volume } 0.04167M\ K_2Cr_2O_7 \text{ solution titrated, mL}}{\text{Volume FAS used in titration, mL}} \times 0.2500$$

e. Mercuric sulfate, $HgSO_4$, crystals or powder.

f. Sulfamic acid: Required only if the interference of nitrites is to be eliminated (see Section 5220A.2 above).

g. Potassium hydrogen phthalate (KHP) standard, $HOOCC_6H_4COOK$: Lightly crush and then dry KHP to constant weight at 110°C. Dissolve 425 mg in distilled water and dilute to 1000 mL. KHP has a theoretical COD[1] of 1.176 mg O_2/mg and this solution has a theoretical COD of 500 µg O_2/ mL. This solution is stable when refrigerated, but not indefinitely. Be alert to development of visible biological growth. If practical, prepare and transfer solution under sterile conditions. Weekly preparation usually is satisfactory.

4. Procedure

a. Treatment of samples with COD of >50 mg O_2/L: Blend sample if necessary and pipet 50.00 mL into a 500-mL refluxing flask. For samples with a COD of >900 mg O_2/L, use a smaller portion diluted to 50.00 mL. Add 1 g $HgSO_4$, several glass beads, and very slowly add 5.0 mL sulfuric acid reagent, with mixing to dissolve $HgSO_4$. Cool while mixing to avoid possible loss of volatile materials. Add 25.00 mL 0.04167*M* $K_2Cr_2O_7$ solution and mix. Attach flask to condenser and turn on cooling water. Add remaining sulfuric acid reagent (70 mL) through open end of condenser. Continue swirling and mixing while adding sulfuric acid reagent. CAUTION: *Mix reflux mixture thoroughly before applying heat to prevent local heating of flask bottom and a possible blowout of flask contents.*

Cover open end of condenser with a small beaker to prevent foreign material from entering refluxing mixture and reflux for 2 h. Cool and wash down condenser with distilled water. Disconnect reflux condenser and dilute mixture to about twice its volume with distilled water. Cool to room temperature and titrate excess $K_2Cr_2O_7$ with FAS, using 0.10 to 0.15 mL (2 to 3 drops) ferroin indicator. Although the quantity of ferroin indicator is not critical, use the same

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volume for all titrations. Take as the end point of the titration the first sharp color change from blue-green to reddish brown that persists for 1 min or longer. Duplicate determinations should agree within 5% of their average. Samples with suspended solids or components that are slow to oxidize may require additional determinations. The blue-green may reappear. In the same manner, reflux and titrate a blank containing the reagents and a volume of distilled water equal to that of sample.

b. Alternate procedure for low-COD samples: Follow procedure of ¶ 4*a*, with two exceptions: (*i*) use standard 0.004167*M* $K_2Cr_2O_7$, and (*ii*) titrate with standardized 0.025*M* FAS. Exercise extreme care with this procedure because even a trace of organic matter on the glassware or from the atmosphere may cause gross errors. If a further increase in sensitivity is required, concentrate a larger volume of sample before digesting under reflux as follows: Add all reagents to a sample larger than 50 mL and reduce total volume to 150 mL by boiling in the refluxing flask open to the atmosphere without the condenser attached. Compute amount of $HgSO_4$ to be added (before concentration) on the basis of a weight ratio of 10:1, $HgSO_4$:Cl^-, using the amount of Cl^- present in the original volume of sample. Carry a blank reagent through the same procedure. This technique has the advantage of concentrating the sample without significant losses of easily digested volatile materials. Hard-to-digest volatile materials such as volatile acids are lost, but an improvement is gained over ordinary evaporative concentration methods. Duplicate determinations are not expected to be as precise as in 5220B.4*a*.

c. Determination of standard solution: Evaluate the technique and quality of reagents by conducting the test on a standard potassium hydrogen phthalate solution.

5. Calculation

$$\text{COD as mg } O_2/\text{L} = \frac{(A - B) \times M \times 8000}{\text{mL sample}}$$

where:

A = mL FAS used for blank,
B = mL FAS used for sample,
M = molarity of FAS, and
8000 = milliequivalent weight of oxygen × 1000 mL/L.

6. Precision and Bias

A set of synthetic samples containing potassium hydrogen phthalate and NaCl was tested by 74 laboratories. At a COD of 200 mg O_2/L in the absence of chloride, the standard deviation was ±13 mg/L (coefficient of variation, 6.5%). At COD of 160 mg O_2/L and 100 mg Cl^-/L, the standard deviation was ±14 mg/L (coefficient of variation, 10.8%).

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5220 C. Closed Reflux, Titrimetric Method

1. General Discussion

a. Principle: See Section 5220B.1*a*.

b. Interferences and limitations: See Section 5220A.2. Volatile organic compounds are more completely oxidized in the closed system because of longer contact with the oxidant. Before each use inspect culture-tube caps for breaks in the TFE liner. Select culture-tube size according to block heater capacity and degree of sensitivity desired. Use the 25- × 150-mm tube for samples with low COD content because a larger volume sample can be treated.

This procedure is applicable to COD values between 40 and 400 mg/L. Obtain higher values by dilution. Alternatively, use higher concentrations of dichromate digestion solution to determine greater COD values. COD values of 100 mg/L or less can be obtained by using a more dilute dichromate digestion solution or a more dilute FAS titrant. Overall accuracy can be improved by using an FAS titrant which is less than the 0.10*M* solution specified below. Higher dichromate concentrations or reduced FAS concentrations probably require titrations to be done in a separate vessel, rather than in the digestion vessel, because of the volumes of titrant required.

2. Apparatus

a. Digestion vessels: Preferably use borosilicate culture tubes, 16- × 100-mm, 20- × 150-mm, or 25- × 150-mm, with TFE-lined screw caps. Alternatively, use borosilicate ampules, 10-mL capacity, 19- to 20-mm diam.

Digestion vessels with premixed reagents and other accessories are available from commercial suppliers. Contact supplier for specifications.*#(104)

b. Block heater or similar device to operate at 150 ± 2°C, with holes to accommodate digestion vessels. Use of culture tubes probably requires the caps to be outside the vessel to

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protect caps from heat. CAUTION: *Do not use an oven because of the possibility of leaking samples generating a corrosive and possibly explosive atmosphere. Also, culture tube caps may not withstand the 150°C temperature in an oven.*

c. Microburet.

d. *Ampule sealer*: Use only a mechanical sealer to insure strong, consistent seals.

3. Reagents

a. Standard potassium dichromate digestion solution, 0.01667*M*: Add to about 500 mL distilled water 4.903 g $K_2Cr_2O_7$, primary standard grade, previously dried at 150°C for 2 h, 167 mL conc H_2SO_4, and 33.3 g $HgSO_4$. Dissolve, cool to room temperature, and dilute to 1000 mL.

b. Sulfuric acid reagent: See Section 5220B.3*b*.

c. Ferroin indicator solution: See Section 5220B.3*c*. Dilute this reagent by a factor of 5 (1 + 4).

d. Standard ferrous ammonium sulfate titrant (FAS), approximately 0.10*M*: Dissolve 39.2 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water. Add 20 mL conc H_2SO_4, cool, and dilute to 1000 mL. Standardize solution daily against standard $K_2Cr_2O_7$ digestion solution as follows:

Pipet 5.00 mL digestion solution into a small beaker. Add 10 mL reagent water to substitute for sample. Cool to room temperature. Add 1 to 2 drops diluted ferroin indicator and titrate with FAS titrant.

Molarity of FAS solution

$$= \frac{\text{Volume } 0.01667M\ K_2Cr_2O_7 \text{ solution titrated, mL}}{\text{Volume FAS used in titration, mL}} \times 0.1000$$

e. Sulfamic acid: See Section 5220B.3*f*.

f. Potassium hydrogen phthalate standard: See Section 5220B.3*g*.

4. Procedure

Wash culture tubes and caps with 20% H_2SO_4 before first use to prevent contamination. Refer to Table 5220:I for proper sample and reagent volumes. Make volumetric measurements as accurate as practical; use Class A volumetric ware. The most critical volumes are of the sample and digestion solution. Use a microburet for titrations. Measure H_2SO_4 to ±0.1 mL. The use of hand-held pipettors with non-wetting (polyethylene) pipet tips is practical and adequate. Place sample in culture tube or ampule and add digestion solution. Carefully run sulfuric acid reagent down inside of vessel so an acid layer is formed under the sample-digestion solution layer. Tightly cap tubes or seal ampules, and invert each several times to mix completely. CAUTION: *Wear face shield and protect hands from heat produced when contents of vessels are mixed. Mix*

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thoroughly before applying heat to prevent local heating of vessel bottom and possible explosive reaction.

Place tubes or ampules in block digester preheated to 150°C and reflux for 2 h behind a protective shield. CAUTION: *These sealed vessels may be under pressure from gases generated during digestion. Wear face and hand protection when handling. If sulfuric acid is omitted or reduced in concentration, very high and dangerous pressures will be generated at 150°C.* Cool to room temperature and place vessels in test tube rack. Some mercuric sulfate may precipitate out but this will not affect the analysis. Remove culture tube caps and add small TFE-covered magnetic stirring bar. If ampules are used, transfer contents to a larger container for titrating. Add 0.05 to 0.10 mL (1 to 2 drops) ferroin indicator and stir rapidly on magnetic stirrer while titrating with standardized 0.10*M* FAS. The end point is a sharp color change from blue-green to reddish brown, although the blue-green may reappear within minutes. In the same manner reflux and titrate a blank containing the reagents and a volume of distilled water equal to that of the sample.

5. Calculation

$$\text{COD as mg } O_2/L = \frac{(A-B) \times M \times 8000}{\text{mL sample}}$$

where:

A = mL FAS used for blank,
B = mL FAS used for sample,
M = molarity of FAS, and
8000 = milliequivalent weight of oxygen × 1000 mL/L.

Preferably analyze samples in duplicate because of small sample size. Samples that are inhomogeneous may require multiple determinations for accurate analysis. Results should agree within ±5% of their average unless the condition of the sample dictates otherwise.

6. Precision and Bias

Sixty synthetic samples containing potassium hydrogen phthalate and NaCl were tested by six laboratories. At an average COD of 195 mg O_2/L in the absence of chloride, the standard deviation was ±11 mg O_2/L (coefficient of variation, 5.6%). At an average COD of 208 mg O_2/L and 100 mg Cl^-/L, the standard deviation was ±10 mg O_2/L (coefficient of variation, 4.8%).

5220 D. Closed Reflux, Colorimetric Method

1. General Discussion

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a. Principle: See Section 5220B.1*a*. When a sample is digested, the dichromate ion oxidizes COD material in the sample. This results in the change of chromium from the hexavalent (VI) state to the trivalent (III) state. Both of these chromium species are colored and absorb in the visible region of the spectrum. The dichromate ion ($Cr_2O_7^{2-}$) absorbs strongly in the 400-nm region, where the chromic ion (Cr^{3+}) absorption is much less. The chromic ion absorbs strongly in the 600-nm region, where the dichromate has nearly zero absorption. In 9*M* sulfuric acid solution, the approximate molar extinction coefficients for these chromium species are as follows: Cr^{3+} – 50 L/mole cm at 604 nm; $Cr_2O_7^{2-}$ – 380 L/mole cm at 444 nm; Cr^{3+} – 25 L/mole cm at 426 nm. The Cr^{3+} ion has a minimum in the region of 400 nm. Thus a working absorption maximum is at 420 nm.

For COD values between 100 and 900 mg/L, increase in Cr^{3+} in the 600-nm region is determined. Higher values can be obtained by sample dilution. COD values of 90 mg/L or less can be determined by following the decrease in $Cr_2O_7^{2-}$ at 420 nm. The corresponding generation of Cr^{3+} gives a small absorption increase at 420 nm, but this is compensated for in the calibration procedure.

b. Interferences and limitations: See Section 5220C.1*b*.

For this procedure to be applicable, all visible light-absorbing interferents must be absent or be compensated for. This includes insoluble suspended matter as well as colored components. If either type of interference occurs, the test is not necessarily lost because COD can be determined titrimetrically as in 5220C.

2. Apparatus

a. See Section 5220C.2. Ensure that reaction vessels are of optical quality. Other types of absorption cells with varying path lengths may be used. Use the extinction coefficients of the ions of interest for this approach.

b. Spectrophotometer, for use at 600 nm and/or 420 nm with access opening adapter for ampule or 16-, 20-, or 25-mm tubes. Verify that the instrument operates in the region of 420 nm and 600 nm. Values slightly different from these may be found, depending on the spectral bandpass of the instrument.

3. Reagents

a. Digestion solution, high range: Add to about 500 mL distilled water 10.216 g $K_2Cr_2O_7$, primary standard grade, previously dried at 150°C for 2 h, 167 mL conc H_2SO_4, and 33.3 g $HgSO_4$. Dissolve, cool to room temperature, and dilute to 1000 mL.

b. Digestion solution, low range: Prepare as in 3*a*, but use only 1.022 g potassium dichromate.

c. Sulfuric acid reagent: See Section 5220B.3*b*.

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d. Sulfamic acid: See Section 5220B.3*f*.

e. Potassium hydrogen phthalate standard: See Section 5220B.3*g*.

4. Procedure

a. Treatment of samples: Measure suitable volume of sample and reagents into tube or ampule as indicated in Table 5220:I. Prepare, digest, and cool samples, blank, and one or more standards as directed in Section 5220C.4. *Note the safety precautions.* It is critical that the volume of each component be known and that the total volume be the same for each reaction vessel. If volumetric control is difficult, transfer digested sample, dilute to a known volume, and read. Premixed reagents in digestion tubes are available commercially.

b. Measurement of dichromate reduction: Cool sample to room temperature slowly to avoid precipitate formation. Once samples are cooled, vent, if necessary, to relieve any pressure generated during digestion. Mix contents of reaction vessels to combine condensed water and dislodge insoluble matter. Let suspended matter settle and ensure that optical path is clear. Measure absorption of each sample blank and standard at selected wavelength (420 nm or 600 nm). At 600 nm, use an undigested blank as reference solution. Analyze a digested blank to confirm good analytical reagents and to determine the blank COD; subtract blank COD from sample COD. Alternately, use digested blank as the reference solution once it is established that the blank has a low COD.

At 420 nm, use reagent water as a reference solution. Measure all samples, blanks, and standards against this solution. The absorption measurement of an undigested blank containing dichromate, with reagent water replacing sample, will give initial dichromate absorption. Any digested sample, blank, or standard that has a COD value will give lower absorbance because of the decrease in dichromate ion. Analyze a digested blank with reagent water replacing sample to ensure reagent quality and to determine the reagents' contribution to the decrease in absorbance during a given digestion. The difference between absorbances of a given digested sample and the digested blank is a measure of the sample COD. When standards are run, plot differences of digested blank absorbance and digested standard absorbance versus COD values for each standard.

c. Preparation of calibration curve: Prepare at least five standards from potassium hydrogen phthalate solution with COD equivalents to cover each concentration range. Make up to volume with reagent water; use same reagent volumes, tube, or ampule size, and digestion procedure as for samples. Prepare calibration curve for each new lot of tubes or ampules or when standards prepared in ¶ 4*a* differ by $\geq$5% from calibration curve. Curves should be linear. However, some nonlinearity may occur, depending on instrument used and overall accuracy needed.

5. Calculation

If samples, standards, and blanks are run under same conditions of volume and optical path length, calculate COD as follows:

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$$\text{COD as mg } O_2/L = \frac{\text{mg } O_2 \text{ in final volume} \times 1000}{\text{mL sample}}$$

Preferably analyze samples in duplicate because of small sample size. Samples that are inhomogeneous may require multiple determinations for accurate analysis. These should not differ from their average by more than ±5% for the high-level COD test unless the condition of the sample dictates otherwise. In the low-level procedure, results below 25 mg/L may tend to be qualitative rather than quantitative.

6. Precision and Bias

Forty-eight synthetic samples containing potassium hydrogen phthalate and NaCl were tested by five laboratories. At an average COD of 193 mg O_2/L in the absence of chloride, the standard deviation was ±17 mg O_2/L (coefficient of variation 8.7%). At an average COD of 212 mg O_2/L and 100 mg Cl^-/ L, the standard deviation was ±20 mg O_2/L (coefficient of variation, 9.6%). Additional QA/QC data for both high- and low-level procedures may be found elsewhere.[1]

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5310 TOTAL ORGANIC CARBON (TOC)*#(105)

5310 A. Introduction

1. General Discussion

The organic carbon in water and wastewater is composed of a variety of organic compounds in various oxidation states. Some of these carbon compounds can be oxidized further by biological or chemical processes, and the biochemical oxygen demand (BOD), assimilable

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organic carbon (AOC), and chemical oxygen demand (COD) methods may be used to characterize these fractions. Total organic carbon (TOC) is a more convenient and direct expression of total organic content than either BOD, AOC, or COD, but does not provide the same kind of information. If a repeatable empirical relationship is established between TOC and BOD, AOC, or COD for a specific source water then TOC can be used to estimate the accompanying BOD, AOC, or COD. This relationship must be established independently for each set of matrix conditions, such as various points in a treatment process. Unlike BOD or COD, TOC is independent of the oxidation state of the organic matter and does not measure other organically bound elements, such as nitrogen and hydrogen, and inorganics that can contribute to the oxygen demand measured by BOD and COD. TOC measurement does not replace BOD, AOC, and COD testing.

Measurement of TOC is of vital importance to the operation of water treatment and waste treatment plants. Drinking water TOCs range from less than 100 µg/L to more than 25,000 µg/L. Wastewater may contain very high levels of organic compounds (TOC >100 mg/L). Some of these applications may include waters with substantial ionic impurities as well as organic matter.

In many applications, the presence of organic contaminants may degrade ion-exchange capacity, serve as a nutrient source for undesired biological growth, or be otherwise detrimental to the process for which the water is to be utilized. For drinking waters in particular, organic compounds may react with disinfectants to produce potentially toxic and carcinogenic compounds.

To determine the quantity of organically bound carbon, the organic molecules must be broken down and converted to a single molecular form that can be measured quantitatively. TOC methods utilize high temperature, catalysts, and oxygen, or lower temperatures (<100°C) with ultraviolet irradiation, chemical oxidants, or combinations of these oxidants to convert organic carbon to carbon dioxide (CO_2). The CO_2 may be purged from the sample, dried, and transferred with a carrier gas to a nondispersive infrared analyzer or coulometric titrator. Alternatively, it may be separated from the sample liquid phase by a membrane selective to CO_2 into a high-purity water in which corresponding increase in conductivity is related to the CO_2 passing the membrane.

2. Fractions of Total Carbon

The methods and instruments used in measuring TOC analyze fractions of total carbon (TC) and measure TOC by two or more determinations. These fractions of total carbon are defined as: inorganic carbon—the carbonate, bicarbonate, and dissolved CO_2; total organic carbon (TOC)—all carbon atoms covalently bonded in organic molecules; dissolved organic carbon (DOC)—the fraction of TOC that passes through a 0.45-µm-pore-diam filter; suspended organic carbon—also referred to as particulate organic carbon, the fraction of TOC retained by a 0.45-µm filter; purgeable organic carbon—also referred to as volatile organic carbon, the fraction of TOC removed from an aqueous solution by gas stripping under specified conditions; and nonpurgeable organic carbon—the fraction of TOC not removed by gas stripping.

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In most water samples, the inorganic carbon fraction is many times greater than the TOC fraction. Eliminating or compensating for inorganic carbon interferences requires determinations of both TC and inorganic carbon to measure TOC. Inorganic carbon interference can be eliminated by acidifying samples to pH 2 or less to convert inorganic carbon species to CO_2. Subsequent purging of the sample with a purified gas or vacuum degassing removes the CO_2 by volatilization. Sample purging also removes purgeable organic carbon so that the organic carbon measurement made after eliminating inorganic carbon interferences is actually a nonpurgeable organic carbon determination: determine purgeable organic carbon to measure TOC. In many surface and ground waters the purgeable organic carbon contribution to TOC is negligible. Therefore, in practice, the nonpurgeable organic carbon determination is substituted for TOC.

Alternatively, inorganic carbon interference may be compensated for by separately measuring total carbon (TC) and inorganic carbon. The difference between TC and inorganic carbon is TOC.

The purgeable fraction of TOC is a function of the specific conditions and equipment employed. Sample temperature and salinity, gas-flow rate, type of gas diffuser, purging-vessel dimensions, volume purged, and purging time affect the division of TOC into purgeable and nonpurgeable fractions. When separately measuring purgeable organic carbon and nonpurgeable organic carbon on the same sample, use identical conditions for purging during the purgeable organic carbon measurement as in purging to prepare the nonpurgeable organic carbon portion for analysis. Consider the conditions of purging when comparing purgeable organic carbon or nonpurgeable organic carbon data from different laboratories or different instruments.

3. Selection of Method

The high-temperature combustion method (B) is suitable for samples with higher levels of TOC that would require dilution for the various persulfate methods (Method C or Method D). Generally, it also will determine organic carbon from compounds that are chemically refractory and not determined by Method C or Method D. High-temperature combustion may be desirable for samples containing high levels of suspended organic carbon, which may not be efficiently oxidized by persulfate and/or UV methods. Interlaboratory studies have shown biases on the order of 1 mg/L using older high-temperature instruments. With newer instruments, detection limits as low as 10 μg/L have been reported. Some high-temperature combustion instruments are not designed for levels below 1 mg/L. The high-temperature methods accumulate nonvolatile residues in the analyzer, whereas, in Method C, residuals are drained from the analyzer. Method C generally provides better sensitivity for lower-level (<1 mg/L) samples. Persulfate and/or UV oxidation are useful for TOC as low as 10 μg/L. Because the range of sensitivity of the methods overlaps, other factors may dictate method choice in the range of 1 mg/L to 50 mg/L. A method may be chosen on the basis of desired precision, ease of use, cost, etc. Method D generally is equivalent to Method C, but the equipment for Method D is no longer manufactured.

To qualify a particular instrument for use, demonstrate that the single-user precision and bias given in each method can be reproduced. Also, preferably demonstrate the overall precision by

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conducting in-house studies with more than one operator.

Evaluate the selected method to ensure that data quality objectives are attained. Evaluate method detection limit in a matrix as similar as possible to the unknowns as described in Section 1030. Be aware that instrument blanks are handled in a variety of ways in TOC analyzers and that the true magnitude of the blank may not be readily apparent to the analyst. Some instruments ‘‘zero out’’ much of the blank by adjusting the zero on the detector. Others enter blank values in units such as mv responses rather than absolute concentrations, whereas other instruments accumulate the total blank in the system during a blank run. Carefully observe the variability of low-level measurements and check it any time reagents or instrument operations are changed. The following methods note that when a water blank is run there is a contribution to the observed blank value from the level of carbon in the blank water.

The methods show expected single-operator and multiple-laboratory precision. These equations are based on referenced interlaboratory studies that in some cases were performed on older equipment. The range of testing is important to observe because the error and bias generally will be some significant fraction of the low standard. Consult references to determine type of equipment and conditions of the interlaboratory study. Determine the performance of the instrument being used by analyzing waters with matrices similar to those of unknowns, using the procedures outlined in Section 1040B.

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5310 B. High-Temperature Combustion Method

1. General Discussion

The high-temperature combustion method has been used for a wide variety of samples, but its utility is dependent on particle size reduction because it uses small-orifice syringes.

a. Principle: The sample is homogenized and diluted as necessary and a microportion is injected into a heated reaction chamber packed with an oxidative catalyst such as cobalt oxide, platinum group metals, or barium chromate. The water is vaporized and the organic carbon is oxidized to CO_2 and H_2O. The CO_2 from oxidation of organic and inorganic carbon is transported in the carrier-gas streams and is measured by means of a nondispersive infrared analyzer, or titrated coulometrically.

Because total carbon is measured, inorganic carbon must be removed by acidification and sparging or measured separately and TOC obtained by difference.

Measure inorganic carbon by injecting the sample into a reaction chamber where it is acidified. Under acidic conditions, all inorganic carbon is converted to CO_2, which is transferred to the detector and measured. Under these conditions organic carbon is not oxidized and only inorganic carbon is measured.

Alternatively, convert inorganic carbonates to CO_2 with acid and remove the CO_2 by purging before sample injection. The sample contains only the nonpurgeable organic carbon fraction of total carbon: a purgeable organic carbon determination also is necessary to measure TOC.

b. Interference: Removal of carbonate and bicarbonate by acidification and purging with purified gas results in the loss of volatile organic substances. The volatiles also can be lost during sample blending, particularly if the temperature is allowed to rise. Another important loss can occur if large carbon-containing particles fail to enter the needle used for injection. Filtration, although necessary to eliminate particulate organic matter when only DOC is to be determined, can result in loss or gain of DOC, depending on the physical properties of the

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carbon-containing compounds and the adsorption or desorption of carbonaceous material on the filter. Check filters for their contribution to DOC by analyzing a filtered blank. Note that any contact with organic material may contaminate a sample. Avoid contaminated glassware, plastic containers, and rubber tubing. Analyze sample treatment, system, and reagent blanks.

Combustion temperatures above 950°C are required to decompose some carbonates. Systems that use lower temperatures must destroy carbonates by acidification. Elemental carbon may not be oxidized at lower temperatures but generally it is not present in water samples nor is it formed during combustion of dilute samples. The advantage of using lower temperatures (680°C) is that fusion of dissolved salts is minimized, resulting in lower blank values. Gases evolved from combustion, such as water, halide compounds, and nitrogen oxides, may interfere with the detection system. Consult manufacturers' recommendations regarding proper selection of scrubber materials and check for any matrix interferences.

The major limitation to high-temperature techniques is the magnitude and variability of the blank. Instrument manufacturers have developed new catalysts and procedures that yield lower blanks, resulting in lower detection levels.

c. Minimum detectable concentration: 1 mg C/L or less, depending on the instrument used. This can be achieved with most high-temperature combustion analyzers although instrument performance varies. The minimum detectable concentration may be reduced by concentrating the sample, or by increasing the portion taken for analysis.

d. Sampling and storage: If possible, rinse bottles with sample before filling and carry field blanks through sampling procedure to check for any contamination that may occur. Collect and store samples in glass bottles protected from sunlight and seal with TFE-backed septa. Before use, wash bottles with acid, seal with aluminum foil, and bake at 400°C for at least 1 h. Wash uncleaned TFE septa with detergent, rinse repeatedly with organic-free water, wrap in aluminum foil, and bake at 100°C for 1 h. Check performance of new or cleaned septa by running appropriate blanks. Preferably use thick silicone rubber-backed TFE septa with open ring caps to produce a positive seal. Less rigorous cleaning may be acceptable if the concentration range is relatively high. Check bottle blanks with each set of sample bottles to determine effectiveness or necessity of cleaning. Preserve samples that cannot be examined immediately by holding at 4°C with minimal exposure to light and atmosphere. Acidification with phosphoric or sulfuric acid to a pH $\leq$2 at the time of collection is especially desirable for unstable samples, and may be used on all samples: acid preservation, however, invalidates any inorganic carbon determination on the samples.

2. Apparatus

a. Total organic carbon analyzer, using combustion techniques.

b. Sampling, injection, and sample preparation accessories, as prescribed by instrument manufacturer.

c. Sample blender or homogenizer.

d. Magnetic stirrer and TFE-coated stirring bars.

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e. Filtering apparatus and 0.45-µm-pore-diam filters. Preferably use HPLC syringe filters with no detectable TOC blank. Glass fiber or silver membrane filters also can be used. Rinse filters before use and monitor filter blanks.

3. Reagents

a. Reagent water: Prepare reagents, blanks, and standard solutions from reagent water with a TOC value less than 2 × the MDL (see Section 1030 and Section 1080).

b. Acid: Phosphoric acid, H_3PO_4. Alternatively use sulfuric acid, H_2SO_4.

c. Organic carbon stock solution: Dissolve 2.1254 g anhydrous primary-standard-grade potassium biphthalate, $C_8H_5KO_4$, in carbon-free water and dilute to 1000 mL; 1.00 mL = 1.00 mg carbon. Prepare laboratory control standards using any other appropriate organic-carbon-containing compound of adequate purity, stability, and water solubility. Preserve by acidifying with H_3PO_4 or H_2SO_4 to pH ≤2, and store at 4°C.

d. Inorganic carbon stock solution: Dissolve 4.4122 g anhydrous sodium carbonate, Na_2CO_3, in water, add 3.497 g anhydrous sodium bicarbonate, $NaHCO_3$, and dilute to 1000 mL; 1.00 mL = 1.00 mg carbon. Alternatively, use any other inorganic carbonate compound of adequate purity, stability, and water solubility. Keep tightly stoppered. Do not acidify.

e. Carrier gas: Purified oxygen or air, CO_2-free and containing less than 1 ppm hydrocarbon (as methane).

f. Purging gas: Any gas free of CO_2 and hydrocarbons.

4. Procedure

a. Instrument operation: Follow manufacturer's instructions for analyzer assembly, testing, calibration, and operation. Adjust to optimum combustion temperature before using instrument; monitor temperature to insure stability.

b. Sample treatment: If a sample contains gross solids or insoluble matter, homogenize until satisfactory replication is obtained. Analyze a homogenizing blank consisting of reagent water carried through the homogenizing treatment.

If inorganic carbon must be removed before analysis, transfer a representative portion (10 to 15 mL) to a 30-mL beaker, add acid to reduce pH to 2 or less, and purge with gas for 10 min. Inorganic carbon also may be removed by stirring the acidified sample in a beaker while directing a stream of purified gas into the beaker. Because volatile organic carbon will be lost during purging of the acidified solution, report organic carbon as total nonpurgeable organic carbon. Check efficiency of inorganic carbon removal for each sample matrix by splitting a sample into two portions and adding to one portion an inorganic carbon level similar to that of the sample. The TOC values should agree; if they do not, adjust sample container, sample volume, pH, purge gas flow rate, and purge time to obtain complete removal of inorganic carbon.

If the available instrument provides for a separate determination of inorganic carbon

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(carbonate, bicarbonate, free CO_2) and total carbon, omit decarbonation and determine TOC by difference between TC and inorganic carbon.

If dissolved organic carbon is to be determined, filter sample through 0.45-µm-pore-diam filter; analyze a filtering blank.

c. Sample injection: Withdraw a portion of prepared sample using a syringe fitted with a blunt-tipped needle. Select sample volume according to manufacturer's direction. Stir samples containing particulates with a magnetic stirrer. Select needle size consistent with sample particulate size. Other sample injection techniques, such as sample loops, may be used. Inject samples and standards into analyzer according to manufacturer's directions and record response. Repeat injection until consecutive measurements are obtained that are reproducible to within ± 10%.

d. Preparation of standard curve: Prepare standard organic and inorganic carbon series by diluting stock solutions to cover the expected range in samples within the linear range of the instrument. Dilute samples higher than the linear range of the instrument in reagent water. Inject and record peak height or area of these standards and a dilution water blank. Plot carbon concentration in milligrams per liter against corrected peak height or area on rectangular coordinate paper. This is unnecessary for instruments provided with a digital readout of concentration.

With most TOC analyzers, it is not possible to determine separate blanks for reagent water, reagents, and the entire system. In addition, some TOC analyzers produce a variable and erratic blank that cannot be corrected reliably. In many laboratories, reagent water is the major contributor to the blank value. Correcting only the instrument response of standards (which contain reagent water + reagents + system blank) creates a positive error, while also correcting samples (which contain only reagents and system blank contributions) for the reagent water blank creates a negative error. Minimize errors by using reagent water and reagents low in carbon.

Inject samples and procedural blanks (consisting of reagent water taken through any pre-analysis steps—values are typically higher than those for reagent water) and determine sample organic carbon concentrations directly from the readout or measurements by comparing corrected instrument response to the calibration curve. Instruments with coulometric detectors do not require calibration curves. Regularly analyze laboratory control samples to confirm performance of the instrument (see Quality Control, below). These detectors accumulate the system blank; therefore, monitor system blank regularly.

5. Calculations

Calculate corrected instrument response of standards and samples by subtracting the reagent-water blank instrument response from that of the standard and sample. Prepare a standard curve of corrected instrument response vs. TOC concentration. Subtract procedural blank from each sample instrument response and compare to standard curve to determine carbon content. Apply appropriate dilution factor when necessary. Subtract inorganic carbon from total

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carbon when TOC is determined by difference.

NOTE: The reagent water blank may include an instrument contribution not dependent on reagent-water carbon, and a true response due to reagent-water carbon. When reagent-water carbon is a significant fraction of reagent-water blank, a negative error no larger than reagent-water blank is introduced in the sample values. If TOC analyzer design permits isolation of each of the contributions to the total blank, apply appropriate blank corrections to instrument response of standards (reagent blank, water blank, system blank) and sample (reagent blank and system blank).

6. Quality Control

Determine instrument detection limit according to Section 1030.

After every tenth analysis, analyze a blank and a laboratory control sample prepared from a source of material other than the calibration standards, at a level similar to the analytical samples. Preferably prepare the laboratory control sample in a matrix similar to that of the samples. Alternatively, periodically make known additions to samples to ensure recovery from unknown matrices.

7. Precision

The difficulty of sampling particulate matter on unfiltered samples limits the precision of the method to approximately 5 to 10%.

Interlaboratory studies of high-temperature combustion methods have been conducted in the range above 2 mg/L.[1] The resulting equation for single-operator precision on matrix water is:

$$S_o = 0.027x + 0.29$$

Overall precision is:

$$S_t = 0.044x + 1.49$$

where:

S_o = single-operator precision,
S_t = overall precision, and
x = TOC concentration, mg/L.

8. Reference

1. AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1994. Standard Test Method for Total and Organic Carbon in Water by High Temperature Oxidation and by Coulometric Detection. D4129–88. Annual Book of ASTM Standards. American Soc. Testing & Materials, Philadelphia, Pa.

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5310 C. Persulfate-Ultraviolet or Heated-Persulfate Oxidation Method

1. General Discussion

Many instruments utilizing persulfate oxidation of organic carbon are available. They depend either on heat or ultraviolet irradiation activation of the reagents. These oxidation methods provide rapid and precise measurement of trace levels of organic carbon in water.

a. Principle: Organic carbon is oxidized to carbon dioxide, CO_2, by persulfate in the presence of heat or ultraviolet light. The CO_2 produced may be purged from the sample, dried, and transferred with a carrier gas to a nondispersive infrared (NDIR) analyzer, or be coulometrically titrated, or be separated from the liquid stream by a membrane that allows the specific passage of CO_2 to high-purity water where a change in conductivity is measured and related to the CO_2 passing the membrane.

Some instruments utilize an ultraviolet lamp submerged in a continuously gas-purged reactor that is filled with a constant-feed persulfate solution. The samples are introduced serially into the reactor by an autosampler or they are injected manually. The CO_2 produced is sparged continuously from the solution and is carried in the gas stream to an infrared analyzer that is specifically tuned to the absorptive wavelength of CO_2. The instrument's microprocessor calculates the area of the peaks produced by the analyzer, compares them to the peak area of the calibration standard stored in its memory, and prints out a calculated organic carbon value in

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milligrams per liter.

Other UV-persulfate instruments use continuous-flow injection of the sample into the instrument. Removal of inorganic carbon by vacuum degassing is provided optionally. The sample is acidified and persulfate added. Sample flow is split; one channel passes to a delay coil while the other passes through the UV reactor. The CO_2 from each stream is separated from the sample stream by membranes selectively permeable to CO_2 that allow the CO_2 to pass into high-purity water where change in conductivity is measured. CO_2 from the non-UV-irradiated stream represents inorganic carbon. CO_2 from the irradiated stream represents TC. The instrument automatically converts the detector signals to unit of concentration (mg/L or µg/L). The TOC is calculated as the difference between the TC and inorganic carbon channels.

Heated-persulfate instruments utilize a digestion vessel heated to 95 to 100°C. Samples are added by direct injection, loop injection, line injection, or autosampler. After inorganic carbon is removed by acidification and sparging, a measured amount of persulfate solution is added to the sample. After an oxidation period, the resulting CO_2 is sparged from the solution and carried to an infrared analyzer specifically tuned to the absorptive wavelength of CO_2. The instrument's microprocessor converts the detector signal to organic carbon concentrations in mg/L based on stored calibration data.

b. Interferences: See Section 5310B.1. Insufficient acidification will result in incomplete release of CO_2.

The intensity of the ultraviolet light reaching the sample matrix may be reduced by highly turbid samples or with aging of the ultraviolet source, resulting in sluggish or incomplete oxidation. Large organic particles or very large or complex organic molecules such as tannins, lignins, and humic acid may be oxidized slowly because persulfate oxidation is rate-limited. However, oxidation of many large biological molecules such as proteins and monoclonal antibodies proceeds rapidly. Because the efficiency of conversion of organic carbon to CO_2 may be affected by many factors, check efficiency of oxidation with selected model compounds representative of the compounds of interest in a matrix representative of the sample.

Some instruments give low results for certain difficult-to-oxidize compounds under certain conditions. The following compounds are difficult to oxidize, are sufficiently soluble in water, and can be mixed and measured accurately at trace levels: urea, nicotinic acid, pyridine, *n*-butanol, acetic acid, leucine, acetonitrile, octoxynol-9, tartaric acid, 1,10-phenanthroline, 1-glutonic acid, 2-propanol, and sodium dodecylbenzenesulfonate. Use these compounds as matrix additions to evaluate oxidation efficiency.

Persulfate oxidation of organic molecules is slowed in samples containing significant concentrations of chloride by the preferential oxidation of chloride; at concentrations above 0.05% chloride, oxidation of organic matter may be inhibited. To remove this interference add mercuric nitrate*#(106) to the persulfate solution in UV-persulfate instruments, or extend reaction time and/or increase amount of persulfate solution in heated-persulfate instruments.

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With any organic carbon measurement, contamination during sample handling and treatment is a likely source of interference. This is especially true of trace analysis. Take extreme care in sampling, handling, and analysis of samples below 1 mg TOC/L.

c. Minimum detectable concentration: Concentration of 0.01 mg TOC/L can be measured by some instruments if scrupulous attention is given to minimizing sample contamination and method background. See Section 1030 for procedures to evaluate the MDL for a specific instrument. Use the high-temperature combustion method (B) for high concentrations of TOC or dilute the sample, ensuring that the dilution process does not contaminate the sample.

d. Sampling and storage: See Section 5310B.1*d*.

2. Apparatus

a. Total organic carbon analyzer utilizing persulfate oxidation principle.

b. Sampling and injection accessories, as specified by the instrument manufacturer.

3. Reagents

a. Reagents listed in Section 5310B.3.

b. Persulfate solution: Different instrument manufacturers recommend different forms and concentrations of peroxydisulfate. Typical preparations are as follows:

1) *Sodium peroxydisulfate*, 10%: Dissolve 100 g reagent in water; bring volume to 1 L.

2) *Ammonium peroxydisulfate*, 15%: Dissolve 150 g reagent in water; bring volume to 1 L.

3) *Potassium peroxydisulfate*, 2%: Dissolve 20 g reagent in water; bring volume to 1 L.

Check blank values from reagents and, if values are high, purify reagent or use a higher-purity source.

4. Procedure

a. Instrument operation: Follow manufacturer's instructions for assembly, testing, calibration, and operation.

b. Sample preparation: If a sample contains gross particulates or insoluble matter, homogenize until a representative portion can be withdrawn through the syringe needle, autosampler tubing, or sample inlet system of continuous on-line monitor.

If dissolved organic carbon is to be determined, filter sample and a reagent water blank through 0.45-µm filter. HPLC syringe filters have been found to pass water without contamination. Glass fiber or silver membrane filters also can be used. Check filter blanks regularly.

To determine nonpurgeable organic carbon, transfer 15 to 30 mL sample to a flask or test tube and acidify to a pH of 2. Purge according to manufacturer's recommendations. In some instruments this is performed internally. Check efficiency of inorganic carbon removal for each sample matrix by splitting a sample into two portions; to one of the portions, add inorganic carbon to a level similar to that of the sample. The TOC values should agree. If the values do not

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agree, adjust conditions such as sample container, sample volume, pH, purge-gas flow rate, and purge time to obtain complete removal of inorganic carbon.

c. Sample injection: See Section 5310B.4*c*.

d. Standard curve preparation: Prepare an organic carbon standard series over the range of organic carbon concentrations in the samples. Run standards and blanks and record analyzer's response. Determine instrument response for each standard and blank. Unless carbon dioxide is trapped and desorbed, producing consistent peak heights, determinations based on peak height may be inadequate because of differences in the rate of oxidation of standards and samples. Correct instrument response of standards by subtracting reagent water blank and plot organic carbon concentration in milligrams per liter against corrected instrument response. For instruments providing a digital computation of concentration, this is not necessary. Be sure that the instrument's algorithm includes blank correction and linearity of response. Analyze standards having concentrations above and below those determined in the samples, preferably prepared in a similar matrix, to confirm proper instrument operation.

5. Calculation

See Section 5310B.5, or use instrument manufacturer's procedure.

6. Quality Control

See Section 5310B.6.

7. Precision and Bias

Interlaboratory studies of persulfate and/or UV with NDIR detection methods have been conducted in the range of 0.1 mg/L to 4 000 mg/L of carbon.[1] The resulting equation for organic carbon, single-operator precision is:

$$S_o = 0.04x + 0.1$$

Overall precision is expressed as:

$$S_t = 0.08x + 0.1$$

where:

S_o = single-operator precision,
S_t = overall precision, and
x = TOC concentration, mg/L.

An interlaboratory study was conducted for the membrane conductivity method,†#(107) covering samples with 1 to 25 mg/L organic carbon concentrations. The resulting equation for single-operator precision is:

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$$S_o = 0.012x - 0.022$$

Overall precision is expressed as:

$$S_t = 0.027x + 0.09$$

where terms are defined as above.

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1. AMERICAN SOCIETY FOR TESTING AND MATERIALS. 1994. Standard Test Method for Total Carbon in Water by Ultraviolet, or Persulfate Oxidation, or Both, and Infrared Detection. D4839-88. Annual Book of ASTM Standards. American Soc. Testing & Materials, Philadelphia, Pa.

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5310 D. Wet-Oxidation Method

1. General Discussion

The wet-oxidation method is suitable for the analyses of water, water-suspended sediment mixtures, seawaters, brines, and wastewaters containing at least 0.1 mg nonpurgeable organic carbon/L. The method is not suitable for the determination of volatile organic constituents.

a. Principle: The sample is acidified, purged to remove inorganic carbon, and oxidized with persulfate in an autoclave at temperatures from 116 to 130°C. The resultant carbon dioxide (CO_2) is measured by nondispersive infrared spectrometry.

b. Interferences: See Section 5310B.1 and Section 5310C.1.

c. Minimum detectable concentrations: High concentrations of reducing agents may interfere. Concentration of 0.10 mg TOC/L can be measured if scrupulous attention is given to minimizing sample contamination and method background. Use the high-temperature combustion method (B) for high concentrations of TOC.

d. Sampling and storage: See Section 5310B.1*d*.

2. Apparatus

a. Ampules, precombusted, 10-mL, glass.

b. Ampule purging and sealing unit.

c. Autoclave.

d. Carbon analyzer.

e. Homogenizer.

3. Reagents

In addition to the reagents specified in Section 5310B.3*a*, Section 5310B.3*c*, Section 5310B.3*e*, and Section 5310B.3*f*, the following reagents are required:

a. Phosphoric acid solution, H_3PO_4, 1.2*N*: Add 83 mL H_3PO_4 (85%) to water and dilute to 1 L with water. Store in a tightly stoppered glass bottle.

b. Potassium persulfate, reagent-grade, granular. Avoid using finely divided forms.

4. Procedure

Follow manufacturer's instructions for instrument assembly, testing calibration, and

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operation. Add 0.5 mL 1.2N H_3PO_4, solution to precombusted ampules.

To analyze for dissolved organic carbon, follow the filtration procedure in Method B. Homogenize sample to produce a uniform suspension. Rinse homogenizer with reagent water after each use. Pipet water sample (10.0 mL maximum) into an ampule. Adjust smaller volumes to 10 mL with reagent water. Prepare one reagent blank (10 mL reagent water plus acid and oxidant) for every 15 to 20 water samples. Prepare standards covering the range of 0.1 to 40 mg C/L by diluting the carbon standard solution. Immediately place filled ampules on purging and seating unit and purge them at rate of 60 mL/min for 6 min with purified oxygen. Add 0.2 g potassium persulfate using a dipper calibrated to deliver 0.2 g to the ampule. Seal samples according to the manufacturer's instructions. Place sealed samples, blanks, and a set of standards in ampule racks in an autoclave and digest 4 h at temperature between 116 and 130°C.

Set sensitivity range of carbon analyzer by adjusting the zero and span controls in accordance with the manufacturer's instructions. Break combusted ampules in the cutter assembly of the carbon analyzer, sweep CO_2 into the infrared cell with nitrogen gas, and record area of each CO_2 peak. CAUTION: *Because combusted ampules are under positive pressure, handle with care to prevent explosion.*

5. Calculations

Prepare an analytical standard curve by plotting peak area of each standard versus concentration (mg/L) of organic carbon standards. The relationship between peak area and carbon concentration is curvilinear. Define operating curves each day samples are analyzed.

Report nonpurgeable organic carbon concentration as follows: 0.1 mg/L to 0.9 mg/L, one significant figure; 1.0 mg/L and above, two significant figures.

6. Quality Control

See Section 5310B.6.

7. Precision and Bias

Multiple determinations of four different concentrations of aqueous potassium acid phthalate samples at 2.00, 5.00, 10.0, and 40.0 mg C/L resulted in mean values of 2.2, 5.3, 9.9, and 38 mg/L and standard deviations of 0.13, 0.15, 0.11, and 1.4, respectively.

Precision also may be expressed in terms of percent relative standard deviation as follows:

Number of Replicates	Mean *mg/L*	Relative Standard Deviation %
9	2.2	5.9
10	5.3	2.8
10	9.9	1.1

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Number of Replicates	Mean mg/L	Relative Standard Deviation %
10	38.0	3.7

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5320 DISSOLVED ORGANIC HALOGEN*#(108)

5320 A. Introduction

Dissolved organic halogen (DOX) is a measurement used to estimate the total quantity of dissolved halogenated organic material in a water sample. This is similar to literature references to ‘‘total organic halogen’’ (TOX), ‘‘adsorbable organic halogen’’ (AOX), and carbon-adsorbable organic halogen (CAOX). The presence of halogenated organic molecules is indicative of disinfection by-products and other synthetic chemical contamination. Halogenated compounds that contribute to a DOX result include, but are not limited to: the trihalomethanes (THMs); organic solvents such as trichloroethene, tetrachloroethene, and other halogenated alkanes and alkenes; chlorinated and brominated pesticides and herbicides; polychlorinated biphenyls (PCBs); chlorinated aromatics such as hexachlorobenzene and 2,4-dichlorophenol; and high-molecular-weight, partially chlorinated aquatic humic substances. Compound-specific methods such as gas chromatography typically are more sensitive than DOX measurements.

The adsorption-pyrolysis-titrimetric method for DOX measures only the total molar amount of dissolved organically bound halogen retained on the activated carbon adsorbent; it yields no information about the structure or nature of the organic compounds to which the halogens are bound or about the individual halogens present. It is sensitive to organic chloride, bromide, and iodide, but does not detect fluorinated organics.

DOX measurement is an inexpensive and useful method for screening large numbers of samples before specific (and often more complex) analyses; for extensive field surveying for pollution by certain classes of synthetic organic compounds in natural waters; for mapping the

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extent of organohalide contamination in groundwater; for monitoring the breakthrough of some synthetic organic compounds in water treatment processes; and for estimating the level of formation of chlorinated organic by-products after disinfection. When used as a screening tool, a large positive (i.e., above background measurements) DOX test result indicates the need for identifying and quantifying specific substances. In saline or brackish waters the high inorganic halogen concentrations interfere. The possibility of overestimating DOX concentration because of inorganic halide interference always should be considered when interpreting results.

5320 B. Adsorption-Pyrolysis-Titrimetric Method

1. General Discussion

a. Principle: The method consists of four processes. First, dissolved organic material is separated from inorganic halides and concentrated from aqueous solution by adsorption onto activated carbon. Second, inorganic halides present on the activated carbon are removed by competitive displacement by nitrate ions. Third, the activated carbon with adsorbed organic material is introduced into a furnace that pyrolyzes organic carbon to carbon dioxide (CO_2) and the bound halogens to hydrogen halide (HX). Fourth, the HX is transported in a carrier gas stream to a microcoulometric titration cell where the amount of halide is quantified by measuring the current produced by silver-ion precipitation of the halides. The microcoulometric detector operates by maintaining a constant silver-ion concentration in a titration cell. An electric potential is applied to a solid silver electrode to produce silver ions in the cell solution. As hydrogen halide from the pyrolysis furnace enters the cell in the carrier gas, it is partitioned into the acetic acid solution where it precipitates as silver halide. The current that is produced is integrated over the period of the pyrolysis. The integrated area under the curve is proportional to the number of moles of halogen recovered. The mass concentration of organic halides is reported as an equivalent concentration of organically bound chloride in micrograms per liter. Because this DOX procedure relies on activated carbon to adsorb organic halides, it also has been referred to as carbon-adsorbable organic halogen (CAOX). Because of the poor adsorption efficiency of some organic compounds containing halogen and the desorption of some halogen-containing compounds during the removal of adsorbed inorganic halogen, this method does not measure total organic halogen.

When a sample is purged with inert gas before activated carbon adsorption, analysis of that sample determines the nonpurgeable dissolved organic halogen (NPDOX) fraction of DOX. The purgeable organic halogen concentration (POX) may be estimated by subtracting the NPDOX value from the DOX value. Alternatively, the POX fraction may be determined directly by purging the sample with carrier gas and introducing that gas stream and the volatilized organics directly into the pyrolysis furnace. Thus, depending on approach, the analysis of POX, DOX, and NPDOX may be determined directly or by difference. Finally, because the POX often is dominated by the THMs, they may be determined as directed in Section 6200 and used to estimate POX. However, this approach is not included here as a standardized procedure.

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b. Interferences: The method is applicable only to aqueous samples free of visible particulate matter. Different instruments vary in tolerance of small amounts of suspended matter. Inorganic substances such as chloride, chlorite, chlorate, bromate, bromide, and iodide will adsorb on activated carbon to an extent dependent on their original concentration in the aqueous solution and the volume of sample adsorbed.[1] Positive interference will result if inorganic halides are not removed. Treating the activated carbon with a concentrated aqueous solution of nitrate ion causes competitive desorption from the activated carbon of inorganic halide species and washes inorganic halides from other surfaces. However, if the inorganic halide concentration is greater than 10 000 times[2] the concentration of organic halides, the DOX results may be affected significantly. In general, this procedure may not be applicable to samples with inorganic halide concentrations above 500 mg Cl^-/L, based on activated carbon quality testing results. Therefore, consider both the results of mineral analysis for inorganic halides and the results of the activated carbon quality test (see ¶ 5, below) when interpreting results.

Halogenated organic compounds that are weakly adsorbed on activated carbon are recovered only partially. These include certain alcohols and acids (e.g., chloroethanol), and such compounds as chloroacetic acid, that can be removed from activated carbon by the nitrate ion wash. However, for most halogenated organic molecules, recovery is very good; the activated carbon adsorbable organic halide (CAOX) therefore is a good estimate of true DOX.

Failure to acidify samples with nitric acid or sulfuric acid may result in reduced adsorption efficiency for some halogenated organic compounds and may intensify the inorganic halide interference. However, acidification may result in precipitation loss of humic acids and any DOX associated with that fraction. Further, if the water contains residual chlorine, reduce it before adsorption to eliminate positive interference resulting from continued chlorination reactions with organic compounds adsorbed on the activated carbon surface or with the activated carbon surface itself. The sulfite dechlorinating agent may cause decomposition of a small fraction of the DOX if nitric acid is used; this decomposition is avoided if sulfuric acid is used. Do not add acid in great excess.

Highly volatile components of the POX fraction may be lost during sampling, shipment, sample storage, sample handling, and sample preparation, or during sample adsorption. A laboratory quality-control program to ensure sample integrity from time of sampling until analysis is vital. During sample filtration for the analysis of samples containing undissolved solids, major losses of POX can be expected. Syringe-type filtration systems can minimize losses. Analyze for POX before sample filtration and analyze for NPDOX after filtration; the sum of POX and NPDOX is the total DOX. In preparing samples for DOX analysis, process a blank and a standard solution to determine effect of this procedure on DOX measurement. If an insignificant loss of POX occurs during the removal of particulate matter by filtration, DOX may be measured directly.

Granular activated carbon used to concentrate organic material from the sample can be a major source of variability in the analysis and has a dramatic effect on the minimum detectable concentration. Ideally, activated carbon should have a low halide content, readily release

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adsorbed inorganic halides on nitrate washing, be homogeneous, and readily adsorb *all* organic halide compounds even in the presence of large excesses of other organic material. An essential element of quality control for DOX requires testing and monitoring of activated carbon (see ¶ 5 below). Nonhomogeneous activated carbon or activated carbon with a high background value affects the method reliability at low concentrations of DOX. A high and/or variable blank value raises the minimum detectable concentration. Random positive bias, in part because of the ease of activated carbon contamination during use, may necessitate analyzing duplicates of each sample. Because activated carbon from different sources may vary widely in the ease of releasing inorganic halides, test for this quality before using activated carbon. Proper quantification also may be affected by the adsorptive capacity of the activated carbon. If excessive organic loading occurs, some DOX may break through and not be recovered. For this reason, make serial adsorptions of each sample portion and individual analyses.

c. Sampling and storage: Collect and store samples in amber glass bottles with TFE-lined caps. If amber bottles are not available, store samples in the dark. To prepare sample bottles, acid wash, rinse with deionized water, seal with aluminum foil, and bake at 400°C for at least 1 h. If bottle blanks without baking show no detectable DOX, baking may be omitted. Wash septa with detergent, rinse repeatedly in organic-free, deionized water, wrap in aluminum foil, and bake for 1 h at 100°C. Preferably use thick silicone rubber-backed TFE septa and open ring caps to produce a positive seal that prevents loss of POX and contamination. Store sealed sample bottles in a clean environment until use. Completely fill sample bottles but take care not to volatilize any organic halogen compounds. Preserve samples that cannot be analyzed promptly by acidifying with concentrated nitric acid or sulfuric acid to pH 2. Refrigerate samples at 4°C with minimal exposure to light. Reduce any residual chlorine by adding sodium sulfite crystals (minimum: 5 mg/L). Add 4 drops conc H_2SO_4 plus sodium sulfite crystals to bottles shipped to the field. NOTE: Some organic chloramines are not completely dechlorinated by sodium sulfite, particularly at pH > 7. This may affect reported concentrations.[1] Analyze all samples within 14 d.

d. Minimum detectable concentration: For nonsaline waters free of particulate matter, 5 to 10 µg organic Cl^-/L is considered a typical range for detection limits. The minimum detectable concentration may be influenced by the analytical repeatability, equipment used, activated carbon quality, and the analyst. Determine the detection limit for each procedure, instrument, and analyst.

2. Apparatus

a. Adsorption assembly, including gas-tight sample reservoir, activated carbon-packed adsorption columns, column housings, and nitrate solution reservoir. In particular, note the following:

1) *Noncombustible insulating material (microcolumn method only):* Form into plugs to hold activated carbon in columns. NOTE: *Do not touch with fingers.*

2) *Activated carbon columns (microcolumn method only):* Pack 40 ± 5 mg activated carbon

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(¶ 3*k*) into dry glass tubing approximately 2 to 3 mm ID × 6 mm OD × 40 to 50 mm long. NOTE: *Protect these columns from all sources of halogenated organic vapors*. Clean glass tubes before use with a small-diameter pipe cleaner to remove residual carbon, then soak in chromate cleaning solution for 15 min and dry at 400°C. Rinse between steps with deionized water. NOTE: Use prepacked columns with caution, because of occasional reported contamination.

b. Analyzer assembly, including carrier gas source, boat sampler, and pyrolysis furnace, that can oxidatively pyrolyze halogenated organics at a temperature of 800 to 900°C to produce hydrogen halides and deliver them to the titration cell with a minimum overall efficiency of 90% for 2,4,6-trichlorophenol; including a microcoulometric titration system with integrator, digital display, and data system or chart recorder connection; including (optional) purging apparatus.

c. Chart recorder or microprocessor, controlled data system.

d. Batch adsorption equipment: Use instrument manufacturer's purge vessel or similar purging flask, erlenmeyer flasks (100 to 250 mL), and high-speed stirrers.

e. Filtering apparatus and filters: Use 0.45-µm-pore diam filters, preferably HPLC syringe filters or similar, with no detectable DOX blank. Rinsed glass-fiber filters are satisfactory for sample filtration. Preferably use membrane filters for separating activated carbon from aqueous phase.

3. Reagents and Materials

Use chemicals of ACS reagent grade or other grades if it can be demonstrated that the reagent is of sufficiently high purity to permit its use without lessening accuracy of the determination.

a. Carbon dioxide, argon, or nitrogen, as recommended by the equipment manufacturer, purity 99.99%.

b. Oxygen, purity 99.99%.

c. Aqueous acetic acid, 70 to 85%, as recommended by the equipment manufacturer.

d. Sodium chloride standard, NaCl: Dissolve 0.1648 g NaCl and dilute to 100 mL with reagent water; 1 µL = 1 µg Cl^-.

e. Ammonium chloride standard, NH_4Cl: Dissolve 0.1509 g NH_4Cl and dilute to 100 mL with reagent water; 1 µL = 1 µg Cl^-.

f. Trichlorophenol stock solution: Dissolve 1.856 g trichlorophenol and dilute to 100 mL with methanol; 1 µL = 10 µg Cl^-.

g. Trichlorophenol standard solution: Make a 1:20 dilution of the trichlorophenol stock solution with methanol; 1 µL = 0.5 µg Cl^-.

h. Trichloroacetic acid stock solution: Dilute 199.44 mg trichloroacetic acid in 1000 mL reagent water; 1 mL = 130 µg Cl^-.

i. Trichloroacetic acid standard solution: Dilute 2.0 mL trichloroacetic acid stock solution

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into 1000 mL with reagent water; 1 mL = 0.260 µg Cl^-.

j. Chloroform standard solution, $CHCl_3$: Dilute 100 mg $CHCl_3$ to 100 mL with methanol; 1 µL = 1 µg $CHCl_3$.

k. Blank standard: Use reagent water. Reagent water preferably is carbon-filtered, deionized water that has been heated and purged.

l. Nitrate wash solution, 0.08*M*: Dilute 8.2 g KNO_3 to 1000 mL with reagent water. Adjust to pH 2 with HNO_3. 1 L =5000 mg NO_3^-.

m. Activated carbon, 100 to 200 mesh: Ideally use activated carbon having a very low apparent halide background that readily releases adsorbed inorganic halides on nitrate washing, and reliably adsorbs organic halides in the presence of a large excess of other organic compounds.*#(109) See ¶ 5 below for preparation and evaluation of activated carbon. CAUTION: *Protect activated carbon from contact with halogenated organic vapors.*

n. Sodium sulfite, Na_2SO_3, crystals.

o. Nitric acid, HNO_3, conc, or *sulfuric acid*, H_2SO_4, conc.

4. Procedure

Use either the microcolumn (¶ 4*a*) or batch adsorption (¶ 4*b*) method to determine DOX (as CAOX). If present, determine POX separately (¶ 4*c*). The microcolumn method utilizes small glass columns packed with activated carbon through which the sample is passed under positive pressure to adsorb the organic halogen compounds. The batch adsorption method uses a small quantity of activated carbon that is added to the sample. After stirring, activated carbon is removed by filtration, washed with nitrate, and analyzed. The batch adsorption procedure typically is run on samples that have had POX analyzed directly [¶ 4*c*], yielding NPDOX directly as well.

a. Microcolumn procedure:

1) Apparatus setup—Adjust equipment in accordance with the manufacturer's instructions. Make several injections of NaCl solution directly into the titration cell [¶ 5*c*1)] as a microcoulometer/ titration cell check at the start of each day.

2) Sample pretreatment for DOX analysis—If the sample has not been acidified during collection, adjust pH to 2 with HNO_3 or H_2SO_4. If the samples contain undissolved solids, filter through a glass-fiber filter (other means of removing particulate matter may be used, if it can be demonstrated that they do not cause significant interferences). Also filter a blank and standard. Analyze these to determine the contribution of filtration to the organic halogen measurement. Vacuum filtration will cause some loss of volatile organic halogen. Analyze for POX (¶ 4*c*) before filtration and NPDOX after filtration, unless it is shown that POX losses during filtration are insignificant for a specific water type.

3) Sample adsorption—Transfer a representative portion of sample to the cleaned sample

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reservoir with two activated carbon adsorption columns in series attached by the column housings to the reservoir outlet. Seal the reservoir. Adjust to produce a flow rate of about 3mL/min. When the desired volume has been processed, stop the flow, detach the activated carbon housings and columns, and rinse the sample reservoir twice with reagent-grade water. Vary volume processed to produce optimum quantities of adsorbed DOX on the columns. Suggested volumes are as follows:

Volume Processed *mL*	Instrument Optimum Range *µg Cl⁻*	Conc of DOX in Waters *µg/L*
100	0.5–50	5–50
50	12.5–50	250–1000
25	12.5–50	500–2000

If possible, avoid using volumes greater than 100mL because the maximum adsorptive capacity of the activated carbon may be exceeded, leading to adsorbate breakthrough and loss of DOX. Larger sample volumes processed lead to an increased quantity of inorganic halide accumulated on the activated carbon and may result in a positive interference. Do not use a sample less than 25mL to minimize volumetric errors. For samples exceeding 2000µg DOX/L dilute before adsorption. Protect columns from the atmosphere until DOX is determined.

4) Inorganic halide removal—Attach columns through which sample has been processed in series to the nitrate wash reservoir and pass 2 to 5 mL NO_3^- solution through the columns at a rate of approximately 1 mL/min.

5) DOX determination—After concentrating sample on activated carbon and removing inorganic halogens by nitrate washing, pyrolyze contents of each microcolumn and determine organic halogen content. Remove top glass microcolumn from the column housing, taking care not to contaminate the sample with inorganic halides. Using a clean ejector rod, eject the activated carbon and noncombustible insulating material plugs into the sample boat. Prepare sample boat during the preceding 4 h by heating at 400 to 800°C for at least 4 min in an oxygen-rich atmosphere (i.e., in the pyrolysis furnace). Remove residual ash. Place ejector rod on the plug of the effluent end of the carbon microcolumn and place the influent end of the carbon microcolumn in the quartz boat first. Seal sample inlet tube and let instrument stabilize. After NO_3^- wash avoid contact with inorganic halides. Wear latex gloves while carrying out this procedure. Preferably clean work area frequently with deionized water.

Pyrolyze the activated carbon and determine halide content. Repeat for each microcolumn. Check for excess breakthrough (¶ 5*b*) and repeat analysis as necessary.

6) Replicates—When DOX determination is used strictly as a screening tool, total

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replication is not necessary. Single-operator precision (% CV) is expected to be less than 15% for tap water and wastewater (Table 5320:I). If system performance is consistently worse as demonstrated by routine QA duplicates, or if quality objectives dictate, run replicates of each sample by repeating steps 3, 4, and 5.

7) Blanks—Analyze one method blank [¶ 5*e*2)] with each set of ten samples. Preferably analyze the method blank before starting the sample set and run a blank after the last set of the day.

8) Preparation and analysis of calibration standard—Run daily calibration standards in accordance with ¶ 5*c*3) for POX analysis or ¶ 5*c*5) for microcolumn-adsorption DOX analysis. Accompany by a suitable blank [¶ 5*e*3) or ¶ 5*e*]. Be certain that analytical conditions and procedures (e.g., purging temperature) are the same for the analysis of calibration standards as for the analysis of samples.

b. Batch adsorption procedure:

1) Apparatus setup—Adjust equipment in accordance with the manufacturer's instructions.

2) Sample pretreatment—Adjust sample pH to 2 with conc HNO_3 or H_2SO_4 [see ¶ 4*a*2)].

3) Sample adsorption—Prepare carbon suspension by adding high-quality activated carbon to high-purity, deionized, granular activated carbon (GAC)-treated water to produce a uniform suspension of 10 mg carbon/mL. To an erlenmeyer flask, transfer prepurged sample of optimum size from a purging flask standardized in the same manner as the instrument's purging vessel. Add 20 mg activated carbon (2 mL carbon suspension). Using a high-speed mixer (20 000 rpm), stir for 45 min in an organohalide vapor-free environment. Filter through a membrane filter under vacuum or pressure, and collect filtrate. Remove flask containing filtrate. Wash carbon cake and filter with 10 mL NO_3^- wash solution. Add portions of wash solution serially to keep activated carbon and NO_3^- solution in contact for 15 min. Using clean instruments, transfer carbon cake and membrane filter to pyrolysis unit sample boat. Let instrument stabilize, pyrolyze, and determine the halide content of the first serial filter.

Add 20 mg more activated carbon to filtrate in erlenmeyer flask. Repeat carbon mixing, filtering, and washing procedures. Pyrolyze and determine halide content of second serial filter. If the second value is greater than 10% of the total value (first plus second), perform the NPDOX determination on an additional sample portion.

c. POX procedure (optional) (direct purge): Adjust apparatus [¶ 4*a*1)]. Select sample volume by comparing expected POX value (if known) with optimum instrument range. Using a gastight syringe, inject sample through septum into purge vessel, and purge as recommended by equipment manufacturer. Carefully control gas flow rate, sample temperature, and purging time. The maximum POX that can be determined is:

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$$\text{POX}_{max},\ \mu m/L = \frac{0.5 \times 1000}{\text{mL sample} \times 35.5}$$

If replicates are analyzed, sampling from replicate sample bottles may minimize variability due to volatilization losses.

5. Quality Control

a. Activated carbon quality: Purchase activated carbon ready for use or prepare activated carbon by milling and sieving high-quality activated carbon. Use only 100- to 200-mesh carbon in the microcolumn method. During preparation, take care not to expose the activated carbon to organic vapors. Use of a clean room is helpful. Prepare only small quantities (a month's supply or less) at one time. Discard the activated carbon if its DOX background concentration has increased significantly from the time of preparation or if the background is greater than 1 µg apparent organic Cl^-/40 mg activated carbon. Uniformity of activated carbon is important; therefore, after sieving small portions, combine and mix thoroughly. Transfer representative portions to clean glass bottles with ground-glass stoppers or with rubber-backed TFE septa and open ring caps. Store bottles in a gas-purged, evacuated, sealed desiccator.

Test each newly prepared batch of activated carbon to ensure adequate quality before use. Use only activated carbon meeting the guidelines outlined below.

1) Check activated carbon particle size by applying deionized water to two 40-mg activated carbon microcolumns. If flow rate is significantly less than 3 mL/min, resieve activated carbon to remove excess fines.

2) Analyze a pair of method blanks, ¶ 5*e*2). Reject carbon if the apparent organic halogen exceeds 1.2 µg/40 mg activated carbon.

If the activated carbon originated from a previously untested batch from a commercial supplier, test it for adsorption efficiency and inorganic halide rejection.

3) Adsorb replicate 100-mL portions of solutions containing 100, 500, and 1000 mg inorganic Cl^-/L deionized water. Wash with nitrate solution and analyze. The apparent organic halogen yield should not increase by more than 0.50 µg over the value determined in 2) above. A greater increase indicates significant interference at that concentration.

b. Serial adsorption: Each aqueous standard and sample is serially adsorbed on activated carbon in both procedures given above. Of the net organic halide, 90% or more should be adsorbed on the first activated carbon portion and the remaining 10% or less on the second. If, upon separate analysis of the two serial activated carbon portions, the second shows more than 10% of the net (after subtracting the method blank), reanalyze sample. Inorganic halogen interference or organic breakthrough are the most common reasons for a high second activated carbon value. Sample dilution before adsorption may improve recovery on the first activated

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carbon in series, but the minimum detectable concentration will be affected.

c. Standards: The standards used in routine analysis, quality control testing, and isolating specific causes during corrective maintenance include:

1) Sodium chloride standard (¶ 3*d*)—Use to check functioning of the titration cell and microcoulometer by injecting directly into the acetic acid solution of the titration cell. By examining the height and shape of the peak produced on the chart recorder and from the integrated value, problems associated with the cell and coulometer may be isolated. Use this standard at startup each day and after cell cleaning throughout the day. At daily startup consecutive duplicates should be within 3% of the historical mean. Depending on sample loading and number of analyses performed, it may be necessary to clean the titration cell several times per day. After cleaning, cell performance may be very unstable; therefore, inject a single NaCl standard before analyzing an instrument calibration standard [see ¶ 4) below]. Do *not* introduce NaCl standards into the pyrolysis furnace by application to the sample boat.

2) Ammonium chloride standard (¶ 3*e*)—Apply this standard to the sample boat to check for loss of halide in the pyrolysis furnace and entrance of the titration cell. Typically, this may be necessary when injection of a NaCl standard indicates proper titration cell and microcoulometer function but the recovery of the calibration standard is poor: suspect either poor conversion of organic chloride to hydrogen chloride or loss of hydrogen halide after conversion but before partitioning into the cell solution. To isolate the possible loss of hydrogen halides inject NH_4Cl standard directly onto the quartz sample boat. Recovery should be better than 95%, with a single peak of uniform shape produced. Use only a new quartz sample boat free of any residue; an encrusted boat dramatically reduces recovery. Use this standard for corrective maintenance problem isolation but not for routine analyses.

3) Purgeable organic halide calibration standards—For the POX analysis use aqueous chloroform solutions for instrument calibration. Also for POX analysis an aqueous bromoform standard can be used initially to insure acceptable purging conditions. Develop a standard curve over the dynamic range of the microcoulometer and check daily as in ¶ 5*c*5). Recovery of chloroform and bromoform should exceed 90% and 80%, respectively.

4) Instrument calibration standard—Direct injection of trichlorophenol working standard onto the nitrate-washed method blank in concentrations over the working range of the instrument determines linearity and calibration of the analyzer module. After checking for proper microcoulometer function by injecting NaCl standard, pyrolyze duplicate instrument calibration standards and then duplicate method blanks. The net response to the calibration standards should be within 3% of the calibration curve value. If not, check for loss of halide in the pyrolysis furnace using the ammonium chloride standard [¶ 5*c*2)].

5) Nonvolatile organic halide calibration standards—Develop an initial standard curve by analyzing aqueous solutions of 2,4,6-trichlorophenol, trichloroacetic acid (commonly formed during chlorination), or another appropriate halogenated organic compound over the dynamic range of the microcoulometer. This dynamic range typically is from 0.5 to 50 µg chloride, but

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will vary betwen microcoulometers and titration cells. Construct an initial calibration curve using five calibration standards in range of 0.5 to 50 µg organic chloride; recheck calibration curve after changes in an instrument's configuration, such as replacement of a titration cell or major instrument maintenance. Daily, analyze a calibration standard to check proper function of the instrumentation and procedures. Select check standard in the concentration range of samples to be analyzed that day. When sample filtration is used to remove particulate matter, also use this pretreatment with the calibration standard. If DOX recovery is less than 90%, analyze a set of instrument calibration standards [¶ 5*c*4)].

d. Standard addition recovery: During routine analyses, ideally make standard additions to every tenth sample. Where the compounds constituting the DOX are known, use standards of these compounds. Where the compounds constituting the DOX are wholly or partially unknown, use standards reflecting the relative abundance of the halogens, the molecular size, and the volatility of the halogenated compounds presumed to be present. Recovery of 90% or more of the added amount indicates that the analyses are in control. Do not base acceptance of data on standard addition recoveries.

e. Blanks: High precision and accuracy of the background or blank value is important to the accurate measurement of DOX. Make blank measurements daily. Blanks that may be required are:

1) Reagent water blank—Analyze each batch of organic-free reagent water. The blank should have less than the minimum detectable concentration. Use this blank to insure that the standards, equipment, and procedures are not contributing to the DOX. Once reagent water blank is demonstrated, it can be used to determine method blank and POX blank as described below.

2) Method blank—Analyze activated carbon that has been nitrate-washed. Analyze method blanks daily before sample analysis and after at least each 10 to 14 sample pyrolyses.

3) Purgeable organic halogen blank—Analyze organic-free, pre-purged, reagent water to determine the POX blank.

6. Calculation

Calculate the net organic halide content as chloride (C_4) of each replicate of each sample and standard:

$$C_4 = \frac{C_1 - C_3 + C_2 - C_3}{V}$$

where:

C_1 = organic halide as Cl^- on the first activated carbon column or activated carbon cake, µg,

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C_2 = organic halide as Cl^- on the second activated carbon column or activated carbon cake, µg,

C_3 = mean of method blanks on the same day and same instrument, µg X as Cl^-,

C_4 = uncorrected net organic halide as Cl^- of absorbed sample, µg organic halide as Cl^-/L, and

V = volume of sample absorbed, L.

If $C_2 \leq C_3$, then use:

$$C_4 = \frac{C_1 - C_3}{V}$$

If applicable, calculate net purgeable organic halide as Cl^- content (P_3):

$$P_3 = \frac{P_1 - P_2}{V}$$

where:

P_1 = sample purgeable organic halide as Cl^-, µg,

P_2 = blank purgeable organic halide as Cl^-, µg,

P_3 = uncorrected net purgeable organic halide as Cl^-, µg X as Cl^-/L, and

V = volume of sample or standard purged, L.

Report sample results and percent recovery of the corresponding calibration standards [¶ 5*c*3) or ¶ 5*c*5)]. Also report the calibration standard curve if it is significantly nonlinear.

7. Precision and Bias

Precision and bias depend on specific procedures, equipment, and analyst. Develop and routinely update precision and bias data for each procedure, each instrument configuration, and each analyst. Table 5320:I shows sample calculations of precision expressed as the standard deviation among replicates and bias in the recovery of 2,4,6-trichlorophenol.

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5510 AQUATIC HUMIC SUBSTANCES*#(110)

5510 A. Introduction

1. General Discussion

Aquatic humic substances (AHS) are heterogeneous, yellow to black, organic materials that include most of the naturally occurring dissolved organic matter in water. Aquatic humic substances have been shown to produce trihalomethanes (THMs) on chlorination and to affect the transport and fate of other organic and inorganic species through partition/adsorption, catalytic, and photolytic reactions.

Humic substances, the major fraction of soil organic matter, are mixtures; their chemical composition is poorly understood. They have been classified into three fractions based on water ‘‘solubility’’†#(111): humin is the fraction not soluble in water at any pH value; humic acid is

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not soluble under acidic conditions (pH < 2) but becomes soluble at higher pH; and fulvic acid is soluble at all pH conditions.

AHS have the solubility characteristics of fulvic acids but they should not be referred to as such unless they have been fractionated by precipitation at pH < 2. Avoid using the terms ‘‘humic acid’’ and ‘‘tannic acid’’ to describe AHS because they represent other classifications of natural organic materials.

The heterogeneity of AHS requires an operational definition. Isolation by the methods included herein most likely will be incomplete and compounds that are not AHS may be isolated incidentally. Users of these methods are cautioned in the interpretation of results; the bibliography suggests several sources for more information.

Measurement of AHS begins by separation of the sample into dissolved (containing AHS) and particulate organic carbon fractions. Although there is no distinct size that separates these two groups, 0.45 µm is used as the compromise between acceptable flow rate and rejection of small colloidal materials. Low-pressure liquid chromatography serves to concentrate these materials and to isolate them from interfering substances. AHS are quantified by measuring dissolved organic carbon (DOC), Method 5310.

2. Selection of Method

Concentration/isolation of AHS may be achieved by sorption on the nonpolar resin XAD-8 (Method Section 5510C) or by anion-exchange on diethylaminoethyl (DEAE) cellulose (Method Section 5510B). In a collaborative study with seven laboratories using deionized water fortified with about 10 mg AHS/L (previously isolated with XAD), the DEAE method gave better recoveries. Nevertheless, the XAD method has been used extensively; refer to the discussions of interferences and minimum detectable concentrations to assist in method selection. Both methods require further quality control development.

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5510 B. Diethylaminoethyl (DEAE) Method

1. General Discussion

a. Principle: AHS are concentrated by column chromatography on diethylaminoethyl (DEAE) cellulose and measured as dissolved organic carbon (DOC). AHS are weak organic acids that bind to anion-exchange materials, such as DEAE cellulose, at neutral pH values. The method is based on the assumption that AHS are the major dissolved organic acids present.

b. Interferences: Any carbonaceous nonhumic materials that are concentrated and isolated by the chromatographic method will interfere (false positive response). Substances that have been shown to interfere include fatty acids, phenols, surfactants, proteinaceous materials, and DOC leached from cellulose.

c. Minimum detectable concentration: Estimated limit of detection is 1.1 mg/L using a 50-mL water sample. The detection limit can be decreased by increasing sample volume. The major limitation is blank contamination.

d. Standard substance: Eliminate documentation of false negatives by analyses of a sample of known humic concentration at regular intervals (at least once per batch of samples).

2. Apparatus

a. Membrane filtration apparatus: Use an all-glass filtering device and 0.45-µm silver membrane filters. Consult manufacturer's specifications for filter details. Do not use filters that sorb AHS or are contaminated with detergents and other organic material.

b. Glass column, approximately 1 × 20 cm with silanized glass wool.

c. Dye-impregnated paper or strips for approximate pH measurements.

d. Organic carbon analyzer capable of measuring concentrations as low as 0.1 mg/L (see Section 5310).

*e. Buchner funnel and filter paper.**#(112)

3. Reagents

a. Water, DOC-free: Preferably use activated-carbon-filtered, redistilled water.

b. DEAE cellulose, exchange capacity 0.22–1.0 meq/g.†#(113) Do not use high-exchange-capacity cellulose, which may decrease recovery of AHS. Take care not to overload low-exchange-capacity cellulose.

c. Hydrochloric acid, HCl, 0.1*N:* Add 8.3 mL conc HCl to 1000 mL water.

d. Hydrochloric acid, HCl, 0.5*N:* Add 41.5 mL conc HCl to 1000 mL water.

e. Sodium hydroxide, NaOH, 0.1*N:* Dissolve 4.0 g NaOH in 1000 mL water.

f. Sodium hydroxide, NaOH, 0.5*N:* Dissolve 20 g NaOH in 1000 mL water.

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g. DOC standards: See Section 5310.

h. Potassium chloride, KCl, 0.01*N:* Add 0.75 g KCl to 1000 mL water.

i. Phosphoric acid, H_3PO_4, conc.

4. Procedure

a. Sample concentration and preservation: AHS are sensitive to biodegradatio n and photodegradation. Collect and store samples in organic-free glass containers. Filter at least duplicate portions through a 0.45-µm silver membrane filter as soon after collection as possible. Store samples in the dark at 4°C.

Use care to avoid overloading chromatographic columns and losing AHS. A rough guideline for sample volume selection is as follows:

Sample DOC *mg/L*	Sample Volume *mL*
0–2	250
2–10	50
10–50	25

b. Preparation of DEAE cellulose: Add 70 g DEAE cellulose to 1000 mL 0.5*N* HCl and stir gently for 1 h. Rinse cellulose with water in a Buchner funnel until funnel effluent pH is about 4. Resuspend DEAE in 1000 mL 0.5*N* NaOH and stir for 1 h. Rinse in a Buchner funnel with water until pH is about 6. Remove fines by suspending the treated DEAE in a 1000-mL graduated cylinder filled with water. Let mixture stand undisturbed for 1 h, then decant and discard the supernatant. Repeat removal of fines. Filter remaining DEAE using a Buchner funnel and store in a refrigerated glass container. Avoid prolonged storage, which may lead to microbial contamination.

c. Chromatography: Add 10 mL water to about 1 g DEAE to make a slurry. Carefully pipet enough into a 1- × 20-cm column fitted with a small (0.5-cm) glass-wool plug to make a 1-cm-deep column bed. Avoid getting DEAE on the sides of the column. Carefully place another 0.5-cm glass-wool plug on top of the bed. Rinse column with 50 mL 0.01*N* KCl (adjusted to pH 6 with 0.1*N* HCl or NaOH) just before sample concentration.

Adjust sample to pH 6 and pass it through the column at a flow rate of about 2 mL/min. Rinse with 5 mL water (pH 6). Elute AHS by adding about 3 mL 0.1*N* NaOH to the top of the column. Start collecting column effluent when it appears colored. (This will occur after about 1 mL has passed out of the column). Collect eluate in a graduated, conical test tube until it becomes colorless (about 2 mL). Acidify with conc H_3PO_4 to a pH of 2 or less (about 2 to 3 drops) and remove dissolved carbon dioxide (inorganic carbon) by purging with nitrogen for 10

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min. Avoid exposure of alkaline samples to air (i.e. acidify immediately) to minimize contamination with CO_2. Determine volume and DOC of acidified eluate.

Process two portions of water and a second portion of sample by the same procedure. Pack a fresh column of DEAE for each sample and each control (DEAE cannot be reused).

5. Calculation

Calculate the concentration of AHS as:

$$\text{AHS, mg DOC/L} = [(A - B) \times C]/D$$

where:

A = average DOC concentration of the two sample NaOH eluates, mg C/L,
B = average DOC concentration of the two control NaOH eluates, mg C/L,
C = volume of eluate, L, and
D = volume of sample, L.

Multiplication of AHS, mg DOC/L, by 2 converts concentration to AHS, mg/L, if it is assumed that AHS contain 50% carbon. This will be the minimum concentration of AHS because recoveries are less than 100%.

6. Precision and Bias

For seven single-operator analyses, the relative standard deviation of triplicate samples (about 10 mg/L as AHS) ranged from 2.5 to 14.4% with an average of 4.9% ($n = 7$).

For seven single-operator analyses, recoveries ranged from 59.3 to 97.3% with an average of 77.4% and a relative standard deviation of 18.1%.

5510 C. XAD Method

1. General Discussion

a. Principle: AHS are concentrated by column chromatography on XAD resin and measured as dissolved organic carbon (DOC). Acidification of AHS decreases polarity, allowing partition into the nonpolar XAD matrix. The method is based on the assumption that AHS are the major dissolved organic acids present.

b. Interferences: Any carbonaceous nonhumic materials that are concentrated and isolated by the chromatographic method will interfere. This includes fatty acids, phenols, surfactants, proteinaceous materials, and DOC leached from the resin, chromatography pump, or tubing.

c. Minimum detectable concentration: Estimated limit of detection is 1.4 mg/L using a 50-mL water sample. The detection limit can be decreased by increasing sample volume. The major limitation is blank contaminations.

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2. Apparatus

See Section 5510B.2*a*, c, and *d*. In addition, the following are required:

a. Glass column, 0.2 × 25 cm with silanized glass wool.

b. Pump, with inert internal parts and tubing, capable of flow rates of 0.2 to 1.0 mL/min.*#(114)

c. TFE tubing, 0.2 cm ID.

d. Extraction apparatus, Soxhlet.

3. Reagents

In addition to reagents *a*, *c*, *e*, *g*, and *i* of Section 5510B :

a. XAD resin,†#(115) approximately 250-µm size.

b. Hexane.

c. Methanol.

d. Acetonitrile.

4. Procedure

a. Sample collection and preservation: See Section 5510B.4*a*.

b. Preparation of XAD resin: Clean resin by successive washing with 0.1*N* NaOH for 5 d. Extract resin sequentially in a Soxhlet extractor with hexane, methanol, acetonitrile, and methanol, for 24 h each. Pack clean resin into a 0.2- × 25-cm glass column that has a 2-mm length of glass wool in one end. After filling, cap column with another 2-mm length of glass wool.

Wet dry column with methanol. When the air has been displaced, pump distilled water through the column until the effluent concentration of DOC decreases to 0.5 mg/L (approximately 20 bed volumes).

c. Chromatography: Preclean column with three cycles of 0.1*N* NaOH and 0.1*N* HCl just before pumping sample into column. Leave column saturated with 0.1*N* HCl. Acidify sample to pH 2.0 with concentrated HCl, and pump it onto the column at rate of 1.0 mL/min. Save column effluent for DOC analysis. Significant concentrations of DOC in the effluent can indicate that the column was overloaded and that a smaller sample volume should be used. Colored organic acids adsorb to the top of the column. Back-elute (reverse flow) the column with 0.1*N* NaOH at 0.2 mL/min and collect eluate in a graduated, conical test tube until it becomes colorless (about 2 mL). Acidify with conc H_3PO_4 to a pH of 2 or less (about 2 to 3 drops) and remove dissolved carbon dioxide (inorganic carbon) by purging with nitrogen for 10 min. Avoid exposure of alkaline samples to air (i.e. acidify immediately) to minimize contamination with CO_2. Determine volume and DOC of acidified column effluent.

After eluting and collecting AHS from the column with back-elution using 0.1*N* NaOH, continue rinsing with about 20 bedvolumes of the basic solution. Rinse with water for about 20

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bed volumes. Repeat the triplicate acid/base column precleaning procedure described above, then reuse the column to analyze a replicate sample. Process two portions of water by the same procedure to serve as controls.

The XAD column may be reused to analyze subsequent samples and controls if the triplicate acid/base precleaning procedure is repeated immediately before analysis of each replicate. Replace the column if recovery is poor or the resin becomes discolored.

5. Calculation

Calculate the concentration of AHS as given in Section 5510B.5.

6. Precision and Bias

For seven single-operator analyses, the relative standard deviation of triplicate samples (about 10 mg/L as AHS) ranged from 0.9 to 20.7% with an average of 5.4% ($n = 7$).

For seven single-operator analyses, recoveries ranged from 15.1 to 71.0% with an average of 51.6% and a relative standard deviation of 35.1%.

5520 OIL AND GREASE*#(116)

5520 A. Introduction

In the determination of oil and grease, an absolute quantity of a specific substance is not measured. Rather, groups of substances with similar physical characteristics are determined quantitatively on the basis of their common solubility in an organic extracting solvent. ‘‘Oil and grease’’ is defined as any material recovered as a substance soluble in the solvent. It includes other material extracted by the solvent from an acidified sample (such as sulfur compounds, certain organic dyes, and chlorophyll) and not volatilized during the test. The 12th edition of *Standard Methods* prescribed the use of petroleum ether as the solvent for natural and treated waters and *n*-hexane for polluted waters. The 13th edition added trichlorotrifluoroethane as an optional solvent for all sample types. In the 14th through the 17th editions, only trichlorotrifluoroethane was specified. However, because of environmental problems associated with chlorofluorocarbons, an alternative solvent (80% *n*-hexane and 20% methyl-*tert*-butyl ether) was included for gravimetric methods in the 19th edition. In the 20th edition, trichlorotrifluoroethane has been dropped from all gravimetric procedures (retained for 5520C, an infrared method), and replaced by *n*-hexane. Solvent-recovery techniques are included and solvent recycling is strongly recommended.

It is important to understand that, unlike some constituents that represent distinct chemical elements, ions, compounds, or groups of compounds, oils and greases are defined by the method used for their determination. In a detailed study involving many complex organic matrices, it was shown that either *n*-hexane or 80/ 20 *n*-hexane/methyl-*tert*-butyl ether gave results that were

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not statistically different from results obtained with trichlorotrifluoroethane.[1] Although 5520B allows either solvent system for extraction of wastewaters, note that for certain regulatory purposes U.S. EPA currently recommends only *n*-hexane.[2]

The methods presented here are suitable for biological lipids and mineral hydrocarbons. They also may be suitable for most industrial wastewaters or treated effluents containing these materials, although sample complexity may result in either low or high results because of lack of analytical specificity. The method is not applicable to measurement of low-boiling fractions that volatilize at temperatures below 85°C.

1. Significance

Certain constituents measured by the oil and grease analysis may influence wastewater treatment systems. If present in excessive amounts, they may interfere with aerobic and anaerobic biological processes and lead to decreased wastewater treatment efficiency. When discharged in wastewater or treated effluents, they may cause surface films and shoreline deposits leading to environmental degradation.

A knowledge of the quantity of oil and grease present is helpful in proper design and operation of wastewater treatment systems and also may call attention to certain treatment difficulties.

In the absence of specially modified industrial products, oil and grease is composed primarily of fatty matter from animal and vegetable sources and from hydrocarbons of petroleum origin. The portion of oil and grease from each of these two major sources can be determined with Method 5520F. A knowledge of the relative composition of a sample minimizes the difficulty in determining the major source of the material and simplifies the correction of oil and grease problems in wastewater treatment plant operation and stream pollution abatement.

2. Selection of Method

For liquid samples, three methods are presented: the partition-gravimetric method (B), the partition-infrared method (C), and the Soxhlet method (D). Method C is designed for samples that might contain volatile hydrocarbons that otherwise would be lost in the solvent-removal operations of the gravimetric procedure. Method D is the method of choice when relatively polar, heavy petroleum fractions are present, or when the levels of nonvolatile greases may challenge the solubility limit of the solvent. For low levels of oil and grease (<10 mg/L), Method C is the method of choice because gravimetric methods do not provide the needed precision.

Method E is a modification of the Soxhlet method and is suitable for sludges and similar materials. Method F can be used in conjunction with Methods B, C, D, or E to obtain a hydrocarbon measurement in addition to, or instead of, the oil and grease measurement. This method makes use of silica gel to separate hydrocarbons from the total oil and grease on the basis of polarity.

3. Sample Collection, Preservation, and Storage

Collect a representative grab sample in a wide-mouth glass bottle that has been washed with

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soap, rinsed with water, and finally rinsed with solvent to remove any residues that might interfere with the analysis. As an alternative to solvent rinsing, cap bottle with aluminum foil and bake at 200 to 250°C for at least 1 h. Use PTFE-lined caps for sample bottles; clean liners as above, but limit temperature to 110 to 200°C. Collect a separate sample for an oil and grease determination. Do not overfill the sample container and do not subdivide the sample in the laboratory. Collect replicate samples for replicate analyses or known-addition QA checks. Collect replicates either in rapid succession, in parallel, or in one large container with mechanical stirring (in the latter case, siphon individual portions). Typically, collect wastewater samples of approximately 1 L. If sample concentration is expected to be greater than 1000 mg extractable material/L, collect proportionately smaller volumes. If analysis is to be delayed for more than 2 h, acidify to pH 2 or lower with either 1:1 HCl or 1:1 H_2SO_4 and refrigerate. When information is required about average grease concentration over an extended period, examine individual portions collected at prescribed time intervals to eliminate losses of grease on sampling equipment during collection of a composite sample.

In sampling sludges, take every possible precaution to obtain a representative sample. When analysis cannot be made within 2 h, preserve samples with 1 mL conc HCl/80 g sample and refrigerate. Never preserve samples with $CHCl_3$ or sodium benzoate.

4. Interferences

a. Organic solvents have the ability to dissolve not only oil and grease but also other organic substances. Any filterable solvent-soluble substances (e.g., elemental sulfur, complex aromatic compounds, hydrocarbon derivatives of chlorine, sulfur, and nitrogen, and certain organic dyes) that are extracted and recovered are defined as oil and grease. No known solvent will dissolve selectively only oil and grease. Heavier residuals of petroleum may contain a significant portion of materials that are not solvent-extractable. The method is entirely empirical; duplicate results with a high degree of precision can be obtained only by strict adherence to all details.

b. For Methods 5520B, D, E, and F, solvent removal results in the loss of short-chain hydrocarbons and simple aromatics by volatilization. Significant portions of petroleum distillates from gasoline through No. 2 fuel oil are lost in this process. Adhere strictly to sample drying time, to standardize gradual loss of weight due to volatilization. For Methods 5520B, D, E, and F, during the cooling of the distillation flask and extracted material, a gradual increase in weight may be observed, presumably due to the absorption of water if a desiccator is not used. For Method 5520C use of an infrared detector offers a degree of selectivity to overcome some coextracted interferences (¶ 4*a*). For Methods 5520D and E, use exactly the specified rate and time of extraction in the Soxhlet apparatus because of varying solubilities of different greases. For Method 5520F, the more polar hydrocarbons, such as complex aromatic compounds and hydrocarbon derivatives of chlorine, sulfur, and nitrogen, may be adsorbed by the silica gel. Extracted compounds other than hydrocarbons and fatty matter also interfere.

c. Alternative techniques may be needed for some samples if intractable emulsions form that cannot be broken by centrifugation. Such samples may include effluents from pulp/paper

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processing and zeolite manufacturing. Determine such modifications on a case-by-case basis.

d. Some sample matrices can increase the amount of water partitioned into the organic extraction fluid. When the extraction solvent from this type of sample is dried with sodium sulfate, the drying capacity of the sodium sulfate can be exceeded, thus allowing sodium sulfate to dissolve and pass into the tared flask. After drying, sodium sulfate crystals will be visible in the flask. The sodium sulfate that passes into the flask becomes a positive interference in gravimetric methods. If crystals are observed in the tared flask after drying, redissolve any oil and grease with 30 mL of extraction solvent and drain the solvent through a funnel containing a solvent-rinsed filter paper into a clean, tared flask. Rinse the first flask twice more, combining all solvent in the new flask, and treat as an extracted sample.

e. Silica gel fines may give positive interferences in 5520F if they pass through the filter. Use filters with smaller pores if this occurs with a particular batch of silica gel.

5. References

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1995. Report of the Method 1664 Validation Studies. EPA-821-R-95-036, U.S. Environmental Protection Agency, Washington, D.C.
2. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1995. Method 1664. EPA-821-B-94-004B, U.S. Environmental Protection Agency, Washington, D.C.

5520 B. Partition-Gravimetric Method

1. General Discussion

Dissolved or emulsified oil and grease is extracted from water by intimate contact with an extracting solvent. Some extractables, especially unsaturated fats and fatty acids, oxidize readily; hence, special precautions regarding temperature and solvent vapor displacement are included to minimize this effect. Organic solvents shaken with some samples may form an emulsion that is very difficult to break. This method includes a means for handling such emulsions. Recovery of solvents is discussed. Solvent recovery can reduce both vapor emissions to the atmosphere and costs.

2. Apparatus

a. Separatory funnel, 2-L, with TFE*#(117) stopcock.

b. Distilling flask, 125-mL.

c. Liquid funnel, glass.

d. Filter paper, 11-cm diam.†#(118)

e. Centrifuge, capable of spinning at least four 100-mL glass centrifuge tubes at 2400 rpm or more.

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f. Centrifuge tubes, 100-mL, glass.

g. Water bath, capable of maintaining 85°C.

h. Vacuum pump or other source of vacuum.

i. Distilling adapter with drip tip. Setup of distillate recovery apparatus is shown in Figure 5520:1. Alternatively, use commercially available solvent recovery equipment.

j. Ice bath.

k. Waste receptacle, for used solvent.

l. Desiccator.

3. Reagents

a. Hydrochloric or sulfuric acid, 1:1: Mix equal volumes of either acid and reagent water.

b. n-Hexane, boiling point 69°C. The solvent should leave no measurable residue on evaporation; distill if necessary. Do not use any plastic tubing to transfer solvent between containers.

c. Methyl-tert-*butyl ether (MTBE)*, boiling point 55°C to 56°C. The solvent should leave no measurable residue on evaporation; distill if necessary. Do not use any plastic tubing to transfer solvent between containers.

d. Sodium sulfate, Na_2SO_4, anhydrous crystal.

e. Solvent mixture, 80% *n*-hexane/20% MTBE, v/v.

4. Procedure

When a sample is brought into the laboratory, either mark sample bottle at the water meniscus or weigh the bottle, for later determination of sample volume. If sample has not been acidified previously (see Section 5520A.3), acidify with either 1:1 HCl or 1:1 H_2SO_4 to pH 2 or lower (generally, 5 mL is sufficient for 1 L sample). Using liquid funnel, transfer sample to a separatory funnel. Carefully rinse sample bottle with 30 mL extracting solvent (either 100% *n*-hexane, ¶ 3*b*, or solvent mixture, ¶ 3*e*) and add solvent washings to separatory funnel. Shake vigorously for 2 min. Let layers separate. Drain aqueous layer and small amount of organic layer into original sample container. Drain solvent layer through a funnel containing a filter paper and 10 g Na_2SO_4, both of which have been solvent-rinsed, into a clean, tared distilling flask. If a clear solvent layer cannot be obtained and an emulsion of more than about 5 mL exists, drain emulsion and solvent layers into a glass centrifuge tube and centrifuge for 5 min at approximately 2400 rpm. Transfer centrifuged material to an appropriate separatory funnel and drain solvent layer through a funnel with a filter paper and 10 g Na_2SO_4, both of which have been prerinsed, into a clean, tared distilling flask. Recombine aqueous layers and any remaining emulsion or solids in separatory funnel. For samples with <5 mL of emulsion, drain only the clear solvent through a funnel with pre-moistened filter paper and 10 g Na_2SO_4. Recombine aqueous layers and any remaining emulsion or solids in separatory funnel. Extract twice more

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with 30 mL solvent each time, but first rinse sample container with each solvent portion. Repeat centrifugation step if emulsion persists in subsequent extraction steps. Combine extracts in tared distilling flask, and include in flask a final rinsing of filter and Na_2SO_4 with an additional 10 to 20 mL solvent. Distill solvent from flask in a water bath at 85°C for either solvent system. To maximize solvent recovery, fit distillation flask with a distillation adapter equipped with a drip tip and collect solvent in an ice-bath-cooled receiver (Figure 5520:1). When visible solvent condensation stops, remove flask from water bath. Cover water bath and dry flasks on top of cover, with water bath still at 85°C, for 15 min. Draw air through flask with an applied vacuum for the final 1 min. Cool in desiccator for at least 30 min and weigh. To determine initial sample volume, either fill sample bottle to mark with water and then pour water into a 1-L graduated cylinder, or weigh empty container and cap and calculate the sample volume by difference from the initial weight (assuming a sample density of 1.00).

5. Calculation

If the organic solvent is free of residue, the gain in weight of the tared distilling flask is due to oil and grease. Total gain in weight, *A*, of tared flask, less calculated residue from solvent blank, *B*, is the amount of oil and grease in the sample:

$$\text{mg oil and grease/L} = \frac{(A - B) \times 1000}{\text{mL sample}}$$

6. Precision and Bias

Method B with 80:20 hexane/MTBE mixture was tested by a single laboratory on a raw wastewater sample. The oil and grease concentration was 22.4 mg/L. When samples were dosed with 30 mg Fisher Heavy Mineral Oil, recovery of added oil was 84.2% with a standard deviation of 1.2 mg/L. Method B was tested with *n*-hexane as solvent. The method detection limit was determined to be 1.4 mg/L.[1] When reagent water was fortified with hexadecane and stearic acid each at approximately 20 mg/L, initial precision and recovery limit standards were 10% and 83 to 101%, respectively. Acceptable recovery limits for laboratory-fortified matrix/laboratory-fortified matrix duplicate and ongoing laboratory control standards are 79 to 114%, with a relative percent difference limit of 18%.

7. References

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KIRSCHMAN, H.D. & R. POMEROY. 1949. Determination of oil in oil field waste waters. *Anal. Chem.* 21:793.

5520 C. Partition-Infrared Method

1. General Discussion

a. Principle: The use of trichlorotrifluoroethane as extraction solvent allows absorbance of the carbon-hydrogen bond in the infrared to be used to measure oil and grease. Elimination of the evaporation step permits infrared detection of many relatively volatile hydrocarbons. Thus, the lighter petroleum distillates, with the exception of gasoline, may be measured accurately. With adequate instrumentation, as little as 0.2 mg oil and grease/L can be measured.

b. Definitions: A ‘‘known oil’’ is defined as a sample of oil and/or grease that represents the only material of that type used or manufactured in the processes represented by a wastewater. An ‘‘unknown oil’’ is defined as one for which a representative sample of the oil or grease is not available for preparation of a standard.

2. Apparatus

a. Separatory funnel, 2-L, with TFE*#(119) stopcock.

b. Volumetric flask, 100-mL.

c. Liquid funnel, glass.

d. Filter paper, 11-cm diam.†#(120)

e. Centrifuge, capable of spinning at least four 100-mL glass centrifuge tubes at 2400 rpm or more.

f. Centrifuge tubes, 100-mL, glass.

g. Infrared spectrophotometer, double-beam, recording.

h. Cells, near-infrared silica.

3. Reagents

a. Hydrochloric acid, HCl, 1 + 1.

b. Trichlorotrifluoroethane (1,1,2-trichloro-1,2,2-trifluoro ethane), boiling point 47°C. The solvent should leave no measurable residue on evaporation; distill if necessary. Do not use any plastic tubing to transfer solvent between containers.

c. Sodium sulfate, Na_2SO_4, anhydrous, crystal.

d. Reference oil: Prepare a mixture, by volume, of 37.5% isooctane, 37.5% hexadecane, and 25.0% benzene. Store in sealed container to prevent evaporation.

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4. Procedure

Refer to Section 5520B.4 for sample handling and for method of dealing with sample emulsions. After carefully transferring sample to a separatory funnel, rinse sample bottle with 30 mL trichlorotrifluoroethane and add solvent washings to funnel. Shake vigorously for 2 min. Let layers separate. Drain all but a very small portion of the lower trichlorotrifluoroethane layer through a funnel containing a filter paper and 10 g Na_2SO_4, both of which have been solvent-rinsed, into a clean, 100-mL volumetric flask. If a clear solvent layer cannot be obtained and an emulsion of more than about 5 mL exists, see Section 5520B.4. Extract twice more with 30 mL solvent each time, but first rinse sample container with each solvent portion. Repeat centrifugation step if emulsion persists in subsequent extraction steps. Combine extracts in volumetric flask, and include in flask a final rinsing of filter and Na_2SO_4 with an additional 10 to 20 mL solvent. Adjust final volume to 100 mL with solvent.

Prepare a stock solution of known oil by rapidly transferring about 1 mL (0.5 to 1.0 g) of the oil or grease to a tared 100-mL volumetric flask. Stopper flask and weigh to nearest milligram. Add solvent to dissolve and dilute to mark. If the oil identity is unknown (Section 5520C.1*b*) use the reference oil (Section 5520C.3*d*) as the standard. Using volumetric techniques, prepare a series of standards over the range of interest. Select a pair of matched near-infrared silica cells. A 1-cm-path-length cell is appropriate for a working range of about 4 to 40 mg. Scan standards and samples from 3200 cm^{-1} to 2700 cm^{-1} with solvent in the reference beam and record results on absorbance paper. Measure absorbances of samples and standards by constructing a straight base line over the scan range and measuring absorbance of the peak maximum at 2930 cm^{-1} and subtracting baseline absorbance at that point. If the absorbance exceeds 0.8 for a sample, select a shorter path length or dilute as required. Use scans of standards to prepare a calibration curve.

5. Calculation

$$\text{mg oil and grease/L} = \frac{A \times 1000}{\text{mL sample}}$$

where:

A = mg of oil or grease in extract as determined from calibration curve.

6. Precision and Bias

Method C was used by a single laboratory to test a wastewater sample. By this method the oil and grease concentration was 17.5 mg/L. When 1-L sample portions were dosed with 14.0 mg of a mixture of No. 2 fuel oil and Wesson oil, the recovery of added oils was 99% with a standard deviation of 1.4 mg.

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7. Bibliography

GRUENFELD, M. 1973. Extraction of dispersed oils from water for quantitative analysis by infrared spectrophotometry. *Environ. Sci. Technol.* 7:636.

5520 D. Soxhlet Extraction Method

1. General Discussion

Soluble metallic soaps are hydrolyzed by acidification. Any oils and solid or viscous grease present are separated from the liquid samples by filtration. After extraction in a Soxhlet apparatus with solvent, the residue remaining after solvent evaporation is weighed to determine the oil and grease content. Compounds volatilized at or below 103°C will be lost when the filter is dried.

2. Apparatus

a. Extraction apparatus, Soxhlet, with 125-mL extraction flask.

b. Extraction thimble, paper, solvent-extracted.

c. Electric heating mantle.

d. Vacuum pump or other source of vacuum.

e. Vacuum filtration apparatus.

f. Buchner funnel, 12-cm.

g. Filter paper, 11-cm diam.*#(121)

h. Muslin cloth disks, 11-cm diam, solvent-extracted.

i. Glass beads or glass wool, solvent-extracted.

j. Water bath, capable of maintaining 85°C.

k. Distilling adapter with drip tip. See Section 5520B.2*i* and Figure 5520:1.

l. Ice bath.

m. Waste receptacle, for used solvent.

n. Desiccator.

3. Reagents

a. Hydrochloric acid, HCl, 1 + 1.

b. n-Hexane: See Section 5520B.3*b*.

c. Methyl-tert-*butyl ether* (MTBE): See Section 5520B.3*c*.

d. Diatomaceous-silica filter aid suspension,†#(122) 10 g/L distilled water.

e. Solvent mixture, 80% *n*-hexane/20% MTBE, v/v.

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4. Procedure

When sample is brought into the laboratory, either mark sample bottle at the meniscus or weigh bottle for later determination of volume. If sample has not been acidified previously (see Section 5520A.3), acidify with 1:1 HCl or 1:1 H_2SO_4 to pH 2 or lower (generally, 5 mL is sufficient). Prepare filter consisting of a muslin cloth disk overlaid with filter paper. Wet paper and muslin and press down edges of paper. Using vacuum, pass 100 mL filter aid suspension through prepared filter and wash with 1 L distilled water. Apply vacuum until no more water passes filter. Filter acidified sample. Apply vacuum until no more water passes through filter. Using forceps, transfer entire filter to a watch glass. Add material adhering to edges of muslin cloth disk. Wipe sides and bottom of collecting vessel and Buchner funnel with pieces of filter paper soaked in extraction solvent, taking care to remove all films caused by grease and to collect all solid material. Add pieces of filter paper to material on watch glass. Roll all filter material containing sample and fit into an extraction thimble. Add any pieces of material remaining on watch glass. Wipe watch glass with a filter paper soaked in extraction solvent and place in extraction thimble. Dry filled thimble in a hot-air oven at 103°C for 30 min. Fill thimble with glass wool or small glass beads. Weigh extraction flask and add 100 mL extraction solvent (*n*-hexane, ¶ 3*b*, or solvent mixture, ¶ 3*e*). Extract oil and grease in a Soxhlet apparatus, at a rate of 20 cycles/h for 4 h. Time from first cycle. For stripping and recovery of solvent, cooling extraction flask before weighing, and determining initial sample volume, see Section 5520B.4.

5. Calculation

See Section 5520B.5.

6. Precision and Bias

In analyses of synthetic samples containing various amounts of Crisco and Shell S.A.E. No. 20 oil, an average recovery of 98.7% was obtained, with a standard deviation of 1.86%. Ten replicates each of two wastewater samples yielded standard deviations of 0.76 mg and 0.48 mg.

7. Bibliography

HATFIELD, W.D. & G.E. SYMONS. 1945. The determination of grease in sewage. *Sewage Works J.* 17:16.

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ULLMANN, W.W. & W.W. SANDERSON. 1959. A further study of methods for the determination of grease in sewage. *Sewage Ind. Wastes* 31:8.

5520 E. Extraction Method for Sludge Samples

1. General Discussion

Drying acidified sludge by heating leads to low results. Magnesium sulfate monohydrate is

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capable of combining with 75% of its own weight in water in forming $MgSO_4 \cdot 7H_2O$ and is used to dry sludge. After drying, the oil and grease can be extracted with an organic solvent.

2. Apparatus

a. Beaker, 150-mL, glass.

b. Mortar and pestle, porcelain.

c. Extraction apparatus, Soxhlet.

d. Extraction thimble, paper, solvent-extracted.

e. Glass beads or glass wool, solvent-extracted.

f. Electric heating mantle.

g. Vacuum pump or other source of vacuum.

h. Liquid funnel, glass.

i. Grease-free cotton: Extract nonabsorbent cotton with solvent.

j. Water bath, capable of maintaining 85°C.

k. Distilling adapter with drip tip. See Section 5520.2*i* and Figure 5520:1.

l. Ice bath.

m. Waste receptacle, for used solvent.

n. Desiccator.

3. Reagents

a. Hydrochloric acid, HCl, conc.

b. n-Hexane: See Section 5520B.3*b*.

c. Methyl-tert-*butyl ether (MTBE)*: See Section 5520B.3*c*.

d. Magnesium sulfate monohydrate: Prepare $MgSO_4 \cdot H_2O$ by drying a thin layer overnight in an oven at 150°C.

e. Solvent mixture, 80% *n*-hexane/20% MTBE, v/v.

4. Procedure

When sample is brought into the laboratory, if it has not been acidified previously (Section 5520A.3), add 1 mL conc HCl/80 g sample. In a 150-mL beaker weigh out a sample of wet sludge, 20 ± 0.5 g, for which the dry-solids content is known. Acidify to pH 2.0 or lower (generally, 0.3 mL conc HCl is sufficient). Add 25 g $MgSO_4 \cdot H_2O$. Stir to a smooth paste and spread on sides of beaker to facilitate subsequent sample removal. Let stand until solidified, 15 to 30 min. Remove solids and grind in a porcelain mortar. Add powder to a paper extraction thimble. Wipe beaker and mortar with small pieces of filter paper moistened with solvent and add to thimble. Fill thimble with glass wool or small glass beads. Tare extraction flask, and add 100 mL extraction solvent (¶ 3*b* or ¶ 3*e*). Extract in a Soxhlet apparatus at a rate of 20 cycles/h

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for 4 h. If any turbidity or suspended matter is present in the extraction flask, remove by filtering through grease-free cotton into another weighed flask. Rinse flask and cotton with solvent. For solvent stripping and recovery, and cooling the extraction flask before weighing, see Section 5520B.4.

5. Calculation

Oil and grease as % of dry solids

$$= \frac{\text{gain in weight of flask, g} \times 100}{\text{weight of wet solids, g} \times \text{dry solids fraction}}$$

6. Precision

The examination of six replicate samples of sludge yielded a standard deviation of 4.6%.

5520 F. Hydrocarbons

1. General Discussion

Silica gel has the ability to adsorb polar materials. If a solution of hydrocarbons and fatty materials in a nonpolar solvent is mixed with silica gel, the fatty acids are removed selectively from solution. The materials not eliminated by silica gel adsorption are designated hydrocarbons by this test.

2. Apparatus

a. Magnetic stirrer.

b. Magnetic stirring bars, TFE-coated.

c. Liquid funnel, glass.

d. Filter paper, 11-cm diam.*#(123)

e. Desiccator.

3. Reagents

a. n-Hexane: See Section 5520B.3*b*.

b. Trichlorotrifluoroethane: See Section 5520C.3*b*.

c. Silica gel, 100 to 200 mesh.†#(124) Dry at 110°C for 24 h and store in a tightly sealed container.

4. Procedure

Use the oil and grease extracted by Method B, C, D, or E for this test. When only hydrocarbons are of interest, introduce this procedure in any of the previous methods before final

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measurement. When hydrocarbons are to be determined after total oil and grease has been measured, redissolve the extracted oil and grease in trichlorofluoroethane (Method C) or 100 mL *n*-hexane. To 100 mL solvent add 3.0 g silica gel/100 mg total oil and grease, up to a total of 30.0 g silica gel (1000 mg total oil and grease). For samples with more than 1000 mg total oil and grease use a measured volume of the 100 mL solvent dissolved sample, add appropriate amount of silica gel for amount of total oil and grease in the sample portion, and bring volume to 100 mL. Stopper container and stir on a magnetic stirrer for 5 min. For infrared measurement of hydrocarbons no further treatment is required before measurement as described in Method C. For gravimetric determinations, filter solution through filter paper pre-moistened with solvent, wash silica gel and filter paper with 10 mL solvent, and combine with filtrate. For solvent stripping and recovery, and for cooling extraction flask before weighing, see Section 5520B.4.

5. Calculation

Calculate hydrocarbon concentration, in milligrams per liter, as in oil and grease (Method B, C, D, or E).

6. Precision and Bias

The following data, obtained on synthetic samples, are indicative for natural animal, vegetable, and mineral products, but cannot be applied to the specialized industrial products previously discussed.

For hydrocarbon determinations on 10 synthetic solvent extracts containing known amounts of a wide variety of petroleum products, average recovery was 97.2%. Similar synthetic extracts of Wesson oil, olive oil, Crisco, and butter gave 0.0% recovery as hydrocarbons measured by infrared analysis.

Using reagent water fortified with approximately 20 mg/L each of hexadecane and stearic acid, initial hydrocarbon recovery limits based on hexadecane of 83 to 116% were developed, with a precision limit of 13%. Laboratory-fortified matrix/laboratory-fortified matrix duplicate gave recovery limits of 66 to 114% with a relative percent difference of 24%.

7. Bibliography

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1995. Report of the Method 1664 Validation Studies. EPA-821-R-95-036, U.S. Environmental Protection Agency, Washington, D.C.

5530 PHENOLS*#(125)

5530 A. Introduction

Phenols, defined as hydroxy derivatives of benzene and its condensed nuclei, may occur in domestic and industrial wastewaters, natural waters, and potable water supplies. Chlorination of such waters may produce odorous and objectionable-tasting chlorophenols. Phenol removal

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processes in water treatment include superchlorination, chlorine dioxide or chloramine treatment, ozonation, and activated carbon adsorption.

1. Selection of Method

The analytical procedures offered here use the 4-aminoantipyrine colorimetric method that determines phenol, ortho- and meta-substituted phenols, and, under proper pH conditions, those para-substituted phenols in which the substitution is a carboxyl, halogen, methoxyl, or sulfonic acid group. The 4-aminoantipyrine method does not determine those para-substituted phenols where the substitution is an alkyl, aryl, nitro, benzoyl, nitroso, or aldehyde group. A typical example of these latter groups is paracresol, which may be present in certain industrial wastewaters and in polluted surface waters.

The 4-aminoantipyrine method is given in two forms: Method C, for extreme sensitivity, is adaptable for use in water samples containing less than 1 mg phenol/L. It concentrates the color in a nonaqueous solution. Method D retains the color in the aqueous solution. Because the relative amounts of various phenolic compounds in a given sample are unpredictable, it is not possible to provide a universal standard containing a mixture of phenols. For this reason, phenol (C_6H_5OH) itself has been selected as a standard for colorimetric procedures and any color produced by the reaction of other phenolic compounds is reported as phenol. Because substitution generally reduces response, this value represents the minimum concentration of phenolic compounds. A gas-liquid chromatographic procedure is included in Section 6420B and may be applied to samples or concentrates to quantify individual phenolic compounds.

2. Interferences

Interferences such as phenol-decomposing bacteria, oxidizing and reducing substances, and alkaline pH values are dealt with by acidification. Some highly contaminated wastewaters may require specialized techniques for eliminating interferences and for quantitative recovery of phenolic compounds.

Eliminate major interferences as follows (see Section 5530B for reagents):

Oxidizing agents, such as chlorine and those detected by the liberation of iodine on acidification in the presence of potassium iodide (KI)—Remove immediately after sampling by adding excess ferrous sulfate ($FeSO_4$). If oxidizing agents are not removed, the phenolic compounds will be oxidized partially.

Sulfur compounds—Remove by acidifying to pH 4.0 with H_3PO_4 and aerating briefly by stirring. This eliminates the interference of hydrogen sulfide (H_2S) and sulfur dioxide (SO_2).

Oils and tars—Make an alkaline extraction by adjusting to pH 12 to 12.5 with NaOH pellets. Extract oil and tar from aqueous solution with 50 mL chloroform ($CHCl_3$). Discard oil- or tar-containing layer. Remove excess $CHCl_3$ in aqueous layer by warming on a water bath before proceeding with the distillation step.

3. Sampling

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Sample in accordance with the instructions of Section 1060.

4. Preservation and Storage of Samples

Phenols in concentrations usually encountered in wastewaters are subject to biological and chemical oxidation. Preserve and store samples at 4°C or lower unless analyzed within 4 h after collection.

Acidify with 2 mL conc H_2SO_4/L.

Analyze preserved and stored samples within 28 d after collection.

5. Bibliography

ETTINGER, M.B., S. SCHOTT & C.C. RUCHHOFT. 1943. Preservation of phenol content in polluted river water samples previous to analysis. *J. Amer. Water Works Assoc.* 35:299.

CARTER, M.J. & M.T. HUSTON. 1978. Preservation of phenolic compounds in wastewaters. *Environ. Sci. Technol.* 12:309.

NEUFELD, R.D. & S.B. POLADINO. 1985. Comparison of 4-aminoantipyrine and gas-liquid chromatography techniques for analysis of phenolic compounds. *J. Water Pollut. Control Fed.* 57:1040.

5530 B. Cleanup Procedure

1. Principle

Phenols are distilled from nonvolatile impurities. Because the volatilization of phenols is gradual, the distillate volume must ultimately equal that of the original sample.

2. Apparatus

a. Distillation apparatus, all-glass, consisting of a 1-L borosilicate glass distilling apparatus with Graham condenser.*#(126)

b. pH meter.

3. Reagents

Prepare all reagents with distilled water free of phenols and chlorine.

a. Phosphoric acid solution, H_3PO_4, 1 + 9: Dilute 10 mL 85% H_3PO_4 to 100 mL with water.

b. Methyl orange indicator solution.

c. Special reagents for turbid distillates:

1) *Sulfuric acid,* H_2SO_4, 1*N*.

2) *Sodium chloride,* NaCl.

3) *Chloroform,* $CHCl_3$, or *methylene chloride,* CH_2Cl_2.

4) *Sodium hydroxide,* NaOH, 2.5*N:* Dilute 41.7 mL 6*N* NaOH to 100 mL or dissolve 10 g

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NaOH pellets in 100 mL water.

4. Procedure

a. Measure 500 mL sample into a beaker, adjust pH to approximately 4.0 with H_3PO_4 solution using methyl orange indicator or a pH meter, and transfer to distillation apparatus. Use a 500-mL graduated cylinder as a receiver. Omit adding H_3PO_4 and adjust pH to 4.0 with 2.5*N* NaOH if sample was preserved as described in 5530A.4.

b. Distill 450 mL, stop distillation and, when boiling ceases, add 50 mL warm water to distilling flask. Continue distillation until a total of 500 mL has been collected.

c. One distillation should purify the sample adequately. Occasionally, however, the distillate is turbid. If so, acidify with H_3PO_4 solution and distill as described in ¶ 4*b*. If second distillate is still turbid, use extraction process described in ¶ 4*d* before distilling sample.

d. Treatment when second distillate is turbid: Extract a 500-mL portion of original sample as follows: Add 4 drops methyl orange indicator and make acidic to methyl orange with 1*N* H_2SO_4. Transfer to a separatory funnel and add 150 g NaCl. Shake with five successive portions of $CHCl_3$, using 40 mL in the first portion and 25 mL in each successive portion. Transfer $CHCl_3$ layer to a second separatory funnel and shake with three successive portions of 2.5*N* NaOH solution, using 4.0 mL in the first portion and 3.0 mL in each of the next two portions. Combine alkaline extracts, heat on a water bath until $CHCl_3$ has been removed, cool, and dilute to 500 mL with distilled water. Proceed with distillation as described in ¶s 4*a* and b.

NOTE: CH_2Cl_2 may be used instead of $CHCl_3$, especially if an emulsion forms when the $CHCl_3$ solution is extracted with NaOH.

5530 C. Chloroform Extraction Method

1. General Discussion

a. Principle: Steam-distillable phenols react with 4-aminoantipyrine at pH 7.9 ± 0.1 in the presence of potassium ferricyanide to form a colored antipyrine dye. This dye is extracted from aqueous solution with $CHCl_3$ and the absorbance is measured at 460 nm. This method covers the phenol concentration range from 1.0 µg/L to over 250 µg/L with a sensitivity of 1 µg/L.

b. Interference: All interferences are eliminated or reduced to a minimum if the sample is preserved, stored, and distilled in accordance with the foregoing instructions.

c. Minimum detectable quantity: The minimum detectable quantity for clean samples containing no interferences is 0.5 µg phenol when a 25-mL $CHCl_3$ extraction with a 5-cm cell or a 50-mL $CHCl_3$ extraction with a 10-cm cell is used in the photometric measurement. This quantity is equivalent to 1 µg phenol/L in 500 mL distillate.

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2. Apparatus

a. Photometric equipment: A spectrophotometer for use at 460 nm equipped with absorption cells providing light paths of 1 to 10 cm, depending on the absorbances of the colored solutions and the individual characteristics of the photometer.

b. Filter funnels: Buchner type with fritted disk.*#(127)

c. Filter paper: Alternatively use an appropriate 11-cm filter paper for filtering $CHCl_3$ extracts instead of the Buchner-type funnels and anhydrous Na_2SO_4.

d. pH meter.

e. Separatory funnels, 1000-mL, Squibb form, with ground-glass stoppers and TFE stopcocks. At least eight are required.

3. Reagents

Prepare all reagents with distilled water free of phenols and chlorine.

a. Stock phenol solution: Dissolve 100 mg phenol in freshly boiled and cooled distilled water and dilute to 100 mL. CAUTION—*Toxic; handle with extreme care.* Ordinarily this direct weighing yields a standard solution; if extreme accuracy is required, standardize as follows:

1) To 100 mL water in a 500-mL glass-stoppered conical flask, add 50.0 mL stock phenol solution and 10.0 mL bromate-bromide solution. Immediately add 5 mL conc HCl and swirl gently. If brown color of free bromine does not persist, add 10.0-mL portions of bromate-bromide solution until it does. Keep flask stoppered and let stand for 10 min; then add approximately 1 g KI. Usually four 10-mL portions of bromate-bromide solution are required if the stock phenol solution contains 1000 mg phenol/L.

2) Prepare a blank in exactly the same manner, using distilled water and 10.0 mL bromate-bromide solution. Titrate blank and sample with 0.025*M* sodium thiosulfate, using starch solution indicator.

3) Calculate the concentration of phenol solution as follows:

$$\text{mg phenol/L} = 7.842\ [(A \times B) - C]$$

where:

A = mL thiosulfate for blank,
B = mL bromate-bromide solution used for sample divided by 10, and
C = mL thiosulfate used for sample.

b. Intermediate phenol solution: Dilute 1.00 mL stock phenol solution in freshly boiled and cooled distilled water to 100 mL; 1 mL = 10.0 µg phenol. Prepare daily.

c. Standard phenol solution: Dilute 50.0 mL intermediate phenol solution to 500 mL with freshly boiled and cooled distilled water; 1 mL = 1.0 µg phenol. Prepare within 2 h of use.

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d. Bromate-bromide solution: Dissolve 2.784 g anhydrous $KBrO_3$ in water, add 10 g KBr crystals, dissolve, and dilute to 1000 mL.

e. Hydrochloric acid, HCl, conc.

f. Standard sodium thiosulfate titrant, 0.025*M:* See Section 4500-O.C.2*e*.

g. Starch solution: See Section 4500-O.C.2*d*.

h. Ammonium hydroxide, NH_4OH, 0.5*N*: Dilute 35 mL fresh, conc NH_4OH to 1 L with water.

i. Phosphate buffer solution: Dissolve 104.5 g K_2HPO_4 and 72.3 g KH_2PO_4 in water and dilute to 1 L. The pH should be 6.8.

j. 4-Aminoantipyrine solution: Dissolve 2.0 g 4-aminoantipyrine in water and dilute to 100 mL. Prepare daily.

k. Potassium ferricyanide solution: Dissolve 8.0 g $K_3Fe(CN)_6$ in water and dilute to 100 mL. Filter if necessary. Store in a brown glass bottle. Prepare fresh weekly.

l. Chloroform, $CHCl_3$.

m. Sodium sulfate, anhydrous Na_2SO_4, granular.

n. Potassium iodide, KI, crystals.

4. Procedure

Ordinarily, use Procedure *a*; however, Procedure *b* may be used for infrequent analyses.

a. Place 500 mL distillate, or a suitable portion containing not more than 50 µg phenol, diluted to 500 mL, in a 1-L beaker. Prepare a 500-mL distilled water blank and a series of 500-mL phenol standards containing 5, 10, 20, 30, 40, and 50 µg phenol.

Treat sample, blank, and standards as follows: Add 12.0 mL 0.5*N* NH_4OH and *immediately* adjust pH to 7.9 ± 0.1 with phosphate buffer. Under some circumstances, a higher pH may be required.†#(128) About 10 mL phosphate buffer are required. Transfer to a 1-L separatory funnel, add 3.0 mL aminoantipyrine solution, mix well, add 3.0 mL $K_3Fe(CN)_6$ solution, mix well, and let color develop for 15 min. The solution should be clear and light yellow.

Extract immediately with $CHCl_3$, using 25 mL for 1- to 5-cm cells and 50 mL for a 10-cm cell. Shake separatory funnel at least 10 times, let $CHCl_3$ settle, shake again 10 times, and let $CHCl_3$ settle again. Filter each $CHCl_3$ extract through filter paper or fritted glass funnels containing a 5-g layer of anhydrous Na_2SO_4. Collect dried extracts in clean cells for absorbance measurements; do not add more $CHCl_3$ or wash filter papers or funnels with $CHCl_3$.

Read absorbance of sample and standards against the blank at 460 nm. Plot absorbance against micrograms phenol concentra tion. Construct a separate calibration curve for each photometer and check each curve periodically to insure reproducibility.

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b. For infrequent analyses prepare only one standard phenol solution. Prepare 500 mL standard phenol solution of a strength approximately equal to the phenolic content of that portion of original sample used for final analysis. Also prepare a 500-mL distilled water blank.

Continue as described in ¶ a, above, but measure absorbances of sample and standard phenol solution against the blank at 460 nm.

5. Calculation

a. For Procedure *a:*

$$\mu\text{g phenol/L} = \frac{A}{B} \times 1000$$

where:

A = µg phenol in sample, from calibration curve, and
B = mL original sample.

b. For Procedure *b,* calculate the phenol content of the original sample:

$$\mu\text{g phenol/L} = \frac{C \times D \times 1000}{E \times B}$$

where:

C = µg standard phenol solution,
D = absorbance reading of sample,
E = absorbance of standard phenol solution, and
B = mL original sample.

6. Precision and Bias

Because the ‘‘phenol’’ value is based on C_6H_5OH, this method yields only an approximation and represents the minimum amount of phenols present. This is true because the phenolic reactivity to 4-aminoantipyrine varies with the types of phenols present.

In a study of 40 refinery wastewaters analyzed in duplicate at concentrations from 0.02 to 6.4 mg/L the average relative standard deviation was ± 12%. Data are not available for precision at lower concentrations.

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5530 D. Direct Photometric Method

1. General Discussion

a. Principle: Steam-distillable phenolic compounds react with 4-aminoantipyrine at pH 7.9 ± 0.1 in the presence of potassium ferricyanide to form a colored antipyrine dye. This dye is kept in aqueous solution and the absorbance is measured at 500 nm.

b. Interference: Interferences are eliminated or reduced to a minimum by using the distillate from the preliminary distillation procedure.

c. Minimum detectable quantity: This method has less sensitivity than Method C. The minimum detectable quantity is 10 μg phenol when a 5-cm cell and 100 mL distillate are used.

2. Apparatus

a. Photometric equipment: Spectrophotometer equipped with absorption cells providing

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light paths of 1 to 5 cm for use at 500 nm.

b. pH meter.

3. Reagents

See Section 5530C.3.

4. Procedure

Place 100 mL distillate, or a portion containing not more than 0.5 mg phenol diluted to 100 mL, in a 250-mL beaker. Prepare a 100-mL distilled water blank and a series of 100-mL phenol standards containing 0.1, 0.2, 0.3, 0.4, and 0.5 mg phenol. Treat sample, blank, and standards as follows: Add 2.5 mL 0.5*N* NH_4OH solution and immediately adjust to pH 7.9 ± 0.1 with phosphate buffer. Add 1.0 mL 4-aminoantipyrine solution, mix well, add 1.0 mL $K_3Fe(CN)_6$ solution, and mix well.

After 15 min, transfer to cells and read absorbance of sample and standards against the blank at 500 nm.

5. Calculation

a. Use of calibration curve: Estimate sample phenol content from photometric readings by using a calibration curve constructed as directed in Section 5530C.4*a*.

$$\text{mg phenol/L} = \frac{A}{B} \times 1000$$

where:

A = mg phenol in sample, from calibration curve, and
B = mL original sample.

b. Use of single phenol standard:

$$\text{mg phenol/L} = \frac{C \times D \times 1000}{E \times B}$$

where:

C = mg standard phenol solution,
D = absorbance of sample, and
E = absorbance of standard phenol solution.

6. Precision and Bias

Precision and bias data are not available.

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5540 SURFACTANTS*#(129)

5540 A. Introduction

1. Occurrence and Significance

Surfactants enter waters and wastewaters mainly by discharge of aqueous wastes from household and industrial laundering and other cleansing operations. A surfactant combines in a single molecule a strongly hydrophobic group with a strongly hydrophilic one. Such molecules tend to congregate at the interfaces between the aqueous medium and the other phases of the system such as air, oily liquids, and particles, thus imparting properties such as foaming, emulsification, and particle suspension.

The surfactant hydrophobic group generally is a hydrocarbon radical (R) containing about 10 to 20 carbon atoms. The hydrophilic groups are of two types, those that ionize in water and those that do not. Ionic surfactants are subdivided into two categories, differentiated by the charge. An anionic surfactant ion is negatively charged, e.g., $(RSO_3)^{-}Na^{+}$, and a cationic one is positively charged, e.g., $(RMe_3N)^{+}Cl^{-}$. Nonionizing (nonionic) surfactants commonly contain a polyoxyethylene hydrophilic group ($ROCH_2CH_2OCH_2CH_2$......OCH_2CH_2OH, often abbreviated RE, where n is the average number of -OCH_2CH_2- units in the hydrophilic group). Hybrids of these types exist also.

In the United States, ionic surfactants amount to about two thirds of the total surfactants used and nonionics to about one third. Cationic surfactants amount to less than one tenth of the ionics and are used generally for disinfecting, fabric softening, and various cosmetic purposes rather than for their detersive properties. At current detergent and water usage levels the surfactant content of raw domestic wastewater is in the range of about 1 to 20 mg/L. Most domestic wastewater surfactants are dissolved in equilibrium with proportional amounts adsorbed on particulates. Primary sludge concentrations range from 1 to 20 mg adsorbed anionic surfactant per gram dry weight.[1] In environmental waters the surfactant concentration generally is below 0.1 mg/L except in the vicinity of an outfall or other point source of entry.[2]

2. Analytical Precautions

Because of inherent properties of surfactants, special analytical precautions are necessary. Avoid foam formation because the surfactant concentration is higher in the foam phase than in the associated bulk aqueous phase and the latter may be significantly depleted. If foam is formed, let it subside by standing, or collapse it by other appropriate means, and remix the liquid phase before sampling. Adsorption of surfactant from aqueous solutions onto the walls of containers, when concentrations below about 1 mg/L are present, may seriously deplete the bulk aqueous phase. Minimize adsorption errors, if necessary, by rinsing container with sample, and for

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anionic surfactants by adding alkali phosphate (e.g., 0.03*N* KH_2PO_4).[3]

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5540 B. Surfactant Separation by Sublation

1. General Discussion

a. Principle: The sublation process isolates the surfactant, regardless of type, from dilute aqueous solution, and yields a dried residue relatively free of nonsurfactant substances. It is accomplished by bubbling a stream of nitrogen up through a column containing the sample and an overlying layer of ethyl acetate. The surfactant is adsorbed at the water-gas interfaces of the bubbles and is carried into the ethyl acetate layer. The bubbles escape into the atmosphere leaving behind the surfactant dissolved in ethyl acetate. The solvent is separated, dehydrated, and evaporated, leaving the surfactant as a residue suitable for analysis. This procedure is the same as that used by the Organization for Economic Co-operation and Development (OECD),[1] following the development by Wickbold.[2,3]

b. Interferences: The sublation method is specific for surfactants, because any substance preferentially adsorbed at the water-gas interface is by definition a surfactant. Although nonsurfactant substances largely are rejected in this separation process, some amounts will be carried over mechanically into the ethyl acetate.

c. Limitations: The sublation process separates only dissolved surfactants. If particulate matter is present it holds back an equilibrium amount of adsorbed surfactant. As sublation removes the initially dissolved surfactant, the particulates tend to reequilibrate and their adsorbed surfactants redissolve. Thus, continued sublation eventually should remove substantially all adsorbed surfactant. However, if the particulates adsorb the surfactant tightly, as sewage particulates usually do, complete removal may take a very long time. The procedure given herein calls for preliminary filtration and measures only dissolved surfactant. Determine adsorbed surfactant content by analyzing particulates removed by filtration; no standard method is available now.

d. Operating conditions: Make successive 5-min sublations from 1 L of sample containing 5 g $NaHCO_3$ and 100 g NaCl. Under the conditions specified, extensive transfer of surfactant

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occurs in the first sublation and is substantially complete in the second.[2-4]

e. Quantitation: Quantitate the surfactant residue by the procedures in Section 5540C or Section 5540D. Direct weighing of the residue is not useful because the weight of surfactant isolated generally is too low, less than a milligram, and varied amounts of mechanically entrained nonsurfactants may be present. The procedure is applicable to water and wastewater samples.

2. Apparatus

a. Sublator: A glass column with dimensions as shown in Figure 5540:1. For the sintered glass disk use a coarse-porosity frit (designation ‘‘c’’–nominal maximum pore diam 40 to 60 µm as measured by ASTM E-128) of the same diameter as the column internal diameter. Volume between disk and upper stopcock should be approximately 1 L.

b. Gas washing bottle, as indicated in Figure 5540:1, working volume 100 mL or more.

c. Separatory funnel, working volume 250 mL, preferably with inert TFE stopcock.

d. Filtration equipment, suitable for 1-L samples, using medium-porosity qualitative-grade filter paper.

e. Gas flowmeter, for measuring flows up to l L/min.

3. Reagents

a. Nitrogen, standard commercial grade.

b. Ethyl acetate: CAUTION: *Ethyl acetate is flammable and its vapors can form explosive mixtures with air.*

c. Sodium bicarbonate, $NaHCO_3$.

d. Sodium chloride, NaCl.

e. Water, surfactant-free.

4. Procedure

a. Sample size: Select a sample to contain not more than 1 to 2 mg surfactant.[4] For most waters the sample volume will be about 1 L; for wastewater use a smaller volume.

b. Filtration: Filter sample through medium-porosity qualitative filter paper. Wash filter paper by discarding the first few hundred milliliters of filtrate.

c. Assembly: Refer to Figure 5540:1.

Connect nitrogen cylinder through flowmeter to inlet of gas washing bottle. Connect gas outlet at top of sublator to a gas scrubber or other means for disposing of ethyl acetate vapor (e.g., vent to a hood or directly outdoors). In the absence of a flowmeter, ensure proper gas flow rate by measuring volume of gas leaving the sublator, with a water-displacement system.

d. Charging: Fill gas washing bottle about two-thirds full with ethyl acetate. Rinse sublation column with ethyl acetate and discard rinse. Place measured filtered sample in sublator and add 5

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g $NaHCO_3$, 100 g NaCl, and sufficient water to bring the level up to or slightly above the upper stopcock (about 1 L total volume). If sample volume permits, add salts as a solution in 400 mL water or dissolve them in the sample and quantitatively transfer to the sublator. Add 100 mL ethyl acetate by running it carefully down the wall of the sublator to form a layer on top of the sample.

e. Sublation: Start the nitrogen flow, increasing the rate carefully to 1 L/min initially but do not exceed a rate at which the liquid phases begin vigorous intermixing at their interface. Avoid overly vigorous intermixing, which will lead to back-extraction of the surfactant into the aqueous phase and to dissolution of ethyl acetate. Continue sublation for 5 min at 1 L/min. If a lower flow rate is necessary to avoid phase intermixing, prolong sublation time proportionally. If the volume of the upper phase has decreased by more than about 20%, repeat the operation on a new sample but avoid excessive intermixing at the interface. Draw off entire ethyl acetate layer through upper stopcock into the separatory funnel; return any transferred water layer to the sublator. Filter ethyl acetate layer into a 250-mL beaker through a dry, medium-porosity, qualitative filter paper (prewashed with ethyl acetate to remove any adventitious surfactant) to remove any remaining aqueous phase.

Repeat process of preceding paragraph with a second 100-mL layer of ethyl acetate, using the same separatory funnel and filter, and finally rinse sublator wall with another 20 mL, all into the original beaker.

Evaporate ethyl acetate from the beaker on a steam bath in a hood, blowing a gentle stream of nitrogen or air over the liquid surface to speed evaporation and to minimize active boiling. Evaporate the first 100 mL during the second sublation to avoid overfilling the beaker. To avoid possible solute volatilization, discontinue heating after removing the ethyl acetate. The sublated surfactant remains in the beaker as a film of residue.

Draw off aqueous layer in the sublator and discard, using the stopcock just above the sintered disk to minimize disk fouling.

5. Precision and Bias

Estimates of the efficiency of surfactant transfer and recovery in the sublation process include the uncertainties of the analytical methods used in quantitating the surfactant. At present the analytical methods are semiquantitative for surfactant at levels below 1 mg/L in environmental samples.

With various known surfactants at 0.2 to 2 mg/L and appropriate analytical methods, over 90% of added surfactant was recovered in one 5-min sublation from 10% NaCl. Without NaCl, recovery of nonionics was over 90% but recovery of anionics and cationics was only 2 to 25%.[4]

Five laboratories studied the recovery of five anionic surfactant types from concentrations of 0.05, 0.2, 1.0, and 5.0 mg/L in aqueous solutions.[5] The amount in each solution was determined directly by methylene blue analysis and compared with the amount recovered in the sublation process, also analyzed by methylene blue. The overall average recovery was 95.9% with a standard deviation of ± 7.4 (n = 100). The extreme individual values for recovery were 65% and

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115% and the other 98 values ranged from 75% to 109%. Recovery did not depend on surfactant concentration (average recoveries ranging from 94.7% at 5.0 mg/L to 96.8% at 1.0 mg/L) nor on the surfactant type (average recoveries ranging from 94.7% to 96.6%). Average recoveries at the five laboratories ranged from 90.0% to 98.0%.

Application of the sublation method in three laboratories to eight different samples of raw wastewater in duplicate gave the results shown in Table 5540:I. Methylene blue active substances (MBAS) recovery in double sublation averaged 87 ± 16% of that determined directly on the filtered wastewater; these results would have been influenced by any nonsurfactant MBAS that might have been present. Repeating double sublation on the spent aqueous phase yielded another 0.02 mg MBAS and another 0.08 mg cobalt thiocyanate active substances (CTAS). Adding 0.05 to 0.10 mg of known linear alkylbenzene sulfonate (LAS) or 0.50 to 0.67 mg of known linear alcohol-based $C_{12-18}E_{11}$ to the same sublator contents and again running double sublation resulted in over 90% recovery of the amount added.

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5540 C. Anionic Surfactants as MBAS

1. General Discussion

a. Definition and principle: Methylene blue active substances (MBAS) bring about the transfer of methylene blue, a cationic dye, from an aqueous solution into an immiscible organic liquid upon equilibration. This occurs through ion pair formation by the MBAS anion and the methylene blue cation. The intensity of the resulting blue color in the organic phase is a measure of MBAS. Anionic surfactants are among the most prominent of many substances, natural and synthetic, showing methylene blue activity. The MBAS method is useful for estimating the

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anionic surfactant content of waters and wastewaters, but the possible presence of other types of MBAS always must be kept in mind.

This method is relatively simple and precise. It comprises three successive extractions from acid aqueous medium containing excess methylene blue into chloroform ($CHCl_3$), followed by an aqueous backwash and measurement of the blue color in the $CHCl_3$ by spectrophotometry at 652 nm. The method is applicable at MBAS concentrations down to about 0.025 mg/L.

b. Anionic surfactant responses: Soaps do not respond in the MBAS method. Those used in or as detergents are alkali salts of $C_{10–20}$ fatty acids $[RCO_2]^-Na^+$, and though anionic in nature they are so weakly ionized that an extractable ion pair is not formed under the conditions of the test. Nonsoap anionic surfactants commonly used in detergent formulations are strongly responsive. These include principally surfactants of the sulfonate type $[RSO_3]^-Na^+$, the sulfate ester type $[ROSO_3]^-Na^+$, and sulfated nonionics $[REOSO_3]^-Na^+$. They are recovered almost completely by a single $CHCl_3$ extraction.

Linear alkylbenzene sulfonate (LAS) is the most widely used anionic surfactant and is used to standardize the MBAS method. LAS is not a single compound, but may comprise any or all of 26 isomers and homologs with structure $[R'C_6H_4SO_3]^-Na^+$, where R′ is a linear secondary alkyl group ranging from 10 to 14 carbon atoms in length. The manufacturing process defines the mixture, which may be modified further by the wastewater treatment process.

Sulfonate- and sulfate-type surfactants respond together in MBAS analysis, but they can be differentiated by other means. The sulfate type decomposes upon acid hydrolysis; the resulting decrease in MBAS corresponds to the original sulfate surfactant content while the MBAS remaining corresponds to the sulfonate surfactants. Alkylbenzene sulfonate can be identified and quantified by infrared spectrometry after purification.[1] LAS can be distinguished from other alkylbenzene sulfonate surfactants by infrared methods.[2] LAS can be identified unequivocally and its detailed isomer-homolog composition determined by desulfonation-gas chromatography.[3]

c. Interferences: Positive interferences result from all other MBAS species present; if a direct determination of any individual MBAS species, such as LAS, is sought, all others interfere. Substances such as organic sulfonates, sulfates, carboxylates and phenols, and inorganic thiocyanates, cyanates, nitrates, and chlorides also may transfer more or less methylene blue into the chloroform phase. The poorer the extractability of their ion pairs, the more effective is the aqueous backwash step in removing these positive interferences; interference from chloride is eliminated almost entirely and from nitrate largely so by the backwash. Because of the varied extractability of nonsurfactant MBAS, deviations in $CHCl_3$ ratio and backwashing procedure may lead to significant differences in the total MBAS observed, although the recovery of sulfonate- and sulfate-type surfactants will be substantially complete in all cases.

Negative interferences can result from the presence of cationic surfactants and other cationic materials, such as amines, because they compete with the methylene blue in the formation of ion

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pairs. Particulate matter may give negative interference through adsorption of MBAS. Although some of the adsorbed MBAS may be desorbed and paired during the $CHCl_3$ extractions, recovery may be incomplete and variable.

Minimize interferences by nonsurfactant materials by sublation if necessary (Section 5540B). Other countermeasures are nonstandard. Remove interfering cationic surfactants and other cationic materials by using a cation-exchange resin under suitable conditions.[3] Handle adsorption of MBAS by particulates preferably by filtering and analyzing the insolubles. With or without filtration, adsorbed MBAS can be desorbed by acid hydrolysis; however, MBAS originating in any sulfate ester-type surfactant present is destroyed simultaneously.[1] Sulfides, often present in raw or primary treated wastewater, may react with methylene blue to form a colorless reduction product, making the analysis impossible. Eliminate this interference by prior oxidation with hydrogen peroxide.

d. Molecular weight: Test results will appear to differ if expressed in terms of weight rather than in molar quantities. Equimolar amounts of two anionic surfactants with different molecular weights should give substantially equal colors in the $CHCl_3$ layer, although the amounts by weight may differ significantly. If results are to be expressed by weight, as generally is desirable, the average molecular weight of the surfactant measured must be known or a calibration curve made with that particular compound must be used. Because such detailed information generally is lacking, report results in terms of a suitable standard calibration curve, for example ‘‘0.65 mg MBAS/L (calculated as LAS, mol wt 318).’’

e. Minimum detectable quantity: About 10 µg MBAS (calculated as LAS).

f. Application: The MBAS method has been applied successfully to drinking water samples. In wastewater, industrial wastes, and sludge, numerous materials normally present can interfere seriously if direct determination of MBAS is attempted. Most nonsurfactant aqueous-phase interferences can be removed by sublation. The method is linear over an approximate range of 10 to 200 µg of MBAS standard. This may vary somewhat, depending on source of standard material.

2. Apparatus

a. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer,* for use at 652 nm, providing a light path of 1 cm or longer.

2) *Filter photometer,* providing a light path of 1 cm or longer and equipped with a red color filter exhibiting maximum transmittance near 652 nm.

b. Separatory funnels: 500-mL, preferably with inert TFE stopcocks and stoppers.

3. Reagents

a. Stock LAS solution: Weigh an amount of the reference material*#(130) equal to 1.00 g LAS on a 100% active basis. Dissolve in water and dilute to 1000 mL; 1.00 mL = 1.00 mg LAS. Store in a refrigerator to minimize biodegradation. If necessary, prepare weekly.

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b. Standard LAS solution: Dilute 10.00 mL stock LAS solution to 1000 mL with water; 1.00 mL = 10.0 µg LAS. Prepare daily.

c. Phenolphthalein indicator solution, alcoholic.

d. Sodium hydroxide, NaOH, 1*N*.

e. Sulfuric acid, H_2SO_4, 1*N* and 6*N*.

f. Chloroform, $CHCl_3$: CAUTION: *Chloroform is toxic and a suspected carcinogen. Take appropriate precautions against inhalation and skin exposure*.

g. Methylene blue reagent: Dissolve 100 mg methylene blue†#(131) in 100 mL water. Transfer 30 mL to a 1000-mL flask. Add 500 mL water, 41 mL 6*N* H_2SO_4, and 50 g sodium phosphate, monobasic, monohydrate, $NaH_2PO_4 \cdot H_2O$. Shake until dissolved. Dilute to 1000 mL.

h. Wash solution: Add 41 mL 6*N* H_2SO_4 to 500 mL water in a 1000-mL flask. Add 50 g $NaH_2PO_4 \cdot H_2O$ and shake until dissolved. Dilute to 1000 mL.

i. Methanol, CH_3OH. CAUTION: *Methanol vapors are flammable and toxic; take appropriate precautions.*

j. Hydrogen peroxide, H_2O_2, 30%.

k. Glass wool: Pre-extract with $CHCl_3$ to remove interferences.

l. Water, reagent-grade, MBAS-free. Use for making all reagents and dilutions.

4. Procedure

a. Preparation of calibration curve: Prepare an initial calibration curve consisting of at least five standards covering the referenced (¶ 1 *f*) or desired concentration range. Provided that linearity is demonstrated over the range of interest (r = 0.995 or better) run daily check standards at the reporting limit and a concentration above the expected samples' concentration. Check standard results should be within 25% of original value at the reporting limit and 10% of original value for all others. Otherwise, prepare a new calibration curve.

Prepare a series of separatory funnels for a reagent blank and selected standards. Pipet portions of standard LAS solution (¶ 3*b*) into funnels. Add sufficient water to make the total volume 100 mL in each separatory funnel. Treat each standard as described in ¶s 4*d* and e following, and plot a calibration curve of absorbance vs. micrograms LAS taken, specifying the molecular weight of the LAS used.

b. Sample size: For direct analysis of waters and wastewaters, select sample volume on the basis of expected MBAS concentration:

Expected MBAS Concentration *mg/L*	Sample Taken *mL*

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Expected MBAS Concentration *mg/L*	Sample Taken *mL*
0.025–0.080	400
0.08 –0.40	250
0.4 –2.0	100

If expected MBAS concentration is above 2 mg/L, dilute sample containing 40 to 200 µg MBAS to 100 mL with water.

For analysis of samples purified by sublation, dissolve sublate residue (Section 5540B.4*e*) in 10 to 20 mL methanol, quantitatively transfer the entire amount (or a suitable portion if more than 200 µg MBAS is expected) to 25 to 50 mL water, evaporate without boiling until methanol is gone, adding water as necessary to avoid going to dryness, and dilute to about 100 mL with water.

c. Peroxide treatment: If necessary to avoid decolorization of methylene blue by sulfides, add a few drops of 30% H_2O_2.

d. Ion pairing and extraction:

1) Add sample to a separatory funnel. Make alkaline by dropwise addition of 1*N* NaOH, using phenolphthalein indicator. Discharge pink color by dropwise addition of 1*N* H_2SO_4.

2) Add 10 mL $CHCl_3$ and 25 mL methylene blue reagent. Rock funnel vigorously for 30 s and let phases separate. Alternatively, place a magnetic stirring bar in the separatory funnel; lay funnel on its side on a magnetic mixer and adjust speed of stirring to produce a rocking motion. Excessive agitation may cause emulsion formation. To break persistent emulsions add a small volume of isopropyl alcohol (<10 mL); add same volume of isopropyl alcohol to all standards. Some samples require a longer period of phase separation than others. Before draining $CHCl_3$ layer, swirl gently, then let settle.

3) Draw off $CHCl_3$ layer into a second separatory funnel. Rinse delivery tube of first separatory funnel with a small amount of $CHCl_3$. Repeat extraction two additional times, using 10 mL $CHCl_3$ each time. If blue color in water phase becomes faint or disappears, discard and repeat, using a smaller sample.

4) Combine all $CHCl_3$ extracts in the second separatory funnel. Add 50 mL wash solution and shake vigorously for 30 s. Emulsions do not form at this stage. Let settle, swirl, and draw off $CHCl_3$ layer through a funnel containing a plug of glass wool into a 100-mL volumetric flask; filtrate must be clear. Extract wash solution twice with 10 mL $CHCl_3$ each and add to flask through the glass wool. Rinse glass wool and funnel with $CHCl_3$. Collect washings in volumetric flask, dilute to mark with $CHCl_3$, and mix well.

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e. Measurement: Determine absorbance at 652 nm against a blank of $CHCl_3$.

5. Calculation

From the calibration curve (¶ 4*a*) read micrograms of apparent LAS (mol wt _______) corresponding to the measured absorbance.

$$\text{mg MBAS/L} = \frac{\mu\text{g apparent LAS}}{\text{mL original sample}}$$

Report as ''MBAS, calculated as LAS, mol wt _______.''

6. Precision and Bias

A synthetic sample containing 270 µg LAS/L in distilled water was analyzed in 110 laboratories with a relative standard deviation of 14.8% and a relative error of 10.6%.

A tap water sample to which was added 480 µg LAS/L was analyzed in 110 laboratories with a relative standard deviation of 9.9% and a relative error of 1.3%.

A river water sample with 2.94 mg LAS/L added was analyzed in 110 laboratories with a relative standard deviation of 9.1% and a relative error of 1.4%.[4]

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5540 D. Nonionic Surfactants as CTAS

1. General Discussion

a. Definition and principle: Cobalt thiocyanate active substances (CTAS) are those that react with aqueous cobalt thiocyanate solution to give a cobalt-containing product extractable into an organic liquid in which it can be measured. Nonionic surfactants exhibit such activity, as may other natural and synthetic materials; thus, estimation of nonionic surfactants as CTAS is possible only if substantial freedom from interfering CTAS species can be assured.

The method requires sublation to remove nonsurfactant interferences and ion exchange to remove cationic and anionic surfactants, partition of CTAS into methylene chloride from excess aqueous cobalt thiocyanate by a single extraction, and measurement of CTAS in the methylene

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chloride by spectrophotometry at 620 nm. Lower limit of detectability is around 0.1 mg CTAS, calculated as $C_{12-18}E_{11}$. Beyond the sublation step the procedure is substantially identical to that of the Soap and Detergent Association (SDA).[1]

b. Nonionic surfactant responses: For pure individual molecular species the CTAS response is negligible up to about RE_5, where it increases sharply and continues to increase more gradually for longer polyether chains.[2,3] Fewer than about six oxygens in the molecule do not supply enough cumulative coordinate bond strength to hold the complex together. Commercial nonionic surfactants generally range from about RE_7 to RE_{15}; however, each such product, because of synthesis process constraints, is actually a mixture of many individual species ranging from perhaps RE_0 to RE_2 in a Poisson distribution averaging RE.

The hydrophobes used for nonionic surfactants in the U.S. household detergent industry are mainly linear primary and linear secondary alcohols with chain lengths ranging from about 12 to about 18 carbon atoms. Nonionics used in industrial operations include some based on branched octyl- and nonylphenols. These products give strong CTAS responses that may differ from each other, on a weight basis, by as much as a factor of 2. Specifically, eight such products showed responses from 0.20 to 0.36 absorbance units/mg by the SDA procedure.[1]

As with anionic surfactants measured as MBAS, the nonionic surfactants found in water and wastewater might have CTAS responses at least as varied as their commercial precursors because the proportions of the individual molecular species will have been changed by biochemical and physicochemical removal at varied rates, and further because their original molecular structures may have been changed by biodegradation processes.

c. Reference nonionic surfactant: Until it is practical to determine the nature and molecular composition of an unknown mixed CTAS, and to calculate or determine the CTAS responses of its component species, exact quantitation of uncharacterized CTAS in a sample in terms of weight is not possible. Instead, express the analytical result in terms of some arbitrarily chosen reference nonionic surfactant, i.e., as the weight of the reference that gives the same amount of CTAS response. The reference is the nonionic surfactant $C_{12}-{}_{18}E_{11}$, derived from a mixture of linear primary alcohols ranging from 12 to 18 carbon atoms in chain length by reaction with ethylene oxide in a molar ratio of 1:11. $C_{12}-{}_{18}E_{11}$ is reasonably representative of nonionic surfactants in commercial use; its CTAS response is about 0.21 absorbance units/mg.

If the identity of the nonionic surfactant in the sample is known, use that same material in preparing the calibration curve.

d. Interferences: Both anionic and cationic surfactants may show positive CTAS response[1,4] but both are removed in the ion-exchange step. Sublation removes nonsurfactant interferences. Physical interferences occur if some of the CTAS is adsorbed on particulate matter. Avoid such interference by filtering out the particulates for the sublation step; this will measure only dissolved CTAS.

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e. Minimum detectable quantity: About 0.1 mg CTAS, calculated as $C_{12-18}E_{11}$, which corresponds to 0.1 mg/L in a 1-L sample.

f. Application: The method is suitable for determining dissolved nonionic surfactants of the ethoxylate type in most aqueous systems.

2. Apparatus

a. Sublation apparatus: See Section 5540B.2.

b. Ion-exchange column, glass, about 1- × 30-cm. Slurry anion-exchange resin in methanol and pour into column to give a bed about 10 cm deep. Insert plug of glass wool and then add a 10-cm bed of cation-exchange resin on top in the same manner. One column may be used for treating up to six sublated samples before repacking.

c. Spectrophotometer and 2.0-cm stoppered cells, suitable for measuring absorbance at 620 nm.

d. Separatory funnels, 125-mL, preferably with TFE stopcock and stopper.

e. Extraction flasks, Soxhlet type, 150-mL.

3. Reagents

a. Sublation reagents: See Section 5540B.3.

b. Anion-exchange resin, polystyrene-quaternary ammonium-type,*#(132) 50- to 100-mesh, hydroxide form. To convert chloride form to hydroxide, elute with 20 bed volumes of 1*N* NaOH and wash with methanol until free alkali is displaced.

c. Cation-exchange resin, polystyrene-sulfonate type,†#(133) 50- to 100-mesh, hydrogen form.

d. Cobaltothiocyanate reagent: Dissolve 30 g $Co(NO_3)_2 \cdot 6H_2O$ and 200 g NH_4SCN in water and dilute to 1 L. This reagent is stable for at least 1 month at room temperature.

e. Reference nonionic surfactant, $C_{12-18}E_{11}$: Reaction product of C_{12-18} linear primary alcohol with ethylene oxide in 1:11 molar ratio.‡#(134)

f. Reference nonionic surfactant stock solution, methanolic, approximately 2 mg nonionic/mL methanol: Quantitatively transfer entire contents (approximately 1 g nonionic) from preweighed ampule into 500-mL volumetric flask, thoroughly rinse ampule with methanol, make up to volume with methanol, and reweigh dried ampule. Calculate concentration in milligrams per milliter as in ¶ 5*a*. Because of possible phase separation, use all material in the ampule.

g. Reference nonionic surfactant standard solution, methanolic, approximately 0.1 mg nonionic/mL methanol: Dilute 10.00 mL stock solution to 200 mL with methanol. Exact concentration is 1/20 that of the stock solution.

h. Sodium hydroxide, NaOH: 1*N*.

i. Glass wool: Pre-extract with chloroform or methylene chloride.

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j. Methanol, CH_3OH: CAUTION: *Methanol vapors are flammable and toxic; take appropriate precautions.*

k. Methylene chloride, CH_2Cl_2: CAUTION: *Methylene chloride vapors are toxic; take adequate precautions.*

l. Water: Use distilled or deionized, CTAS-free water for making reagents and dilutions.

4. Procedure

a. Purification by sublation: Proceed according to Section 5540B, using sample containing no more than 2 mg CTAS. (NOTE: For samples of known character containing no interfering materials, omit this step.)

b. Ion-exchange removal of anionic and cationic surfactants: Dissolve sublation residue in 5 to 10 mL methanol and transfer quantitatively to ion-exchange column. Elute with methanol at 1 drop/s into a clean, dry 150-mL extraction flask until about 125 mL is collected. Evaporate methanol on a steam bath aided by a gentle stream of clean, dry nitrogen or air, taking care to avoid loss by entrainment; remove from heat as soon as the methanol is completely evaporated. (NOTE: With samples of known character containing no anionic or cationic materials, omit step *b*.)

c. CTAS calibration curve: Into a series of 150-mL extraction flasks containing 10 to 20 mL methanol place 0.00, 5.00, 10.00, 20.00, and 30.00 mL reference nonionic surfactant standard solution and evaporate just to dryness. Continue as in ¶s 4*d* and e, below, and plot a calibration curve of absorbance against milligrams of reference nonionic taken, specifying its identity (e.g., $C_{12-18}E_{11}$ and lot number).

d. Cobalt complexing and extraction: Charge a 125-mL separatory funnel with 5 mL cobaltothiocyanate reagent. With precautions against excessive and variable evaporation of the methylene chloride, dissolve residue from ion-exchange operation, ¶ 4*b*, by adding 10.00 mL methylene chloride and swirling for a few seconds. Immediately transfer by pouring into the separatory funnel. *Do not rinse flask.* (NOTE: Because of the volatility of methylene chloride, rigidly standardize these operations with respect to handling and elapsed time; alternatively, evaporate the methanol in 200-mL erlenmeyer flasks to be stoppered with glass or TFE stoppers during dissolution. Transfer as directed here is incomplete, but in this case it will not introduce error because the loss of nonionics is exactly compensated for by the diminished volume of the organic layer in the extraction.) Shake separatory funnel vigorously for 60 s and let layers separate. Run lower layer into a 2.0-cm cell through a funnel containing a plug of pre-extracted glass wool and stopper. Be sure filtrate is absolutely clear. (NOTE: If desired, clarify by running the lower layer into a 12-mL centrifuge tube, stopper, spin at or above 1000 × *g* for 3 min, and transfer to the cell by a Pasteur pipet; use same procedure for both calibration and samples.)

e. Measurement: Determine absorbance at 620 nm against a blank of methylene chloride. (NOTE: If haze develops in the cell, warm slightly with a hot air gun or heat lamp to clarify.)

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5. Calculations

a. Nonionic surfactant in reference nonionic stock solution ¶ 3 *f*:

mg nonionic/mL methanol = mg reference sample/500 mL

b. Nonionic surfactant in sample: From the calibration curve read milligrams of reference nonionic corresponding to the measured absorbance:

mg CTAS/L = mg apparent nonionic/L sample

Report as ‘‘CTAS, calculated as nonionic surfactant $C_{12-18}E_{11}$.’’

6. Precision and Bias

Twenty-four samples of 6.22% w/v solution of reference nonionic surfactant $C_{12-18}E_{11}$ were analyzed in three laboratories by CTAS alone, without sublation or ion exchange. The overall relative standard deviation was about 3%. Results of the three laboratories individually were:

Laboratory	% w/w ± SD
A	6.08 ± 0.14 (n = 36)
B	6.56 ± 0.17 (n = 6)
C	6.25 ± 0.14 (n = 36)
Overall	6.20 ± 0.19 (n = 78)

Samples of raw wastewater were freed of surfactants by four successive sublations, then 0.50 or 0.67 mg reference nonionic surfactant $C_{12-18}E_{11}$ was added and carried through the entire sequence of sublation, ion exchange, and CTAS extraction. Recoveries averaged 92% with overall standard deviation around 6%:

Laboratory	% Recovery ± SD
A	87 ± 4 (n = 4)
B	97 ± 1 (n = 4)
Overall	92 ± 6 (n = 8)

The above data relate to the bias and precision of the method when applied to a known nonionic surfactant. When the nature of the nonionic surfactant is unknown, there is greater uncertainty. The response of the reference $C_{12-18}E_{11}$ is about 0.21 absorbance units/mg, while that of the eight nonionic types mentioned under ¶ 1*b* ranged from 0.20 to 0.36, and

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environmental nonionics might differ still more. If the nonionic surfactant in the sample has a response of 0.42, the result calculated in terms of milligrams $C_{12-18}E_{11}$ would be double the actual milligrams of the unknown nonionic.

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5550 TANNIN AND LIGNIN*#(135)

5550 A. Introduction

Lignin is a plant constituent that often is discharged as a waste during the manufacture of paper pulp. Another plant constituent, tannin, may enter the water supply through the process of vegetable matter degradation or through the wastes of the tanning industry. Tannin also is applied in the so-called internal treatment of boiler waters, where it reduces scale formation by causing the production of a more easily handled sludge.

5550 B. Colorimetric Method

1. General Discussion

a. Principle: Both lignin and tannin contain aromatic hydroxyl groups that react with Folin phenol reagent (tungstophosphoric and molybdophosphoric acids) to form a blue color suitable for estimation of concentrations up to at least 9 mg/L. However, the reaction is not specific for lignin or tannin, nor for compounds containing aromatic hydroxyl groups, inasmuch as many

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other reducing materials, both organic and inorganic, respond similarly.

b. Applicability: This method is generally suitable for the analysis of any organic chemical that will react with Folin phenol reagent to form measurable blue color at the concentration of interest. However, many compounds are reactive (see ¶ 1*c*) and each yields a different molar extinction coefficient (color intensity). Hence, the analyst must demonstrate conclusively the absence of interfering substances.

c. Interferences: Any substance able to reduce Folin phenol reagent will produce a false positive response. Organic chemicals known to interfere include hydroxylated aromatics, proteins, humic substances, nucleic acid bases, fructose, and amines. Inorganic substances known to interfere include iron (II), manganese (II), nitrite, cyanide, bisulfite, sulfite, sulfide, hydrazine, and hydroxylamine hydrochloride. Both 2 mg ferrous iron/L and 125 mg sodium sulfite/L individually produce a color equivalent to 1 mg tannic acid/L.

d. Minimum detectable concentrations: Approximately 0.025 mg/L for phenol and tannic acid and 0.1 mg/L for lignin with a 1-cm-path-length spectrophotometer.

2. Apparatus

Colorimetric equipment: One of the following is required:

a. Spectrophotometer, for use at 700 nm. A light path of 1 cm or longer yields satisfactory results.

b. Filter photometer, provided with a red filter exhibiting maximum transmittance in the wavelength range of 600 to 700 nm. Sensitivity improves with increasing wavelength. A light path of 1 cm or longer yields satisfactory results.

c. Nessler tubes, matched, 100-mL, tall form, marked at 50-mL volume.

3. Reagents

a. Folin phenol reagent: Transfer 100 g sodium tungstate, $Na_2WO_4 \cdot 2H_2O$, and 25 g sodium molybdate, $Na_2MoO_4 \cdot 2H_2O$, together with 700 mL distilled water, to a 2000-mL flat-bottom boiling flask. Add 50 mL 85% H_3PO_4 and 100 mL conc HCl. Connect to a reflux condenser and boil gently for 10 h. Add 150 g Li_2SO_4, 50 mL distilled water, and a few drops of liquid bromine. Boil without condenser for 15 min to remove excess bromine. Cool to 25°C, dilute to 1 L, and filter. Store finished reagent, which should have no greenish tint, in a tightly stoppered bottle to protect against reduction by air-borne dust and organic materials.

Alternatively, purchase commercially prepared Folin phenol reagent and use before the recommended expiration date.

b. Carbonate-tartrate reagent: Dissolve 200 g Na_2CO_3 and 12 g sodium tartrate, $Na_2C_4H_4O_6 \cdot 2H_2O$, in 750 mL hot distilled water, cool to 20°C, and dilute to 1 L.

c. Stock solution: The nature of the substance present in the sample dictates the choice of chemical used to prepare the standard, because each substance produces a different color

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intensity. Weigh 1.000 g tannic acid, tannin, lignin, or other compound being used for boiler water treatment or known to be a contaminant of the water sample. Dissolve in distilled water and dilute to 1000 mL. If the identity of the compound in the water sample is not known, use phenol and report results as ‘‘substances reducing Folin phenol reagent’’ in mg phenol/L. Interpret such results with caution.

Note that tannin and lignin are not individual chemical species of known molecular weight and structure; rather, they are substances containing a spectrum of chemicals of different molecular weights. Their chemical properties depend on source and method of isolation. If a particular substance is being added to the water, use it to prepare the stock solution.

d. Standard solution: Dilute 10.00 mL or 50.00 mL stock solution to 1000 mL with distilled water; 1.00 mL = 10.0 or 50.0 µg active ingredient.

4. Procedure

Bring 50-mL portions of clear sample and standards to a temperature above 20°C and maintain within a ± 2°C range. Add in rapid succession 1 mL Folin phenol reagent and 10 mL carbonate-tartrate reagent. Allow 30 min for color development. Compare visually against simultaneously prepared standards in matched Nessler tubes or make photometric readings against a reagent blank prepared at the same time. Use the following guide for instrumental measurement at a wavelength of 700 nm:

Tannic Acid in 61-mL Final Volume *µg*	Lignin in 61-mL Final Volume *µg*	Light Path *cm*
50–600	100–1500	1
10–150	30–400	5

Report results in mg/L of the compound known to be present or as ‘‘substances reducing Folin phenol reagent’’ in mg phenol/L.

5. Precision and Bias

In a single laboratory analyzing seven replicates for phenol at 0.1 mg/L the precision was ± 7% and recovery was 107%.

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5560 ORGANIC AND VOLATILE ACIDS*#(136)

5560 A. Introduction

The measurement of organic acids, either by adsorption and elution from a chromatographic column or by distillation, can be used as a control test for anaerobic digestion. The chromatographic separation method is presented for organic acids (B), while a method using distillation (C) is presented for volatile acids. Alternative methods using GC or IC are available in the literature and may provide better speciation information for specific situations, but have not yet been recommended as standard methods.

Volatile fatty acids are classified as water-soluble fatty acids that can be distilled at atmospheric pressure. These volatile acids can be removed from aqueous solution by distillation, despite their high boiling points, because of co-distillation with water. This group includes water-soluble fatty acids with up to six carbon atoms.

The distillation method is empirical and gives incomplete and somewhat variable recovery. Factors such as heating rate and proportion of sample recovered as distillate affect the result, requiring the determination of a recovery factor for each apparatus and set of operating conditions. However, it is suitable for routine control purposes. Removing sludge solids from the sample reduces the possibility of hydrolysis of complex materials to volatile acids.

5560 B. Chromatographic Separation Method for Organic Acids

1. General Discussion

a. Principle: An acidified aqueous sample containing organic acids is adsorbed on a column of silicic acid and the acids are eluted with *n*-butanol in chloroform ($CHCl_3$). The eluate is collected and titrated with standard base. All short-chain (C_1 to C_6) organic acids are eluted by this solvent system and are reported collectively as total organic acids.

b. Interference: The $CHCl_3$-butanol solvent system is capable of eluting organic acids other than the volatile acids and also some synthetic detergents. Besides the so-called volatile acids, crotonic, adipic, pyruvic, phthalic, fumaric, lactic, succinic, malonic, gallic, aconitic, and oxalic acids; alkyl sulfates; and alkyl-aryl sulfonates are adsorbed by silicic acid and eluted.

c. Precautions: Basic alcohol solutions decrease in strength with time, particularly when exposed repeatedly to the atmosphere. These decreases usually are accompanied by the

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appearance of a white precipitate. The magnitude of such changes normally is not significant in process control if tests are made within a few days of standardization. To minimize this effect, store standard sodium hydroxide (NaOH) titrant in a tightly stoppered borosilicate glass bottle and protect from atmospheric carbon dioxide (CO_2) by attaching a tube of CO_2-absorbing material, as described in the inside front cover. For more precise analyses, standardize titrant or prepare before each analysis.

Although the procedure is adequate for routine analysis of most sludge samples, volatile-acids concentrations above 5000 mg/L may require an increased amount of organic solvent for quantitative recovery. Elute with a second portion of solvent and titrate to reveal possible incomplete recoveries.

2. Apparatus

a. Centrifuge or filtering assembly.

b. Crucibles, Gooch or medium-porosity fritted-glass, with filtering flask and vacuum source. Use crucibles of sufficient size (30 to 35 mL) to hold 12 g silicic acid.

c. Separatory funnel, 1000-mL.

3. Reagents

a. Silicic acid, specially prepared for chromatography, 50 to 200 mesh: Remove fines by slurrying in distilled water and decanting supernatant after settling for 15 min. Repeat several times. Dry washed acid in an oven at 103°C until *absolutely dry,* then store in a desiccator.

b. Chloroform-butanol reagent: Mix 300 mL reagent-grade $CHCl_3$, 100 mL *n*-butanol, and 80 mL 0.5*N* H_2SO_4 in a separatory funnel. Let water and organic layers separate. Drain off lower organic layer through a fluted filter paper into a dry bottle. CAUTION: *Chloroform has been classified as a cancer suspect agent. Use hood for preparation of reagent and conduct of test.*

c. Thymol blue indicator solution: Dissolve 80 mg thymol blue in 100 mL absolute methanol.

d. Phenolphthalein indicator solution: Dissolve 80 mg phenolphthalein in 100 mL absolute methanol.

e. Sulfuric acid, H_2SO_4, conc.

f. Standard sodium hydroxide, NaOH, 0.02*N:* Dilute 20 mL 1.0*N* NaOH stock solution to 1 L with absolute methanol. Prepare stock in water and standardize in accordance with the methods outlined in Section 2310B.3*d*.

4. Procedure

a. Pretreatment of sample: Centrifuge or vacuum-filter enough sludge to obtain 10 to 15 mL clear sample in a small test tube or beaker. Add a few drops of thymol blue indicator solution, then conc H_2SO_4 dropwise, until definitely red to thymol blue (pH = 1.0 to 1.2).

b. Column chromatography: Place 12 g silicic acid in a Gooch or fritted-glass crucible and

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apply suction to pack column. Tamp column while applying suction to reduce channeling when the sample is applied. With a pipet, distribute 5.0 mL acidified sample as uniformly as possible over column surface. Apply suction momentarily to draw sample into silicic acid. Release vacuum as soon as last portion of sample has entered column. Quickly add 65 mL $CHCl_3$-butanol reagent and apply suction. Discontinue suction just before the last of reagent enters column. Do not reuse columns.

c. Titration: Remove filter flask and purge eluted sample with N_2 gas or CO_2-free air immediately before titrating. (Obtain CO_2-free air by passing air through a CO_2 absorbant.*#(137))

Titrate sample with standard 0.02*N* NaOH to phenolphthalein end point, using a fine-tip buret and taking care to avoid aeration. The fine-tip buret aids in improving accuracy and precision of the titration. Use N_2 gas or CO_2-free air delivered through a small glass tube to purge and mix sample and to prevent contact with atmospheric CO_2 during titration.

d. Blank: Carry a distilled water blank through steps ¶s 4*a* through 4*c*.

5. Calculation

$$\text{Total organic acids (mg as acetic acid/L)} = \frac{(a - b) \times N \times 60\,000}{\text{mL sample}}$$

where:

a = mL NaOH used for sample,
b = mL NaOH used for blank, and
N = normality of NaOH.

6. Precision

Average recoveries of about 95% are obtained for organic acid concentrations above 200 mg as acetic acid/L. Individual tests generally vary from the average by approximately 3%. A greater variation results when lower concentrations of organic acids are present. Titration precision expressed as the standard deviation is about ±0.1 mL (approximately ±24 mg as acetic acid/L).

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5560 C. Distillation Method

1. General Discussion

a. Principle: This technique recovers acids containing up to six carbon atoms. Fractional recovery of each acid increases with increasing molecular weight. Calculations and reporting are on the basis of acetic acid. The method often is applicable for control purposes. Because it is empirical, carry it out exactly as described. Because the still-heating rate, presence of sludge solids, and final distillate volume affect recovery, determine a recovery factor.

b. Interference: Hydrogen sulfide (H_2S) and CO_2 are liberated during distillation and will be titrated to give a positive error. Eliminate this error by discarding the first 15 mL of distillate and account for this in the recovery factor. Resides on glassware from some synthetic detergents have been reported to interfere; use water and dilute acid rinse cycles to prevent this problem.

2. Apparatus

a. Centrifuge, with head to carry four 50-mL tubes or 250-mL bottles.

b. Distillation flask, 500-mL capacity.

c. Condenser, about 76 cm long.

d. Adapter tube.

e. pH meter or recording titrator: See Section 2310B.2*a*.

f. Distillation assembly: Use a conventional distilling apparatus. To minimize fluctuations in distillation rate, supply heat with a variable-wattage electrical heater.

3. Reagents

a. Sulfuric acid, H_2SO_4, 1 + 1.

b. Standard sodium hydroxide titrant, 0.1*N:* See Section 2310B.3*c*.

c. Phenolphthalein indicator solution.

d. Acetic acid stock solution, 2000 mg/L: Dilute 1.9 mL conc CH_3COOH to 1000 mL with deionized water. Standardize against 0.1*N* NaOH.

4. Procedure

a. Recovery factor: To determine the recovery factor, *f*, for a given apparatus, dilute an appropriate volume of acetic acid stock solution to 250 mL in a volumetric flask to approximate

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the expected sample concentration and distill as for a sample. Calculate the recovery factor

$$f = \frac{a}{b}$$

where:

a = volatile acid concentration recovered in distillate, mg/L, and
b = volatile acid concentration in standard solution used, mg/L.

b. Sample analysis: Centrifuge 200 mL sample for 5 min. Pour off and combine supernatant liquors. Place 100 mL supernatant liquor, or smaller portion diluted to 100 mL, in a 500-mL distillation flask. Add 100 mL distilled water, four to five clay chips or similar material to prevent bumping, and 5 mL H_2SO_4. Mix so that acid does not remain on bottom of flask. Connect flask to a condenser and adapter tube and distill at the rate of about 5 mL/ min. Discard the first 15 mL and collect exactly 150 mL distillate in a 250-mL graduated cylinder. Titrate with 0.1N NaOH, using phenolphthalein indicator, a pH meter, or an automatic titrator. The end points of these three methods are, respectively, the first pink coloration that persists on standing a short time, pH 8.3, and the inflection point of the titration curve (see Section 2310). Titration at 95°C produces a stable end point.

Distill and analyze a blank and reference standard with each sample batch to insure system performance.

5. Calculation

$$\text{mg volatile acids as acetic acid/L} = \frac{\text{mL NaOH} \times N \times 60\,000}{\text{mL sample} \times f}$$

where:

N = normality of NaOH, and
f = recovery factor.

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5710 FORMATION OF TRIHALOMETHANES AND OTHER DISINFECTION BY-PRODUCTS*#(138)

5710 A. Introduction

Trihalomethanes (THMs) are produced during chlorination of water. Only four THM compounds normally are found: chloroform ($CHCl_3$), bromodichloromethane ($CHBrCl_2$), dibromochloromethane ($CHBr_2Cl$), and bromoform ($CHBr_3$). Additional chlorination by-products can be formed (including haloacetic acids and halonitriles; for example, see 5710D) during the relatively slow organic reactions that occur between free chlorine and naturally occurring organic precursors such as humic and fulvic acids. The formation potentials of these additional by-products also can be determined, but different quenching agents and different analytical procedures may be needed. Predictive models for estimating/calculating THM formation exist, but because eventual THM concentrations cannot be calculated precisely from conventional analyses, methods to determine the potential for forming THMs are useful in evaluating water treatment processes or water sources or for predicting THM concentrations in a distribution system.

To obtain reproducible and meaningful results, control such variables as temperature, reaction time, chlorine dose and residual, and pH. THM formation is enhanced by elevated temperatures and alkaline pH and by increasing concentrations of free chlorine residuals, although THM formation tends to level off at free chlorine residuals of 3 mg/L and above; a longer reaction time generally increases THM formation.[1,2]

Low concentrations of bromide exist in most natural waters and are responsible for the formation of brominated organic compounds. Figure 5710:1 shows that an oxidant ratio of about 40 times more chlorine than bromine (on a molar basis, = 40 on the *x* axis) is required to form equimolar amounts of substituted organic chloride and bromide (= 1 on the *y* axis); small amounts of bromide also can increase the molar yield of THMs.[3]

The possible addition of organic precursors contained in reagent solutions cannot be accounted for accurately without a great deal of extra work; therefore, sample dilutions resulting from reagent additions (approximately 2%) are ignored in the final calculations. However, sample dilution may need to be taken into account if other volumes are used. Sample dilution also changes the concentrations of bromide and organic matter, potentially leading to speciation changes.

1. Definition of Terms

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See Figure 5710:2a and Figure 5710:2b for the relationship among the following definitions.

Total trihalomethane (*$TTHM_T$*) is the sum of all four THM compound concentrations (see Section 5710B.5) produced at any time *T* (usually days). $TTHM_0$ is the total THM concentration at the time of sampling. $TTHM_0$ concentrations can range from nondetectable, which usually means the sample has not been chlorinated, to several hundred micrograms per liter if the sample has been chlorinated. $TTHM_7$ is the sum of all four THM compound concentrations produced during reactions of sample precursors with excess free chlorine over a 7-d reaction time.

Standard reaction conditions (see Section 5710B) are as follows: free chlorine residual at least 3 mg/L and not more than 5 mg/L at the end of a 7-d reaction (incubation) period, with sample incubation temperature of 25 ± 2°C, and pH controlled at 7.0 ± 0.2 with phosphate buffer. Standard conditions are not intended to simulate water treatment processes but are most useful for estimating the concentration of THM precursors, as well as for measuring the effectiveness of water-treatment options for reducing levels of THM precursors in the raw water.

Special applications permit different test conditions, but they must be stated explicitly when reporting results.

Trihalomethane formation potential (*THMFP or Δ THMFP*) is the difference between the final $TTHM_T$ concentration and the initial $TTHM_0$ concentration. If sample does not contain chlorine at the time of collection, $TTHM_0$ will be close to zero and the term THMFP may be used. If sample does contain chlorine at the time of collection, because of formation of THMs, use the term Δ THMFP (the increase of THM concentration during storage) when reporting the difference between TTHM concentrations.

The term ''THMFP'' often has been equated to the final TTHM concentration, even if the sample had contained chlorine when collected. To use this definition, explicitly define the term when reporting data.

Simulated distribution system trihalomethane (*SDS-THM*), Section 5710C, is the concentration of TTHMs in a sample that has been disinfected comparably to finished drinking water and under the same conditions and time as in a water distribution system. It includes pre-existing THMs plus those produced during storage. This method can be used in conjunction with laboratory, pilot, or full-scale studies of treatment processes to estimate expected concentration of THMs in a distribution system. Do not use SDS-THM to estimate the precursor removal efficiency of a treatment process, because THM yields are highly variable at low chlorine residual concentrations. For SDS-type testing, low chlorine residuals (< 1 mg Cl_2/L) are often encountered, thereby resulting in lower THM formation than would be obtained for higher chlorine residuals. THM yields at higher chlorine concentrations (> 3 mg/ L) tend to level off and become relatively independent of variations in free chlorine residuals.

2. Sampling and Storage

Collect samples in 1-L glass bottles sealed with TFE-lined screw caps. If multiple tests will

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be performed for each sample, or if many different analyses will be performed for each sample (see Section 5710D), collect 4 L instead. Further, if multiple reaction time periods will be used to study reaction rates, process a separate sample bottle (taken from one large sample) for each time period. One liter is enough sample to determine chlorine demand and duplicate THM analyses. Use only freshly collected samples and process immediately. If this is not possible, store samples at 4°C and analyze as soon as possible. Significant sample degradation can occur in unpreserved samples within 24 h.

If the sample has been chlorinated previously, collect the sample with minimum turbulence and fill the sample bottle completely to avoid loss of THMs already present. Determine the zero-time THM concentration ($TTHM_0$), if desired, on another sample collected at the same time and dechlorinated immediately with fresh sodium sulfite solution, crystals, or sodium thiosulfate.

3. References

1. STEVENS, A.A. & J.M. SYMONS. 1977. Measurement of trihalomethanes and precursor concentration changes. *J. Amer. Water Works Assoc.* 69:546.
2. SYMONS, J.M., A.A. STEVENS, R.M. CLARK, E.E. GELDREICH, O.T. LOVE, JR. & J. DEMARCO. 1981. Treatment Techniques for Controlling Trihalomethanes in Drinking Water. EPA-600/2-81-56, U.S. Environmental Protection Agency, Cincinnati, Ohio.
3. SYMONS, J.M., S.W. KRASNER, L. SIMMS & M. SCLIMENTI. 1993. Measurement of trihalomethane and precursor concentrations revisited: The impact of bromide ion. *J. Amer. Water Works Assoc.* 85(1):51.

5710 B. Trihalomethane Formation Potential (THMFP)

1. General Discussion

a. Principle: Under standard conditions, samples are buffered at pH 7.0 ± 0.2, chlorinated with an excess of free chlorine, and stored at 25 ± 2°C for 7 d to allow the reaction to approach completion. As a minimum, pH is buffered at a defined value and a free chlorine residual of 3 to 5 mg Cl_2/L exists at the end of the reaction time. THM concentration is determined by using liquid-liquid extraction (see Section 6232B) or purge and trap (see Section 6200).

b. Interference: If the water was exposed to free chlorine before sample collection (e.g., in a water treatment plant), a fraction of precursor material may have been converted to THM. Take special precautions to avoid loss of volatile THMs by minimizing turbulence and filling sample bottle completely.

Interference will be caused by any organic THM-precursor materials present in the reagents or adsorbed on glassware. Heat nonvolumetric glassware to 400°C for 1 h, unless routine analysis of blanks demonstrates that this precaution is unnecessary. Reagent impurity is difficult to control. It usually is traceable to reagent water containing bromide ion or organic impurities.

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Use high-grade reagent water as free of organic contamination and chlorine demand as possible. If anion exchange is used to remove bromide or organic ions, follow such treatment by activated carbon adsorption (see Section 1080).

Other interferences include volatile organic compounds (VOCs), including THMs and chlorine-demanding substances. VOCs may co-elute with THMs during analysis. THMs or other interfering substances that are present as the result of a chemical spill, etc., will bias the results.

Nitrogenous species and other constituents may interfere in the determination of free residual chlorine. Add enough free chlorine to oxidize chorine-demanding substances and leave a free chlorine residual of at least 3 mg/L, but not more than 5 mg/L, at the end of the incubation period. A free chlorine residual of at least 3 mg/L decreases the likelihood that a combined residual will be mistaken for a free residual and assures that THM formation occurs under conditions that are reasonably independent of variations in chlorine residual concentrations.

c. Minimum detectable quantity: The sensitivity of the method is determined by the analytical procedure used for THM.

2. Apparatus

a. Incubator, to maintain temperature of 25 ± 2°C.

b. Bottles, glass, with TFE-lined screw caps to contain 245 to 255 mL, 1-L, 4-L.

c. Vials, glass, 25- or 40-mL with TFE-lined screw caps.

d. pH meter, accurate to within ± 0.1 unit.

3. Reagents

Prepare aqueous reagents in organic-free water (¶ 3*e* below) unless chlorine-demand-free water (¶ 3 *f* below) is specified.

a. Standardized stock hypochlorite solution: Dilute 1 mL, using a 1-mL volumetric pipet, 5% aqueous sodium hypochlorite (NaOCl, to be referred to as stock hypochlorite) solution to approximately 25 mL with chlorine-demand-free water (see ¶ 3 *f* below), mix well, and titrate to a starch-iodide end point using 0.100*N* sodium thiosulfate titrant (see Section 4500-Cl.B). Calculate chlorine concentration of the stock hypochlorite solution as:

$$\text{Stock hypochlorite, mg Cl}_2\text{/mL} = \frac{N \times 35.45 \times \text{mL titrant}}{\text{mL stock hypochlorite added}}$$

where *N* is the normality of the titrant (= 0.100). Use at least 10 mL titrant; if less is required, standardize 2 mL stock hypochlorite solution. Measure chlorine concentration each time a dosing solution (¶ 3*b* below) is made; discard stock hypochlorite solution if its chlorine concentration is less than 20 mg Cl_2/mL.

b. Chlorine dosing solution, 5 mg Cl_2/mL: Calculate volume of stock hypochlorite solution

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required to produce a chlorine concentration of 5 mg Cl_2/mL:

$$\text{mL required} = \frac{1250}{\text{stock hypochlorite conc. mg } Cl_2/\text{mL}}$$

Dilute this volume of stock hypochlorite solution in a 250-mL volumetric flask to the mark with chlorine-demand-free water. Mix and transfer to an amber bottle, seal with a TFE-lined screw cap, and refrigerate. Keep away from sunlight. Discard if the chlorine concentration drops below 4.7 mg Cl_2/mL; this will occur if the ‘‘initial chlorine concentration’’ (as determined in ¶ 4*a* below) drops below 94 mg Cl_2/L.

c. Phosphate buffer: Dissolve 68.1 g potassium dihydrogen phosphate (anhydrous), KH_2PO_4, and 11.7 g sodium hydroxide, NaOH, in 1 L water. Refrigerate when not in use. If a precipitate develops, filter through a glass fiber filter. After buffer is added to a sample, a pH of 7.0 should result. Check before use with a sample portion that can be discarded.

d. Sodium sulfite solution: Dissolve 10 g sodium sulfite, Na_2SO_3, in 100 mL water. Use for dechlorination: 0.1 mL will destroy about 5 mg residual chlorine. Make fresh every 2 weeks. NOTE: More dilute solutions oxidize readily.

e. Organic-free water: Pass distilled or deionized water through granular-activated-carbon columns. A commercial system may be used.*#(139) Special techniques such as preoxidation, activated carbon adsorption (perhaps accompanied by acidification and subsequent reneutralization), or purging with an inert gas to remove THMs may be necessary.

f. Chlorine-demand-free water: Follow the procedure outlined in Section 4500-Cl.C.3*m*, starting with organic-free water. After residual chlorine has been destroyed completely, purge by passing a clean, inert gas through the water until all THMs have been removed.

g. DHBA solution: Dissolve 0.078 g anhydrous 3,5-dihydroxy-benzoic acid (DHBA) in 2 L chlorine-demand-free water. This solution is not stable; make fresh before each use.

h. Nitric or hydrochloric acid, HNO_3 or HCl, concentrations of 1:1, 1.0*N*, and 0.1*N*.

i. Sodium hydroxide, NaOH, 1.0*N* and 0.1*N*.

j. Borate buffer (optional): Dissolve 30.9 g anhydrous boric acid, H_3BO_3, and 10.8 g sodium hydroxide, NaOH, in 1 L water. Filter any precipitate that may form with a glass fiber filter. This solution will keep sample pH at 9.2; check before using. NOTE: Waters containing significant amounts of calcium may precipitate calcium phosphate (or carbonate) at higher pH values.

k. Mixed buffer (optional): Mix equal amounts of phosphate and borate buffer solutions, then adjust pH as desired before using with samples. Determine the amount of acid or base needed on a separate sample that can be discarded. This mixed buffer is reasonably effective in the pH range of 6 to 11.

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4. Procedure

a. Chlorine demand determination: Determine or accurately estimate the 7-d sample chlorine demand. A high chlorine dose is specified below to drive the reaction close to completion quickly. The following procedure yields only a rough estimate of chlorine demand; other techniques may be used.

Pipet 5 mL chlorine dosing solution into a 250-mL bottle, fill completely with chlorine-demand-free water, and cap with a TFE-lined screw cap. Shake well. Titrate 100 mL with 0.025*N* sodium thiosulfate to determine the initial chlorine concentration (C_I). This should be about 100 mg Cl_2/L. Pipet 5 mL phosphate buffer and 5 mL chlorine dosing solution into a second 250-mL bottle, fill completely with sample, and seal with a TFE-lined screw cap. Store in the dark for at least 4 h at 25°C. After storage, determine chlorine residual (C_R). Calculate chlorine demand (D_{Cl}) as follows:

$$D_{Cl} = C_I - C_R$$

where:

D_{Cl} = chlorine demand, mg Cl_2/L,
C_R = chlorine residual of sample after at least 4 h storage, mg Cl_2/L, and
C_I = initial (dosed) chlorine concentration, mg Cl_2/L.

b. Sample chlorination: If sample contains more than 200 mg/L alkalinity or acidity, adjust pH to 7.0 ± 0.2 using 0.1 or 1.0*N* HNO_3, HCl, or NaOH and a pH meter. With a graduated pipet, transfer appropriate volume of the 5 mg Cl_2/mL chlorine dosing solution, V_D, into sample bottle:

$$V_D = \frac{D_{Cl} + 3}{5} \times \frac{V_S}{1000}$$

where:

V_S = volume of sample bottle, mL, and
V_D = volume of dosing solution required, mL.

Add 5 mL phosphate buffer solution if using a 250-mL sample bottle (or 1 mL buffer/50 mL sample) and fill completely with sample. Immediately seal with a TFE-lined screw cap, shake well, and store in the dark at 25 ± 2°C for 7 d. Analyze a reagent blank (¶ 4*d*) with each batch of samples. To increase the likelihood of achieving the desired chlorine residual concentration (3 to 5 mg/L) at the end of the 7-d reaction period, dose several sample portions to provide a range of

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chlorine concentrations, with each chlorine dose differing in increments of 2 mg Cl_2/L.

c. Sample analysis: After the 7-d reaction period, place 0.1 mL sulfite reducing solution in a 25-mL vial and gently and completely fill vial with sample. If free chlorine residual has not been determined previously, measure it using a method accurate to 0.1 mg/L and able to distinguish free and combined chlorine (see Section 4500-Cl). Adjust pH to the value required by the method chosen for chlorine analysis. [NOTE: If other by-products are to be measured, a different quenching agent may be needed (see 5710D.4). Also, if sample portions have been dosed with different chlorine concentrations, first determine the free chlorine residual and select only that portion having the required chlorine residual concentration of 3 to 5 mg/L for further processing.] If THMs will not be analyzed immediately, lower the pH to <2 by adding 1 or 2 drops of 1:1 HCl to the reduced sample in the vial. Seal vial with TFE-lined screw cap. Store samples at 4°C until ready for THM analysis (preferably no longer than 7 d). Let sample reach room temperature before beginning analysis.

d. Reagent blank: Add 1 mL chlorine dosing solution to 50 mL phosphate buffer, mix, and completely fill a 25-mL vial, seal with a TFE-lined screw cap, and store with samples. (NOTE: This reagent blank is for quality control of reagent solutions only and is not a true blank, because the reagent concentrations in this blank are considerably higher than those in samples. THM concentrations in the reagent blank will be biased high and cannot be subtracted from sample values. Make no further dilutions before the reaction because the reagent water itself might contribute to THM formation.) After reaction for 7 d, pipet 1 mL sulfite reducing solution into a 250-mL bottle and add, without stirring, 5.0 mL reacted reagent mixture. Immediately fill bottle with organic-free water that has been purged free of THMs and seal with a TFE-lined screw cap. Mix. Analyze a portion of this reagent blank for THMs using the same method used for samples. The sum of all THM compounds in the reagent blank should be less than 5 μg TTHM as $CHCl_3$/L.

The reagent blank is a rough measure of THMs contributed by reagents added to the samples, but it cannot be used as a correction factor. If the reagent blank is greater than 5% of the sample value or greater than 5 μg TTHM/L, whichever is larger, additional treatment for reagent water is necessary. See Section 1080. It also may be necessary to obtain reagents of higher purity. Analyze a reagent blank each time samples are analyzed and each time fresh reagents are prepared.

5. Calculation

Report concentration of each of the four common THM compounds separately because it is desirable to know their relative concentrations. Larger amounts of bromine-substituted compounds, relative to chlorine-substituted compounds, indicate a higher concentration of dissolved bromide in the water (see Figure 5710:1). Also report free chlorine concentration at end of reaction time along with the incubation time, temperature, and pH.

THM concentrations may be reported as a single value as micrograms $CHCl_3$ per liter (μg

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$CHCl_3$/L), or micromoles per liter (μ*M*). Do not use the simple sum of mass units micrograms per liter except when required for regulatory reporting. Compute TTHM concentration using one of the following equations:

To report TTHM in units of μg $CHCl_3$/L:

$$TTHM = A + 0.728B + 0.574C + 0.472D$$

where:

A = μg $CHCl_3$/L,

B = μg $CHBrCl_2$/L,

C = μg $CHBr_2Cl$/L, and

D = μg $CHBr_3$/L.

To report TTHM in units of μ*M* as $CHCl_3$:

$$TTHM = \frac{TTHM,\ \mu g\ CHCl_3/L}{119}$$

To report TTHM on a weight basis as μg/L (not used except for regulatory purposes):

$$TTHM = A + B + C + D$$

To report a change of TTHM concentration over 7 d:

$$\Delta\ THMFP = TTHM_7 - TTHM_0$$

Finally, if $TTHM_0 = 0$, then:

$$THMFP = TTHM_7 = \Delta\ THMFP$$

Do not make blank correction or a correction for sample dilution resulting from addition of reagents. If conditions differ from pH of 7, 25°C, 7-d reaction time, and 3 to 5 mg/L chlorine residual, report these nonstandard test conditions with the results. Nonstandard test conditions may mimic water quality conditions in a specific distribution system or may be relevant to other investigations (see Section 5710C).

6. Quality Control

a. Use dihydroxy-benzoic acid solution (DHBA) as a quality-control check, especially for the presence of interfering bromides in reagents or reagent water.

Dilute 1.0 mL chlorine dosing solution to 1000 mL with chlorine-demand-free water

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(diluted chlorine dosing solution). Pipet 5 mL phosphate buffer solution (pH = 7.0) into each of two 250-mL bottles; add 1.00 mL DHBA solution to one bottle and fill both bottles completely with diluted chlorine dosing solution; seal with TFE-lined screw caps. Store in the dark for 7 d at 25 ± 2°C, and analyze as directed in ¶ 4*c*.

b. The THM concentration of the solution containing the added DHBA minus the THM concentration of the blank (i.e., the bottle that does not contain added DHBA, which is a true blank for this application only and differs from the reagent blank discussed in ¶ 4*b* above) should be about 119 µg/L THM as $CHCl_3$, with essentially no contribution from bromide-containing THMs. If there is a significant contribution from brominated THMs, 10% or more of the total THM, it may be necessary to remove bromide from the reagent water or to obtain higher-purity reagents containing less bromide. Determine source of bromide and correct the problem. If the THM concentration of the water blank exceeds 20 µg/L, treat reagent water to reduce contamination.

7. Precision and Bias

The precision of this method is determined by the analytical precision and bias of the method used for measuring THM as well as the control of variables such as pH, chlorine residual, temperature, sample homogeneity, etc. Method bias can be determined only for synthetic solutions (e.g., the DHBA solution), because THM formation potential is not an intrinsic property of the sample but rather a quantity defined by this method.

Table 5710:I presents single-operator precision and bias data for samples processed under standard conditions. The values were obtained by analyzing DHBA solutions and blanks. The expected value for the samples listed is 116 µg/L TTHM (as $CHCl_3$), rather than 119 µg/L, because the DHBA reagent used was only 97% pure. Percent recovery was calculated by the formula:

$$\%\ \text{recovery} = \frac{\text{DHBA sample} - \text{average blank}}{116} \times 100$$

Table 5710:II presents the same data set, except that the pH of samples and blanks was adjusted to 9.2 with borate buffer. Also included are results for single-operator precision with filtered river-water samples that had been diluted with 2 parts organic-free water to 1 part filtrate, again using borate-buffered samples at a pH of 9.2.

5710 C. Simulated Distribution System Trihalomethanes (SDS-THM)

1. General Discussion

a. Principle: The SDS-THM testing method uses bench-scale techniques to provide an

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estimate of the THMs formed in a distribution system after disinfection.[1] It may be used to estimate the THM concentration at any point in a distribution system or to evaluate the formation of other disinfectant by-products (5710D). However, to measure efficiency of any unit treatment process for precursor removal, see Section 5710B.

The term ''disinfection,'' rather than ''chlorination,'' is used because free chlorine residuals are not necessarily provided in all distribution systems. For example, monochloramine residuals may be used.

SDS-THM concentrations measured by this procedure generally will be lower than THM concentrations measured by procedures in 5710B because disinfectant concentrations used in SDS-type samples are intended to mimic conditions in a distribution system and are almost always lower than disinfectant concentrations used with standardized formation potential procedures.

Two types of SDS procedures may be used: (1) a simple storage method that requires only the addition, at the end of the desired storage time, of a quenching agent, sodium sulfite, to a sample collected from the entry to a distribution system; and (2) a comprehensive method that involves one or more steps described in Section 5710B, with appropriate modifications.

SDS procedures are not standard procedures in the traditional sense. Test variables are modified to mimic local distribution-system conditions with bench-top procedures. These conditions include temperature, pH, disinfectant dose and residual, bromide ion concentration, and reaction time (corresponding to the residence time of water within the distribution system). However, the method used to simulate a distribution system can be standardized according to specific needs.

2. Apparatus

See Section 5710B.2.

Temperature control: Appropriate equipment, such as a water bath or incubator, to control sample storage temperature, capable of a range of temperature adjustments required.

3. Reagents

See Section 5710B.3, and also Section 5710D.3, if applicable.

4. Procedure

a. Simple storage procedure: Measure and report both initial and final values for all variables, especially pH, temperature, $TTHM_0$ (if desired), and residual disinfectant concentration using a method accurate to 0.1 mg/L and able to distinguish among the various disinfectant forms—see Section 4500-Cl for chlorine analyses.

Collect treated, disinfected water from the clearwell of a treatment plant or other suitable location in either a 1-L or a 250-mL completely filled bottle. Seal with a TFE-lined screw cap and store at selected temperature for selected length of time. The storage time frequently chosen is the maximum residence time within the distribution system, and the temperature is

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representative of the distribution system.

A second sample for immediate quenching provides an initial THM concentration if desired ($TTHM_0$). Quench by adding 2 drops (0.1 mL) sodium sulfite solution to a 25-mL glass vial, and gently and completely fill vial with sample. [NOTE: If other by-products will be analyzed, a different quenching agent may be needed (see Section 5710D.4).]

At end of storage period, quench a portion of stored sample with sodium sulfite solution. Report all values (pH, temperature, and residual disinfectant concentration) together with THM results. Ideally, residual disinfectant concentration after storage equals residual disinfectant concentration found in the distribution system.

b. Bench-top procedure: Use any or all steps given in Section 5710B, except that all variables, such as disinfectant dose, residual concentrations, temperature, pH, and storage time are adjusted to simulate distribution system conditions. For better sample pH control, use a buffer such as the mixed-buffer solution (Section 5710B.3*k*). If a buffer is used, adjust buffer pH to the appropriate value before adding to the sample. Add buffer to a sample portion to be discarded after titrating to the desired pH with either NaOH or HCl solution; add this determined amount of acid or base to the buffer solution before adding to actual samples. When measuring disinfectant residuals, adjust sample pH to that required by the analytical method for the residual disinfectant, because the buffer capacity of the sample may be greater than the amount of buffer required by the method. For example, DPD Methods 4500-Cl.F and 4500-Cl.G require control of the pH within the range from 6.2 to 6.5 for stable color development, but the sample pH might be buffered to a value of 8.3, requiring adjustment of the sample pH to the appropriate range by addition of mineral acid before color development with DPD reagents.

Also add disinfectant to sample if it does not already contain enough to provide the desired disinfectant residual for the chosen storage time and temperature. Use the chlorine demand procedure (Section 5710B.4*a*) as a guide, or develop correlations between disinfectant use and TOC or other variables.

Process sample generally following procedures in 5710B. Measure and report both initial and final values of all test variables, especially temperature, pH, and residual disinfectant concentration, as well as all THM results.

5. Calculation

See Section 5710B.5. Report $TTHM_T$ values for SDS-type samples in any appropriate units, except that the concept of ‘‘formation potential’’ is not applicable. Use prefix ‘‘SDS-’’ to distinguish between ‘‘SDS-TTHM’’ and ‘‘THMFP’’ results. Also report each compound concentration (e.g., ‘‘µg/L SDS_T-$CHCl_3$’’).

6. Quality Control

See Section 5710B.6 for check on reagent purity or as a check on analytical precision and control by using a reaction with a pure, organic compound under more stable, standardized conditions. (This applies only to the formation of THM, using free chlorine.)

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7. Precision and Bias

No data are available. More variability of results is expected for SDS-type testing than for samples tested with the standard conditions of Section 5710B. A larger number of replicates will be required for SDS procedures, as opposed to standard conditions, to obtain reliable estimates of effects of treatment changes and techniques.

8. Reference

1. KOCH, B., S.W. KRASNER, M.J. SCLIMENTI & W.K. SCHIMPFF. 1991. Predicting the formation of DBPs by the simulated distribution system. *J. Amer. Water Works Assoc.* 83 (10):62.

5710 D. Formation of Other Disinfection By-Products (DBPs)

1. General Discussion

a. Principle: The techniques and principles discussed in Section 5710A through C may be applied to other disinfection by-products (DBPs) and total/dissolved organic halogen (TOX/DOX) as well as for trihalomethanes (THMs). Although all the DBPs listed in this method may result from chlorination reactions, some may be formed by disinfectants other than free chlorine.

This method may be extended to cover formation potentials (Section 5710B) and distribution system simulations (Section 5710C) for additional by-products formed by reactions between other disinfectants (ozone, chlorine dioxide, chloramines, etc.) with dissolved organic matter. Some of the commonly found DBPs are listed in this method, but many cannot be determined because well-defined analytical methods are not yet available.

The procedures by which formation potentials and behavior in distribution systems of other DBPs can be measured are exactly as described in 5710A through C, except that different quenching agents may be required for different compounds.

In general, by-product concentrations increase with reaction time, but exceptions exist and different variables may produce different results. For example, at high pH values, THM concentrations increase with time, but if the pH is high enough, trichloroacetic acid will not form at all; at high pH, however, the concentrations of dihaloacetonitriles (DHANs) quickly reach a maximum value in a relatively short time and then decrease because of hydrolysis reactions. Further, some compounds, such as brominated haloacetic acids, are not stable and can degrade during storage—either during a long reaction time (7 d may be too long for some compounds) or after the reaction has been quenched (even if stored at 4°C).

Small concentrations of bromide ion may have significant effects. If a compound contains more than one halogen atom in its molecular formula, compounds containing all the possible combinations of mixed chloro-/bromo- formulas might also be formed.

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The most common other disinfection by-products are: trihaloacetic acids (THAAs), including trichloroacetic acid (TCAA), bromodichloroacetic acid (BDCAA), dibromochloroacetic acid (DBCAA), and tribromoacetic acid (TBAA); dihaloacetic acids (DHAAs), including dichloroacetic acid (DCAA), bromochloroacetic acid (BCAA), and dibromoacetic acid (DBAA); monohaloacetic acids (MHAAs), including monochloroacetic acid (MCAA) and monobromoacetic acid (MBAA); chloral hydrate (CH); dihaloacetonitriles (DHANs), including dichloroacetonitrile (DCAN), bromochloroacetonitrile (BCAN), and dibromoacetonitrile (DBAN); 1,1,1-trichloropropanone (111-TCP); chloropicrin (CP), which may be produced either with free or combined chlorine; cyanogen chloride, formed either with free chlorine or monochloramine (and is more stable in solutions containing monochloramine); and dissolved organic halide (DOX) (see Section 5320). Total organic halide (TOX) also may be determined if the sample is not filtered.

The present method differs from those described in 5710B and C, in the means by which reactions are quenched: the different by-products formed require different quenching agents to stop reactions. Sodium sulfite is used to quench the THM reaction, but it may react with and degrade other compounds formed, such as DHANs. The procedure below lists the recommended quenching steps. Several portions of the same sample may require different quenching agents, depending upon the by-products to be determined. Use appropriate analytical methods to determine the different types of by-products formed.

2. Apparatus

See Section 5710B.2.

Vials: 40-mL glass vials with TFE-lined screw caps.

3. Reagents

In addition to the reagents listed in Section 5710B.3, the following reagents also may be needed, depending upon the by-products to be measured:

a. Ammonium chloride solution: Weigh 5 g NH_4Cl and dissolve in 100 mL organic-free water.

b. Nitric acid solution (approx. 3.5*N*): Dilute 80 mL conc HNO_3 (CAUTION: *strong oxidant*) to 250 mL with organic-free water.

c. Methyl tert-*butyl ether* (*MTBE*), highest purity.

d. Other reagents: Those required by analytical methods for determination of specific by-product concentrations.

4. Procedure

Procedures for the formation of by-products during reactions between disinfectants and dissolved organic matter have been described in Section 5710A through C, but the quenching agents needed to stop these reactions depend on the specific compounds to be analyzed. For THMs, the quenching agent is sodium sulfite, and its use has already been described (Section

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5710B.4.*c*). For the other by-products listed above, substitute as described below. Store all samples headspace-free and sealed with TFE-lined screw caps.

a. Chloral hydrate (CH): This compound may be analyzed with THMs. Use sulfite reducing solution to quench. Adjust sample pH 6 to 7 with (1.0*N* or 0.1*N*) HCl. Determine amount of acid to be added with a separate portion containing the same amount of sulfite reducing solution added to sample. Analyze by liquid-liquid extraction, capillary column, GC/electron capture analysis.[1]

b. DHANs, CP, and 111-TCP: Add 4 drops (0.2 mL) ammonium chloride solution to a 40-mL vial and nearly fill with sample. Add a predetermined amount of HCl that will adjust sample pH to 6 to 7 (see ¶ a above) and fill completely. (Add 3 drops of phosphate buffer solution for more control of sample pH, if desired, before determining amount of acid solution needed for pH adjustment.) NH_4Cl quenches the reaction by converting free chlorine to monochloramine. Analyze by liquid-liquid extraction, capillary column, GC/electron capture analysis.[1]

c. Haloacetic acids (HAAs), mono-, di-, and trihaloacetic acids: Add 4 drops (0.2 mL) NH_4Cl solution to a 250-mL bottle, and fill bottle completely with sample. Before acidifying and extracting sample, add 1 mL sodium sulfite solution to the 250-mL sample, mix well, and analyze by liquid-liquid extraction, capillary column, GC/electron capture analysis (see Section 6251 or equivalent methods[2]). Sodium sulfite can slowly degrade some of the brominated haloacetic acids during storage; do not add until just before acidification. If a GC/MS method is used, remove water in the sample by drying with sodium sulfate crystals before methylation.

d. Cyanogen chloride: Analyze by GC/MS purge and trap method,[3] which uses ascorbic acid to dechlorinate samples. Also see Section 4500-CN-J. Hydrolysis of cyanogen chloride to cyanate occurs rapidly in the pH range of 8.5 to 9.0 (within 30 min), but the reaction is much slower at pH values of 7.0 and below.

e. DOX: Add 1 mL sodium sulfite solution to a 250-mL bottle and nearly fill with sample. Add sufficient 3.5*N* HNO_3 to lower pH to 2.0 (approximately 12 drops, or 0.6 mL) and fill completely. Analyze according to Section 5320. Total organic halogen (TOX) also may be determined by the same method if the sample is not filtered.

f. Other disinfection by-products not mentioned above: Disinfectants, such as chlorine, ozone, monochloramine, chlorine dioxide, etc., may form other disinfection by-products. Formation potentials or SDS-type data also can be determined for these additional compounds.

5. Calculation

Report concentrations of each compound separately in μg/L. Compare concentrations of different compounds on a micromolar basis (micromoles/L, or μ*M*):

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$$\text{Compound, } \mu M = \frac{\text{compound concentration, } \mu g/L}{MW}$$

where:

MW = molecular weight of the compound.

Concentrations of a series of compounds that can be grouped together may sometimes be reported as one value. For example, the dihaloacetic acid group (DHAAs) includes DCAA, BCAA and DBAA and may be reported in terms of a group concentration, obtained by adding the molar concentrations of the separate compounds within the class:*#(140)

$$\text{DHAA, } \mu M = \frac{\text{DCAA, } \mu g/L}{129} + \frac{\text{BCAA, } \mu g/L}{173} + \frac{\text{DBAA, } \mu g/L}{219}$$

Alternatively, report in terms of µg/L as DCAA by multiplying the molar concentration by the molecular weight of DCAA:

$$\text{DHAA, } \mu g/L \text{ as DCAA} = \text{DHAA, } \mu M \times 129$$

or, for SDS testing:

$$\text{SDS-DHAA, } \mu g/L \text{ as DCAA} = \text{SDS-DHAA, } \mu M \times 129$$

The definitions given in Section 5710A through C also are valid. For example, if the initial concentration of disinfectant by-products (DHAAs, for instance) is zero or insignificant, then:

$$\text{DHAAFP} = \text{DHAA}_7.$$

Alternatively, if there is a significant concentration of initial by-product, then:

$$\Delta\text{DHAAFP} = \text{DHAA}_7 - \text{DHAA}_0.$$

6. Quality Control

See Section 5710B.6 for check on reagent purity or as a check on analytical precision and control by using a reaction with a pure, organic compound under more stable, standardized conditions. The test detailed in Section 5710B.6 applies only to THM formation using excess free chlorination conditions.

7. Precision and Bias

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Precision and bias measurements depend, in part, on the analytical procedure used to measure each specific disinfectant by-product concentration. These measurements also depend upon compound properties such as stability toward oxidation and biodegradation. In general, however, formation potential reactions should be reproducible to the extent indicated in Section 5710B for chlorination reactions. SDS-type reactions (5710C) would not, however, be expected to be as accurate or as precise, although such reactions should predict distribution system concentrations reasonably well.

8. References

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5910 UV-ABSORBING ORGANIC CONSTITUENTS*#(141)

5910 A. Introduction

1. Applications

Some organic compounds commonly found in water and wastewater, such as lignin, tannin, humic substances, and various aromatic compounds, strongly absorb ultraviolet (UV) radiation. UV absorption is a useful surrogate measure of selected organic constituents in fresh waters,[1-3] salt waters,[4-6] and wastewater.[7,8] Strong correlations may exist between UV absorption and organic carbon content, color, and precursors of trihalomethanes (THMs) and other disinfection by-products.[9,10] UV absorption also has been used to monitor industrial wastewater effluents[11] and to evaluate organic removal by coagulation,[10] carbon adsorption,[12,13,14] and other water treatment processes.[10] Specific absorption, the ratio of UV absorption to organic carbon concentration, has been used to characterize natural organic matter.[10,15,16]

Although UV absorption can be used to detect certain individual organic contaminants after separation (e.g., by HPLC), as described in Part 6000, the method described here is not suitable for detection of trace concentrations of individual chemicals. It is intended to be used to provide an *indication* of the aggregate concentration of UV-absorbing organic constituents.

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5910 B. Ultraviolet Absorption Method

1. General Discussion

a. Principle: UV-absorbing organic constituents in a sample absorb UV light in proportion to their concentration. Samples are filtered to control variations in UV absorption caused by particles. Adjustment of pH before filtration is optional.

UV absorption is measured at 253.7 nm (often rounded off to 254 nm). The choice of wavelength is arbitrary. Historically, 253.7 nm has been used as the standard wavelength; however, experienced analysts may choose a wavelength that minimizes interferences from compounds other than those of interest while maximizing absorption by the compound(s) of interest. If a wavelength other than 253.7 nm is used, state that wavelength when reporting results.

b. Interferences: The primary interferences in UV-absorption measurements are from colloidal particles, UV-absorbing organics other than those of interest, and UV-absorbing inorganics, notably ferrous iron, nitrate, nitrite, and bromide. Certain oxidants and reducing agents, such as ozone, chlorate, chlorite, chloramines, and thiosulfate, also will absorb ultraviolet light at 253.7 nm. Many natural waters and waters processed in drinking water treatment plants have been shown to be free of these interferences.

Evaluate and correct for UV absorption contributed by specific interfering substances. If cumulative corrections exceed 10% of the total absorption, select an alternate wavelength and/or use another method. Because UV absorption by organic matter may vary at pH values below 4 or above 10, avoid these values.[1]

A UV absorption scan from 200 to 400 nm can be used to determine presence of interferences. Typical absorption scans of natural organic matter are featureless curves of increasing absorption with decreasing wavelength. Sharp peaks or irregularities in the absorption scan may be indicative of inorganic interferences or unexpected organic contaminants. Because many organic compounds in water and wastewater (e.g., carboxylic acids and carbohydrates) do not absorb significantly in the UV wavelengths, correlate UV absorption to dissolved organic carbon (DOC) or soluble chemical oxygen demand (COD). However, use such correlations with care because they may vary from water to water, seasonally on the same water, and between raw and treated waters. In addition, chemical oxidation (e.g., ozonation, chlorination) of the organic material may reduce UV absorption without removing the organics and thus may change correlations. Because UV absorption and correlations with UV absorption are site-specific, they may not be comparable from one water source to another.

c. Minimum detectable concentration: The minimum detectable concentration cannot be determined rigorously because this is a nonspecific measurement. For precise measurement, select cell path length to provide an absorbance of approximately 0.005 to 0.900. Alternatively, dilute high-strength samples. The minimum detectable concentration of a particular constituent

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depends on the relationship between UV absorption, the desired characteristic (e.g., trihalomethane formation potential or DOC), and any interfering substances.

2. Apparatus

a. Spectrophotometer, for use between 200 and 400 nm with matched quartz cells providing a light path of 1 cm. For low-absorbance samples use a path length of 5 or 10 cm. A scanning spectrophotometer is useful.

b. Filter: Use a glass-filter*#(142) without organic binder. Other filters that neither sorb UV-absorbing organics of interest nor leach interfering substances (e.g., nitrate or organics) into the water may be used, especially if colloidal matter must be removed. Alternatively use filters of TFE, polycarbonate, or silver. Prerinse filter with sample of organic-free water to remove soluble impurities. If alternate separation techniques, filters or filter preparations are used, demonstrate that equivalent results are produced. Filter pore size will influence test results, especially in raw waters.

c. Filter assembly, glass, TFE, or stainless steel, capable of holding the selected filters.

3. Reagents

a. Organic-free water: Reagent water (see Section 1080) or equivalent water containing less than 0.05 mg DOC/L.

b. Hydrochloric acid (optional), HCl, 0.1*N*.

c. Sodium hydroxide (optional), NaOH, 0.1*N*.

d. Phosphate buffer (optional): Dissolve 4.08 g dried anhydrous KH_2PO_4 and 2.84 g dried anhydrous Na_2HPO_4 in 800 mL organic-free water. Verify that pH is 7.0 and dilute to 1 L with organic-free water. Store in brown glass bottle at 4°C. Prepare fresh weekly or more frequently if microbial growth is observed.

4. Procedure

a. Sample volume: Select sample volume on basis of the cell path length or dilution required to produce a UV absorbance between 0.005 and 0.900. For most applications a 50-mL sample is adequate. Use 100 mL sample if a 10-cm cell path length is required.

b. Sample preparation: Wash filter and filter assembly by passing at least 50 mL organic-free water through the filter. For specific applications and correlations, sample pH may be adjusted with HCl or NaOH. In poorly buffered samples an appropriate non-UV-absorbing buffer system such as a phosphate buffer may be used. Take care to avoid precipitate formation during pH adjustment. UV absorbance of fulvic acid solutions apparently remains constant between pH 4 and 10.[1] Report sample pH value used with recorded absorbance. Once sample pH has been adjusted and/or measured, filter sample. Prepare an organic-free water blank and the sample in an identical manner.

c. Spectrophotometric measurement: Let spectrophotometer equilibrate according to

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manufacturer's instructions. Set wavelength to 253.7 nm and adjust spectrophotometer to read zero absorbance with the organic-free water blank. Measure UV absorbance at 253.7 nm of at least two filtered portions of sample at room temperature.

5. Calculation

Report mean UV absorption in units of cm^{-1} using the following notation. To report units in m^{-1} multiply the equation by one hundred.

$$UV_{\lambda}^{pH} = \left[\frac{\bar{A}}{b}\right] D$$

where:

UV_{λ}^{pH} = mean UV absorption, cm^{-1} (subscript denotes wavelength used, nm, and superscript denotes pH used if other than 7.0),

b = cell path length, cm,

$\bar{A}$ = mean absorbance measured, and

D = dilution factor resulting from pH adjustment and/or dilution with organic-free water.

$$D = \frac{\text{final sample volume}}{\text{initial sample volume}}$$

Correct results for absorption contributed by known interfering substances. If UV absorption contributed by interfering substances exceeds 10% of the total UV absorption do not use UV absorption at 253.7 nm as an indicator of organics.

6. Quality Control

a. Replicate measurements: Use at least two portions of filtered sample.

b. Duplicate analyses: Analyze every tenth sample in duplicate (i.e., duplicating the entire procedure) to assess method precision.

c. Baseline absorbance: Check system baseline UV absorbance at least after every 10 samples by measuring the absorbance of an organic-free water blank. A non-zero absorbance reading for the blank may indicate need for cell cleaning, problems with the reference cell if a dual-beam instrument is being used, or variation in the spectrophotometer response caused by heating or power fluctuations over time.

d. Spectrophotometer check: Difficulties in comparing UV absorption data from different spectrophotometers have been reported. Potassium hydrogen phthalate (KHP), also known as

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potassium biphthalate, standards were prepared in pH 7, phosphate-buffered (3*d*) reagent water without acidification (see Section 5310B.3*c*) and analyzed in five laboratories. The results are shown in Table 5910:I; these data suggest acceptable precision. These data also are useful for checking spectrophotometer results with KHP standards commonly used for TOC and/or COD analysis. A correlation equation for this 40-sample data set is:

$$UV_{254} = 0.0144\ KHP + 0.0018$$

with correlation coefficient (r^2) = 0.987, UV_{254} expressed in cm^{-1}, and KHP expressed as mg/L as C.

This equation can assist in verifying spectrophotometer performance. For example, if a set of UV_{254} analyses is performed and the results are in the 0.010 range, prepare a KHP standard of 0.5 mg/L as C. The projected UV_{254} of this KHP standard would be 0.009 cm^{-1}. If the measured UV_{254} is outside 13% relative standard deviation (RSD) of 0.009 cm^{-1}, the spectrophotometer may be suspect and require maintenance. The correlation between UV_{254} and KHP standards is presented solely as a useful means of verifying spectrophotometer performance.

7. Precision and Bias

Table 5910:I shows interlaboratory precision data for 40 KHP samples. The percent relative standard deviations (% RSD) ranged from 9.38 to 12.8.

Single-operator precision data are presented in Table 5910:II for fulvic acid solutions.[2] The % RSD ranged from 0.9 to 6%. Because UV absorption is an aggregate measure of organic carbon, true standards are not available and bias cannot be determined.

8. References

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Figures

Figure 5520:1. Distillate recovery apparatus.

Figure 5540:1. Sublation apparatus.[1] See Section 5540B.2*a* and *b* and 4*c*. Bottom stopcock: TFE plug, 4-mm bore; side stopcocks: TFE plug, 2-mm bore.

Figure 5710:1. Effect of changing molar oxidant ratios of free chlorine: free bromine on

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molar ratios of substituted organic chloride: organic bromide, using four different precursor substrates. Reaction times varied between 1 and 7 d. Standard conditions were used at pH=7.0, except that the free chlorine residual after 7 d storage for the surface water was 17 mg/L instead of the 3 to 5 mg/L range for the other three substrates.

Figure 5710:2a. Relationships between definitions used in the formation potential test, for a sample that did not contain free chlorine at the time of sampling. Total THM concentration at the time of sampling ($TTHM_0$) was very close to or equal to zero; therefore, the THM formation potential for the 7-d reaction time (THMFP, with a free chlorine residual of at least 3 mg/L) was essentially equal to the total THM concentration in the sample at the end of the reaction storage time ($TTHM_7$).

Figure 5710:2b. Relationships between definitions used in the formation potential test, for a sample that already contained free chlorine at the time of sampling. Total THM concentration at the time of sampling ($TTHM_0$) was a significant fraction of the final value obtained after 7-d storage ($TTHM_7$) with an excess of at least 3 mg/L free chlorine. Δ THMFP is the difference between these two values.

Tables

TABLE 5210:I. UBOD RESULTS FOR WASTEWATER SAMPLE

Day	(1) Average DO* mg/L	(2) Average Blank DO† mg/L	(3) Accumulated DO Consumed by Sample‡ mg/L	(4) Average NO_3-N mg/L	(5) NBOD mg/L§	(6) CBOD mg/L‖
0	8.1	—	0	0.0	0	0
3	5.6	—	2.5	—	0	2.5
5	3.5/8.0	—	4.6	0.0	0	4.6
7	6.2	—	6.4	—	0.23	6.2
10	3.2/8.2	—	9.4	0.10	0.46	8.9
15	4.3	—	13.3	—	0.58	12.7
18	2.7/8.1	—	14.9	0.15	0.69	14.2
20	6.6	—	16.4	—	0.80	15.6
25	5.4	—	17.6	0.20	0.92	16.7
30	2.6/8.2	—	20.4	—	0.92	19.5
40	5.3	—	23.3	0.20	0.92	22.4
50	3.1/8.0	—	25.5	—	0.92	24.6
60	4.5	—	29.0	—	0.92	28.1

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Day	(1) Average DO* *mg/L*	(2) Average Blank DO† *mg/L*	(3) Accumulated DO Consumed by Sample‡ *mg/L*	(4) Average NO_3-N *mg/L*	(5) NBOD *mg/L*§	(6) CBOD *mg/L*‖
70	3.3/8.1	—	30.2	—	0.92	29.3
90	5.4	—	32.9	0.20	0.92	32.0

*Two readings indicate concentrations before and after reaeration.
†None was used.
‡Column (1)–blank correction (none needed in the example).
§Column (4) × 4.57 (linear interpolation between values).
‖[Column (3)–Column (5)] × dilution factor.
Ultimate CBOD = 34.5 mg/L; CBOD decay rate = 0.03/d (calculated with first-order equation from 5210C.4).

TABLE 5220:I. SAMPLE AND REAGENT QUANTITIES FOR VARIOUS DIGESTION VESSELS

Digestion Vessel	Sample *mL*	Digestion Solution *mL*	Sulfuric Acid Reagent *mL*	Total Final Volume *mL*
Culture tubes:				
16 × 100 mm	2.50	1.50	3.5	7.5
20 × 150 mm	5.00	3.00	7.0	15.0
25 × 150 mm	10.00	6.00	14.0	30.0
Standard 10-mL ampules	2.50	1.50	3.5	7.5

TABLE 5320:I. INTRALABORATORY, SINGLE-OPERATOR, DISSOLVED ORGANIC HALOGEN (MICROCOLUMN PROCEDURE)—PRECISION AND BIAS DATA

Characteristic of Analysis	Tap Water	Tap Water + 43.5 µg Organic Chloride	Ground Water (50:1)	Wastewater	Waste-wate + 1000 µg Organic Chloride
Concentration determined, µg Cl^-/L:					
Replicate 1	38.5	89.0	123.6	186.0	1178.0
Replicate 2	36.7	90.9	124.8	195.0	1183.0
Replicate 3	43.1	88.4	125.2	195.0	1185.5

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Characteristic of Analysis	Tap Water	Tap Water + 43.5 μg Organic Chloride	Ground Water (50:1)	Wastewater	Waste-wate + 1000 μg Organic Chloride
Replicate 4	35.9	90.1	123.3	204.0	1196.5
Replicate 5	41.1	91.7	125.3	185.0	1183.0
Replicate 6	48.5	93.0	127.0	236.5	1204.0
Replicate 7	52.8	97.0	123.5	204.0	1138.0
Mean, μg Cl^-/L	42.37	91.5	124.7	200.8	1181.1
Standard deviation:>/RO>					
μg Cl^-/L	±6.29	±3.0	±1.3	±17.47	±21.04
%	15	3	1	9	2
Value of blank + standard addition, μg Cl^-/L	—	85.87	—	—	1200.8
Recovery, %	—	107	—	—	98
Error, %	—	7	—	—	2

TABLE 5540:I. SURFACTANT RECOVERY BY SUBLATION

Variable	MBAS	CTAS
Sample volume, mL	200–300	500
Concentration without sublation, mg/L	2.2–4.7	—
Concentration found in sublate,* mg/L	1.8–4.4	0.3–0.6
Recovery in sublate, %	87 ± 16†	—
Amount in second sublate,‡ mg	0.02 ± 0.02†	0.08 ± 0.01†
Amount added, mg	0.05–0.10§	0.50–0.67
Recovery in sublation,# %	94 ± 17†	92 ± 6†

* Two 5-min sublations.
† Average ± SD (*n* = 8).
‡ Two more 5-min sublations.
§ Reference LAS.
‖ Linear alcohol ethoxylate $C_{12–18}E_{11}$.
Fifth and sixth 5-min sublations.

TABLE 5710:I. SINGLE-OPERATOR PRECISION AND BIAS DATA FOR THMFP*

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§ Reference LAS.
‖ Linear alcohol ethoxylate $C_{12-18}E_{11}$.
Fifth and sixth 5-min sublations.

TABLE 5710:I. SINGLE-OPERATOR PRECISION AND BIAS DATA FOR THMFP*

Sample	THM µg/L				THMFP µg/L as $CHCl_3$	Recove %
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$		
Blank 1	0.8	—	—	—	0.8	—
Blank 2	1.9	—	—	—	1.9	—
Blank 3	0.1	0.1	—	—	0.2	—
Blank 4	0.7	—	—	—	0.7	—
Blank 5	0.5	—	—	—	0.5	—
Blank 6	0.7	—	—	—	0.7	—
Average					0.8	—
Standard deviation					± 0.6	—
DHBA 1	114.1	0.1	—	—	114.2	97.8
DHBA 2	113.2	—	—	—	113.2	96.9
DHBA 3	107.8	—	—	—	107.8	92.2
DHBA 4	108.3	—	—	—	108.3	92.7
DHBA 5	109.6	0.1	—	—	109.7	93.9
DHBA 6	111.8	0.1	—	—	111.9	95.8
DHBA 7	112.6	—	—	—	112.6	96.4
Average					111.1†	95.1
Standard deviation					± 2.5	2.2

* Source: MOORE, L., Unpublished data. U.S. Environmental Protection Agency, Cincinnati, Ohio.
† Expected value = 116.

TABLE 5710:II. SINGLE-OPERATOR PRECISION AND BIAS DATA FOR TTHM (pH = 9.2)*

Sample	THM µg/L				TTHM µg $CHCl_3$/L
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$	
Blank 1	3.0	0.3	—	—	3.2

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Sample	THM µg/L				TTHM µg $CHCl_3$/L
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$	
Blank 2	1.7	0.1	—	—	1.8
Blank 3	1.3	0.1	—	—	1.4
Blank 4	1.6	0.1	—	—	1.7
Blank 5	2.3	0.2	—	—	2.4
Blank 6	2.6	0.1	—	—	2.7
Blank 7	2.5	0.2	—	—	2.6
Average					2.3
Standard deviation (relative standard deviation)					± 0.6 (± 26.1°
DHBA 1	45.4	3.3	0.1	—	47.9
DHBA 2	51.0	3.9	0.1	—	53.9
DHBA 3	39.2	3.0	0.1	—	41.4
DHBA 4	48.3	3.6	0.1	—	51.0
DHBA 5	47.6	3.7	0.1	—	50.4
DHBA 6	43.4	3.2	0.1	—	45.8
DHBA 7	46.0	3.6	0.1	—	48.7
Average					48.4
Standard deviation (relative standard deviation)					± 3.7 (± 7.6%
RWS 1†	33.1	17.2	11.3	0.5	52.3
RWS 2	31.7	16.1	10.6	0.5	49.7
RWS 3	38.7	18.4	11.7	0.6	59.1
RWS 4	35.1	18.0	11.7	0.8	55.3
RWS 5	36.0	17.9	11.7	0.6	56.0
RWS 6	38.7	18.7	11.7	0.6	59.3
RWS 7	37.7	18.1	11.2	0.6	57.6
Average					55.6
Standard deviation (relative standard deviation)					± 3.3 (± 5.9%

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* Source: MOORE, L., Unpublished data. U.S. Environmental Protection Agency, Cincinnati, Ohio.
† RWS = raw water sample, filtered and diluted 1 part filtrate, 2 parts organic-free water.

TABLE 5910:I. PRECISION OF UV ANALYSES AND CORRELATION TO KHP SAMPLES

Analysis	UV_{254} Result for Given KHP Sample Concentration* cm^{-1}							
	0.54	0.93	1.79	4.87	9.61	25.0	50.0	1
Laboratory 1	0.008	0.015	0.034	0.079	0.158	0.323	0.638	1
Laboratory 2	0.009	0.016	0.026	0.070	0.134	0.401	0.803	1
Laboratory 3	0.010	0.017	0.027	0.081	0.161	0.353	0.695	1
Laboratory 4	0.007	0.020	0.033	0.070	0.132	0.319	0.750	1
Laboratory 5	0.009	0.018	0.030	0.087	0.140	0.394	0.643	1
Mean	0.0086	0.0142	0.0300	0.0774	0.1450	0.3580	0.7058	1
Standard deviation	0.0011	0.0019	0.0035	0.0074	0.0136	0.0384	0.0708	0
% Relative standard deviation†	12.8	11.1	11.7	9.56	9.38	10.7	10.0	10

* KHP sample concentration mg/L as C, measured as in Section 5310C.† The percent relative standard deviation is given by:

$$\% \; RSD = \left[\frac{\text{standard deviation } (S)}{\text{mean } (\bar{X})} \right] \times 100$$

TABLE 5910:II. SINGLE-OPERATOR PRECISION FOR UV ABSORPTION MEASUREMENTS OF FULVIC ACID SOLUTIONS

Replicate No.	Result cm^{-1}		
	DOC = 2.5 mg/L	DOC = 4.9 mg/L	DOC = 10.0 mg/L
1	0.110	0.240	0.480
2	0.120	0.230	0.480
3	0.110	0.240	0.470
4	0.100	0.230	0.480
5	0.110	0.240	0.480
6	0.100	0.240	0.470

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Replicate No.	Result cm^{-1} DOC = 2.5 mg/L	DOC = 4.9 mg/L	DOC = 10.0 mg/L
7	0.110	0.240	0.480
8	0.110	0.230	0.480
9	0.120	0.240	0.480
10	0.110	0.240	0.480
Mean	0.110	0.237	0.478
Standard deviation	0.00667	0.00483	0.00422
% Relative standard deviation	6.06	2.05	0.882

Part 6000 INDIVIDUAL ORGANIC COMPOUNDS

6010 INTRODUCTION

6010 A. General Discussion

The methods presented in Part 6000 are intended for the determination of individual organic compounds. Methods for determination of aggregate concentrations of groups of organic compounds are presented in Part 5000.

Most of the methods presented herein are highly sophisticated instrumental methods for determining very low concentrations of the organic constituents. Stringent quality control requirements are given with each method and require careful attention.

Many compounds are determinable by two or more of the methods presented in Part 6000. Table 6010:I shows the specific analytical methods applicable to each compound. Guidance on selection of method is provided in the introduction to each section.

6010 B. Sample Collection and Preservation

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1. Volatile Organic Compounds

Use 25- or 40-mL vial equipped with a screw cap with a hole in the center*#(143) and TFE-faced silicone septum.†#(144) Wash vials, caps, and septa with detergent, rinse with tap and distilled water, and dry at 105°C for 1 h before use in an area free of organic vapors. NOTE—Do not heat seals for extended periods of time (> 1 h) because the silicone layer slowly degrades at 105°C. When bottles are cool, seal with TFE seals. Alternatively purchase precleaned vials free from volatile organics.

Collect all samples in duplicate and prepare replicate field reagent blanks with each sample set. A sample set is all samples collected from the same general sampling site at approximately the same time. Prepare field reagent blanks in the laboratory by filling a minimum of two sample bottles with reagent water, sealing, and shipping to the sampling site along with empty sample bottles.

Fill sample bottle just to overflowing without passing air bubbles through sample or trapping air bubbles in sealed bottle. When sampling from a water tap, open tap and flush until water temperature has stabilized (usually about 10 min). Adjust flow rate to about 500 mL/min and collect duplicate samples from flowing stream. When sampling from an open body of water, fill a 1-L, wide-mouth bottle or breaker with a representative sample and carefully fill duplicate sample bottles from the container.

Preservation of samples is highly dependent on target constituents and sample matrix. Ongoing research indicates the following areas of concern: rapid biodegradation of aromatic compounds, even at low temperatures;[1] dehydrohalogenation reactions such as conversion of pentachloroethane to tetrachloroethane;[2] reactions of alkylbenzenes in chlorinated samples, even after acidification; and possible interactions among preservatives and reductants when dechlorination is used to prevent artifact formation, especially in samples potentially containing many target compounds.

There is as yet no single preservative that can be recommended. Ideally, maintain samples chilled (preferably at 4°C) and analyze immediately. In practice, delays between sampling and analysis often necessitate preservation. The recommended preservation techniques are summarized in Table 6010:II.

1) For samples and field blanks that contain volatile constituents but do not contain residual chlorine, add HCl (4 drops 6*N* HCl/40 mL) to prevent biodegradation and dehydrohalogenation. NOTE: HCl may contain traces of organic solvents. Verify freedom from contamination before using a specific lot for preservation.

2) For samples and field blanks that contain residual chlorine, also add a reducing agent. Ascorbic acid (25 mg/40 mL) appears to be optimal, but demonstrate that this reductant is appropriate for the specific sample matrix. Sodium thiosulfate (3 mg/40 mL) or sodium sulfite (3 mg /40 mL) also may be appropriate reducing agents, but when either of these is added in the presence of HCl, SO_2 formation may interfere with certain packed-column gas chromatographic or GC/MS techniques.

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In all cases, run reagent blanks to insure absence of interferences. Add either ascorbic acid or HCl to the sample bottle immediately before shipping it to the sample site or immediately before filling sample bottle. When both preservatives are needed, add only one before filling the sample bottle, to prevent interactions between the acid and the reductant. Add the second preservative once the bottle is almost full. However, if there is evidence that interactions of acid and reducing agent will not create analytical or preservation problems, they may be added simultaneously.

Tightly seal sample bottles, TFE face down. After sampling and preservation invert several times to mix. Chill samples to 4°C immediately after collection and hold chilled in an atmosphere free of organic solvent vapors until analysis. Normally analyze all samples within 14 d of collection. Shorter or longer holding times may be appropriate, depending on constituents and sample matrix. Develop data to show that alternate holding times are appropriate.

2. Other Organic Compounds

See individual methods for sampling and preservation requirements.

3. References

1. BELLAR, T. & J. LICHTENBERG. 1978. Semi-automated headspace analysis of drinking waters and industrial waters for purgeable volatile organic compounds. *In C. E. Van Hall, ed. Measurement of Organic Pollutants in Water and Wastewater. STP 686, American Soc. Testing & Materials. Philadelphia, Pa.*
2. BELLAR, T. & J. LICHTENBERG. 1985. The Determination of Synthetic Organic Compounds in Water by Purge and Sequential Trapping Capillary Column Gas Chromatography. U.S. Environmental Protection Agency, Cincinnati, Ohio.

4. Bibliography

KEITH, L. H., ed. 1988. Principles of Environmental Testing. American Chemical Soc., Washington, D.C.

6010 C. Analytical Methods

1. General Discussion

The methods presented in Part 6000 for identification and quantitation of trace organic constituents in water generally involve isolation and concentration of the organics from a sample by solvent or gas extraction (see Section 6040 and individual methods), separation of the components, and identification and quantitation of the compounds with a detector.

2. Gas Chromatographic Methods

Gas chromatographic (GC) methods are highly sophisticated microanalytical procedures. They should be used only by analysts experienced in the techniques required and competent to evaluate and interpret the data.

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a. Gas chromatograph:

1) Principle—In gas chromatography a mobile phase (a carrier gas) and a stationary phase (column packing or capillary column coating) are used to separate individual compounds. The carrier gas is nitrogen, argon-methane, helium, or hydrogen. For packed columns, the stationary phase is a liquid that has been coated on an inert granular solid, called the column packing, that is held in borosilicate glass tubing. The column is installed in an oven with the inlet attached to a heated injector block and the outlet attached to a detector. Precise and constant temperature control of the injector block, oven, and detector is maintained. Stationary-phase material and concentration, column length and diameter, oven temperature, carrier-gas flow, and detector type are the controlled variables.

When the sample solution is introduced into the column, the organic compounds are vaporized and moved through the column by the carrier gas. They travel through the column at different rates, depending on differences in partition coefficients between the mobile and stationary phases.

2) Interferences—Some interferences in GC analyses occur as a result of sample, solvent, or carrier gas contamination, or because large amounts of a compound may be injected into the GC and linger in the detector. Methylene chloride, chloroform, and other halocarbon and hydrocarbon solvents are ubiquitous contaminants in environmental laboratories. Make strenuous efforts to isolate the analytical system from laboratory areas where these or other solvents are in use. An important sample contaminant is sulfur, which is encountered generally only in base/neutral extracts of water, although anaerobic groundwaters and certain wastewaters and sediment/sludge extracts may contain reduced sulfur compounds, elemental sulfur, or polymeric sulfur. Eliminate this interference by adding a small amount of mercury or copper filings to precipitate the sulfur as metallic sulfide. Sources of interference originating in the chromatograph, and countermeasures, are as follows:

- Septum bleed—This occurs when compounds used to make the septum on the injection port of the GC bleed from the heated septum. These high-molecular-weight silicon compounds are distinguished readily from compounds normally encountered in environmental samples. Nevertheless, minimize septum bleed by using septum sweep, in which clean carrier gas passes over the septum to flush out the ‘‘bleed’’ compounds.
- Column bleed—This term refers to loss of column coating or breakdown products when the column is heated. This interference is more prevalent in packed columns, but also occurs to a much lesser extent in capillary columns. It occurs when the column temperature is high or when water or oxygen are introduced into the system. Solvent injection can damage the stationary phase by displacing it. Certain organic compounds acting as powerful solvents, acids, or bases can degrade the column coating. Injection of large amounts of certain surface-active agents may destroy GC columns.
- Ghost peaks—These peaks occur when an injected sample contains either a large amount of a given compound, or a compound that adsorbs to the column coating or injector parts (e.g., septum). When a subsequent sample is injected, peaks can appear as a result of the previous

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injection. Eliminate ghost peaks by injecting a more dilute sample, by producing less reactive derivatives of a compound that may interact strongly with the column material, by selecting a column coating that precludes these interactions, or by injecting solvent blanks between samples.

b. Detectors: Various detectors are available for use with gas chromatographic systems. See individual methods for recommendations on appropriate detectors.

1) Electrolytic conductivity detector—The electrolytic conductivity detector is a sensitive and element-specific detector that has gained considerable attention because of its applicability to the gas chromatographic analysis of environmentally significant compounds. It is utilized in the analysis of purgeable halocarbons, pesticides, herbicides, pharmaceuticals, and nitrosamines. This detector is capable of operation in each of four specific modes: halogen (X), nitrogen (N), sulfur (S), and nitrosamine (NO). Only organic compounds containing these elements will be detected.

Compounds eluting from a gas chromatographic column enter a reactor tube heated to 800°C. They are mixed with a reaction gas, hydrogen for X, N, or NO modes, and air for the S mode. The hydrogen catalytically reduces the compounds while the air oxidizes them. The gaseous products are transferred to the detector through a conditioned ion exchange resin or scrubber. In the halogen mode, only HX is detected, while NH_3 or H_2S are eliminated on the resin. In the nitrogen or nitrosamine mode, the NH_3 formed is ionized while HX and H_2S, if present, are eliminated with a KOH/quality wool scrubber. The sulfur mode produces SO_2 or SO_3, which is ionized while HX is removed with a silver wire scrubber. All other products either are not ionizable or are produced in such low yield that they are not detectable.

The electrolytic conductivity detector contains reference and analytical electrodes, a gas-liquid contactor, and a gas-liquid separator. The conductivity solvent enters the cell and flows by the reference electrode. It combines with the gaseous reaction products in the gas-liquid contactor. This heterogeneous mixture is separated into gas and liquid phases in the gas-liquid separator, with the liquid phase flowing past the analytical electrode. The electrometer monitors the difference in conductivity at the reference electrode (solvent) and the analytical electrode (solvent + carrier + reaction products).

2) Electron capture detector—The electron capture detector (ECD) usually is used for the analysis of compounds that have high electron affinities, such as chlorinated pesticides, drugs, and their metabolites. This detector is somewhat selective in its response, being highly sensitive toward molecules containing electronegative groups: halogens, peroxides, quinones, and nitro groups. It is insensitive toward functional groups, such as amines, alcohols, and hydrocarbons.

The detector is operated by passing the effluent from the gas chromatographic column over a radioactive beta particle emitter, usually nickel-63 or tritium adsorbed on platinum or titanium foil. An electron from the emitter ionizes the carrier gas, preferably nitrogen, and produces a burst of electrons. About 100 secondary electrons are produced for each initial beta particle. After further collisions, the energy of these electrons is reduced to the thermal level and they can

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be captured by electrophilic sample molecules.

The electron population in the ECD cell is collected periodically by applying a short voltage pulse to the cell electrodes and the resulting current is compared with a reference current. The pulse interval is adjusted automatically to keep the cell current constant, even when some of the electrons are being captured by the sample. The change in the pulse rate when a sample enters the ECD is then related to the sample concentration. The ECD offers linearity in the range of 10^4 and subpicogram detection limits for compounds with high electron affinities.

3) Flame ionization detector—The flame ionization detector (FID) is widely used because of its high sensitivity to organic carbon-containing compounds. The detector consists of a small hydrogen/air diffusion flame burning at the end of a jet. When organic compounds enter the flame from the column, electrically charged intermediates are formed. These are collected by applying a voltage across the flame. The resulting current is amplified by an electrometer and measured. The response of the detector is directly proportional to the total mass entering the detector per unit time and is independent of the concentration in the carrier gas.

The FID is perhaps the most widely used detector for gas chromatography because of several advantages: (*a*) it responds to virtually all organic carbon-containing compounds with high sensitivity (approximately 10^{-13}g/mL); (*b*) it does not respond to common carrier gas impurities such as water and carbon dioxide; (*c*) it has a large linear response range (approximately 10^7) and excellent baseline stability; (*d*) it is relatively insensitive to small column flow-rate changes during temperature programming; (*e*) it is highly reliable, rugged, and easy to use; and (*f*) it has low detector dead volume effects and fast response. Its limitations include: (*a*) it gives little or no response to noncombustible gases and all noble gases; and (*b*) it is a destructive detector that changes the physical and chemical properties of the sample irreversibly.

4) Photoionization detector—Photoionization occurs when a molecular species absorbs a photon of light energy and dissociates into a parent ion and an electron. The photoionization detector (PID) detects organic and some inorganic species in the effluent of a gas chromatograph with detection limits as low as the picogram range. The PID is equipped with a sealed ultraviolet light source that emits photons which pass through an optically transparent window (made of LiF, MgF_2, NaF, or sapphire) into an ionization chamber where photons are absorbed by the eluted species. Compounds having ionization potential less than the UV source energy are ionized. A positively biased high-voltage electrode accelerates the resulting ions to a collecting electrode and the resulting current is measured by an electrometer. This current is proportional to the concentration.

The PID has high sensitivity, low noise (approximately 10^{-14}A), and excellent linearity (10^7), is nondestructive, and can be used in series with a second detector for more selective detection. The PID can be operated as a universal detector or a selective detector by simply changing the photon energy of the ionization source. Tables of ionization potentials are used to select the appropriate UV source for a given measurement.

5) Mass spectrometer—The mass spectrometer (MS) has the ability to detect a wide variety

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of compounds, coupled with a capacity to deduce compound structures from fragmentation patterns. Among the different types of mass spectrometers, the quadrupole has become the most widely used in water and wastewater analysis.

The mass spectrometer detects compounds by ionizing molecules into charged species with a 70-eV beam. The ions are accelerated toward the quadrupole mass filter through a series of lenses held at 0 to 200 V. The differently sized, charged fragments are separated according to mass-to-charge ratio (related to molecular weight) by means of the quadrupole, which uses varying electric and radiofrequency (rf) fields. The quadrupole is connected to a computer, which varies these fields so that only fragments of one particular mass-to-charge ratio (±0.5) can traverse the quadrupole at any one time. As the ions leave the quadrupole they are attracted to the electron multiplier through an electrical potential of several thousand volts. The charge fragments, in turn, are detected by the electron multiplier. Because the electric and the rf fields are cycled every few seconds, a fragmentation pattern is obtained. Each cycle is called a mass scan. Most chemicals have unique fragmentation patterns, called mass spectra. The computer contains, and can search, a library of known mass spectra to identify tentatively an unknown compound exhibiting a particular spectrum. Use authentic compounds for confirmation after tentative identifications are made.

Background mass interference can result from the ability of the mass spectrometer to detect any ions created in its ion volume (up to a specified mass). Any compounds continuously present in the source will be detected. Some mass ions always present are due to air components that leak into the system, such as oxygen (masses 16 and 32), nitrogen (masses 14 and 28), carbon dioxide (mass 44), argon (mass 40), and water (mass 18), or to helium carrier gas (masses 4 and 8), or to diffusion pump oil vapors.

3. High-Performance Liquid Chromatographic (HPLC) Methods

a. Principle: HPLC is an analytical technique in which a liquid mobile phase transports a sample through a column containing a liquid stationary phase. The interaction of the sample with the stationary phase selectively retains individual compounds and permits separation of sample components. Detection of the separated sample compounds is achieved mainly through the use of absorbance detectors for organic compounds and through conductivity and electrochemical detectors for metal and inorganic components.

b. Detectors:

1) Photodiode array detector (PDAD)—The PDAD measures the absorbance of a sample from an incident light source (UV-VIS). After passing through the sample cell, the light is directed through a holographic grating that separates the beam into its component wavelengths reflected on a linear array of photodiodes. This permits the complete absorbance spectrum to be obtained in 1 s or less and simultaneous multiwavelength analysis.

The PDAD is subject to the interference encountered with all absorbance detectors. Of special concern for HPLC is the masking of the absorbance region of the HPLC mobile phase and its additives. This may reduce the range and sensitivity of the detector to the sample

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components. Most interferences occur in monitoring the shorter wavelengths (200–230 nm). In this region, many organic compounds absorb light energy and can be sources of interference.

2) Post column reactor (PCR)—The PCR consists of in-line sample derivatizing/reacting equipment that permits chemical alteration of certain organic compounds. This equipment is used to enhance detection by attaching a chromophore to the compound(s) of interest. Sensitivity and selectivity of compounds that were initially undetectable are altered to make them detectable.

Interferences from this technique usually arise from the impurities in the reagents used in the reaction. When this technique is coupled with a selective detector such as fluorescence, these interferences are minimized. Generally, only compounds of the same class as the compounds of interest will cause interference.

3) Fluorescence detector—The fluorescence detector is an absorbance detector in which the sample is energized by a monochromatic light source. Compounds capable of absorbing the light energy do so and release it as fluorescence emission. Filters permit the detector to respond only to the fluorescent energy. The fluorescence detector is the most sensitive of the current HPLC detectors available and often is used in conjunction with a post column reactor.

Because of instrument sensitivity, minute quantities of contaminants can cause interferences to fluorescence detectors. Contamination can happen from glassware, mobile phase solvents, post-column reagents, etc. These sources will raise the background signal and thus narrow the range of the detector. Interference from individual compounds is minimal because of detector specificity (i.e., all interferences must fluoresce).

6020 QUALITY ASSURANCE/QUALITY CONTROL FOR ORGANIC COMPOUNDS

6020 A. Introduction

Quality assurance (QA) and quality control (QC) for organic compound analysis include the operating principles stated in Section 1020 Quality Assurance, Section 1030 Data Quality, and Section 1040 Method Development and Evaluation. This section consolidates the additional requirements common to the methods in Part 6000. The requirements are recommended minimum QA/QC activities; they should be followed unless the individual method gives different, but comparable, specifications. Some methods may have additional QA/QC requirements. Others may have broader acceptance criteria because of the unique difficulties associated with the determination of a constituent, e.g., the extraction efficiency for phenols.

6020 B. Quality Control Practices

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This section describes the elements of a quality control program deemed necessary to maintain proper control for organic analyses. The related data quality objectives (DQOs), i.e., the rationale for sampling and analyses, should define which elements are necessary for each individual analytical design.

1. Calibration

a. Initial calibration: Perform initial calibration with a minimum of five concentrations of analytical standards for the analyte(s) of interest (for example: 1, 5, 10, 20, 40). The lowest concentration should be at the minimum reporting level; for example, if the minimum reporting level is 1, the lowest concentration should be 1. The highest concentration should be near the upper end of the calibration range; for example, if the upper end of the calibration range is 40, the high standard should be 40. Concentration ranges should reflect concentrations in actual samples. Choose calibration concentrations with no more than one order of magnitude between concentrations; for example, for a calibration range of 1 to 1000, choose concentrations of 1, 10, 100, and 1000.

Use any of the following calibration functions, as appropriate: response factor for internal standard calibration, calibration factor for external standard calibration, or calibration curve. Calibration curves may be linear or quadratic, and may or may not pass through the origin. Use the following recommended acceptance criteria for the various calibration functions.

If response factors or calibration factors are used, relative standard deviation (RSD) for each analyte should be less than 20%. If RSD is not less than 20% for any analyte, then identify and correct source of lack of linearity before sample quantitation. When using response factors (i.e., for GC/MS analysis), check performance or sensitivity of instrument for the analyte against minimum acceptance values for response factors. Refer to specific analytical method for the acceptance criteria for response factors for each analyte.

If a linear regression is used, the correlation coefficient should be >0.995. If a calibration curve has been constructed, recalculate each calibration point compared to curve. Values should be within ±20%. If any of the recalculated values are not within ±20%, identify and correct source of outlier(s) before sample quantitation.

Use initial calibration with any of the above functions (response factor, calibration factor, or calibration curve) for quantitation of analytes in samples. Use continuing calibration, ¶ b below, only for checks on initial calibration and not for sample quantitation. Perform initial calibration when instrument is set up and whenever continuing calibration criteria are not met.

b. Continuing calibration: Continuing calibration is the periodic verification, by analysis of a calibration standard, that instrument performance has not changed significantly from initial calibration. Perform continuing calibration every 10 samples for GC analysis, every 20 samples for GC/MS analysis, or every 12 h, whichever is more frequent. Perform continuing calibration with one or more of the concentrations of analytical standards in the initial calibration, varying actual concentration of continuing calibration standard over the calibration range. The acceptance criteria for continuing calibration should be ±20% (80 to 120% recovery) compared

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to the known or expected value of the calibration standard. If the acceptance criteria are not met, reanalyze continuing calibration standard or repeat initial calibration. When using response factors (i.e., GC/MS analysis), check performance or sensitivity of instrument for analytes against minimum acceptance values for the response factors. Refer to the specific analytical method for acceptance criteria for response factors for each analyte.

c. Closing standard: Finish all runs with a laboratory-fortified blank (LFB) for VOC analyses or closing standard (for methods with procedural standards) to demonstrate that performance was still acceptable for last sample analyzed. A LFB is a reagent blank to which a known concentration of analytes has been added. See ¶ 3*b* below. All samples must be bracketed by acceptable continuing calibrations.

2. Initial Quality Control

a. Initial demonstration of capability: Before analysis of any sample, require each analyst to demonstrate proficiency with the method of choice. Include at least the analysis of a laboratory reagent blank (LRB) and four laboratory-fortified blanks (LFBs) that have added concentrations between 5 times the minimum reporting level and the midpoint of the calibration curve. The blank should not contain any analyte at a concentration greater than one-fourth the minimum quantitation level. The precision and percent recovery calculated from the four LFBs should be at least as good as the values listed in the method of choice.

b. Method detection level: Determine method detection level (MDL) before any samples are analyzed, using procedure described in Section 1030C or other specified method[1] required for the type of samples the laboratory is intending to analyze. As a starting point for determining concentration to use in performing the laboratory's MDL calculation, try about five times the estimated instrument detection level or refer to the selected method. Determine MDL as an iterative process. Repeat determinations if calculated MDL is not within a factor of 10 of the fortified value. Determine MDL at least annually. Analyze samples for MDL determination over a 3- to 5-d period to generate a more realistic value. Include all applicable sample preparatory techniques in MDL determinations.

c. Minimum quantitation level (MQL): The MQL is the lowest level that can be quantitated accurately. MQL is defined as four times the MDL. Report samples containing compounds of interest at a level less than the MQL as <MQL. Report samples containing compounds of interest at a level less than the MDL as ND (not detected).

e. Sample (batch) set: A sample or batch set is defined as those samples extracted in a single day, not to exceed 20 samples.

f. Analytical day: An analytical day is defined as a 12-h analytical period.

3. Batch Quality Control

a. Reagent blank: A reagent blank consists of all reagents and preservatives that normally contact a sample when it is carried through the entire analytical procedure. Use a reagent blank to determine contribution of reagents and preparative analytical steps to error in the observed

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value. No analyte of interest should be present in a reagent blank at a level greater than one fourth the MQL. Include a minimum of one reagent blank with each sample set or batch.

b. Laboratory-fortified blank (LFB): An LFB, also known as spiked blank, is a reagent blank containing all the same reagents and preservatives as samples and to which a known concentration of analytes has been added. Use LFB to evaluate laboratory performance and analyte recovery in a blank matrix. Make addition concentration at least 5 times the MQL or the midpoint of the calibration curve, and use to calculate recovery limits and to plot control charts as in Section 1020B. Prepare known-addition solution for blanks and samples from a different primary mix than that used to develop working standard mix. Include a minimum of one LFB with each sample set or batch. Ensure that LFB meets performance criteria in the method of choice.

c. Internal standard (IS): An internal standard is a compound of known concentration added to each standard and sample extract just before sample analysis. This compound should have chromatographic characteristics similar to those of the analytes of interest. Use IS to monitor retention time, relative response, and quantity of analytes in each extract. When quantifying by the internal standard method, measure all analyte responses relative to this standard. Internal standard response should be in the range of ±30% compared to calibration curve response. The retention time of this compound should separate from all analytes of interest and elute in a representative area of the chromatogram. If a single compound cannot be found to meet these criteria, use additional compounds.

d. Surrogate standard: A surrogate standard is a compound of a known concentration added to each environmental and blank sample before extraction. Use compound(s) that have characteristics similar to those of the analytes of interest and that are unlikely to be found in environmental samples. Carry surrogate standard through entire sample extraction and analytical process to monitor extraction efficiency of the method for each sample. Refer to method of choice for specific surrogates and acceptance criteria.

e. Quality control sample: Analyze an externally generated quality control sample of known quantity as a laboratory-fortified blank at least quarterly or whenever new stock solutions are prepared. This sample is used to validate the laboratory's standards both qualitatively and quantitatively.

f. Laboratory-fortified sample (LFS): A laboratory-fortified sample, also known as laboratory-fortified matrix or matrix spike, is another portion of a sample fortified with the analytes of interest at a concentration at least 5 times the MQL or around the midpoint of the calibration range. Include a minimum of one LFS with each sample set (batch). Make LFSs of sufficient concentrations that sample background levels do not adversely affect the recovery calculations. (Adjust addition concentrations if this is a known sample to be about five times background level.) Base sample batch acceptance on results of LFBs rather than on LFSs, because the sample matrix may interfere with method performance. Prepare addition solution for blanks and samples from a different primary mix than that used to develop working standard mix.

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g. Laboratory-fortified sample duplicates (LFSDs): A laboratory-fortified sample duplicate, also known as laboratory-fortified matrix duplicate, spiked sample duplicate, or matrix spike duplicate, is a second portion of the sample to which a known amount of analyte is added. If sufficient sample volume is collected, add to a second portion of fortified sample and compare to first. If sufficient sample volume is not collected, use a second sample to obtain results on two separate LFSs rather than LFSDs. Include a minimum of one LFSD with each sample set (batch). Compare precision and bias to those listed in the method. Base sample batch acceptance on results of reagent blank additions rather than laboratory-fortified sample duplicates.

4. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1995. Definition and procedure for the determination of the method detection limit, revision 1.11. 40 CFR Part 136, Appendix B. *Federal Register* 51:23703.

6040 CONSTITUENT CONCENTRATION BY GAS EXTRACTION*#(145)

6040 A. Introduction

The ability to analyze ultratrace levels of organic pollutants in water has been limited, in part, by the concentration technique. With the development of closed-loop stripping analysis (CLSA) (Method B), organic compounds of intermediate volatility and molecular weight, i.e., from the heavier volatiles to the lighter polynuclear aromatic hydrocarbons, can be extracted from water and concentrated to allow quantitative and semiquantitative analysis (depending on the compound) at nanograms-per-liter levels. This extract can be analyzed on a gas chromatograph (GC) connected to one of several detectors. A CLSA technique coupled with gas chromatographic/mass spectrometric (GC/MS) analysis for the determination of trace organic compounds is presented here. It is applicable to both treated and natural waters.

The purge and trap technique (Method C) is a valuable concentration method applicable to volatile organic compounds. The compounds are concentrated by bubbling an inert gas through the sample followed by collection in, and desorption from, a sorbent trap. This extract may be analyzed by GC or GC/MS methods. The technique is applicable to both water and wastewater.

6040 B. Closed-Loop Stripping, Gas Chromatographic/Mass Spectrometric Analysis

1. General Discussion

a. Principle: This CLSA-GC/MS procedure is suitable for the analysis of a broad spectrum of organic compounds in water. It can be used for the identification and quantitation of specific

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compounds, such as earthy-musty-smelling compounds [e.g., 2-methylisoborneol (MIB) and geosmin][1-3] or U.S. Environmental Protection Agency (EPA) priority pollutants.[4,5] The method is suitable for other taste- and odor-causing compounds (e.g., geranyl acetone 6-methylhept-5-en-2-one, β-ionone, and β-cyclocitral).[6]

In closed-loop stripping, volatile organic compounds of intermediate molecular weight are stripped from water by a recirculating stream of air. The organics are removed from the gas phase by an activated carbon filter. They are extracted from the filter with carbon disulfide (CS_2) or methylene chloride. A portion of the extract is injected into a capillary-column GC/MS for identification of the organic compounds by retention time and spectrum matching; quantification is done by single-ion current integration. Alternatively, analysis may be made by capillary-column GC equipped with a flame ionization detector with identification by retention times on primary and secondary capillary columns.

b. Interference: Organic compounds that are stripped during this procedure may coelute with the compounds of interest. The uniqueness of the mass spectrum of each target compound makes it possible to confirm compound identity with a high probability when coeluting components are present. Problems may arise if several isomers of a compound are present that are not resolvable chromatographically.

c. Detection levels: Trace organics can be detected at low nanogram-per-liter levels. The CLSA-GC/MS detection limits are affected by many factors; especially important are the stripping efficiency and the condition of the GC/MS. Stripping efficiencies can be improved by using an elevated stripping temperature and/ or the salting-out technique. The stripping and extraction portion of the method can be evaluated independently of the instrumentation portion. As an option, add internal standard after stripping and extraction, and transfer extract quantitatively.

The method detection levels for five earthy-musty-smelling compounds are shown in Table 6040:I. Detection levels for the salted CLSA method are less than half those for the unsalted method for each compound. Using the elevated stripping temperature rather than the salting-out technique produces comparable recoveries[7] and similar detection levels. Detection levels for various organic compounds of interest, obtained with an elevated stripping temperature/salting-out technique, ranged from 0.1 to 100 ng/L (see Table 6040:II).[8]

2. Apparatus

Use clean glassware in sample collection and calibration standard preparation. Wash with soapy water, rinse with tap water, with demineralized water, and finally with reagent-grade acetone. As an alternative to acetone rinse, bake glassware in an annealing oven for 1 h at 400°C. Air-dry and bake at 180°C for 6 to 12 h. Do not bake sample bottle caps or volumetric ware. After drying and baking, store inverted or cover mouths with aluminum foil to prevent accumulation of dust or other contaminants.

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a. Sample bottles, 1-L capacity or larger, glass, with TFE-lined screw caps.

b. CLSA apparatus, equipped with the following components (Figure 6040:1) or their equivalents.*#(146)

1) *Stripping bottle,* with mark at 1-L level and stainless-steel quick-connect stems (Figure 6040:2) or unpolished spherical glass joints sealed with TFE-covered silicone rubber O-rings and secured with metal clamps.†#(147) Immediately after use, clean stripping bottle by rinsing twice with demineralized water and once with organic-free water. For particularly adherent impurities, clean with acetone and bake at 180°C for at least 2 h. Turbid samples may cause a film to deposit on the stripping bottle and frit and may require washing with acid detergent.

2) *Gas heater,* with aluminum heating cylinder and soldering iron (25 W) controlled by a variable transformer (Figure 6040:3). Alternatively, use a temperature-controlled heater block to maintain a fixed temperature at the filter that is 10 to 20°C above temperature of the thermostatic water bath.

3) *Filter holder,* stainless steel or glass.‡#(148) If glass is used, also use an auxiliary heating device, e.g., an infrared light, to maintain proper filter temperature.

4) *Pump,* with stainless-steel bellows,§#(149) providing air flow in the range of 1 to 1.5 L/min. When using the salting-out technique, periodically disassemble the pump and clean. Salt will leave deposits on the pump bellows. If there is a noticeable drop in pump performance, clean valve assembly with acetone or replace.

5) *Automatic timer* (optional), connected to pump.

6) *Circuit,* with stainless-steel parts: 1/8-in. (0.3-cm)-OD tubing, 4-in. (10.2-cm) × 1/4-in. (0.6-cm)-OD flexible tubing, tube fittings, and quick-connect bodies;‖#(150) or glass joints described in ¶ 1) above. Glass sample lines can be used except where circuit enters and exits pump. Use TFE ferrules in making connections to glass and flexible metal tubing. Whenever sample carryover is observed, clean circuit and pump as follows: Connect fittings to the quick-connect bodies on both ends of the circuit to open system. Turn on pump and flush with approximately 100 mL each organic-free water, acetone, and methanol. After last rinse, dry with a heat gun with pump still running (or flush with nitrogen) until there is no residual methanol. (NOTE: Overheating quick-connect units can cause deterioration of internal O-rings.)

7) *Thermostatic water bath,* with 222-mm-OD × 457-mm chromatography jar and thermoregulating system accurate to at least ±0.5°C. When the ambient temperature of the laboratory is greater than 25°C, maintain water bath at 25°C by inserting a coil of copper tubing connected to a cold water tap to recirculate cold water.

8) *Filters,* with 1.5 mg activated carbon##(151) (Figure 6040:4). Use a set of filters matched in solvent flow resistance and performance for each group of samples and calibration standards. Determine filter resistance by measuring solvent flow rate through a cleaned, solvent-wetted filter. First fill the longer glass tube above the charcoal with organic-free water and let flow by gravity through filter disk. Rinse once with acetone, rinse twice with elution solvent, rinse twice with acetone, but on the final rinse, measure time necessary to empty the solvent (0.3-mL

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volume) from top of filter tube to surface of carbon. Rates for new, commercially prepared filters vary significantly, and decrease with use. Flow rates also depend on the solvent used. Determine optimal flow rate range from analyte recoveries. Preferably, verify filter performance by preparing check standards. Figure 6040:5 shows the reduction in air flow caused by using a ‘‘slow’’ filter. Figure 6040:6 shows the effect of filter resistance on recovery of earthy-musty odorants and one of the internal standards. Clean filter as soon as possible after use. Fill glass tube with organic-free water and let flow through filter. Repeat once with acetone, twice with elution solvent, and twice with acetone. Measure flow on final acetone rinse. If solvent flow is slow because of salt deposits, pull 1*N* HNO_3 through filter, using a vacuum connection. After acid washing, rinse with distilled water and acetone and continue with cleaning as above. After final rinse, remove residual solvent by connecting filter to a vacuum for approximately 5 min. Clean auxiliary filter after 40 uses or 2 weeks, whichever comes first.

If the salting-out technique is used, Na_2SO_4 may be carried over and ultimately may clog the filter. The initial water rinse is necessary to remove deposited salts and may be avoided if salt is not used.

c. Stirrer (optional), with 5-cm-long TFE stirring bar.

d. Microsyringes, 5-, 10-, 25-, and 50-µL capacity. Use a 25-µL gastight syringe with electrotapered (blunt-end) tip**#(152) for transferring extract.

e. Receivers, 50-µL capacity (Figure 6040:4). Receivers can be produced by a custom glass-blowing company: use a 1.6-mm-ID precision-bore capillary glass and grind to 5 mm OD, then heat-constrict to close off bore at approximately 29 mm from top. Mark at 20-µL level with glass scribe. To clean receivers and extract storage vials (¶ g, below), rinse seven times with elution solvent and bake at 180°C overnight or rinse with acetone and bake in annealing oven at 400°C for 1 h. Rinse receivers several times with elution solvent before using.

f. TFE sleeve, 5-mm-ID TFE flexible tubing approximately 19 mm long. If a 5-mm-OD receiver is not prepared as described above, then connect filter and receiver with a piece of heat-shrink TFE tubing that is custom-shrunk to the dimensions of the filter and receiver. Rinse sleeve with acetone after each use and store in acetone until ready to use.

g. Extract storage vials, 100-µL capacity conical-shaped vials with TFE-lined septum screw cap or crimp cap.††#(153) Transfer sample extracts from receiver to storage vial for extract storage. The storage vials are compatible with various autosamplers.

h. Gas chromatograph (GC)/mass spectrometer (MS)/data system, equipped with:

1) *Capillary injector,* Grob-designed split-splitless injector or equivalent with 2.5-mm-ID glass insert or nonvaporizing, septumless, cold on-column injector.

2) *Capillary column,* 30-m or 60-m × 0.25-mm-ID DB-1 or DB-5 fused silica or other capillary column capable of producing adequate and reproducible resolution. If using a Grob on-column injector, use a 0.32-mm-ID column for injection when a stainless-steel needle is used, or use a 0.25-mm-ID column with a fused-silica needle.

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For other injector types, a 1-m precolumn of uncoated, deactivated 0.53-mm-ID fused silica is recommended. Connect precolumn to analytical column with a zero-dead-volume union.

3) *Microsyringes,* 5- and 10-µL capacity, with 75-mm-long needles. Use 0.23-mm-OD stainless-steel or 0.17-mm-OD fused-silica needle for on-column injection.

4) *Mass spectrometer analyzer:* See Section 6200B.2 for suggested specifications.

5) *Data system,* with software capable of performing reverse-library searches (optional).

6) *Autosampler injector* (recommended for improved precision of analysis). When methylene chloride is the elution solvent, manual injection is recommended.

3. Reagents

Use reagent-grade solvents or better and obtain purest standards available.

a. Carbon disulfide, CS_2: Use only after gas chromatographic verification of purity to ensure that the solvent does not contain components that coelute with the compounds of interest. CAUTION: *Use proper safety procedures; explosive, toxic, and occasionally allergenic.*

b. Acetone.

c. Methylene chloride, high-resolution grade. Preferably use for analyses conducted at high altitudes. MIB has been shown to be unstable in methylene chloride; extract with CS_2 whenever possible.[9]

d. Carrier gas: Helium gas, ultrapurified grade, moisture- and oxygen-free.

e. Internal standards and surrogates:

1) *1-Chlorooctane.*

2) *1-Chlorodecane.*

3) *1-Chlorododecane.*

4) *1-Chlorohexane, 1-chlorohexadecane,* and *1-chlorooctadecane* can be added for broad-spectrum analysis.

f. Stock internal standard and surrogate solutions: Dissolve 1 mL of each internal standard‡‡#(154) in acetone and dilute to 25 mL with acetone in a volumetric flask; 1 µL = 35 µg.

g. Combination internal standard and surrogate solution: Combine 7.2 µL of each stock solution and dilute to 25 mL with acetone; 1 µL = 10 ng each.

h. Reference standards: Compounds of interest may be available commercially.§§#(155) Deuterated geosmin and methylisoborneol can be synthesized.[10]

i. Stock reference solutions: Dissolve 20 mg of each target compound in acetone and dilute to 10 mL with acetone in a volumetric flask; 1 µL = 2 µg.

j. Combination reference standards solution: Combine 10 µL of each stock reference solution and dilute to 5 mL with acetone; 1 µL = 4 ng each.

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k. Organic-free water: Prepare water by treating with activated carbon, mixed-bed deionization, and filtration through a membrane filter.‖ ‖#(156) Irradiate under ultraviolet light (185 and 254 nm) for 1 h (optional) and prestrip in the CLSA apparatus for 1 h (optional if laboratory blanks are consistently free of interferences), using a clean activated carbon filter or, alternatively, prestrip large quantities of water with nitrogen (ultra-high-purity grade) just before use. Store in a closed bottle tightly capped with TFE-lined screw cap, under nitrogen (optional), in a refrigerator and away from solvent contamination for not longer than 1 week.

l. Sodium sulfate, Na_2SO_4 (optional), granular, anhydrous. Bake at 400°C for 2 h before use; store at room temperature in desiccator.

4. Procedure

a. Sample collection and storage: Rinse sample bottle with sample, fill to overflowing (with no air bubbles), and cap tightly. Collect duplicate samples and in the field keep in an insulated container stocked with ice. In the laboratory store at 4°C, but analyze as soon as possible, preferably within 3 d. For holding longer than 3 d, add 40 mg $HgCl_2$/L to inhibit biological activity. Adding a dechlorinating agent is optional, because disinfection by-products may be affected. CAUTION: *$HgCl_2$-containing samples must be disposed of as hazardous waste. See Section 1090 for precautions.*

b. Treatment of samples:

1) Stripping—Rinse stripping bottle with sample and fill to the 1-L mark, wetting the glass joint with sample. Fill stripping bottle slowly, with minimal aeration, to prevent loss of volatile compounds. Add 10 μL combination internal standard and surrogate solution with the syringe needle tip immersed. Stopper tightly and attach springs. Place in thermostatic water bath at 25°C with glass joint below water level and connect bottle to the circuit. Operate gas heater at 45 to 50°C. Put an ‘‘auxiliary’’ carbon filter in the holder and prestrip for 10 s to flush air contaminants from system. If air quality in the room is demonstrated to be free of interference by analysis of method blank, eliminate prestrip step. Exchange auxiliary filter for a clean one and strip for 2 h (pump flow rate of 1 to 1.5 L/min). Auxiliary filter may be reused many times before cleaning. If stripping bottle has a smaller height-to-diameter ratio than shown in Figure 6040:2, more than 2 h may be required for stripping. Optionally use an automatic timer to terminate each stripping run. Strip time is adjustable, but strip calibration standards and samples under the same conditions.

If sample contains a large amount of algae or turbidity or foaming agents, use only 900 mL sample and 9 μL combination internal standard solution. Because this additional headspace can result in different stripping efficiencies, comparably analyze a calibration standard. Alternatively, dilute concentrated or foaming samples with organic-free water.

2) Alternate stripping techniques—To improve stripping recovery, use a combination of a), b), and c) below to reduce stripping time. Optimize combination, depending on compounds to be analyzed.

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a) Elevated stripping temperature—Increase temperature of thermostatic water bath to 45°C to increase recovery of many organic compounds.[5,7] Raise temperature of gas heater to at least 55°C (for a 45°C stripping temperature) to avoid condensation of water vapor on the activated carbon filter. Further increases in stripping temperature reduce recovery.[5]

b) Salting-out technique—Raising the sample ionic strength with Na_2SO_4 before stripping increases the stripping rate of many organic compounds.[3] Bring sample to room temperature before analysis by immersing it in a water bath at 25°C for approximately 15 min. Transfer 800 mL to the stripping bottle and add stirring bar. Using a glass funnel and with the stirring bar (at intermediate setting) in motion, add 72 g Na_2SO_4. Remove funnel and replace with a glass stopper. Continue stirring until Na_2SO_4 has dissolved (not more than 1 min). Remove stopper and stirring bar, then add remaining 100 mL of sample,###(157) rinsing and wetting inside neck of bottle. Add 9 µL combination internal standard solution and strip at 25°C as described in standard stripping procedure above. If analysis is only for MIB and geosmin, a 1-h strip time is adequate. If additional target compounds are being analyzed, verify strip time needed for adequate recoveries of each target compound. Alternatively, combine salt and sample by pouring salt directly into 900 mL sample, stopper tightly, shake vigorously, let stand for several minutes, and add internal standards.

c) Alternative analysis–Use deuterium-labeled geosmin and MIB as internal standards for the determination of geosmin and MIB.[11] Table 6040:III shows a comparison of quantitation and monitoring ions between the 1-chlorodecane internal standard and the MIB-d_3 and geosmin-d_3 internal standards. Variations in stripping or water temperature do not affect the accuracy of the analysis when labeled internal standards are used because MIB and geosmin will strip at the same rate as MIB-d_3 and geosmin-d_3.[11] In addition, labeled standards, if added in the field, will document degradation of the target compound within a 3- to 4-week period. The labeled standards compensate for losses of analyte by physical, chemical, and biological processes during***#(158) sample storage.[11]

3) Extracting the filter—Remove activated carbon filter from holder. In a fume hood, extract with CS_2 as indicated in Figure 6040:4. Keep solvents well within the hood to avoid inhalation by analyst or contamination of stripping apparatus. Add 2 µL elution solvent to a clean receiver and connect filter and receiver with a TFE sleeve so as to leave no dead space between glass parts. Place 10 µL elution solvent above the carbon, taking care not to touch carbon with the needle. Warm receiver with hands and alternately pass elution solvent across carbon 10 times. Cool receiver with ice, taking care not to freeze the elution solvent, in order to draw the elution solvent below the carbon. Tap the filter/vial assembly gently on a hard surface to complete transfer of elution solvent to bottom of vial.

Repeat filter extraction with a 10-µL and a 5-µL portion of elution solvent. Separate vial from filter, adjust volume precisely to the 20-µL mark,†††#(159) and, using a gastight electrotapered tip syringe, transfer extract to a clean, conical-shaped storage vial. Label and store

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at −20°C until analysis.

Filter may be extracted while maintaining tight seal between filter and vial during the procedure. Using ice chips, cool closed volume in the vial; solvent accumulates on lower side of the filter disk. Push solvent back to upper side by warming the closed vial between two fingers. Repeat and then extract with more solvent as above.

c. Gas chromatography/mass spectrometry:

1) ‘‘Hot-needle’’ injection technique—To reduce discrimination against higher-boiling compounds by distillation from the needle, use a hot-needle injection technique when the injector is a hot vaporizing type. (Do not use the following procedure for cold on-column injection.) Wet syringe needle and barrel with solvent and expel as much as possible. Pull syringe plunger back, leaving an air gap. Pull up approximately 1.5 µL sample and pull sample totally into syringe barrel. Close the split on the GC injector, wait 10 s, insert syringe needle into the injector, and let needle warm up for 1 to 2 s (optimize time by experience). Rapidly push plunger to bottom of syringe barrel to inject sample. Remove syringe and rinse well with solvent. Open split valve consistently at same time (suggested time 30 s) after the injection.

2) On-column injection technique—To more fully reduce discrimination against higher-boiling compounds, use an on-column injector. A cold on-column injector also can be used to avoid decomposition of thermally labile compounds, e.g., dimethyl polysulfides.[12] Determine thermally labile compounds quantitatively by using a cold on-column injector or an inactive, vaporizing injector.

With an on-column injector, increase sensitivity by injecting large sample volumes (up to 8 µL). To prevent problems from a heavy condensation of solvent with such large-volume injections, use a 2-m retention gap (an empty, deactivated piece of 0.53-mm ID fused silica tubing connected to the head of the column with a zero-dead-volume connector).[13] To preclude backpressure from large-volume injections, inject slowly at about 1 µL/5 s. Keep initial column temperature at 10°C above boiling point of solvent for a full solvent effect and to produce sharp peaks (narrow peak widths).[13] Because the entire injection is deposited directly into the head of the column, the column can develop active sites after as few as 50 to 80 injections. Check activity by injecting a polarity test mixture at least weekly. Breaking approximately 30 cm off the head of the column can restore inertness.

3) Operating conditions for GC/MS—After initial installation of the capillary column, condition it according to the manufacturer’s instruction. Daily, make a conditioning run with a CS_2 injection or method blank extraction injection before injecting any samples (optional). Typical instrument conditions are given in Table 6040:IV.

d. Calibration standard: The method is semiquantitative for a large number of compounds, but has been shown to be quantitative for many of the compounds listed in this section. Prepare a 20-ng/L target-compound calibration standard by dosing 1 L organic-free water in the stripping bottle with 10 µL combination internal standard solution plus 5 µL combination reference standards solution. (Internal standards concentration is 100 ng/L each.) If the salting-out

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technique is used, add 72 g Na_2SO_4 to a total volume of 900 mL organic-free water before dosing with 9 µL combination internal standard solution plus 4.5 µL combination reference standards solution. Analyze as directed above. Inject the calibration standard extract, preferably daily, to determine GC/MS response factors and verify spectra.

Verify working linear range by analyzing standards and representative samples with added organics at different concentrations. Calibrate at least every two weeks. Use calibration levels that bracket the levels found in samples. At minimum, use a three-level calibration curve.

e. Blanks: Run a procedural blank daily to assess contamination from reagents, apparatus, and other sources. Run a blank immediately after analyzing any very high-level sample or after installing new parts in the system. Analyze organic-free water with internal standards under the same conditions as samples.

5. Calculations

a. Identification: Identify a compound by matching both retention time and spectra of sample and standard. If they are available, use both a reverse-search computer program with a target-compound library and a forward-search program with the National Institute of Standards and Technology library for tentative identification of other compounds present.

1) Retention times—Use each internal standard to calculate relative retention times for all compounds in the same part of the chromatogram (Table 6040:V). For compounds eluting on the solvent tail, use an early-eluting internal standard (e.g., 1-chlorophexane). Sample retention times should match predicted retention times within ±15 s.

$$\text{Predicted } T_{z,x} = \frac{T_{z,s}}{T_{I,s}} \times T_{I,x}$$

where:

$T_{z,x}$ = retention time of target compound in sample analysis,
$T_{z,s}$ = retention time of target compound in calibration standard analysis,
$T_{I,s}$ = retention time of internal standard in calibration standard analysis, and
$T_{I,x}$ = retention time of internal standard in sample analysis.

2) Spectra—Peaks of at least three characteristic ions should all maximize at the same retention time and have standard intensity ratios (spectra) within ±20% of those of the calibration-standard compounds. Characteristic ions and their typical relative intensities for three of the internal standards and two earthy-musty-smelling compounds are given in Table 6040:V. Preferably, use reference spectra of 10 to 14 key masses. Determine reference spectra by analysis of standards; verify these frequently. The spectra of MIB are particularly dependent on instrument condition; both 107 and 95 amu have been reported as base peaks (Figure 6040:7).

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Figure 6040:8 shows the mass spectrum for geosmin.

b. Quantitation: Determine concentrations by comparison of peak areas of specific quantitation ions. A quantitation ion should be relatively intense in the mass spectrum, yet be free from interference problems caused by closely eluting compounds (see Table 6040:V). Calculate a response factor for each compound from CLSA of a calibration standard as follows:

$$R_z = \frac{A_z \times C_I}{C_z \times A_I}$$

where:

R_z = response factor for target compound z,
A_z = peak area of target compound z,
A_I = peak area of internal standard,
C_I = concentration of internal standard, and
C_z = concentration of target compound z.

Alternatively, a calibration fit can be used.

Compound concentration in the sample (x) is:

$$C_{z,x}, \text{ ng/L} = \frac{A_{z,x} \times C_{I,x}}{R_z \times A_{I,x}}$$

where:

$C_{z,x}$ = concentration of target compound in sample,
$A_{z,x}$ = peak area of target compound in sample,
$C_{I,x}$ = concentration of internal standard in sample, and
$A_{I,x}$ = peak area of internal standard in sample.

Use the internal standard 1-chlorodecane for determining response factors. Use other internal standards as a check on the system; calculated values should be to ±20%. Computerized reverse-search spectral matching and automatic quantitation are recommended to improve identification in complex matrices and to facilitate data processing.

Where calibration standards are unavailable, estimate concentrations by comparing the total ion current of the compounds to that of the internal standard 1-chlorodecane.

6. Quality Assurance/Quality Control

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The CLSA method is semiquantitative for some compounds because of the variability of stripping efficiencies. However, quantitative data are obtainable for compounds that are reproducibly stripped (e.g., MIB and geosmin).[1-3,7] Follow general quality assurance/quality control requirements (e.g., calibration, initial quality control, and batch quality control) described in Section 6020.

Analyze a replicate sample at least once per 10 samples to check precision. If unusual or unexpected results are obtained, analyze a replicate to confirm. Typically, single-analyst determinations for a relatively simple matrix have a coefficient of variation less than or equal to 10%. Otherwise, precision is usually within 20%. For compounds that are poorly stripped, a higher coefficient of variation may be obtained.

Analyze a sample with a known addition at least once per 10 samples to check accuracy and recovery. If matrix problems exist, this will confirm the accuracy of results. Adjust these recoveries against the calibration standards results. Even when absolute recoveries are less than 50%, standard adjusted recoveries, which correct for stripping efficiencies, are usually between 80 and 120%.

Internal standard response should equal ± 40% of the daily standard. An unacceptable internal standard response requires extract reinjection. If the reinjection is still unacceptable, investigate cause, restrip sample, and reanalyze. If consecutive samples fail the internal standard acceptance criterion, immediately analyze a calibration standard. If the calibration standard internal standard response also is unacceptable, recalibrate the instrument.

Ideally prepare and analyze an intralaboratory check sample monthly. Prepare from an independent stock solution of the standards.

7. Precision and Bias

Precision and bias data are given in Table 6040:VI and Table 6040:VII for the analysis of earthy-musty-smelling compounds. Table 6040:VIII shows recovery and precision data for selected pollutants.

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6040 C. Purge and Trap Technique

For applications of this technique to analyses for volatile organics, volatile aromatic organics, and volatile halocarbons, see Section 6200.

6200 VOLATILE ORGANIC COMPOUNDS*#(160)

6200 A. Introduction

1. Source and Significance

Many organic compounds have been detected in ground and surface waters. While most groundwater contamination episodes are traceable to leaking underground fuel or solvent storage vessels, landfills, agriculture practices, and wastewater disposal, the most probable cause for contamination of some aquifers and surface waters has never been firmly established. Contamination may be due to past practices of on-site (leach field) disposal of domestic and industrial wastes or to illegal discharges. Organohalides, particularly the trihalomethanes, are present in most chlorinated water systems, especially those using surface waters as a source of supply. Toxicological studies on animal models have shown that some of these organics have the potential for teratogenesis or carcinogenesis in human beings. To minimize these health risks, sensitive detection and accurate and reproducible quantitation of organics is of paramount importance.

2. Selection of Method

Two capillary gas chromatographic methods for purgeable organic compounds are presented. The scope of analytes is detector-dependent. Method B is a gas chromatographic/mass spectrometric (GC/MS) technique. Method C combines GC with photoionization detection (PID)/electrolytic conductivity detection (ELCD) in series. Methods B and C are applicable to a wide range of purgeable organics. Both methods can be applied to finished drinking water, drinking water in any stage of treatment, source water, or wastewater.

The methods presented are highly sophisticated micro-analytical procedures that should be used only by analysts experienced in chromatography and data evaluation and interpretation. While the methods are similar, they are not interchangeable from a regulatory point of view.[1,2]

3. Scope

Table 6200:I lists the compounds that can be determined by these methods. All are determinable by both Method B and Method C. Other compounds may be amenable to these methods.

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4. Sampling and Storage

See Section 6010B.1

5. Method-Required Quality Control Criteria

a. Initial quality control:

1) Initial demonstration of capability—Conduct initial demonstration of capability study at least once, before analysis of any sample, by each analyst, to demonstrate proficiency with the method of choice. Include at least analysis of a reagent blank and four reagent blank samples fortified at a concentration between 10 times the minimum reporting level and the midpoint of the calibration curve. The blank should not contain any compound of interest at a concentration greater than minimum reporting level. Mean percent recovery for each compound calculated from the four fortified samples should be 80% to 120%, and the relative standard deviation (RSD) should be <20%.

2) Method detection level (MDL)—The MDL is a statistical determination of the minimum concentration that can be measured by the method with a confidence level of 99% that the analyte concentration is greater than zero. Determine MDL before any samples are analyzed, using the procedure described in Section 1030 or other appropriate procedure[3] as required for each matrix to be analyzed. For MDL calculation, start with a concentration about five times the estimated instrument detection level. Perform MDL determination as an iterative process. The values listed in Table 6200:III were generated using a concentration of 0.5 µg/L. Conduct MDL determination at least annually. Analyze samples for MDL determination over a 3- to 5-d period to generate a more realistic value.

3) Quality-control sample—Analyze an externally generated quality-control sample as a laboratory fortified blank at least quarterly or whenever new stock solutions are generated. Obtain this sample from sources external to the immediate laboratory, and use it to validate the laboratory's standards both qualitatively and quantitatively. Acceptance criteria are supplied by the manufacturer. If all criteria are not met, determine cause of error, and correct it before continuing.

4) Minimum quantitation level (MQL)—The MQL is the lowest level that can be quantified accurately. The MQL is defined as four times the MDL.

b. Calibration:

1) Initial calibration—Perform initial calibration with a minimum of five concentrations of analytical calibration standards (CALs) for the compound(s) of interest. The lowest concentration should be at the working reporting level; the highest concentration should be at the upper end of the calibration range. Do not report values that are outside of the defined calibration range. For the calibration concentrations, there should be no more than one order of magnitude between concentrations.

Use any of the following calibration functions, as appropriate: response factor for internal standard calibration, calibration factor for external standard calibration, or calibration curve.

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Calibration curves may be linear through the origin, linear not through the origin, or quadratic through or not through the origin. Use the following recommended acceptance criteria for the various calibration functions.

If using response factors or calibration factors, relative standard deviation (RSD) for each compound of interest should be less than 20%. If the RSD is not less than 20% for any compound of interest, then identify and correct source of lack of linearity before sample quantitation. When using response factors (i.e., for GC/MS analysis), check performance or sensitivity of the instrument for the compound of interest against minimum acceptance values for the response factors. See specific analytical method for the acceptance criteria for the response factors for each compound.

For a linear regression, the correlation coefficient should be >0.994. Recalculate each calibration point compared to curve. Resulting values should be within ±20%. If any of the recalculated values are not within ±20%, identify and correct source of outlier(s) before sample quantitation.

Use initial calibration, with any of the above functions (response factor, calibration factor, or calibration curve) for quantitation of the analytes of interest in samples. Use continuing calibration, described in ¶ 2) below, only for checks on initial calibration and not for sample quantitation. Perform initial calibration when instrument is set up and whenever continuing calibration criteria are not met.

2) Continuing calibration—Continuing calibration (CCAL) is the periodic analysis of a calibration standard used to verify that the instrument response has not changed significantly from the initial calibration. Perform continuing calibration every 10 samples for GC analysis, every 20 samples for GC/MS analysis, or every 12 h, whichever is more frequent. Perform continuing calibration with one or more of the concentrations of analytical standards in the initial calibration. Vary actual concentration of continuing calibration standard over calibration range, with a minimum concentration greater than two times the reporting limit. The acceptance criterion for continuing calibration is 70% to 130% recovery compared to the known or expected value of the calibration standard (at the analyst's discretion, the acceptance criterion for the gases may be extended to 60% to 140% recovery). If the acceptance criteria are not met, re-analyze continuing calibration standard or repeat initial calibration. When using response factors, check performance or sensitivity of instrument for analytes of interest against minimum acceptance values for response factors.

3) Closing standard—Finish all sample sets with a closing standard to demonstrate that performance was still acceptable for the last sample analyzed. Use acceptance criteria as for the CCAL.

c. Batch quality control:

1) Analytical day—An analytical day is defined as a 12-h analytical period.

2) Sample set (batch)—A sample set (batch) is defined as those samples extracted in an analytical day, not to exceed 20 samples.

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3) Laboratory reagent blank (LRB)—A LRB is a blank sample consisting of all reagents that normally contact a sample when carried through the entire analytical procedure. Use reagent blank to determine contribution of reagents and preparative analytical steps to observed value. No compound of interest should be present in reagent blank at a level greater than the MQL. Include a minimum of one reagent blank with each sample set (batch).

4) Laboratory-fortified blank (LFB)—See ¶ b2) above. NOTE: For this method the LFB and CCAL are the same.

5) Internal standard (IS)—An internal standard is a compound of known concentration added to each standard and sample just before sample analysis. Because of the nature of purge and trap analysis, the IS is taken through the entire analytical process, just as is the surrogate standard [see ¶ 6) below]. However, the IS is used for quantitation, whereas the surrogate standard is used to monitor ongoing purge recovery. Use IS to monitor retention time, relative response, and concentration of analytes in each sample. When quantifying by the internal standard method, measure all compound responses relative to this standard. Internal standard area response should be in the range of ±30% compared to the mean calibration curve area response. The IS compound should mimic the chromatographic conditions of the analytes of interest. The retention time of this compound should separate from all analytes of interest and elute in a representative area of the chromatogram. If a specific compound cannot be found to meet these criteria, use additional compounds to satisfy analytical needs.

6) Surrogate standard (SS)—A surrogate standard is a compound added to each standard and sample at a known concentration before extraction. Choose a compound(s) that is chemically similar to the analytes and that is unlikely to be found in environmental samples. Carry surrogate standard through entire sample extraction and analytical process to monitor extraction recovery for each sample. Surrogate recovery should remain reasonably constant over time. Recovery should not vary more than 30% from the known value. Refer to method of choice for specific surrogates.

7) Laboratory-fortified sample (LFS)—A LFS is an additional portion of a sample to which the analytes of interest have been added at a concentration at least two times the MRL or around the middle of the calibration range. Include a minimum of one LFS with each sample set (batch). Make LFSs at sufficient concentrations that sample background levels do not adversely affect recovery calculations. (If this is a known sample, adjust addition concentrations to be about five times background level). Base sample batch acceptance on results of CCALs and LFBs rather than on LFSs, because the matrix of the sample may interfere with method performance. Prepare fortifying solution for blanks and samples from a different primary mix than that used to develop working standard mix.

8) LFS duplicates—A LFS duplicate is a second LFS used to evaluate the precision of the method in a matrix sample. If sufficient sample volume is collected, fortify a large enough volume to yield two sample portions for analysis. If sufficient sample volume is not collected, use a second bottle of the same sample fortified to the same concentration as the first. Include a minimum of one LFS duplicate with each sample set (batch). Compare precision and bias to

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those listed in the method. Base sample batch acceptance on results of CCAL and LFB additions rather than LFS duplicates.

6. References

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1987. National Primary Drinking Water Regulations—synthetic organic chemicals; monitoring for unregulated contaminants; final rule. 40 CFR 141 & 142; *Federal Register* 52, No. 130.
2. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1986. Guidelines establishing test procedures for the analysis of pollutants under the Clean Water Act. 40 CFR Part 136; *Federal Register* 51, No. 125.
3. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1995. Definition and procedure for the determination of the method detection limit, revision 1.11. 40 CFR Part 136, Appendix B.

6200 B. Purge and Trap Capillary-Column Gas Chromatographic/Mass Spectrometric Method

This method[1] is applicable to the determination of a wide range of purgeable organic compounds (see Table 6200:I). The method can be extended to include other volatile organic compounds, provided that all performance criteria are met. It should be used only by analysts experienced in the operation of GC/MS systems and in evaluation and interpretation of mass spectra.

1. General Discussion

a. Principle: Volatile organic compounds are transferred efficiently from the aqueous to the gaseous phase by bubbling an inert gas (e.g., helium) through a water sample contained in a specially designed purging chamber at ambient temperature. The vapor is swept through a sorbent trap that adsorbs the analytes of interest. After purging is complete, the trap is heated and back-flushed with the same inert gas to desorb the compounds onto a gas chromatographic column. The gas chromatograph is temperature-programmed to separate the compounds. The detector is a mass spectrometer. See Section 6010C for discussion of gas chromatographic and mass spectrometric principles.

b. Interferences: Impurities in the purge gas and organic compounds outgassing from the plumbing upstream of the trap account for most contamination problems. Demonstrate that the system is free from contamination under operational conditions by analyzing laboratory reagent blanks daily. (NOTE: Use blanks for monitoring only; corrections for blank values are unacceptable.) Avoid using non-TFE plastic tubing, non-TFE thread sealants, or flow controllers with rubber components in the purge and trap system. Ensure that the analytical area is not subject to contamination from laboratory solvents, particularly methylene chloride and methyl

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tert-butyl ether (MtBE).

Samples can be contaminated by diffusion of volatile organics (particularly fluorocarbons and methylene chloride) through the septum seal during shipment and storage. Use a field reagent blank prepared from reagent water and carried through the sampling, handling, and shipping procedures as a check on such contamination.

Contamination by carryover can occur whenever high-level and low-level concentration samples are analyzed sequentially. To reduce carryover, rinse purging device and sample syringe with reagent water between samples. Follow analysis of an unusually high concentration sample with a LRB to check for carryover contamination. For samples containing large amounts of water-soluble materials, suspended solids, high boiling compounds, or high levels of volatile compounds, wash purging device with a detergent solution, rinse it with distilled water, and dry it in an oven at 105°C between analyses. The trap and other parts of the system also are subject to contamination; therefore, frequently bake and purge entire system.

c. Detection levels: Method detection levels (MDLs) are compound-dependent and vary with purging efficiency and instrument response. In a single laboratory using reagent water and known-addition concentrations of 0.5 µg/L, observed MDLs were in the range of 0.025 to 0.450 µg/L. The applicable calibration range of this method is compound- and instrument-dependent, but is approximately 0.2 to 200 µg/L. Compounds that are inefficiently purged from water will not be detected when present at low concentrations, but they can be measured with acceptable bias and precision when present at sufficient concentration. Determination of some geometrical isomers (e.g., xylenes) may be hampered by co-elution.

d. Safety: The toxicity or carcinogenicity of each analyte has not been precisely defined. Benzene, carbon tetrachloride, bis(1-chloroisopropyl)ether, 1,4-dichlorobenzene, 1,2-dichloroethane, hexachlorobutadiene, 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane, chloroform, 1,2-dibromoethane, tetrachloroethene, trichloroethene, and vinyl chloride have been classified tentatively as known or suspect carcinogens. Handle pure standard materials and stock standard solutions of these compounds in a hood and wear a NIOSH/MESA-approved toxic gas respirator when handling high concentrations.

2. Apparatus

a. Purge and trap system: The purge and trap system consists of purging device, trap, and desorber. Several complete systems are available commercially.

1) *Purging device,* designed to accept 25-mL samples with a water column at least 5 cm deep. A smaller 5-mL purging device is acceptable if required method detection levels and performance criteria are met. Keep gaseous headspace between water column and trap to a total volume of less than 15 mL. Pass purge gas through water column as finely divided bubbles with a diameter of less than 3 mm at the origin. Introduce purge gas no more than 5 mm from base of water column. The purging device illustrated in Figure 6200:1 meets these criteria.

Needle spargers may be used instead of the glass frit shown in Figure 6200:1; however, in either case, introduce purge gas at a point <5 mm from base of water column.

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2) *Trap,* at least 25 cm long and with an inside diameter of at least 3 mm, packed with the following minimum lengths of adsorbents: 1.0 cm methyl silicone coated packing, 7.7 cm 2,6-diphenylene oxide polymer, 7.7 cm silica gel, and 7.7 cm coconut charcoal. If analysis is not to be made for dichlorodifluoromethane, the charcoal may be eliminated and the polymer section lengthened to 15 cm. Alternative sorbents may be used provided that all quality control criteria are met. Various sorbent traps are available commercially**#(161); ensure that trap keeps total purge gas volume and purge time constant (i.e., 40 mL/min for 11 min) and that performance will meet all quality control criteria. The minimum specifications for the trap are illustrated in Figure 6200:2.

Methyl silicone coated packing is recommended, but not mandatory. The packing protects the diphenylene oxide polymer adsorbent from aerosols, re-coating any active site that may develop during the heating process, and ensures that the polymer is fully enclosed within the heated zone of the trap, thus eliminating potential cold spots. Alternatively, silanized glass wool may be used as a spacer in the trap inlet.

Before initial use, condition trap overnight following manufacturer's instructions. Vent trap effluent to the room, not to analytical column. Before daily use, condition trap for 10 min with back-flushing. Optimally, vent trap to analytical column during daily conditioning; however, run column through temperature program before sample analysis.

b. Gas chromatograph (GC)†#(162)*:* Use a temperature-programmable GC, suitable for on-column injection. Deactivate all glass components (e.g., injector liners) with a silanizing agent.

c. Capillary GC columns: Use any capillary GC column that meets all performance criteria. Ensure that desorb flow rate is compatible with the column of choice. Four examples of acceptable columns are listed below.

1) *Column 1:* 60-m-long × 0.75-mm-ID VOCOL‡#(163) wide-bore capillary column with 1.5-µm film thickness.

2) *Column 2:* 30-m-long × 0.53-mm-ID DB-624[§]#(164) mega-bore capillary column with 3-µm film thickness.

3) *Column 3:* 30-m-long × 0.32-mm-ID DB-5[§] capillary column with 1-µm film thickness.

4) *Column 4:* 30-m-long × 0.25-mm-ID DB-624[§] capillary column with 1.4-µm film thickness.

d. Mass spectrometer, capable of scanning from 35 to 300 amu every 2 s or less, utilizing 70 eV (nominal) electron energy in the electron impact ionization mode, and producing a mass spectrum that meets all criteria in Table 6200:II when 25 ng or less of 4-bromofluorobenzene is introduced into GC. To ensure sufficient precision, the desired scan rate permits acquisition of at least five spectra while a sample component elutes from the GC.

e. Purge and trap − GC/MS interface: Use an open-split or direct-split interface, depending on which column is used. Alternatively, if the narrow-bore column (4) is used, a capillary

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concentrator preceding the GC may be necessary. This interface condenses desorbed materials onto an uncoated fused silica pre-column and when flash-heated transfers compounds onto the capillary column. The uncoated section of column is cooled to −150°C during desorption and heated to 250°C to transfer condensed materials.

f. Data system: To the mass spectrometer attach a computer that allows continuous acquisition and storage of all mass spectra obtained throughout the chromatographic program. Computer software should allow for a search of all acquired spectra for specific *m/z* (masses) and the plot of such *m/z* abundances versus time or scan number. This type of plot is an extracted ion current profile (EICP). Software also should allow the integration of the abundances in any EICP over a specified time or scan limit.

g. Syringes, 0.5-, 1.0-, 5-, and 25-mL glass hypodermic with detachable tip.‖#(165)

h. Syringe valves, two-way, with detachable tip.‖

i. Microsyringes, 10-, 25-, and 100-µL with a 5-cm × 0.15-mm-ID and 220 bevel needle.##(166)

j. Bottles, 40-mL with TFE-lined screw cap.

3. Reagents

a. Reagent water, in which no interferent is observed at or above the MDL of the constituents of interest. Prepare by passing tap water through a carbon filter bed containing about 0.5 kg activated carbon, by distillation, or by using a water purification system.**#(167)

b. Trap packing materials:

1) *2,6-Diphenylene oxide polymer,* 60/80 mesh, chromatographic grade.

2) *Methyl silicone packing,* 3 OV-1.

3) *Silica gel,* 35/60 mesh.

c. Methanol, purge-and-trap grade.

d. Hydrochloric acid: HCl, 1 + 1.

e. Vinyl chloride, 99.9% pure.

f. Ascorbic acid.

g. Stock standard solutions: Prepare from pure standard materials or purchase as certified solutions. Prepare stock standard solutions in methanol using assayed liquids or gases as appropriate. CAUTION: *Toxic substances.* See ¶ 1*d*.

Place about 9.8 mL methanol in a 10-mL ground-glass-stoppered volumetric flask. Let stand unstoppered for about 10 min or until all alcohol-wetted surfaces have dried. Weigh flask to nearest 0.1 mg.

Add assayed reference materials as follows: For liquids, using a 100-µL syringe or disposable capillary-tip glass pipet, immediately add two or more drops of assayed reference material to flask, then reweigh. Ensure that the drops fall directly into the alcohol without contacting flask neck. For halocarbon gases that boil below 30°C (bromomethane, chloroethane,

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chloromethane, dichlorofluoromethane, trichlorofluoromethane, vinyl chloride), attach a vinyl plastic††#(168) tube to port of gas bottle containing reference material, with open end bubbling into a beaker of methanol showing flow through the tubing; insert needle of 5-mL valved gastight syringe into tube and pull gas into syringe slowly to 5.0-mL mark. Lower syringe needle to within 5 mm of methanol surface and slowly force gas onto surface. The gas will dissolve into the methanol and will be seen as a vortex as it dissolves into the solvent. Reweigh flask (difference is amount of gas dissolved into methanol), dilute to volume, stopper, and mix by inverting several times. Calculate concentration in micrograms per microliter from net gain in weight. When compound purity is assayed to be 96% or greater, calculate concentration of stock standard from uncorrected weight. Preferably use commercially prepared stock standards at any concentration if they are certified by the manufacturer or an independent source. Transfer stock standard solution into a TFE-sealed screw-cap bottle. Store with minimum headspace at −10 to −20°C away from light.

h. Secondary dilution standards: Using stock standard solutions, prepare in methanol secondary dilution standards that contain the compounds of interest, either singly or mixed together. Prepare secondary dilution standards at concentrations that will permit aqueous calibration standards (¶ j below) to bracket working range of the analytical system. Store secondary dilution standards with minimal headspace in a freezer and check frequently for signs of evaporation (which would indicate need for regeneration). Always bring to room temperature before preparing calibration standards. Prepare standards fresh weekly for gases. Replace all other standards monthly, or sooner if comparison with check standards indicates a problem.

i. Internal standard/surrogate standard known addition: Prepare a solution containing fluorobenzene (internal standard) and 1,2-dichlorobenzene-d_4 (surrogate) in methanol. Alternate internal standard and surrogate compounds may be used, provided that they meet method criteria and do not interfere with any method analyte(s). Prepare secondary dilution standard at a concentration of 5 μg/mL of each compound. Adding 5.0 μL standard to 25.0 mL sample or calibration standard yields a concentration equivalent to 1.0 μg/L. Alternate secondary standard concentrations can be used if addition volume is adjusted accordingly and all internal standard criteria are met. Add this mixture to each sample, standard, and blank.

j. Calibration standards: Prepare at least five concentration levels for each compound by adding appropriate amounts of secondary standard solution to reagent water and inverting water sample twice. Prepare one standard at a concentration near, but above, the MDL (i.e., 4 × MDL for potable-water-type samples) or a level that defines the low end of the working range and the others to correspond to the expected range of sample concentrations or to define the detector working range. Aqueous calibration standards can be stored up to 24 h if held in sealed vials with zero headspace. Otherwise, discard within 1 h. Alternatively, prepare calibration standards by injecting, with a solvent flush, an appropriate amount of a standard mix dilution and internal standard/surrogate mix, directly into a 25-mL syringe filled with reagent water; immediately inject water standard into purge vessel.

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4. Procedure

a. Operating conditions: Table 6200:III summarizes recommended operating conditions for the gas chromatograph and gives estimated retention times and MDLs that can be achieved under these conditions. An example of the separations obtained with the specified column is shown in Figure 6200:3. Other chromatographic columns or conditions can be used if the quality control criteria are met.

b. GC/MS performance tests: At beginning of each day on which analyses are to be performed, check GC/MS system by a performance test with BFB before any samples, blanks, or standards are analyzed. Performance tests require the following instrument parameters:

Electron energy: 70 eV (nominal)
Mass range: 35 to 300 amu
Scan time: at least 5 scans/peak but not more than 2 s/scan

Inject 25 ng BFB directly on GC column. If direct injection is not easily performed, add 1 µL 25-µg/mL BFB solution to 25 mL reagent water in syringe used for sample transfer to purge device and analyze as a sample. Obtain a background-corrected mass spectrum of BFB and confirm that all key *m/z* criteria in Table 6200:II are achieved. If all criteria are not achieved, re-tune mass spectrometer and repeat test until all criteria are met.

c. Calibration: Calibrate system as follows:

1) System setup—Condition trap initially overnight at 180°C by back-flushing with inert gas at 20 mL/min. Condition trap daily for 10 min at manufacturer's suggested temperature. Connect purge and trap system to GC using recommended temperature program and flow-rate conditions. Calibrate system by either the internal or the external standard technique.

2) Internal standard calibration technique—Select one or more internal standards similar in analytical behavior to the compounds of interest. Fluorobenzene is a recommended internal standard compound. Demonstrate that measurement of internal standard is not affected by method or matrix interference. Because of such limitations, no one internal standard may be applicable to all samples. The compounds used as surrogates (e.g., 1,2-dichlorobenzene-d_4) for quality control also can be used successfully as internal standards. Prepare calibration standards at a minimum of five concentration levels for each compound as described in ¶ 3 *j* above. Prepare a secondary dilution standard containing each of the internal standards (¶ 3*i* above). Analyze each calibration standard according to procedure for samples, adding internal standard solution directly to syringe. Tabulate peak height or area responses against concentration for each compound and internal standard, and calculate response factors (RF) for each compound as follows:

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$$RF = \frac{(A_s)(C_{is})}{(A_{is})(C_s)}$$

where:

A_s = response for compound to be measured,
A_{is} = response for internal standard,
C_{is} = concentration for internal standard, and
C_s = concentration of compound to be measured.

Average RF can be used if RSD is less than 20%.

3) External standard calibration technique—Prepare standards as directed in ¶ 3 *j*. Analyze each calibration standard and tabulate peak area responses versus concentration. Prepare calibration curve for each compound. Alternatively, if ratio of response to concentration (calibration factor) is a constant over the working range (<20% RSD), assume linearity through the origin and use average calibration factor in place of a calibration curve.

4) Calibration check—See ¶ A.5*b*2).

d. Sample analysis: Bring sample to ambient temperature. Remove plunger from 25-mL syringe and close attached valve. Open sample bottle and carefully pour sample into syringe barrel to just short of overflowing. Replace syringe plunger, invert syringe, and open valve. Vent any air and adjust sample volume to 25.0 mL, in duplicate if sufficient sample is available (once sample cap has been removed, sample cannot be stored, because of headspace). Add an appropriate amount of surrogate/internal standard through valve bore, and close valve. Attach to purge device, open valves, and inject sample into purge vessel. Close valves and purge sample for 11.0 min at ambient temperature at a flow rate of 40 mL/min (helium or nitrogen). If water vapor causes problems in the mass spectrometer, use a 3-min dry purge and/or a moisture control module.

Desorb trapped materials onto head of chromatographic column at 180°C while back-flushing trap for 4 min with inert gas at a flow rate compatible with the column of choice, and begin GC temperature program.

Set system auto-drain to empty purge chamber while trap is being desorbed into GC, or alternatively, use sample syringe to empty vessel. Washing chamber with two 25-mL flushes of reagent water is useful if highly contaminated samples are being analyzed. Be sure all areas wetted during purging are also wetted during rinsing to maximize flushing.

Recondition trap by baking at conditioning temperature for 5 to 7 min. Let trap cool to ambient before introduction of next sample into purge vessel. When all sample compounds have been eluted from chromatographic column, end data acquisition and store data files. Use data system software to display full range mass spectra and appropriate extracted ion current profiles

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(EICP). If any ion abundances exceed system working range, dilute sample in second syringe with reagent water and analyze. NOTE: *Take care with sample because compounds can be very volatile and can be lost if sample is reopened.* Estimate amount of dilution needed and expel excess sample from second syringe, inject that portion into purge vessel, and with a second syringe, add necessary reagent water to a total of 25.0 mL in purge vessel.

5. Calculation

When compounds have been identified, base quantitation on integrated area abundance from the EICP of the primary characteristic *m/z* given in Table 6200:III. If sample produces an interference for the primary *m/z*, calculate a response factor or calibration curve using a secondary characteristic *m/z*, and use secondary *m/z* to quantitate. Report results in micrograms per liter. Report all quality control data with sample results.

6. Quality Control

See Section 6200A.5.

7. Precision and Bias

Typical single-laboratory precision and bias data are shown in Table 6200:IV.

8. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1992. Methods for the Determination of Organic Compounds in Finished Drinking Water and Raw Source Water. U.S. Environmental Protection Agency, Environmental Monitoring & Support Lab., Cincinnati, Ohio.

6200 C. Purge and Trap Capillary-Column Gas Chromatographic Method

This method[1] is applicable to the determination of purgeable halocarbons and aromatic organic compounds (Table 6200:I) in finished drinking water, raw source water, drinking water in any treatment stage, and wastewater.

1. General Discussion

a. Principle: See Section 6200B.1*a*.

b. Interferences: See Section 6200B.1*b*.

c. Detection levels: In a single laboratory using reagent water and known additions of 0.5 µg/L, calculated method detection levels (MDLs) for these compounds were in the range of 0.01 to 0.05 µg/L, depending on the compound. Some laboratories may not be able to achieve these detection levels because results depend on instrument sensitivity and matrix effects. Analysis of complex mixtures containing partially resolved compounds may be hampered by concentration differences larger than a factor of 10. This problem commonly occurs in analyses of finished

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drinking waters because of the relatively high trihalomethane content.

d. Safety: The toxicity or carcinogenicity of each reagent has not been defined precisely. Carbon tetrachloride, 1,2-dichloroethane, 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane, chloroform, 1,2-dibromoethane, tetrachloroethene, trichloroethene, and vinyl chloride have been classified tentatively as known or suspected human or mammalian carcinogens. Prepare primary standards of these compounds in a hood and wear a NIOSH/MESA-approved toxic gas respirator when handling high concentrations.

2. Apparatus

a. Purge and trap system: The purge and trap system consists of three separate pieces of equipment: purging device, trap, and desorber. Several complete systems are commercially available.

1) *Purging device*—See Section 6200B.2*a*1).

2) *Trap*—See Section 6200B.2*a*2). If only compounds boiling above 35°C are to be analyzed, both silica gel and charcoal can be eliminated and polymer increased to fill entire trap. Trap failure is characterized by a pressure drop above 21 kPa across trap during purging or by poor bromoform sensitivities.

3) *Assembly*—See Figure 6200:1 and Figure 6200:2.

b. Gas chromatograph: See Section 6200B.2*b*.

1) *Column*—See Section 6200B.2*c*.

2) *Electrolytic conductivity or microcoulometric detector*— Halogen-specific systems eliminate misidentifications due to non-organohalides that may be coextracted during purging.

3) *Photoionization detector*—A high-temperature detector equipped with a 10.2-eV (nominal) lamp.*#(169) Insert between analytical column and halide detector to analyze simultaneously for aromatic and unsaturated volatile organic compounds (see Table 6200:I).

c. Syringes, 5-mL glass hypodermic with detachable tip.†#(170)

d. Other equipment: See Section 6200B.2*g* through *j*.

3. Reagents

See Section 6200B.3*a* through *h*.

4. Procedure

a. Operating conditions: Table 6200:V summarizes recommended operating conditions for the gas chromatograph, estimated retention times, and method detection levels. Examples of separations obtained with the specified column are shown in Figure 6200:4 and Figure 6200:5.

b. Calibration: See Section 6200A.5*b*. Use either internal or external calibration technique. If using internal standard technique, prepare a dilution standard as described in Section 6200B.3*i*.

c. Instrument performance: See Section 6200A.5. Ensure that all peaks in standard

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chromatograms are sharp and symmetrical. Correct any peak tailing significantly in excess of that shown in method chromatograms. Tailing problems generally are traceable to active sites on the GC column or to detector operation. If only compounds eluting before chloroform give random responses or unusually wide peak widths, are poorly resolved, or are missing, the problem usually is traceable to the trap/desorber. If only brominated compounds show poor peak geometry or do not respond properly at low concentrations, replace trap. Excessive detector reactor temperatures also can cause low bromoform response. If negative peaks appear in the chromatogram, replace both ion-exchange column and electrolyte in detector. Check precision between replicate analyses. A properly operating system shows an average relative standard deviation of less than 10%. Poor precision generally is traceable to pneumatic leaks, especially around sample purger and detector reactor inlet and exit, electronic problems, or sampling and storage problems. Monitor retention times for each compound using calibration standards and laboratory control standard. If individual retention times vary by more than 10% over an 8-h period or do not fall within 10% of an established norm, locate and correct source of retention data variance.

d. Sample analysis: See Section 6200B.5.

5. Calculation

Identify each organohalide in sample chromatogram by comparing retention time of suspect peak to retention times generated by calibration standards and laboratory control standard. Determine concentrations of individual compounds. If external standard calibration procedure is used, calculate concentration of compound being measured from peak response using calibration curve or calibration factor previously determined.

If internal standard calibration procedure is used, calculate concentration using response factor [*RF*, ¶ B.4*c*2)] by the following equation:

$$\text{Concentration, } \mu\text{g/L} = \frac{A_s \times C_{is}}{A_{is} \times RF}$$

where:

A_s = response for compound to be measured,
A_{is} = response for internal standard, and
C_{is} = concentration of internal standard.

Report results in micrograms per liter without correction for recovery. Report quality control data with sample results.

6. Quality Control

See Section 6200A.5.

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7. Precision and Bias

See Table 6200:VI.

8. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1991. Volatile organic compounds in water by purge and trap capillary column gas chromatography with photoionization and electrolytic conductivity detectors in series. Method 502.2 *in* Methods for the Determination of Organic Compounds in Finished Drinking Water and Raw Source Water. U.S. Environmental Protection Agency, Environmental Monitoring & Support Lab., Cincinnati, Ohio.

6211 METHANE*#(171)

6211 A. Introduction

1. Occurrence and Significance

Methane (CH_4) is a colorless, odorless, tasteless combustible gas occasionally found in groundwaters. Escape of this gas from water may cause an explosive atmosphere not only in a utility's tanks, pumphouses, and other facilities, but also on the consumer's property, particularly where water is sprayed through poorly ventilated spaces such as public showers.

The explosive limits of CH_4 in air are 5 to 15% by volume. At sea level, a 3.95% CH_4 concentration in air theoretically could be reached in a poorly ventilated space sprayed with hot (68°C) water having a CH_4 concentration of only 0.7 mg/L. At higher water temperatures, the vapor pressure of water is so great that no explosive mixture can form. At lower barometric pressures, the theoretical hazardous concentration of methane in water will be reduced proportionately. In an atmosphere of N_2 or other inert gas, at least 12.8% O_2 must be present for there to be an explosion hazard.

Methane also is produced from wastewater and may be present in sewers and wastewater treatment plants (see Section 2720).

2. Selection of Method

The combustible-gas indicator method (B) offers the advantages of simplicity, speed, and great sensitivity. The volumetric method (C) can be made more accurate for concentrations of 4 to 5 mg/L and higher, but will not be satisfactory for very low concentrations. The volumetric method also can be applied to differentiate between CH_4 and other gases, as when a water supply is contaminated by liquid petroleum gas or other volatile combustible materials.

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Methane also may be determined with the gas chromatograph as described in Sludge Digester Gas, Section 2720. This method permits differentiation between H_2 and CH_4, and/or its higher homologs.

6211 B. Combustible-Gas Indicator Method

1. General Discussion

a. Principle: An equilibrium according to Henry's law is established between CH_4 in solution and the partial pressure of CH_4 in the gas phase above the solution. The partial pressure of CH_4 can be determined with a combustible-gas indicator. The operation of the instrument is based on the catalytic oxidation of a combustible gas on a heated platinum filament that is made a part of a Wheatstone bridge. The heat generated by the oxidation of the gas increases the electrical resistance of the filament. The resulting imbalance of the electrical circuit causes deflection of a milliammeter that may be calibrated in terms of percentage of CH_4 or percentage of the lower explosive limit of the gas sampled.

b. Interference: Small amounts of ethane usually are associated with CH_4 in natural gas and presumably would be present in water that contains methane. Hydrogen gas has been observed in well waters and would behave similarly to CH_4 in this procedure. Hydrogen sulfide may interfere if the pH of the water is low enough for an appreciable fraction of the total sulfide to exist in the unionized form. The vapors of combustible oils also may interfere. In general, these interferences are of no practical importance because primary interest is in calculating the explosion hazard to which all combustible gases and vapors contribute.

Interference due to H_2S can be reduced by the addition of solid NaOH to the container before sampling.

c. Minimum detectable concentration: The limit of sensitivity of the test is approximately 0.2 mg/L.

d. Sampling: If the water is supersaturated with CH_4, a representative sample cannot be obtained unless the water is under sufficient pressure to keep all of the gas dissolved. Operate wells long enough to insure sampling water coming directly from the aquifer. Representative samples can be expected only when the well is equipped with a pump operating at sufficient submergence to assure that no gas escapes from the water.

2. Apparatus

*a. Combustible-gas indicator:**#(172) Connect a three-way stopcock to the inlet to zero instrument on atmospheric air immediately before obtaining sample reading. For laboratory use, replace the suction bulb with a filter pump throttled to draw gas through the instrument at a rate of approximately 600 mL/min. See Figure 6211:1.

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b. Laboratory filter pump.

c. Glass bottle, 4-L, fitted with a two-hole rubber stopper. Extend inlet tube to within 1 cm of bottom. End outlet tube approximately 1 cm below stopper. Use metal or glass tubes, each fitted with stopcocks or with short (approximately 5-cm) lengths of rubber tubing and pinchcocks. The entire assembly should be capable of holding a low vacuum for several hours. Determine volume of assembly by filling with water and measuring volume, or weight, of water contained.

3. Reagent

Sodium hydroxide, NaOH, pellets.

4. Procedure

a. Rough estimation of CH_4 concentration: Fill bottle about half full of water, using a rubber tube connecting sampling tap and inlet tube, with outlet tube open. With both inlet and outlet tubes closed, shake bottle vigorously for approximately 15 s and let stand for approximately 1 min. Sample gas phase by withdrawing gas from the outlet, leaving inlet open to admit air. If the needle swings rapidly to a high level on the meter and then drops to zero, the CH_4-air mixture is too rich to burn; take a smaller sample for the final test. If needle deflection is too small to be read accurately, take a larger volume of water.

b. Accurate determination: If the water contains H_2S, add approximately 0.5 g NaOH pellets to empty bottle to suppress interference. Evacuate bottle, using filter pump. Fill bottle not more than three-quarters full by connecting inlet tube to sampling cock, with outlet tube closed. After collecting desired volume of water, let bottle fill with air through inlet tube. Close inlet cock, shake bottle vigorously for 60 s, and let stand for at least 2 h. Sample gas phase through outlet tube with inlet cock open. Take reading as rapidly as possible before the entering air has diluted sample appreciably. Measure volume of water sampled.

5. Calculation

The weight of CH_4 (w), in mg, in the sample is given by the equation:

$$w = P\left(\frac{1.928\ V_g}{T + 273} + \frac{890\ V_1}{H}\right)$$

where:

P = partial pressure of CH_4, kPa,

T = temperature, °C,

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V_g = volume of gas phase, mL,
V_1 = volume of liquid phase, mL, and
H = Henry's law constant, kPa/mole CH_4/mole of water.

Values for Henry's constant are as follows:

Temperature °C	Henry's Constant H*	Temperature °C	Henry's Constant H*
0	2.265	40	5.261
5	2.625	45	5.577
10	3.010	50	5.846
15	3.413	60	6.342
20	3.804	70	6.749
25	4.181	80	6.911
30	4.544	90	7.013
35	4.926	100	7.106

*Multiply given values by 10^6.

For most determinations, it may be assumed that atmospheric pressure is 100 kPa, and that the temperature is 20°C. The concentration of CH_4 in the sample is then given by:

$$\text{mg CH}_4/\text{L} = Rf\left(6.7\,\frac{V_0 - V_1}{V_1} + 0.24\right)$$

where:

R = scale reading,
V_0 = total volume of sample bottle, mL,
V_1 = volume of water sampled, mL, and
f = factor depending on instrument used.

If the instrument reads directly in percentage of methane, = 1.00. If the instrument reads in percentage of the lower explosive limit of CH_4, = 0.05. For instruments that require additional

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factors, consult the manufacturer. For example, one commercial instrument with a scale that reads in percentage of the lower explosive limit of combustible gases requires an additional factor of 0.77 for CH_4. Hence, the value of in the above equation would be 0.77 × 0.05, or 0.0385.

For more accurate work, or in locations where normal barometric pressure is significantly lower than 100 kPa, use the equation:

$$\text{mg } CH_4/L = RBf\left(19.277\,\frac{V_0 - V_1}{TV_1} + \frac{8900}{H}\right)$$

where:

B = barometric pressure, kPa,

and other symbols are as above.

6. Accuracy

The accuracy of the determination is limited by the accuracy of the instrument used. Errors of approximately 10% may be expected. Calibration of instrument on known CH_4-air mixtures will improve accuracy.

7. Bibliography

ROSSUM, J.R., P.A. VILLARRUZ & J.A. WADE. 1950. A new method for determining methane in water. *J. Amer. Water Works Assoc.* 42:413.

6211 C. Volumetric Method

1. General Discussion

a. Principle: If CH_4 is slowly mixed with an excess of O_2 in the presence of a platinum coil heated to yellow incandescence, most of the CH_4 will be converted to CO_2 and H_2O in a smooth reaction. Several passes of the mixed gases may be needed to burn substantially all the CH_4. An excess of O_2 is mixed with the sample before passage through the assembly. By differential absorption and volumetric changes the product CO_2 is measured.

b. Interference: Low-boiling hydrocarbons other than ethane and vapors from combustible oils interfere. These substances, however, are not likely to be present in water in sufficiently high concentration to affect the results significantly.

c. Minimum detectable concentration: This method is not satisfactory for determining CH_4 in

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water where the concentration is less than 2 mg/L.

d. Sampling: Collect sample as directed in Method B and observe the same precautions to obtain representative samples (Section 6211B.1*d*). Omit NaOH pellets and fill sample bottle with water up to 90% of capacity.

2. Methane Determination

See Section 2720B for a description of apparatus, reagents, procedure, calculation, and precision and bias.

Use percentage of CH_4 found by this method with Henry's law to obtain the CH_4 concentration in original sample. Substitute CH_4 percentage for *R* (scale reading) and = 1 in the calculation given under Section 6211B.5 preceding.

3. Bibliography

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6231 1,2-DIBROMOETHANE (EDB) AND 1,2-DIBROMO-3-CHLOROPROPANE (DBCP)*#(173)

6231 A. Introduction

1. Sources and Significance

Dibromoethane and dibromochloropropane have been found in groundwater supplies in many areas of the United States; typically they are found in agricultural areas where these compounds have been applied in the past as fumigants. Toxicological studies suggest that they may have detrimental effects on human health, and therefore many states have established maximum contaminant levels for them.

2. Selection of Method

The liquid-liquid extraction gas chromatographic (GC) method (Section 6231B) uses a microextraction and capillary columns and is the preferred method. In addition, these compounds can be detected by the purge and trap gas chromatographic/mass spectrometric (GC/MS) and GC

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methods (Section 6200B and Section 6200C), and dibromoethane by closed-loop stripping analysis (see Section 6040). For additional information on applicability, sensitivity, precision, and bias, see specific methods.

6231 B. Liquid-Liquid Extraction Gas Chromatographic Method

This method[1-3] is applicable to the determination of 1,2-dibromoethane (EDB) and 1,2-dibromo-3-chloropropane (DBCP) in drinking water and untreated groundwater.

1. General Discussion

a. Principle: The sample is extracted with hexane and injected into a gas chromatograph equipped with a linearized electron capture detector for separation and analysis. Identification is confirmed by analyzing the sample with a dissimilar column. See Section 6010C for discussion of gas chromatographic principles.

b. Interferences: Impurities in the extracting solvent usually account for most analytical problems. Analyze solvent blanks on each new bottle of solvent before use. Obtain indirect daily checks on the extracting solvent by monitoring sample blanks; whenever an interference is noted, reanalyze the extracting solvent. If necessary, remove interference by distillation or column chromatography[3] or, more simply, obtain a new source solvent. Interference-free solvent contains less than 0.1 µg/L individual compound interference. Store solvents in an area free of organochlorine solvents.

Accidental sample contamination can occur through diffusion of volatile organics through the septum seal into the sample bottle during shipment and storage. Sample blanks monitor this.

EDB at low concentrations may be masked by very high levels of dibromochloromethane (DBCM) when the confirmation column is used.

For further information on interferences in gas chromatographic methods, see Section 6010C.

c. Detection levels: The method detection levels (MDL)[4] for EDB and DBCP are 0.01 µg/L. The method is useful over a concentration range from approximately 0.03 to 200 µg/L. Actual detection limits are highly dependent on the characteristics of the gas chromatographic system used.

d. Safety: The toxicity or carcinogenicity of each reagent has not been defined precisely. EDB and DBCP have been classified tentatively as known or suspected human or mammalian carcinogens. Handle pure standard materials and stock standard solutions in a hood or glovebox and wear a NIOSH/MESA-approved toxic gas respirator when handling high concentrations.

2. Sampling and Storage

Collect all samples in duplicate and prepare replicate field blanks with each sample set. A sample set is all of the samples collected from the same general sampling site at approximately the same time. Prepare the field reagent blanks in the laboratory by filling a minimum of two sample bottles with reagent water, sealing, and shipping to the sampling site along with sample

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bottles.

Fill sample bottle to overflowing without air bubbles. When sampling from a water tap, open tap and flush until water temperature has stabilized (usually about 10 min). Adjust flow rate to about 500 mL/min and collect duplicate samples from the flowing stream. When sampling from a well, fill a wide-mouth bottle or beaker with sample, and carefully fill duplicate 40-mL sample bottles.

Keep samples chilled in an atmosphere free of organic solvent vapors, from day of collection until analysis. Do not add sodium thiosulfate as a dechlorinating agent nor acidify.

Analyze all samples within 28 d of collection.

3. Apparatus

a. Sample containers, 40-mL screw-cap vials*#(174) each with a TFE-faced silicone septum.†#(175) Wash vials and septa with detergent and rinse with tap and distilled water before using. Let vials and septa air dry at room temperature, place in a 105°C oven for 1 h, then remove and let cool in an area free of organics.

b. Vials, auto sampler, screw cap with septa, 1.8 mL.‡#(176)

c. Microsyringes, 10- and 100-µL.

d. Microsyringe, 25-µL with a 51- by 0.15-mm needle.§#(177)

e. Pipets, 2.0- and 5.0-mL transfer.

f. Volumetric flasks, 10- and 100-mL, glass stoppered.

g. Standard solution storage containers, 15-mL bottles with TFE-lined screw caps.

h. Gas chromatograph:‖#(178) See Section 6200B.2*b*. The system is equipped with a linearized electron capture detector and a capillary column splitless injector.

Two gas chromatography columns are recommended. Column 1 is a highly efficient column that provides separations for EDB and DBCP without interferences from trihalomethanes. Use Column 1 as the primary analytical column unless routinely occurring compounds are not adequately resolved. Use Column 2 as a confirmatory column when GC/MS confirmation is not available.

1) *Column 1,* 30 m long × 0.32-mm ID fused silica capillary with dimethyl silicone mixed phase.##(179) See Table 6231:I. Injector temperature: 200°C; detector temperature: 290°C. See Figure 6231:1 for a sample chromatogram.

2) *Column 2* (confirmation column), 30 m long × 0.32-mm ID fused silica capillary with methyl polysiloxane phase.**#(180) See Table 6231:I. Injector temperature: 200°C; detector temperature: 290°C.

4. Reagents

a. Reagent water: See Section 6200B.3*a*.

b. Hexane extraction solvent, UV grade.††#(181)

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c. Methanol, pesticide quality or equivalent.

d. Sodium chloride, NaCl: Before using, pulverize and place in a muffle furnace at room temperature. Increase temperature to 400°C for 30 min. Store in capped bottle.

e. 1,2-Dibromoethane, 99%.‡‡#(182)

f. 1,2-Dibromo-3-chloropropane, 99.4%.§§#(183)

g. Standard stock solutions: See Section 6200B.3*g*. Store in 15-mL bottles with TFE-lined screw caps. Methanol solutions prepared from liquid standard materials are stable for at least 4 weeks when stored at 4°C.

h. Secondary dilution standards: See Section 6200B.3*h*. Dilution standards are as stable as stock solutions.

5. Procedure

a. Operating conditions: Table 6231:I summarizes recommended operating conditions for the gas chromatograph and estimated retention times.

b. Calibration: Prepare calibration standards as directed in Section 6200B.3 *j* and analyze according to ¶ 5*d* below. Follow rest of calibration procedure in Section 6200A.5*b*), but limit variations from predicted response to ± 15% rather than ± 20%.

c. Instrument performance: See Section 6200C.4*c*.

d. Sample analysis: Let samples and standards come to room temperature. For samples and field blanks, open bottle, discard 5 mL using a 5-mL transfer pipet, and replace container cap. Weigh to nearest 0.1 g; record weight for subsequent volume determination. For calibration standards, QC check standards, and reagent blank, measure 35 mL using a 50-mL graduated cylinder and transfer to a 40-mL sample container.

Remove container cap and add 7 g NaCl. Add 2.0 mL hexane with a transfer pipet. Recap and shake vigorously by hand for 1 min. Let water and hexane phases separate. (If sample is stored at this stage, keep container upside down). Carefully transfer 0.5 mL of hexane layer into an autosampler vial using a disposable glass pipet. Transfer remaining hexane phase, but not any of the water phase, into a second autosampler vial. Hold second vial at 4°C for reanalysis if necessary. Transfer first sample vial to an autosampler set up to inject 2.0-µL portions into the gas chromatograph. Alternatively, manually inject 2-µL portions.

To determine sample volume for samples and field blanks, remove cap and discard remaining sample/hexane mixture. Shake off remaining drops using short, brisk wrist movements. Reweigh empty container with original cap and calculate net weight of sample by difference to the nearest 0.1 g. This net weight is equivalent to the volume of water (in mL) extracted. Alternatively, weigh vial before collection and reweigh full vial. Sample volume then equals gross weight (g) – [tare weight (g) + 5 g].

6. Calculation

Identify EDB and DBCP in sample chromatogram by comparing retention time of suspect

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peak to retention times generated by calibration and laboratory control standards. Retention times of samples should be within ± 0.1 min of standard for positive identification.

Use calibration curve or calibration factor to calculate uncorrected concentration (C_i) of each compound (e.g., calibration factor × response). Calculate sample volume (V_s) as equal to the net sample weight:

$$V_s = \text{gross weight} - \text{bottle tare}$$

The corrected sample concentration is:

$$\text{Concentration, } \mu\text{g/L} = C_i \times \frac{35}{V_s}$$

Round off results to the nearest 0.1 µg/L or two significant figures.

7. Quality Control

Follow procedures given in Section 6200A.5.

8. Precision and Bias

Single-laboratory precision and bias at several concentrations in tap water are presented in Table 6231:II [5].

9. References

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6231 C. Purge and Trap Gas Chromatographic/Mass Spectrometric Method

See Section 6200B for capillary-column method.

6231 D. Purge and Trap Gas Chromatographic Method

See Section 6200C for capillary-column method.

6232 TRIHALOMETHANES AND CHLORINATED ORGANIC SOLVENTS*#(184)

6232 A. Introduction

1. Sources and Significance

The trihalomethane (THM) compounds have been found in most chlorinated water supplies in the United States; typically they are produced in the treatment process as a result of chlorination. The formation of these compounds is a function of precursor concentration, contact time, chlorine dose, and pH. Toxicological studies suggest that chloroform is a potential human carcinogen. Consequently, total trihalomethanes are being regulated in potable waters. Chlorinated organic solvents are found in many raw waters because of industrial contamination.

2. Selection of Method

Several methods are available for measurement of the trihalomethanes and chlorinated organic solvents. Some of these are specific for these compounds and others have a much broader spectrum. Method 6232B is a simple liquid-liquid extraction gas chromatographic (GC) method that is highly sensitive and very precise for these compounds and certain other chlorinated solvents. Method C refers to purge and trap gas chromatographic/mass spectrometric (GC/MS) methods that can detect not only THMs but also a wide variety of other compounds. Method D refers to purge and trap GC methods with similar target compounds. All of these methods have approximately the same sensitivity for the trihalomethanes; method choice depends on availability of equipment, operator choice, and the list of desired target compounds. In addition, closed-loop stripping analysis can be used for several of these compounds (see Section 6040).

6232 B. Liquid-Liquid Extraction Gas Chromatographic Method

This method[1-3] is applicable to the determination of four trihalomethanes (THMs), i.e., chloroform, bromodichloromethane, dibromochloromethane, and bromoform, and the selected chlorinated solvents in finished drinking water, drinking water during intermediate stages of

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treatment, and in both surface and ground water. For other compounds or sample matrices, collect precision and bias data on actual samples[4] and provide qualitative confirmation of results by gas chromatography/mass spectrometry (GC/MS) to demonstrate the usefulness of the method. Retain documentation to demonstrate method performance. This method is particularly useful when only a few compounds are being monitored.

1. General Discussion

a. Principle: Sample is extracted once with pentane and the extract is injected into a gas chromatograph equipped with a linearized electron capture detector (ECD) for separation and analysis. Extraction and analysis time is 10 to 30 min per sample, depending on analytical conditions.

Confirmatory evidence, where necessary, is obtained by using dissimilar columns, other selective detectors, or mass spectrometry. Component concentrations must be sufficiently high (i.e., >50 µg/L) for confirmatory analyses using a mass spectrometer. See other methods in this section for alternative means of confirming positive results.

Standards added to organic-free water and samples are extracted and analyzed in the same manner, under identical conditions. This step is essential to adjust for the less-than-100% extraction efficiency of the simplified extraction technique. Extreme differences in ionic strength or organic content between standards and samples can result in different equilibria of sample constituents with the extracting solvent and a method bias may result. Monitor known additions recoveries on various matrices for bias.

Where required, sum the concentrations of the four trihalomethanes and report as total trihalomethanes in micrograms per liter.

See Section 6010 for discussion of gas chromatographic principles.

b. Interferences: Impurities contained in the extracting solvent account for many analytical problems. Maintain records of the reagent's manufacturer, lot number, purity, date bottle was opened, and expiration date. Analyze solvent blanks before using a new bottle of solvent. Make indirect daily checks on the extracting solvent by monitoring the sample blanks. Whenever an interference is noted in the method blank, analyze a solvent blank. Discard (or use for another purpose) extraction solvent if a high level of interference is traced to it. Low-level interferences can be removed by distillation or column chromatography;[5] however, it usually is more economical to obtain new solvent or select an approved alternative solvent. Interference-free solvent is defined as a solvent containing less than the laboratory determined detection limit of interference for each constituent. Protect interference-free solvents by storing in an area known to be free of organohalogen solvents. *Do not subtract blank values from sample analysis results as a correction for contamination.*

Sample contamination has been attributed to diffusion of volatile organics through the septum seal on a sample bottle during shipment and storage. Use the trip blank to monitor for this problem.

Contamination also may occur whenever equipment and materials used to store, extract, or

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analyze samples are inadequately cleaned, prepared, tested, or stored. There are many sources of contamination including contamination of reagents during storage and contamination of equipment reused in the sequential extraction of samples and standards. Maintain records of dates of preparation and cleaning and inclusive dates of use of reagents, standards, bottles, and equipment. Test all reagents and standards before initial use. To reduce possibility of carryover contamination, always clean equipment thoroughly after each use. Where equipment contamination is a concern, processing and analysis of additional method blanks beyond the minimum requirements of this method may be useful. Start by placing reagent water in a sample vial of the same lot that was used for samples and add preservative as was done for samples. Process this method blank in conjunction with samples using the same reagents, materials, and equipment. Where analysis of method blank indicates contamination, investigate possible sources and isolate the cause. Take and document corrective action. Following analysis of a sample containing late-eluting interferences, or containing over-range concentrations of constituents of interest, analyze a solvent blank or method blank to demonstrate freedom from carry-over.

This liquid-liquid extraction technique efficiently extracts a wide boiling range of nonpolar organic compounds and also extracts polar organic components with varying efficiencies. To analyze rapidly for trihalomethanes and chlorinated solvents with sensitivities in the low microgram-per-liter range in the presence of these other organic compounds use the semi-specific electron capture detector. Trihalomethanes are primarily products of the chlorination process and seldom appear in raw unchlorinated source water. The absence of peaks with retention times similar to the trihalomethanes in raw source water analysis is supporting evidence of an interference-free finished drinking water analysis. Because of possible interferences, analysis of a representative raw source water when analyzing finished drinking water provides evidence of freedom from this interference source. When potential interferences are noted in the raw source water, use the alternate chromatographic columns to reanalyze the sample set. If interferences still are noted, make confirmatory qualitative identifications as directed in ¶ 1*a*. If the peaks are determined to be other than the constituents of interest and they add significantly to the constituents' value in the finished drinking water, analyze sample set by the purge and trap method.[6]

Where chlorinated solvents are present in finished drinking water the most likely source is the raw water. Analyze individual raw water samples to isolate the source of contamination. Always consider the possibility of coeluting interferences. Analysis using capillary chromatography minimizes this possibility. Analysis using dissimilar columns may confirm the presence of interferences through differences in retention time between the constituent of interest and the unknown compound in the sample; however, the most definitive confirmation routinely available is GC/MS.[7]

Because the simplified extraction technique depends on equilibria between solvent and water, because extraction is not 100% efficient, and because efficiency is dependent on concentration, it is important to: extract samples and standards in the same manner; monitor

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matrix recoveries to assess differences in equilibria; and, where the concentration is found to be above the linear range of the method, to either dilute samples carefully before extraction, or prepare standards in water at the estimated sample concentration and carefully dilute *both* sample and standard extracts. Always process standards and samples together and hold constant such variables as water temperature, solvent temperature, room temperature, extraction time, and separation times.

For further information on interferences in gas chromatographic methods, see Section 6010C.

c. Detection levels: The method is useful for trihalomethane and selected chlorinated solvents at concentrations from approximately 0.1 to 200 µg/L. Actual detection levels are highly dependent on the characteristics of the gas chromatographic system used, the ratio of solvent to water, and interferences present in the solvent. See Section 1030.

2. Sampling and Storage

See Section 6010B.1 and Section 5710.

If trihalomethane formation potential is to be measured, do not add any preservatives at the time of sample collection. If chemical stabilization is not used at time of sampling, add the reducing agent just before extracting the sample or add it at the time specified in the formation potential method for quenching the sample.[8,9]

The raw source water sample history should resemble that of the finished drinking water. Take into account the average retention time of the finished drinking water within the water plant when sampling the raw source water.

Store blanks and samples, collected at a given site (sample set), together in a protected area known to be free from contamination. At a water treatment plant, duplicate raw source water, duplicate finished water, and duplicate sample blanks comprise the minimum sample set. When samples are collected and stored under conditions specified in 6010B.1, no measurable loss has been detected over extended periods of time.[8] Analyze samples within 14 d of collection.

For samples collected soon after chlorination, quenching with reducing agent may not be sufficient to prevent further formation of THMs completely, because of hydrolysis of intermediates. In that case, acidification is necessary and consistent with the recommended preservation techniques.

3. Apparatus

a. Sample storage vials: Clean, baked 40-mL glass open screw-top TFE-faced septum VOA vials or equivalent. See Section 6010B for additional information on cleaning, storage, and preparation.

b. Microsyringes, 10, 25, and 100-µL. Microsyringes with extended barrels are suggested for proper injection of methanolic standards when preparing aqueous standards in volumetric flasks.

c. Volumetric flasks, *glass-stoppered*, 10, 25, 50, 100, 250, 500, and 1000 mL, Class A. Choose size according to final volume of aqueous standard required and concentration of

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methanolic standards.

d. Extraction vessels: Use sample storage vials. Optionally, where samples are transferred to smaller extraction vessels, select an extraction container on the basis of specific requirements for final extract volume, solvent-to-water volume ratio, and availability. If a separate vessel is used for extraction, place standards along with samples in sample storage vials and follow identical procedures for preservation and transfer to the extraction vessel. Use the same lots of vessels for sample and standard extraction. Use of clean, oven-baked glass vessels and TFE-faced septa is critical.

e. Extract storage vials: 1.8-mL autosampler vials with open screw-top caps and TFE septa, or equivalent.

f. Gas chromatograph, preferably temperature-programmable with linearized electron-capture detector.

*g. Chromatographic columns:**#(185)

1) 0.32-mm ID × 30 m fused silica capillary, 1 µm DB-5,†#(186) or equivalent, at linear velocity of 20 cm/s. Temperature program: 35°C for 5 min, ramp 10°C/min to 70°C, then 20°C/min to 200°C. See Figure 6232:1 for a typical standard chromatogram.

2) 0.53-mm ID × 30 m, 1.5 µm DB-5†#(187) or equivalent, at 25 cm/s. Starting at 30°C for 1 min, ramp 6°C/min to 150°C.

3) 2-mm ID × 2 m long glass packed with 1% SP-1000‡#(188) on Carbopack B (60/80) operated at 50°C with 60 mL/min flow, or, if temperature-programmable GC is available, 45°C for 1 min, ramp 8°C/min to 240°C.

4) 2-mm ID × 2 m long glass packed with 10% squalene on Chromosorb WAW (80/100 mesh) operated at 67°C with 25 mL/min flow.

5) 2-mm ID × 3 m long glass packed with 6% OV-11/4% SP-2100 on Supelcoport (100/120 mesh); temperature program 45°C for 12 min, then 1°C to 70°C with 25-mL/min flow rate.

h. Mechanical shaker: Optionally, a rotary platform shaker.

i. Solvent pipetor: 2-mL transfer pipet, pipetor, or all-glass and TFE repipetor that attaches to the pentane storage bottle.

j. Transfer pipets, 5 mL. Preferably use a pipetor with disposable tips, cleaned and dried as recommended for TFE septa.

k. Analytical balance, capable of measuring to ± 0.01 g.

4. Reagents

a. Extraction solvent: See ¶ 1*b*. For capillary column split injection technique, preferably use only pentane. For other techniques, recommended solvent is pentane; alternatively, use hexane, methylcyclohexane, methyl-*tert*-butyl ether (MtBE) or 2,2,4-trimethylpentane. For alternative solvents, collect and document precision and bias data, evaluate extraction efficiency and effect of constituent concentration on efficiency, and maintain documents demonstrating applicability.

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Demonstrate that solvent is free of compounds for which the analysis is being performed.

b. Methyl alcohol, demonstrated to be free of interferences.

c. Neat standard materials: Use materials of 96% purity or greater for:

1) *Calibration standards:* bromoform ($CHBr_3$), bromodichloromethane (BDCM), dibromochloromethane (DBCM), chloroform ($CHCl_3$), 1,1,1-trichloroethane (TCA), tetrachloroethene (PCE), trichloroethene (TCE), and carbon tetrachloride (CCl_4).

2) *Internal standard:* 1,2 dibromopropane. A compound selected as an internal standard should have baseline resolution to separate it from constituents of interest and any interferences. Because this requirement is highly dependent on the samples and the analytical conditions and equipment used, no single internal standard is universally applicable. 1,2-dibromopropane has been found to be generally useful.

d. Reagent water: Generate VOC-free water, defined as water free of interference when used in the procedure described herein, by passing tap water through a carbon filter. Alternatively, prepare VOC-free water as follows: boil water for 15 min, then maintain at 90°C while bubbling a contaminant-free inert gas through water at 100 mL/min for 1 h. While water is still hot, transfer to a narrow-mouth screw-cap bottle with a TFE seal. Test VOC-free water each day before use by analyzing a method blank for constituents of interest. If any chlorine residual remains after such treatment, destroy it. See Section 1080 for additional information and general discussion of reagent-grade water.

e. Stock standard solutions: See Section 6200B.3*g*. Alternatively, purchase prepared standard solutions in methanol.

CAUTION: *Trihalomethanes and chlorinated solvents are toxic and may be carcinogenic: prepare primary stock solutions in a hood and wear appropriate personal protective equipment.*

f. Secondary dilution standards: From standard stock solutions, prepare multi-component secondary standards in methyl alcohol so that standards over the working range of the instrument can be prepared using no more than 20 µL methanolic standard solution /100 mL reagent water. See Section 6200B.3*h*.

g. Internal standard solution: Prepare stock solution from neat material in hexane. Make secondary dilution directly into storage container of pentane extracting solvent to produce a concentration of 30 µg internal standard /L pentane.

h. Aqueous calibration standards: Construct a calibration curve for each constituent using a minimum of three different concentrations, but preferably use five to seven concentrations. Bracket each sample with two of the concentrations. Use one concentration near, but above, the laboratory-determined detection limit. Where a sample component exceeds the range bracketed by standards, dilute a fresh volume of sample and re-extract, or prepare new standards in reagent water to bracket the concentration and dilute sample and standard extracts to bring them into the linear range of the detector. To prepare calibration standards, rapidly inject the required volume of alcoholic standard into the expanded area of a reagent-water-filled volumetric flask. Using an

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extended barrel syringe, inject the methanol *well below* the reagent water surface. Preferably incline the volumetric flask at an approximately 45-deg angle while injecting the standard. Remove syringe and stopper flask. Mix aqueous standards by gently inverting flask three times only. Discard to waste the contents in the neck of flask before transferring standards to sample vials. Add any preservatives to both samples and standards before extraction. Process standards through extraction in conjunction with sample sets. Aqueous standards, when stored with a headspace, are not stable; discard after 1 h. When stored in headspace-free sample storage vials, aqueous standards may be used for 24 h.

Avoid standard preparation procedures that require delivery of less than 10 µL of methanolic standards into volumetric flasks. Instead, use a larger volumetric flask and a larger volume of methanolic standard.

i. Quality control (QC) check standards: Obtain concentrate in methanol from USEPA or NIST for each compound, or if not available, from a second source vendor. If no second source is available, prepare stock standards separately from neat materials used for calibration standards. Prepare a mixed secondary dilution standard containing each compound and then an aqueous QC check standard at a concentration approximating the midlevel calibration standard.

5. Procedure

a. Extraction: Let samples and standards come to room temperature. Open each sample vial and remove 5 mL of sample and discard to waste, preferably using a transfer pipetor with disposable tips. Replace cap, weigh vial to nearest 0.1 g, and record weight.

Using a clean, dedicated volumetric measurement device (¶ 3*i*) carefully measure 2.00 mL pentane and add to sample vial. *Vigorously* shake by hand for 1 min or use a rotary platform shaker set at 60 to 100 rpm.

Let phases separate for at least 2 min. Where emulsions do not separate on standing, centrifuge or transfer entire emulsion to a separate vial and cool extract below 4°C to promote separation. Using a disposable glass pipet, transfer at least 1 mL of upper pentane extract to extract storage vials. Optionally, transfer half of the pentane extract to each of two vials to provide for reanalysis where necessary. Protect pentane extracts from warm temperatures and minimize extract holding time at room temperature. Store extracts at 4°C.

Empty sample/extraction vial to waste, rinse, and shake dry. Reweigh empty container with original cap to nearest 0.1 g and record weight. Calculate weight of sample extracted to the nearest 0.1 g by subtraction of vial-only weight from sample-plus-vial weight. For an assumed density of 1 g /mL, weight of sample extracted is equal to volume of sample extracted, in milliliters. Convert volume in milliliters to liters and record.

b. Sample and standard analysis: Before extraction of samples or standards prepare and analyze a method blank to verify freedom from interferences. Once extracts have been prepared, analyze standards and calculate a calibration curve or calibration factor as outlined in 6. Calculations. Inject 1 to 5 µL of standard extract depending on the configuration of the instrument and the required sensitivity. Inject exactly the same volume of extract each time,

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preferably using an autosampler. To test that injection volumes are repeatable, inject replicates of a single standard extract, and determine the standard deviation. Percent relative standard deviation (%RSD) should not be more than 5%. If this precision is not routinely achievable, use the internal standard calibration procedure.

After standardization, analyze the method blank, samples, and quality control samples. Extract and analyze a quality-control check standard each twentieth analysis and at the end of the analytical sequence. The percent recovery for the QC check standard should be between 80 and 120%. Develop historical mean control charts of QC check standard recovery for each compound and use the 99% confidence about historical data as the control criteria for rejection of QC check standards validity. Where criteria are failed, repeat analysis of any samples analyzed since the last QC check standard was in control.

c. Internal standard analysis procedure: Add the internal standard to the pentane solvent in the storage container at the concentration specified, and proceed with extraction and analysis of samples and standards as outlined above.

d. Compound identification: Identification of compounds in samples is based on comparison of retention times (RT) of suspect peaks to the confidence limits for RT of the authentic compounds in standards. Using the retention times of the standards analyzed, determine the average retention time for each compound and the standard deviation of the retention time. Tentatively identify peaks in sample chromatograms as compounds on basis of the 99% confidence interval around the calculated mean value using the calculated standard deviation. Nominally, the retention time window would be expected to be no wider than 0.25 min (packed column) and 0.05 min (capillary) before and after the average retention time calculated for the standards. When the 99% confidence limits for the data set are wider than the nominal value, institute corrective action.

Additional evidence of compound identity may be obtained by adding standard material to the suspect extract (standard addition) and reanalyzing. Presence of separate peaks in the extract with the known addition confirms that the suspect peak is not the compound of interest.

If chromatographic data systems are used to identify compounds, follow manufacturer's specifications. If the RT windows calculated by a computerized system are wider than the nominal values, investigate sources of retention time variability and take corrective action.

6. Calculations

a. External standard procedure: Use this procedure only if the volume of the injection can be held constant. Calculate individual response factors (RFs) for each standard analyzed as follows:

$$RF = \frac{\text{Nominal amount compound extracted, } \mu\text{g}}{\text{Response (peak area or peak height)}}$$

Calculate the amount of compound for each standard as:

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$$W_s = V_s \times C_s$$

where:

W_s = amount of compound, μg,
V_s = volume of standard extracted, L, and
C_s = concentration of prepared standard, μg/L.

For each compound determine average RF and standard deviation of the RFs using all calibration standards analyzed. If the percent relative standard deviation [%RSD = (SD/mean RF) × 100] is less than 10% use average RF to calculate sample concentration.

If the %RSD is greater than 10%, plot a calibration curve of amount injected versus response. Use the graph to determine the amount of compound present in each sample. Then determine the concentration by dividing amount, μg, by the volume, L, of sample extracted. Optionally use a data system to prepare a linear regression and use the linear regression equation to calculate compound amounts in samples from response values.

Where average RF is used, determine sample concentration as follows:

$$C_x = \frac{RF \times R_x}{V_x}$$

where:

C_x = compound concentration, μg/L,
R_x = sample response (mm, area, etc.), and
V_x = volume of sample extracted, L.

Round all final sample results to two significant figures.

b. Internal standard procedure: For all analyses made in a given analytical sequence, determine average internal standard response and standard deviation of the internal standard response. Calculate percent relative standard deviation. If the %RSD is greater than 25% take corrective action to improve method precision. Establish the 99% confidence interval for the internal standard response using the calculated mean and standard deviation for the sample set. Reject analyses where the internal standard response is outside these confidence limits, and reanalyze. After analysis of calibration standards, calculate individual relative response factors (RRF) for each compound in each standard as follows:

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$$RRF = \frac{R_s \times C_i}{R_i \times C_s}$$

where:

R_s, R_i = responses for calibration standard and internal standards,respectively, and
C_s, C_i = compound concentrations in calibration and internal standards, respectively.

Calculate average RRF for each compound, standard deviation of the RRFs, and %RSD. If %RSD is less than 10% use the average RRF; if it is greater, develop a calibration curve or a linear regression equation as outlined in the external standard procedure.

When using the average internal standard RRF, calculate concentration in samples as follows:

$$C_x = \frac{R_x \times C_i}{R_i \times RRF}$$

where:

C_x = compound concentration in sample, μg/L, and
R_x = sample response.

c. Total trihalomethane concentration: Calculate total trihalomethane concentration by summing the concentration of the four individual trihalomethanes in each sample. This is required for USEPA reporting purposes but it is preferable to report only individual THMs.

7. Quality Control

A minimum program of quality control consists of an initial demonstration of proficiency for each analyst and each instrument system and an ongoing program of quality control analysis. Record initial quality by documenting initial performance relative to published performance criteria. Maintain records of performance by comparing ongoing quality control checks to performance criteria and objectives for data quality. Document this performance as outlined in Section 1010, Section 1030, and Section 6020.

a. Analyst proficiency: The analyst should be experienced in the operation of a GC/ECD and produce an initial demonstration of proficiency in accordance with the procedure outlined in Section 6200A.5*a*1).

b. Method blanks: Prepare and analyze method blanks. Concentrations of compounds in the method blank should not exceed the experimentally determined method detection limit. If the method blank is out of control isolate the source of contamination, apply corrective action, and

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process a new method blank. Under no circumstances subtract method blank values from the sample result.

c. Quality control (QC) check standards: Preferably obtain QC standards from a separate source and prepare independently from calibration standards. Analyze QC check standards as though they were samples. Compare results to known concentration of the check standard and calculate percent recovery. Percent recovery nominally should be between 80 and 120%. Develop mean recovery control charts of QC check standards results and use historical 99% confidence limits to accept or reject the ongoing calibration. Where historical confidence limits are wider than the nominal limits, investigate standard materials, preparation and storage procedures, and other potential sources of error. Take and document corrective actions.

d. Detector sensitivity: Maintain a log of detector response, in area counts or peak height, using one standard that is analyzed each day, to monitor changes in detector sensitivity. Optionally, plot these data to observe trends in detector sensitivity. Note the sensitivity at which method detection limit studies were performed and replace or repair detectors where minimum detectable quantities are significantly affected by declining detector sensitivity.

e. Laboratory-fortified samples with known additions: In a laboratory analyzing more than 10 samples daily, extract and analyze a known addition on each tenth sample. Be sure this is representative of different sample types because there is some evidence of matrix effects with liquid-liquid extraction methods. See Section 1020B.6. In a laboratory analyzing fewer than 10 samples daily, each time sample extractions are performed, extract and analyze at least one laboratory-generated known-addition sample. Chart percent recovery as outlined in Section 1020B.12 using a means chart. To evaluate method bias see Section 1030.

f. Duplicate analysis: Randomly select, then extract and analyze in duplicate, 10% of all samples. Maintain an up-to-date log on bias and precision data collected on known-addition samples and duplicate samples. Evaluate results as outlined in Section 1030. If results are significantly different from those cited in ¶ 8 below, check entire analytical scheme to determine why the laboratory's precision and bias limits are excessive.

g. Laboratory control standards (performance evaluation standards): Quarterly, add an external reference laboratory evaluation standard to organic-free water, extract, and analyze. Preferably obtain this standard from a governing agency or other authoritative source. The results from this sample should agree within 20% of the true value for each compound. If not, check each step in preparation and analysis to isolate the problem. Document external reference standard results and any corrective action taken.

8. Precision and Bias

The single-laboratory precision and bias data in Table 6232:I were generated by adding known amounts of trihalomethanes and chlorinated organic solvents to organic-free water. The mixtures were analyzed as true unknowns.

9. References

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6232 C. Purge and Trap Gas Chromatographic/Mass Spectrometric Method

See Section 6200B for capillary-column method.

6232 D. Purge and Trap Gas Chromatographic Method

See Section 6200C for capillary column method.

6251 DISINFECTION BY-PRODUCTS: HALOACETIC ACIDS AND

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TRICHLOROPHENOL*#(189)

6251 A. Introduction

1. Sources and Significance

The haloacetic acids (HAAs) are formed by the chlorination of natural organic (humic and fulvic) matter. Utilities using chlorine as a water disinfectant generate haloacetic acids, usually as the second most prevalent group of known disinfection by-products[1]; the primary group formed is usually the trihalomethanes. Toxicological studies indicate that dichloroacetic acid and trichloroacetic acid are animal carcinogens.[2] The USEPA has proposed a maximum level for the sum of six haloacetic acids,[3] and requires large utilities to monitor drinking water for specified haloacetic acids.[4]

2. References

1. U.S. ENVIRONMENTAL PROTECTION AGENCY & ASSOCIATION OF METROPOLITAN WATER AGENCIES. 1989. Disinfection By-Products in U.S. Drinking Water. Vol. 1 Report, James M. Montgomery Consulting Engineers, Pasadena, Calif.
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6251 B. Micro Liquid-Liquid Extraction Gas Chromatographic Method

This method[1] was developed to analyze simultaneously for monochloroacetic acid (MCAA), monobromoacetic acid (MBAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), bromochloroacetic acid (BCAA), dibromoacetic acid (DBAA), and 2,4,6-trichlorophenol (TCPh) in treated and untreated drinking water. Additional haloacetic acids may be present and analyzed by this method; however, standards for these compounds are not readily available and are less stable.

1. General Discussion

a. Principle: The sample is extracted with methyl *tertiary*-butyl ether (MtBE) at an acidic pH to extract the nondissociated acidic compounds to be determined. A salting agent is added to increase extraction efficiency. The extracted compounds are methylated with diazomethane solution to produce methyl ester or ether derivatives that can be separated chromatographically.

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A temperature-programmable gas chromatograph using a fused silica capillary column and an electron capture detector (ECD) is used for analysis. Simultaneous analysis and confirmation using a single injection can be effected by setting up both the analytical column and the confirmation column to share a common injection port. Alternatively, use separate analytical and confirmation columns. Alternative detectors may be used if QC criteria can be met. Aqueous calibration standards are extracted, methylated, and analyzed in the same manner to compensate for less than 100% extraction and derivatization efficiencies.

b. Interferences: Impurities in extraction solvent and on glassware and other equipment can interfere. Follow specifications and cleaning procedures carefully to minimize interference. As shown in Figure 6251:1, the analysis separates peaks of haloacetic acids from those of other common disinfection by-products. Use of two columns is recommended because for waters with high carbonate contents, false-positive MCAA peaks have been observed on the column described in ¶ 3*h*4).

c. Detection levels: Method detection levels (MDL) are given in Table 6251:I.[2] The method has been shown to be useful for haloacetic acids over a working range of 0.5 to 30 µg/L (1.0 to 30 µg/L for MCAA) and 0.25 to 15 µg/L for TCPh. The calibration range can be extended, depending on the compound and detector characteristics.

d. Safety: The toxicity and carcinogenicity of each reagent has not been defined precisely. Minimize exposure to these chemicals and use them only in a properly operating ventilation hood.

Avoid exposure to DCAA and TCAA because they are carcinogens.[3] Avoid contact with the other haloacetic acids and their solutions.

MNNG (1-methyl-3-nitro-1-nitrosoguanidine) is carcinogenic. Keep in properly labelled plastic containers, containing activated carbon, with tight-fitting lids and store in a refrigerator used only for chemical storage. Store spatulas and glassware for the handling of MNNG in specially labelled plastic containers and use only for MNNG.

Diazomethane is toxic, carcinogenic, and an explosion hazard.[4] Follow special precautions whenever handling this material. Use only in a properly operating fume hood; CAUTION: *Do not breathe vapors.* To avoid explosions, do not heat above 90°C and do not use glassware with ground-glass surfaces (e.g., ground-glass joints, sleeve bearings) or glass stirrers. Special glassware for diazomethane generation and handling, as well as screw-cap volumetric flasks, are available commercially. Always use a safety shield when generating diazomethane. Always quench excess diazomethane with silica gel. Do not store diazomethane/ether solutions; they are extremely hazardous and tend to become contaminated.

Store ether in tightly-closed amber bottles in an explosion-safe or -proof refrigerator. Store only with compatible chemicals. Eliminate all sources of ignition; keep away from heat, sparks, and flames. Handle ether only in a hood and avoid direct physical contact. Do not breathe vapors. If ether is spilled or leaks, evacuate area, ventilate, and absorb on vermiculite or similar material. Wear appropriate OSHA equipment before entering spill area. Also see Section 1090.

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2. Sampling and Storage

See Section 6010B.1.

Preferably collect grab samples in quadruplicate to allow sufficient volume for replicates and known additions. Flush sampling tap until water temperature stabilizes and stagnant lines are cleared. Collect samples in nominal 40- or 60-mL vials containing approximately 65 mg crystalline NH_4Cl (bake overnight at >100°C to eliminate contaminants), which converts free chlorine to a combined chlorine residual, and sealed with TFE-faced septa and screw caps. To minimize aeration, fill vials so that no air bubbles pass through the sample. Do not rinse with sample and do not let vial overfill. Seal sample vials with no headspace.

Analyze samples as soon as possible after collection. Store dechlorinated samples at 4°C, but for no more than 9 d;[1,5] check compound stability in any unknown sample matrix. Sample extracts can be held in a freezer at −11°C for 21 d.

3. Apparatus

Preferably dedicate all analytical glassware used in this method to this procedure.

a. Sample containers and extraction vials,*#(190) 40- or 60-mL screw-cap vials with TFE-faced silicone septa. Clean vials by washing with detergent, rinsing thoroughly with tap water, rinsing with 1:10 HCl, rinsing again with tap water, and finally rinsing with reagent water. Heat in an oven at 180° for at least 1 h. Clean caps and septa by rinsing with acetone, then hexane. Heat at 80°C for not more than 1 h in a clean, forced-air convection oven.

b. Microsyringes, 5, 10, 25, 50, 100, 250, 500, and 1000 µL.

c. Syringe, 30-mL glass hypodermic, metal luer-lok tip with 8.9-cm- (3.5-in.-) long × 17 gauge stainless steel pipetting needle (alternatively use a 30-mL volumetric pipet). See ¶ a above for glassware cleaning procedure.

d. Micro volumetric flasks,†#(191) TFE-lined screw-cap: 2-mL, 5-mL, and 10-mL. Immediately after use, rinse three times with methanol. Invert to drain. Let air-dry completely in a ventilation hood.

e. Mechanical shaker,‡#(192) to automate MtBE extraction. Insert vials into a wooden holding block (20 vial capacity) made from laminated plywood with drilled holes to accept vials, dimensioned to fit snugly onto the shaker table.

f. Extract and standard solution storage container, 1.8-mL clear glass, 7- and 14-mL amber glass screw-cap vials with TFE-lined silicone septa. For cleaning procedures, see ¶ a above.

g. Transfer pipets, 14.6- and 23-cm (5.75- and 9-in.) disposable glass pasteur pipets. See ¶ a above for glassware cleaning procedure.

h. Gas chromatograph, temperature-programmable (preferably with multiple ramp capability) with injector. Optimally use an autosampler for sample injection and a computer data system for peak integration and quantitation. (A detector base that can mount two electron capture detectors is ideal.)

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1) *Gas handling equipment:* Use carrier (helium) and makeup (nitrogen) gases of high purity (99.999%) grade that pass through indicating calcium sulfate, molecular sieve 5A, activated charcoal, and an oxygen purifying cartridge. Use two-stage metal diaphragm high-purity regulators at the compressed gas sources. Use flow controllers to regulate carrier gas flow. Make all gas lines 0.3-cm (1/8-in.) copper (or stainless steel) tubing; rinse with acetone and bake before use.

2) *Injector*, split/splitless (using straight open bore insert).

3) *Analytical column*,§#(193) 30 m long × 0.25 mm ID, fused silica capillary column with a 0.25-µm film thickness or equivalent.

4) *Confirmation column*, 30 m long × 0.25 mm ID, fused silica capillary column‖#(194) with a 0.25-µm film thickness or a 30 m long × 0.25 mm ID, fused silica capillary column##(195) with a 0.5-µm film thickness.

5) *Detectors,* a constant-current pulse-modulated ^{63}Ni ECD with standard size cell (use two ECDs for simultaneous confirmation analysis).

i. Salt scoops for sodium sulfate, made from stainless steel 1.3-cm- (0.5-in.-) diam bar stock drilled out to a volume of 1.73 mL so that each level scoopful contains 3 g. Alternatively, weigh the salt.

j. Pipetting dispensers, adjustable 5- and 2-mL sizes with TFE transfer lines, that can be mounted on the supplier's reagent bottles. Use for dispensing H_2SO_4 and MtBE. Alternatively use a 3-mL volumetric pipet and a 5-mL graduated pipet with manual pipet bulbs.

k. Diazomethane generator: Use millimole-size generator with ''o''-ring joint (Figure 6251:2).**#(196) Immediately after use, rinse inner tube twice with 20% NaOH, then rinse twice with tap water. Immediately add 1 g silica gel to the outside tube to quench any residual diazomethane solution, rinse twice with methanol, and twice with tap water. Rinse both inner and outer tubes with reagent water three times. Bake at 180°C until dry in a clean, forced-air convection oven.

Alternatively use the millimole-size diazomethane generator shown in Figure 6251:3.††#(197) To clean, rinse with reagent water and then with methanol, invert, and let air-dry.

l. pH-strips, pH indicating strips, 0 to 2.5 range.

4. Reagents

a. Extraction solvent, 99+ % MtBE, preservative-free.‡‡#(198)

b. Sodium sulfate reagents:

1) *Granular sodium sulfate*, Na_2SO_4, reagent grade, suitable for pesticide analysis. Heat at 400°C overnight in a shallow stainless steel pan covered with aluminum foil. Store in a 1-L glass bottle with TFE-lined polypropylene cap.

2) *Acidified sodium sulfate:* To 100 g anhydrous sodium sulfate, heated as above and cooled,

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add diethyl ether to just cover the solid; make a slurry. Add 0.1 mL conc sulfuric acid and mix thoroughly. Remove ether under low vacuum. Mix 1 g acidified sodium sulfate with 5 mL reagent water and check that pH is less than 4. Store remainder of reagent at 130°C.

c. Methanol, pesticide grade or equivalent.

d. Ammonium chloride, NH_4Cl.

e. Reagents for diazomethane generation with the apparatus shown in Figure 6251:2.

1) *Sodium hydroxide solution,* NaOH, 20%: Dissolve 200 g ACS low-carbonate-grade pellets in 800 mL reagent water.

2) *1-methyl-3-nitro-1-nitrosoguanidine,* MNNG.[§§]#(199)

f. Reagents for diazomethane generation with apparatus shown in Figure 6251:3.

1) *Diethylene glycol monoethyl ether.*‖ ‖#(200)

2) *N-methyl-N-nitroso-p-toluene sulfonamide.*###(201)

3) *Ethyl ether,* absolute.

4) *N-methyl-N-nitroso-p-toluene sulfonamide solution:* Mix 10 g reagent *f*2) in 100 mL 1:1 (v:v) solution of ethyl ether and reagent *f*1). Solution is stable for 1 month when stored at 4°C in an amber-colored bottle with a TFE-lined screw cap.

5) *Potassium hydroxide solution,* KOH: Dissolve 37 g in 100 mL reagent water.

g. Silica gel, 35/60 mesh activated at 180°C and stored in a desiccator.

h. Sulfuric acid, H_2SO_4, conc.

i. Copper (II) sulfate pentahydrate, $CuSO_4 \cdot 5H_2O$.

j. Standard material, see Table 6251:II for source and physical information.

1) *Individual haloacetic acid standard stock solutions:* Prepare individual haloacetic acid and the trichlorophenol stock solutions as follows: Weigh 0.150 g of each acid. Dilute each standard in MtBE to 10 mL in a screw-top volumetric flask. (NOTE: Do not use methanol for dilution, because spontaneous methylation of the haloacetic acid may occur in methanolic solution.[7] Transfer each to a separate clean 14-mL amber vial and store in a freezer at −11°C. Stock standards are usable for 6 months.

2) *Multicomponent haloacetic acid additive solution:* Prepare a six-component additive solution using individual haloacetic acid stock solutions. Dilute 16.7 µL of each stock standard into a 10-mL volumetric flask containing 9 mL MtBE, but use 8.4 µL of 2,4,6-trichlorophenol solution. After adding all stock solutions, dilute to volume with MtBE. This gives 25 µg/mL of each HAA and 12.5 µg/mL for 2,4,6-trichlorophenol. The additive solution is usable for 3 months when stored at less than −10°C. Alternatively, prepare known-addition solution monthly in methanol and store at 4°C.

Measure microliter volumes with a gastight syringe using the solvent flush delivery technique. Do solvent flush with a 25-µL syringe by first drawing up 2.5 µL of solvent and then

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drawing the syringe plunger to the 5-µL mark with air. From the 5-µL mark measure amount of stock solution desired and then deliver the entire contents to the volumetric flask.

3) *Individual haloester standard stock solutions:* Prepare individual methyl ester stock solutions as follows: Weigh 0.1 × (molecular weight of ester/molecular weight of acid) g of each methyl ester in a 10-mL volumetric flask and dilute to mark with MtBE. Prepare methyl ester for dibromoacetic acid by derivatizing 1 mL of a 20 000-µg/mL acid solution with 100 µL methanol (follow derivatization steps in 5*e* below, but substitute dibromoacetic acid stock solution as the solution added to the outer tube for collection of the diazomethane). After derivatizing, transfer ester quantitatively to a 2-mL volumetric flask with a TFE-lined screw cap and dilute to mark with MtBE. Stock standards are usable for 6 months when stored at less than −10°C.

4) *Multicomponent haloester additive solution:* Prepare a multi-component additive solution by diluting 10 µL of each haloester stock standard, but use 5 µL of 2,4,6-trichloroanisole (methyl ether of the phenol), in a 10-mL volumetric flask and bring to volume with MtBE. This will yield a mixture containing approximately 10 µg/mL each, except for 2,4,6-trichloroanisole, which will be approximately 5 µg/mL. Additive solution is usable for 3 months when stored at less than −10°C.

5) *Direct injection haloester standards:* Prepare direct injection standards using 10 µg/mL multicomponent haloester additive solution, a 30-µg/mL internal standard additive solution [see ¶ 4*k*2)], and a 10-µg/mL methanol solution of methyl-2,3-dibromopropionate [surrogate ester, see ¶ 4*l*4)]. Prepare direct injection standards by diluting appropriate volumes of multicomponent haloester additive mix, internal standard additive solution, and surrogate ester solution with enough MtBE to give a final volume of 1.0 mL.

k. Internal standard, 1,2,3-trichloropropane (IS-TCP), 98% pure (alternatively use 1,2-dibromopropane (IS-DBP).

1) *Internal standard stock solutions:* Weigh 50 mg into a 10-mL volumetric flask and bring to volume with methanol. This will yield a 5000-µg/mL stock solution. Stock standards are usable for 6 months when stored at less than −10°C.

2) *Internal standard additive solution,* 30 µg/mL: Deliver 60 µL internal standard stock solution into a 10-mL volumetric flask and dilute to volume with methanol. Divide evenly among six 1.8-mL vials and store at −11°C. Additive solution is usable for 3 months.

Add 20 µL of internal standard additive solution to each 2 mL extract, yielding internal standard of 300 ng/mL.

l. Surrogate, (DBPA-SUR) 2,3-dibromopropionic acid, 99% pure or 2,3,5,6-tetrafluorobenzoic acid (TFBA-SUR).

1) *Surrogate stock solution,* 20 000 µg/mL: Weigh 0.2000 g SUR acid into a 10-mL screw-cap volumetric flask and dilute to mark with MtBE. Stock solutions are usable for 6 months when stored at less than −10°C.

2) *Surrogate additive solution:*

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a) *DBPA-SUR,* 10 µg/mL: Deliver 5 µL DBPA-SUR stock solution into a 10-mL volumetric flask and dilute to volume with methanol. Divide evenly among six 1.8-mL vials and store at −11°C. Additive solutions are usable for 3 months.

Add 30 µL DBPA-SUR additive solution to each 30-mL sample portion, yielding DBPA-SUR 10 µg/L.

b) *TFBA-SUR*, 20 µg/mL: Deliver 300 µL TFBA-SUR stock solution into 1-L volumetric flask and dilute to volume with reagent water. Add 0.5 mL TFBA-SUR additive solution to each 30-mL sample, yielding 100 µg/L.

3) *Esterified surrogate stock solution,* 10 000 µg/mL: Derivatize 1 mL SUR stock solution and 100 µL methanol, according to derivatization steps in ¶ 5*e*, but substitute SUR stock solution and 100 µL methanol for MtBE. After derivatizing, transfer quantitatively to a 2-mL volumetric flask with a TFE-lined screw cap and dilute to mark with MtBE. Stock solutions are usable for 6 months when stored at less than −10°C.

4) *Surrogate ester additive solution,* 10 µg/mL: Deliver 10 µL SUR ester stock solution into a 10-mL volumetric flask and dilute to volume with methanol. Additive solutions are usable for 3 months when stored at less than −10°C.

Add 10 µL DBPA-SUR or 100 µL TFBA-SUR ester additive solution to each 1 mL of direct injection standard yielding 100 ng/mL DBPA-SUR or 1000 ng/mL TFBA-SUR.

m. Reagent water: See Section 1080.

n. Calibration standards: Prepare aqueous calibration standards in reagent water by injecting a measured amount of the multicomponent haloacetic acid solution directly into water using the solvent flush technique. Prepare five different concentration levels from 0.5 to 30 µg/L for the HAAs and 0.25 to 15 µg/L for 2,4,6-trichlorophenol in 40-mL TFE-lined screw-top bottles containing 30 mL reagent water. Extract these standards and process the same way as samples, using the procedure given below.

5. Procedure

a. Sample preparation: Remove samples and standards from storage and let equilibrate to room temperature. Each time a new sample matrix is analyzed check that the amount of H_2SO_4 added will reduce pH to less than 0.5 before beginning microextraction. Test a separate 10-mL portion by adding 1 g $CuSO_4$, 4 g granular Na_2SO_4 [¶ 4*b*1)], and 0.5 mL conc H_2SO_4, mix until salt dissolves, then test using pH indicating strips.

b. Microextraction: Transfer 30 mL from the sample container to a 40- or 60-mL vial with TFE-faced septum and screw cap.

Add surrogate additive solution as indicated in ¶ 4*l*2) to each sample, including standards and blanks. Add haloacetic acid additive solution at this step for known additions.

Take one vial at a time and add the following in sequence: 1.5 mL conc H_2SO_4, 3 g $CuSO_4$,

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12 g baked Na_2SO_4 [¶ 4*b*1)], and 3 mL MtBE. Immediately cap and shake briefly by hand to break up any salt clumps.

When using automated extraction, place vials in mechanical shaker wooden holding block. Shake vials at fast speed (approximately 300 cycles/min) for 9 min; alternatively shake manually for 2 min until salt is dissolved.

Remove vials, place upright, and let stand for at least 3 min until the phases separate.

c. Preparation of diazomethane[6]: Using the apparatus in Figure 6251:2, add approximately 130 mg MNNG to the inside tube of the generator. Add 0.5 mL reagent water to the MNNG and secure cap and septum. Add 2 mL MtBE to the outside tube. Place butyl o-ring in glass joint, place inside tube firmly on top of o-ring, and clamp securely with a screw-type pinch clamp.

Place generator in an ice water bath containing enough ice to keep diazomethane MtBE solution at 0°C until used.

Add 600 µL 20% NaOH (1 drop/5 s) using a 1-mL gastight syringe (22-gauge needle) through the generator septum (check that the syringe needle is on the opposite side of the vapor exit hole). Let derivatization continue for 30 min after adding NaOH; use product as soon as possible. Add more ice if necessary to maintain temperature.

If more diazomethane is needed, prepare two or more batches and combine before use.

Alternatively use the apparatus in Figure 6251:3 to prepare diazomethane. Add enough ethyl ether to tube 1 to cover the first impinger. Add 10 mL MtBE to 15-mL collection vial. Set nitrogen flow at 5 to 10 mL/min. Add 4 mL sulfonamide solution and 3 mL 37% KOH solution to the second impinger. Connect tubing as shown and let nitrogen flow purge diazomethane from the reaction vessel into the collection vial for 30 min. Cap vial when collection is complete and hold at 0°C. When stored at 0°C diazomethane solution may be used over a period of 48 h.

d. Separation and concentration: NOTE: Ensure that all items that come into contact with the sample prior to methylation have been washed with a dilute solution of sulfuric acid.

The drying step included here may be used if excess diazomethane is required to maintain the persistent yellow color of the sample (5*e*). It is not necessary in every case and may be used at the discretion of the analyst. Plug a small disposable pipet with a small amount of acid-washed glass wool. Add approximately 1 g acidified Na_2SO_4 [¶ 4*b*2)] to the pipet and pass exactly 2 mL of the top MtBE sample extract through the salt, being careful not to transfer any of the aqueous phase. Rinse the salt in the pipet with two 250-µL volumes of solvent and collect together in a receiver ampule (a 2-mL volumetric flask with TFE-lined screw cap) for subsequent concentration and methylation.

Concentrate MtBE extract to approximately 1.7 mL using a moderate stream of nitrogen blowing on the surface of the extract.

e. Derivatization: Add 20 µL of 30 µg/mL internal standard additive solution to each concentrated extract. (The internal standard is added at this time to minimize manipulation in the presence of diazomethane.) Cool in an explosion-safe freezer or in an ice bath for 7 min and add

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diazomethane (cooling extracts is unnecessary if diazomethane is generated by apparatus shown in Figure 6251:3).

Uncap one volumetric flask and add 250 µL of cold diazomethane/MtBE solution. Cap immediately with TFE-lined screw cap; mix gently by inverting once. Repeat for remaining extracts. A persistent yellow color after addition of diazomethane indicates that an excess is available for esterification. If necessary add more diazomethane solution.

Hold for 15 min at 4°C in an explosion-safe or explosion-proof refrigerator. Alternatively, keep extracts in an ice bath (cooling is unnecessary if diazomethane was generated by the apparatus shown in Figure 6251:3).

After holding 15 min, place extracts in a hood and let stand another 15 min until they reach room temperature. Dilute to mark with MtBE and invert flask to mix. If using an autosampler, transfer each extract evenly between two labeled autosampler vials containing approximately 0.01 g silica gel with a 23-cm (9-in.) disposable pasteur pipet to quench excess diazomethane. Keep each extract in contact with diazomethane for approximately the same amount of time before quenching. Store extra autosampler vial in freezer at −10°C as a backup extract. Alternatively, add silica gel to volumetric flask after derivatization is complete.

f. Gas chromatography: Typical operating conditions for the chromatograph are as follows:

Injector temperature 160°C; split valve opened at 0.5 min.

Temperature program: 37°C for 21 min, rising 11°C/min to 136°C, holding 3 min at 136°C, rising 20°C/min to 236°C, holding 3 min at 236°C.

Detector temperature: 300°C.

Carrier gas flow: 30 cm/s at 37°C.

Makeup gas flow: 23 mL/min.

At the beginning of each analytical run, inject two MtBE solvent blanks to condition the GC and to verify that interferences are absent. A 2-µL extract is injected in splitless mode. Always inject same sample volume and use sample dilution to obtain response in the calibration range. If levels greater than the highest standard are obtained, reanalyze diluted sample extract and readjust internal standard concentration. Calculate concentration only for those compounds that were at levels higher than the calibration curve; for other compounds use values obtained from the undiluted sample extract. See Figure 6251:4 for a chromatogram of an extracted and derivatized 30-µg/L standard on the analytical column. See Table 6251:III for retention times. A direct standard may be injected after the MtBE solvent blanks to verify continued system performance.

g. Calibration: Use five levels of calibration standards to define the quantitation range. The lowest standard should be near the limit of quantitation (LOQ) (see Table 6251:IV) for each compound. Use other standards to bracket the expected range of sample concentrations; do not exceed linear range of detector. Prepare standards by adding haloacetic acids and trichlorophenol to reagent water and then extract with the same solvent and derivatize with the same batch of diazomethane as used for the samples. Use the same extraction/esterification procedure for both

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standards and samples to correct for recovery characteristics. Analyze calibration standards under the same chromatographic conditions as samples.

Analyze calibration standard at one or more levels with each sample set to verify the working calibration curve. If the calibration standard is within ± 15% of the expected value, a new five-level calibration curve is not needed.

6. Calculation

A 2-µL injection of each calibration level will provide peak area (A_a) data for each compound and an internal standard peak area (A_i) for each level; use these peak areas to calculate relative response for each compound.

$$\text{Relative response} = A_a/A_i$$

A calibration curve passing through zero is generated from the plotted points for each compound using the relative response versus standard concentration. Use the internal standard quantitation method to determine unknown concentrations by a linear, quadratic, or point-to-point curve fit.

7. Quality Control

a. Quality control program: Because sample preparation requires many manipulations, chances for errors are increased. Consequently, at least follow minimum quality control requirements to monitor and maintain method performance. Include method blanks, an initial demonstration of laboratory capability and detection limits, assessment of the internal standard recovery, determination of surrogate compound recoveries, evaluation of calibration data and curves, sample matrix additions, and precision of replicate sample analysis. Additional quality control measures may be used.

b. Method blanks: Process a method blank (30 mL reagent water) with each set of samples. If the blank produces any peak within the retention time window of a compound that would prevent its determination, seek out and eliminate the source of contamination and reanalyze samples.

c. Initial demonstration of capability: To demonstrate an adequate level of performance, conduct the following operations before analyzing samples and whenever any major analytical change, such as new analyst or switch in type of column, is made.

1) Accuracy as percent recovery—Establish a calibration curve as in ¶ 5*g* and select a representative additive concentration (5 µg/L is convenient) for each target compound. Using a syringe, add the appropriate amount of stock standard mix to each of a minimum of seven 30-mL portions of reagent water and analyze.

Calculate average percent recovery (P) and standard deviation of the recovery (S_r). Compare results to the single-laboratory recovery and precision data in Table 6251:V. Compare precision at similar concentrations, that is $P \pm 30\%$ of the additive level. Acceptable mean recovery values are within the interval $P \pm 30\%$. For compounds not meeting this criterion, repeat with another

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seven samples until satisfactory performance has been demonstrated.

2) Absolute recovery—Use direct injection haloester standards, ¶ 4 *j*5), to check absolute recoveries of extracted and derivatized haloacetic acids. Calculate absolute recoveries by comparing the ratio (area for compound/area for internal standard) for samples in ¶ 7*c*1) with the area ratio for direct injection standard at a tenfold concentration to account for the extraction (expressed as the haloacetic acid). Typical absolute recoveries are given in Table 6251:VI; acceptable absolute recoveries are within ± 30%. Recoveries outside this range may indicate insufficient shaking or poor methylation, possibly due to water in the extracts. Correct problem and produce acceptable absolute recoveries before analyzing samples.

Because of rapid advances in chromatography, columns and conditions may be modified to improve separation or to lower cost. Repeat procedure in 7*c* for each modification.

d. Internal standard assessment: The internal standard corrects for any deviation in sample volume injected. A sample injection is acceptable if the area counts of the internal standard peak do not vary more than ± 20% from the daily calibration standard(s) IS response.

Reinject an extract exceeding the ± 20% range. If reanalysis does not produce acceptable results, reextract and reanalyze. If the reextracted sample results are not acceptable or if samples have exceeded holding time, resample and reanalyze or record results as suspect and out-of-control.

e. Evaluating surrogate recovery: The surrogate is added directly to all samples before acidification and extraction. If the surrogate area is low or absent, it is likely that there has been a derivatization problem (e.g., water in extract) or extraction problem (e.g., water insufficiently acidified).

An extract is acceptable if the area counts of the surrogate standard recovery are ± 30% from the surrogate standard recovery for the daily calibration standard(s).

When surrogate recovery is not acceptable, check the following: locate possible errors in calculations or procedure, degradation of standard solution, contamination sources, and instrument performance. If these steps do not reveal the problem, reanalyze the extract. If reanalysis does not produce acceptable results, reextract and reanalyze samples. If the reextracted sample results are not acceptable or if samples have exceeded holding time, record results as suspect and out-of-control.

f. Extracted standard calibration: Quantitation is done by internal standard referencing with relative areas. Produce a minimum five-level extracted standard calibration curve for sample quantitation.

If the response for any compound falls outside the predicted response by more than 15% from a previous calibration, make a new calibration standard and analyze it until an acceptable curve is obtained.

Analyze calibration standards with each sample set after an acceptable five-level calibration curve is generated. If the continuing calibration standards are not within ± 15%, check for errors or degradation of standards and construct a new calibration curve.

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g. Matrix additions: Add each target compound into one sample per sample set (a sample set is all samples extracted within a 24-h period) or 10% of the samples, whichever is greater.

The added concentration should be near to or greater than the background. Take care, particularly with dichloroacetic acid, to ensure that the addition plus background concentration does not exceed calibration range (extract dilution may be needed).

Analyze one sample portion to determine the background concentration (B) of each compound. Add working standard mix to a second sample portion and analyze to determine the concentration of each compound (A). Calculate percent recovery (P_i) as $100\,(A - B)/T$, where T is the known concentration of the material added to the sample.

Compare percent recovery (P_i) for each compound with established QC acceptance criteria. Establish QC criteria by initially analyzing seven samples with additions and calculating the average percent recovery (P) and the standard deviation of the percent recovery (S_r).

Calculate QC acceptance criteria as follows:

$$\text{Upper control limit (UCL)} = P + 3S_r$$
$$\text{Lower control limit (LCL)} = P - 3S_r$$

The data generated during the initial demonstration of capability, ¶ 7*c*1), may be used to set the initial upper and lower control limits.

Monitor all data from dosed samples. Compound recoveries must fall within established control limits. After 10 new recovery measurements, recalculate P and S_r using all the data, and construct new control limits. When the total number of data points reaches 20, update control limits by calculating P and S_r using only the most recent 20 data points.

Compare percent recovery (P_i) for each compound with the QC acceptance criteria established by the control limits. If recovery of any compound falls outside the designated range, performance is judged to be out of control. Seek source of problem immediately and resolve before continuing the analysis.

However, if recovery of a compound meets calibration, blank, internal standard, surrogate, and replicate quality control, laboratory performance is in control, and the recovery problem is matrix-related. Label result for that compound in the sample as suspect/matrix.

h. Replicate analysis: Analyze sample duplicates to monitor precision. Analyze duplicates on at least 10% of all samples randomly selected.

Determine control limits by calculating the range as a function of the relative standard deviation. The range, R, is the absolute difference of the duplicate values, X_1 and X_2, as follows:

$$R = |X_1 - X_2|$$

The normalized range (R_n) is calculated by dividing the range by the average of the duplicate

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values (X_m):

$$R_n = \frac{R}{X_m}$$

$$X_m = \frac{X_1 + X_2}{2}$$

Calculate mean normalized range (R_m) for 20 pairs of duplicate data points initially and 20 pairs of points quarterly:

$$R_m = \frac{\sum R_n}{n}$$

where:

n = number of duplicate pairs.

$$\text{Variance} = S^2 = \frac{\sum (R_n - R_m)^2}{n - 1}$$

The standard deviation (s) is the square root of the variance. Upper and lower control limits are $R_m + 3s$ and zero, respectively. Acceptable duplicates fall within the control limits. The upper warning limit is $R_m + 2s$. If an R_n value is outside the warning limit, a potential problem is indicated and investigated before the analysis is out of control. Recalculate control limits quarterly using the most recent 20 points not including any data points that are out of control. Recalculate control limits when any major analytical changes are made and after at least 20 points have been collected.

Analyze any problem and correct it. If the duplicate is not acceptable, reextract only for those compounds out of control. If the duplicates are still unacceptable or the sample holding time has been exceeded, resample and reanalyze. If this is not possible record results as suspect and out of control. Do not use such data in range calculations.

i. Additional quality control: Each quarter, analyze QC check standards from an external source. Independent confirmation may include interlaboratory split sampling for comparison. Analyze performance evaluation samples, preferably from USEPA or appropriate state agency, at least once a year. Results for each target compound must be within established acceptance limits.

Shipping blanks are containers filled with reagent water containing appropriate amount of NH_4Cl (see ¶ 2), shipped to all sample locations with sample bottles, and returned with the

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samples. They are used to assess contamination during sampling and transit. Analyze a shipping blank with each sample set. If the shipping blank contains reportable levels, compare with the laboratory reagent blank. If contamination is not detected in the laboratory reagent blank, the sampling or transportation practices may have caused the contamination. Discard all samples in the set and resample.

Direct ester standards (concentration based on corresponding acid) may be injected at the start of each set to verify the sensitivity, chromatography, and retention times on the gas chromatograph.

Make an instrument check of the entire analytical system daily using data gathered from analyses of reagent blanks, standards, and replicate samples.

8. Precision and Bias

Single-laboratory method detection limits (MDL) and extracted recovery data from reagent water are presented in Table 6251:I. Data for absolute recoveries in reagent water are given in Table 6251:VI. Laboratory data from two different laboratories showing duplicate precision and matrix additions recoveries are presented in Table 6251:VII and Table 6251:VIII.

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6252 DISINFECTION BY-PRODUCTS: ALDEHYDES (PROPOSED)*#(202)

6252 A. Introduction

1. Sources and Significance

Ozone reactions during water treatment are complex and often produce a wide range of unstable oxidation by-products, usually oxygenated and polar. Among the intermediate products formed, when ozone attacks the organic matter present in raw waters, are low-molecular-weight by-products such as aldehydes. If oxidized further, these aldehydes can produce aldo-acids and carboxylic acids. Formaldehyde, a ubiquitous component of the environment, may be introduced into drinking water by ozone treatment, natural metabolism, and commercial processes.

There are two postulated mechanisms for aldehyde formation during ozone treatment. The first involves a two-step Criegee attack at unsaturated C-C bonds by molecular ozone with ozonides or epoxides formed as intermediates.[1] The second involves an indirect reaction of OH radicals.[2] Although the levels of aldehyde formation are usually a function of ozone dose, their concentrations are often controlled in water treatment by increasing the pH and thus the alkalinity of the water.

Aldehydes are unlikely to pose a serious health hazard to the consumer at microgram-per-liter concentrations as usually encountered in drinking water treatment. However, they react with nucleophiles even at these low levels and can therefore be a potential threat.[3] Thus, for example, formaldehyde, acetaldehyde, and crotonaldehyde are known animal carcinogens. Formaldehyde is a known human carcinogen.[4] Aldehydes also may serve as important components of assimilable organic carbon in promoting undesirable bioactivity.

2. Selection of Method

The most effective method for the determination of aldehydes in aqueous solutions involves the use of *o*-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBHA)†#(203) as a derivatizing agent. PFBHA reacts with low-molecular-weight carbonyl compounds, including aldehydes, to form the corresponding oximes. Unless the carbonyl compound is a symmetrical ketone or formaldehyde, two geometric isomers of the oxime derivatives are formed. These derivatives are extractable with organic solvents and are highly sensitive to analysis by gas chromatography with electron capture detection (GC/ECD) and gas chromatography with selective ion mass

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spectrometric detection (GC/SIM-MS).

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6252 B. PFBHA Liquid-Liquid Extraction Gas Chromatographic Method

This method measures straight-chain, low-molecular-weight aldehydes in raw and treated drinking water and simultaneously can analyze for C_1–C_{10} mono-carbonyl saturated aliphatic aldehydes, benzaldehyde, the dialdehyde glyoxal, and the keto-aldehyde methyl glyoxal.[1] The effectiveness of the derivatizing agent (PFBHA) in its reactions with these carbonyl compounds has been reviewed.[2]

1. General Discussion

a. Principle: Samples at room temperature are buffered to pH 6, PFBHA is added, and the samples are placed in a constant-temperature water bath. The carbonyl compounds are converted to their corresponding oximes during reaction with PFBHA. Sulfuric acid is used to quench excess PFBHA and the oxime derivatives are extracted with hexane. After H_2SO_4 cleanup, the organic extract is analyzed by gas chromatography where the volatile derivatives are easily separated in a temperature-programmable gas chromatograph equipped with a fused-silica capillary column and either an electron capture detector or selective ion mass spectrometer. Simultaneous analysis and confirmation with a single injection can be effected by setting up both the analytical column and the confirmation column to share a common injection port. Alternatively, use separate analytical and confirmation columns. Aqueous calibration standards similarly are derivatized, extracted, and analyzed. A surrogate recovery standard is added to the samples before derivatization to indicate any variation in derivatization and extraction efficiency.

With the exception of symmetrical ketones and formaldehyde, most carbonyl compounds form two geometrical isomers of oxime derivative. Methyl glyoxal, however, produces only one

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prominent isomer.

The method described may be used if appropriate quality control can be demonstrated for quantification of all C_1–C_{10} mono-carbonyl, saturated aliphatic aldehydes, benzaldehyde, glyoxal, and methyl glyoxal, but precision and quality control data are presented only for the most commonly found ozonation by-products, namely, formaldehyde, acetaldehyde, heptanal, benzaldehyde, glyoxal, and methyl glyoxal.

b. Interferences: Dissolved ozone, residual chlorine, and other oxidizing substances interfere with the PFBHA reaction. Quantitative addition of sodium thiosulfate as a reducing agent before derivatization or the addition of ammonium chloride or sulfate at the time of sampling (KI if ozone is present) prevents this interference. Ketones and quinones or large quantities of aldehydes may deplete the PFBHA reagent excess necessary to ensure complete reaction. Waters with high sulfide content inhibit the derivatization of carbonyl compounds. The occurrence of artifacts by aldehyde formation from thermal decomposition of water components is a potential positive interference. Because formaldehyde is used as a preservative for membranes, purified water produced by reverse osmosis is also a potential positive interference. In addition, formaldehyde and acetaldehyde are air pollutants and some formaldehyde in the air can be traced to certain insulation materials.

c. Detection levels: The method detection levels (MDL) and precision data for those aldehydes most commonly found in ozonated waters are given in Table 6252:I. These levels were evaluated for the extracted oximes in hexane from aldehyde-free water. The minimum reporting levels (MRL) for these aldehydes are usually set at five times the MDL. In effect, the MRL for all aldehydes analyzed by this method is 0.5 μg/L except for acetaldehyde and glyoxal, where the value is 1 μg/L. Formaldehyde is a ubiquitous contaminant. Method blanks may contain formaldehyde at trace levels. If it cannot be eliminated, raise the MRL. The precision data presented may be matrix-sensitive. Use known standard additions to the matrix if oxime standards are not available.

This method is useful for detecting carbonyl compounds such as short-chain aldehydes (C_1–C_{10}), benzaldehyde, glyoxal, and methyl glyoxal in the range of 1 to 100 μg/L. A clean laboratory reagent water blank, free of these contaminants, is essential.

d. Safety: The toxicity or carcinogenicity of each reagent used in this method has not been defined precisely. Acetaldehyde contains material that can cause cancer in animals, formaldehyde contains material that can cause cancer in humans, and crotonaldehyde causes respiratory tract and eye burns if inhaled or swallowed. Observe proper ventilation and handling procedures. Wear quantitatively-fitted negative-pressure respirators with charcoal air-purifying filter canisters, gloves (such as butyl but not natural rubber, latex, or nitrile[2]), and protective garments resistant to the degrading effects and permeation of these chemicals. Glyoxal and methyl glyoxal are mutagenic in in-vitro tests and the former has subchronic oral toxicity. Take care when handling high concentrations of aldehydes during preparation of primary standards. When handling hexane solutions of the oxime derivatives, wear nitrile gloves (not butyl or

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latex).

2. Sampling and Storage

See Section 6010B.1 and note the following additional requirements:

Seal sample vials with TFE-lined polypropylene screw caps.*#(204) Do not use bakelite black caps made from a formulation containing phenol and formaldehyde. Do not add HCl for this method. Reduce residual free chlorine according to the following method. Additional reagents are required for ozonated samples as described below. If free chlorine is present in the samples, add ammonium chloride or sulfate (0.1 mL of a 20% solution /40 mL sample) before sample collection. Monochloramine may form but will not change the aldehyde concentration of samples subsequently stored at 4°C. If residual ozone is present, the levels of aldehydes may change as the ozone-natural organic matter reaction continues. To prevent this, quantitatively add sodium thiosulfate to the empty vials. Alternatively quench residual ozone by adding 0.1 mL of a 3 g KI/L solution to each 40-mL vial.

Prepare field reagent blanks from organic-free reagent water (¶ 4*e*).

Ideally, derivatize samples for aldehyde analysis immediately after collection. If this is not feasible, complete derivatization and extraction within 48 h of collection.

3. Apparatus

a. Sample containers and extraction vials: 40-mL screw-top, glass sample vials with aldehyde-free caps. (NOTE: Do not use the thermoset phenol-formaldehyde or urea-formaldehyde.) Prepare these, together with the 14-mL amber vials for storing stock solutions, as follows: Wash with detergent, rinse with tap water, soak in 10% HNO_3 for at least 30 min, rinse with tap water, rinse with laboratory organic-free water (¶ 4*e* below), and oven dry at 180°C for at least 1 h.

Clean caps and septa by rinsing with methanol, then with hexane, and dry at 80°C for no more than 1 h in a clean, forced-air convection oven.

b. Microsyringes or Eppendorf micro-pipets with glass tips, to measure the following volumes: 5, 10, 25, 50, 100, 250, 500, and 1000 µL.

c. Volumetric flasks, 5, 10, and 25 mL, borosilicate glass. Prepare initially by the method of ¶ 3*a*, except that after rinsing with organic-free water, rinse with methanol and invert to drain. Air-dry only. *Do not dry in oven.*

d. Syringe: 20-mL glass hypodermic, metal luer lock tip with 8.9-cm- (3.5-in.-) long × 17 gauge stainless steel pipetting needle (alternatively, use a 20-mL volumetric pipet). Clean as above.

e. Automatic pipet dispensers: To simplify batch processing, add reagent by use of these dispensers. Preferably use adjustable 1-mL and 4-mL sizes with PTFE transfer lines that can be mounted on the suppliers' reagent bottles. If these are not available use 1-mL and 4-mL volumetric pipets.

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f. Constant-temperature water bath or incubator, capable of holding multiple 40-mL sample vials and maintaining 45°C ± 0.5°C.

g. Pasteur pipets: Have a selection of short-tipped (14.6-cm or 5.75-in.) and long-tipped (23-cm or 9-in.) pipets.

h. Mechanical shaker,†#(205) to automate hexane extraction (see Section 6251B.3*e*). Alternatively, use a vortex mixer or manual shaking for 1 min.

i. Storage vials: 7-mL glass, screw-cap vials with PTFE-lined silicone septa cleaned as described above.

j. Gas chromatograph, with capillary columns, temperature programmable, and supplied with a temperature-controlled injector and electron-capture detector.

1) *Gas handling equipment:* Use carrier (helium) and make-up (nitrogen or 95% argon/5% methane) gases of high purity (99.999%) that pass through indicating calcium sulfate, molecular sieve 5A, activated charcoal, and an oxygen-purifying cartridge. Use two-stage metal diaphragm high-purity regulators at the compressed gas sources. Use flow controllers to regulate carrier gas flow. Ensure that all gas lines use 0.3-cm (0.125-in.) copper (or stainless steel) tubing; rinse with high-purity acetone, and bake before use.

2) *Injector*, split/splitless (using straight open-bore insert).

3) *Analytical column*,‡#(206) 30 m long × 0.25 mm ID, fused silica capillary column with a 0.25-µm film thickness.

4) *Confirmation column,*§#(207) 30 m long × 0.25 mm ID, fused silica capillary column with a 0.25-µm film thickness.

5) *Detectors*, a constant-current pulse-modulated ^{63}Ni ECD with standard size cell (use two ECDs for simultaneous confirmation analysis).

4. Reagents

a. Extraction solvent, UV-grade, glass-distilled hexane.‖#(208)

b. Solvent for standard preparation, reagent-grade methanol, free of the target aldehydes.##(209)

c. Preservation agents, ammonium chloride, NH_4Cl, or sulfate, $(NH_4)_2SO_4$, or sodium thiosulfate, $Na_2S_2O_3$.

d. Sulfuric acid, H_2SO_4, conc and 0.2*N*.

e. Organic-free reagent water: Treat water prepared in commercially available water systems to remove all traces of aldehydes. Two methods have been demonstrated: Either expose reagent water produced by a laboratory purification system**#(210) to UV irradiation for 1 h or distill reagent water from acidified potassium permanganate (500 mL water with 64 mg potassium permanganate and 1 mL conc H_2SO_4).

Alternative purification techniques, such as addition of another granular activated carbon

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filtration step, may be used if they can be shown to effectively eliminate background levels of aldehydes. *Do not use a reagent water with formaldehyde contamination to quantify formaldehyde in aqueous samples.*

f. Buffer pH 6 reagent: Prepare in a 200-mL volumetric flask by mixing 100 mL 0.5*M* potassium hydrogen phthalate solution in aldehyde-free water with 43.6 mL of 1*M* NaOH and bringing to volume. Add 1 mL of this solution/20 mL aqueous sample before derivatization.

Prepare an alternative buffer solution by dissolving 0.2 g proprietary buffer salt.††#(211)

g. Derivatizing agent, PFBHA:‡‡#(212) Weigh *o*-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine hydrochloride into organic-free water to give a solution concentration of 15 mg/mL. Prepare fresh daily. Prepare enough to add 1 mL/20 mL sample.

h. Standard materials: See Table 6252:II for source and physical characteristics of the standards. Obtain purity assay of each purchased standard before use.

1) *Individual aldehyde standard stock solutions:* Prepare by weighing aldehyde standards in methanol. Weigh between 20 and 70 mg of each standard into a 10-mL volumetric flask. Weigh solid standards directly into the empty flask, then fill with methanol. Add liquid standards to the flask, which has been filled to the neck previously with methanol. Place on a weighing balance and stabilize the weight. Inject liquid standard with a microsyringe directly into the bulk of the methanol and determine exact weight after the addition. Because some aldehyde standards are supplied as aqueous solutions, evaluate weight of actual standard component and make an approximate determination of the required volume to be added from either the density (for pure liquids) or the percentage by weight (for solutions). Due to the high volatility of acetaldehyde, keep in the refrigerator at all times and place measurement syringe in a freezer for 10 min before preparing the stock solution. After diluting to 10 mL with methanol, cap flask and invert three times to mix. Transfer stock solutions to separate 14-mL amber vials with screw caps and PTFE liners and store at 4°C bound with self-adhesive film.§§#(213) Stock solutions (except formaldehyde), are usable for up to 3 months. Let them come to room temperature before pipetting. Overcome presence of turbidity or a precipitate by ultrasonication in warm water. If a precipitate persists, make a new stock solution. Prepare formaldehyde stock solutions each month.

Verify the aldehyde concentrations of the aqueous solutions after filtering through 0.45-µm PTFE filters by the sodium bisulfite-iodine titration method.[3]

2) *Multicomponent aldehyde additive standards*: Prepare additive standards solution using individual stock solutions of those aldehydes of interest. Make mixture weekly. The concentration of each component in this additive standard solution should be about 10 mg/L when added to 20-mL aqueous samples. When preparing a calibration curve in 100 mL of organic-free water, prepare two or more multicomponent additive standards from which a volume in the range 10 to 100 µL can be injected directly into the water. For example, if the stock solution concentration is 50 mg in 10 mL or 5 g/L, 20 µL in 10 mL methanol is required to

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produce a 10-mg/L additive standard. This is best achieved by first filling the 10-mL volumetric flask to just above the neck with methanol. Inject required volume of each of the stock solutions, using a clean microsyringe for each component, into the bulk of the methanol. After adding all stock solutions, fill to the mark with methanol. Cap and invert three times to mix.

i. Standards derivatives: To determine reaction and extraction efficiency of each aldehyde in different matrices compare the chromatographic response of the derivatized standard in the matrix to that of authentic standards of the oximes. The surrogate standard used to establish optimum conditions for derivatization and laboratory-synthesized oximes for six of the aldehydes have been used to verify recovery of derivatized aldehydes from organic-free water. See Table 6252:III. Unforeseen matrix effects can occur, and because PFBHA-derivatized aldehyde standards (oximes) are not available commercially, some representative syntheses of these derivatives are available.[4]

j. Internal standard, 1,2-dibromopropane and decafluorobiphenyl, 98% purity.‖ ‖#(214)

1) *Internal standard stock solution:* Weigh 50 mg into a 10-mL volumetric flask containing methanol up to the neck. Fill to mark with methanol. This 5-g/L stock solution can be used for up to 6 months when stored as described in ¶ h1) above.

2) *Internal standard working solvent*, 100 µg/L in hexane: Deliver 20 µL internal standard stock solution directly into 1 L hexane in the solvent bottle to be used in the extraction. Cap bottle and invert three times to ensure thorough mixing. This dilution can be used for 4 weeks. To ensure suitability for extraction, run a sample of this working solvent on the GC before extraction of aqueous samples. Before processing samples, provide enough working solvent to extract all calibration and aqueous samples to be analyzed. *Never make up fresh working solvent for use during sample processing.*

k. Surrogate (SUR), 2,3,5,6-tetrafluorobenzaldehyde, 98% pure.‖ ‖#(215)

1) *Surrogate stock solution*, 20 g/L: Weigh 0.2 g SUR into a 10-mL volumetric flask containing methanol up to the neck. After determining the weight difference, fill to mark with methanol. Stock solutions can be used for up to 6 months if stored as described in ¶ h1) above.

2) *Surrogate additive solution*, 20 mg/L: Deliver 10 µL SUR stock solution into a 10-mL volumetric flask and dilute to volume with methanol. This solution can be used for up to 3 months when stored at 4°C. At the beginning of sample processing, add 10-µL surrogate additive solution to each 20-mL sample portion, yielding a surrogate concentration of 10 µg/L.

l. Calibration standards: Prepare aqueous calibration standards in 100 mL organic-free water by injecting a measured amount of the multicomponent aldehyde additive standard solution directly into the water using the solvent flush technique. Prepare five different concentration levels within the expected sample range. These would normally be in the range 0.5 to 30 µg/L.

5. Procedure

a. Sample preparation: Remove samples and standard solutions from storage and let reach room temperature.

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b. Derivatization: Withdraw 20 mL sample from sample vial using a 20-mL glass syringe or glass pipet. Discard remaining sample, shake the vial dry by hand, and return the syringe contents to the vial. Add 10 µL surrogate additive solution using either a microsyringe or automatic pipettor, to all samples and standards. Add 1 mL pH 6 KHP/NaOH buffer to each portion with an automatic pipettor and swirl to mix. Add 1 mL freshly prepared 15-mg/mL PFBHA solution to each vial by automatic pipet, secure cap, and swirl to mix gently. Place all samples in a constant-temperature water bath set at 45 ± 0.5°C for 1 h and 45 min. Remove vials and cool to room temperature for 15 min.

c. Microextraction: To each vial add 0.05 mL (approximately 2 drops) conc H_2SO_4 to quench the derivatization reaction and then add 4 mL hexane working solvent containing the internal standard. When using automated extraction, place vials in a mechanical wooden shaker box. Shake vials on fast setting (approximately 300 cycles) for 3.5 min. Alternatively, shake manually for approximately 3 min. Remove vials and place upright. Let stand for approximately 5 min to permit phases to separate.

d. Extraction cleanup: Draw off top hexane layer using a clean 14.6-cm (5.75-in.) disposable pasteur pipet for each sample into a smaller 7-mL clear vial containing 3 mL 0.2*N* H_2SO_4. Shake for 30 s by hand and let stand for approximately 5 min for phase separation. Draw off top hexane layer using another clean 14.6-cm (5.75-in.) disposable pasteur pipet for each sample and place in two 1.8-mL autosampler vials per sample. Store extra autosampler vials in a refrigerator at 4°C as a backup extract.

e. Gas chromatography: Use the following operating conditions for the gas chromatograph: injector temperature 180°C; split valve open at 0.5 min; split flow at 50 mL/min; temperature program: 50°C for 1 min, rising at 4°C/min to 220°C and then at 20°C/min to 250°C; detector temperature: 300°C; carrier gas flow: 1.5 mL/min at 100°C; make-up gas flow: 27 mL/min.

At the beginning of each analysis, inject one hexane solvent blank to condition the GC and to verify that there are no interferences present. Inject 1 µL onto the splitless injector. See Figure 6252:1 and Figure 6252:2 for examples of chromatograms obtained with the above GC conditions for both the analytical and confirmation columns. If dual-column analysis is unavailable, use the column specified in ¶ 3 *j*3) above, but be aware of possible interferences. Table 6252:IV lists retention times for both columns.

f. Calibration: The use of five levels of calibration defines the quantitation range. The lowest standard is based on the lowest level of quantitation for each component. Prepare standard levels by adding the aldehydes to reagent-grade water and derivatize with the same PFBHA solution as the samples. This corrects for any recovery characteristics inherent in the method. Analyze the calibration standards under the same GC conditions as the samples.

6. Calculation

a. Standards procedure: Use this procedure when evaluating external, internal, surrogate, and calibration standards. Calculate individual response factor (*RF*) for each standard as follows:

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$$RF = \frac{A}{W}$$

where:

A = response (peak area), and
W = amount of compound, µg.

For each compound, determine the average *RF* and standard deviation of the *RF* values for all the standards. If the percent relative standard deviation (%*RSD*) is greater than 10%, take corrective action to improve method precision. When %*RSD* is less than 10%, then the mean *RF* is acceptable for use in calculating the relative response ratio of the surrogate/calibration standard *RF* to the internal standard *RF*.

b. Determination of constituent concentration: Construct calibration curve for each target constituent from the total relative response factor of all isomers (compared to one of the internal standards). Calculate the individual relative response factor (*RRF*) for each component as follows:

$$RRF = \frac{R_s}{R_i}$$

where:

R_s = total area of the calibration standard E- and Z-isomers and
R_i = area of the internal standard.

Calculate the average *RRF* for each compound, the standard deviation of the *RRF* values, and the %*RSD* from triplicate sample analyses. If %*RSD* is less than 10%, use the average *RRF* in linear regression (plotting the *RRF* against standard concentrations) and a linear regression equation:

$$RRF_a = m(C_x) + b$$

where:

RRF_a = relative response factor of constituent,
C_x = calibration standard concentration in samples, µg/L,
m = slope of line, and
b = intercept of the y axis.

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Use this internal standard calibration curve to determine concentrations in samples from the *RRF* values for each target constituent.

7. Quality Control

a. General considerations: PFBHA is a highly reactive *o*-substituted hydroxylamine. Like hydroxylamine, PFBHA reacts readily with a variety of carbonyl functional groups to produce corresponding oximes. The ease with which PFBHA reacts with carbonyl-containing compounds makes the potential contamination of samples a serious concern. Lower-molecular-weight aldehydes are commonly found in laboratory and outside air and can ultimately contaminate water samples, leading to incorrect calculation of aldehyde concentrations. As a further concern, PFBHA, especially in moist laboratory environments, can react to form oximes when directly exposed to aldehydes in air. For these reasons, exercise care to reduce the sources and exposure of samples, standard solutions, and PFBHA reagents to aldehyde contaminants. Consider storing PFBHA in a desiccator under an inert atmosphere, drying laboratory solvents with molecular sieves, using purified water, and making fresh derivatizing stocks on a regular basis. If, after analysis of appropriate sample blanks, contamination remains a problem, the source of the problem may be in the PFBHA reagent and solutions. Recrystallization of PFBHA may be necessary to remove oximes formed as a result of reagent contamination.

The effects of chromatographic and analytical conditions on E/Z ratios of the oximes have not been fully explored. The possibility of changing E/Z ratios under differing analytical conditions, such as injection temperature, requires that analytical conditions be carefully controlled. E/Z ratios may change as a function of time; therefore analyze samples as soon as possible after preparation and within groups. Use the sum of the isomer peak areas for each constituent for both calibration and quantification. With dicarbonyl species such as glyoxal, E/Z isomerism occurs from oxime formation with both carbonyl groups, increasing the number of possible isomers. Formation of the mono-derivatives from these di-carbonyl species may pose a problem if analytical conditions do not favor the complete derivatization of both carbonyl groups. Mono-derivatives have been shown to have similar retention and mass spectral characteristics as single carbonyl-containing oxime derivatives, potentially leading to incorrect identification and underestimation of the amounts of di-carbonyl species present in water samples. This method has been validated for the recovery of oxime derivatives of aldehydes from organic-free water; the recovery of the surrogate standard from this matrix appears to reflect method performance. Consequently, compare the *RRF* of surrogate standard extracted from aqueous samples to the value obtained when building the calibration curve. If these values are outside the range for accepted mean recovery values of 30% (see Section 6251B.7*c*), authentic oxime standards may have to be used to validate the method for the new matrix. In this case, if pure standards are unavailable, recognize that analyses of aldehydes are semi-quantitative; report as such.

b. Monitoring for interferences:

1) Solvent blank—Analyze each reagent bottle of hexane containing internal standard before

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it is used. If there are any spurious peaks in the chromatogram, solvent purity has been compromised. Remake the working solvent.

2) Method blank—See Section 6251B.7*b*.

3) Travel or shipping blanks—Prepare blanks for each sampling location in the laboratory by filling 40-mL vials, as described above, with organic-free water and containing the same reagents present (if any) in the sample vials. Ship to the sampling site and back to the laboratory with the sample bottles. Do not open these bottles in the field.

c. Internal standard assessment: Injections of the hexane extracts are acceptable if the area counts of the internal standard peak do not vary more than ± 20% from the mean of all the samples analyzed with the same batch of PFBHA. Reanalyze samples that do not meet this precision. If, after reinjection, criteria are still not met, the sample holding time has not been exceeded, and the same working solvent used for constructing the calibration curve is still available, the second vial may be analyzed.

d. Surrogate standard recovery: Add the surrogate (2,3,5,6-tetrafluorobenzaldehyde) directly to the 20-mL aqueous sample portions before reagent addition to monitor constituent recovery from the sample matrix. If the surrogate area is low or absent, there is likely to be a problem with derivatization or extraction that needs to be resolved before quantification can be undertaken (see Section 6251B.7*e*). A sample extract is acceptable if the area counts of the surrogate peak (or the *RRF* values compared to an acceptable internal standard area) do not vary more than ± 30% from other samples analyzed with the same batch of PFBHA.

e. Sample quantification: See Section 6251B.7*f*.

f. Matrix additions: See Section 6251B.7*g*.

g. Replicate analysis: See Section 6251B.7*h*.

8. References

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6410 EXTRACTABLE BASE/NEUTRALS AND ACIDS*#(216)

6410 A. Introduction

1. Sources and Significance

The semivolatile compounds covered by this section include many classes of compounds, each characterized by different sources. The compounds include polynuclear aromatic hydrocarbons, often as by-products of petroleum processing or combustion; phthalates, used as plasticizers; phenolics, found most often in wood preservatives; organochlorine pesticides, found most often in agricultural runoff or in wastewaters draining such areas; and PCBs (also see Section 6431A). Many of the listed compounds are toxic or carcinogenic. However, they generally are relatively insoluble in water so they do not occur frequently in potable waters or most wastewaters.

2. Selection of Method

Method Section 6410B is a broad-spectrum gas chromatographic/mass spectrometric (GC/MS) packed- or capillary-column method for detection of these compounds following liquid-liquid extraction. Although this method can be used to determine all the listed compounds, it is not the most sensitive method for individual classes of compounds, which are detected at lower concentrations by GC methods such as those listed in Section 6420C (phenols), Section 6440B and Section 6440C (polynuclear aromatic hydrocarbons), and Section 6630C and Section 6630D (organochlorine pesticides and PCBs). In some cases, notably the pesticides, the GC method is substantially more sensitive than the GC/MS method. In other cases, such as the phenols, there is less difference between the methods.

6410 B. Liquid-Liquid Extraction Gas Chromatographic/Mass Spectrometric Method

This method[1] is applicable to the determination of organic compounds that are partitioned into an organic solvent and are amenable to gas chromatography,*#(217) in municipal and industrial discharges.

1. General Discussion

a. Principle: A measured volume of sample is extracted serially with methylene chloride at a pH above 11 and again at a pH below 2. The extract is dried, concentrated, and analyzed by GC/MS.[2,3] Qualitative compound identification is based on retention time and relative abundance of three characteristic masses (*m*/*z*). Quantitative analysis uses internal-standard

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techniques with a single characteristic *m*/*z*.

b. Interferences:

1) General precautions—See Section 6010C. Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample-processing hardware that lead to discrete artifacts and/or elevated base lines in detector output. Routinely demonstrate that all materials are free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 6200A.5*c*3).

Clean all glassware thoroughly[4] as soon as possible after use by rinsing with the last solvent used in it, followed by detergent washing with hot water and rinsing with tap water and distilled water. Drain glassware dry and heat in a muffle furnace at 400°C for 15 to 30 min. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide-quality hexane may be substituted for the baking. Thorough rinsing with such solvents usually eliminates PCB interference. Do not heat volumetric ware in a muffle furnace. After drying and cooling, seal and store glassware in a clean environment to prevent accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

Use high-purity reagents and solvents to minimize interference. Purification of solvents by distillation in all-glass systems may be required.

Matrix interferences may be caused by coextracted contaminants. The extent of matrix interferences will vary considerably depending on the sample.

2) Special precautions—Benzidine can be lost by oxidation during solvent concentration. Under the alkaline conditions of the extraction step, α-BHC, γ-BHC, endosulfan I and II, and endrin are subject to decomposition. Hexachlorocyclopentadiene is subject to thermal decomposition in the inlet of the gas chromatograph, chemical reaction in acetone solution, and photochemical decomposition. *N*-nitrosodimethylamine is difficult to separate from the solvent under the chromatographic conditions described. *N*-nitrosodiphenylamine decomposes in the gas chromatographic inlet and cannot be separated from diphenylamine. Other methods may be preferred for these compounds.[1]

The base-neutral extraction may cause significantly reduced recovery of phenol, 2-methylphenol, and 2,4-dimethylphenol. Results obtained under these conditions are minimum concentrations.

The packed gas chromatographic columns recommended for the basic fraction may not be able to resolve certain isomeric pairs including the following: anthracene and phenanthrene; chrysene and benzo(a)anthracene; and benzo(b)fluoranthene and benzo(k)fluoranthene because retention time and mass spectra for these pairs are not sufficiently different to make unambiguous identification possible. Use alternative techniques, such as the method for polynuclear aromatic hydrocarbons (Section 6440B), to identify and quantify these compounds.

In samples containing many interferences, use chemical ionization (CI) mass spectrometry to make identification easier. Table 6410:I and Table 6410:II give characteristic CI ions for most compounds covered by this method. Use of CI mass spectrometry to support electron ionization

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(EI) mass spectrometry is encouraged but not required.

c. Detection levels: The method detection level (MDL) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[5] The MDL concentrations listed in Table 6410:I and II were obtained with reagent water.[6] The MDL actually obtained in a given analysis will vary, depending on instrument sensitivity and matrix effects.

d. Safety: The toxicity or carcinogenicity of each reagent has not been defined precisely. Benzo(a)anthracene, benzidine, 3,3′-dichlorobenzidine, benzo(a)pyrene, α-BHC, β-BHC, δ-BHC, γ-BHC, dibenzo(a,h)anthracene, *n*-nitrosodimethylamine, 4,4′-DDT, and polychlorinated biphenyls (PCBs) have been tentatively classified as known or suspected, human or mammalian carcinogens. Prepare primary standards of these compounds in a hood and wear a NIOSH/MESA-approved toxic gas respirator when handling high concentrations.

2. Sampling and Storage

Collect grab samples in 1-L amber glass bottles fitted with a screw cap lined with TFE. Foil may be substituted for TFE if the sample is not corrosive. If amber bottles are not available, protect samples from light. Wash and rinse bottle and cap liner with acetone or methylene chloride, and dry before use. Follow conventional sampling practices[7] but do not rinse bottle with sample. Collect composite samples in refrigerated glass containers. Optionally, use automatic sampling equipment as free as possible of plastic tubing and other potential sources of contamination; incorporate glass sample containers for collecting a minimum of 250 mL. Refrigerate sample containers at 4°C and protect from light during compositing. If the sampler includes a peristaltic pump, use a minimum length of compressible silicone rubber tubing, but before use, thoroughly rinse it with methanol and rinse repeatedly with distilled water to minimize contamination. Use an integrating flow meter to collect flow-proportional composites.

Fill sample bottles and, if residual chlorine is present, add 80 mg sodium thiosulfate per liter of sample and mix well. Ice all samples or refrigerate at 4°C from time of collection until extraction.

Extract samples within 7 d of collection and analyze completely within 40 d of extraction.

3. Apparatus

a. Separatory funnel, 2-L, with TFE stopcock.

b. Drying column, chromatographic, 400 mm long × 19 mm ID, with coarse frit filter disk.

c. Concentrator tube, Kuderna-Danish, 10-mL, graduated.†#(218) Check calibration at volumes used. Use ground-glass stopper to prevent evaporation.

d. Evaporative flask, Kuderna-Danish, 500-mL.‡#(219) Attach to concentrator tube with springs.

e. Snyder column, Kuderna-Danish, three-ball macro.[§]#(220)

f. Snyder column, Kuderna-Danish, two-ball micro.‖#(221)

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g. Vials, 10- to 15-mL, amber glass, with TFE-lined screw cap.

h. Continuous liquid-liquid extractor, equipped with TFE or glass connecting joints and stopcocks requiring no lubrication.##(222)

i. Boiling chips, approximately 10/40 mesh. Heat to 400°C for 30 min or extract in a Soxhlet extractor with methylene chloride.

j. Water bath, heated, with concentric ring cover and temperature control to ±2°C. Use bath in a hood.

k. Balance, analytical, capable of accurately weighing 0.0001 g.

*l. Gas chromatograph:***#(223) An analytical system complete with a temperature-programmable gas chromatograph and all required accessories including syringes, analytical columns, and gases. Use chromatograph with the injection port designed for on-column injection when packed columns are used and for splitless injection when capillary columns are used.

1) *Column for base/neutrals*, 1.8 m long × 2 mm ID glass, packed with 3% SP-2250 on Supelcoport (100/200 mesh) or equivalent. This column was used to develop the detection limit and precision and bias data presented herein. Guidelines for the use of alternate columns (e.g., DB-5 fused silica capillary) are provided in ¶ 5*b*.

2) *Column for acids*, 1.8 m long × 2 mm ID glass, packed with 1% SP-1240DA on Supelcoport (100/120 mesh) or equivalent. The detection limit and precision and bias data presented herein were developed with this column. For guidelines for the use of alternate columns (e.g., DB-5 fused silica capillary) see ¶ 5*b*.

m. Mass spectrometer, capable of scanning from 35 to 450 amu every 7 s or less, utilizing 70-V (nominal) electron energy in the electron impact ionization mode, and producing a mass spectrum that meets all the criteria in Table 6410:III when 50 ng of decafluorotriphenyl phosphine [DFTPP; bis(perfluorophenyl) phenyl phosphine] is injected through the GC inlet.

n. GC/MS interface: Any GC to MS interface that gives acceptable calibration points at 50 ng or less per injection for each of the compounds of interest and achieves all acceptable performance criteria may be used. GC to MS interfaces constructed of all glass or glass-lined materials are recommended. Glass can be deactivated by silanizing with dichlorodimethylsilane.

o. Data system: See Section 6200B.2 *f*.

4. Reagents

a. Reagent water: See Section 6200B.3*a*.

b. Sodium hydroxide solution, NaOH, 10*N:* Dissolve 40 g NaOH in reagent water and dilute to 100 mL.

c. Sodium sulfate, Na_2SO_4, granular, anhydrous. Purify by heating at 400°C for 4 h in a shallow tray.

d. Sodium thiosulfate, $Na_2S_2O_3 \cdot 5H_2O$, granular.

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e. Sulfuric acid, H_2SO_4, 1 + 1: Slowly add 50 mL conc H_2SO_4 to 50 mL reagent water.

f. Acetone, methanol, methylene chloride, pesticide quality or equivalent.

g. Stock standard solutions: Prepare from pure standard materials or purchase as certified solutions. Prepare by accurately weighing about 0.0100 g of pure material, dissolve in pesticide-quality acetone or other suitable solvent, and dilute to volume in a 10-mL volumetric flask; 1 µL = 1.00 µg compound. When compound purity is assayed to be 96% or greater, use the weight without correction to calculate concentration of the stock standard. Use commercially prepared stock standards at any concentration if certified by the manufacturer or by an independent source.

Transfer stock standard solutions into TFE-sealed screw-cap bottles. Store at 4°C and protect from light. Check stock standard solutions frequently for signs of degradation or evaporation, especially just before preparing calibration standards. Replace stock standard solutions after 6 months, or sooner if comparison with check standards indicates a problem.

h. Surrogate standard known-addition solution: Select a minimum of three surrogate compounds from Table 6410:IV. Prepare a surrogate standard solution containing each selected surrogate compound at a concentration of 100 µg/mL in acetone. Adding 1.00 mL to 1000 mL sample is equivalent to a concentration of 100 µg/L of each surrogate standard. Store at 4°C in TFE-sealed glass container. Check solution frequently for stability. Replace solution after 6 months, or sooner if comparison with quality-control check standards indicates a problem.

i. DFTPP standard: Prepare a 25-µg/mL solution of DFTPP in acetone.

j. Calibration standards: Prepare calibration standards at a minimum of three concentration levels for each compound by adding appropriate volumes of one or more stock standards to a volumetric flask. To each calibration standard or standard mixture, add a known constant amount of one or more internal standards (such as those listed in Table 6410:IV), and dilute to volume with acetone. Prepare one calibration standard at a concentration near, but above, the MDL and others corresponding to the expected range of sample concentrations or defining the working range of the GC/MS system.

k. Quality control (QC) check sample concentrate: Obtain a check sample concentrate containing each compound at a concentration of 100 µg/mL in acetone. Multiple solutions may be required. PCBs and multicomponent pesticides may be omitted. If such a sample is not available from an external source, prepare using stock standards prepared independently from those used for calibration.

5. Procedure

a. Extraction: Extraction by means of a separatory funnel, ¶ 1), is most common, but if emulsions will prevent acceptable solvent recovery, use continuous extraction, ¶ 2).

1) Separatory funnel extraction—Normally use a sample volume of 1 L. For sample volumes of 2 L, use 250-, 100-, and 100-mL volumes of methylene chloride for the serial extraction of the base/neutrals and 200-, 100-, and 100-mL volumes of methylene chloride for the acids.

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Mark water meniscus on side of sample bottle for later determination of sample volume. Pour entire sample into a 2-L separatory funnel. Pipet 1.00 mL surrogate standard solution into separatory funnel and mix well. Check pH with wide-range pH paper and adjust to pH > 11 with NaOH solution.

Add 60 mL methylene chloride to sample bottle, seal, and shake for 30 s to rinse inner surface. Transfer solvent to separatory funnel and extract sample by shaking for 2 min with periodic venting to release excess pressure. Let organic layer separate from water phase for a minimum of 10 min. If emulsion interface between layers is more than one-third the volume of the solvent layer, use mechanical techniques to complete phase separation. The optimum technique depends on the sample, but may include stirring, filtering emulsion through glass wool, centrifuging, or other physical methods. Collect methylene chloride extract in a 250-mL erlenmeyer flask.

If the emulsion cannot be broken (recovery of less than 80% of the methylene chloride, corrected for water solubility of methylene chloride) in the first extraction, transfer sample, solvent, and emulsion into extraction chamber of a continuous extractor and proceed as described in ¶ 2) below.

Add a second 60-mL volume of methylene chloride to sample bottle and repeat extraction procedure, combining extracts in the erlenmeyer flask. Perform a third extraction in the same manner.

After the third extraction, adjust pH of aqueous phase to <2 using H_2SO_4. Serially extract acidified aqueous phase three times with 60-mL portions of methylene chloride. Collect and combine extracts in a 250-mL erlenmeyer flask and label combined extracts as the acid fraction.

For each fraction, assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used if the requirements of ¶ 7 are met.

Pour combined extract through a solvent-rinsed drying column containing at least 10 cm anhydrous Na_2SO_4 or more and collect extract in concentrator. Rinse erlenmeyer flask and column with 20 to 30 mL methylene chloride to complete transfer.

Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet Snyder column by adding about 1 mL methylene chloride to the top. Place K-D apparatus on a hot water bath (60 to 65°C) in a hood so that concentrator tube is partially immersed in the hot water and entire lower rounded surface of flask is bathed with hot vapor. Adjust vertical position of apparatus and water temperature as required to complete concentration in 15 to 20 min. At proper rate of distillation the column balls actively chatter but the chambers are not flooded with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove K-D apparatus and let drain and cool for at least 10 min.

Remove Snyder column and rinse flask and its lower joint into the concentrator tube with 1 to 2 mL methylene chloride, preferably using a 5-mL syringe.

Add another one or two clean boiling chips to concentrator tube for each fraction and attach a

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two-ball micro-Snyder column. Prewet Snyder column by adding about 0.5 mL of methylene chloride to the top. Place K-D apparatus on a hot-water bath (60 to 65°C) so that concentrator tube is partially immersed in hot water and continue concentrating as directed above without further solvent addition until apparent volume of liquid reaches about 0.5 mL. After cooling, remove Snyder column and rinse flask and its lower joint into the concentrator tube with approximately 0.2 mL acetone or methylene chloride. Adjust final volume to 1.0 mL with solvent. Stopper concentrator tube and store refrigerated if further processing will not be done immediately. If extract is to be stored longer than 2 d, transfer to a TFE-sealed screw-cap vial and label base/neutral or acid fraction as appropriate.

Determine original sample volume by refilling sample bottle to mark and transferring liquid to a 1000-mL graduated cylinder. Record sample volume to nearest 5 mL.

2) Continuous extraction—Mark water meniscus on side of sample bottle, and determine sample volume later as described in ¶ 1). Check pH with wide-range pH paper and adjust to pH > 11 with NaOH solution. Transfer sample to continuous extractor and, using a pipet, add 1.00 mL surrogate standard solution and mix well. Add 60 mL methylene chloride to sample bottle, seal, and shake for 30 s to rinse inner surface. Transfer solvent to extractor. Repeat rinse with an additional 50- to 100-mL portion methylene chloride and add rinse to extractor.

Add 200 to 500 mL methylene chloride to distilling flask, add sufficient reagent water to ensure proper operation, and extract for 24 h. Let cool and detach distilling flask. Dry, concentrate, and seal extract as in ¶ 1) above.

Charge a clean distilling flask with 500 mL methylene chloride and attach it to continuous extractor. Carefully, while stirring, adjust pH of aqueous phase to less than 2 with H_2SO_4. Extract for 24 h. Dry, concentrate, and seal extract as in ¶ 1) above.

b. GC/MS operating conditions: Table 6410:I summarizes the recommended gas chromatographic operating conditions for the base/neutral fraction and Table 6410:II for the acid fraction. Included in these tables are retention times and MDLs that can be achieved under these conditions. Examples of the separations obtained with these columns are shown in Figure 6410:1, Figure 6410:2, Figure 6410:3, Figure 6410:4, Figure 6410:5, Figure 6410:6, Figure 6410:7, Figure 6410:8, Figure 6410:9, Figure 6410:10, Figure 6410:11, and Figure 6410:12. Other packed or capillary (open-tubular) columns or chromatographic conditions may be used if the requirements of ¶ 7 are met.

c. GC/MS performance tests: At the beginning of each day on which analyses are to be performed, check GC/MS system to see if acceptable performance criteria are achieved for DFTPP.[8] Each day that benzidine is to be determined, the tailing factor criterion described in ¶ 2) must be achieved. Each day that the acids are to be determined, the tailing factor criterion described in ¶ 3) must be achieved.

These performance tests have the requirements given in Section 6200B.4*b*, but use following conditions:

Electron energy: 70 V (nominal)

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Mass range: 35 to 450 amu

Scan time: To give at least 5 scans per peak but not to exceed 7 s per scan.

1) DFTPP performance test—At beginning of each day, inject 2 µL (50 ng) DFTPP standard solution. Obtain a background-corrected mass spectrum of DFTPP and confirm that all the key *m/z* criteria in Table 6410:III are achieved. If not, retune mass spectrometer and repeat test until all criteria are achieved. Meet performance criteria before any samples, blanks, or standards are analyzed. The tailing factor tests in ¶s 2) and 3) may be performed simultaneously with the DFTPP test.

2) Column performance test for base/neutrals—At beginning of each day that base/neutral fraction is to be analyzed for benzidine, calculate benzidine tailing factor. Inject 100 ng benzidine either separately or as a part of a standard mixture that may contain DFTPP, and calculate tailing factor, which must be less than 3.0. Calculation of the tailing factor is illustrated in Figure 6410:13.[9] Replace column packing if tailing factor criterion cannot be met.

3) Column performance test for acids—At beginning of each day that acids are to be determined, inject 50 ng pentachlorophenol either separately or as a part of a standard mix that may contain DFTPP. The tailing factor for pentachlorophenol must be less than 5. Calculation of the tailing factor is illustrated in Figure 6410:13.[9] Replace column packing if tailing factor criterion cannot be met.

d. Calibration of GC/MS system: Calibrate system daily after performance tests.

Select three or more internal standards similar in analytical behavior to the compounds of interest. Demonstrate that the measurement of the internal standards is not affected by method or matrix interferences. Some recommended internal standards are listed in Table 6410:IV. Use base peak *m/z* as the primary *m/z* for quantification. If interferences are noted, use one of the next two most intense *m/z* quantities for quantification. Using injections of 2 to 5 µL, analyze each calibration standard according to ¶ e below and tabulate area of primary characteristic *m/z* (Table 6410:I and Table 6410:II) against concentration for each compound and internal standard. Calculate response factors (RF) for each compound by the equation given in Section 6200B.4*c*2). If the RF value over the working range is a constant (<35% RSD), it can be assumed to be invariant; use the average RF for calculations. Alternatively, use the results to plot a calibration curve of response ratios, A_s / A_{is} vs. RF.

Verify working calibration curve or RF on each working day by measuring one or more calibration standards. If the response for any compound varies from the predicted response by more than 20%, repeat test using a fresh calibration standard. Alternatively, prepare a new calibration curve for that compound.

e. Sample analysis: Add internal standard to sample extract, mix thoroughly, and immediately inject 2 to 5 µL of sample extract or standard into GC/MS system using solvent-flush technique[10] to minimize losses due to adsorption, chemical reaction, or evaporation. Smaller (1.0-µL) volumes may be injected if automatic devices are used. Record

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volume injected to nearest 0.05 µL. If response for any *m*/*z* exceeds the working range of the GC/MS system, dilute extract and reanalyze. Make all qualitative and quantitative measurements as described below and in ¶ 6. When extract is not being used, store at 4°C, protected from light, in screw-cap vial equipped with unpierced TFE-lined septum.

Obtain EICPs for the primary *m*/*z* and the two other masses listed in Table 6410:I and Table 6410:II. See ¶ d for masses to be used with internal and surrogate standards. Use the following criteria to make a qualitative identification:

- The characteristic masses of each compound maximize in the same or within one scan of each other.
- The retention time falls within ±30 s of the retention time of the authentic compound.
- The relative peak heights of the three characteristic masses in the EICPs fall within ±20% of the relative intensities of these masses in a reference mass spectrum obtained from a standard analyzed in the GC/MS system or from a reference library.

Structural isomers that have very similar mass spectra and less than 30 s difference in retention time can be identified explicitly only if the resolution between authentic isomers in a standard mix is acceptable. Acceptable resolution is achieved if the baseline to valley height between the isomers is less than 25% of the sum of the two peak heights. Otherwise, structural isomers are identified as isomeric pairs.

f. Screening procedure for 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD): CAUTION: *In screening a sample for 2,3,7,8-TCDD, do not handle reference material without taking extensive safety precautions.* It is sufficient to analyze the base/neutral extract by selected ion monitoring (SIM) GC/MS techniques, as follows:

Concentrate base/neutral extract to a final volume of 0.2 mL. Adjust temperature of base/neutral column to 220°C. Operate mass spectrometer to acquire data in the SIM mode using the ions at *m*/*z* 257, 320, and 322 and a dwell time no greater than 333 ms/mass. Inject 5 to 7 µL of base/neutral extract. Collect SIM data for a total of 10 min. The possible presence of 2,3,7,8-TCDD is indicated if all three masses exhibit simultaneous peaks at any point in the selected ion current profiles. For each occurrence where the possible presence of 2,3,7,8-TCDD is indicated, calculate and retain the relative abundances of each of the three masses. False positives may be caused by the presence of single or coeluting combinations of compounds whose mass spectra contain all of these masses. Conclusive results of the presence and concentration level of 2,3,7,8-TCDD can be obtained only from a properly equipped laboratory using a specialized test method.[11]

6. Calculation

When a compound has been identified, base quantitation on the integrated abundance from the EICP of the primary characteristic *m*/*z* given in Table 6410:I and Table 6410:II. Use base peak *m*/*z* for internal and surrogate standards. If sample produces an interference for the primary *m*/*z*, use a secondary characteristic *m*/*z* to quantitate.

Calculate sample concentration using the response factor (RF) determined in ¶ 5*d* and the

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equation:

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)\ (I_s)}{(A_{is})\ (RF)\ (V_e)}$$

where:

A_s = area of characteristic *m/z* for compound or surrogate standard to be measured,
A_{is} = area of characteristic *m/z* for internal standard,
I_s = amount of internal standard added to each extract, µg, and
V_e = volume of water extracted, L.

Report results in µg/L without correction for recovery. Report all QC data with sample results.

7. Quality Control

a. Quality control program: See Section 6200A.5.

b. Initial quality control: Proceed according to Section 6200A.5*a*1) and Section 6200A.5*a*2). Use Table 6410:V for acceptance criteria.

c. Analyses of samples with known additions: Use quality acceptance criteria given in Table 6410:V.

d. Quality-control check standard analysis: Proceed as in Section 6200A.5*a*3); prepare QC check standard with 1.0 mL QC check standard concentrate and 1 L reagent water.

e. Bias assessment and records: Assess method bias and maintain records. For example, after the analysis of five wastewater samples, calculate the average percent recovery (P) and the standard deviation of the percent recovery (s_p). Express bias assessment as a percent recovery interval from $P - 2s_p$ to $P + 2s_p$. If $P = 90\%$ and $s_p = 10\%$, the recovery interval is expressed as 70–110%. Update bias assessment for each compound regularly, (e.g., after each five to ten new accuracy measurements).

f. Use of surrogate compounds: As a quality control check, make known additions to all samples of surrogate standard solution as described in ¶ 5*a*1), and calculate percent recovery of each surrogate compound.

g. Additional quality-assurance practices: Other desirable practices depend on the needs of the laboratory and the nature of the samples. Analyze field duplicates to assess precision of environmental measurements. Whenever possible, analyze standard reference materials and participate in relevant performance evaluation studies. Certain compounds, such as phthalates, are common laboratory contaminants. When these are measured above the detection limits in

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sample blanks, locate their source and repeat the analysis after taking corrective action.

8. Precision and Bias

This method was tested by 15 laboratories using reagent water, drinking water, surface water, and industrial wastewaters with additions at six concentrations over the range 5 to 1300 µg/L.[3] Single-operator precision, overall precision, and method bias were found to be related directly to the compound concentration and essentially independent of the sample matrix. Linear equations describing these relationships are presented in Table 6410:VI.

9. References

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6420 PHENOLS*#(224)

6420 A. Introduction

1. Sources and Significance

Phenols are found in many wastewaters and some raw source waters in the United States. They generally are traceable to industrial effluents or landfills. These compounds have a low taste threshold in potable waters and also may have a detrimental effect on human health at higher levels.

2. Selection of Method

For methods of determining total phenols in water and wastewater, see Section 5530.

The methods presented in this section are intended for the determination of individual phenolic compounds. For specific compounds covered, see each method. Method 6420B is a gas chromatographic (GC) method using liquid-liquid extraction and either flame ionization detection (FID) or derivatization and electron capture detection (ECD) to determine a wide variety of phenols at relatively low concentrations. In addition, Method 6420C, a liquid-liquid extraction gas chromatographic/mass spectrometric (GC/MS) method, can be used to determine the phenols at slightly higher concentrations.

6420 B. Liquid-Liquid Extraction Gas Chromatographic Method

This method[1] is applicable to the determination of phenol and certain substituted phenols*#(225) in municipal and industrial discharges. When analyzing unfamiliar samples for any or all of these compounds, support the identifications by at least one additional qualitative technique. Alternatively, use the derivatization, cleanup, and electron capture detector gas chromatography (ECD/GC) procedure to confirm measurements made by the flame ionization detector gas chromatographic (FID/GC) procedure. The method for base/neutrals and acids (Section 6410B) provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for qualitative and quantitative confirmation of results using the extract produced.

1. General Discussion

a. Principle: See Section 6010C for discussion of gas chromatographic principles. A measured volume of sample is acidified and extracted with methylene chloride. The extract is dried and exchanged to 2-propanol during concentration. The extract is separated by gas chromatography and phenols are measured with a flame ionization detector.[2]

The method provides for a derivatization and column chromatography cleanup procedure to aid in the elimination of interferences.[2,3] Derivatives are analyzed by an electron capture

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detector.

b. Interferences:

1) General precautions—See Section 6410B.1*b*.

2) Other countermeasures—The cleanup procedure in ¶ 5*c* can be used to overcome many of these inteferences, but unique samples may require additional cleanup to achieve the method detection limits.

The basic sample wash (¶ 5*a*) may cause low recovery of phenol and 2,4-dimethylphenol. Results obtained under these conditions are minimum concentrations.

c. Detection levels: The method detection level (MDL) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[4] The MDL concentrations listed in Table 6420:I and Table 6420:II were obtained by using reagent water.[5] Similar results were achieved with representative wastewaters. The MDL actually obtained in a given analysis will vary, depending on instrument sensitivity and matrix effects.

d. Safety: The toxicity or carcinogenicity of each reagent used in this method has not been defined precisely. Take special care in handling pentafluorobenzyl bromide, which is a lachrymator, and 18-crown-6-ether, which is highly toxic.

2. Sampling and Storage

See Section 6410B.2.

3. Apparatus

Use all the apparatus specified in Section 6410B.3*a* – *g* and Section 6410B.3*i* – *k*, and in addition:

a. Chromatographic column, 100 mm long × 10 mm ID, with TFE stopcock.

b. Reaction flask, 15- to 25-mL round-bottom, with standard tapered joint, fitted with a water-cooled condenser and U-shaped drying tube containing granular calcium chloride.

c. Gas chromatograph:†#(226) An analytical system complete with a temperature-programmable gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. Preferably use a data system for measuring peak areas.

1) *Column for underivatized phenols*, 1.8 m long × 2 mm ID glass, packed with 1% SP1240DA on Supelcoport (80/100 mesh) or equivalent. The detection limit and precision and bias data presented herein were developed with this column. For guidelines for the use of alternate columns (e.g., capillary or megabore) see ¶ 5*b*1).

2) *Column for derivatized phenols*, 1.8 m long × 2 mm ID, glass, packed with 5% OV-17 on Chromosorb W-AW-DMCS (80/100 mesh) or equivalent. This column was used to develop the detection limit and precision and bias data presented herein. For guidelines for the use of alternate columns (e.g., capillary or megabore) see¶ 5*b*1).

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3) *Detectors*, flame ionization (FID) and electron capture (ECD). Use the FID to determine parent phenols. Use the ECD when determining derivatized phenols. For guidelines for use of alternative detectors see ¶ 5*b*1).

4. Reagents

Use reagents listed in Section 6410B.4*a-f*, and in addition:

a. Sodium hydroxide solution, NaOH, 1*N:* Dissolve 4 g NaOH in reagent water and dilute to 100 mL.

b. Sulfuric acid, H_2SO_4, 1*N:* Slowly add 58 mL conc H_2SO_4 to 500 mL reagent water and dilute to 1 L.

c. Potassium carbonate, K_2CO_3, powdered.

d. Pentafluorobenzyl bromide (α-bromopentafluorotoluene), 97% minimum purity. (CAUTION: *This chemical is a lachrymator*.)

e. 18-Crown-6-ether (1,4,7,10,13,16-hexaoxacyclooctadecane), 98% minimum purity. (CAUTION: *This chemical is highly toxic*.)

f. Derivatization reagent: Add 1 mL pentafluorobenzyl bromide and 1 g 18-crown-6-ether to a 50-mL volumetric flask and dilute to volume with 2-propanol. Prepare fresh weekly. Prepare in a hood. Store at 4°C and protect from light.

g. Acetone, hexane, methanol, methylene chloride, 2-propanol, toluene, pesticide quality or equivalent.

h. Silica gel, 100/200 mesh.‡#(227) Activate at 130°C overnight and store in a desiccator.

i. Stock standard solutions: Prepare from pure standard materials or purchase as certified solutions. Prepare as directed in Section 6410B.4*g*, but dissolve material in 2-propanol.

j. Calibration standards: Prepare standards appropriate to chosen means of calibration.

1) *External standards:* Prepare at a minimum of three concentration levels for each compound by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with 2-propanol. Prepare one standard at a concentration near, but above, the MDL (see Table 6420:I or Table 6420:II) and the others to correspond to the expected range of sample concentrations or to define the working range of the detector.

2) *Internal standards:* Prepare at a minimum of three concentration levels for each compound by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with 2-propanol. Prepare one standard at a concentration near, but above, the MDL and the others to correspond to the expected range of sample concentrations or to define the working range of the detector.

k. Quality control (QC) check sample concentrate: Obtain a check sample concentrate[§]#(228) containing each compound at a concentration of 100 µg/mL in 2-propanol. If such a sample is not available from an external source, prepare using stock standards prepared

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independently from those used for calibration.

5. Procedure

a. Extraction: Mark water meniscus on side of sample bottle for later determination of volume. Pour entire sample into a 2-L separatory funnel. For samples high in organic content, solvent wash sample at basic pH as prescribed in next paragraph, to remove potential interferences. During wash, avoid prolonged or exhaustive contact with solvent, which may result in low recovery of some phenols, notably phenol and 2,4-dimethylphenol. For relatively clean samples, omit wash and extract directly.

To wash, adjust pH to 12.0 or greater with NaOH solution. Add 60 mL methylene chloride and shake the funnel for 1 min with periodic venting to release excess pressure. Discard solvent layer. Repeat wash up to two additional times if significant color is being removed.

Before extraction, adjust to pH of 1 to 2 with H_2SO_4. Extract three times with methylene chloride as directed in Section 6410B.5*a*1). Assemble Kuderna-Danish apparatus, concentrate extract to 1 mL, and remove, drain, and cool K-D apparatus as directed in Section 6410B.5*a*1).

Increase temperature of hot water bath to 100°C. Remove Snyder column and rinse flask and its lower joint into concentrator tube with 1 to 2 mL 2-propanol. Preferably use a 5-mL syringe for this operation. Attach a two-ball micro-Snyder column to concentrator tube and prewet column by adding about 0.5 mL 2-propanol to the top. Place micro-K-D apparatus on water bath so that concentrator tube is partially immersed in hot water. Adjust vertical position of apparatus and water temperature so as to complete concentration in 5 to 10 min. (CAUTION: If temperature is raised too quickly the sample may be blown out of the K-D apparatus). At proper rate of distillation the column balls actively chatter but the chambers are not flooded. When the apparent volume of liquid reaches 2.5 mL, remove K-D apparatus and let drain and cool for at least 10 min. Add 2 mL 2-propanol through top of micro-Snyder column and resume concentrating as before. When the apparent volume of liquid reaches 0.5 mL, remove K-D apparatus and let drain and cool for at least 10 min.

Remove micro-Snyder column and rinse lower joint into concentrator tube with a minimum amount of 2-propanol. Adjust extract volume to 1.0 mL. Stopper concentrator tube and store at 4°C if further processing will not be done immediately. If extract is to be stored longer than 2 d, transfer to a TFE-sealed screw-cap vial. If sample extract requires no further cleanup, proceed with chromatographic analysis (¶ b). If sample requires further cleanup, proceed to ¶ c.

Determine original sample volume by refilling sample bottle to mark and transferring liquid to a 1000-mL graduated cylinder. Record sample volume to nearest 5 mL.

b. Flame ionization detector gas chromatography (FID/GC):

1) Operating conditions— Table 6420:I summarizes the recommended operating conditions for the gas chromatograph and gives retention times and MDLs that can be achieved under these conditions. An example of the separations obtained with this column is shown in Figure 6420:1. Other packed or capillary (open-tubular) columns, chromatographic conditions, or detectors may be used if the requirements of ¶ 7 are met.

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2) Calibration—To calibrate the system for underivatized phenols, establish gas chromatographic operating conditions equivalent to those given in Table 6420:I. Calibrate using the external or the internal standard technique as follows:

a) External standard calibration procedure—Prepare standards as directed in ¶ 4 *j*1) and follow the procedure of ¶ 3) below. Tabulate data and obtain calibration curve or calibration factor as directed in Section 6200B.4*c*3).

b) Internal standard calibration procedure—Prepare samples as directed in ¶ 4 *j*2) and follow the procedure of ¶ 3) below. Tabulate data and calculate response factors as directed in Section 6200B.4*c*2).

Verify working calibration curve, calibration factor, or RF on each working day by measuring one or more calibration standards. If the response for any compound varies from the predicted response by more than ±15%, prepare a new calibration curve for that compound.

3) Sample analysis—If the internal standard calibration procedure is used, add internal standard to sample extract and mix thoroughly immediately before injecting 2 to 5 µL sample extract or standard into gas chromatograph using the solvent-flush technique.[6] Smaller (1.0-µL) volumes may be injected if automatic devices are used. Record volume injected to nearest 0.05 µL and resulting peak size in area or peak height units.

Identify compounds in sample by comparing peak retention times with peaks of standard chromatograms. Base width of retention time window used to make identifications on measurements of actual retention time variations of standards over the course of a day. To calculate a suggested window size, use three times the standard deviation of a retention time for a compound. Analyst’s experience is important in interpreting chromatograms.

If the response for a peak exceeds the working range of the system, dilute extract and reanalyze.

If peak response cannot be measured because of interferences, use the alternative gas chromatographic procedure (¶ c below).

c. Derivatization and electron capture detector gas chromatography (ECD/GC):

1) Derivatization—Pipet 1.0 mL of the 2-propanol solution of standard or sample extract into a glass reaction vial. Add 1.0 mL derivatizing reagent (¶ 4 *f*); this is sufficient to derivatize a solution having a total phenolic content not exceeding 0.3 mg/mL. Add about 3 mg K_2CO_3 and shake gently. Cap mixture and heat for 4 h at 80°C in a hot water bath. Remove from hot water bath and let cool. Add 10 mL hexane and shake vigorously for 1 min. Add 3.0 mL distilled, deionized water and shake for 2 min. Decant a portion of the organic layer into a concentrator tube and cap with a glass stopper.

2) Cleanup—Place 4.0 g silica gel in a chromatographic column. Tap column to settle silica gel and add about 2 g anhydrous Na_2SO_4 to the top. Pre-elute column with 6 mL hexane. Discard eluate and just before exposing Na_2SO_4 layer to air, pipet onto the column 2.0 mL hexane solution, ¶ 1) above, that contains the derivatized sample or standard. Elute column with

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10.0 mL hexane and discard eluate. Elute column, in order, with 10.0 mL 15% toluene in hexane (Fraction 1), 10.0 mL 40% toluene in hexane (Fraction 2), 10.0 mL 75% toluene in hexane (Fraction 3), and 10.0 mL 15% 2-propanol in toluene (Fraction 4). Prepare all elution mixtures on a volume:volume basis. Elution patterns for the phenolic derivatives are shown in Table 6420:II. Fractions may be combined as desired, depending on the specific phenols of interest or level of interferences.

3) Operating conditions— Table 6420:II summarizes the recommended operating conditions for the gas chromatograph and gives retention times and MDLs that can be achieved under these conditions. An example of the separations obtained with this column is shown in Figure 6420:2.

4) Calibration—Calibrate system daily by preparing a minimum of three 1-mL portions of calibration standards, ¶ 4 *j*1), containing each of the phenols of interest and derivatized as above. Analyze 2 to 5 µL of each column eluate collected as in ¶ 5) below and tabulate peak height or area responses against calculated equivalent mass of underivatized phenol injected. Prepare a calibration curve for each compound.

Before using any cleanup procedure, process a series of calibration standards through the procedure to validate elution patterns and to assure absence of interferences from the reagents.

5) Sample analysis—Inject 2 to 5 µL column fractions into the gas chromatograph using the solvent-flush technique. Smaller (1.0-µL) volumes can be injected if automatic devices are used. Record volume injected to nearest 0.05 µL and resulting peak size in area or peak height units. If peak response exceeds linear range of system, dilute extract and reanalyze.

6. Calculation

a. FID/GC analysis: Determine concentration of individual compounds. If the external standard calibration procedure is used, calculate amount of material injected from peak response using calibration curve or calibration factor determined previously. Calculate sample concentration from the equation:

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)\ (V_t)}{(V_i)\ (V_s)}$$

where:

A = amount of material injected, ng,
V_i = volume of extract injected, µL,
V_t = volume of total extract, µL, and
V_s = volume of water extracted, mL.

If the internal standard calibration procedure is used, calculate concentration in sample using the response factor (RF) determined above and the equation:

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$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)\ (I_s)}{(A_{is})\ (RF)\ (V_o)}$$

where:

A_s = response for compound to be measured,
A_{is} = response for internal standard,
I_s = amount of internal standard added to each extract, µg, and
V_o = volume of water extracted, L.

b. Derivatization and ECD/GC analysis: To determine concentration of individual compounds in the sample, use the equation:

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)\ (V_t)\ (B)\ (D)}{(V_i)\ (V_s)\ (C)\ (E)}$$

where:

A = mass of underivatized phenol represented by area of peak in sample chromatogram, determined from calibration curve in ¶ 5*c*4), ng,
V_i = volume of eluate injected, µL,
V_t = total volume of column eluate or combined fractions from which V_i was taken, µL,
V_s = volume of water extracted in ¶ 5*a*, mL,
B = total volume of hexane added in ¶ 5*c*1), mL,
C = volume of hexane sample solution added to cleanup column in ¶ 5*c*2), mL,
D = total volume of 2-propanol extract before derivatization, mL, and
E = volume of 2-propanol extract carried through derivatization in ¶ 5*c*1), mL.

Report results in µg/L without correction for recovery. Report QC data with sample results.

7. Quality Control

a. Quality control program: See Section 6200A.5.

b. Initial quality control: To establish the ability to generate data with acceptable bias and precision, perform the following operations:

Using a pipet, prepare QC check samples at a concentration of 100 µg/L by adding 1.00 mL of 100 µg/mL QC check sample concentrate to each of four 1-L portions reagent water. Analyze

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check samples according to the method of ¶ 5 and proceed with the check described in Section 6200A.5*a*1) and Section 6200A.5*a*2). Use acceptance criteria given in Table 6420:III.

c. Analyses of laboratory-fortified samples: On an ongoing basis, make known additions to at least 10% of the samples from each sample site being monitored. For laboratories analyzing one to ten samples per month, analyze at least one such sample with a known addition per month. Use the procedure detailed in Section 6200A.5*c*7) and Section 6200A.5*c*8), but use an addition of 100 µg/L rather than 20 µg/L and compare percent recovery for each compound with the corresponding QC acceptance criteria found in Table 6420:III. If the known addition was at a concentration lower than 100 µg/L, use either the QC acceptance criteria in Table 6420:III or optional QC acceptance criteria calculated for the specific addition concentration based on the equations in Table 6420:IV.

d. Quality-control check standard analysis: If analysis of any compound fails to meet the acceptance criteria for recovery, prepare and analyze a QC check standard containing each compound that failed. NOTE: The frequency for the required analysis of a QC check standard will depend on the number of compounds being tested for simultaneously, the complexity of the sample matrix, and the performance of the laboratory.

Prepare the QC check standard by adding 1.0 mL of QC check sample concentrate to 1 L reagent water and proceed as in Section 6200A.5*a*3) using Table 6420:III.

e. Bias assessment and records: Assess method bias and maintain records as directed in Section 6410B.7*e*.

8. Precision and Bias

This method was tested by 20 laboratories using reagent water, drinking water, surface water, and three industrial wastewaters with known additions at six concentrations over the range 12 to 450 µg/L.[7] Single-operator precision, overall precision, and method bias were found to be related directly to compound concentration and essentially independent of sample matrix. Linear equations describing these relationships for a flame ionization detector are presented in Table 6420:IV.

9. References

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3. KAWAHARA, F.K. 1968. Microdetermination of derivatives of phenols and mercaptans by means of electron capture gas chromatography. *Anal. Chem.* 40:100.
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6420 C. Liquid-Liquid Extraction Gas Chromatographic/Mass Spectrometric Method

See Section 6410B.

6431 POLYCHLORINATED BIPHENYLS (PCBS)*#(229)

6431 A. Introduction

1. Sources and Significance

The polychlorinated biphenyls (PCBs) are found principally in water supplies contaminated by transformer oils in which PCBs were originally used as a heat-exchange medium. Although the use of these compounds has been banned, there are still numerous transformers in existence that contain PCBs, which results in their occasional discharge into potable water or wastewater. These compounds are toxic, bioaccumulative, and extremely stable, and thus there is a need to monitor them in wastewaters.

2. Selection of Method

The liquid-liquid extraction (LLE) gas chromatographic (GC) method is used to monitor both the PCBs and the organochlorine pesticides simultaneously. This method has excellent sensitivity. The LLE gas chromatographic/mass spectrometric (GC/MS) method also can be used to detect PCBs, but with substantially less sensitivity.

PCBs usually are measured as commercial mixtures of isomers rather than as individual isomers (congeners).

6431 B. Liquid-Liquid Extraction Gas Chromatographic Method

See Section 6630B and Section 6630C.

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6431 C. Liquid-Liquid Extraction Gas Chromatographic/Mass Spectrometric Method

See Section 6410B.

6440 POLYNUCLEAR AROMATIC HYDROCARBONS*#(230)

6440 A. Introduction

1. Sources and Significance

The polynuclear aromatic hydrocarbons (PAHs) often are by-products of petroleum processing or combustion. Many of these compounds are highly carcinogenic at relatively low levels. Although they are relatively insoluble in water, their highly hazardous nature merits their monitoring in potable waters and wastewaters.

2. Selection of Method

Method 6440B encompasses both a high-performance liquid chromatographic (HPLC) method with UV and fluorescence detection and a gas chromatographic (GC) method using flame ionization detection. Method 6440C is a gas chromatographic/mass spectrometric (GC/MS) method that also can detect these compounds at somewhat higher concentrations. Certain of these compounds may also be measured by closed-loop stripping analysis, Section 6040.

6440 B. Liquid-Liquid Extraction Chromatographic Method

This method[1] is applicable to the determination of certain polynuclear aromatic hydrocarbons (PAH)*#(231) in municipal and industrial discharges. When analyzing unfamiliar samples for any or all of these compounds, support the identifications by at least one additional qualitative technique. The method for base/neutrals and acids (Section 6410B) provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for qualitative and quantitative confirmation of results using the extract produced.

1. General Discussion

a. Principle: A measured volume of sample is extracted with methylene chloride. The extract is dried, concentrated, and separated by the high-performance liquid chromatographic (HPLC) or gas chromatographic (GC) method. If other analyses having essentially the same extraction and concentration steps are to be performed, extraction of a single sample will be sufficient for all the determinations. Ultraviolet (UV) and fluorescence detectors are used with HPLC to identify

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and measure the PAHs. A flame ionization detector is used with GC.[2]

The method provides a silica gel column cleanup to aid in eliminating interferences. When cleanup is required, sample concentration levels must be high enough to permit separate treatment of subsamples before the solvent-exchange steps.

Chromatographic conditions (¶ 5*d*) appropriate for the simultaneous measurement of combinations of these compounds may be selected but they do not adequately resolve the following four pairs of compounds: anthracene and phenanthrene; chrysene and benzo(a)anthracene; benzo(b)fluoranthene and benzo(k)fluoranthene; and dibenzo(a,h)anthracene and indeno(1,2,3-cd)pyrene. Unless reporting the sum of an unresolved pair is acceptable, use the liquid chromatographic method, which does resolve all 16 listed PAHs.

b. Interferences: See Section 6410B.1*b* for precautions concerning glassware, reagent purity, and matrix interferences. Interferences in liquid chromatographic techniques have not been assessed fully. Although HPLC conditions described allow for unique resolution of specific PAHs, other PAH compounds may interfere.

c. Detection levels: The method detection level (MDL) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[3] The MDL concentrations listed in Table 6440:I were obtained with reagent water.[4] Similar results were achieved with representative wastewaters. MDLs for the GC method were not determined. The MDL actually obtained in a given analysis will vary, depending on instrument sensitivity and matrix effects. This method has been tested for linearity of known-addition recovery from reagent water and has been demonstrated to be applicable over the concentration range from 8 × MDL to 800 × MDL,[4] with the following exception: benzo(ghi)perylene recovery at 80 × and 800 × MDL were low (35% and 45%, respectively).

d. Safety: The toxicity or carcinogenicity of each reagent has not been defined precisely. The following compounds have been classified tentatively as known or suspected, human or mammalian carcinogens: benzo(a)anthracene, benzo(a)pyrene, and dibenzo(a,h)anthracene. Prepare primary standards of these compounds in a hood and wear NIOSH/MESA-approved toxic gas respirator when handling high concentrations.

2. Sampling and Storage

For collection and general storage requirements, see Section 6410B.2. Because PAHs are light-sensitive, store samples, extracts, and standards in amber or foil-wrapped bottles to minimize photolytic decomposition.

3. Apparatus

Use all the apparatus specified in Section 6410B.3*a* - *g* and Section 6410B.3*i* - *k*, and in addition:

a. Chromatographic column, 250 mm long × 10 mm ID with coarse frit filter disk at bottom and TFE stopcock.

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b. High-performance liquid chromatograph (HPLC): An analytical system complete with column supplies, high-pressure syringes, detectors, and compatible strip-chart recorder. Preferably use a data system for measuring peak areas and retention times.

1) *Gradient pumping system*, constant flow.

2) *Reverse phase column*, HC-ODS Sil-X, 5-µm particle diam, in a 25-cm × 2.6-mm ID stainless steel column.†#(232) This column was used to develop MDL and precision and bias data presented herein. For guidelines for the use of alternate column packings see ¶ 5*d*1).

3) *Detectors*, fluorescence and/or UV. Use the fluorescence detector for excitation at 280 mm and emission greater than 389 nm cutoff.‡#(233) Use fluorometers with dispersive optics for excitation utilizing either filter or dispersive optics at the emission detector. Operate the UV detector at 254 nm and couple it to the fluorescence detector. These detectors were used to develop MDL and precision and bias data presented herein. For guidelines for the use of alternate detectors see ¶ 5*d*1).

c. Gas chromatograph:[§]#(234) An analytical system complete with temperature-programmable gas chromatograph suitable for on-column or splitless injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. Preferably use a data system for measuring peak areas.

1) *Column*, 1.8 m long × 2 mm ID glass, packed with 3% OV-17 on Chromosorb W-AW-DCMS (100/120 mesh) or equivalent. This column was used to develop the retention time data in Table 6440:II. For guidelines for the use of alternate columns (e.g. capillary or megabore) see ¶ 5*d*2).

2) *Detector*, flame ionization. This detector is effective except for resolving the four pairs of compounds listed in ¶ 1*a*. With the use of capillary columns, these pairs may be resolved with GC. For guidelines for the use of alternate detectors see ¶ 5*d*2).

4. Reagents

a. Reagent water: See Section 6200B.3*a*.

b. Sodium thiosulfate, $Na_2S_2O_3 \cdot 5H_2O$, granular.

c. Cyclohexane, methanol, acetone, methylene chloride, pentane, pesticide quality or equivalent.

d. Acetonitrile, HPLC quality, distilled in glass.

e. Sodium sulfate, Na_2SO_4, granular, anhydrous. Purify by heating at 400°C for 4 h in a shallow tray.

f. Silica gel, 100/200 mesh, desiccant.‖#(235) Before use, activate for at least 16 h at 130°C in a shallow glass tray, loosely covered with foil.

g. Stock standard solutions: Prepare as directed in Section 6410B.4*g*, using acetonitrile as the solvent.

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h. Calibration standards: Prepare standards appropriate to chosen means of calibration following directions in Section 6420B.4 *j*, except that acetonitrile is the diluent instead of 2-propanol. See Table 6440:I for MDLs.

i. Quality control (QC) check sample concentrate: Obtain a check sample concentrate##(236) containing each compound at the following concentrations in acetonitrile: 100 µg/mL of any of the six early-eluting PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, and anthracene); 5 µg/mL of benzo(k)fluoranthene; and 10 µg/mL of any other PAH. If such a sample is not available from an external source, prepare using stock standards prepared independently from those used for calibration.

5. Procedure

a. Extraction: Mark water meniscus on side of sample bottle for later determination of volume. Pour entire sample into a 2-L separatory funnel and extract as directed in Section 6410B.5*a*1) without any pH adjustment.

After extraction, concentrate by adding one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet Snyder column by adding about 1 mL methylene chloride to the top. Place K-D apparatus on a hot water bath (60 to 65°C) in a hood so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of flask is bathed with hot vapor. Adjust vertical position of apparatus and water temperature as required to complete the concentration in 15 to 20 min. At proper rate of distillation the column balls actively chatter but the chambers are not flooded with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove K-D apparatus and let drain and cool for at least 10 min.

Remove Snyder column and rinse flask and its lower joint into concentrator tube with 1 to 2 mL methylene chloride. Preferably use a 5-mL syringe for this operation. Stopper concentrator tube and store refrigerated if further processing will not be done immediately. If extract is to be stored longer than 2 d, transfer to a TFE-sealed screw-cap vial and protect from light. If sample extract requires no further cleanup, proceed with gas or liquid chromatographic analysis (¶s c through *f* below). If sample requires further cleanup, first follow procedure of ¶ b before chromatographic analysis.

Determine original sample volume by refilling sample bottle to mark and transferring liquid to a 1000-mL graduated cylinder. Record sample volume to nearest 5 mL.

b. Cleanup and separation: Use procedure below or any other appropriate procedure; however, first demonstrate that the requirements of ¶ 7 can be met.

Before using silica-gel cleanup technique, exchange extract solvent to cyclohexane. Add 1 to 10 mL sample extract (in methylene chloride) and a boiling chip to a clean K-D concentrator tube. Add 4 mL cyclohexane and attach a two-ball micro-Snyder column. Prewet column by adding 0.5 mL methylene chloride to the top. Place micro-K-D apparatus on a boiling (100°C) water bath so that concentrator tube is partially immersed in hot water. Adjust vertical position of apparatus and water temperature so as to complete concentration in 5 to 10 min. At proper rate

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of distillation the column balls actively chatter but the chambers are not flooded. When apparent volume of liquid reaches 0.5 mL, remove K-D apparatus and let drain and cool for at least 10 min. Remove micro-Snyder column and rinse its lower joint into concentrator tube with a minimum amount of cyclohexane. Adjust extract volume to about 2 mL.

To perform silica-gel column cleanup, make a slurry of 10 g activated silica gel in methylene chloride and place in a 10-mm-ID chromatographic column. Tap column to settle silica gel and elute with methylene chloride. Add 1 to 2 cm anhydrous Na_2SO_4 to top of silica gel. Pre-elute with 40 mL pentane. Elute at rate of about 2 mL/min. Discard eluate and just before exposure of Na_2SO_4 layer to the air, transfer all the cyclohexane sample extract onto column using an additional 2 mL cyclohexane. Just before exposure of Na_2SO_4 layer to air, add 25 mL pentane and continue elution. Discard this pentane eluate. Next, elute column with 25 mL methylene chloride/pentane (4 + 6) (v/v) into a 500-mL K-D flask equipped with a 10-mL concentrator tube. Concentrate collected fraction to less than 10 mL as in ¶ 5*a*. After cooling, remove Snyder column and rinse flask and its lower joint with pentane.

c. Reconcentration: Concentrate further as follows:

1) For high-performance liquid chromatography—To extract in a concentrator tube, add 4 mL acetonitrile and a new boiling chip. Attach a two-ball micro-Snyder column and concentrate solvent as in ¶ 5*a* (but set water bath at 95 to 100°C.) After cooling, remove micro-Snyder column and rinse its lower joint into the concentrator tube with about 0.2 mL acetonitrile. Adjust extract volume to 1.0 mL.

2) For gas chromatography—To achieve maximum sensitivity with this method, concentrate extract to 1.0 mL. Add a clean boiling chip to methylene chloride extract in concentrator tube. Attach a two-ball micro-Snyder column. Prewet column by adding about 0.5 mL methylene chloride to the top. Place micro-K-D apparatus on a hot water bath (60 to 65°C) and continue concentration as in ¶ 5*b*. Remove micro-Snyder column and rinse its lower joint into concentrator tube with a minimum amount of methylene chloride. Adjust final volume to 1.0 mL and stopper concentrator tube.

d. Operating conditions:

1) High-performance liquid chromatography— Table 6440:I summarizes the recommended operating conditions for HPLC and gives retention times, capacity factors, and MDLs that can be achieved under these conditions. Preferably use the UV detector for determining naphthalene, acenaphthylene, acenapthene, and fluorene and the fluorescence detector for the remaining PAHs. Examples of separations obtained with this HPLC column are shown in Figure 6440:1 and Figure 6440:2. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of ¶ 7 are met.

2) Gas chromatography— Table 6440:II summarizes the recommended operating conditions for the gas chromatograph and gives retention times that were obtained under these conditions. An example of the separations is shown in Figure 6440:3. Other packed or capillary (open-tubular) columns, chromatographic conditions, or detectors may be used if the

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requirements of ¶ 7 are met.

e. Calibration: Calibrate system daily using either external or internal standard procedure.

1) External standard calibration procedure—Prepare standards as directed in¶ 4*h* and follow either procedure of ¶ f below. Tabulate data and obtain calibration curve or calibration factor as directed in Section 6200B.4*c*3).

2) Internal standard calibration procedure—Prepare standards as directed in¶ 4*h* and follow either procedure of ¶ f below. Tabulate data and calculate response factors as directed in Section 6200B.4*c*2).

Verify working calibration curve, calibration factor, or RF on each working day by measuring one or more calibration standards. If the response for any compound varies from the predicted response by more than ± 15%, repeat test using a fresh calibration standard. Alternatively, prepare a new calibration curve for that compound.

Before using any cleanup procedure, process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

f. Sample analysis:

1) High-performance liquid chromatography—If the internal standard calibration procedure is being used, add internal standard to sample extract and mix thoroughly. Immediately inject 5 to 25 µL sample extract or standard into HPLC using a high-pressure syringe or a constant-volume sample injection loop. Record volume injected to nearest 0.1 µL and resulting peak size in area or peak height units. Re-equilibrate HPLC column at initial gradient conditions for at least 10 min between injections.

Identify compounds in sample by comparing peak retention times with peaks of standard chromatograms. Base width of retention time window used to make identifications on measurements of actual retention time variations of standards over the course of a day. To calculate a suggested window size use three times the standard deviation of a retention time for a compound. Analyst’s experience is important in interpreting chromatograms.

If the response for a peak exceeds the working range of the system, dilute extract with acetonitrile and reanalyze.

If peak response cannot be measured because of interferences, further cleanup is required.

2) Gas chromatography—See Section 6420B.5*b*3). If peak response cannot be measured because of interferences, further cleanup is required.

6. Calculation

Determine concentration of individual compounds using the procedures given in Section 6420B.6*a*. Report results in µg/L without correction for recovery. Report QC data with sample results.

7. Quality Control

a. Quality-control program: See Section 6200A.5.

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b. Initial quality control: To establish the ability to generate data with acceptable precision and bias, proceed as follows: Using a pipet, prepare QC check samples at test concentrations shown in Table 6440:III by adding 1.00 mL of QC check sample concentrate (¶ 4*i*) to each of four 1-L portions of reagent water. Analyze QC check samples according to the method of ¶ 5. Calculate average recovery and standard deviation of the recovery, compare with acceptance criteria, and evaluate and correct system performance as directed in Section 6200A.5*a*1) and Section 6200A.5*a*2), using acceptance criteria given in Table 6440:III.

c. Analyses of samples with known additions: See Section 6420B.7*c*. Prepare QC check sample concentrate according to ¶ 4*i* and use Table 6440:III and Table 6440:IV. On an ongoing basis, make known additions to at least 10% of the samples from each sample site being monitored. For laboratories analyzing one to ten samples per month, analyze at least one such sample with a known addition per month. Use the procedure described in Section 6200A.5*c*7) and Section 6200A.5*c*8).

d. Quality-control check standard analysis: See Section 6420B.7*d*. Prepare QC check standard according to ¶ 4*i* and use Table 6440:III. If all compounds in Table 6440:III are to be measured in the sample in ¶ c above, it is probable that the analysis of a QC check standard will be required; therefore, routinely analyze the QC check standard with the known-addition sample.

e. Bias assessment and records: See Section 6410B.7*e*.

8. Precision and Bias

This method was tested by 16 laboratories using reagent water, drinking water, surface water, and three industrial wastewaters with known additions at six concentrations over the range 0.1 to 425 µg/L.[5] Single-operator precision, overall precision, and method bias were found to be related directly to compound concentration and essentially independent of sample matrix. Linear equations describing these relationships are presented in Table 6440:IV.

9. References

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PB84-211614, Springfield, Va.

6440 C. Liquid-Liquid Extraction Gas Chromatographic/Mass Spectrometric Method

See Section 6410B.

6610 CARBAMATE PESTICIDES*#(237)

6610 A. Introduction

1. Selection of Method

This method is appropriate for the determination of certain *N*-methylcarbamoyloxime and *N*-methylcarbamate pesticides in natural ground and surface waters. The procedure is based on high-performance liquid chromatography (HPLC) in conjunction with a post-column derivatization system and a fluorescence detector.

Carbamate pesticides are heat-sensitive and labile, and hence not amenable to analysis by gas chromatography. HPLC is the method of choice, but without preconcentration the usual ultraviolet detector is not adequate because of low sensitivity. A procedure to separate seven carbamate pesticides by HPLC followed by post-column alkaline hydrolysis has been reported.[1] A fluorescent adduct[2–4] is produced and is measured with a fluorescence detector.[5–8]

2. References

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4. KRAUSE, R.T. 1979. Resolution, sensitivity and selectivity of a high-performance liquid chromatographic post-column fluorometric labeling technique for determination of carbamate insecticides. *J. Chromatogr.* 185:615.
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6. FOERST, D.C. & H.A. MOYE. 1985. Aldicarb and related compounds in drinking water via direct aqueous injection HPLC with post-column derivatization. *In* Advances in Water Analysis and Treatment, Proc. 12th Annu. AWWA Water Quality Technology Conf., Dec. 2–5, 1984, Denver, Colo., p. 189. American Water Works Assoc., Denver, Colo.
7. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1991. Method 531.1— Measurement of *N*-methylcarbamoyloximes and *N*-methylcarbamates in water by direct aqueous injection HPLC with post-column derivatization. *In* Methods for the Determination of Organic Compounds in Drinking Water. EPA-600/4-88-039, rev. July 1991, U.S. Environmental Protection Agency, Cincinnati, Ohio.
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6610 B. High-Performance Liquid Chromatographic Method

1. General Discussion

a. Principle: This HPLC method is applicable to the determination of certain carbamate pesticides in water.*#(238) The sample is filtered and injected into a reverse-phase HPLC column. The constituents are separated by gradient elution chromatography. After elution the *N*-methyl compounds are hydrolyzed with sodium hydroxide. The resulting methylamine is reacted with *o*-phthalaldehyde (OPA) and 2-mercaptoethanol (MERC) to form a highly fluorescent isoindole product that is detected with a fluorescence detector.

b. Interferences: The post-column reaction generally is sensitive to primary amines and will form fluorescent adducts that may cause interference depending on their elution time and fluorescence intensity. Interferences also may be caused by contaminants in solvents, reagents, glassware, and sample-processing equipment. Specific sources of contamination have not been identified. Demonstrate that reagents and apparatus are free from interferences under the conditions of the analysis by analyzing laboratory method blanks. Use only high-purity reagents and solvents.

High chlorine concentrations, which may be encountered near a chlorine injection point, cause interference and loss of some constituents. Collect samples before chlorination or as far as practical from an injection point. If a raw water source is to be evaluated, collect sample before chlorination.

Matrix interferences may be caused by contaminants in the sample. They vary considerably from source to source. Severe interferences are not expected from most ground and surface waters, but the method probably is not suitable for waste streams, landfill leachate, or

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wastewater effluents. Confirmatory analysis may be used to increase the confidence of identification and quantitation for compounds determined by the primary analysis.

Interfering contamination in the analysis may occur when a low-level sample is analyzed immediately after a sample containing relatively high concentrations of test compounds. To minimize contamination use disposable syringes, filters, and sample vials. Analyze a reagent water blank after analyzing a sample containing high concentrations of carbamate pesticides, to demonstrate that no carryover contamination is present. As an alternative check for carryover, reanalyze any positive samples that immediately followed a high-concentration sample.

c. Detection levels: This method has been validated in a multiple laboratory test. Estimated detection levels (EDLs) are listed in Table 6610:I. Observed detection levels may vary between laboratories and samples, depending on interferences and specific instrumentation.

2. Sampling and Storage

Fill bottle only to shoulder with water to be sampled, leaving space for expansion on freezing. If residual chlorine is present, add 8 mg sodium thiosulfate/100 mL sample before collecting the sample. Keep samples cold (4°C) from time of collection until receipt in the laboratory. Oxamyl, 3-hydrocarbofuran, and carbaryl can degrade quickly in water at room temperature. This short-term degradation is of concern during sample processing and holding at room temperature in autosampler trays. Preserve samples targeted for the analysis of these three compounds by adjusting to pH 3 with monochloroacetic acid buffer solution (1.5 mL buffer/50 mL sample). For maximum protection, add buffer to sample bottle before taking sample. In the laboratory, store samples at 4°C until analysis. Samples are stable for at least 28 d when adjusted to pH 3 and stored at 4°C (see Table 6610:II).

3. Apparatus

a. High-performance liquid chromatograph (HPLC): An analytical system complete with column supplies, high-pressure syringes, detectors, and compatible strip-chart recorder. Preferably use a data system for measuring peak areas and retention times. Use system capable of injecting 200- to 500-µL portions and of performing binary linear gradients at a constant flow rate. See Figure 6610:1.

1) *Primary column:* 150 mm long × 3.9 mm ID stainless steel packed with 4 µm Novapak C18.†#(239) If other columns, conditions, or detectors are used, demonstrate that acceptable results are obtained.

2) *Alternate column:* 250 mm long × 4.6 mm ID stainless steel packed with 5 µm Beckman Ultrasphere ODS.† Data presented herein were obtained with this column. However, newer manufactured columns have not been able to resolve aldicarb sulfone from oxamyl.

3) *Confirmatory column:* 250 mm long × 4.6 mm ID stainless steel packed with 5 µm Supelco LC-1.†#(240) This is a trimethylsilyl bonded silica column.

4) *Post-column reactor:* Use a post-column reactor capable of mixing reagents into the mobile phase and equipped with pumps to deliver 0.1 to 1.0 mL/min of each reagent. Use a

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delivery rate of 0.5 mL/min for both sodium hydroxide and OPA solution. Use PTFE tubing‡#(241) for coils and other post-column lines. Stainless steel, polyetheretherketone (PEEK), and/or nickel lines have been used successfully.

5) *Detector:* Use fluorescence detector capable of excitation at 230 nm and detection of emission energies greater than 418 nm.

b. Filters: For macrofiltration of derivatization solutions and mobile phases, use 47-mm filters.§#(242) For microfiltration of samples before HPLC analysis, use 13-mm filter holder‖#(243) and 13-mm-diam 0.2-µm polyester filters.##(244) If disposable filters**#(245) and syringes are to be used, test and verify that comparable results are obtained.

c. Analytical balance, capable of weighing to the nearest 0.0001 g.

d. Sample bottles, 120-mL (or other convenient size) screw-cap polypropylene bottles. Less preferably, use polyethylene bottles or glass containers.

4. Reagents

Use reagent-grade chemicals of high purity and HPLC-grade (tested on HPLC and verified to give no impurity peaks) solvents or equivalent.

a. Methanol, CH_3OH, HPLC grade, or equivalent.

b. Reagent water: Generate by using a water purification system.††#(246) Alternatively, purchase HPLC-grade water commercially. For additional alternatives, see Section 1080.

c. Sodium hydroxide, NaOH, 0.05*N:* Dissolve 2.0 g NaOH in 1.0 L reagent water. Filter and degas with helium before use.

d. 2-Mercaptoethanol solution: Mix 10.0 mL 2-mercaptoethanol and 10.0 mL acetonitrile. Cap. Store in refrigerator. (CAUTION: *Stench.*)

e. Sodium borate solution: Dissolve 19.1 g $Na_2B_4O_7 \cdot 10H_2O$ in reagent water. Dilute to 1 L. Prepare a day before use to insure complete dissolution.

f. OPA reaction solution: Dissolve 0.100 g *o*-phthalaldehyde in 10 mL methanol. Add to 1.0 µL sodium borate solution. Mix, filter, and degas with helium. Add 100 µL 2-mercaptoethanol solution and mix. If protected from oxygen, solution is stable for at least 3 d; otherwise, prepare daily.

g. Monochloroacetic acid, 2.5*M*: Dissolve 236 g monochloroacetic acid in 1 L reagent water.

h. Potassium acetate solution, 2.5*M:* Dissolve 245 g potassium acetate in 1 L reagent water.

i. Monochloroacetic acid buffer: Mix 156 mL 2.5*M* monochloroacetic acid with 100 mL 2.5*M* potassium acetate solution. CAUTION: *Handle with care; long-term health effects from exposure have not been determined.*

j. Stock pesticide solutions: Prepare stock standard solutions (1.00 µg/µL) by accurately weighing approximately 0.0100 g of each compound into separate 10-mL volumetric flasks. Dissolve in about 5 mL methanol and dilute to mark with methanol. Larger volumes may be used

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at the convenience of the analyst. If compound purity is certified at 96% or greater, use uncorrected weight in calculations. These solutions are stable for several months when stored in a freezer at −10°C.

k. Calibration standards:

1) *Internal standard:* Prepare an internal standard solution by accurately weighing approximately 0.0010 g 4-bromo-3,5-dimethylphenyl *N*-methyl carbamate (BDMC), dissolving in methanol, and diluting to volume in a 10-mL volumetric flask. Add 5 µL of internal standard solution to 50 mL sample to give an internal standard concentration of 10 µg/L.

2) *Stock calibration standard:* Prepare a mixed concentrated standard of the compounds listed in Table 6610:I by adding appropriate amounts of each stock pesticide solution to methanol to yield a concentration of about 1.0 µg/mL each.

3) *Calibration standard:* Prepare working calibration standards by additional dilutions of the stock calibration standard with pH 3 buffered water to yield solutions containing approximately 100, 40, 10, and 2 µg/L of each compound. Any similar series of concentrations is satisfactory. The lowest standard concentration should be near (but above) the EDL (see Table 6610:I). Add internal standard to these working calibration standards to give a final concentration of 10 µg BDMC/L in each.

l. Sodium thiosulfate, $Na_2S_2O_3$.

5. Procedure

a. Liquid chromatography: Table 6610:III summarizes the recommended operating conditions for the liquid chromatograph, including retention times. An example of the separations achieved under these conditions is shown in Figure 6610:2.

Completely thaw frozen samples before analysis. If it has not already been done, adjust standards and samples to pH 3 by adding 1.5 mL monochloroacetic acid buffer/50 mL sample or standard. Fill a 50-mL volumetric flask to the mark with sample. Add internal standard (BDMC) and mix. Rinse the syringe and filter with 5 mL reagent water and then with 5 mL of prepared sample; discard the filtrate. Filter 5 to 10 mL of prepared sample and inject up to 500 µL of filtrate. Record peak sizes in area units. If the peak response exceeds the working range of the system, dilute sample with pH 3 buffered water and reinject.

b. Calibration:

1) *Internal standard calibration:* Using prepared working calibration standards, inject 500 µL of each standard. The lowest calibration standard should represent compound concentrations near, but above, their respective EDLs. The remaining calibration standards should bracket expected concentration range of samples or should define the working range of the detector. Tabulate peak height or area responses against concentration for each compound and the internal standard. Calculate response factors (*RF*) for each compound, using the equation:

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$$RF = \frac{A_s \times C_{is}}{A_{is} \times C_s}$$

where:

A_s = peak area or peak height for compound in working standard,
A_{is} = peak area or peak height of internal standard,
C_{is} = concentration of internal standard, µg/L, and
C_s = concentration of compound in working standard, µg/L.

If the *RF* value over the working range is constant (≤ 20% RSD), use average *RF* for calculations.

Alternately, plot a calibration curve using (A_s/A_{is}) vs. C_s.

Verify working calibration curve or RF for each work session by measuring one or more calibration standards. If the response for any compound varies from the predicted response by more than ± 20%, repeat the test using a fresh calibration standard and, if necessary, prepare a new calibration curve.

Verify calibration standards at least quarterly by analyzing a standard prepared using reference material obtained from an independent source.

2) *External standard calibration:* Use working calibration standards (¶ 4*k*3). Starting with the lowest concentration, analyze each working calibration standard, using 500-µL injections. Tabulate responses, peak height or area versus compound concentration in the standard. Prepare a calibration curve for each compound. Alternately, if the ratio of response to concentration (calibration factor) is constant (≤ 20%) over the working range of the curve, use a calibration factor in place of the calibration curve.

Verify calibration curve each working day by analysis of a working calibration standard at the beginning of the day and a different concentration at its end. For periods of analysis greater than 8 h, intersperse calibration standards with samples at regular intervals.

6. Calculations

Determine the concentrations of individual compounds using the following equations:

For internal standard quantitation,

$$C_x = \frac{A_x \times C_{is}}{A_{is} \times RF}$$

and for external standard quantitation,

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$$C_x = \frac{A_x \times C_s}{A_s}$$

where:

C_x = compound concentration, µg/L,
A_x = response of sample compound (peak area or height), and other terms are as defined previously.

7. Quality Control

A minimum quality control program should include the following quality assurance elements: initial demonstration of laboratory capability, laboratory control standards, monitoring of systems performance, blank samples, quality control check samples, duplicate analyses, demonstration of adequate recoveries, assessment of matrix effects, and demonstration of storage stability. Also see Part 1000 for general quality control requirements.

8. Precision and Bias

Single-laboratory recovery data are summarized in Table 6610:II. Comparable results should be obtained in other laboratories. Results are comparable if the calculated percent relative standard deviation (RSD) does not exceed 3 times the single-laboratory RSD or 30%, whichever is greater, and the mean recovery lies within the interval R ± 3SD or R ± 30%, whichever is greater.

Results of an eight-laboratory collaborative test are shown in Table 6610:IV. Test involved six concentrations for each compound (overall range 1 to 109 µg/L).

6630 ORGANOCHLORINE PESTICIDES*#(247)

6630 A. Introduction

1. Sources and Significance

The organochlorine pesticides commonly occur in waters that have been affected by agricultural discharges. Some of the listed compounds are degradation products of other pesticides detected by this method. Several of the pesticides are bioaccumulative and relatively stable, as well as toxic or carcinogenic; thus they require close monitoring.

2. Selection of Method

Methods 6630B and C consist of gas chromatographic (GC) procedures following

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liquid-liquid extraction of water samples. They are relatively sensitive methods that can be used to detect numerous pesticides. Differences between the methods are minimal after extraction. Method 6630D is a gas chromatographic/ mass spectrometric (GC/MS) method that can detect all of the target compounds, but at much higher concentrations. All these methods also are useful for determination of polychlorinated biphenyls (PCBs) (also see Section 6431A).

6630 B. Liquid-Liquid Extraction Gas Chromatographic Method I

1. General Discussion

a. Application: This gas chromatographic procedure is suitable for quantitative determination of the following specific compounds: BHC, lindane (γ-BHC), heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin, captan, DDE, DDD, DDT, methoxychlor, endosulfan, dichloran, mirex, and pentachloronitrobenzene. Under favorable circumstances, strobane, toxaphene, chlordane (tech.), and others also may be determined when relatively high concentrations of these complex mixtures are present and the chromatographic fingerprint is recognizable in packed or capillary column analysis. Trifluralin and certain organophosphorus pesticides, such as parathion, methylparathion, and malathion, which respond to the electron-capture detector, also may be measured. However, the usefulness of the method for organophosphorus or other specific pesticides must be demonstrated before it is applied to sample analysis.

b. Principle: In this procedure the pesticides are extracted with a mixed solvent, diethyl ether/hexane or methylene chloride/hexane. The extract is concentrated by evaporation and, if necessary, is cleaned up by column adsorption-chromatography. The individual pesticides then are determined by gas chromatography. Although procedures detailed below refer primarily to packed columns, capillary column chromatography also may be used. See Section 6010C.2*a*1) for discussion of gas chromatographic principles and Section 6010C.2*b*2) for discussion of electron-capture detector.

As each component passes through the detector a quantitatively proportional change in electrical signal is measured on a strip-chart recorder. Each component is observed as a peak on the recorder chart. The retention time is indicative of the particular pesticide and peak height/peak area is proportional to its concentration.

Variables may be manipulated to obtain important confirmatory data. For example, the detector system may be selected on the basis of the specificity and sensitivity needed. The detector used in this method is an electron-capture detector that is very sensitive to chlorinated compounds. Additional confirmatory identification can be made from retention data on two or more columns where the stationary phases are of different polarities. A two-column procedure that has been found particularly useful is specified. If sufficient pesticide is available for detection and measurement, confirmation by a more definitive technique, such as mass spectrometry, is desirable.

c. Interference: See Section 6010C.2*a*2) and Section 6010C.2*b*2). Some compounds other

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than chlorinated compounds respond to the electron-capture detector. Among these are oxygenated and unsaturated compounds. Sometimes plant or animal extractives obscure pesticide peaks. These interfering substances often can be removed by auxiliary cleanup techniques. A magnesia-silica gel column cleanup and separation procedure is used for this purpose. Such cleanup usually is not required for potable waters.

1) Polychlorinated biphenyls (PCBs)—Industrial plasticizers, hydraulic fluids,and old transformer fluids that contain PCBs are a potential source of interference in pesticide analysis. The presence of PCBs is suggested by a large number of partially resolved or unresolved peaks that may occur throughout the entire chromatogram. Particularly severe PCB interference will require special separation procedures.

2) Phthalate esters—These compounds, widely used as plasticizers, cause electron-capture detector response and are a source of interferences. Water leaches these esters from plastics, such as polyethylene bottles and plastic tubing. Phthalate esters can be separated from many important pesticides by the magnesia-silica gel column cleanup. They do not cause response to halogen-specific detectors such as microcoulometric or electrolytic conductivity detectors.

d. Detection limits: The ultimate detection limit of a substance is affected by many factors, for example, detector sensitivity, extraction and cleanup efficiency, concentrations, and detector signal-to-noise level. Lindane (γ-BHC) usually can be determined at 10 ng/L in a sample of relatively unpolluted water; the DDT detection limit is somewhat higher, 20 to 25 ng/L. Increased sensitivity is likely to increase interference with all pesticides.

e. Sample preservation: Some pesticides are unstable. Transport under iced conditions, store at 4°C until extraction, and do not hold more than 7 d. When possible, extract upon receipt in the laboratory and store extracts at 4°C until analyzed. Analyze extracts within 40 d.

2. Apparatus

Clean thoroughly all glassware used in sample collection and pesticide residue analyses. Clean glassware as soon as possible after use. Rinse with water or the solvent that was last used in it, wash with soapy water, rinse with tap water, distilled water, redistilled acetone, and finally with pesticide-quality hexane. As a precaution, glassware may be rinsed with the extracting solvent just before use. Heat heavily contaminated glassware in a muffle furnace at 400°C for 15 to 30 min. High-boiling-point materials, such as PCBs, may require overnight heating at 500°C, but no borosilicate glassware can exceed this temperature without risk. Do not heat volumetric ware. Clean volumetric glassware with special reagents.*#(248) Rinse with water and pesticide-quality hexane. After drying, store glassware to prevent accumulation of dust or other contaminants. Store inverted or cover mouth with aluminum foil.

a. Sample bottles: 1-L capacity, glass, with TFE-lined screw cap. Bottle may be calibrated to minimize transfers and potential for contamination.

b. Evaporative concentrator, Kuderna-Danish, 500-mL flask and 10-mL graduated lower tube fitted with a 3-ball Snyder column, or equivalent.

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c. Separatory funnels, 2-L capacity, with TFE stopcock.

d. Graduated cylinders, 1-L capacity.

e. Funnels, 125-mL.

f. Glass wool, filter grade.

g. Chromatographic column, 20 mm in diam and 400 mm long, with coarse fritted disk at bottom.

h. Microsyringes, 10- and 25-µL capacity.

i. Hot water bath.

j. Gas chromatograph, equipped with:

1) *Glass-lined injection port.*

2) *Electron-capture detector.*

3) *Recorder:* Potentiometric strip chart, 25-cm, compatible with detector and associated electronics.

4) *Borosilicate glass column,* 1.8 m × 4-mm ID or 2-mm ID.

Variations in available gas chromatographic instrumentation necessitate different operating procedures for each. Therefore, refer to the manufacturer's operating manual as well as gas chromatography catalogs and other references (see Bibliography). In general, use equipment with the following features:

- Carrier-gas line with a molecular sieve drying cartridge and a trap for removal of oxygen from the carrier gas. A special purifier†#(249) may be used. Use only dry carrier gas and insure that there are no gas leaks.
- Oven temperature stable to ±0.5°C or better at desired setting.
- Chromatographic columns—A well-prepared column is essential to an acceptable gas chromatographic analysis. Obtain column packings and pre-packed columns from commercial sources or prepare column packing in the laboratory.

It is inappropriate to give rigid specifications on size or composition to be used because some instruments perform better with certain columns than do others. Columns with 4-mm ID are used most commonly. The carrier-gas flow is approximately 60 mL/min. When 2-mm-ID columns are used, reduce carrier-gas flow to about 25 mL/min. Adequate separations have been obtained by using 5% OV-210 on 100/120 mesh dimethyl-dichlorosilane-treated diatomaceous earth‡#(250) in a 2-m column. The 1.5% OV-17 and 1.95% QF-1 column is recommended for confirmatory analysis. Two additional column options are included: 3% OV-1 and mixed-phase 6% QF-1 + 4% SE-30, each on dimethyl-dichlorosilane-treated diatomaceous earth, 100-120 mesh. OV-210, which is a refined form of QF-1, may be substituted for QF-1. A column is suitable when it effects adequate and reproducible resolution. Sample chromatograms are shown in Figure 6630:1, Figure 6630:2, Figure 6630:3, and Figure 6630:4.

Alternately, use fused silica capillary[§]#(251) columns, 30 m long with a 0.32-mm ID and

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0.25-µm film thickness, or equivalent. See Figure 6630:V. To confirm identification use a column of different polarity.‖#(252)

3. Reagents

##(253) Use solvents, reagents, and other materials for pesticide analysis that are free from interferences under the condition of the analysis. Specific selection of reagents and distillation of solvents in an all-glass system may be required. ‘‘Pesticide quality’’ solvents usually do not require redistillation; however, always determine a blank before use.

a. Hexane.

b. Petroleum ether, boiling range 30 to 60°C.

c. Diethyl ether: CAUTION: *Explosive peroxides tend to form.* Test for presence of peroxides**#(254) and, if present, reflux over granulated sodium-lead alloy for 8 h, distill in a glass apparatus, and add 2% methanol. Use immediately or, if stored, test for peroxides before use.

d. Ethyl acetate.

e. Methylene chloride.

f. Magnesia-silica gel,††#(255) PR grade, 60 to 100 mesh. Purchase activated at 676°C and store in the dark in glass container with glass stopper or foil-lined screw cap; do not accept in plastic container. Before use, activate each batch overnight at 130°C in foil-covered glass container.

g. Sodium sulfate, Na_2SO_4, anhydrous, granular: Do not accept in plastic container. If necessary, bake in a muffle furnace to eliminate interferences.

h. Silanized glass wool.

i. Column packing:

1) Solid support—Dimethyl dichlorosilane-treated diatomaceous earth,‡‡#(256) 100 to 120 mesh.

2) Liquid phases—OV-1, OV-210, 1.5% OV-17 (SP 2250) + 1.95% QF-1 (SP 2401), and 6% QF-1 + 4% SE-30, or equivalent.

j. Carrier gas: One of the following is required:

1) *Nitrogen gas,* purified grade, moisture- and oxygen-free.

2) *Argon-methane* (95 + 5%) for use in pulse mode.

k. Pesticide reference standards: Obtain purest standards available (95 to 98%) from gas chromatographic and chemical supply houses.

l. Stock pesticide solutions: Dissolve 100 mg of each pesticide in ethyl acetate and dilute to 100 mL in a volumetric flask; 1.00 mL = 1.00 mg.

m. Intermediate pesticide solutions: Dilute 1.0 mL stock solution to 100 mL with ethyl acetate; 1.0 mL = 10 µg.

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n. Working standard solutions for gas chromatography: Prepare final concentration of standards in hexane solution as required by detector sensitivity and linearity.

4. Procedure

a. Preparation of chromatograph:

1) Packing the column—Use a column constructed of silanized borosilicate glass because other tubing materials may catalyze sample component decomposition. Before packing, rinse and dry column tubing with solvent, e.g., methylene chloride, then methanol. Pack column to a uniform density not so compact as to cause unnecessary back pressure and not so loose as to create voids during use. Do not crush packing. Fill column through a funnel connected by flexible tubing to one end. Plug other end of column with about 1.3 cm silanized glass wool and fill with aid of *gentle* vibration or tapping but do not use an electric vibrator because it tends to fracture packing. Optionally, apply a vacuum to plugged end. Plug open end with silanized glass wool.

2) Conditioning—Proper thermal and pesticide conditioning are essential to eliminate column bleed and to provide acceptable gas chromatographic analysis. The following procedure provides excellent results: Connect packed column to the injection port. *Do not* connect column to detector; however, maintain gas flow through detector by using the purge-gas line, or in dual-column ovens, by connecting an unpacked column to the detector. Adjust carrier-gas flow to about 50 mL/min and slowly (over a 1-h period) raise oven temperature to 230°C. After 24 to 48 h at this temperature the column is ready for pesticide conditioning.

Adjust oven temperature and carrier-gas flow rate to approximate operating levels. Make six consecutive 10-µL injections of a concentrated pesticide mixture through column at about 15-min intervals. Prepare this injection mixture from lindane (γ-BHC), heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin, and *p,p′*-DDT, each compound at a concentration of 200 ng/µL. After pesticide conditioning, connect column to detector and let equilibrate for at least 1 h, preferably overnight. Column is then ready for use.

3) Injection technique

a) Develop an injection technique with constant rhythm and timing. The ‘‘solvent flush’’ technique described below has been used successfully and is recommended to prevent sample blowback or distillation within the syringe needle. Flush syringe with solvent, then draw a small volume of clean solvent into syringe barrel (e.g., 1 µL in a 10-µL syringe). Remove needle from solvent and draw 1 µL of air into barrel. For packed columns, draw 3 to 4 µL of sample extract into barrel. Remove needle from sample extract and draw approximately 1 µL air into barrel. Record volume of sample extract between air pockets. Rapidly insert needle through inlet septum, depress plunger, withdraw syringe. After each injection thoroughly clean syringe by rinsing several times with solvent.

b) Inject standard solutions of such concentration that the injection volume and peak height of the standard are approximately the same as those of the sample.

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b. Treatment of samples:

1) Sample collection—Fill sample bottle to neck. Collect samples in duplicate.

2) Extraction of samples—Shake sample well and accurately measure all the sample in a 1-L graduated cylinder in two measuring operations if necessary (or use a precalibrated sample bottle to avoid transfer operation). Pour sample into a 2-L separatory funnel. Rinse sample bottle and cylinder with 60 mL 15% diethyl ether or methylene chloride in hexane, pour this solvent into separatory funnel, and shake vigorously for 2 min. Let phases separate for at least 10 min.

Drain water phase from separatory funnel into sample bottle and carefully pour organic phase through a 2-cm-OD column containing 8 to 10 cm of Na_2SO_4 into a Kuderna-Danish apparatus fitted with a 10-mL concentrator tube. Pour sample back into separatory funnel.

Rinse sample bottle with 60 mL mixed solvent, use solvent to repeat sample extraction, and pass organic phase through Na_2SO_4. Complete a third extraction with 60 mL of mixed solvent that was used to rinse sample bottle again, and pass organic phase through Na_2SO_4. Wash Na_2SO_4 with several portions of hexane and drain well. Fit Kuderna-Danish apparatus with a three-ball Snyder column and reduce volume to about 7 mL in a hot water bath (90 to 95°C). At this point all methylene chloride present in the initial extracting solvent has been distilled off. Cool, remove concentrator tube from Kuderna-Danish apparatus, rinse ground-glass joint, and dilute to 10 mL with hexane. Make initial gas chromatographic analysis at this dilution.

3) Gas chromatography—Inject 3 to 4 µL of extract solution into a packed column. Always inject the same volume. Inspect resulting chromatogram for peaks corresponding to pesticides of concern and for presence of interferences.

a) If there are presumptive pesticide peaks and no significant interference, rechromatograph the extract solution on an alternate column.

b) Inject standards frequently to insure optimum operating conditions. If necessary, concentrate or dilute (*do not use methylene chloride*) the extract so that peak size of pesticide is very close to that of corresponding peaks in standard. (See dilution factor, ¶ 5*a*).

c) If significant interference is present, separate interfering substances from pesticide materials by using the cleanup procedure described in the following paragraph.

4) Magnesia-silica gel cleanup—Adjust sample extract volume to 10 mL with hexane. Place a charge of activated magnesia-silica gel[§§]#(257) (weight determined by lauric-acid value, see Appendix ¶ 4) in a chromatographic column. After settling gel by tapping column, add about 1.3 cm anhydrous granular Na_2SO_4 to the top. Pre-elute column, after cooling, with 50 to 60 mL petroleum ether. Discard eluate and just before exposing sulfate layer to air, quantitatively transfer sample extract into column by careful decantation and with subsequent petroleum ether washings (5 mL maximum). Adjust elution rate to about 5 mL/min and, separately, collect the eluates in 500-mL Kuderna-Danish flasks equipped with 10-mL receivers.

Make first elution with 200 mL 6% ethyl ether in petroleum ether, and the second with 200 mL 15% ethyl ether in petroleum ether. Make third elution with 200 mL 50% ethyl

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ether-petroleum ether and the fourth with 200 mL 100% ethyl ether. Follow with 50 to 100 mL petroleum ether to insure removal of all ethyl ether from the column. Alternatively, to separate PCBs elute initially with 0% ethyl ether in petroleum ether and proceed as above to yield four fractions.

Concentrate eluates in Kuderna-Danish evaporator in a hot water bath as in ¶ 4*b*2) preceding, dilute to appropriate volume, and analyze by gas chromatography.

Eluate composition—By use of an equivalent quantity of any batch of magnesia-silica gel as determined by its lauric acid value (see Appendix) the pesticides will be separated into the eluates indicated below:

	6% Ethyl Ether Eluate	
Aldrin	Heptachlor	Pentachloro-nitrobenzene
BHC	Heptachlor epoxide	Strobane
Chlordane	Lindane (γ-BHC)	Toxaphene
DDD	Methoxychlor	Trifluralin
DDE	Mirex	PCBs
DDT		

15% Ethyl Ether Eluate	50% Ethyl Ether Eluate
Endosulfan I	Endosulfan II
Endrin	Captan
Dieldrin	
Dichloran	
Phthalate esters	

If present, certain thiophosphate pesticides will occur in each of the above fractions as well as in the 100% ether fraction. For additional information regarding eluate composition and the procedure for determining the lauric acid value, refer to the FDA Pesticide Analytical Manual (see Bibliography). For elution pattern test procedure see Appendix, ¶ 4.

5) Determination of extraction efficiency—Add known amounts (at concentrations similar to those expected in samples) of pesticides in ethyl acetate solution to 1 L water sample and carry through the same procedure as for samples. Dilute an equal amount of intermediate pesticide solution (¶ 3*m* above) to the same final volume. Call peak height from standard ‘‘*a*’’ and peak height from sample to which pesticide was added ‘‘*b*,’’ whereupon the extraction efficiency equals *b/a*. Periodically determine extraction efficiency and a control blank to test the procedure.

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Also analyze one set of duplicates with each series of samples as a quality-control check.

5. Calculation

a. Dilution factor: If a portion of the extract solution was concentrated, the dilution factor, *D*, is a decimal; if it was diluted, the dilution factor exceeds 1.

b. Determine pesticide concentrations by direct comparison to a single standard when the injection volume and response are within 10% of those of the sample pesticide of interest (Table 6630:I). Calculate concentration of pesticide:

$$\mu g/L = \frac{A \times B \times C \times D}{E \times F \times G}$$

where:

A = ng standard pesticide,
B = peak height of sample, mm, or area count,
C = extract volume, µL,
D = dilution factor,
E = peak height of standard, mm, or area count,
F = volume of extract injected, µL, and
G = volume of sample extracted, mL.

Typical chromatograms of representative pesticide mixtures are shown in Figure 6630:1, Figure 6630:2, Figure 6630:3, Figure 6630:4 and Figure 6630:5.

Report results in micrograms per liter without correction for efficiency.

6. Precision and Bias

Ten laboratories in an interlaboratory study selected their own water samples and added four representative pesticides to replicate samples, at two concentrations in acetone. The added pesticides came from a single source. Samples were analyzed with and without magnesia-silica gel cleanup. Precision and recovery data are given in Table 6630:II.

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Appendix—Standardization of Magnesia-Silica Gel*#(258) Column by Weight Adjustment Based on Adsorption of Lauric Acid

A rapid method for determining adsorptive capacity of magnesia-silica gel is based on adsorption of lauric acid from hexane solution. An excess of lauric acid is used and the amount not adsorbed is measured by alkali titration. The weight of lauric acid adsorbed is used to calculate, by simple proportion, equivalent quantities of gel for batches having different adsorptive capacities.

1. Reagents

a. Ethyl alcohol, USP or absolute, neutralized to phenolphthalein.

b. Hexane, distilled from all-glass apparatus.

c. Lauric acid solution: Transfer 10.000 g lauric acid to a 500-mL volumetric flask, dissolve in hexane, and dilute to 500 mL; 1.00 mL = 20 mg.

d. Phenolphthalein indicator: Dissolve 1 g in alcohol and dilute to 100 mL.

e. Sodium hydroxide, 0.05*N:* Dilute 25 mL 1*N* NaOH to 500 mL with distilled water. Standardize as follows: Weigh 100 to 200 mg lauric acid into 125-mL erlenmeyer flask; add 50 mL neutralized ethyl alcohol and 3 drops phenolphthalein indicator; titrate to permanent end point; and calculate milligrams lauric acid per milliliter NaOH (about 10 mg/mL).

2. Procedure

Transfer 2.000 g magnesia-silica gel to a 25-mL glass-stoppered erlenmeyer flask. Cover

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loosely with aluminum foil and heat overnight at 130°C. Stopper, cool to room temperature, add 20.0 mL lauric acid solution (400 mg), stopper, and shake occasionally during 15 min. Let adsorbent settle and pipet 10.0 mL supernatant into a 125-mL erlenmeyer flask. Avoid including any gel. Add 50 mL neutral alcohol and 3 drops phenolphthalein indicator solution; titrate with 0.05*N* NaOH to a permanent end point.

3. Calculation of Lauric Acid Value and Adjustment of Column Weight

Calculate amount of lauric acid adsorbed on gel as follows:

$$\text{Lauric acid value} = \text{mg lauric acid/g gel} = 200 - (\text{mL required for titration} \times \text{mg lauric acid/mL } 0.05N \text{ NaOH}).$$

To obtain an equivalent quantity of any batch of gel, divide 110 by lauric acid value for that batch and multiply by 20 g. Verify proper elution of pesticides by the procedure given below.

4. Test for Proper Elution Pattern and Recovery of Pesticides

Prepare a test mixture containing aldrin, heptachlor epoxide, *p,p′*-DDE, dieldrin, parathion, and malathion. Dieldrin and parathion should elute in the 15% eluate; all but a trace of malathion in the 50% eluate, and the others in the 6% eluate.

6630 C. Liquid-Liquid Extraction Gas Chromatographic Method II

This method[1] is applicable to the determination of organochlorine pesticides and PCBs*†#(259)# in municipal and industrial discharges. When analyzing unfamiliar samples for any or all of these compounds, support the identifications by at least one additional qualitative technique. This method includes analytical conditions for a second, confirmatory gas chromatographic column. Alternatively, analyze by a gas chromatographic/mass spectrometric (GC/MS) method for base/neutrals and acids using the extract produced by this method.

Additional PCB congeners can be determined if standards are included.

1. General Discussion

a. Principle: A measured volume of sample is extracted with methylene chloride. The extract is dried and exchanged to hexane during concentration. If other determinations having essentially the same extraction and concentration steps are to be performed, a single sample extraction is sufficient. The extract is separated by gas chromatography and the compounds are measured with an electron capture detector.[2] See Section 6010C for discussion of gas chromatographic principles.

The method provides procedures for magnesia-silica gel column cleanup and elemental sulfur removal to aid in the elimination of interferences. When cleanup is required, sample concentration levels must be high enough to permit separate treatment of subsamples. Chromatographic conditions appropriate for the simultaneous measurement of combinations of

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compounds may be selected.

b. Interferences: See Section 6410B.1*b*1) for precautions concerning glassware, reagent purity, and matrix interferences.

Phthalate esters may interfere in pesticide analysis with an electron capture detector. These compounds generally appear in the chromatogram as large, late-eluting peaks, especially in the 15 and 50% fractions from magnesia-silica gel. Common flexible plastics contain phthalates that are easily extracted during laboratory operations. Cross-contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Minimize interferences from phthalates by avoiding use of plastics. Exhaustive cleanup of reagents and glassware may be required to eliminate phthalate contamination.[3,4] Phthalate ester interference can be avoided by using a microcoulometric or electrolytic conductivity detector.

c. Detection levels: The MDL is the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.[5] The MDL concentrations listed in Table 6630:III were obtained by using reagent water.[6] Similar results were achieved with representative wastewaters. The MDL actually obtained in a given analysis will vary, depending on instrument sensitivity and matrix effects. This method has been tested for linearity of known-addition recovery from reagent water and is applicable over the concentration range from 4 × MDL to 1000 × MDL with the following exceptions: Chlordane recovery at 4 × MDL was low (60%); toxaphene recovery was linear over the range of 10 × MDL to 1000 × MDL.[6] It is difficult to determine MDLs for mixtures such as these. To calculate the MDLs given, a few of the GC peaks in each mixture were used. Depending on the particular peaks selected, these results may or may not be reproducible in other laboratories.

d. Safety: The toxicity or carcinogenicity of each reagent has not been defined precisely. The following compounds have been classified tentatively as known or suspected, human or mammalian carcinogens: 4,4′-DDT, 4,4′-DDD, the BHCs, and the PCBs. Prepare primary standards of these compounds in a hood and wear a NIOSH/MESA-approved toxic gas respirator when handling high concentrations. Treat and dispose of Hg used for sulfur removal as a hazardous waste.

2. Sampling and Storage

For collection and storage requirements, see Section 6410B.2. If samples will not be extracted within 72 h of collection, adjust pH to the range 5.0 to 9.0 with NaOH or H_2SO_4. Record volume of acid or base used. If aldrin is to be determined, add sodium thiosulfate when residual chlorine is present.

3. Apparatus

Use apparatus specified in Section 6410B.3*a* - Section 6410B.3*e*, Section 6410B.3*g*, and Section 6410B.3*i* - Section 6410B.3*k*.

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In addition:

a. Chromatographic column, 400 mm long × 22 mm ID, with TFE stopcock and coarse frit filter disk.‡#(260)

b. Gas chromatograph:§#(261) An analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, and strip-chart recorder. Preferably use a data system for measuring peak areas.

1) *Column 1*, 1.8 m long × 4 mm ID, glass, packed with 1.5% SP-2250/1.95% SP-2401 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the detection limit and precision and bias data presented herein. For guidelines for the use of alternate column packings see ¶ 5*c*.

Although procedures detailed below refer primarily to packed columns, capillary columns may be used if equivalent results can be demonstrated.

2) *Column 2*, 1.8 m long × 4 mm ID, glass, packed with 3% OV-1 on Supelcoport (100/120 mesh) or equivalent.

3) *Detector*, electron-capture. This detector was used to develop the detection limit and precision and bias data presented herein. For use of alternate detectors see ¶ 5*c*.

4. Reagents

This method requires reagents described in Section 6410B.4*a* – e, and in addition:

a. Acetone, hexane, isooctane, methylene chloride, pesticide quality or equivalent.

b. Ethyl ether, nanograde redistilled in glass if necessary. Demonstrate before use freedom from peroxides by means of test strips.‖#(262) Remove peroxides by procedures provided with the test strips. After cleanup, add 20 mL ethyl alcohol preservative per liter of ether.

c. Magnesia-silica gel,##(263) 60/100 mesh. Purchase activated at 1250°F and store in the dark in glass containers with ground-glass stoppers or foil-lined screw caps. Before use, activate each batch for at least 16 h at 130°C in a foil-covered glass container; let cool.

d. Mercury, triple-distilled.

e. Copper powder, activated.

f. Stock standard solutions: Prepare as directed in Section 6410B.4*g*, using isooctane as the solvent.

g. Calibration standards: See Section 6420B.4 *j*. Dilute with isooctane and use MDL values from Table 6630:III.

h. Quality control (QC) check sample concentrate: Obtain a check sample concentrate**#(264) containing each compound at the following concentrations in acetone: 4,4′-DDD, 10 µg/mL; 4,4′-DDT, 10 µg/mL; endosulfan II, 10 µg/mL; endosulfan sulfate, 10 µg/mL; endrin, 10 µg/mL; any other single-component pesticide, 2 µg/mL. If this method will be used only to analyze for PCBs, chlordane, or toxaphene, the QC check sample concentrate should contain the most representative multicomponent compound at a concentration of 50

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μg/mL in acetone. If such a sample is not available from an external source, prepare using stock standards prepared independently from those used for calibration.

5. Procedure

a. Extraction: Mark water meniscus on side of sample bottle for later determination of volume. Pour entire sample into a 2-L separatory funnel and extract with methylene chloride as directed in Section 6410B.5*a*1), without any pH adjustment or solvent wash.

After extracting and concentrating with a three-ball Snyder column, increase temperature of hot water bath to about 80°C. Momentarily remove Snyder column, add 50 mL hexane and a new boiling chip, and reattach Snyder column. Concentrate extract as before but use hexane to prewet column. Complete concentration in 5 to 10 min.

Remove Snyder column and rinse flask and its lower joint into the concentrator tube with 1 to 2 mL hexane. Preferably use a 5-mL syringe for this operation. Stopper concentrator tube and store refrigerated if further processing will not be done immediately. If extract is to be stored longer than 2 d, transfer to a TFE-sealed screw-cap vial. If extract requires no further cleanup, proceed with gas chromatographic analysis. If further cleanup is required, follow procedure of ¶ b before chromatographic analysis.

Determine original sample volume by refilling sample bottle to mark and transferring liquid to a 1000-mL graduated cylinder. Record sample volume to nearest 5 mL.

b. Cleanup and separation: Use either procedure below or any other appropriate procedure; however, first demonstrate that the requirements of ¶ 7 can be met. The magnesia-silica gel column allows for a select fractionation of compounds and eliminates polar interferences. Elemental sulfur, which interferes with the electron-capture gas chromatography of certain pesticides, can be removed by the technique described below.

1) Magnesia-silica gel column cleanup—Place a weight of magnesia-silica gel (nominally 20 g) predetermined by calibration, ¶ d3), into a chromatographic column. Tap column to settle gel and add 1 to 2 cm anhydrous Na_2SO_4 to the top. Add 60 mL hexane to wet and rinse. Just before exposure of the Na_2SO_4 layer to air, stop elution of hexane by closing stopcock on column. Discard eluate. Adjust sample extract volume to 10 mL with hexane and transfer it from K-D concentrator tube onto column. Rinse tube twice with 1 to 2 mL hexane, adding each rinse to the column. Place a 500-mL K-D flask and c lean concentrator tube under chromatographic column. Drain column into flask until Na_2SO_4 layer is nearly exposed. Elute column with 200 mL 6% ethyl ether in hexane (v/v) (Fraction 1) at a rate of about 5 mL/min. Remove K-D flask and set aside. Elute column again, using 200 mL 15% ethyl ether in hexane (v/v) (Fraction 2), into a second K-D flask. Elute a third time using 200 mL 50% ethyl ether in hexane (v/v) (Fraction 3). The elution patterns for the pesticides and PCBs are shown in Table 6630:IV. Concentrate fractions for 15 to 20 min as in ¶ a, using hexane to prewet the column, and set water bath temperature at about 85°C. After cooling, remove Snyder column and rinse flask and its lower joint into concentrator tube with hexane. Adjust volume of each fraction to 10 mL with

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hexane and analyze by gas chromatography, ¶s c through *e* below.

2) Sulfur interference removal—Elemental sulfur usually will elute entirely in Fraction 1 of the magnesia-silica gel column cleanup. To remove sulfur interference from this fraction or the original extract, pipet 1.00 mL concentrated extract into a clean concentrator tube or TFE-sealed vial. Add 1 to 3 drops of mercury and seal.[7] Mix for 15 to 30 s. If prolonged shaking (2 h) is required, use a reciprocal shaker. Alternatively, use activated copper powder for sulfur removal.[8] Analyze by gas chromatography.

c. Gas chromatography operating conditions: Table 6630:III summarizes the recommended operating conditions for the gas chromatograph and gives retention times and MDLs that can be achieved under these conditions. Examples of separations obtained with Column 1 are shown in Figure 6630:6, Figure 6630:7, Figure 6630:8, Figure 6630:9, Figure 6630:10, Figure 6630:11, Figure 6630:12, Figure 6630:13, Figure 6630:14 and Figure 6630:15. Other packed or capillary (open-tubular) columns,[9] chromatographic conditions, or detectors may be used if the requirements of ¶ 7 are met.

d. Calibration: Calibrate system daily by either external or internal procedure. NOTE: For quantification and identification of mixtures such as PCBs, chlordane, and toxaphene, take extra precautions.[9-11]

1) External standard calibration procedure—Prepare standards as directed in ¶ 4*g* and follow procedure of Section 6420B.5*b*3). Tabulate data and obtain calibration curve or calibration factor as directed in Section 6200B.4*c*3).

2) Internal standard calibration procedure—Prepare standards as directed in ¶ 4*g* and follow procedure of Section 6420B.5*b*3). Tabulate data and calculate response factors as directed in Section 6200B.4*c*2).

Verify working calibration curve, calibration factor, or RF on each working day by measuring one or more calibration standards. If the response for any compound varies from the predicted response by more than ±15%, repeat test using a fresh calibration standard. Alternatively, prepare a new calibration curve for that compound.

3) Magnesia-silica gel standardization—Gel from different batches or sources may vary in adsorptive capacity. To standardize the amount used, use the lauric acid value[12], which measures the adsorption from a hexane solution of lauric acid (mg/g gel). Determine the amount to be used for each column by dividing 110 by this ratio and multiplying the quotient by 20 g.

Before using any cleanup procedure, process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

e. Sample analysis: See Section 6420B.5*b*3). If peak response cannot be measured because of interferences, further cleanup is required.

6. Calculation

Determine concentration of individual compounds using procedures given in Section 6420B.6*a*.

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If it is apparent that two or more PCB (Aroclor) mixtures are present, the Webb and McCall procedure[13] may be used to identify and quantify the Aroclors, depending on the Aroclors present. Other techniques also are available.

For multicomponent mixtures (chlordane, toxaphene, and PCBs) match peak retention times in standards with peaks in sample. Quantitate every identifiable peak unless interference with individual peaks persists after cleanup. Add peak height or peak area of each identified peak in chromatogram. Calculate as total response in sample versus total response in standard. Environmental degradation of these compounds may make identification difficult. This method is suitable only for intact mixtures such as the original Aroclor or pesticide formulation and it is not suitable for the other altered mixtures that are sometimes found in the environment. In these instances the GC peak pattern would not match the standard.

Report results in micrograms per liter without correction for recovery data. Report QC data with the sample results.

7. Quality Control

a. Quality control program: See Section 6200A.5.

b. Initial quality control: To establish the ability to generate data with acceptable precision and bias, proceed as follows: Using a pipet, prepare QC check samples at test concentrations shown in Table 6630:V by adding 1.00 mL of QC check sample concentrate (¶ 4*h*) to each of four 1-L portions of reagent water. Analyze QC check samples according to the method beginning in ¶ 5*a*. Calculate average recovery and standard deviation of the recovery, compare with acceptance criteria and evaluate and correct system performance as directed in Section 6200A.5*a*1) and Section 6200A.5*a*2).

c. Analyses of samples with known additions: See Section 6420B.7*c*. Prepare QC check sample concentrates according to ¶ 4*h* and use Table 6630:III and Table 6630:IV.

d. Quality-control check standard analysis: See Section 6420B.7*d*. Prepare QC check standard according to ¶ 4*h* and use Table 6630:V. If all compounds in Table 6630:V are to be measured in the sample in ¶ c above, it is probable that the analysis of a QC check will be required; therefore, routinely analyze the QC check standard with the known-addition sample.

e. Bias assessment and records: See Section 6410B.7*e*.

8. Precision and Bias

This method was tested by 20 laboratories using reagent water, drinking water, surface water, and industrial wastewaters with known additions at six concentrations over the range 0.5 to 30 µg/L for single-component pesticides and 8.5 to 400 µg/L for multicomponent samples.[14] Single-operator precision, overall precision, and method bias were found to be related directly to the compound concentration and essentially independent of sample matrix. Linear equations describing these relationships are presented in Table 6630:VI.

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6630 D. Liquid-Liquid Extraction Gas Chromatographic/Mass Spectrometric Method

See Section 6410B.

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6640 ACIDIC HERBICIDE COMPOUNDS*#(265)

6640 A. Introduction

1. Sources and Significance

Agricultural chemicals used for weed control can be found as contaminants in various aquatic systems.[1] Although formerly only two chlorinated phenoxy acid herbicides have been among the substances regulated by the U.S. Environmental Protection Agency in drinking water, and thus were routinely measured, several other types of carboxylic acid compounds used for their toxic effects now are under regulatory control.[2]

2. Selection of Method

The micro liquid-liquid extraction gas chromatographic method presented here does not require drying and concentration of large volumes of solvent extract, requires less equipment, and has fewer analytical steps than other methods.[3] A simple procedure for producing diazomethane has been included. The method also provides for simultaneous dual-column confirmation using two electron-capture detectors and dual-channel data acquisition. An optional simplified procedure for alkaline solvent wash of difficult sample matrices allows for additional cleanup of extraneous organics and hydrolysis of the sample.

3. Sampling and Storage

Collect grab samples in quadruplicate. Flush sampling tap until water temperature stabilizes and stagnant lines are cleared. Collect samples in nominal 40-mL vials containing 3.2 mg sodium thiosulfate (if residual chlorine is present) and seal with a TFE-faced septum and screw cap. Fill vials so that no air bubbles pass through the sample. Do not rinse vial before filling and do not overfill. Seal vials with no headspace.

Add diluted hydrochloric acid at the sampling site to adjust to pH ≤ 2; check pH using short-range (0 to 3) pH paper. Samples can be held in ice away from light or refrigerated at 4°C for 14 d; sample extracts can be held at 4°C for 14 d.[3] Verify stability of each constituent in any unknown sample matrix.

4. References

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3. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1989. Method 515.1, Revision 4.0, Determination of Chlorinated Acids by Gas Chromatography with an Electron Capture Detector. Environmental Monitoring Systems Lab., Off. Research & Development, Cincinnati, Ohio.

6640 B. Micro Liquid-Liquid Extraction Gas Chromatographic Method

1. General Discussion

a. Application: This method can be used to measure various acidic organic compounds and their corresponding acid salts although the form of each acid is not differentiated and the calculated amount of each is expressed as free acid. The method can be applied to groundwater, finished drinking water, raw source waters, and other sample matrices provided that it can meet the necessary quality control objectives. This method measures a wide range of carboxylic acid compounds including, but not limited to, acidic agricultural chemicals such as the acid herbicides (halogenated and nonhalogenated), haloacetic acids, and trichlorophenol. Method performance data have been developed for nine herbicide compounds. The U.S. Environmental Protection Agency (USEPA) currently requires monitoring of seven of these compounds, namely, 2,2-dichloropropanoic acid (dalapon), 3,6-dichloro-2-methoxybenzoic acid (dicamba), (2,4-dichlorophenoxy)-acetic acid (2,4-D), pentachlorophenol (pcp), (2,4,5-trichlorophenoxy)-propionic acid (2,4,5-TP/silvex), 2-(1-methylpropyl)-4,6-dinitrophenol (dinoseb), and 4-amino-3,5,6-trichloropicolinic acid (picloram). The other two herbicides are 3-(methylethyl)-1H-2,2,3-benzothiadiazin-4(3H)-one 2,3-dioxide (bentazon), which must be monitored in the State of California, and (2,4,5-trichlorophenoxy)-acetic acid (2,4,5-T), which is used in the EPA performance evaluation sample as a laboratory performance check compound.

b. Principle: A 30-mL sample portion is extracted with 3 mL methyl *tertiary*-butyl ether (MtBE), at an acidic pH to extract the nondissociated acid, and with a salting agent to increase extraction efficiency. Extracted compounds are esterified with diazomethane solution to produce methyl ester derivatives that can be chromatographed. Analyze on a temperature-programmable gas chromatograph (GC) using a fused silica capillary column and an electron capture detector (ECD). Analysis and confirmation are simultaneous; the analytical column and the confirmation column share a common injection port. Alternatively, use separate injections for analysis and confirmation.

c. Interferences: Use HPLC-grade MtBE to minimize interferences from any impurities in the extraction solvent. No further distillation or cleanup of the solvent is necessary.

Clean glassware is essential to eliminate contamination and background interferences. Preferably dedicate all analytical glassware used in this procedure. Clean all glassware, except volumetric flasks and diazomethane generators, as follows: Wash with detergent, rinse three times with tap water, and rinse twice with reagent-grade water. Heat at 180°C for at least 1 h in a

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clean convection oven. Rinse all caps and septa with methanol immediately after use. Heat at 80°C for not more than 1 h in a clean convection oven or air-dry overnight.

Clean millimole diazomethane generators immediately after use. Rinse the inside tube three times with methanol and twice with tap water. Rinse the outer tube three times with methanol, into a waste container containing 11 g silica gel, then twice with tap water. Rinse both inner and outer tubes with reagent water three times. Bake at 180°C in a clean convection oven for at least 1 h until dry.

Immediately after use of volumetric flasks, rinse three to five times with methanol. Invert flasks to drain on a rack and let air-dry in a ventilation hood.

An optional procedure for simplified alkaline solvent wash of difficult matrices is given in ¶ 4*b*, below.

CAUTION: *Verification of identity is essential. Be aware of possible interferents, and always confirm with second column and also with GC/MS whenever possible.*

d. Minimum detectable concentration: The method detection levels (Table 6640:I) were calculated from the analysis of seven laboratory fortified blanks.[1] The actual method working range will be matrix dependent; however, as a general guideline the acid herbicides can be measured over a practical working range from approximately five to ten times the MDL for the lower limit to an upper limit of at least 100 times the MDL. The calibration range can be extended, depending on the compound characteristics and instrument response. Level 1 calibration standard in Table 6640:IV is a suggested starting point for a practical low-level quantitation concentration.

e. Safety: Toxicity and carcinogenicity of all chemicals used in this method have not been fully evaluated; therefore, treat each as a potential health hazard. Minimize exposure and use only in a properly operating ventilation hood. Make material safety data sheets (MSDS) available. Establish a laboratory safety program to meet Occupational Safety and Health Act (OSHA) regulations.

For precautions in handling diazomethane and ether, see Section 6251B.1*d*.

2. Apparatus

Use apparatus listed in Section 6251B.3, with the following exceptions: Only 2- and 10-mL micro volumetric flasks are needed; only 14-mL amber glass vials are needed. NOTE: In using the generator shown in Figure 6251:2, inside tube will contain *N*-methyl-*N*-nitroso-*p*-toluene sulfonamide and methanol, not MNNG.

3. Reagents

All reagents listed in Section 6251B.4*a*, Section 6251B.4*b*, Section 6251B.4*c*, Section 6251B.4*e*1), Section 6251B.4 *f*2), Section 6251B.4*g*, Section 6251B.4*h*, and Section 6251B.4*i*, and in addition:

a. Sodium thiosulfate, $Na_2S_2O_4$, anhydrous.

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b. Standard material: See Table 6640:II for source and physical information.

1) *Individual herbicide stock solutions:* Weigh 0.0500 g of each compound on an analytical balance. Dilute each in MtBE to the 10-mL mark in a screw-top volumetric flask. Transfer each solution to a separate clean 14-mL amber vial and store in a freezer at −11°C. Alternatively purchase solutions as certified standards. The 5000-µg/mL stock standards are usable for 6 months.

2) *Stock herbicide mix:* Dilute an appropriate amount of each stock standard into a 10-mL volumetric flask containing approximately 7 mL methanol. After all stock solutions have been added, dilute to 10-mL mark with methanol. Solution is usable for 3 months. See Table 6640:III for example solution concentrations.

3) *Herbicide known-addition mix:* Add 200 µL stock herbicide mix to a 2-mL volumetric flask containing about 1.5 mL methanol; dilute to 2-mL mark with methanol (see Table 6640:III).

c. Internal standard (IS): 1,2,3-trichloropropane, 99% pure.

1) *Internal standard stock solution:* Weigh 0.1000 g IS into a 10-mL volumetric flask and bring to volume with methanol. This will yield a 10-mg/mL stock solution. Stock standards are usable for 1 year.

2) *MtBE plus internal standard solution*, (*MtBE+IS*), MtBE + 3 µg IS/mL: Mix 240 µL IS stock solution in 800 mL MtBE. Screw 5-mL pump-type dispenser directly on top of the vendor's 1-L bottle and set it to deliver 3 mL. The sample extraction solvent is the same as the internal standard known addition solution.

d. Surrogate (*SUR*): 2,3,5,6-tetrafluorobenzoic acid, 99% pure.

1) *Surrogate stock solution,* 10 mg SUR/mL: Weigh 0.1000 g SUR acid into a 10-mL screw-cap volumetric flask and dilute to mark with methanol. Stock solutions are usable for 1 year.

2) *Surrogate known-addition solution,* 30 µg SUR/mL: Add 30 µL SUR stock solution to a 10-mL volumetric flask and dilute to mark with methanol.

e. Reagent water: Purify the water by several stages of cartridge-type purification to filter, demineralize, and trap organic compounds, then distill with an all-glass distillation system.

f. Calibration standards: Using a 2-mL screw-cap volumetric flask, add 1.7 mL MtBE for each external standard to be prepared. When using the internal standard method add MtBE+IS to the 2-mL mark and concentrate solvent to approximately 1.7 mL with a gentle stream of nitrogen. Inject a measured amount of herbicide known-addition mix solution and 20 µL SUR known-addition solution to each volumetric flask and place in a freezer at −11°C for 11 min before adding 0.25 mL cold diazomethane/MtBE solution to each standard. Let stand at least 23 min to react and dilute to 2 mL with MtBE. The solution should remain yellow after diazomethane/MtBE addition. Make a minimum of three calibration levels, one near the quantitation limit and the others bracketing actual sample concentrations to be measured. Three

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low-level standards are suggested in Table 6640:IV to make a short-range calibration curve.

*g. Trimethylsilyl-diazomethane (TMSD)**#(266) (optional), 2*M* in hexane.

4. Procedure

a. Sample preparation: See Section 6251B.5*a*.

b. Microextraction: Transfer 30 mL from sample container to a 40-mL vial with TFE-faced septum and screw cap.

Add 30 µL SUR known additions solution to each sample, including standards and blanks. Add herbicide known additions mix at this step.

Simplified alkaline solvent wash and extraction—To the 30-mL sample, add 9 g Na_2SO_4 and immediately shake vigorously until salt dissolves, then add enough (approximately 100 µL) 20% NaOH to produce pH ≥12. Hold at room temperature for 1 h; shake periodically. Add 3 mL MtBE and shake vigorously for 1 min, then decant as much of the MtBE as possible with a disposable pasteur pipet (taking a little water is acceptable) and discard. Add 1.5 mL conc H_2SO_4, 3 g $CuSO_4 \cdot 5H_2O$, and 3 g Na_2SO_4, then shake until salt dissolves. Add 3 mL MtBE (for external standard calibration) or MtBE+IS (for internal standard calibration). Immediately cap vial and shake briefly by hand to break up any salt clumps. Lay vial on its side before continuing to next sample. The internal standard can be added later if other quality-control measures are desired.

Place each vial in the wooden holding block. Once all the vials have been inserted, place block immediately on the mechanical shaker. Shake vials at fast speed (approximately 300 cycles/s) for 9 min. (Alternatively, shake manually for about 3 min.)

Remove vials from holder, place upright, and let stand for at least 3 min until the phases separate. If an emulsion forms it can be broken up by briefly sonicating for 10 s in an ultrasonic water bath, then letting the phases separate for at least 3 min.

c. Preparation of diazomethane:[2] Also see ¶ 4*d* below. With the generator specified, use at least 0.37 g *N*-methyl-*N*-nitroso-*p*-toluene sulfonamide (diazald) (this is enough for derivatizing 10 sample extracts) to initiate a good reaction; if more is needed add about 37 mg more diazald per sample to inside tube of generator. Add enough methanol to diazald cover the yellow crystals, then add 0.3 mL excess methanol before securing cap and septum.

Add up to 5.5 mL MtBE to outside tube of generator, allowing 0.25 mL MtBE per sample extract to be derivatized, then add 0.5 mL MtBE in excess, thereby preparing enough diazomethane/MtBE solution to derivatize at least 20 sample extracts. For example, if derivatizing 10 sample extracts, add 3 mL MtBE to outside tube.

Place butyl-o-ring in the glass joint, and twist inside tube firmly on top of the o-ring, then clamp securely with a screw-type pinch clamp.

Place generator and its contents in an ice-water bath containing enough ice to keep solution at 0°C.

Measure at least 0.7 mL 20% NaOH solution to initiate a good reaction, using a 1-mL

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gastight syringe with a 22-gauge needle. If a larger batch of diazomethane is being prepared add about 50 µL extra 20% NaOH solution per sample. Working behind a safety shield, slowly add NaOH through the top generator septum (check that syringe needle is on opposite side of vapor exit hole). Add NaOH at rate of 1 mL/3 s. Swirl briefly to mix. Allow derivatization to continue for 30 min. If more diazomethane is needed, prepare two or more batches and combine just before use.

A strong yellow-colored solution in the outer tube indicates that sufficient diazomethane gas has been generated and has dissolved into the MtBE. If the solution remains clear or only faintly yellow, generate another batch of diazomethane until a strong yellow color is obtained. If poor generation is due to old or decomposed diazald, replace with fresh reagent. Other causes are poor sealing of the diazomethane generator tubes or water present in the MtBE. Alternatively, use the procedure described in Section 6251B.5*c*.

d. Separation and concentration: See Section 6251B.5*d*. After concentration, internal standard can be added if desired. If not analyzing for dalapon, consider using TMSD (¶ 3*g* above) in lieu of diazomethane for derivatization because it is less hazardous and is stable in storage.

If using TMSD, after hydrolysis, extraction, and drying of the sample concentrate the 2-mL extract to 1.5 mL so that 0.5 mL of methanol (20% of the sample) may be added. (TMSD produces the most effective methylation of the acid herbicides in a 20% methanol, 80% MtBE solution.) Add approximately 30 µL of 2*M* TMSD solution to the 2-mL sample extract. Place tube containing extract in a heating block at 50°C and maintain for 1 h. Let extract cool to room temperature. Add 60 µL of 2*M* acetic acid in methanol to react any excess TMSD. Internal standard may be added at this time. Proceed with analysis and measurement according to ¶ 4*f*. Verify all quality control requirements as outlined in ¶ 6.

e. Derivatization: Cool extracts in an explosion-safe freezer at approximately −11°C for 11 min and add diazomethane. Uncap one volumetric flask and add 250 µL cold diazomethane/MtBE solution. Cap immediately with a TFE-lined screw cap; mix gently by inverting once. Repeat for the remaining extracts. A persistent faint yellow color should be present after addition of the diazomethane solution, indicating an excess available for esterification.

If using an autosampler, prepare two 1.8-mL vials for each sample. Label vials and add approximately 0.01 g silica gel to quench excess diazomethane.

Let stand in a laboratory fume hood for 23 min to reach room temperature. Dilute to 2-mL mark with MtBE and invert flask to mix. Keep each extract in contact with diazomethane for approximately the same amount of time before quenching. If using an autosampler, transfer each extract evenly between two labeled autosampler vials containing silica gel using a 23-cm (9-in.) disposable pasteur pipet. Store MtBE extract in the freezer at −11°C until analyzed.

f. Gas chromatography: Typical operating conditions are as follows:

Injector temperature 225°C; split valve opened at 0.75 min; split flow 70 mL/min; injection

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volume 4.0 µL; slow injection rate 1.3 µL/s.

Temperature program: 40°C for 7 min, rising 20°C/min to 160°C, rising 2°C/min to 195°C, and rising 10°C/min to 225°C, holding 6 min at 225°C.

Detector temperature: 300°C; range setting 10.

Carrier gas (helium) flow: 33 cm/s at 37°C.

Makeup gas (nitrogen) flow: 20 mL/min.

At beginning of each analytical run, inject two MtBE solvent blanks to condition GC and to verify that no solvent interferences are present. If available, analyze an extra calibration standard to ensure retention times and response of GC system. Always inject the same amount of sample (3.7 µL), and use sample dilution if necessary to obtain response in the calibration range. See Figure 6640:1 and Figure 6640:2 for sample chromatograms of a single injection onto both the analytical and confirmation columns (gas chromatograph is set up to inject simultaneously onto two columns). See Table 6640:V for retention times.

g. Calibration: Use at least three levels of calibration standards to define the quantitation range of analysis (see Table 6640:IV). Use standards that bracket the expected range of sample concentrations and do not exceed the linear range of the detector. Prepare standards as described in ¶ 3 *f*. Analyze each standard by injecting the same volume as each sample extract, under the same analytical conditions. Either internal or external standard calibration can be used for quantitation.

Internal standard calibration requires use of a response factor calculated from the peak area and internal standard area for each constituent in the standard. Use the following equation to calculate the response factor:

$$RF = \frac{(A_H)(C_I)}{(A_I)(C_H)}$$

where:

RF = response factor,
A_H = area of herbicide peak,
A_I = area of internal standard peak,
C_I = concentration of internal standard, µg/L, and
C_H = concentration of herbicide, µg/L.

External standard calibration uses a calibration factor calculated as the ratio of herbicide area response to the concentration in the standard.

5. Calculation

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When using internal standard calibration, calculate compound concentration in a sample with the following equation:

$$C = \frac{(A_H)(IS)}{(A_I)(RF)(V)}$$

where:

C = concentration of compound, µg/L,
A_H = area of herbicide peak,
IS = internal standard amount, µg,
A_I = area of internal standard peak,
RF = response factor from calibration standards in ¶ 4*g*, and
V = volume of water extracted, L.

The external standard calibration procedure uses the area response of sample compound divided by the calibration factor in ¶ 4*g* to calculate concentration in a sample.

If possible, use a computer with chromatographic software to calculate the best-fit second-order calibration equation. Calculate sample concentration with the best-fit equation.

6. Quality Control

a. Quality control program:[3] Follow minimum quality control (QC) requirements to monitor and maintain method performance. The basic QC program incorporates method blanks, an initial demonstration of laboratory capability, determination of acceptable surrogate response in each sample and blank, sample matrix with known additions, laboratory fortified blanks, and QC samples. Additional quality control measures may be used.

b. Method blanks: Analyze a 30-mL portion of reagent water with the same procedure as an actual sample. Each time a set of samples is extracted or reagents are changed, process a laboratory method blank. If the blank produces any peak within the retention time window of a constituent that would prevent its determination, eliminate source of contamination and reanalyze affected samples.

c. Initial demonstration of capability: To demonstrate an adequate level of performance, determine bias as percent recovery before analyzing unknown samples.

Use a known concentration near the levels given in Table 6640:VI for each target constituent. Using a syringe, add the appropriate amount of herbicide mix to each of a minimum of four 30-mL portions of reagent water and analyze.

Calculate average percent recovery (R) and standard deviation of the percent recovery (S_r) for all four samples.

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Compare results obtained to the single-laboratory recovery and precision data found in Table 6640:VI. Compare precision data at similar concentrations (i.e., within two times the addition level shown in Table 6640:VI). The mean recovery values for each compound must be within the interval R ± 30% using the values in Table 6640:VI. Results that meet these criteria are acceptable. For results that fail these criteria, correct the problem and repeat the procedure using another four samples, until satisfactory performance has been demonstrated.

d. Method modifications: The analyst may modify GC columns, GC conditions, detectors, continuous extraction techniques, concentration techniques (i.e., evaporation techniques), internal standard and surrogate compounds. Each time such modifications to the method are made, repeat the procedure in ¶ c above.

e. Evaluating surrogate recovery: The surrogate is added directly into all water samples before acidification and extraction. If the surrogate area is low or absent, there has been a derivatization problem (e.g., water in extract) or extraction problem (e.g., water insufficiently acidified).

An extract is acceptable if the surrogate recovery does not exceed 100 ± 30% compared to the average daily calibration standard. If surrogate recovery is unacceptable, check the following and correct: possible errors in calculations or procedure, degradation of standard solution, contamination sources, and instrument performance. If the cause of the problem cannot be determined, reanalyze the extract. If surrogate recovery is acceptable, report only data for the reanalyzed extract. If sample extract continues to fail, report all data for that sample as suspect.

If a blank extract reanalysis fails the recovery criterion, identify the problem and correct it before continuing.

f. Internal standard assessment: If the IS procedure is used, monitor the IS area of each sample during each analysis day. The IS area should not deviate from the average daily calibration standards by more than 30%; if it does, optimize instrument performance and inject a second portion of that extract.

If the reinjection is acceptable report the results. If the IS area is still over 30%, re-extract sample if more is available and the holding period has not expired; otherwise report results as suspect.

If consecutive samples fail the IS response immediately analyze a calibration standard. If the standard response factor is within 20% of the predicted value, begin with optimizing instrument performance as described above for each sample failing the IS response. If the standard is above the 20% predicted value, recalibrate as specified in ¶ 4*g*.

g. Laboratory fortified blank: Analyze at least one laboratory fortified blank (LFB) with every 20 samples or at least one per sample set (all samples extracted within a 24-h period). The concentration of each compound in the LFB should be within two times the Table 6640:VI addition amounts. Calculate accuracy as percent recovery. If recovery of any compound falls outside the control limits, identify the source of the problem and correct it before continuing analyses.

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Initially, assess the LFB with the results from the initial demonstration of capability in (¶ c above) and Table 6640:VII. When a minimum of 20 LFB have been analyzed, use these data to develop control charts with the mean percent recovery (X), and the standard deviation (s) of the percent recovery. The upper control limit is $X + 3s$ and the lower control limit is $X - 3s$. After 5 to 10 new measurements, calculate new control limits using only the most recent 20 to 30 data points.

Periodically determine and document the laboratory's detection limit capabilities.[3]

At least quarterly, analyze a QC sample from an outside source.

h. Matrix additions: Add each target constituent into a minimum of 10% of the routine samples at a concentration near or greater than the background concentration. Ensure that the addition plus background concentration does not exceed the calibration range. Eventually, make additions to all routine samples.

Analyze one sample portion to determine the background concentration (b) of each constituent. Add herbicide mix to a second sample portion and analyze to determine the total matrix concentration (t) for each constituent. Calculate percent recovery of each constituent (R_i) as $100 (t - b)/A$, where A is known concentration of the additions solution.

Compare percent recovery (R_i) for each constituent with established QC acceptance criteria. QC criteria for samples containing no background are established by initially analyzing four reagent water samples with known additions and calculating average percent recovery (R) and standard deviation of the percent recovery (s_r).

If the untreated sample has no background concentrations and the additions are the same as an LFB then the appropriate control limits are those established for the LFB.

If sample does contain background concentrations, determine mean concentration and standard deviation (s_b) for that background level by performing replicate analyses. With replicate analyses measure total matrix addition concentration (background + added amount) and calculate mean concentration (C_t) and standard deviation (s_t). Calculate mean percent recovery (R_t) of the total matrix addition concentration as follows:

$$R_t = \frac{100(C_t)}{b + A}$$

The control limits are defined as $R_t \pm 3s_s$, where:

$$s_s = (s_t^2 + s_b^2)^{1/2}/A$$

When recovery of any compound falls outside the control limits and the LFB is in control, the recovery problem is matrix-related; label the result for that compound ''suspect/matrix.''

i. Laboratory performance check sample: Monitor instrument performance daily by

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analyzing the laboratory performance check (LPC) sample. The LPC sample contains compounds that indicate instrument sensitivity and column performance. LPC components and performance criteria are given in Table 6640:VIII. If performance criteria cannot be met, reevaluate the instrument system. If the laboratory MDLs differ from those in Table 6640:I, adjust the concentrations of the LPC components to be compatible with the laboratory MDLs.

j. Calibration standards: When using internal-standard calibration, if *RF* values are less than or equal to 20% RSD, either the average *RF* or a calibration curve can be used for quantitation. Verify the calibration or *RF* each working shift by measuring one or more calibration standards. If the response is more than ±20%, repeat the test using a fresh calibration standard. If the repeat test fails, make a new calibration curve with freshly prepared standards. Single-point calibration can be used if the sample extract response is within 20% of the standard.

When using external standard calibration and the calibration factor is less than or equal to 20% RSD, either the average calibration factor or a calibration curve can be used for quantitation. Verify the calibration curve or calibration factor on each working day by measuring at least two calibration standards, one at the beginning and one at the end of the analysis day. Use two standards with different concentrations to verify the calibration curve. If the response is more than ±20%, analyze a fresh calibration standard. If the test fails, make a new calibration curve with freshly prepared standards. Single-point calibration can be used if the sample extract response is within 20% of the standard.

At least quarterly, check the calibration standards against reference material obtained from an independent source; the results must be within the routine calibration criteria.

7. Precision and Bias

Single-laboratory method detection levels[3] and extracted recovery data from reagent water are presented in Table 6640:I. Laboratory data showing single-laboratory accuracy and precision data at two different concentration levels are presented in Table 6640:VI and Table 6640:VII. Matrix addition recoveries are presented in Table 6640:IX for three different sample matrix types. The interlaboratory comparison data for an Environmental Protection Agency performance evaluation sample with acceptance limits calculated from other participating laboratories[3] are shown in Table 6640:X. Additional quality-control data for a typical laboratory fortified blank are shown in Table 6640:XI.

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Acids by Gas Chromatography with an Electron Capture Detector. Method 515.1, revision 4.0, Environmental Monitoring Systems Lab., Off. Research & Development, Cincinnati, Ohio.

6651 GLYPHOSATE HERBICIDE*#(267)

6651 A. Introduction

1. Sources and Significance

Glyphosate[*N*-(phosphonomethyl)glycine] is a broad-spectrum, nonselective, postemergence herbicide that has found widespread agricultural and domestic use. It is sold as a terrestrial and aquatic herbicide under the trade names Roundup® and Rodeo®. Because of low mammalian toxicity (LD50 = 1568 mg/kg rats; oral) there is less concern about water and food contamination than with other pesticides, but the nonselectivity of the herbicide can make nontarget phytotoxicity a problem. Glyphosate's (GLYPH) major metabolite is aminomethylphosphonic acid (AMPA). Contamination of water can occur through runoff and spray drift.

2. Selection of Method

Several methods for determination of GLYPH and AMPA in environmental samples have been developed; those using liquid chromatography are the most precise and accurate. GLYPH and AMPA are not good chromophores or fluorophores and their electrochemical or conductometric detection have not been demonstrated. Sensitive and selective detection has been achieved with the post-column reaction/fluorometric method.[1-3] The absence of a sensitive liquid chromatography technique for confirmation necessitates the use of two different stationary phases.

The liquid chromatographic method presented in 6651B is accurate and precise[3] and includes confirmation by using two columns. Gas chromatography/mass spectrometry confirmation[4] has been used when structural confirmation is required, but the method has not been tested on residues in natural waters.

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6651 B. Liquid Chromatographic Post-Column Fluorescence Method

1. General Discussion

a. Principle: GLYPH and AMPA are separated by anion- or cation-exchange chromatography and measured by post-column fluorescence derivatization. The post-column reactions consist of oxidation of GLYPH (a secondary amine) to glycine (a primary amine) by hypochlorite solution. Glycine then reacts with an *o*-phthalaldehyde (OPA) and mercaptoethanol (MERC) mixed reagent to form an isoindole that is measured fluorometrically. AMPA (a primary amine) reacts directly with the OPA/MERC reagent and is detected (with decreased sensitivity) in the presence of hypochlorite.

b. Interferences: No matrix interferences in water are known. GLYPH degrades in chlorinated water. GLYPH also is known to sorb strongly to minerals and glass surfaces.

c. Minimum detectable concentration: Minimum detection using this method is 25 µg/L for GLYPH and AMPA by direct injection and 0.5 µg/L with the concentration step.

2. Sampling and Storage

Collect a 500-mL representative sample in a polypropylene container. Treatment of sample to remove residual chlorine will prevent glyphosate losses during storage. Destroy chlorine by adding 100 mg/L sodium thiosulfate. Store samples at 4°C away from light and analyze within 2 weeks.

3. Apparatus

a. High-performance liquid chromatograph (HPLC): An analytical system with pump, injector, detectors, and compatible strip chart recorder. Preferably use a data system for measuring peak areas and retention times. Use system capable of injecting 200-µL portions. See

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Figure 6651:1.

1) *Analytical columns:* Use either a cation exchange resin[1] or an anion-exchange resin[2] packed in a 4.6-mm × 25- to 30-cm column.*#(268) Heat columns to between 50 and 60°C to obtain maximum efficiency.

2) *Post-column reactor:* Use system consisting of two separate pumps capable of delivering reaction solutions at 0.1 to 0.5 mL/min and able to withstand pressures of up to 2000 kPa. Include two woven 1-mL TFE reaction coils[3] (0.5-mm ID × 1.4-mm OD × 5 m) with one maintained at 40°C. Turnkey post-column reactor systems are available commercially.

3) *Fluorescence detector:* Use filter or grating fluorimeter capable of sensitively and selectively measuring the isoindole derivative, with excitation wavelength 230 nm (deuterium), 340 nm (quartz halogen or xenon), and emission wavelength 420 to 455 nm.

4. Reagents

a. Reagent water: See Section 1080.

b. Phosphoric acid, H_3PO_4, conc.

c. Sulfuric acid, H_2SO_4, conc. Prepare anion-exchange mobile phase by adding 26 mL conc H_3PO_4 and 2.7 mL conc H_2SO_4 to 5 L water.

d. Hydrochloric acid, HCl, conc.

e. Methanol, CH_3OH, tested on HPLC and verified to give no impurity peaks.

f. Potassium dihydrogen phosphate, KH_2PO_4. Prepare cation-exchange mobile phase by dissolving 0.68 g KH_2PO_4 in 1 L methanol-water (4:96). Adjust to pH 2.1 with conc H_3PO_4. Filter through a 0.22- or 0.45-m membrane filter and degas.

g. Disodium ethylenediamine tetraacetate dihydrate, EDTA sodium salt solutions: Prepare a 0.001*M* solution by dissolving 0.37 g EDTA dihydrate in 1.0 L water and filter through a 0.22- or 0.45-µm filter. Prepare a 0.03*M* solution by dissolving 11.2 g EDTA dihydrate in 1.0 L water and filtering through a 0.33- or 0.45-µm filter.

h. Sodium chloride, NaCl.

i. Sodium hydroxide, NaOH.

j. Calcium hypochlorite, $Ca(OCl)_2$, 70.9% available chlorine.

k. Oxidation reagent: Dissolve 0.5 g$Ca(OCl)_2$ in 500 mL water with rapid magnetic stirring for 45 min. In a 1.0-L volumetric flask, dissolve 1.74 gK H_2PO_4, 11.6 g NaCl, 0.4 g NaOH, and 10 mL stock $Ca(OCl)_2$ solution. Dilute to volume, mix well, and filter through a 0.22 or 0.45 µm filter.

l. o-phthalaldehyde, $C_6H_4(CHO)_2$, OPA.

m. 2-mercaptoethanol, $HSCH_2CH_2OH$, MERC.

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n. Boric acid powder, H_3BO_3.

o. Potassium hydroxide, KOH.

p. Fluorogenic labeling reagent: Dissolve 100 g boric acid and 72 g KOH in about 700 mL water in a 1.0-L flask. This takes 1 to 2 h. Add 0.8 g OPA dissolved in 5 mL methanol. Add 2.0 mL MERC. Mix well.

q. Glyphosate analytical standard, N-(phosphonomethyl) glycine, 99% or greater.

r. Aminomethylphosphonic acid analytical standard, 99% or greater.

s. Glyphosate and AMPA fortification standards: Prepare a solution containing both 0.1 mg GLYPH/mL and 0.1 mg AMPA/mL in water. Make working solutions of 10.0 and 1.0 µg/mL by serial dilution of this stock solution. Store in a refrigerator, in a polypropylene bottle. Prepare fresh monthly.

t. Glyphosate and AMPA HPLC calibration standards: Prepare a solution containing both 0.1 mg GLYPH/mL and 0.1 mg AMPA/mL in 0.001*M* disodium EDTA solution. Make working solutions of 1.00, 0.50, 0.10, 0.05, and 0.025 µg/mL by serial dilution. Store in a refrigerator, in polypropylene bottle. Prepare fresh monthly.

5. Procedure

a. HPLC operation: Equilibrate column at 50°C with mobile-phase flow rate of 0.5 mL/min (see Figure 6651:1). Use an approximate flow rate of 0.5 mL/min for the oxidant and 0.3 mL/min for the OPA-MERC reagent but adjust rates to obtain maximum response. While GLYPH reaches a maximum response at some flow rate of oxidative solution, the AMPA response decreases with any addition of this reagent. Thus, an oxidative reagent flow rate that gives an equal response for both GLYPH and AMPA simultaneously is considered optimum for simultaneous measurements. Reagent flow rates will differ for different mobile phases. Establish a standard curve by injecting calibration standards.

Approximate retention times for GLYPH and AMPA are 13.5 and 10.0 min on the anion-exchange column and 21.5 and 30.0 min on the cation-exchange column.

b. Sample preparation: No concentration is needed for samples containing 25 µg/L or more. Fortify a 9.9-mL sample portion with 0.1 mL 0.10*M* EDTA, filter through a 0.22- or 0.45-µm filter, and inject 200 µL. Fortify portions of the same samples with known amounts of GLYPH and AMPA to determine recovery. Perform duplicate injections on at least 10% of the samples with measurable GLYPH and AMPA or 10% of fortified samples to determine precision.

To concentrate samples containing less than the detection limit, transfer 250 mL to a 500-mL round-bottom flask. If suspended matter is present, filter sample through coarse filter paper.†#(269) For samples used to assess recovery, make known additions. Add 5 mL conc HCl to flask and 5 mL to sample remaining in original container. Concentrate on a rotary evaporator by slowly increasing temperature from 20 to 60°C. Before the first portion is completely evaporated, add remaining sample and two 5-mL rinses of the sample bottle. Evaporate to dryness, and if necessary, remove final traces of water with a stream of dry nitrogen. Dissolve

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residue in 2.9 mL of mobile phase (adjust pH to 2 if necessary) and 0.10 mL 0.03*M* EDTA solution. Filter through 0.45-µm filter to a test tube and inject into the HPLC system.

6. Calculations

Determine concentration of GLYPH and AMPA by regression analysis of the standard curve. Multiply results for samples that were concentrated by the concentration factor, 166.7 (500 mL original sample/3.0 mL), to determine the original water concentration. Report results in milligrams per liter. Report percent recovery but do not correct for recovery.

7. Quality Control

See Section 6020.

8. Precision and Bias

For six single-operator analyses, the relative standard deviation of duplicate samples (with additions from 0.5 to 5000 µg/L) ranged from 12.1 to 20% with an average of 14.9% for glyphosate. The relative standard deviation for identical AMPA concentrations ranged from 6.5 to 28.8% with an average of 14.5%.[4]

For six single-operator analyses, recoveries of glyphosate (with additions from 0.5 to 5000 µg/L) ranged from 94.6 to 120% with an average of 104.0%. Recoveries of AMPA ranged from 86.0 to 100% with an average of 93.1%.[4]

9. References

1. COWELL, J.E., J.L. KUNSTMAN, P.J. NORD, J.R. STEINMETZ & G.R. WILSON. 1986. Validation of an analytical residue method for analysis of glyphosate and metabolite: An interlaboratory study. *J. Agric. Food Chem.* 34:955.
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3. SELVAKA, C.M., K.S. JAIO & I.S. KRULL. 1987. Construction and comparison of open tubular reactors for postcolumn reaction detection in liquid chromatography. *Anal. Chem.* 59:2221.
4. OPPENHUIZEN, M.E. & J.E. COWELL. 1991. Liquid chromatographic determination of glyphosate and (aminomethyl)phosphonic acid in environmental water. *J. Assoc. Offic. Anal. Chem.* 74:317.

Figures

Figure 6040:1. Schematic of closed-loop stripping apparatus (not to scale). Source:

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KRASNER, S.W., C.J. HWANG & M.J. MCGUIRE. 1983. A standard method for quantification of earthy-musty odorants in water. *Water Sci. Technol.* 15(6/7):127.

Figure 6040:2. One-liter ''tall form'' stripping bottle. Source: KRASNER, S.W., C.J. HWANG & M.J. MCGUIRE. 1983. A standard method for quantification of earthy-musty odorants in water. *Water Sci. Technol.* 15(6/7):127. (To obtain dimensions in inches, divide dimensions in millimeters by 25.4.)

Figure 6040:3. Gas heater.

Figure 6040:4. Extraction of filter. Source: KRASNER, S.W., C.J. HWANG & M.J. MCGUIRE. 1981. Development of a closed-loop stripping technique for the analysis of taste- and odor-causing substances in drinking water. *In* L.H. Keith, ed. Advances in the Identification and Analysis of Organic Pollutants in Water, Vol. 2. Ann Arbor Science Publishers, Ann Arbor, Mich.

Figure 6040:5. Flow rate through 1.5-mg carbon filter. Air flow rate with no filter is 0.86 L/min.

Figure 6040:6. Effect of filter resistance, measured as flow, on recovery of earthy-musty odorants and C_1–C_{10} internal standard. Reprinted with permission from: HWANG, C.J., S.W. KRASNER, M.J. MCGUIRE, M.S. MOYLAN & M.S. DALE. 1984. Determination of subnanogram per liter levels of earthy-musty odorants in water by the salted closed-loop stripping method. *Environ. Sci. Technol.* 18:535. Copyright 1984, American Chemical Society.

Figure 6040:7. Mass spectrum of 2-methylisoborneol.

Figure 6040:8. Mass spectrum of geosmin.

Figure 6200:1. Purging device.

Figure 6200:2. Trap packings and construction to include desorb capability.

Figure 6200:3. GC/MS chromatogram. Column: J&W DB-624, 30 m, 0.25 mm ID, 1.4 µm film; temperature program: 35°C for 4 min; 4°C/min; 50°C, 0 min; 10°C/min; 175°C, 4 min.:

Figure 6200:4. PID chromatogram. GC conditions: Column: Supelco VOCOL, 60 m, 0.75 mm ID, 1.5 µm film; temperature program: 0° C, 8 min: 4° C/min; 185° C, 1.5 min.

Figure 6200:5. ELCD chromatogram. GC conditions: Column: Supelco VOCOL, 60 m, 0.75 mm ID, 1.5 µm film; temperature program: 0° C, 8 min; 4° C/ min; 185° C, 1.5 min.

Figure 6211:1. Combustible gas indicator circuit and flow diagram.

Figure 6231:1. Extract of reagent water with 0.114 µg/L added EDB and DBCP. Column: fused silica capillary; liquid phase: Durawax-DX3; film thickness: 0.25 µm; column dimensions: 30 m × 0.317 mm ID.

Figure 6232:1. Chromatogram for THMs and chlorinated organic solvents. Concentration was 50 µg/L for each compound; primary column DB-5. Retention times are shown in

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parentheses.

Figure 6251:1. Haloacetic acids separation from other commonly produced disinfection by-products on a DB-1701 column. Chromatogram produced by mixing the methyl esters of each haloacetic acid with other disinfection by-products in MtBE (disinfection by-products tentatively identified).

Figure 6251:2. Easy-to-use diazomethane generator apparatus for preparing small amounts of diazomethane in methyl *tertiary*-butyl ether (MtBE).

Figure 6251:3. Easy-to-use alternative diazomethane generator for preparing small amounts of diazomethane in MtBE.

Figure 6251:4. Chromatogram produced by reagent water with known additions: 30 μg/L extracted standard on DB-1701 column.

Figure 6252:1. Results for analytical column.

Figure 6252:2. Results for confirmation column.

Figure 6410:1. Gas chromatogram of base/neutral fraction. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

Figure 6410:2. Gas chromatogram of acid fraction. Column: 1% SP-1240DA on Supelcoport; program: 70°C for 2 min, 8°C/min to 200°C; detector: mass spectrometer.

Figure 6410:3. Gas chromatogram of pesticide fraction. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

Figure 6410:4. Gas chromatogram of chlordane. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

Figure 6410:5. Gas chromatogram of toxaphene. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

Figure 6410:6. Gas chromatogram of PCB-1016. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

Figure 6410:7. Gas chromatogram of PCB-1221. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

Figure 6410:8. Gas chromatogram of PCB-1232. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

Figure 6410:9. Gas chromatogram of PCB-1242. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

Figure 6410:10. Gas chromatogram of PCB-1248. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

Figure 6410:11. Gas chromatogram of PCB-1254. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

Figure 6410:12. Gas chromatogram of PCB-1260. Column: 3% SP-2250 on Supelcoport; program: 50°C for 4 min, 8°C/min to 270°C; detector: mass spectrometer.

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Figure 6410:13. Tailing factor calculation. Example calculation:
Peak height = DE = 100 mm
10% peak height = BD = 10 mm
Peak width at 10% peak height = AC = 23 mm
AB = 11 mm BC = 12 mm
Tailing factor = 12/11 = 1.1

Figure 6420:1. Gas chromatogram of phenols. Column: 1% SP-1240DA on Supelcoport; program: 80°C at injection, immediate 8°C/ min to 150°C; detector: flame ionization.

Figure 6420:2. Gas chromatogram of PFB derivatives of phenols. Column: 5% OV-17 on Chromosorb W-AW-DMCS; temperature: 200°C; detector: electron capture.

Figure 6440:1. Liquid chromatogram of polynuclear aromatic hydrocarbons. Column: HC—ODS SIL-X; mobile phase: 40% to 100% acetonitrile in water; detector: ultraviolet at 254 nm.

Figure 6440:2. Liquid chromatogram of polynuclear aromatic hydrocarbons. Column: HC—ODS SIL-X; mobile phase: 40% to 100% acetonitrile in water; detector: fluorescence.

Figure 6440:3. Gas chromatogram of polynuclear aromatic hydrocarbons. Column: 3% OV-17 on Chromosorb W-AW-DCMS; program: 100°C for 4 min, 8°C/min to 280°C; detector: flame ionization.

Figure 6610:1. Schematic of post-column reaction HPLC system.

Figure 6610:2. Schematic HPLC-PCD chromatogram of carbamate mix indicating relative response, separations, and retention.

Compound	Concentration μg/L
Aldicarb sulfoxide	8.6
Aldicarb sulfone	7.6
Oxamyl	12.6
Methomyl	4.7
3-Hydroxycarbofuran	13.8
Aldicarb	6.5
Baygon	11.6
Carbofuran	11.3
Carbaryl	6.1
Methiocarb	20.4

Figure 6630:1. Results of gas chromatographic procedure for organochlorine pesticides.

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Column packing: 1.5% OV-17 + 1.95% QF-1; carrier gas: argon/methane at 60 mL /min; column temperature: 200°C; detector: electron capture in pulse mode.

Figure 6630:2. Results of gas chromatographic procedure for organochlorine pesticides. Column packing: 5% OV-210; carrier gas: argon/methane at 70 mL /min; column temperature: 180°C; detector: electron capture.

Figure 6630:3. Chromatogram of pesticide mixture. Column packing: 6% QF-1 + 4% SE-30; carrier gas; argon/methane at 60 mL / min; column temperature: 200°C; detector: electron capture.

Figure 6630:4. Chromatogram of pesticide mixture. Column packing: 3% OV-1; carrier gas: argon/methane at 70 mL /min; column temperature: 180°C; detector: electron capture.

Figure 6630:5. Chromatogram of pesticide mixture. Column DB-5, 30 m long, multilevel program temperature, electron-capture detector.

Figure 6630:6. Gas chromatogram of pesticides. Column: 1.5% SP-2250/ 1.95% SP-2401 on Supelcoport; temperature: 200°C; detector: electron capture.

Figure 6630:7. Gas chromatogram of chlordane. Column: 1.5% SP-2250/ 1.95% SP-2401 on Supelcoport; temperature: 200°C; detector: electron capture.

Figure 6630:8. Gas chromatogram of toxaphene. Column: 1.5% SP-2250/ 1.95% SP-2401 on Supelcoport; temperature: 200°C; detector: electron capture.

Figure 6630:9. Gas chromatogram of PCB-1016. Column: 1.5% SP-2250/ 1.95% SP-2401 on Supelcoport; temperature: 160°C; detector: electron capture.

Figure 6630:10. Gas chromatogram of PCB-1221. Column: 1.5% SP-2250/ 1.95% SP-2401 on Supelcoport; temperature: 160°C; detector: electron capture.

Figure 6630:11. Gas chromatogram of PCB-1232. Column: 1.5% SP-2250/ 1.95% SP-2401 on Supelcoport; temperature: 160°C; detector: electron capture.

Figure 6630:12. Gas chromatogram of PCB-1242. Column: 1.5% SP-2250/ 1.95% SP-2401 on Supelcoport; temperature: 160°C; detector: electron capture.

Figure 6630:13. Gas chromatogram of PCB-1248. Column: 1.5% SP-2250/ 1.95% SP-2401 on Supelcoport; temperature: 160°C; detector: electron capture.

Figure 6630:14. Gas chromatogram of PCB-1254. Column: 1.5% SP-2550/ 1.95% SP-2401 on Supelcoport; temperature: 200°C; detector: electron capture.

Figure 6630:15. Gas chromatogram of PCB-1260. Column: 1.5% SP-2250/ 1.95% SP-2401 on Supelcoport; temperature: 200°C; detector: electron capture.

Figure 6640:1. Herbicide standard chromatogram on analytical capillary column. Chromatograms in Figure 6640:1 and Figure 6640:2 generated simultaneously with a single injection using specified analytical conditions; all acidic compounds analyzed as methyl esters.

Figure 6640:2. Herbicide standard chromatogram on confirmatory capillary column.

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Chromatograms in Figure 6640:1 and Figure 6640:2 generated simultaneously with a single injection using specified analytical conditions; all acidic compounds analyzed as methyl esters.

Figure 6651:1. Schematic of post-column reaction HPLC system.

Tables

TABLE 6010:I. ANALYSIS METHODS FOR SPECIFIC ORGANIC COMPOUNDS*

Compound	Analysis Methods (section number)
Acenaphthene	6040B; 6410B; 6440B
Acenaphthylene	6410B; 6440B
Acetaldehyde	6252B
Aldicarb	6610B
Aldicarb sulfone	6610B
Aldicarb sulfoxide	6610B
Aldrin	6410B; 6630B,C
Aminomethylphosphonic acid (AMPA)	6651B
Anthracene	6040B; 6410B; 6440B
Baygon	6610B
Bentazon	6640B
Benzaldehyde	6252B
Benzene	6200B,C
Benzidine	6410B
Benzo(a)anthracene	6040B; 6410B; 6440B
Benzo(a)pyrene	6410B, 6440B
Benzo(b)fluoranthene	6410B, 6440B
Benzo(ghi)perylene	6410B, 6440B
Benzo(k)fluoranthene	6410B, 6440B
BHC(s)	6410B; 6630C
Bromobenzene	6040B; 6200B,C
Bromochloroacetic acid	6251B
Bromochloromethane	6200B,C
Bromodichloromethane	6040B; 6200B,C; 6232B
Bromoform	6040B; 6200B,C; 6232B

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Compound	Analysis Methods (section number)
Bromomethane	6200B,C
Bromophenoxybenzene	6040B
Bromophenyl phenyl ether	6410B
Butyl benzyl phthalate	6410B
Butylbenzene(s)	6200B,C
Captan	6630B
Carbaryl	6610B
Carbofuran	6610B
Carbon tetrachloride	6200B,C
Chlordane	6410B; 6630B,C
Chlorobenzene	6040B; 6200B,C
Chloroethane	6200B,C
Chloroethoxy methane	6040B; 6410B
Chloroethyl ether	6040B; 6410B
Chloroethylvinyl ether	6200B,C
Chloroform	6200B, C; 6232B
Chloroisopropyl ether	6410B
Chloromethane	6200B,C
Chloromethyl benzene	6040B
Chloromethylphenol	6410B; 6420B
Chloronaphthalene(s)	6040B; 6410B
Chlorophenol(s)	6410B; 6420B
Chlorophenoxy benzene	6040B
Chlorophenyl phenyl ether	6410B
Chlorotoluene	6200B,C
Chrysene	6040B; 6410B; 6440B
2,4-D (dichlorophenoxyacetic acid)	6640B
Dalapon	6640B
DDD	6410B; 6630B,C
DDE	6410B; 6630B,C
DDT	6410B; 6630B,C
Dibenzo(a,h) anthracene	6410B; 6440B

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Compound	Analysis Methods (section number)
Dibromoacetic acid (DBAA)	6251B
Dibromochloromethane	6040B; 6200B,C; 6232B
Dibromochloropropane	6200B,C; 6231B
Dibromoethane	6040B; 6200B,C; 6231B
Dibromomethane	6200B,C
Dibutyl phthalate	6410B
Dicamba	6640B
Dichloran	6630B
Dichloroacetic acid (DCAA)	6251B
Dichlorobenzene(s)	6040B; 6200B,C; 6410B
Dichlorobenzidine	6410B
Dichlorodifluoromethane	6200B,C
Dichloroethane	6200B,C
Dichloroethene(s)	6200B,C
Dichlorophenol(s)	6410B; 6420B
Dichloropropane(s)	6200B,C
Dichloropropene	6040B; 6200B,C
Dieldrin	6410B; 6630B,C
Diethyl phthalate	6040B; 6410B
Dimethyl phthalate	6410B
Dimethylphenol(s)	6410B; 6420B
Dinitrophenol(s)	6410B; 6420B
Dinitrotoluene(s)	6410B
Dinoseb	6640B
Di-*n*-octyl phthalate	6410B
Diphenyl hydrazine	6040B
Endosulfan	6410B; 6630B,C
Endosulfan sulfate	6410B; 6630C
Endrin	6410B; 6630B,C
Endrin aldehyde	6410B; 6630C
Ethenyl benzene (styrene)	6040B
Ethylbenzene	6040B; 6200B, C

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Compound	Analysis Methods (section number)
Ethylhexyl phthalate	6410B
Fluoranthene	6040B; 6410B; 6440B
Fluorene	6040B; 6410B; 6440B
Formaldehyde	6252B
Geosmin	6040B
Glyoxal	6252B
Glyphosate	6651B
Heptachlor	6410B; 6630B,C
Heptachlor epoxide	6410B; 6630B,C
Heptaldehyde	6252B
Hexachlorobenzene	6040B; 6410B
Hexachlorobutadiene	6040B; 6200B, C; 6410B
Hexachlorocyclopentadiene	6410B
Hexachloroethane	6040B; 6410B
3-Hydroxycarbofuran	6610B
Indeno(1,2,3-cd)pyrene	6410B; 6440B
Isobutylmethoxy pyrazine	6040B
Isophorone	6410B
Isopropylbenzene	6200B, C
Isopropyl methoxy pyrazine	6040B
Isopropyltoluene	6200B, C
Lindane (γ-BHC)	6630B
Malathion	6630B
Methane	6211
Methiocarb	6610B
Methoxychlor	6630B
Methyldinitrophenol(s)	6410B; 6420B
Methylene chloride	6200B, C
Methyl glyoxal	6252B
Methylisoborneol	6040B
Methyl parathion	6630B
Methomyl	6610B

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Compound	Analysis Methods (section number)
Mirex	6630B
Monobromoacetic acid (MBAA)	6251B
Monochloroacetic acid (MCAA)	6251B
Naphthalene	6040B; 6200B, C; 6410B; 6440B
Nitrobenzene	6410B
Nitrophenol(s)	6410B; 6420B
Nitrosodi-*n*-propylamine	6410B
Nitrosodimethylamine	6410B
Nitrosodiphenylamine	6410B
Oxamyl	6610B
Parathion	6630B
PCB-1016, 1221, 1232, 1242, 1248, 1254, 1260	6410B, 6630C
Pentachloronitrobenzene	6630B
Pentachlorophenol	6410B; 6420B; 6640B
Phenanthrene	6040B; 6410B; 6440B
Phenol	6410B; 6420B
Phenylbenzamine	6040B
Picloram	6640B
Propylbenzene	6040B; 6200B,C
Pyrene	6040B; 6410B; 6440B
Silvex (trichlorophenoxy propionic acid)	6640B
Strobane	6630B
Styrene (ethenyl benzene)	6200B,C
2,4,5-T (trichlorophenoxy acetic acid)	6640B
2,4,5-TP	6640B
Tetrachloroethane(s)	6040B; 6200B,C
Tetrachloroethene	6040B; 6200B,C
Toluene	6200B,C
Toxaphene	6410B; 6630B,C
Trichloroanisole	6040B
Trichloroacetic acid (TCAA)	6251B
Trichlorobenzene(s)	6040B; 6200B,C; 6410B

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Compound	Analysis Methods (section number)
Trichloroethane(s)	6040B; 6200B,C
Trichloroethene	6040B; 6200B,C
Trichlorofluoromethane	6200B,C
Trichlorophenol	6251B; 6410B; 6420B
Trichloropropane	6200B,C
Trifluralin	6630B
Trimethylbenzene(s)	6200B,C
Vinyl chloride	6200B,C
Xylene(s)	6040B; 6200B,C

* Compounds are listed under the names by which they are most commonly known and called in specific methods.

TABLE 6010:II. RECOMMENDED PRESERVATION FOR VOLATILES

Constituents	Chlorinated Matrix	Non-Chlorinated Matrix
Halocarbons	HCl + reducing agent	HCl
Aromatics	HCl + reducing agent	HCl
THMs	Reducing agent (HCl optional)*	None required
EDB/DBCP	None required	None required

* See 6232B.2.

TABLE 6040:I. METHOD DETECTION LEVELS FOR EARTHY-MUSTY SMELLING COMPOUNDS BY CLSA-GC/MS

	Detection Level *ng/L**	
	Unsalted Method[1]	Salting-Out Technique[3]
Geosmin	2	0.8
2-Methylisoborneol	2	0.8
2-Isopropyl-3-methoxy pyrazine	2	0.8

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	Detection Level *ng/L**	
	Unsalted Method[1]	Salting-Out Technique[3]
2-Isobutyl-3-methoxy pyrazine	2	0.8
2,3,6-Trichloroanisole	5	0.8

* Stripping at 25°C. Selective ion monitoring.

TABLE 6040:II. METHOD DETECTION LEVELS FOR SELECTED ORGANIC COMPOUNDS BY CLSA-GC/MS[8]

Compound	Detection Level *ng/L**	Compound	Detection Level *ng/L*†
1,1,1-Trichloroethane	2.0	1,3,5-Trichlorobenzene	0.1
Trichloroethene	100	1,2,4-Trichlorobenzene	10
Dichlorobromomethane	5.0	1,2,3-Trichlorobenzene	2.0
1,3-Dichloropropene	2.0	*bis*(2-Chloro-ethoxy)methane	10
1,1,2-Trichloroethane	2.0	Methylisoborneol (MIB)	0.5
Chlorodibromomethane	1.0	Geosmin	0.2
1,2-Dibromoethane	2.0	Naphthalene	100
Tetrachloroethene	100	1,1,2,3,4,4-Hexachloro-1,3-butadiene	2.0
Chlorobenzene	10	1-Chloronaphthalene	0.5
Ethylbenzene	50	2-Chloronaphthalene	0.5
m,p-Xylene	100	Acenaphthene	0.5
Bromoform	1.0	Fluorene	2.0
Ethylbenzene	5.0	Diethylphthalate	100
o-Xylene	50	1-Chloro-4-phenoxybenzene	0.5
1,1,2,2-Tetrachloroethane	50	*N*-Phenylbenzamine	20
Bromobenzene	0.5	1,2-Diphenylhydrazine (as azobenzene)	1.0
Propylbenzene	0.5	1-Bromo-4-phenoxybenzene	0.5
1-Chloro-3-methylbenzene	0.5	Hexachlorobenzene	1.0
bis (2-Chloroethyl)ether	1.0	Phenanthrene	10
o-Dichlorobenzene	0.1	Anthracene	50
m-Dichlorobenzene	10	Fluoranthene	20

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Compound	Detection Level *ng/L**	Compound	Detection Level *ng/L†*
p-Dichlorobenzene	10	Pyrene	20
Hexachloroethane	20	Chrysene	50
N-Nitrosodi-*n*-propylamine	5.0	Benzo(a)anthracene	50

* Elevated stripping temperature and salting-out both utilized.
† Instrument detection limit based on a 2:1 signal:noise ratio (where a background interference existed, the target compound was required to be at least twice background.)

TABLE 6040:III. COMPARISON OF MONITORING AND QUANTITATION IONS FOR CHLORODECANE AND DEUTERATED MIB AND GEOSMIN INTERNAL STANDARDS[11]

Compound	Ions (*m/z*) Used for Quantitation		Ions (*m/z*) Monitored	
	A	B	A	B
1-Chlorodecane	91	—	43, 91, 93	—
MIB	95	168	93, 107, 135	168
Geosmin	112	112, 182	111, 112, 125	112, 182
MIB-d_3	—	171	—	171
Geosmin-d_3	—	115, 185	—	115, 185

A = 1-Chlorodecane as internal standard.
B = MIB-d_3 and geosmin-d_3 as internal standard.

TABLE 6040:IV. TYPICAL OPERATING CONDITIONS FOR GC/MS ANALYSIS OF CLSA EXTRACTS

Variable	Description or Value
Column	30- or 60-m × 0.25-mm-ID DB-1 or DB-5 fused silica capillary column*
Column temperature program	35°C, 1 min; 35 to 130°C 4°C/min; 130 to 220°C 10°C/min; 1 min
Carrier gas	Helium
Carrier gas flow rate	1 mL/min
Sample size	About 1.5 µL (splitless injection)

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Variable	Description or Value
Injector temperature	Cold, on-column
Transfer line temperature	280°C
Ionizer temperature	280°C
Source pressure	About 7×10^4 Pa
Electron energy	70 eV
Mass range scanned	41 to 453 amu
Scan time	1 s

* JW Scientific, Inc., or equivalent.

TABLE 6040:V. GC/MS DATA FOR THREE INTERNAL STANDARDS AND TWO EARTHY-MUSTY SMELLING COMPOUNDS

Compound	Retention Time* *min*	Quantification Mass *amu*	Characteristic Ions (with relative intensities)
1-Chlorooctane	30.8	91	43 (100), 91 (86), 93 (27)
2-Methylisoborneol	36.4	95, 107†	95 (100), 107 (26), 135 (9)
1-Chlorodecane	39.8	91	43 (100), 91 (87), 93 (28)
Geosmin	45.1	112	112 (100), 111 (28), 125 (18)
1-Chlorododecane	47.2	91	43 (100), 91 (61), 93 (19), 85 (12)

* See Table 6040:IV for GC conditions. Data accumulated using 30-m DB-5 capillary column.
† Quantify using two different masses and obtain an average value.

TABLE 6040:VI. SINGLE-LABORATORY BIAS FOR SELECTED ORGANIC COMPOUNDS CAUSING TASTE AND ODOR

Stripping Technique Compound	Dose Level *ng/L*	Number of Samples*	Mean Recovery† %	Standard Deviation %
Unsalted method‡				
2-Isobutyl-3-methoxy pyrazine	4–20	23	89	21
2-Isobutyl-3-methoxy pyrazine	4–20	22	101	28
2-Methylisoborneol	4–120	30	101	21
2,3,6-Trichloroanisole	4–20	22	88	27
Geosmin	4–120	28	109	20

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Stripping Technique Compound	Dose Level *ng/L*	Number of Samples*	Mean Recovery† %	Standard Deviation %
Salting-out method‡				
2-Isobutyl-3-methoxy pyrazine	4–20	44	120	24
2-Isobutyl-3-methoxy pyrazine	4–20	48	106	18
2-Methylisoborneol	4–20	48	106	15
2,3,6-Trichloroanisole	4–20	45	99	22
Geosmin	4–20	48	105	15

* Finished and natural surface waters.
† Standard-adjusted recovery.
‡ Stripping at 25°C.
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TABLE 6040:VII. PRECISION DATA FOR SELECTED ORGANIC COMPOUNDS CAUSING TASTE AND ODOR*

Compound	Dose Level *ng/L*	Multiple Laboratories† Mean *ng/L*	Standard Deviation *ng/L*	Coefficient of Variation %	Me *ng*
Sample A§					
2-Isopropyl-3-methoxy pyrazine	5.9	5.6	1.6	28	6.
2-Isobutyl-3-methoxy pyrazine	3.0	3.0	0.7	24	3.
2-Methylisoborneol	4.3	4.8	1.0	20	5.
2,3,6-Trichloroanisole	8.7	7.3	3.1	43	9.
Geosmin	2.9	3.3	0.9	27	3.
Sample B§					
2-Isopropyl-3-methoxy pyrazine	25	22	8.0	36	2
2-Isobutyl-3-methoxy pyrazine	15	14	4.2	30	1
2-Methylisoborneol	20	18	6.2	34	2
2,3,6-Trichloroanisole	35	32	9.7	30	3
Geosmin	16	16	5.9	37	2

* Stripping at 25°C, unsalted method.
† Five analysts at three laboratories.
‡ Three analysts at one laboratory.
§ Organic-free water dosed with taste and odor compounds.

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TABLE 6040:VIII. RECOVERY AND PRECISION DATA FOR SELECTED PRIORITY POLLUTANTS*

Compound	Amount *ng*	Mean Recovered Amount† *ng*	Range	Recovery Efficiency %	RSD
Thiophene	25	9	7–11	35	16
Dibromochloromethane	29	17	13–21	57	13
Styrene	22	17	16–20	80	7
Isopropylbenzene	24	26	24–29	107	8
2-Chlorotoluene	26	23	22–27	90	8
bis(2-Chloroethyl)ether	24	3	3–4	12	11
α-Methylstyrene	22	19	18–22	90	6
1,4-Dichlorobenzene	20	18	16–21	93	8
2-Ethyl-1,3-dimethylbenzene	24	22	21–25	92	6
4-Chloro-*o*-xylene	26	22	20–26	85	9
1,1-Dimethylindan	22	24	22–26	110	7
p-Methylphenol	27	ND‡			
Tetrahydronaphthalene	23	23	20–26	99	10
1,2,4-Trichlorobenzene	23	19	18–21	83	7
Hexachloro-1,3-butadiene	27	31	28–34	114	9
2-Methylbiphenyl	24	25	22–27	101	8
1,6-Dimethylnaphthalene	24	10	8–11	39	12
2-Isopropylnaphthalene	23	21	19–22	91	6
Pentachlorobenzene	26	12	11–14	48	11
Hexachlorobenzene	20	6	5–7	31	13
2,2′,4,4′6,6′-Hexachlorobipheny	27	28	26–32	104	7
2,2′,4,5,5′-Pentachlorobiphenyl	28	23	18–28	82	14

* Stripping at 40°C, unsalted method.
† Based on six purging analyses using single ion quantification.
‡ ND = not detected.
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TABLE 6200:I. COMPOUNDS DETERMINABLE BY GAS CHROMATOGRAPHIC METHODS FOR PURGEABLE ORGANIC COMPOUNDS

Analyte	Chemical Abstract Services Registry Number	Analyte	Chemica Ser Registry
Benzene	71-43-2	2,2-Dichloropropane	59
Bromobenzene*	108-86-1	1,1-Dichloropropene*	56
Bromochloromethane	74-97-5	*cis*-1,3-Dichloropropene*	1006
Bromodichloromethane	75-27-4	*trans*-1,3-Dichloropropene*	1006
Bromoform	75-25-2	Ethylbenzene*	10
Bromomethane	74-83-9	Hexachlorobutadiene*	8
n-Butylbenzene*	104-51-8	Isopropylbenzene*	9
sec-Butylbenzene*	135-98-8	*p*-Isopropyltoluene*	9
tert-Butylbenzene*	98-06-6	Methyl *t*-butyl ether*	163
Carbon tetrachloride	56-23-5	Methylene chloride	7
Chlorobenzene*	108-90-7	Naphthalene*	9
Chloroethane	75-00-3	*n*-Propylbenzene*	10
Chloroform	67-66-3	Styrene*	10
Chloromethane	74-87-3	1,1,1,2-Tetrachloroethane	63
2-Chlorotoluene*	95-49-8	1,1,2,2-Tetrachloroethane	7
4-Chlorotoluene*	106-43-4	Tetrachloroethene*	12
Dibromochloromethane	124-48-1	Toluene*	10
1,2-Dibromo-3-chloropropane	96-12-8	1,2,3-Trichlorobenzene*	8
1,2-Dibromoethane	106-93-4	1,2,4-Trichlorobenzene*	12
Dibromomethane	74-95-3	1,1,1-Trichloroethane	7
1,2-Dichlorobenzene*	95-50-1	1,1,2-Trichloroethane	7
1,3-Dichlorobenzene*	541-73-1	Trichloroethene*	7
1,4-Dichlorobenzene*	106-46-7	Trichlorofluoromethane	7
Dichlorodifluoromethane	75-71-8	1,2,3-Trichloropropane	9
1,1-Dichloroethane	75-34-3	1,2,4-Trimethylbenzene*	9
1,2-Dichloroethane	107-06-2	1,3,5-Trimethylbenzene*	10
1,1-Dichloroethene*	75-35-4	Vinyl chloride*	7
cis-1,2-Dichloroethene*	156-59-4	*o*-Xylene*	9
trans-1,2-Dichloroethene*	156-60-5	*m*-Xylene*	10
1,2-Dichloropropane	78-87-5	*p*-Xylene*	10

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Analyte	Chemical Abstract Services Registry Number	Analyte	Chemica Ser Registry
1,3-Dichloropropane	142-28-9		

* Compound can be determined using Method 6200C with PID only.

TABLE 6200:II. BFB KEY M/Z ABUNDANCE CRITERIA

Mass	*m/z* Abundance Criteria
50	15 to 40% of mass 95
75	30 to 60% of mass 95
95	Base peak, 100% relative abundance
96	5 to 9% of mass 95
173	<2% of mass 174
174	>50% of mass 95
175	5 to 9% of mass 174
176	95 to 101% of mass 174
177	5 to 9% of mass 176

TABLE 6200:III. PRIMARY QUANTITATION ION, RETENTION TIMES AND METHOD DETECTION LEVELS

Analyte	Retention Time *min*	MDL μg/*L*	Primary *m/z*
Dichlorodifluoromethane	1.49	0.190	85
Chloromethane	1.71	0.150	50
Vinyl chloride	1.79	0.120	62
Bromomethane	2.16	0.220	94
Chloroethane	2.28	0.230	64
Trichlorofluoromethane	2.57	0.059	101
1,1-Dichloroethene	3.22	0.130	96
1,1,2-Trichloro-1,2,2trifluoroethane	3.25	0.065	101
Methylene chloride	3.96	0.099	49

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Analyte	Retention Time *min*	MDL µg/*L*	Primary *m*/*z*
trans-1,2-Dichloroethene	4.40	0.200	96
Methyl *t*-butyl ether	4.45	0.450	73
1,1-Dichloroethane	5.14	0.047	63
cis-1,2-Dichloroethene	6.30	0.130	96
2,2-Dichloropropane	6.24	0.041	77
Bromochloromethane	6.77	0.032	128
Chloroform	7.00	0.126	83
1,1,1-Trichloroethane	7.24	0.043	97
1,1-Dichloropropene	7.67	0.040	75
Carbon tetrachloride	7.65	0.042	117
Benzene	8.07	0.036	78
1,2-Dichloroethane	8.14	0.055	62
Trichlorethene	9.44	0.045	95
1,2-Dichloropropane	9.85	0.053	63
Dibromomethane	10.07	0.035	93
Bromodichloromethane	10.47	0.112	83
cis-1,3-Dichloropropene	11.29	0.048	75
Toluene	11.81	0.047	91
trans-1,3-Dichloropropene	12.27	0.051	75
1,1,2-Trichloroethane	12.56	0.043	83
1,3-Dichloropropane	12.83	0.090	76
Tetrachloroethene	12.77	0.047	166
Dibromochloromethane	13.24	0.133	129
1,2-Dibromoethane	13.35	0.133	107
Chlorobenzene	14.21	0.052	112
1,1,1,2-Tetrachloroethane	14.37	0.048	131
Ethylbenzene	14.42	0.032	91
m,p-Xylene	14.63	0.038	91
o-Xylene	15.27	0.038	91
Styrene	15.30	0.031	104
Bromoform	15.60	0.131	173

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Analyte	Retention Time *min*	MDL µg/*L*	Primary *m/z*
Isopropylbenzene	15.90	0.074	105
Bromobenzene	16.34	0.140	156
1,1,2,2-Tetrachloroethane	16.41	0.066	83
1,2,3-Trichloropropane	16.44	0.072	75
n-Propylbenzene	16.57	0.260	91
2-Chlorotoluene	16.68	0.042	126
4-Chlorotoluene	16.86	0.040	126
1,3,5-Trimethylbenzene	16.88	0.035	105
tert-Butylbenzene	17.38	0.100	119
sec-Butylbenzene	17.46	0.025	105
1,2,4-Trimethylbenzene	17.74	0.046	105
4-Isopropyltoluene	17.99	0.037	119
1,3-Dichlorobenzene	17.89	0.045	146
1,4-Dichlorobenzene	18.04	0.033	146
1,2-Dichlorobenzene	18.64	0.031	146
n-Butylbenzene	18.65	0.028	91
Hexachlorobutadiene	21.69	0.033	225
1,2,4-Trichlorobenzene	21.34	0.043	180
Naphthalene	21.80	0.049	128
1,2,3-Trichlorobenzene	22.32	0.047	180

GC conditions: Column-JW DB-624, 30 m, 0.25 mm ID, 1.4 µm film; Temperature program-35°C, 4 min; 4°C/min; 50°C, 0 min; 10°C/min; 175°C, 4 min.

TABLE 6200:IV. SINGLE-LABORATORY BIAS AND PRECISION DATA IN REAGENT WATER*

Analyte	Recovery %	Standard Deviation	Relative Standard Deviation %
Benzene	107	0.046	9
Bromobenzene	111	0.034	6
Bromochloromethane	88	0.052	12
Bromodichloromethane	104	0.036	7

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Analyte	Recovery %	Standard Deviation	Relative Standard Deviation %
Bromoform	107	0.042	8
Bromomethane	89	0.049	11
n-Butylbenzene	115	0.048	8
sec-Butylbenzene	113	0.043	8
tert-Butylbenzene	116	0.057	10
Carbon tetrachloride	119	0.048	8
Chlorobenzene	108	0.033	6
Chloroethane	115	0.073	13
Chloroform	108	0.043	8
Chloromethane	74	0.036	10
2-Chlorotoluene	111	0.045	8
4-Chlorotoluene	112	0.049	9
Dibromochloromethane	108	0.042	8
1,2-Dibromoethane	102	0.042	8
Dibromomethane	132	0.113	17
1,2-Dichlorobenzene	106	0.043	8
1,3-Dichlorobenzene	108	0.052	10
1,4-Dichlorobenzene	106	0.045	8
Dichlorodifluoromethane	80	0.058	15
1,1-Dichloroethane	109	0.049	9
1,2-Dichloroethane	102	0.031	6
1,1-Dichloroethene	99	0.059	12
cis-1,2-Dichloroethene	103	0.062	12
trans-1,2-Dichloroethene	113	0.045	8
1,2-Dichloropropane	129	0.064	10
1,3-Dichloropropane	107	0.046	9
2,2-Dichloropropane	106	0.049	9
1,1-Dichloropropene	110	0.044	8
cis-1,3-Dichloropropene	99	0.044	9
trans-1,3-Dichloropropene	101	0.038	7
Ethylbenzene	109	0.049	9

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Analyte	Recovery %	Standard Deviation	Relative Standard Deviation %
Hexachlorobutadiene	112	0.053	9
Isopropylbenzene	112	0.044	8
4-Isopropyltoluene	117	0.046	8
Methylene chloride	85	0.050	12
Methyl *t*-butyl ether	81	0.017	11
Naphthalene	121	0.068	11
n-Propylbenzene	107	0.048	9
Styrene	101	0.039	8
1,1,1,2-Tetrachloroethane	113	0.037	7
1,1,2,2-Tetrachloroethane	104	0.053	10
Tetrachloroethene	106	0.046	9
Toluene	106	0.045	8
1,2,3-Trichlorobenzene	118	0.054	9
1,2,4-Trichlorobenzene	109	0.049	9
1,1,1-Trichloroethane	106	0.040	8
1,1,2-Trichloroethane	97	0.041	9
Trichloroethene	105	0.041	8
Trichlorofluoromethane	105	0.045	9
1,2,3-Trichloropropane	104	0.034	6
1,1,2-Trichloro1,2,2-trifluoroethane	113	0.042	7
1,2,4-Trimethylbenzene	116	0.044	8
1,3,5-Trimethylbenzene	110	0.051	9
Vinyl chloride	85	0.037	9
m,p-Xylene	110	0.057	10
o-Xylene	106	0.044	8

* For all analytes, seven samples, each of 0.5 μg/L concentration, were analyzed.

TABLE 6200:V. RETENTION TIMES AND METHOD DETECTION LEVELS

	Method Detection Level

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* For all analytes, seven samples, each of 0.5 μg/L concentration, were analyzed.

TABLE 6200:V. RETENTION TIMES AND METHOD DETECTION LEVELS

		Method Detection Level	
Analyte	Retention Time *min*	Electrolytic Conductivity Detector μg/*L*	Photo-ionization Detector μg/*L*
Dichlorodifluoromethane	6.22	0.037	—
Chloromethane	7.09	0.041	—
Vinyl chloride	7.68	0.025	0.088
Bromomethane	9.45	0.103	—
Chloroethane	9.76	0.025	—
Trichlorofluoromethane	11.04	0.042	—
1,1-Dichloroethene	13.59	0.018	0.035
1,1,2-Trichloro-1,2,2-trifluoroethane	13.07	0.047	—
Methlyene chloride	15.83	0.068	—
Methyl *t*-butyl ether	16.49	—	0.411
trans-1,2-Dichloroethene	16.78	0.015	0.015
1,1-Dichloroethane	18.49	0.015	—
2,2-Dichloropropane	20.27	0.220	—
cis-1,2-Dichloroethene	20.54	0.012	0.032
Chloroform	21.04	0.017	—
Bromochloromethane	21.53	0.025	—
1,1,1-Trichloroethane	22.14	0.014	—
1,1-Dichloropropene	22.57	0.019	0.008
Carbon tetrachloride	22.80	0.022	—
Benzene	23.38	—	0.017
1,2-Dichloroethane	23.62	0.074	—
Trichloroethene	25.30	0.012	0.014
1,2-Dichloropropane	25.92	0.021	—
Bromodichloromethane	26.63	0.040	—
cis-1,3-Dichloropropene	28.38	0.067	0.041
Dibromomethane	28.40	0.057	—

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Analyte	Retention Time *min*	Method Detection Level	
		Electrolytic Conductivity Detector µg/*L*	Photo-ionization Detector µg/*L*
Toluene	29.16	—	0.023
trans-1,3-Dichloropropene	30.00	0.029	0.046
1,1,2-Trichloroethane	30.39	0.042	—
Tetrachloroethene	31.04	0.013	0.014
1,3-Dichloropropane	31.18	0.020	—
Dibromochloromethane	31.86	0.039	—
1,2-Dibromoethane	32.36	0.070	—
Chlorobenzene	33.67	0.029	0.027
Ethylbenzene	33.89	—	0.028
1,1,1,2-Tetrachloroethane	33.91	0.020	—
m,p-Xylene	34.08	—	0.021
o-Xylene	35.44	—	0.024
Styrene	35.67	—	0.027
Isopropylbenzene	36.64	—	0.018
Bromoform	36.72	0.023	—
1,1,2,2-Tetrachloroethane	37.43	0.034	—
1,2,3-Trichloropropane	37.88	0.048	—
n-Propylbenzene	37.94	—	0.023
Bromobenzene	37.98	0.026	0.026
1,3,5-Trimethylbenzene	38.44	—	0.019
2-Chlorotoluene	38.48	0.017	0.017
4-Chlorotoluene	38.63	0.026	0.028
1,2,4-Trimethylbenzene	39.61	—	0.030
tert-Butylbenzene	39.76	—	0.018
sec-Butylbenzene	40.34	—	0.018
4-Isopropyltoluene	40.80	—	0.019
1,3-Dichlorobenzene	41.01	0.017	0.028
1,4-Dichlorobenzene	41.40	0.059	0.061
n-Butylbenzene	42.21	—	0.028
1,2-Dichlorobenzene	42.62	0.023	0.031

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Analyte	Retention Time *min*	Method Detection Level: Electrolytic Conductivity Detector µg/*L*	Photo-ionization Detector µg/*L*
1,2,4-Trichlorobenzene	48.21	0.019	0.028
Hexachlorobutadiene	48.75	0.026	0.019
Naphthalene	49.05	—	0.043
1,2,3-Trichlorobenzene	49.92	0.018	0.032

GC conditions: Column-Supelco VOCOL, 60 m, 0.75 mm ID, 1.5 µm film;Temperature program-0°C, 8 min; 4°C/min; 185°C, 1.5 min.

TABLE 6200:VI. SINGLE-LABORATORY BIAS AND PRECISION DATA IN REAGENT WATER*

Analyte	Photoionization Detector: Recovery %	Standard Deviation	Relative Standard Deviation %	Electrolytic Cc: Recovery %	S D
Benzene	70	0.006	2	—	
Bromobenzene	—	—	—	89	
Bromochloromethane	—	—	—	83	
Bromodichloromethane	—	—	—	135	
Bromoform	—	—	—	81	
Bromomethane	—	—	—	73	
n-Butylbenzene	63	0.009	3	—	
sec-Butylbenzene	65	0.009	3	—	
tert-Butylbenzene	72	0.006	2	—	
Carbon tetrachloride	—	—	—	79	
Chlorobenzene	70	0.009	2	97	
Chloroethane	—	—	—	64	
Chloroform	—	—	—	83	
Chloromethane	—	—	—	96	
2-Chlorotoluene	—	—	—	91	
4-Chlorotoluene	73	0.009	2	81	
Dibromochloromethane	—	—	—	88	

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Analyte	Photoionization Detector Recovery %	Photoionization Detector Standard Deviation	Photoionization Detector Relative Standard Deviation %	Electrolytic Cc Recovery %	S D
1,2-Dibromoethane	—	—	—	139	
Dibromomethane	—	—	—	79	
1,2-Dichlorobenzene	67	0.010	3	93	
1,3-Dichlorobenzene	70	0.009	3	95	
1,4-Dichlorobenzene	70	0.019	6	91	
Dichlorodifluoromethane	—	—	—	71	
1,1-Dichloroethane	—	—	—	82	
1,2-Dichloroethane	—	—	—	78	
1,1-Dichloroethene	61	0.011	4	81	
cis-1,2-Dichloroethene	61	0.010	3	76	
trans-1,2-Dichloroethene	79	0.005	1	77	
1,2-Dichloropropane	—	—	—	85	
1,3-Dichloropropane	—	—	—	148	
2,2-Dichloropropane	—	—	—	74	
1,1-Dichloropropene	54	0.003	1	74	
cis-1,3-Dichloropropene	57	0.013	5	78	
trans-1,3-Dichloropropene	63	0.015	5	78	
Ethylbenzene	70	0.009	3	—	
Hexachlorobutadiene	55	0.006	2	76	
Isopropylbenzene	67	0.006	2	—	
4-Isopropyltoluene	65	0.006	2	—	
Methylene chloride	—	—	—	83	
Methyl *t*-butyl ether†	75	0.130	3	—	
Naphthalene	73	0.014	4	—	
n-Propylbenzene	70	0.007	2	—	
Styrene	70	0.009	3	—	
1,1,1,2-Tetrachloroethane	—	—	—	83	
1,1,2,2-Tetrachloroethane	—	—	—	88	
Tetrachloroethene	54	0.005	2	79	
Toluene	69	0.007	2	—	

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Analyte	Photoionization Detector			Electrolytic Cc	
	Recovery %	Standard Deviation	Relative Standard Deviation %	Recovery %	S D
1,2,3-Trichlorobenzene	72	0.010	3	84	
1,2,4-Trichlorobenzene	70	0.009	3	94	
1,1,1-Trichloroethane	—	—	—	79	
1,1,2-Trichloroethane	—	—	—	118	
Trichloroethene	57	0.004	2	80	
Trichlorofluoromethane	—	—	—	70	
1,2,3-Trichloropropane	—	—	—	87	
1,1,2-Trichloro-1,2,2-trifluoroethan	—	—	—	79	
1,2,4-Trimethylbenzene	65	0.010	3	—	
1,3,5-Trimethylbenzene	70	0.006	2	—	
Vinyl chloride	73	0.022	6	67	
m,p-Xylene	73	0.007	2	—	
o-Xylene	68	0.008	2	—	

* For all analytes, seven samples, each at a concentration of 0.5 μg/L (unless otherwise noted), were analyzed.
† Sample concentration 5 μg/L.

TABLE 6231:I. CHROMATOGRAPHIC CONDITIONS FOR 1,2-DIBROMOETHANE (EDB) AND 1,2-DIBROMO-3-CHLOROPROPANE (DBCP)

Compound	Retention Time *min*	
	Column 1	Column 2
EDB	9.5	8.9
DBCP	17.3	15.0

Column 1 conditions: Durawax-DX 3 (0.25-μm film thickness) in a 30 m long × 0.32-mm ID fused silica capillary column with helium carrier gas at linear velocity of 25 cm/s. Column temperature held isothermal at 40°C for 4 min, then programmed at 8°C/minto 180°C for final hold.
Column 2 conditions: DB-1 (0.25 μm film thickness) in a 30 m long × 0.32-mmID fused silica capillary column with helium carrier gas at linear velocity of 25 cm/s.Column temperature held isothermal at 40°C for 4 min, then programmed at10°C/min to 270°C for final hold.

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TABLE 6231:II. SINGLE-LABORATORY PRECISION AND BIAS FOR EDB AND DBCPin Tap Water

Compound	Number of Samples	Addition µg/L	Average Bias %	Relative Standard Deviation %
1,2-Dibromoethane	7	0.03	114	9.5
	7	0.24	98	11.8
	7	50.0	95	4.7
1,2-Dibromo-3-chloropropane	7	0.03	90	11.4
	7	0.24	102	8.3
	7	50.0	94	4.8

TABLE 6232:I. PRECISION AND BIAS DATA FOR THM-CHLORINATED ORGANIC SOLVENT METHOD, DB-5 COLUMN

Compound	Added Amount	Amount Recovered µg/L					
		A	B	C	D	E	F
Chloroform ($CHCl_3$)	20.0	18.7	18.6	19.4	19.5	19.2	18.5
Bromodichloromethane (BDCM)	20.0	19.8	20.2	20.7	20.8	20.3	19.7
Dibromochloromethane (DBCM)	20.0	18.7	19.4	20.0	20.2	19.7	18.7
Bromoform ($CHBr_3$)	20.0	17.4	18.5	18.7	19.2	19.3	17.9
Trichloroethane (TCA)	20.0	18.5	18.8	19.7	19.9	19.8	18.5
Carbon tetrachloride (CCl_4)	20.0	20.1	20.4	20.0	20.2	20.6	20.0
Trichloroethene (TCE)	20.0	17.9	18.3	18.9	19.2	19.1	17.9
Tetrachloroethene (PCE)	20.0	19.8	20.4	20.6	20.9	20.7	19.7
Internal standard	100.0	99.0	95.0	100.0	102.0	101.0	99.0

TABLE 6251:I. METHOD DETECTION LEVELS AND PRECISION DATA*

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TABLE 6251:I. METHOD DETECTION LEVELS AND PRECISION DATA*

Compound	Added Conc µg/L	Found Conc. µg/L	Standard Deviation µg/L	Relative Standard Deviation %	Method Detection Level µg/L
Monochloroacetic acid	0.50	0.54	0.026	4.8	0.082
Monobromoacetic acid	0.50	0.80	0.028	3.4	0.087
Dichloroacetic acid	0.50	0.5	0.017	3.5	0.054
Trichloroacetic acid	0.50	0.5	0.017	3.4	0.054
Bromochloroacetic acid	0.50	0.49	0.015	3.1	0.04
Dibromoacetic acid	0.50	0.47	0.021	4.4	0.065
2,4,6-Trichlorophenol	0.25	0.27	0.011	4.1	0.034

* Based on the analysis of seven portions of reagent water with known additions.[2]

TABLE 6251:II. ANALYTICAL STANDARDS

Compound*	Purity %	Molecular Weight	Boiling Point °C mm†
MCAA	99	94.5	183
MBAA	99+	138.95	208
DCAA	99+	128.94	194
TCAA	98	163.39	198
BCAA	97	173.39	215‡
DBAA	99	217.86	195 250
TCPh	95	197.45	246
IS-DBP	95	201.9	140–142
IS-TCP§	99	147.43	156
SUR-DBPA	99	231.88	160 20
SUR-TFBA	99	194.09	—

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Compound*	Purity %	Molecular Weight	Boiling Point °C *mm*†
MeCA	99+	108.52	130 740
MeBA	98	152.98	144
MeDCA	99+	142.97	143
MeTCA	99	177.42	152–153
MeBCAA	98	187.42	155
MeDBA	—	—	182
TCAn	99	211.48	132 28

* Sources: BCAA, Radian Corp., Austin, TX; DBAA, Fluka Chemika-BioChemika,Switzerland; TCPh obtainable from Chem Service, Inc., Westchester, PA; MeDBAand SUR ester, derivatized acid at Metropolitan Water District of Southern California laboratory; other compounds from Aldrich Chemical Company, Inc., Milwaukee, WI.
† °C at reduced pressure in mm Hg.
‡ Decomposes.
§ Ensure that TCP is not a contaminant when it is used as IS.

TABLE 6251:III. RETENTION TIMES

Compound	Retention Time *min*		
	DB-1701 Column	DB-5 Column	DB-210 Column
Methyl chloroacetate (MeCA)	11.11	5.80	10.97
Methyl bromoacetate (MeBA)	18.80	9.23	13.03
Methyl dichloroacetate (MeDCA)	20.53	10.11	12.72
Methyl trichloroacetate (MeTCA)	24.78	18.94	14.37
Methyl bromochloroacetate	26.23	19.19	15.11
Methyl dibromoacetate (MeDBA)	28.64	25.51	16.83
2, 4, 6-Trichloroanisole (TCAn)	~34.7	33.74	22.08
1,2-Dibromopropane (internal standard)	15.97	10.78	—
1,2,3-Trichloropropane (internal standard)	25.87	18.43	13.87
Methyl-2,3-dibromopropionate (surrogate ester)	30.74	28.60	—

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Compound	Retention Time *min*		
	DB-1701 Column	DB-5 Column	DB-210 Column
Methyl-2,3,6,7- tetrafluoro-benzoicate (surrogate)	29.19	26.84	—

TABLE 6251:IV. RECOMMENDED QUANTITATION LIMITS

Compound	Recommended Quantitation Limit *µg/L*
Monochloroacetic acid (MCAA)	1.0
Monobromoacetic acid (MBAA)	0.5
Dichloroacetic acid (DCAA)	0.6
Trichloroacetic acid (TCAA)	0.6
Bromochloroacetic acid (BCAA)	0.8
Dibromoacetic acid (DBAA)	0.6
2,4,6-Trichlorophenol (TCPh)	0.4

TABLE 6251:V. ADDITIVE RECOVERY IN REAGENT WATER*

Compound	Conc. Added *µg/L*	Mean Conc. Recovered *µg/L*	Standard Deviation *µg/L*	Relative Standard Deviation %	Mean Recovery %
Monochloroacetic acid	5.00	4.90	0.19	3.88	98.0
Monobromoacetic acid	5.00	4.95	0.13	2.67	99.0
Dichloroacetic acid	5.00	4.95	0.15	3.11	99.0
Trichloroacetic acid	5.00	5.06	0.16	3.06	101
Dibromoacetic acid	5.00	4.98	0.16	3.11	99.6
2,4,6-Trichlorophenol	2.50	2.51	0.075	2.99	100

* Based on the analysis of seven portions of reagent water with known additions in a single laboratory.

TABLE 6251:VI. ABSOLUTE RECOVERY DATA FOR REAGENT WATER WITH KNOWN

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ADDITIONS

Compound	Conc. Added µg/L	Mean Conc. Recovered µg/L	Standard Deviation µg/L	Relative Standard Deviation %	Mean Recovery %
Monochloroacetic acid	1.00	0.789	0.047	5.92	78.9
Monobromoacetic acid	1.00	0.706	0.034	4.76	70.6
Dichloroacetic acid	1.00	1.10	0.048	4.38	110
Trichloroacetic acid	1.00	0.927	0.051	5.49	92.7
Bromochloroacetic acid	0.50	0.49	0.015	3.07	98
Dibromoacetic acid	1.00	1.16	0.032	2.75	116
2,4,6-Trichlorophenol	0.50	0.523	0.030	5.89	105

TABLE 6251:VII. SAMPLE DUPLICATE DATA FROM TWO LABORATORIES

Laboratory	Compound	Numer of Pairs of Replicates	Average Difference Between Duplicates %	Standard Deviation of Difference Between Duplicates %
A	MCAA	5	7.6	10.6
	MBAA	3	1.9	1.5
	DCAA	7	1.5	0.8
	TCAA	6	1.4	1.0
	DBAA	5	6.0	6.0
	BCAA	11	1.8	1.3
B	MCAA	10	16.7	14.8
	MBAA	3	8.9	8.4
	DCAA	11	8.5	10.6
	TCAA	11	5.5	3.6
	DBAA	5	5.4	4.0
	BCAA	10	5.3	4.3

TABLE 6251:VIII. FIELD SAMPLE RECOVERY WITH KNOWN ADDITIONS TO DRINKING WATER, IN TWO LABORATORIES

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TABLE 6251:VIII. FIELD SAMPLE RECOVERY WITH KNOWN ADDITIONS TO DRINKING WATER, IN TWO LABORATORIES

Laboratory	Compound	Added Conc. µg/L	Number of Samples	Mean Recovery %	Relative Standard Deviation %
A	MCAA	5.0	7	99	4
	MBAA	5.0	7	101	4
	DCAA	5.0	7	96	4
	TCAA	5.0	7	100	3
	BCAA	10.0	14	96	5
	DBAA	5.0	7	102	5
	TCPh	2.5	7	100	6
B	MCAA	5.0	13	101	8
	DCAA	4.0	14	103	7
	TCAA	4.0	14	103	6
	MBAA	5.0	14	97	8
	BCAA	2.0	14	106	8
	DBAA	4.0	14	102	7
	TCPh	0.4	14	104	15

TABLE 6252:I. METHOD DETECTION LEVELS (MDL) AND PRECISION DATA*

Compound	Added Conc. µg/L	Found Conc. µg/L	Standard Deviation µg/L	Relative Standard Deviation %	Method Detection Level µg/L
Formaldehyde	0.550	0.518	0.026	5.04	0.082
Heptanal	0.255	0.191	0.025	13.1	0.079
Benzaldehyde	0.245	0.227	0.022	9.9	0.079
Glyoxal	0.445	0.315	0.073	23	0.228
Methyl glyoxal	0.420	0.355	0.033	9.4	0.105

* Based on the analysis of seven portions of organic-free water with known additions.

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TABLE 6252:II. ANALYTICAL STANDARDS OF CARBONYL COMPOUNDS USED IN THE PFBHA METHOD

Compound	Purity %	Molecular Weight *mg/mmol*	Boiling Point °*C*	Density *g/mL*
Formaldehyde*	†	33.03	96	1.083
Acetaldehyde‡	99.9	44.05	21	0.788
Propanal (propionaldehyde)*	97	58.08	46–50	0.805
Butanal (*n*-butyraldehyde)‡	99.9	72.11	75	0.800
Pentanal(*n*-valeraldehyde)*	99	86.13	103	0.810
Hexanal (caproaldehyde)*	98	100.16	131	0.834
Heptanal (heptaldehyde)*	95	114.19	153	0.818
Octanal (caprylic aldehyde)*	99	128.22	171	0.821
Nonanal (nonyl aldehyde)‡	99.9	142.24	93	0.827
Decanal (decyl aldehyde)‡	99.9	156.27	207–209	0.830
Benzaldehyde‡	99.9	106.12	178–179	1.044
Glyoxal (ethanedial)‡	§	58.04	50	1.14
Methyl glyoxal (pyruvic aldehyde or 2-oxopropionaldehyde)*	§	72.06	72	1.045

* Aldrich Chemical Company, Inc., Milwaukee, WI, or equivalent.
† Available in 37% solution (by weight) in water.
‡ ChemService, Inc., West Chester, PA, or equivalent.
§ Available in 40% solutions (by weight) in water.

TABLE 6252:III. RECOVERY OF TRIPLICATE IN-SITU DERIVATIZED ALDEHYDES COMPARED TO THE RECOVERY OF PURE OXIME DERIVATIVES FROM ORGANIC-FREE WATER

Derivatized Aldehyde	Conc. of Oxime Added* µ*g/L*	Mean Conc. Recovered† µ*g/L*	Standard Deviation µ*g/L*	Relative Standard Deviation %	Mean Recovery‡ %
Formaldehyde	19.2	21.2	0.118	0.56	90.4
Acetaldehyde	19.3	19.3	0.095	0.49	100
Heptaldehyde	19.9	23.6	0.104	0.44	84.3
Benzaldehyde	19.8	18.6	0.138	0.74	107
Glyoxal	19.4	23.7	0.235	0.99	82.0
Methyl glyoxal	20.5	22.1	0.194	0.88	92.5

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Derivatized Aldehyde	Conc. of Oxime Added* µg/L	Mean Conc. Recovered† µg/L	Standard Deviation µg/L	Relative Standard Deviation %	Mean Recovery‡ %

* Amount of standard oxime added to OPW.
† Evaluated by comparing the relative response of the extracted standard oximes to the calibration curve using in-situ derivatized aldehydes.
‡ Calculated recovery of in-situ derivatized aldehydes compared to standard oximes.

TABLE 6252:IV. RETENTION TIMES (RTS) FOR DERIVATIZED ALDEHYDES, SURROGATE AND INTERNAL STANDARDS ON ELECTRON-CAPTURE DETECTOR

Compound	Retention Time on Column of ¶ 3*j*3) *min*	Retention Time on Column of ¶ 3*j*4) *min*
1,2-Dibromopropane*	5.21	6.25
SUR - TFB†	7.27	10.88
Formaldehyde (HCHO-PFBO)	9.61	11.21
E-Acetaldehyde (E-CH_3CHO-PFBO)‡	12.88	14.42
Z-Acetaldehyde (Z-CH_3CHO-PFBO)‡	13.15	14.70
E-Heptanal (E-C_6H_{13}CHO-PFBO)‡	27.82	28.95
Z-Heptanal (Z-C_6H_{13}CHO-PFBO)‡	27.93	29.09
SUR - TFB-PFBO§	32.37	32.77
Benzaldehyde (C_6H_5CHO-PFBO)‡	31.41	33.78
E-Glyoxal (E-OHCCHO-PFBO)‡	39.09	43.87
Z-Glyoxal (Z-OHCCHO-PFBO)‡	39.48	44.09
Methyl glyoxal (OCH_3CCHO-PFBO)	41.09	45.72

* Internal standard.
† Underivatized surrogate aldehyde TFB (2,3,5,6-tetrafluorobenzaldehyde).
‡ These aldehydes form E- and Z-PFBO isomers in assumed order of elution but has not been confirmed (-PFBO = pentafluorobenzyloxime).
§ Derivatized surrogate aldehyde.

TABLE 6410:I. CHROMATOGRAPHIC CONDITIONS, METHOD DETECTION LIMITS, AND CHARACTERISTIC MASSES FOR BASE/NEUTRAL EXTRACTABLES

	Characteristic M

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† Underivatized surrogate aldehyde TFB (2,3,5,6-tetrafluorobenzaldehyde).
‡ These aldehydes form E- and Z-PFBO isomers in assumed order of elution but has not been confirmed (-PFBO = pentafluorobenzyloxime).
§ Derivatized surrogate aldehyde.

TABLE 6410:I. CHROMATOGRAPHIC CONDITIONS, METHOD DETECTION LIMITS, AND CHARACTERISTIC MASSES FOR BASE/NEUTRAL EXTRACTABLES

			Characteristic M		
			Electron Impact		
Compound	Retention Time *min*	Method Detection Limit *µg/L*	Primary	Secondary	Secondary
1,3-Dichlorobenzene	7.4	1.9	146	148	113
1,4-Dichlorobenzene	7.8	4.4	146	148	113
Hexachloroethane	8.4	1.6	117	201	199
bis(2-Chloroethyl) ether	8.4	5.7	93	63	95
1,2-Dichlorobenzene	8.4	1.9	146	148	113
bis(2-Chloroisopropyl) ether*	9.3	5.7	45	77	79
N-Nitrosodi-*n*-propylamine			130	42	101
Nitrobenzene	11.1	1.9	77	123	65
Hexachlorobutadiene	11.4	0.9	225	223	227
1,2,4-Trichlorobenzene	11.6	1.9	180	182	145
Isophorone	11.9	2.2	82	95	138
Naphthalene	12.1	1.6	128	129	127
bis(2-Chloroethoxy) methane	12.2	5.3	93	95	123
Hexachlorocyclopentadiene†	13.9		237	235	272
2-Chloronaphthalene	15.9	1.9	162	164	127
Acenaphthylene	17.4	3.5	152	151	153
Acenaphthene	17.8	1.9	154	153	152
Dimethyl phthalate	18.3	1.6	163	194	164
2,6-Dinitrotoluene	18.7	1.9	165	89	121
Fluorene	19.5	1.9	166	165	167
4-Chlorophenyl phenyl ether	19.5	4.2	204	206	141

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			Characteristic M[truncated]		
			Electron Impact		
Compound	Retention Time *min*	Method Detection Limit *µg/L*	Primary	Secondary	Secondary
2,4-Dinitrotoluene	19.8	5.7	165	63	182
Diethyl phthalate	20.1	1.9	149	177	150
N-Nitrosodiphenylamine†	20.5	1.9	169	168	167
Hexachlorobenzene	21.0	1.9	284	142	249
α-BHC†	21.1		183	181	109
4-Bromophenyl phenyl ether	21.2	1.9	248	250	141
γ-BHC†	22.4		183	181	109
Phenanthrene	22.8	5.4	178	179	176
Anthracene	22.8	1.9	178	179	176
β-BHC	23.4	4.2	181	183	109
Heptachlor	23.4	1.9	100	272	274
δ-BHC	23.7	3.1	183	109	181
Aldrin	24.0	1.9	66	263	220
Dibutyl phthalate	24.7	2.5	149	150	104
Heptachlor epoxide	25.6	2.2	353	355	351
Endosulfan I†	26.4		237	338	341
Fluoranthene	26.5	2.2	202	101	100
Dieldrin	27.2	2.5	79	263	279
4,4′-DDE	27.2	5.6	246	248	176
Pyrene	27.3	1.9	202	101	100
Endrin†	27.9		81	263	82
Endosulfan II†	28.6		237	339	341
4,4′-DDD	28.6	2.8	235	237	165
Benzidine†	28.8	44	184	92	185
4,4′-DDT	29.3	4.7	235	237	165
Endosulfan sulfate	29.8	5.6	272	387	422
Endrin aldehyde			67	345	250

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Compound	Retention Time *min*	Method Detection Limit *μg/L*	Characteristic M		
			Electron Impact		
			Primary	Secondary	Secondary
Butyl benzyl phthalate	29.9	2.5	149	91	206
bis(2-Ethylhexyl) phthalate	30.6	2.5	149	167	279
Chrysene	31.5	2.5	228	226	229
Benzo(a)anthracene	31.5	7.8	228	229	226
3,3′-Dichlorobenzidine	32.2	16.5	252	254	126
Di-*n*-octyl phthalate	32.5	2.5	149		
Benzo(b)fluoranthene	34.9	4.8	252	253	125
Benzo(k)fluoranthene	34.9	2.5	252	253	125
Benzo(a)pyrene	36.4	2.5	252	253	125
Indeno(1,2,3-cd)pyrene	42.7	3.7	276	138	277
Dibenzo(a,h)anthracene	43.2	2.5	278	139	279
Benzo(ghi)perylene	45.1	4.1	276	138	277
N-Nitrosodimethylamine†			42	74	44
Chlordane‡	19–30		373	375	377
Toxaphene‡	25–34		159	231	233
PCB 1016‡	18–30		224	260	294
PCB 1221‡	15–30	30	190	224	260
PCB 1232‡	15–32		190	224	260
PCB 1242‡	15–32		224	260	294
PCB 1248‡	12–34		294	330	262
PCB 1254‡	22–34	36	294	330	362
PCB 1260‡	23–32		330	362	394

* The proper chemical name is 2,2-oxybis(1-chloropropane).
† See introductory section of text.
‡ These compounds are mixtures of various isomers. (See Figure 6410:2, Figure 6410:3, Figure 6410:4, Figure 6410:5, Figure 6410:6, Figure 6410:7, Figure 6410:8, Figure 6410:9, Figure 6410:10, Figure 6410:11, Figure 6410:12.)>

Column conditions: Supelcoport (100/120 mesh) coated with 3% SP-2250 packed in a 1.8 m long × 2 mm ID glass column with helium carrier gas at 30 mL/min flowrate. Column temperature held isothermal at 50°C for 4 min, then programmed at 8°C/min to 270°C and held for 30 min.

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TABLE 6410:II. CHROMATOGRAPHIC CONDITIONS, METHOD DETECTION LIMITS, AND CHARACTERISTIC MASSES FOR ACID EXTRACTABLES

			Characteristic M		
			Electron Impact		
Compound	**Retention Time** *min*	**Method Detection Limit** *µg/L*	**Primary**	**Secondary**	**Secondary**
2-Chlorophenol	5.9	3.3	128	64	130
2-Nitrophenol	6.5	3.6	139	65	109
Phenol	8.0	1.5	94	65	66
2,4-Dimethylphenol	9.4	2.7	122	107	121
2,4-Dichlorophenol	9.8	2.7	162	164	98
2,4,6-Trichlorophenol	11.8	2.7	196	198	200
4-Chloro-3-methylphenol	13.2	3.0	142	107	144
2,4-Dinitrophenol	15.9	42	184	63	154
2-Methyl-4,6-dinitrophenol	16.2	24	198	182	77
Pentachlorophenol	17.5	3.6	266	264	268
4-Nitrophenol	20.3	2.4	65	139	109

Column conditions: Supelcoport (100/120 mesh) coated with 1% SP-1240DA packed in a 1.8 m long × 2 mm ID glass column with helium carrier gas at 30 mL/minflow rate. Column temperature held isothermal at 70°C for 2 min then programmed at 8°C/min to 200°C.

TABLE 6410:III. DFTPP KEY MASSES AND ABUNDANCE CRITERIA

Mass	*m/z* Abundance Criteria
51	30–60% of mass 198
68	Less than 2% of mass 69
70	Less than 2% of mass 69
127	40–60% of mass 198
197	Less than 1% of mass 198
198	Base peak, 100% relative abundance
199	5–9% of mass 198
275	10–30% of mass 198
365	Greater than 1% of mass 198
441	Present but less than mass 443

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Mass	*m/z* Abundance Criteria
442	Greater than 40% of mass 198
443	17–23% of mass 442

TABLE 6410:IV. SUGGESTED INTERNAL AND SURROGATE STANDARDS

Base/Neutral Fraction	Acid Fraction
Aniline-d_5	2-Fluorophenol
Anthracene-d_{10}	Pentafluorophenol
Benzo(a)anthracene-d_{12}	Phenol-d_5
4,4′-Dibromobiphenyl	2-Perfluoromethyl phenol
4,4′-Dibromooctafluorobiphenyl	
Decafluorobiphenyl	
2,2′-Difluorobiphenyl	
4-Fluoroaniline	
1-Fluoronaphthylene	
2-Fluoronaphthylene	
Naphthalene-d_8	
Nitrobenzene-d_5	
2,3,4,5,6-Pentafluorobiphenyl	
Phenanthrene-d_{10}	
Pyridine-d_5	

TABLE 6410:V. QC ACCEPTANCE CRITERIA*

Compound	Test Concentration μg/L	Limits for *s* μg/L	Range for $\bar{X}$ μg/L	Range for *P*, P_s %
Acenaphthene	100	27.6	60.1–132.3	47–145
Acenaphthylene	100	40.2	53.5–126.0	33–145
Aldrin	100	39.0	7.2–152.2	D–166
Anthracene	100	32.0	43.4–118.0	27–133
Benzo(a)anthracene	100	27.6	41.8–133.0	33–143

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Compound	Test Concentration μg/L	Limits for *s* μg/L	Range for $\bar{X}$ μg/L	Range for *P*, P_S %
Benzo(b)fluoranthene	100	38.8	42.0–140.4	24–159
Benzo(k)fluoranthene	100	32.3	25.2–145.7	11–162
Benzo(a)pyrene	100	39.0	31.7–148.0	17–163
Benzo(ghi)perylene	100	58.9	D–195.0	D–219
Benzyl butyl phthalate	100	23.4	D–139.9	D–152
δ-BHC	100	31.5	41.5–130.6	24–149
β-BHC	100	21.6	D–100.0	D–110
bis(2-Chloroethyl) ether	100	55.0	42.9–126.0	12–158
bis(2-Chloroethoxy) methane	100	34.5	49.2–164.7	33–184
bis(2-Chloroisopropyl) ether†	100	46.3	62.8–138.6	36–166
bis(2-Ethylhexyl) phthalate	100	41.1	28.9–136.8	8–158
4-Bromophenyl phenyl ether	100	23.0	64.9–114.4	53–127
2-Chloronaphthalene	100	13.0	64.5–113.5	60–118
4-Chlorophenyl phenyl ether	100	33.4	38.4–144.7	25–158
Chrysene	100	48.3	44.1–139.9	17–168
4,4′-DDD	100	31.0	D–134.5	D–145
4,4′-DDE	100	32.0	19.2–119.7	4–136
4,4′-DDT	100	61.6	D–170.6	D–203
Dibenzo(a,h)anthracene	100	70.0	D–199.7	D–227
Di-*n*-butyl phthalate	100	16.7	8.4–111.0	1–118
1,2-Dichlorobenzene	100	30.9	48.6–112.0	32–129
1,3-Dichlorobenzene	100	41.7	16.7–153.9	D–172
1,4-Dichlorobenzene	100	32.1	37.3–105.7	20–124
3,3-Dichlorobenzidine	100	71.4	8.2–212.5	D–262
Dieldrin	100	30.7	44.3–119.3	29–136
Diethyl phthalate	100	26.5	D–100.0	D–114
Dimethyl phthalate	100	23.2	D–100.0	D–112
2,4-Dinitrotoluene	100	21.8	47.5–126.9	39–139
2,6-Dinitrotoluene	100	29.6	68.1–136.7	50–158
Di-*n*-octylphthalate	100	31.4	18.6–131.8	4–146
Endosulfan sulfate	100	16.7	D–103.5	D–107

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Compound	Test Concentration µg/L	Limits for *s* µg/L	Range for $\bar{X}$ µg/L	Range for *P*, P_S %
Endrin aldehyde	100	32.5	D–188.8	D–209
Fluoranthene	100	32.8	42.9–121.3	26–137
Fluorene	100	20.7	71.6–108.4	59–121
Heptachlor	100	37.2	D–172.2	D–192
Heptachlor epoxide	100	54.7	70.9–109.4	26–155
Hexachlorobenzene	100	24.9	7.8–141.5	D–152
Hexachlorobutadiene	100	26.3	37.8–102.2	24–116
Hexachloroethane	100	24.5	55.2–100.0	40–113
Indeno(1,2,3-cd)pyrene	100	44.6	D–150.9	D–171
Isophorone	100	63.3	46.6–180.2	21–196
Naphthalene	100	30.1	35.6–119.6	21–133
Nitrobenzene	100	39.3	54.3–157.6	35–180
N-Nitrosodi-*n*-propylamine	100	55.4	13.6–197.9	D–230
PCB-1260	100	54.2	19.3–121.0	D–164
Phenanthrene	100	20.6	65.2–108.7	54–120
Pyrene	100	25.2	69.6–100.0	52–115
1,2,4-Trichlorobenzene	100	28.1	57.3–129.2	44–142
4-Chloro-3-methylphenol	100	37.2	40.8–127.9	22–147
2-Chlorophenol	100	28.7	36.2–120.4	23–134
2,4-Dichlorophenol	100	26.4	52.5–121.7	39–135
2,4-Dimethylphenol	100	26.1	41.8–109.0	32–119
2,4-Dinitrophenol	100	49.8	D–172.9	D–191
2-Methyl-4,6-dinitrophenol	100	93.2	53.0–100.0	D–181
2-Nitrophenol	100	35.2	45.0–166.7	29–182
4-Nitrophenol	100	47.2	13.0–106.5	D–132
Pentachlorophenol	100	48.9	38.1–151.8	14–176
Phenol	100	22.6	16.6–100.0	5–112
2,4,6-Trichlorophenol	100	31.7	52.4–129.2	37–144

**s* = standard deviation for four recovery measurements,*X* = average recovery for four recovery measurements,*P*, P_S = percent recovery measured, and*D* = detected; results must be greater than zero. † The proper chemical name is 2,2-oxybis(1-chloropropane).NOTE: These criteria are based directly upon the method performance data in Table 6410:VI. Where necessary, the limits for recovery were broadened to assure applicability of thelimits to concentrations below those used to develop

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Table 6410:VI.

TABLE 6410:VI. METHOD BIAS AND PRECISION AS FUNCTIONS OF CONCENTRATION*

Compound	Bias as Recovery, X' µg/L	Single-Analyst Precision, s_r' µg/L	Overall Precision, S' µg/L
Acenaphthene	$0.96C + 0.19$	$0.15\bar{X} - 0.12$	$0.21\bar{X} - 0.67$
Acenaphthylene	$0.89C + 0.74$	$0.24\bar{X} - 1.06$	$0.26\bar{X} - 0.54$
Aldrin	$0.78C + 1.66$	$0.27\bar{X} - 1.28$	$0.43\bar{X} + 1.13$
Anthracene	$0.80C + 0.68$	$0.21\bar{X} - 0.32$	$0.27\bar{X} - 0.64$
Benzo(a)anthracene	$0.88C - 0.60$	$0.15\bar{X} + 0.93$	$0.26\bar{X} - 0.28$
Benzo(b)fluoranthene	$0.93C - 1.80$	$0.22\bar{X} + 0.43$	$0.29\bar{X} + 0.96$
Benzo(k)fluoranthene	$0.87C - 1.56$	$0.19\bar{X} + 1.03$	$0.35\bar{X} + 0.40$
Benzo(a)pyrene	$0.90C - 0.13$	$0.22\bar{X} + 0.48$	$0.32\bar{X} + 1.35$
Benzo(ghi)perylene	$0.98C - 0.86$	$0.29\bar{X} + 2.40$	$0.51\bar{X} - 0.44$
Benzyl butyl phthalate	$0.66C - 1.68$	$0.18\bar{X} + 0.94$	$0.53\bar{X} + 0.92$
β-BHC	$0.87C - 0.94$	$0.20\bar{X} - 0.58$	$0.30\bar{X} - 1.94$
δ-BHC	$0.29C - 1.09$	$0.34\bar{X} + 0.86$	$0.93\bar{X} - 0.17$
bis(2-Chloroethyl) ether	$0.86C - 1.54$	$0.35\bar{X} - 0.99$	$0.35\bar{X} + 0.10$
bis(2-Chloroethoxy) methane	$1.12C - 5.04$	$0.16\bar{X} + 1.34$	$0.26\bar{X} + 2.01$
bis(2-Chloroisopropyl) ether†	$1.03C - 2.31$	$0.24\bar{X} + 0.28$	$0.25\bar{X} + 1.04$
bis(2-Ethylhexyl) phthalate	$0.84C - 1.18$	$0.26\bar{X} + 0.73$	$0.36\bar{X} + 0.67$
4-Bromophenyl phenyl ether	$0.91C - 1.34$	$0.13\bar{X} + 0.66$	$0.16\bar{X} + 0.66$
2-Chloronaphthalene	$0.89C + 0.01$	$0.07\bar{X} + 0.52$	$0.13\bar{X} + 0.34$
4-Chlorophenyl phenyl ether	$0.91C + 0.53$	$0.20\bar{X} - 0.94$	$0.30\bar{X} - 0.46$
Chrysene	$0.93C - 1.00$	$0.28\bar{X} + 0.13$	$0.33\bar{X} - 0.09$
4,4′-DDD	$0.56C - 0.40$	$0.29\bar{X} - 0.32$	$0.66\bar{X} - 0.96$
4,4′-DDE	$0.70C - 0.54$	$0.26\bar{X} - 1.17$	$0.39\bar{X} - 1.04$
4,4′-DDT	$0.79C - 3.28$	$0.42\bar{X} + 0.19$	$0.65\bar{X} - 0.58$
Dibenzo(a,h)anthracene	$0.88C + 4.72$	$0.30\bar{X} + 8.51$	$0.59\bar{X} + 0.25$
Di-*n*-butyl phthalate	$0.59C + 0.71$	$0.13\bar{X} + 1.16$	$0.39\bar{X} + 0.60$
1,2-Dichlorobenzene	$0.80C + 0.28$	$0.20\bar{X} + 0.47$	$0.24\bar{X} + 0.39$
1,3-Dichlorobenzene	$0.86C - 0.70$	$0.25\bar{X} + 0.68$	$0.41\bar{X} + 0.11$

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Compound	Bias as Recovery, X' μg/L	Single-Analyst Precision, s_r' μg/L	Overall Precision, S' μg/L
1,4-Dichlorobenzene	$0.73C - 1.47$	$0.24\bar{X} + 0.23$	$0.29\bar{X} + 0.36$
3,3-Dichlorobenzidine	$1.23C - 12.65$	$0.28\bar{X} + 7.33$	$0.47\bar{X} + 3.45$
Dieldrin	$0.82C - 0.16$	$0.20\bar{X} - 0.16$	$0.26\bar{X} - 0.07$
Diethyl phthalate	$0.43C + 1.00$	$0.28\bar{X} + 1.44$	$0.52\bar{X} + 0.22$
Dimethyl phthalate	$0.20C + 1.03$	$0.54\bar{X} + 0.19$	$1.05\bar{X} - 0.92$
2,4-Dinitrotoluene	$0.92C - 4.81$	$0.12\bar{X} + 1.06$	$0.21\bar{X} + 1.50$
2,6-Dinitrotoluene	$1.06C - 3.60$	$0.14\bar{X} + 1.26$	$0.19\bar{X} + 0.35$
Di-*n*-octylphthalate	$0.76C - 0.79$	$0.21\bar{X} + 1.19$	$0.37\bar{X} + 1.19$
Endosulfan sulfate	$0.39C + 0.41$	$0.12\bar{X} + 2.47$	$0.63\bar{X} - 1.03$
Endrin aldehyde	$0.76C - 3.86$	$0.18\bar{X} + 3.91$	$0.73\bar{X} - 0.62$
Fluoranthene	$0.81C + 1.10$	$0.22\bar{X} - 0.73$	$0.28\bar{X} - 0.60$
Fluorene	$0.90C - 0.00$	$0.12\bar{X} + 0.26$	$0.13\bar{X} + 0.61$
Heptachlor	$0.87C - 2.97$	$0.24\bar{X} - 0.56$	$0.50\bar{X} - 0.23$
Heptachlor epoxide	$0.92C - 1.87$	$0.33\bar{X} - 0.46$	$0.28\bar{X} + 0.64$
Hexachlorobenzene	$0.74C + 0.66$	$0.18\bar{X} - 0.10$	$0.43\bar{X} - 0.52$
Hexachlorobutadiene	$0.71C - 1.01$	$0.19\bar{X} + 0.92$	$0.26\bar{X} + 0.49$
Hexachloroethane	$0.73C - 0.83$	$0.17\bar{X} + 0.67$	$0.17\bar{X} + 0.80$
Indeno(1,2,3-cd)pyrene	$0.78C - 3.10$	$0.29\bar{X} + 1.46$	$0.50\bar{X} + 0.44$
Isophorone	$1.12C + 1.41$	$0.27\bar{X} + 0.77$	$0.33\bar{X} + 0.26$
Naphthalene	$0.76C + 1.58$	$0.21\bar{X} - 0.41$	$0.30\bar{X} - 0.68$
Nitrobenzene	$1.09C - 3.05$	$0.19\bar{X} + 0.92$	$0.27\bar{X} + 0.21$
N-Nitrosodi-*n*-propylamine	$1.12C - 6.22$	$0.27\bar{X} + 0.68$	$0.44\bar{X} + 0.47$
PCB-1260	$0.81C - 10.86$	$0.35\bar{X} + 3.61$	$0.43\bar{X} + 1.82$
Phenanthrene	$0.87C - 0.06$	$0.12\bar{X} + 0.57$	$0.15\bar{X} + 0.25$
Pyrene	$0.84C - 0.16$	$0.16\bar{X} + 0.06$	$0.15\bar{X} + 0.31$
1,2,4-Trichlorobenzene	$0.94C - 0.79$	$0.15\bar{X} + 0.85$	$0.21\bar{X} + 0.39$
4-Chloro-3-methylphenol	$0.84C + 0.35$	$0.23\bar{X} + 0.75$	$0.29\bar{X} + 1.31$
2-Chlorophenol	$0.78C + 0.29$	$0.18\bar{X} + 1.46$	$0.28\bar{X} + 0.97$
2,4-Dichlorophenol	$0.87C + 0.13$	$0.15\bar{X} + 1.25$	$0.21\bar{X} + 1.28$
2,4-Dimethylphenol	$0.71C + 4.41$	$0.16\bar{X} + 1.21$	$0.22\bar{X} + 1.31$

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Compound	Bias as Recovery, X' µg/L	Single-Analyst Precision, s_r' µg/L	Overall Precision, S' µg/L
2,4-Dinitrophenol	0.81C – 18.04	0.38$\bar{X}$ + 2.36	0.42$\bar{X}$ + 26.29
2-Methyl-4,6-dinitrophenol	1.04C – 28.04	0.10$\bar{X}$ + 42.29	0.26$\bar{X}$ + 23.10
2-Nitrophenol	1.07C – 1.15	0.16$\bar{X}$ + 1.94	0.27$\bar{X}$ + 2.60
4-Nitrophenol	0.61C – 1.22	0.38$\bar{X}$ + 2.57	0.44$\bar{X}$ + 3.24
Pentachlorophenol	0.93C + 1.99	0.24$\bar{X}$ + 3.03	0.30$\bar{X}$ + 4.33
Phenol	0.43C + 1.26	0.26$\bar{X}$ + 0.73	0.35$\bar{X}$ + 0.58
2,4,6-Trichlorophenol	0.91C – 0.18	0.16$\bar{X}$ + 2.22	0.22$\bar{X}$ + 1.81

*X' = expected recovery for one or more measurements of a sample containing a concentration of C,
s_r' = expected single-analyst standard deviation of measurements at an average concentration found of X,
S' = expected interlaboratory standard deviation of measurements at an average concentration found of X,
C = true value for the concentration, and
$\bar{X}$ = average recovery found for measurements of samples containing a concentration of C.
† The proper chemical name is 2,2-oxybis(1-chloropropane).

TABLE 6420:I. CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LIMITS

Compound	Retention Time *min*	Method Detection Limit µg/L
2-Chlorophenol	1.70	0.31
2-Nitrophenol	2.00	0.45
Phenol	3.01	0.14
2,4-Dimethylphenol	4.03	0.32
2,4-Dichlorophenol	4.30	0.39
2,4,6-Trichlorophenol	6.05	0.64
4-Chloro-3-methylphenol	7.50	0.36
2,4-Dinitrophenol	10.00	13.0
2-Methyl-4,6-dinitrophenol	10.24	16.0
Pentachlorophenol	12.42	7.4
4-Nitrophenol	24.25	2.8

Column conditions: Supelcoport (80/100 mesh) coated with 1% SP-1240DA packed ina 1.8 m long × 2 mm ID glass column with nitrogen carrier gas at 30 mL/min flowrate. Column temperature was 80°C at injection, programmed immediately at 8°C/min to150°C final temperature. MDLs determined with an FID.

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TABLE 6420:II. SILICA GEL FRACTIONATION AND ELECTRON CAPTURE GAS CHROMATOGRAPHY OF PFBB DERIVATIVES

Parent Compound	Percent Recovery By Fraction* 1	2	3	4	Retention Time *min*	Method Detection Limit *µg/L*
2-Chlorophenol	—	90	1	—	3.3	0.58
2-Nitrophenol	—	—	9	90	9.1	0.77
Phenol	—	90	10	—	1.8	2.2
2,4-Dimethylphenol	—	95	7	—	2.9	0.63
2,4-Dichlorophenol	—	95	1	—	5.8	0.68
2,4,6-Trichlorophenol	50	50	—	—	7.0	0.58
4-Chloro-3-methylphenol	—	84	14	—	4.8	1.8
Pentachlorophenol	75	20	—	—	28.8	0.59
4-Nitrophenol	—	—	1	90	14.0	0.70

Column conditions: Chromosorb W-AW-DMCS (80/100 mesh) coated with 5% OV-17packed in a 1.8 m long × 2.0 mm ID glass column with 5% methane/95% argoncarrier gas at 30 mL/min flow rate. Column temperature held isothermal at 200°C. MDLsdetermined with an ECD.
* Eluent composition:
Fraction 1 - 15% toluene in hexane.
Fraction 2 - 40% toluene in hexane.
Fraction 3 - 75% toluene in hexane.
Fraction 4 - 15% 2-propanol in toluene.

TABLE 6420:III. QC ACCEPTANCE CRITERIA*

Compound	Test Conc. *µg/L*	Limit for *s* *µg/L*	Range for *X* *µg/L*	Range for *P, P_S* %
4-Chloro-3-methylphenol	100	16.6	56.7–113.4	49–122
2-Chlorophenol	100	27.0	54.1–110.2	38–126
2,4-Dichlorophenol	100	25.1	59.7–103.3	44–119
2,4-Dimethylphenol	100	33.3	50.4–100.0	24–118
4,6-Dinitro-2-methylphenol	100	25.0	42.4–123.6	30–136
2,4-Dinitrophenol	100	36.0	31.7–125.1	12–145
2-Nitrophenol	100	22.5	56.6–103.8	43–117
4-Nitrophenol	100	19.0	22.7–100.0	13–110

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Compound	Test Conc. μg/L	Limit for *s* μg/L	Range for *X* μg/L	Range for *P*, P_S %
Pentachlorophenol	100	32.4	56.7–113.5	36–134
Phenol	100	14.1	32.4–100.0	23–108
2,4,6-Trichlorophenol	100	16.6	60.8–110.4	53–119

* *s* = standard deviation of four recovery measurements,
$\bar{X}$ = average recovery for four recovery measurements, and
P, P_S = percent recovery measured.

NOTE: These criteria are based directly upon the method performance data in Table 6420:IV. Where necessary, the limits for recovery were broadened to assure applicability of thelimits to concentrations below those used to develop Table 6420:IV.

TABLE 6420:IV. METHOD BIAS AND PRECISION AS FUNCTIONS OF CONCENTRATION*

Compound	Bias, as Recovery, *X′* μg/L	Single-Analyst Precision, s_r' μg/L	Overall Precision, *S′* μg/L
4-Chloro-3-methylphenol	0.87*C* – 1.97	$0.11\bar{X} - 0.21$	$0.16\bar{X} + 1.41$
2-Chlorophenol	0.83*C* – 0.84	$0.18\bar{X} + 0.20$	$0.21\bar{X} + 0.75$
2,4-Dichlorophenol	0.81*C* + 0.48	$0.17\bar{X} - 0.02$	$0.18\bar{X} + 0.62$
2,4-Dimethylphenol	0.62*C* – 1.64	$0.30\bar{X} - 0.89$	$0.25\bar{X} + 0.48$
4,6-Dinitro-2-methylphenol	0.84*C* – 1.01	$0.15\bar{X} + 1.25$	$0.19\bar{X} + 5.85$
2,4-Dinitrophenol	0.80*C* – 1.58	$0.27\bar{X} - 1.15$	$0.29\bar{X} + 4.51$
2-Nitrophenol	0.81*C* – 0.76	$0.15\bar{X} + 0.44$	$0.14\bar{X} + 3.84$
4-Nitrophenol	0.46*C* + 0.18	$0.17\bar{X} + 2.43$	$0.19\bar{X} + 4.79$
Pentachlorophenol	0.83*C* + 2.07	$0.22\bar{X} - 0.58$	$0.23\bar{X} + 0.57$
Phenol	0.43*C* + 0.11	$0.20\bar{X} - 0.88$	$0.17\bar{X} + 0.77$
2,4,6-Trichlorophenol	0.86*C* – 0.40	$0.10\bar{X} + 0.53$	$0.13\bar{X} + 2.40$

* *X′* = expected recovery for one or more measurements of a sample containing a concentration of *C*,
s_r' = expected single-analyst standard deviation of measurements at an average concentration found of $\bar{X}$,
S′ = expected interlaboratory standard deviation of measurements at an average concentration found of $\bar{X}$,
C = true value for the concentration, and,
$\bar{X}$ = average recovery found for measurements of samples containing a concentration of *C*.

TABLE 6440:I. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY CONDITIONS AND METHOD DETECTION LIMITS

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C = true value for the concentration, and,
$\bar{X}$ = average recovery found for measurements of samples containing a concentration of C.

TABLE 6440:I. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY CONDITIONS AND METHOD DETECTION LIMITS

Compound	Retention Time *min*	Column Capacity Factor *k′*	Method Detection Limit *μg/L**
Naphthalene	16.6	12.2	1.8
Acenaphthylene	18.5	13.7	2.3
Acenaphthene	20.5	15.2	1.8
Fluorene	21.2	15.8	0.21
Phenanthrene	22.1	16.6	0.64
Anthracene	23.4	17.6	0.66
Fluoranthene	24.5	18.5	0.21
Pyrene	25.4	19.1	0.27
Benzo(a)anthracene	28.5	21.6	0.013
Chrysene	29.3	22.2	0.15
Benzo(b)fluoranthene	31.6	24.0	0.018
Benzo(k)fluoranthene	32.9	25.1	0.017
Benzo(a)pyrene	33.9	25.9	0.023
Dibenzo(a,h)anthracene	35.7	27.4	0.030
Benzo(ghi)perylene	36.3	27.8	0.076
Indeno(1,2,3-cd)pyrene	37.4	28.7	0.043

HPLC column conditions: Reverse phase HC-ODS Sil-X, 5 μm particle size, in a 25 cm× 2.6 mm ID stainless steel column. Isocratic elution for 5 min using acetonitrile/water(4 + 6), then linear gradient to 100% acetonitrile over 25 min at 0.5 mL/min flow rate. If columns having other internal diameters are used, adjust flow rate to maintain a linear velocity of 2 mm/s.
* The MDL for naphthalene, acenaphthylene, acenaphthene, and fluorene were determined using a UV detector. All others were determined using a fluorescence detector.

TABLE 6440:II. GAS CHROMATOGRAPHIC CONDITIONS AND RETENTION TIMES

Compound	Retention Time *min*
Naphthalene	4.5
Acenaphthylene	10.4
Acenaphthane	10.8

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Compound	Retention Time *min*
Fluorene	12.6
Phenanthrene	15.9
Anthracene	15.9
Fluoranthene	19.8
Pyrene	20.6
Benzo(a)anthracene	24.7
Chrysene	24.7
Benzo(b)fluoranthene	28.0
Benzo(k)fluoranthene	28.0
Benzo(a)pyrene	29.4
Dibenzo(a,h)anthracene	36.2
Indeno(1,2,3-cd)pyrene	36.2
Benzo(ghi)perylene	38.6

GC column conditions: Chromosorb W-AW-DCMS (100/120 mesh) coated with 3% OV-17 packed in a 1.8 × 2 mm ID glass column with nitrogen carrier gas at 40 mL/min flow rate. Column temperature held at 100°C for 4 min, then programmed at 8°C/min to a final hold at 280°C.

TABLE 6440:III. QC ACCEPTANCE CRITERIA*

Compound	Test Conc. μg/L	Limit for s μg/L	Range for $\bar{X}$ μg/L	Range for P, P_S %
Acenaphthene	100	40.3	D–105.7	D–124
Acenaphthylene	100	45.1	22.1–112.1	D–139
Anthracene	100	28.7	11.2–112.3	D–126
Benzo(a)anthracene	10	4.0	3.1– 11.6	12–135
Benzo(a)pyrene	10	4.0	0.2– 11.0	D–128
Benzo(b)fluoranthene	10	3.1	1.8– 13.8	6–150
Benzo(ghi)perylene	10	2.3	D– 10.7	D–116
Benzo(k)fluoranthene	5	2.5	D– 7.0	D–159
Chrysene	10	4.2	D– 17.5	D–199
Dibenzo(a,h)anthracene	10	2.0	0.3– 10.0	D–110

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Compound	Test Conc. µg/L	Limit for *s* µg/L	Range for $\bar{X}$ µg/L	Range for *P*, P_S %
Fluoranthene	10	3.0	2.7– 11.1	14–123
Fluorene	100	43.0	D–119	D–142
Indeno(1,2,3-cd) pyrene	10	3.0	1.2– 10.0	D–116
Naphthalene	100	40.7	21.5–100.0	D–122
Phenanthrene	100	37.7	8.4–133.7	D–155
Pyrene	10	3.4	1.4– 12.1	D–140

* *s* = standard deviation of four recovery measurements,
$\bar{X}$ = average recovery for four recovery measurements,
P, P_S = percent recovery measured, and
D = detected; result must be greater than zero.
NOTE: These criteria are based directly upon the method performance data in Table 6440:IV. Where necessary, the limits for recovery were broadened to assure applicability of thelimits to concentrations below those used to develop Table 6440:IV.

TABLE 6440:IV. METHOD BIAS AND PRECISION AS FUNCTIONS OF CONCENTRATION*

Compound	Bias as Recovery, *X′* µg/L	Single-Analyst Precision, s_r µg/L	Overall Precision, *S′* µg/L
Acenaphthene	0.52*C* + 0.54	$0.39\bar{X} + 0.76$	$0.53\bar{X} + 1.32$
Acenaphthylene	0.69*C* – 1.89	$0.36\bar{X} + 0.29$	$0.42\bar{X} + 0.52$
Anthracene	0.63*C* – 1.26	$0.23\bar{X} + 1.16$	$0.41\bar{X} + 0.45$
Benzo(a)anthracene	0.73*C* + 0.05	$0.28\bar{X} + 0.04$	$0.34\bar{X} + 0.02$
Benzo(a)pyrene	0.56*C* + 0.01	$0.38\bar{X} + 0.01$	$0.53\bar{X} - 0.01$
Benzo(b)fluoranthene	0.78*C* + 0.01	$0.21\bar{X} + 0.01$	$0.38\bar{X} - 0.00$
Benzo(ghi)perylene	0.44*C* + 0.30	$0.25\bar{X} + 0.04$	$0.58\bar{X} + 0.10$
Benzo(k)fluoranthene	0.59*C* + 0.00	$0.44\bar{X} - 0.00$	$0.69\bar{X} + 0.01$
Chrysene	0.77*C* – 0.18	$0.32\bar{X} - 0.18$	$0.66\bar{X} - 0.22$
Dibenzo(a,h)anthracene	0.41*C* + 0.11	$0.24\bar{X} + 0.02$	$0.45\bar{X} + 0.03$
Fluoranthene	0.68*C* + 0.07	$0.22\bar{X} + 0.06$	$0.32\bar{X} + 0.03$
Fluorene	0.56*C* – 0.52	$0.44\bar{X} - 1.12$	$0.63\bar{X} - 0.65$
Indeno(1,2,3-cd)pyrene	0.54*C* + 0.06	$0.29\bar{X} + 0.02$	$0.42\bar{X} + 0.01$
Naphthalene	0.57*C* – 0.70	$0.39\bar{X} - 0.18$	$0.41\bar{X} + 0.74$

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Compound	Bias as Recovery, X' µg/L	Single-Analyst Precision, s_r µg/L	Overall Precision, S' µg/L
Phenanthrene	$0.72C - 0.95$	$0.29\bar{X} + 0.05$	$0.47\bar{X} - 0.25$
Pyrene	$0.69C - 0.12$	$0.25\bar{X} + 0.14$	$0.42\bar{X} - 0.00$

* X' = expected recovery for one or more measurements of a sample containing a concentration of C,
s_r = expected single-analyst standard deviation of measurements at an average concentration found of $\bar{X}$,
S' = expected interlaboratory standard deviation of measurements at an average concentration found of $\bar{X}$,
C = true value for concentration, and
X = average recovery found for measurements of samples containing a concentration of C.

Table 6610:I. RECOVERY OF KNOWN ADDITIONS OF SELECTED CARBAMATES FROM WATER AND ESTIMATED DETECTION LIMITS (EDL)

Constituent	Addition µg/L	No. Data Points	Recovery Average %	Standard Deviation µg/L	Relative Standard Deviation %	EDL* µg/L
Aldicarb	1.0	8	107	0.07	7	1.0
Aldicarb sulfoxide	2.0	8	47	0.20	21	2.0
Aldicarb sulfone	2.0	8	83	0.34	20	2.0
Baygon	1.0	7	101	0.32	32	1.0
Carbaryl	2.0	8	97	0.44	23	2.0
Carbofuran	1.5	7	90	0.17	12	1.5
3-Hydroxycarbofuran	2.0	8	108	0.63	29	2.0
Methiocarb	4.0	8	82	0.64	19	4.0
Methomyl	0.50	7	102	0.09	18	0.50
Oxamyl	2.0	8	82	0.29	7	2.0

*EDL = estimated detection limit in sample; calculate by multiplying standard deviation times the Student's t value appropriate for a 99% confidence level and a standard deviation estimate with $n - 1$ degrees of freedom, or a level of compound in sample yielding a peak with a signal-to-noise ratio of approximately 5, whichever value is higher. There were no detectable apparent residues of any constituent in the blank water.

TABLE 6610:II. STORAGE STABILITY OF SAMPLES WITH KNOWN ADDITIONS

Constituent	Added Concentration µg/L	Day	Storage Temperature °C	Average Recovery %	Relative Standard Deviation %

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Constituent	Added Concentration μg/L	Day	Storage Temperature °C	Average Recovery %	Relative Standard Deviation %
Aldicarb	5.0	0	—	100	9
		14	–10	100	4
			+4	110	2
		28	–10	100	6
			+4	83	1
Aldicarb sulfone	10	0	—	99	9
		14	–10	93	3
			+4	99	3
		28	–10	97	0
			+4	86	8
Aldicarb sulfoxide	10	0	—	100	9
		14	–10	91	6
			+4	100	2
		28	–10	98	2
			+4	91	5
Baygon	5.0	0	—	98	10
		14	–10	91	2
			+4	100	4
		28	–10	88	2
			+4	93	9
Carbaryl	10	0	—	100	6
		14	–10	92	4
			+4	95	3
		28	–10	99	18
			+4	89	5
Carbofuran	7.5	0	—	100	9
		14	–10	95	3
			+4	110	7
		28	–10	95	2
			+4	93	3

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Constituent	Added Concentration µg/L	Day	Storage Temperature °C	Average Recovery %	Relative Standard Deviation %
3-Hydroxycarbofuran	10	0	—	95	9
		14	–10	89	6
			+4	100	3
		28	–10	100	8
			+4	95	11
Methiocarb	20	0	—	110	4
		14	–10	100	1
			+4	98	1
		28	–10	99	0
			+4	94	6
Methomyl	2.5	0	—	110	12
		14	–10	90	6
			+4	96	5
		28	–10	93	2
			+4	96	5
Oxamyl	10	0	—	98	6
		14	–10	85	4
			+4	95	5
		28	–10	100	11
			+4	94	9

Conditions: Samples preserved by adjustment to pH 3 with monochloroacetic acid buffer, and protected from light.

TABLE 6610:III. PRIMARY AND CONFIRMATORY CHROMATOGRAPHIC CONDITIONS AND RETENTION TIMES FOR SELECTED CARBAMATE PESTICIDES

	Retention Time		
Constituent	Primary System *min*	Confirmatory System *min*	Alternate System *min*
Aldicarb sulfoxide	6.80	8.5	14.94
Aldicarb sulfone	7.77	8.57	15.23

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Constituent	Retention Time Primary System *min*	Retention Time Confirmatory System *min*	Retention Time Alternate System *min*
Oxamyl	8.20	10.03	17.36
Methomyl	8.94	10.39	18.39
3-Hydroxycarbofuran	13.65	12.51	23.32
Aldicarb	16.35	14.11	27.01
Baygon	18.86	16.02	29.25
Carbofuran	19.17	16.33	29.61
Carbaryl	20.29	17.17	30.78
Methiocarb	24.74	20.45	34.93
BDMC (internal standard)	25.28	20.58	35.50

Primary conditions:

- Column: 150-mm-long × 3.9-mm-ID Waters NovaPak C18 (4 μm)
- Mobile phase: Linear gradient from 10:90 methanol:water, hold 2 min, then linear gradient to 80:20 methanol:water in 25 min
- Flow rate: 1.0 mL/min
- Injection volume: 500 μL
- Detector: Fluorescence; excitation 230 nm; emission >418 nm

Confirmatory conditions:

- Column: 250-mm-long × 4.6-mm-ID Supelco LC-1 (5 μm)
- Mobile phase: Linear gradient from 15:85 methanol:water to 100% methanol in 32 min
- Flow rate: 1.0 mL/min
- Injection volume: 500 μL
- Detector: Fluorescence; excitation 230 nm; emission >418 nm

Alternate conditions:

- Column: 250-mm-long × 4.6-mm-ID Beckman Ultrasphere ODS (5 μm)
- Mobile phase: Linear gradient from 15:85 methanol:water to 100% methanol in 32 min
- Flow rate: 1.0 mL/min
- Injection volume: 500 μL
- Detector: Fluorescence; excitation 230 nm; emission >418 nm

Post-column reactor condition for all detectors:

- Hydrolysis: NaOH (0.05*N*), flow rate 0.5 mL/min, 1.0-mL reaction coil at 95°C
- Derivatization: OPA solution, flow rate 0.5 mL/min, 1.0-mL reaction coil at ambient temperature

TABLE 6610:IV. MEAN RECOVERY, SINGLE-ANALYST STANDARD DEVIATION, AND OVERALL STANDARD DEVIATION FOR COLLABORATIVE STUDY DATA*

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TABLE 6610:IV. MEAN RECOVERY, SINGLE-ANALYST STANDARD DEVIATION, AND OVERALL STANDARD DEVIATION FOR COLLABORATIVE STUDY DATA*

	Reagent Water			
Compound	**Mean Recovery μg/L**	**Single-Analyst Standard Deviation μg/L**	**Overall Standard Deviation μg/L**	**Mean Recove μg/L**
Aldicarb	$0.926C + 0.202$	0.32†	$0.022\bar{X} + 0.370$‡	1.032
Aldicarb sulfone	$0.942C + 0.446$	$0.025\bar{X} + 0.382$	$0.062\bar{X} + 0.132$	0.968
Aldicarb sulfoxide	$0.941C + 0.876$§	$0.040\bar{X} + 0.103$	$0.058\bar{X} + 0.211$	0.952
Baygon	$0.916C + 0.360$	$0.040\bar{X} + 0.092$	$0.058\bar{X} + 0.230$	0.994
Carbaryl	$0.949C + 0.542$	$0.016\bar{X} + 0.480$	$0.058\bar{X} + 0.219$	0.958
Carbofuran	$0.923C + 0.636$	$0.022\bar{X} + 0.322$	$0.006\bar{X} + 0.564$‡	0.970
3-Hydroxycarbofuran	$0.940C + 0.438$	$0.013\bar{X} + 0.697$‡	$0.038\bar{X} + 0.578$	0.979
Methiocarb	$0.923C + 0.887$	$0.005\bar{X} + 1.839$	$0.035\bar{X} + 2.286$	0.958
Methomyl	$0.976C + 0.043$	$0.053\bar{X} + 0.069$	$0.048\bar{X} + 0.133$	0.988
Oxamyl	$0.936C + 0.659$	1.04†	$0.038\bar{X} + 0.699$	0.998

*C = true concentration, μg/L;
$\bar{X}$ = mean recovery, μg/L.
†Weighted linear regression equation had negative slope; average precision is reported.
‡Coefficient of determination of weighted equation was weak (<0.5).
§Lowest addition recovery not used for this regression.
Source: Edgell, K.W., L.A. Biederman J.E. Longbottom. 1991. Measurement of *n*-Methylcarbamoyloximes and *n*-Methylcarbamates in Water by Direct Aqueous Injection HPLCwith Post Column Derivatization: Collaborative Study. *J. Assoc. Offic. Anal. Chem.* 74:309.

TABLE 6630:I. RETENTION RATIOS OF VARIOUS ORGANOCHLORINE PESTICIDESRELATIVE TO ALDRIN

Liquid phase*	1.5% OV-17 + 1.95% QF-1	5% OV-210	3% OV-1	6% QF-1 + 4% SE-30
Column Temperature	200°C	180°C	180°C	200°C
Argon/methane carrier flow	60 mL/min	70 mL/min	70 mL/min	60 mL/min
Pesticide	RR	RR	RR	RR

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Liquid phase*	1.5% OV-17 + 1.95% QF-1	5% OV-210	3% OV-1	6% QF-1 + 4% SE-30
Column Temperature	**200°C**	**180°C**	**180°C**	**200°C**
Argon/methane carrier flow	**60 mL/min**	**70 mL/min**	**70 mL/min**	**60 mL/min**
Pesticide	**RR**	**RR**	**RR**	**RR**
α-BHC	0.54	0.64	0.35	0.49
PCNB	0.68	0.85	0.49	0.63
Lindane (γ-BHC)	0.69	0.81	0.44	0.60
Dichloran	0.77	1.29	0.49	0.70
Heptachlor	0.82	0.87	0.78	0.83
Aldrin	1.00	1.00	1.00	1.00
Heptachlor epoxide	1.54	1.93	1.28	1.43
Endosulfan I	1.95	2.48	1.62	1.79
p,p′-DDE	2.23	2.10	2.00	1.82
Dieldrin	2.40	3.00	1.93	2.12
Captan	2.59	4.09	1.22	1.94
Endrin	2.93	3.56	2.18	2.42
o,p′-DDT	3.16	2.70	2.69	2.39
p,p′-DDD	3.48	3.75	2.61	2.55
Endosulfan II	3.59	4.59	2.25	2.72
p,p′-DDT	4.18	4.07	3.50	3.12
Mirex	6.1	3.78	6.6	4.79
Methoxychlor	7.6	6.5	5.7	4.60
Aldrin (Min absolute)	3.5	2.6	4.0	5.6

* All columns glass, 180 cm × 4 mm ID, solid support Gas-Chrom Q (100/200 mesh).

TABLE 6630:II. PRECISION AND BIAS DATA FOR SELECTED ORGANOCHLORINE PESTICIDES

Pesticide	Level Added *ng/L*	Pretreatment	Mean Recovery *ng/L*	Recovery %	Precision* *ng/L* S_T	S_O

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Pesticide	Level Added *ng/L*	Pretreatment	Mean Recovery *ng/L*	Recovery %	Precision* *ng/L* S_T	S_o
Aldrin	15	No cleanup	10.42	69	4.86	2.59
	110		79.00	72	32.06	20.19
	25	Cleanup†	17.00	68	9.13	3.48‡
	100		64.54	65	27.16	8.02‡
Lindane (γ-BHC)	10	No cleanup	9.67	97	5.28	3.47
	100		72.91	73	26.23	11.49‡
	15	Cleanup†	14.04	94	8.73	5.20
	85		59.08	70	27.49	7.75‡
Dieldrin	20	No cleanup	21.54	108	18.16	17.92
	125		105.83	85	30.41	21.84
	25	Cleanup	17.52	70	10.44	5.10‡
	130		84.29	65	34.45	16.79‡
DDT	40	No cleanup	40.30	101	15.96	13.42
	200		154.87	77	38.80	24.02
	30	Cleanup†	35.54	118	22.62	22.50
	185		132.08	71	49.83	25.31

* S_T = overall precision and S_o = single-operator precision.
† Use of magnesia-silica gel column cleanup before analysis.
‡ $S_o < S_T/2$.

TABLE 6630:III. CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LIMITS*

Compound	Retention Time *min* Column 1	Column 2	Method Detection Limit *μg/L*
α-BHC	1.35	1.82	0.003

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Compound	Retention Time *min*		Method Detection Limit *μg/L*
	Column 1	Column 2	
γ-BHC	1.70	2.13	nd
β-BHC	1.90	1.97	nd
Heptachlor	2.00	3.35	0.003
δ-BHC	2.15	2.20	0.009
Aldrin	2.40	4.10	0.004
Heptachlor epoxide	3.50	5.00	0.083
Endosulfan I	4.50	6.20	0.014
4,4′-DDE	5.13	7.15	0.004
Dieldrin	5.45	7.23	0.002
Endrin	6.55	8.10	0.006
4,4′-DDD	7.83	9.08	0.011
Endosulfan II	8.00	8.28	0.004
4,4′-DDT	9.40	11.75	0.012
Endrin aldehyde	11.82	9.30	0.023
Endosulfan sulfate	14.22	10.70	0.066
Chlordane	mr	mr	0.014
Toxaphene	mr	mr	0.24
PCB-1016	mr	mr	nd
PCB-1221	mr	mr	nd
PCB-1232	mr	mr	nd
PCB-1242	mr	mr	0.065
PCB-1248	mr	mr	nd
PCB-1254	mr	mr	nd
PCB-1260	mr	mr	nd

Column 1 conditions: Supelcoport (100/120 mesh) coated with 1.5% SP-2250/1.95%SP-2401 packed in a 1.8 m long × 4 mm ID glass column with 5% methane/95% argoncarrier gas at 60 mL/min flow rate. Column temperature held isothermal at 200°C, exceptfor PCB-1016 through PCB-1248 at 160°C.

Column 2 conditions: Supelcoport (100/120 mesh) coated with 3% OV-1 packed in a1.8 m long × 4 mm ID glass column with 5% methane/95% argon carrier gas at 60mL/min flow rate. Column temperature held isothermal at 200°C for the pesticides; at140°C for PCB-1221 and 1232; and at 170°C for PCB-1016 and 1242 to 1268.

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*mr = multiple peak response. See Figure 6630:2, Figure 6630:3, Figure 6630:4, Figure 6630:5, Figure 6630:6, Figure 6630:7, Figure 6630:8, Figure 6630:9, Figure 6630:10.
nd = not determined.

TABLE 6630:IV. DISTRIBUTION OF CHLORINATED PESTICIDES AND PCBS INTO MAGNESIA-SILICA GEL COLUMN FRACTIONS[5]

Compound	Recovery by Fraction* %		
	1	2	3
Aldrin	100	—	—
α-BHC	100	—	—
β-BHC	97	—	—
δ-BHC	98	—	—
γ-BHC	100	—	—
Chlordane	100	—	—
4,4′-DDD	99	—	—
4,4′-DDE	98	—	—
4,4′-DDT	100	—	—
Dieldrin	0	100	—
Endosulfan I	37	64	—
Endosulfan II	0	7	91
Endosulfan sulfate	0	0	106
Endrin	4	96	—
Endrin aldehyde	0	68	26
Heptachlor	100	—	—
Heptachlor epoxide	100	—	—
Toxaphene	96	—	—
PCB-1016	97	—	—
PCB-1221	97	—	—
PCB-1232	95	4	—
PCB-1242	97	—	—
PCB-1248	103	—	—
PCB-1254	90	—	—
PCB-1260	95	—	—

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* Eluent composition:
Fraction 1–6% ethyl ether in hexane
Fraction 2–15% ethyl ether in hexane
Fraction 3–50% ethyl ether in hexane

TABLE 6630:V. QC ACCEPTANCE CRITERIA*

Compound	Test Conc. μg/L	Limit for s μg/L	Range for $\bar{X}$ μg/L	Range for $\bar{P}$, P_S %
Aldrin	2.0	0.42	1.08–2.24	42–122
α-BHC	2.0	0.48	0.98–2.44	37–134
β-BHC	2.0	0.64	0.78–2.60	17–147
δ-BHC	2.0	0.72	1.01–2.37	19–140
γ-BHC	2.0	0.46	0.86–2.32	32–127
Chlordane	50	10.0	27.6–54.3	45–119
4,4′-DDD	10	2.8	4.8–12.6	31–141
4,4′-DDE	2.0	0.55	1.08–2.60	30–145
4,4′-DDT	10	3.6	4.6–13.7	25–160
Dieldrin	2.0	0.76	1.15–2.49	36–146
Endosulfan I	2.0	0.49	1.14–2.82	45–153
Endosulfan II	10	6.1	2.2–17.1	D–202
Endosulfan sulfate	10	2.7	3.8–13.2	26–144
Endrin	10	3.7	5.1–12.6	30–147
Heptachlor	2.0	0.40	0.86–2.00	34–111
Heptachlor epoxide	2.0	0.41	1.13–2.63	37–142
Toxaphene	50	12.7	27.8–55.6	41–126
PCB-1016	50	10.0	30.5–51.5	50–114
PCB-1221	50	24.4	22.1–75.2	15–178
PCB-1232	50	17.9	14.0–98.5	10–215
PCB-1242	50	12.2	24.8–69.6	39–150
PCB-1248	50	15.9	29.0–70.2	38–158
PCB-1254	50	13.8	22.2–57.9	29–131
PCB-1260	50	10.4	18.7–54.9	8–127

*s = standard deviation of four recovery measurements,
$\bar{X}$ = average recovery for four recovery measurements,

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P, P_S = percent recovery measured, and

D = detected; result must be greater than zero.

NOTE: These criteria are based directly on the method performance data in Table 6630:VI. Where necessary, the limits for recovery were broadened to assure applicability ofthe limits to concentrations below those used to develop Table 6630:VI.

TABLE 6630:VI. METHOD PRECISION AND BIAS AS FUNCTIONS OF CONCENTRATION*

Compound	Bias, as Recovery, X' µg/L	Single-Analyst Precision, s_r µg/L	Overall Precision, S' µg/L
Aldrin	$0.81C + 0.04$	$0.16\bar{X} - 0.04$	$0.20\bar{X} - 0.01$
α-BHC	$0.84C + 0.03$	$0.13\bar{X} + 0.04$	$0.23\bar{X} - 0.00$
β-BHC	$0.81C + 0.07$	$0.22\bar{X} - 0.02$	$0.33\bar{X} - 0.05$
δ-BHC	$0.81C + 0.07$	$0.18\bar{X} + 0.09$	$0.25\bar{X} + 0.03$
γ-BHC	$0.82C - 0.05$	$0.12\bar{X} + 0.06$	$0.22\bar{X} + 0.04$
Chlordane	$0.82C - 0.04$	$0.13\bar{X} + 0.13$	$0.18\bar{X} + 0.18$
4,4′-DDD	$0.84C + 0.30$	$0.20\bar{X} - 0.18$	$0.27\bar{X} - 0.14$
4,4′-DDE	$0.85C + 0.14$	$0.13\bar{X} + 0.06$	$0.28\bar{X} - 0.09$
4,4′-DDT	$0.93C - 0.13$	$0.17\bar{X} + 0.39$	$0.31\bar{X} - 0.09$
Dieldrin	$0.90C + 0.02$	$0.12\bar{X} + 0.19$	$0.16\bar{X} + 0.16$
Endosulfan I	$0.97C + 0.04$	$0.10\bar{X} + 0.07$	$0.18\bar{X} + 0.08$
Endosulfan II	$0.93C + 0.34$	$0.41\bar{X} - 0.65$	$0.47\bar{X} - 0.20$
Endosulfan sulfate	$0.89C - 0.37$	$0.13\bar{X} + 0.33$	$0.24\bar{X} + 0.35$
Endrin	$0.89C - 0.04$	$0.20\bar{X} + 0.25$	$0.24\bar{X} + 0.25$
Heptachlor	$0.69C + 0.04$	$0.06\bar{X} + 0.13$	$0.16\bar{X} + 0.08$
Heptachlor epoxide	$0.89C + 0.10$	$0.18\bar{X} - 0.11$	$0.25\bar{X} - 0.08$
Toxaphene	$0.80C + 1.74$	$0.09\bar{X} + 3.20$	$0.20\bar{X} + 0.22$
PCB-1016	$0.81C + 0.50$	$0.13\bar{X} + 0.15$	$0.15\bar{X} + 0.45$
PCB-1221	$0.96C + 0.65$	$0.29\bar{X} - 0.76$	$0.35\bar{X} - 0.62$
PCB-1232	$0.91C + 10.79$	$0.21\bar{X} - 1.93$	$0.31\bar{X} + 3.50$
PCB-1242	$0.93C + 0.70$	$0.11\bar{X} + 1.40$	$0.21\bar{X} + 1.52$
PCB-1248	$0.97C + 1.06$	$0.17\bar{X} + 0.41$	$0.25\bar{X} - 0.37$
PCB-1254	$0.76C + 2.07$	$0.15\bar{X} + 1.66$	$0.17\bar{X} + 3.62$
PCB-1260	$0.66C + 3.76$	$0.22\bar{X} - 2.37$	$0.37\bar{X} - 4.86$

*X' = expected recovery for one or more measurements of a sample containing a concentration of C,

s_r = expected single-analyst standard deviation of measurements at an average concentration found of $\bar{X}$,

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S′ = expected interlaboratory standard deviation of measurements at an average concentration found of $\bar{X}$,
C = true value for the concentration, and
$\bar{X}$ = average recovery found for measurements of samples containing a concentration of C.

TABLE 6640:I. METHOD DETECTION LEVELS*

Compound	Amount Added µg/L	Average Recovery %	Mean Amount Found µg/L	Standard Deviation µg/L	Relative Standard Deviation %	Method Detection Level µg/L
Dalapon	2.0	100	2.00	0.031	1.56	0.1
Dicamba	0.4	122	0.48	0.007	1.55	0.02
2,4-D	2.0	98.1	1.9	0.036	1.85	0.1
Pentachlorophenol	0.2	101	0.20	0.004	2.40	0.02
2,4,5-TP	0.2	119	0.24	0.003	1.58	0.01
2,4,5-T	0.2	97.8	0.20	0.005	2.73	0.02
Dinoseb	0.4	111	0.44	0.012	2.86	0.04
Bentazon	0.4	94.6	0.38	0.032	8.68	0.01
Picloram	0.4	123	0.49	0.011	2.29	0.04
Surrogate				11.3	5.92	
Internal standard				3.74	1.18	

* Based on the analysis of seven portions with known additions.[1]

TABLE 6640:II. ANALYTICAL STANDARDS

Compound*	Molecular Weight	Melting Point
Dalapon	142.97	90–92/14 mm†
Dicamba	221.04	114–116
2,4-D	221.04	136–140
Pentachlorophenol (PCP)	266.34	188–191
2,4,5-TP (silvex)	269.51	175–177
2,4,5-T	255.49	154–158
Dinoseb	240.22	38–42
Bentazon	240.28	137–139
Picloram	241.48	218–219
Internal standard (TCP) 1,2,3-trichloropropane	147.43	–14
Surrogate (TFBA) 2,3,5,6-tetrafluorobenzoic acid	194.09	150–152

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* AccuStandard, Inc., New Haven, Conn.
† Boiling point/pressure.

TABLE 6640:III. SUGGESTED HERBICIDE SOLUTION CONCENTRATIONS

Compound	Stock* Conc µL/mL	Stock† Amount Added µL	Stock‡ Herbicide Mix µg/mL	Herbicide§ Addition Mix µg/mL
Dalapon	5000	200	100	10
Dicamba	5000	40	20	2
2,4-D	5000	200	100	10
Pentachlorophenol	5000	20	10	1
2,4,5-TP	5000	20	10	1
2,4,5-T	5000	20	10	1
Dinoseb	5000	40	20	2
Bentazon	5000	40	20	2
Picloram	5000	40	20	2

* Individual stock solutions.
† Amount of individual stock added to 10 mL of methanol to make the stock herbicide mix.
‡ Concentration of each compound in stock herbicide mix.
§ Herbicide addition mix is made by adding 200 µL stock herbicide mix to 2 mL methanol.

TABLE 6640:IV. SHORT-RANGE CALIBRATION STANDARDS*

Compound	Level 1 µg/L	Level 2 µg/L	Level 3 µg/L
Dalapon	1.0	2.0	4.0
Dicamba	0.2	0.4	0.8
2,4-D	1.0	2.0	4.0
Pentachlorophenol	0.1	0.2	0.4
2,4,5-TP	0.1	0.2	0.4
2,4,5-T	0.1	0.2	0.4
Dinoseb	0.2	0.4	0.8
Bentazon	0.2	0.4	0.8
Picloram	0.2	0.4	0.8

* Levels 1, 2, and 3 are prepared by adding 2 µL, 4 µL, and 8 µL of herbicide addition mix, respectively, into 2 mL MtBE.

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TABLE 6640:V. RETENTION TIMES

Compound	Retention Time *min* DB-1701 Column	DB-5 Column
Methyl ester of dalapon	10.32	9.34
Internal standard (TCP)	11.58	10.37
Methyl ester of surrogate (TFBA)	13.20	12.51
4-Nitroanisole	17.87	17.22
Methyl-3,5-dichlorobenzoate	20.25	17.32
Methyl ester of dicamba	20.80	18.60
Methyl ester of 2,4-D	25.08	21.26
Pentachloroanisole	25.64	23.82
Methyl ester of 2,4,5-TP (silvex)	28.33	24.73
Methyl ester of 2,4,5-T	30.52	25.72
Methyl ester of dinoseb	34.27	28.05
Methyl ester of bentazon	35.04	29.2
Methyl ester of picloram	38.39	31.40

TABLE 6640:VI. SINGLE-LABORATORY BIAS AND PRECISION DATA,* LEVEL 1 ADDITION REAGENT WATER

Compound	Added Amount µg/L	Mean Recovery %	Mean Amount Found µg/L	Standard Deviation µg/L	Relative Standard Deviation %
Dalapon	10	114	11	0.72	6.4
Dicamba	1.0	84	0.83	0.02	2.6
2,4-D	5.0	75	4.0	0.22	5.5
Pentachlorophenol	2.0	107	2.1	0.04	1.7
2,4,5-TP	0.5	107	0.5	0.02	3.0
2,4,5-T	0.5	104	0.50	0.02	3.7
Dinoseb	2.0	90	1.8	0.1	5.4
Bentazon	1.0	84	0.85	0.020	2.3
Picloram	2.0	80	1.60	0.11	7.0

* Based on analysis of four portions of reagent water with known additions.

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TABLE 6640:VII. SINGLE-LABORATORY BIAS AND PRECISION DATA,* LEVEL 2 ADDITION REAGENT WATER

Compound	Added Amount μg/L	Mean Recovery %	Mean Amount Found μg/L	Standard Deviation μg/L	Relative Standard Deviation %
Dalapon	30	99.3	29.8	0.21	0.71
Dicamba	3.0	92.2	2.77	0.06	2.2
2,4-D	15	90.5	13.6	0.15	1.10
Pentachlorophenol	6.0	95.8	5.75	0.09	1.5
2,4,5-TP	1.5	98.5	1.48	0.02	1.3
2,4,5-T	1.5	86.4	1.30	0.02	1.3
Dinoseb	6.0	90.0	5.40	0.03	5.1
Bentazon	3.0	103	3.09	0.05	1.6
Picloram	6.0	86.0	5.16	0.18	3.5

* Based on analysis of four portions of reagent water with known additions.

TABLE 6640:VIII. LABORATORY PERFORMANCE CHECK SOLUTION

Test	Test Compound	Concentration μg/mL	Requirements
Sensitivity	Dinoseb	0.004	Detection of compound S/N > 3
Chromatographic performance	4-Nitrophenol	1.6	0.70 <PGF<1.05*
Column performance	3,5-Dichlorobenzoic acid	0.6	Resolution >0.40†
	4-Nitrophenol	1.6	

* PGF = peak Gaussian factor. Calculate using the equation:

$$PGF = \frac{1.83 \times W(1/2)}{W(1/10)}$$

where W(1/2) is the peak width at half height and W(1/10) is the peak width at tenth height.

† Resolution between the two peaks as defined by the equation:

$$R = \frac{t}{W}$$

where t is the difference in elution times between the two peaks and W is the average peak width, at the base line, of the two peaks.

Source: U.S. ENVIRONMENTAL PROTECTION AGENCY. 1989. Determination of Chlorinated Acids by Gas Chromatography with an Electron Capture Detector. Method 515.1, rev.4.0. Environmental Monitoring Systems Lab., Off. Research & Development, Cincinnati, Ohio.

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TABLE 6640:IX. SAMPLE MATRIX ADDITION RECOVERIES*

Compound	Added Amount µg/L	Source 1 Amount Found µg/L	Source 1 % Found	Source 2 Amount Found µg/L	Source 2 % Found	Treated Source Amount Found µg/L	Treated Source % Found
Dalapon	10	11.6	116	12.1	121	12.5	125
Dicamba	1.0	0.9	91	0.9	94	0.8	83
2,4-D	5.0	4.0	80	4.4	88	4.3	86
Pentachlorophenol	2.0	2.2	110	2.3	113	2.1	105
2,4,5-TP	0.5	0.5	106	0.6	114	0.6	114
2,4,5-T	0.5	0.5	104	0.6	120	0.6	128
Dinoseb	2.0	2.1	105	2.2	110	1.9	94
Bentazon	1.0	0.9	86	0.9	94	0.9	87
Picloram	2.0	1.5	77	1.8	88	2.3	115

* Source 1 is Colorado River water, Source 2 is California State Project Water, and the Treated Source water is the chlorinated effluent from the Weymouth Filtration Plant,La Verne, Calif.

TABLE 6640:X. INTERLABORATORY QUALITY CONTROL COMPARISON Data*

Sample No.	Compound	Microextraction Results µg/L	True Value µg/L	Acceptance Limits† µg/L
1	2,4-D	32.8	46.3	15.1–59.1
	Silvex	20.4	18.1	7.47–24.4
2	Dalapon	10.58	12.3	DL–18.4
	Pentachlorophenol	2.28	2.35	DL–3.92
	Dinoseb	10.9	8.63	DL–11.9
	Picloram	11.0	6.88	DL–12.5

* Source: U.S. ENVIRONMENTAL PROTECTION AGENCY, Water Supply Study Performance Evaluation Study 27 (WS027), February 4, 1991.
† DL = detection limit.

TABLE 6640:XI. RESULTS FOR LABORATORY QUALITY CONTROL WITH LABORATORY FORTIFIED BLANK

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TABLE 6640:XI. RESULTS FOR LABORATORY QUALITY CONTROL WITH LABORATORY FORTIFIED BLANK

Compound	Addition Amount µg/L	Amount Found µg/L	Recovery %
Dalapon	10	9.6	96
Dicamba	1.0	0.82	82
2,4-D	5.0	3.4	68
Pentachlorophenol	2.0	2.1	105
2,4,5-TP	0.5	0.51	102
2,4,5-T	0.5	0.38	76
Dinoseb	2.0	1.70	85
Bentazon	1.0	0.82	82
Picloram	2.0	1.51	76

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Endnotes

1 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
2 (Popup - Footnote)
* Dionex P/N 37041 or equivalent.
3 (Popup - Footnote)
† Dionex P/N 37042 or equivalent.
4 (Popup - Footnote)
‡ Dionex P/N 037072 (micro membrane—high capacity/low volume—suppressor) or equivalent.
5 (Popup - Footnote)
Water dip occurs because water conductivity in sample is less than eluent conductivity (eluent is diluted by water).
6 (Popup - Footnote)
* Waters P/N 84560 or equivalent.
7 (Popup - Footnote)
† Waters P/N 07355 or equivalent.
8 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
9 (Popup - Footnote)
*APPROVED BY STANDARD METHODS COMMITTEE, 1997.
10 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
11 (Popup - Footnote)
* Waters Corp. or equivalent.
12 (Popup - Footnote)
* Waters Corp. or equivalent.
13 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1993.
14 (Popup - Footnote)
* Vycor, manufactured by Corning Glass Works, or equivalent.
15 (Popup - Footnote)
† Eastman No. 1179 or equivalent.
16 (Popup - Footnote)
‡ Whatman No. 30 or equivalent.
17 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
18 (Popup - Footnote)
* Teflon or equivalent.
19 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
20 (Popup - Footnote)

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* See also Alkalinity, Section 2320.
21 (Popup - Footnote)
† Copies of the nomographs in Figure 4500-CO_2:1–4, enlarged to several times the size shown here, may be obtained as a set from Standard Methods Manager, The American Water Works Association, 6666 West Quincy Ave., Denver, CO 80235.
22 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
23 (Popup - Footnote)
* Orion Model 94-06A or equivalent.
24 (Popup - Footnote)
* Orion Model 95-10, EIL Model 8002-2, Beckman Model 39565, or equivalent.
25 (Popup - Footnote)
* Amberlite® XAD-7, or equivalent.
26 (Popup - Footnote)
* Teflon or equivalent.
27 (Popup - Footnote)
† MICRO DIST, Lachat Instruments, Milwaukee, WI.
28 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1993.
29 (Popup - Footnote)
* Titration may be delayed up to 10 min without appreciable error if H_3PO_4 is not added until immediately before titration.
30 (Popup - Footnote)
* Kimax 17110-F, 5 mL, Kimble Products, Box 1035, Toledo, OH, or equivalent.
31 (Popup - Footnote)
* Eastman chemical No. 7102 or equivalent.
32 (Popup - Footnote)
† Available from Gallard-Schlesinger Chemical Mfg. Corp., 584 Mineola Avenue, Carle Place, NY 11514, or equivalent.
33 (Popup - Footnote)
* Aldrich No. 17, 753-9, Aldrich Chemical Company, Inc., 1001 West St. Paul Ave., Milwaukee, WI 53233, or equivalent.
34 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
35 (Popup - Footnote)
* Brij 35, available from ICI Americas, Wilmington, DE, or equivalent.
36 (Popup - Footnote)
* Teflon or equivalent.
37 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1993.

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38 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
39 (Popup - Footnote)
* Also known as 1,2 cyclohexylenedinitrilotetraacetic acid.
40 (Popup - Footnote)
* J.T. Baker Catalog number J-112 or equivalent.
41 (Popup - Footnote)
† Brij-35, available from ICI Americas, Wilmington, DE, or equivalent.
42 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1996.
43 (Popup - Footnote)
† p designates $-\log_{10}$ of a number.
44 (Popup - Footnote)
* Teflon or equivalent.
45 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
46 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
47 (Popup - Footnote)
* Eastman chemical No. 3651 or equivalent.
48 (Popup - Footnote)
† Oxone, E.I. duPont de Nemours and Co., Inc., Wilmington, DE, or equivalent.
49 (Popup - Footnote)
* Triton X-100, Catalog No. T9284, Sigma-Aldrich Corp., P.O. Box 14508, St. Louis, MO 63178.
50 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
51 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
52 (Popup - Footnote)
* 18-415, Comar, Inc., Vineland, NJ, or equivalent.
53 (Popup - Footnote)
† Brij-35, available from ICI Americas, Inc., Wilmington, DE, or equivalent.
54 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
55 (Popup - Footnote)
* Orion Model 95-12, EIL Model 8002-2, Beckman Model 39565, or equivalent.
56 (Popup - Footnote)
* Brij-35, available from ICI Americas, Wilmington, DE.
57 (Popup - Footnote)
* Teflon or equivalent.

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58 (Popup - Footnote)
† Clorox, The Clorox Company, Pleasanton, CA, or equivalent.
59 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1993.
60 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
61 (Popup - Footnote)
* Orion Model 90-02, or equivalent.
62 (Popup - Footnote)
† Orion Model 93-07, Corning Model 476134, or equivalent.
63 (Popup - Footnote)
* Tudor Scientific Glass Co., 555 Edgefield Road, Belvedere, SC 29841, Cat. TP-1730, or equivalent.
64 (Popup - Footnote)
† EM Laboratories, Inc., 500 Exec. Blvd., Elmsford, NY, Cat. 2001, or equivalent.
65 (Popup - Footnote)
* Brij-35, available from ICI Americas, Inc., Wilmington, DE, or equivalent.
66 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
67 (Popup - Footnote)
* Rotary kjeldahl digestion unit, Kontes, Model 551000-0000, or equivalent.
68 (Popup - Footnote)
† ASTM E-147 or equivalent.
69 (Popup - Footnote)
* Regular Clorox, The Clorox Company, Pleasanton, CA, or equivalent.
70 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1993.
71 (Popup - Footnote)
* Fundamentally, the current is directly proportional to the activity of molecular oxygen.[7]
72 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
73 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
74 (Popup - Footnote)
* GFS Chemical Co., Columbus, OH, or equivalent.
75 (Popup - Footnote)
* Whatman No. 42 or equivalent.
76 (Popup - Footnote)
† Darco G60 or equivalent.
77 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.

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78 (Popup - Footnote)
* Millipore or equivalent.
79 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
80 (Popup - Footnote)
* Eastman No. 360 has been found satisfactory.
81 (Popup - Footnote)
* Teflon or equivalent.
82 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
83 (Popup - Footnote)
* Eastman catalog No. 5672 has been found satisfactory for this purpose.
84 (Popup - Footnote)
* Orion 941600 or equivalent.
85 (Popup - Footnote)
† Orion 948201 or equivalent.
86 (Popup - Footnote)
‡ EG&G Princeton Applied Research K0066, K0060, G0028, or equivalent.
87 (Popup - Footnote)
* Lachat Instruments MICRO DIST or equivalent.
88 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1996.
89 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
90 (Popup - Footnote)
* ‘‘Desicote’’ (Beckman), or equivalent.
91 (Popup - Footnote)
* Constant weight is defined as a change of not more than 0.5 mg in two successive operations consisting of heating, cooling in desiccator, and weighing.
92 (Popup - Footnote)
* Hach 2100 A.
93 (Popup - Footnote)
* Ion-exchange resin Bio-Rex 70, 20-50 mesh, sodium form, available from Bio-Rad Laboratories, Richmond, CA 94804, or equivalent.
94 (Popup - Footnote)
† Eastman Organic Chemicals, Rochester, NY 14615. No. 8068 3′,3″ bis [*N,N*-bis(carboxymethyl)-aminolmethyl] thymolsulfonphthalein pentasodium salt.
95 (Popup - Footnote)
* Aldrich 24, 511-9, or equivalent.
96 (Popup - Footnote)
† BioRex 70 or equivalent.

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97 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
98 (Popup - Footnote)
* Nitrification Inhibitor, Formula 2533, Hach Co., Loveland, CO, or equivalent.
99 (Popup - Footnote)
* Some analysts have reported satisfactory results with 2-chloro-6-(trichloromethyl) pyridine (Nitrification Inhibitor, Formula 2533, Hach Co., Loveland, CO, or equivalent).
100 (Popup - Footnote)
† Wheaton 2-L BOD bottle No. 227580, 1000 North Tenth St., Millville, NJ, or equivalent.
101 (Popup - Footnote)
* Formula 2533, Hach Chemical Co., Loveland, CO, or equivalent. NOTE: Some commercial formulations are not pure TCMP. Check with supplier to verify compound purity and adjust dosages accordingly.
102 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
103 (Popup - Footnote)
* GFS Chemicals, Inc., Columbus, OH, or equivalent.
104 (Popup - Footnote)
* Hach Co., Bioscience, Inc., or equivalent.
105 (Popup - Footnote)
*APPROVED BY STANDARD METHODS COMMITTEE, 1996.
106 (Popup - Footnote)
*NOTE: If mercuric nitrate is used to complex the chloride, use an appropriate disposal method for the treated waste to prevent mercury contamination.
107 (Popup - Footnote)
†Data may be obtained from *Standard Methods* manager, American Water Works Association.
108 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
109 (Popup - Footnote)
* Westvaco or Calgon Filtrasorb 400 or equivalent.
110 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1993.
111 (Popup - Footnote)
† ‘‘Solubility’’ is here used as a general description of whether or not the material can be uniformly dispersed in an aqueous phase rather than as an expression of equilibrium between a pure solute and its aqueous solution.
112 (Popup - Footnote)
* Whatman No. 1 or equivalent.
113 (Popup - Footnote)
† Whatman pre-swollen microgranular DE 52 or DE 51, or equivalent.
114 (Popup - Footnote)

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* Pump parts may be of stainless steel or TFE.
115 (Popup - Footnote)
† XAD-7 or equivalent.
116 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
117 (Popup - Footnote)
* Teflon or equivalent.
118 (Popup - Footnote)
† Whatman No. 40 or equivalent.
119 (Popup - Footnote)
* Teflon or equivalent.
120 (Popup - Footnote)
† Whatman No. 40 or equivalent.
121 (Popup - Footnote)
* Whatman No. 40 or equivalent.
122 (Popup - Footnote)
† Hyflo Super-Cel, Manville Corp., or equivalent.
123 (Popup - Footnote)
* Whatman No. 40 or equivalent.
124 (Popup - Footnote)
† Davidson Grade 923 or equivalent.
125 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1993.
126 (Popup - Footnote)
* Corning No. 3360 or equivalent.
127 (Popup - Footnote)
* 15-mL Corning No. 36060 or equivalent.
128 (Popup - Footnote)
† For NPDES permit analyses, pH 10 ± 0.1 is required.
129 (Popup - Footnote)
*APPROVED BY STANDARD METHODS COMMITTEE, 1993.
130 (Popup - Footnote)
* For sources of suitable reference material, contact *Standard Methods* manager.
131 (Popup - Footnote)
† Eastman No. P573 or equivalent.
132 (Popup - Footnote)
* Bio-Rad, AGl-X2, or equivalent.
133 (Popup - Footnote)
† Bio-Rad AG 50W-X8, or equivalent.
134 (Popup - Footnote)
‡ For sources of suitable reference material, contact *Standard Methods* manager.

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135 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1993.
136 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1996.
137 (Popup - Footnote)
* Ascarite or equivalent.
138 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1994.
139 (Popup - Footnote)
* Milli-Q, Millipore Corp., or equivalent.
140 (Popup - Footnote)
* Federal regulations may require a simple sum in terms of mass units/L.
141 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1994.
142 (Popup - Footnote)
* Whatman grade 934AH; Gelman type A/E; Millipore type AP40; ED Scientific Specialties grade 161; or other products that give demonstrably equivalent results. Practical filter diameters are 2.2 to 4.7 cm.
143 (Popup - Footnote)
* Pierce 13075 or equivalent.
144 (Popup - Footnote)
† Pierce 12722 or equivalent.
145 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
146 (Popup - Footnote)
* Model CLS 1, Tekmar, Cincinnati, OH; Brechbuhler AG, 8952 Schlieren ZH, Switzerland, available from Chromapon, Whittier, CA; or equivalent.
147 (Popup - Footnote)
† Rotulex Sovirel, Brechbuhler AG or equivalent.
148 (Popup - Footnote)
‡ Brechbuhler AG or equivalent.
149 (Popup - Footnote)
§ Metal Bellows Model MB-21, Sharon, MA, or equivalent.
150 (Popup - Footnote)
‖ Swagelok fittings or equivalent.
151 (Popup - Footnote)
Brechbuhler AG, Chromapon, Inc., or equivalent.
152 (Popup - Footnote)
** Hamilton Model 1805N electrotapered tip, Reno, NV.
153 (Popup - Footnote)
†† Pierce Chemical Company #13100 or equivalent.

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154 (Popup - Footnote)
‡‡ 1-Chlorohexadecane and 1-chlorooctadecane solidify upon refrigeration. Warm before removing a portion.
155 (Popup - Footnote)
§§ Geosmin and 2-methylisoborneol are available from Wako Bioproducts, Wako Chemicals USA, Inc., 1600 Bellwood Rd., Richmond, VA 23237, or Dalton Chemical Lab, Inc., 4700 Keele St., Suite 119, FARQ, North York, Ontario, Canada M3J 1P3. NOTE: This synthetic geosmin is racemic and includes (+)-geosmin, which has an odor intensity different from that of the natural (−) compound. This precludes its use in quantitative sensory analysis; however, its GC/MS characteristics (i.e., retention time and spectrum) are the same as those of natural geosmin.
156 (Popup - Footnote)
‖‖ Millipore Milli-QUV Plus or equivalent.
157 (Popup - Footnote)
A total sample volume of 900 mL is preferred to minimize foaming-over due to salt addition.
158 (Popup - Footnote)
*** NOTE: Because synthetic labeled geosmin is racemic, best results for the compensation of prolonged biological processes are achieved by monitoring the degradation of natural (−)-geosmin using (−)-geosmin-d_3.
159 (Popup - Footnote)
††† Calibration mark needs to be verified periodically.
160 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1997.
161 (Popup - Footnote)
** Tekmar VOCARB 4000 or equivalent.
162 (Popup - Footnote)
† Gas chromatographic methods are extremely sensitive to the materials used. Mention of trade names by *Standard Methods* does not preclude the use of other existing or as-yet- undeveloped products that give *demonstrably* equivalent results.
163 (Popup - Footnote)
‡ Supelco, Inc. or equivalent.
164 (Popup - Footnote)
§ J&W or equivalent.
165 (Popup - Footnote)
‖ Luerlok or equivalent.
166 (Popup - Footnote)
Hamilton # 702 or equivalent
167 (Popup - Footnote)
** Millipore Super Q or equivalent.
168 (Popup - Footnote)
†† Tygon or equivalent.

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169 (Popup - Footnote)
* Tracor Model 703 or equivalent.
170 (Popup - Footnote)
† Luerlok or equivalent.
171 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1996.
172 (Popup - Footnote)
* Marketed under the following trade names: ‘‘Explosimeter,’’ ‘‘Methane Gas Detector,’’ and ‘‘Methane Tester,’’ all manufactured by Mine Safety Appliance Co., Pittsburgh, PA 15235, and ‘‘J-W Combustible Gas Indicator,’’ manufactured by Bacharach Instrument Co., Mountain View, CA 94043, or equivalent.
173 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1993.
174 (Popup - Footnote)
* Pierce #13075 or equivalent.
175 (Popup - Footnote)
† Pierce #12722 or equivalent.
176 (Popup - Footnote)
‡ Varian #96-000099-00 or equivalent.
177 (Popup - Footnote)
§ Hamilton 702N or equivalent.
178 (Popup - Footnote)
‖ Gas chromatographic methods are extremely sensitive to the materials used. Mention of trade names by *Standard Methods* does not preclude the use of other existing or as-yet-undeveloped products that give *demonstrably* equivalent results.
179 (Popup - Footnote)
Durawax-DX3, 0.25-µm film, or equivalent.
180 (Popup - Footnote)
** DB-1, 0.25-µm film, or equivalent.
181 (Popup - Footnote)
†† Burdick and Jackson #216 or equivalent.
182 (Popup - Footnote)
‡‡ Such as that available from Aldrich Chemical Company.
183 (Popup - Footnote)
§§ Such as that available from AMVAC Chemical Corporation, Los Angeles, CA.
184 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1993.
185 (Popup - Footnote)
* Chromatographic methods are extremely sensitive to the materials used. Mention of trade names by *Standard Methods* does not preclude the use of other existing or as-yet-undeveloped

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products that give *demonstrably* equivalent results.
186 (Popup - Footnote)
† J&W Scientific.
187 (Popup - Footnote)
† J&W Scientific.
188 (Popup - Footnote)
‡ Supelco.
189 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1994.
190 (Popup - Footnote)
* Wheaton: Industrial Glassware, Millville, NJ; or equivalent.
191 (Popup - Footnote)
† Kontes or equivalent.
192 (Popup - Footnote)
‡ Eberbach or equivalent.
193 (Popup - Footnote)
§ Durabond-1701, J&W Scientific, or equivalent.
194 (Popup - Footnote)
‖ Durabond-5, J&W Scientific, or equivalent.
195 (Popup - Footnote)
Durabond-210, J&W Scientific, or equivalent.
196 (Popup - Footnote)
** Aldrich or equivalent.
197 (Popup - Footnote)
†† Paxton Woods Glass, Cincinnati, OH, or equivalent.
198 (Popup - Footnote)
‡‡ Omnisolv, manufactured by EM Science, Gibbstown, NJ, or equivalent.
199 (Popup - Footnote)
§§ Aldrich or equivalent.
200 (Popup - Footnote)
‖‖ Carbitol (Aldrich), or equivalent.
201 (Popup - Footnote)
Diazald (Aldrich), or equivalent.
202 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1994.
203 (Popup - Footnote)
† This reagent is known under various synonyms. The more common are *o*-(2,3,4,5,6-pentafluorophenyl)methylhydroxylamine hydrochloride with CAS RN 57981-02-9 and pentafluorobenzyloxylamine hydrochloride (PFBOA). It also has appeared with the acronym PFBHOX.
204 (Popup - Footnote)

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* I-Chem Research, Hayward, CA, or equivalent.
205 (Popup - Footnote)
† Eberbach Corp., or equivalent.
206 (Popup - Footnote)
‡ Durabond-5, J&W Scientific, or equivalent.
207 (Popup - Footnote)
§ Durabond-1701, J&W Scientific, or equivalent.
208 (Popup - Footnote)
‖ Burdick & Jackson, Muskegon, MI, or equivalent.
209 (Popup - Footnote)
Sigma Chemical Co., St. Louis, MO, or equivalent.
210 (Popup - Footnote)
** Milli-Q, Millipore Corp., Bedford, MA, or equivalent.
211 (Popup - Footnote)
†† Hydrion, Micro Essential Lab., Inc., Brooklyn, NY.
212 (Popup - Footnote)
‡‡ Aldrich Chemical Co., or equivalent.
213 (Popup - Footnote)
§§ Parafilm®, American Can Co., Greenwich, CT, or equivalent.
214 (Popup - Footnote)
‖‖ Aldrich Chemical Company, Inc., Milwaukee, WI, or equivalent.
215 (Popup - Footnote)
‖‖ Aldrich Chemical Company, Inc., Milwaukee, WI, or equivalent.
216 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1993.
217 (Popup - Footnote)

* *Base/neutral extractables*: acenaphthene, acenaphthylene, anthracene, aldrin, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, benzyl butyl phthalate, β-BHC, δ-BHC, bis(2-chloroethyl) ether, bis(2-chloroethoxy) methane, bis(2-ethylhexyl) phthalate, bis(2-chloroisopropyl) ether more correctly known as 2,2-oxybis (1-chloropropane), 4-bromophenyl phenyl ether, chlordane, 2-chloronaphthalene, 4-chlorophenyl phenyl ether, chrysene, 4,4′-DDD, 4,4′-DDE, 4,4′-DDT, dibenzo(a,h)anthracene, di-*n*-butylphthalate, 1,3-dichlorobenzene, 1,2-dichlorobenzene, 1,4-dichlorobenzene, 3,3′-dichlorobenzidine, dieldrin, diethyl phthalate, dimethyl phthalate, 2,4-dinitrotoluene, 2,6-dinitrotoluene, di-*n*-octylphthalate, endosulfan sulfate, endrin aldehyde, fluoranthene, fluorene, heptachlor, heptchlor epoxide, hexachlorobenzene, hexachlorobutadiene, hexachloroethane, indeno(1,2,3-cd)pyrene, isophorone, naphthalene, nitrobenzene, *N*-nitrosodi-*n*-propylamine, PCB-1016, PCB-1221, PCB-1232, PCB-1242, PCB-1248, PCB-1254, PCB-1260, phenanthrene, pyrene, toxaphene, 1,2,4-trichlorobenzene. *Acid extractables*: 4-chloro-3-methylphenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4-dimethylphenol,

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2,4-dinitrophenol, 2-methyl-4,6-dinitrophenol, 2-nitrophenol, 4-nitrophenol, pentachlorophenol, phenol, 2,4,6-trichlorophenol.

The method may be extended to include the following compounds: benzidine, α-BHC, γ-BHC, endosulfan I, endosulfan II, endrin, hexachlorocyclopentadiene, *N*-nitrosodimethylamine, *N*-nitrosodiphenylamine.

218 (Popup - Footnote)
† Kontes K-570050-1025 or equivalent.
219 (Popup - Footnote)
‡ Kontes K-570001-0500 or equivalent.
220 (Popup - Footnote)
§ Kontes K-503000-0121 or equivalent.
221 (Popup - Footnote)
|| Kontes K-569001-0219 or equivalent.
222 (Popup - Footnote)
Hershberg-Wolf Extractor, Ace Glass Co., Vineland, NJ, P/N 6841-10, or equivalent.
223 (Popup - Footnote)
** Gas chromatographic methods are extremely sensitive to the materials used. Mention of trade names by *Standard Methods* does not preclude the use of other existing or as-yet-undeveloped products that give *demonstrably* equivalent results.
224 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1993.
225 (Popup - Footnote)
* 4-Chloro-3-methylphenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4-dimethylphenol, 2,4-dinitrophenol, 2-methyl-4,6-dinitrophenol, 2-nitrophenol, 4-nitrophenol, pentachlorophenol, phenol, 2,4,6-trichlorophenol.
226 (Popup - Footnote)
† Gas chromatographic methods are extremely sensitive to the materials used. Mention of trade names by *Standard Methods* does not preclude the use of other existing or as-yet-undeveloped products that give *demonstrably* equivalent results.
227 (Popup - Footnote)
‡ Davison grade 923 or equivalent.
228 (Popup - Footnote)
§ For U.S. federal permit-related analyses, use samples obtainable from U.S. EPA Environmental Monitoring and Support Laboratory, Cincinnati, OH.
229 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1993.
230 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1993.
231 (Popup - Footnote)

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* Acenaphthene, acenaphthylene, anthracene, benzo-(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(ghi)perylene, benzo(k)fluoranthene, chrysene, dibenzo(a,h)anthracene, fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, naphthalene, phenanthrene, and pyrene.

232 (Popup - Footnote)

† Perkin Elmer No. 089-0716 or equivalent.

233 (Popup - Footnote)

‡ Corning 3-75 or equivalent.

234 (Popup - Footnote)

§ Gas chromatographic methods are extremely sensitive to the materials used. Mention of trade names by *Standard Methods* does not preclude the use of other existing or as-yet-undeveloped products that give *demonstrably* equivalent results.

235 (Popup - Footnote)

‖ Davison, grade 923 or equivalent.

236 (Popup - Footnote)

For U.S. federal permit-related analyses, use samples obtainable from U.S. EPA Environmental Monitoring and Support Laboratory, Cincinnati, OH.

237 (Popup - Footnote)

* APPROVED BY STANDARD METHODS COMMITTEE, 1996.

238 (Popup - Footnote)

* Aldicarb sulfoxide, aldicarb sulfone, oxamyl, methomyl, 3-hydroxycarbofuran, aldicarb, baygon, carbofuran, carbaryl, and methiocarb.

239 (Popup - Footnote)

† HPLC methods are extremely sensitive to the materials used. Mention of trade names by *Standard Methods* does not preclude the use of other existing or as-yet-undeveloped products that give *demonstrably* equivalent results.

240 (Popup - Footnote)

‡ Kratos URS 051 and URA 100, or equivalent.

241 (Popup - Footnote)

§ Millipore Type HA, 0.45 µm, for water, and Millipore Type FH, 0.5 µm, for organics, or equivalent.

242 (Popup - Footnote)

§ Millipore Type HA, 0.45 µm, for water, and Millipore Type FH, 0.5 µm, for organics, or equivalent.

243 (Popup - Footnote)

‖ Millipore stainless steel XX300/200, or equivalent.

244 (Popup - Footnote)

Nuclepore 180406, 7035 Commerce Circle, Pleasanton, CA 94566–3294, or equivalent.

245 (Popup - Footnote)

** Gelman Sciences Acro LC 13, 0.45-µm disposable filter assembly, or equivalent, for aqueous

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samples.
246 (Popup - Footnote)
†† Millipore, Super-Q, or equivalent.
247 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1993.
248 (Popup - Footnote)
* No Chromix, Godax, 6 Varick Place, New York, NY, or equivalent.
249 (Popup - Footnote)
† Hydrox, Matheson Gas Products, P. O. Box E, Lyndhurst, NJ, or equivalent.
250 (Popup - Footnote)
‡ Gas Chrom Q, Applied Science Labs., Inc., P. O. Box 440, State College, PA, or equivalent.
251 (Popup - Footnote)
§ J & W Scientific, DB-5, DB-1701, or equivalent.
252 (Popup - Footnote)
‖ J & W Scientific, DB-1, or equivalent.
253 (Popup - Footnote)
Gas chromatographic methods are extremely sensitive to the materials used. Mention of trade names by *Standard Methods* does not preclude the use of other existing or as-yet-undeveloped products that give *demonstrably* equal results.
254 (Popup - Footnote)
** Use E. M. Quant™, MCB Manufacturing Chemists, Inc., 2909 Highland Ave., Cincinnati, OH, or equivalent.
255 (Popup - Footnote)
†† Florisil™ or equivalent.
256 (Popup - Footnote)
‡‡ Gas-Chrom Q™, Supelcoport, or equivalent.
257 (Popup - Footnote)
§§ Florisil™ or equivalent.
258 (Popup - Footnote)
* Florisil™ or equivalent.
259 (Popup - Footnote)
* Aldrin, α-BHC, β-BHC, δ-BHC, γ-BHC, chlordane, 4,4′-DDD, 4,4′-DDE, 4,4′-DDT, dieldrin, endosulfan I, endosulfan II, endosulfan sulfate, endrin, endrin aldehyde, heptachlor, heptachlor epoxide, toxaphene, PCB-1016, PCB-1221, PCB-1232, PCB-1242, PCB-1248, PCB-1254, PCB-1260.
260 (Popup - Footnote)
‡ Kontes K-42054 or equivalent.
261 (Popup - Footnote)
§ Gas chromatographic methods are extremely sensitive to the materials used. Mention of trade names by *Standard Methods* does not preclude the use of other existing or as-yet-undeveloped

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products that give *demonstrably* equivalent results.
262 (Popup - Footnote)
‖ E. Merck, EM Science Quant or equivalent.
263 (Popup - Footnote)
#Florisil or equivalent.
264 (Popup - Footnote)
** For U.S. federal permit-related analyses, use samples obtainable from U.S. EPA Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.
265 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1994.
266 (Popup - Footnote)
* Aldrich Chemical or equivalent.
267 (Popup - Footnote)
* APPROVED BY STANDARD METHODS COMMITTEE, 1996.
268 (Popup - Footnote)
* Aminex, BioRad Labs, A-9 cation exchange and A-27 anion exchange resins, or equivalent.
269 (Popup - Footnote)
† Whatman #1 or equivalent.

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