U.S. FOOD & DRUG ADMINISTRATION

Direct Injection Gas Chromatography Mass Spectrometry (GC-MS) Method for the Detection of Listed Impurities in Hand Sanitizers

Background: For proper hand hygiene, the Centers for Disease Control and Prevention (CDC) recommends the use of alcohol-based hand sanitizers that contain at least 60% alcohol when soap and water are not available. FDA has recently published guidances communicating temporary policies for preparing certain alcohol-based hand sanitizer products for the duration of the COVID-19 public health emergency. Here, the FDA has developed a laboratory analytical procedure to assess the quality of finished hand sanitizer products. The analytical procedure (with appropriate validation) can be used to evaluate products formulated with either alcohol (ethanol) or isopropyl alcohol (also called isopropanol or 2-propanol) as the labeled active ingredient and can screen for potentially harmful impurities, as described in the FDA guidances. This analytical procedure was developed in support of the guidances and provides a methodology to help assure hand sanitizer products contain the correct amount of the labeled ingredients and do not contain harmful levels of impurities.

Conclusions:
A Gas Chromatography-Mass Spectrometry (GC-MS) method was developed and validated for the quality assessment of hand sanitizers. The method can be used to assay for % alcohol (ethanol and isopropanol) and detect targeted impurities listed in the FDA hand sanitizer guidances. Impurities referenced in Attachment 1, Table 1 of the FDA guidances will be referred to as level 1 impurities while those listed in Attachment 1, Table 2 of the guidance will be denoted as level 2. Tables 1 and 2 below list and identify the level 1 and level 2 impurities, their interim limits, [1] the GC-MS method limits of detection (LODs) for level 1 impurities, and the concentration ranges for each impurity assessed by this method.

Table 1. List of level 1 impurities that can be detected in described method.

Impurity	Interim Limit Listed in FDA Guidances (ppm)	Concentration Ranges for this Method (µg/mL)	CDER Determined LODs (ppm)
Methanol	NMT 630	15.82 – 791	29
Benzene	NMT 2	0.044 – 2.19	0.1
Acetaldehyde	NMT 50	1.18 – 58.88	9
1,1-diethoxyethane (Acetal)	NMT 50	1.25 – 62.25	8

[1] FDA is are aware that some consumers and health care personnel are currently experiencing difficulties accessing alcohol-based hand sanitizers, and that the CDC recommends consumers use hand sanitizer containing at least 60% alcohol when soap and water are unavailable. Therefore, FDA is working with industry to ensure that harmful levels of impurities are not present in ethanol used in hand sanitizer.
As part of temporary policies described in the referenced guidances, we are temporarily providing flexibility with respect to certain impurities at the levels established in Table 1 and Table 2 in Attachment 1 of the guidances. Based on our review of available data, we have determined these interim impurity levels can be tolerated for a relatively short period of time, given the emphasis on hand hygiene during the COVID-19 public health emergency and to avoid exacerbating access issues for alcohol-based hand sanitizer.

Table 2. List of level 2 impurities that can be detected in described method.

Impurity	Interim Limit Listed in FDA Guidances (ppm)	Concentration Ranges for this Method (µg/mL)	Concentration Ranges for this Method (ppm)
Acetone	NMT 4400	15.80 – 632	619 - 24784
1-Propanol	NMT 1000	16.08 – 643	631 - 25216
Ethyl Acetate	NMT 2200	18.04 – 722	707 – 28298
2-Butanol	NMT 6200	16.16 – 646	634 – 25349
Isobutanol	NMT 21700	16.06 – 642	630 - 25192
1-Butanol	NMT 1000	16.20 – 648	635 – 25412
3-Methyl-1-Butanol	NMT 4100	16.18 – 647	635 – 25380
Amyl Alcohol	NMT 4100	16.22 – 649	636 - 25443

Purpose

To develop and validate an analytical procedure as an example of a suitable approach to determine % assay for labeled alcohol and also assess finished hand sanitizer products for the targeted impurities listed in the FDA guidances. Level 1 impurities will be assessed quantitatively while level 2 impurities will be evaluated using a limit test approach.

Principle

The USP <467> Residual Solvents Procedure A method was modified from flame ionization detection (FID) to utilize MS detection for the FDA guidance-listed impurities. This allowed for not only chromatographic separation, but also mass spectral separation based on *m/z* utilizing extracted ion chromatograms should any impurities have overlapping retention times. The temperature gradient of the USP method was also modified to reduce run time. Identification of the impurities is based on retention time matching to a standard, mass spectral matching to the NIST17 Mass Spectral Library, and mass spectral matching to the standard. Quantitation of the impurities is performed by comparing peak area of an impurity in a sample chromatogram to the peak area of that impurity in an external calibration standard solution containing the reference standard of that impurity. In addition, spike and recovery studies are conducted on each hand sanitizer sample tested to evaluate matrix effects and provide added confidence in the quantitative data reported.

Table 3. List of reagents needed for analysis.

Reagent	Manufacturers	P/N	Lot #
Methanol	Fisher Scientific	A456-4	194144
Ethanol	Sigma Aldrich	493538-1L	SHBL7739
Isopropanol	Sigma Aldrich	I9030-500ML	SHBL8958
Glycerin	Sigma Aldrich	G2289-500ML	SHBL8723
Hydrogen Peroxide	Sigma Aldrich	216763-100ML	MKCK2976
Benzene	Sigma Aldrich	270709-100ML	SHBL4231
Acetaldehyde	Sigma Aldrich	00070-100ML	SHBK4141
Acetal	Sigma Aldrich	A902-100ML	STBJ7268
Acetone	Sigma Aldrich	A949SK-4	177959

1-Propanol	Sigma Aldrich	34871-1L	SHBM0245
Ethyl Acetate	Sigma Aldrich	34858-1L	SHBL1333
2-Butanol	Sigma Aldrich	19440-1L	BCCB7567
Isobutanol	Sigma Aldrich	270466-1L	SHBM1741
1-Butanol	Sigma Aldrich	281549-1L	SHBL3797
3-Methyl-1-Butanol	Sigma Aldrich	M32658-1L	SHBL1515
Amyl Alcohol	Sigma Aldrich	76929-1L	BCCB5755

Equipment/Instrument

- Agilent 7010B GC-TQ equipped with a 7693 AutoSampler and an 8890 GC
- DB-624 30m x 0.25mm x 1.4µm
- Volumetric Glassware: Class A
- Eppendorf Pipets
- Airtight gas syringe (500µL)
- 2mL HPLC vials

Diluent and Blank: Acetonitrile

Table 4. Mixed Stock Standard preparation. Prepare a mixed stock standard solution in acetonitrile with the following concentrations.

Impurity/Analyte	Density (µg/µL)	Volume of Impurity (µL)	Dilution Volume (mL)	Concentration (µg/mL)	Concentration (ppm)
Methanol	791	200	100	1582	62039
Benzene	874	10		4.4	171
Acetaldehyde	785	15		117.8	4618
Acetal	831	15		124.5	4888
Ethanol	789	500		3945	154706
Isopropanol	785	500		3925	153922
Acetone	790	200		1580	61961
1-Propanol	804			1608	63059
Ethyl Acetate	902			1804	70745
2-Butanol	808			1616	63373
Isobutanol	803			1606	62980
1-Butanol	810			1620	63529
3-Methyl-1-Butanol	809			1618	63451
Amyl Alcohol	811			1622	63608
Note: Benzene is first diluted in 10mL and then 0.5mL of that solution is diluted in the stock 100mL standard. Concentration (ppm) is total concentration in a bottle of hand sanitizer and a sampling volume of 0.3mL. Density of hand sanitizer compounded as described in the FDA Guidances is ~0.85g/mL.					

Standard Preparation

Transfer 1mL of the mixed stock standard via Class A volumetric pipette to a 10mL volumetric flask containing approximately 8mL of acetonitrile. Dilute to volume with acetonitrile and mix well. **Prepare fresh daily.**

Spiked Recovery Standard Preparation
Transfer 1mL of the mixed stock standard via a Class A volumetric pipette to a 100mL volumetric flask containing approximately 80mL of acetonitrile. Dilute to volume with acetonitrile and mix.

Hand Sanitizer Sample Preparation
Transfer 0.3mL of hand sanitizer sample via air displacement pipette to a 10mL volumetric flask containing approximately 8mL of acetonitrile. Dilute to volume with acetonitrile and mix.

Spiked Recovery Hand Sanitizer Sample Preparation
Transfer 0.3mL of hand sanitizer sample via air displacement pipette to a 15mL conical centrifuge tube and add 10mL of Spiked Recovery Standard via Class A pipette. Cap and mix well.

Unspiked Sample Preparation
Transfer 0.3mL of hand sanitizer sample via air displacement pipette to a 15mL conical centrifuge tube and add 10mL of acetonitrile via Class A pipette. Cap and mix well.

Spiking Solution Preparation
Transfer 0.3mL of acetonitrile via air displacement pipette to a 15mL conical centrifuge tube and add 10mL of Spiked Recovery Standard via Class A pipette. Cap and mix well.

Hand Sanitizer Density Determination
Density of the hand sanitizer sample needs to be determined. This may be done utilizing a pycnometer or utilizing a graduated cylinder and an analytical balance to measure out and weigh 1mL of hand sanitizer.

Chromatographic Conditions

- Carrier Gas: Helium
- Run Time: 15.667min
- Flow Rate: 1.0mL/min
- Injector Temp: 250^{o}C
- Injection Vol.: 1.0µL
- Injection Type: Pulsed Split (50:1 split w/ 25psi pulse for 0.5min)
- Oven Temp Gradient: 40^{o}C(5min) → 240^{o}C at 30^{o}C/min (4min)
- MSD Source Temp: 230^{o}C
- MSD Quad Temp: 150^{o}C
- Ionization Mode: EI (70eV)
- MSD Solvent Delay: No solvent delay
- MSD m/z settings: See Table 5

Table 5. MSD m/z settings

Time (min)	Scan Range or SIM Ions	Gain	Dwell Time (ms)
0 - 2.6	29 – 45	5	100
2.6 – 4	29 – 65	1	100
4 - 6.8	29 – 105	1	100
6.8 - 7.3	78, 74, 51, 43 (Unit Resolution)	2	100,50,50,50
7.3 - 15.667	29 – 105	1	100
6.8min to 7.3min is operated in SIM mode. Ions 78(quantifier) and 51(qualifier) are for benzene and 74(qualifier) and 43(quantifier) are for isobutanol. Other time ranges are scan mode.			

Spiked Recovery

Injection Order

- Inject blank (use diluent) at least once at the beginning of a sequence. A blank injection can be done between standards and samples or different samples if carryover is observed.
- Inject standard solution for six replicates. Verify system suitability.
- Inject blank.
- Inject unspiked sample preparation followed by spiked recovery hand sanitizer sample preparation followed by Spiking Solution preparation.

System Suitability Criteria

- The % RSD of the peak area for each listed impurity for all injections of standard solution should be no more than 10%.

Data Processing

- Peak assignment should be based upon retention time matching to the reference standard as well as mass spectral matching to the reference standard's mass spectrum for each impurity.
- Peak areas for all impurities excluding benzene, isobutanol, and ethyl acetate should be acquired from the total ion chromatogram for the sample. Benzene, isobutanol and ethyl acetate should be quantitated from the peak areas in the EIC for *m/z* 78.0 for benzene and 43.1 for isobutanol and ethyl acetate.

Calculation

$$\%Recovery_{Impurity} = (Peak\ Area_{Spike} - Peak\ Area_{Unspike}) \div Peak\ Area_{SpikingSolution} \times 100$$

Reporting

- Report the %RecoveryImpurity to one unit. %Recovery should be within the range of 80 – 120.

FDA Guidance Level 1 Impurities Determination

Injection Order

- Inject Blank (use diluent) at least once at the beginning of a sequence. A blank injection can be done between standards and samples or different samples if carryover is observed.
- Inject Standard solution for three consecutive times before the injection of the first sample.
- Inject Standard solution once every six injections of samples and at the end of a sequence. If the total number of standard solution injections is less than six, inject as many times as is necessary to reach six injections at the end of the run.

Table 6. Example sequence for standards and samples.

Order	Solution	No. of Injections
1	Blank	1
2	Standard	3
3	Blank	1
4	Sample 1	1
5	Sample 2	1
6	Sample 3	1
7	Sample 4	1
8	Sample 5	1
9	Sample 6	1
10	Standard	3
…	…	…

System Suitability Criteria

- The % RSD of the peak area for each listed impurity for all injections of standard solution should be no more than 10%.

Data Processing

- Peak assignment should be based upon retention time matching to the reference standard as well as mass spectral matching to the reference standard's mass spectrum for each impurity.
- Peak areas for all impurities excluding benzene should be acquired from the total ion chromatogram for the sample. Benzene should be quantitated from the peak area in the EIC for *m/z* 78.0.

Calculation

Weight of hand sanitizer in original container (g) $= V_L \times$ *Density*

Where: V_L = Labeled volume (mL)
Density = Calculated density for the hand sanitizer (g/mL)

$$\textit{Hand sanitizer impurity (ppm)} = \textit{Peak Area}_{Sample} \div \textit{Peak Area}_{Std} \times C_S \times V_{Extract} \times V_{Container} \div 0.3mL \div W$$

Where: $\text{Peak Area}_{Sample}$ = Area of the impurity peak in the sample solution
Peak Area_{Std} = Average area (n = 2) of the impurity peak from the standards bracketing the sample
C_s = Concentration of the impurity in the standard solution in µg/mL
W = Weight of total volume of hand sanitizer in container (g)
$V_{Extract}$ = Volume of sample dilution (mL)
$V_{Container}$ = Total volume of hand sanitizer in original container (mL)

Reporting

- Report the impurity content in ppm to one decimal point.
- Report 'not detected' if no impurity is detected or the value is < LOD. The LODs should be individually determined for this analytical procedure at each laboratory and for each instrument utilized.

FDA Guidance Level 2 Impurities Limit Test

Injection Order

- No new injections need to be made. Sample and Standard same as "*FDA Guidance Level 1 Impurities Determination*."

Data Processing

- Peak assignment should be based upon retention time matching to the reference standard as well as mass spectral matching to the reference standard's mass spectrum for each impurity.
- Peak areas for all impurities excluding isobutanol and ethyl acetate should be acquired from the total ion chromatogram for the sample. Isobutanol and ethyl acetate should be quantitated utilizing the peak area from EIC for *m/z* 43.1.

Calculation

Table 7. Conversion factors for each impurity for the limit test.

Impurity	Limit Conversion Factor
Acetone	0.71
1-Propanol	0.16
Ethyl Acetate	0.31
2-Butanol	0.98
Isobutanol	3.45
1-Butanol	0.16
3-Methyl-1-Butanol	0.65
Amyl Alcohol	0.64

Amount of Level 2 Impurity = Peak Area Impurity × Limit Conversion Factor

Where: Peak Area Impurity = Peak Area from that impurity in the standard solution.
Limit Conversion Factor = Value for each impurity shown in Table 7

Reporting

- Report the impurity amount to the units place.
- If the peak area of the impurity in the sample is greater than the Amount of Level 2 Impurity, report "Failed." If it is less, report "Pass."

%Assay for Labeled Alcohol (Ethanol or Isopropanol)

Assay Sample Preparation:

If the ethanol or isopropanol peak for the impurity sample is more than 5x greater than the ethanol or isopropanol peak in the standard, dilute the impurity sample so that the resulting peak area should be approximately 0.5x the standard's peak area for those alcohols. If the peak area is less than 5x greater, then utilize the values from the impurity testing. **Prepare fresh daily.**

Injection Order

- Inject Blank (use diluent) at least once at the beginning of a sequence. A blank injection can be done between standards and samples or different samples if carryover is observed.
- If sample is being run the same day as the impurity sample, inject standard solution once before the sample. If it is within 10% of the average calculated for the impurity study system suitability, then run the sample. If not, inject for three consecutive times before the injection of the first sample. **If run on a different day, prepare a new standard and follow Injection order for the Impurity Study**.
- Inject Standard solution once every six injections of samples and at the end of a sequence. If run on the same day as the impurity study, as long as system suitability below is met, just bracket the samples with two standard injections. If run the following day, the total number of standard solution injections needs to be at least six.

System Suitability Criteria

- For same day runs, the % RSD of the peak area for ethanol or isopropanol for all injections of standard solution when compared to the % RSD in the impurity study standard should be no more than 10%.
- For runs on different days, the % RSD of the peak area for ethanol or isopropanol for all injections of standard solution that day ($n \geq 6$) should be no more than 10%.

Data Processing

- Peak assignment should be based upon retention time matching to the reference standard as well as mass spectral matching to the reference standard's mass spectrum for each impurity.
- Isopropanol should be quantitated from the peak area in the extracted ion chromatogram (EIC) for *m/z* 45.1. Ethanol should be quantitated from the peak area from the TIC.

Calculation

$$\%\ Alcohol\ (v/v) = Peak\ Area_{Sample} \div Peak\ Area_{Std} \times C_S \times Dilution\ Factor \times V_{Extract} \div 1x10^6 \div Density_{Alcohol} \div 0.3mL \times 100$$

Where: Peak $Area_{Sample}$ = Area of the alcohol* peak in the sample solution

Peak Area$_{Std}$ = Average area (n = 2) of the alcohol* peak from the standards bracketing the sample
C_s = Concentration of alcohol* in the standard solution in µg/mL
$V_{Extract}$ = Volume of sample dilution (mL)
Density$_{Alcohol}$ = 0.789 g/mL for ethanol and 0.785 g/mL for isopropanol

**alcohol refers to ethanol or isopropanol, acceptable alcohols used in the production of hand sanitizers.*

%Assay = % Alcohol ÷ Label Claim x 100

Where: Label Claim is the v/v % alcohol content of the hand sanitizer being tested

Reporting

- Report the alcohol content as % label claim.

Reference

1. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research. *Temporary Policy for Preparation of Certain Alcohol-Based Hand Sanitizer Products During the Public Health Emergency (COVID-19) Guidance for Industry*, March 2020, Updated August 7, 2020
2. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research. *Policy for Temporary Compounding of Certain Alcohol-Based Hand Sanitizer Products During the Public Health Emergency*, March 2020, Updated August 7, 2020.

Example Chromatograms

Figures 1 and 2 illustrate example chromatograms for the detection of ethanol (EtOH), isopropanol (IPA) and potential impurities (e.g., MeOH, acetaldehyde) in hand sanitizers as presented in the FDA hand sanitizer guidance.

Figure 1

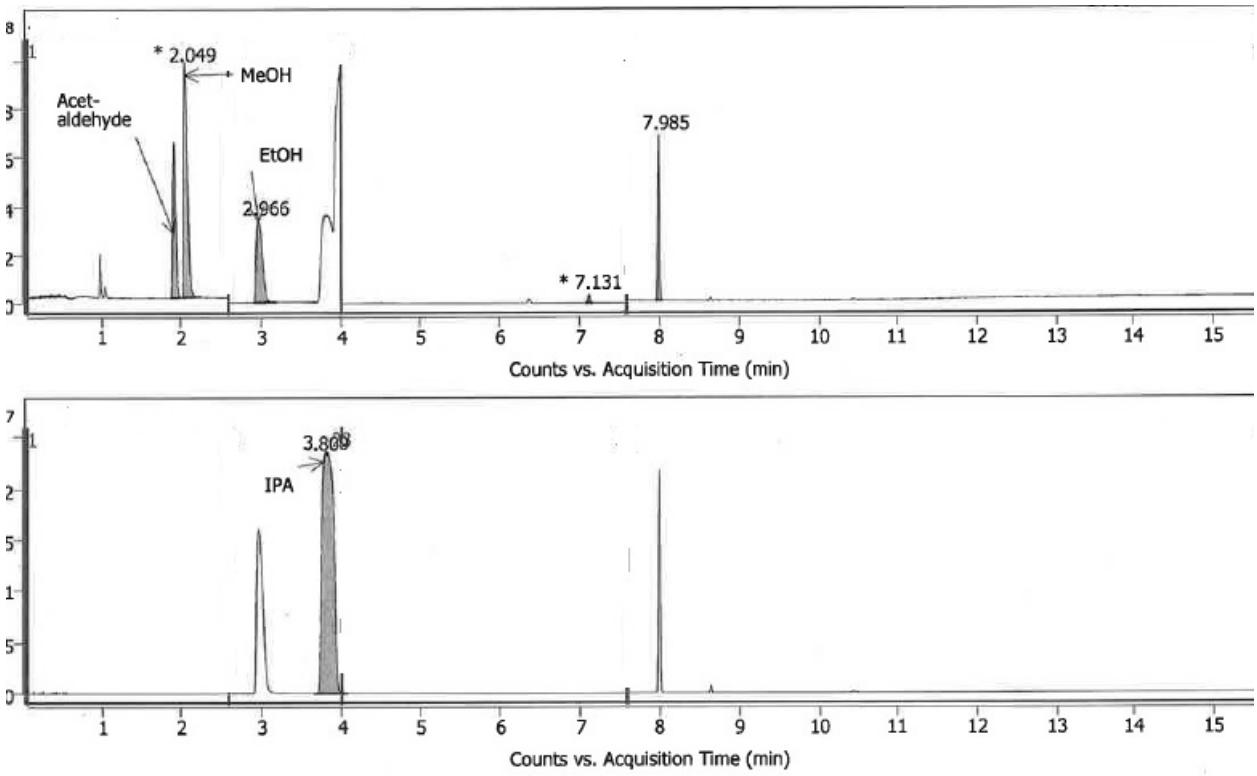
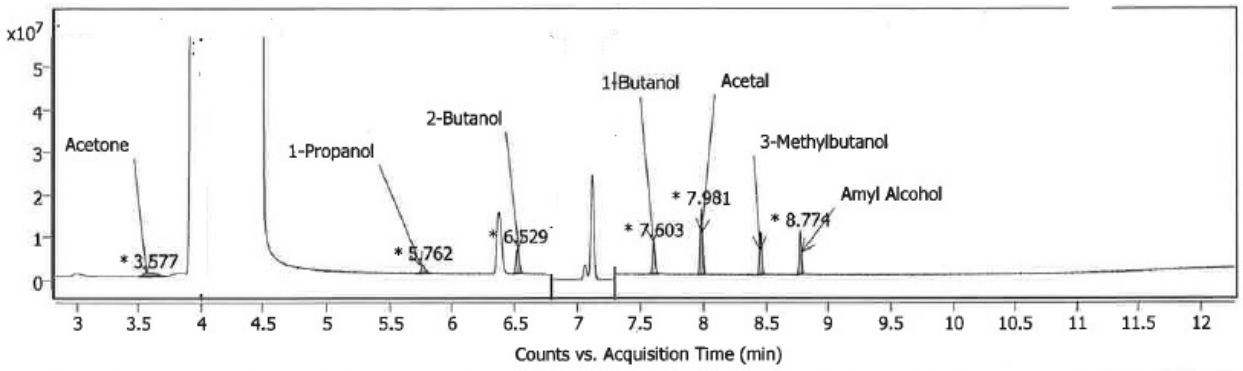
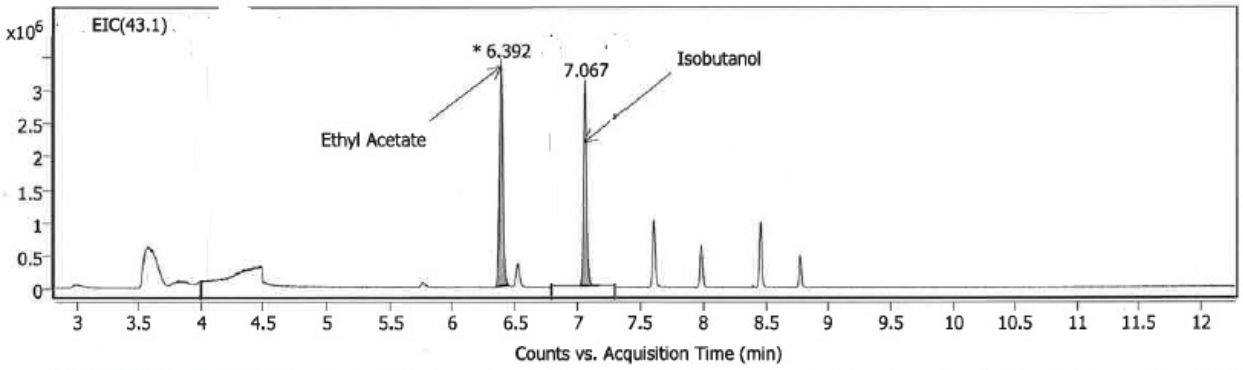
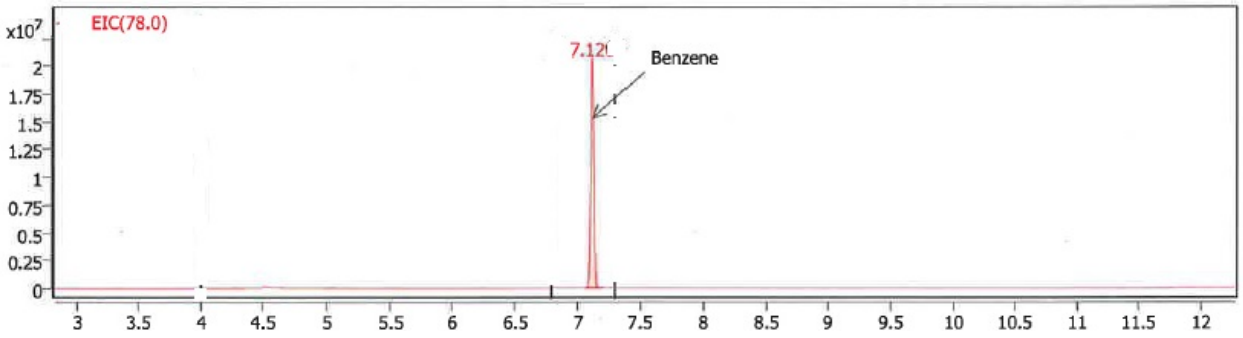

* 2.049
MeOH
Acet-
aldehyde
EtOH
2.966
7.985
* 7.131
Counts vs. Acquisition Time (min)
3.809
IPA
Counts vs. Acquisition Time (min)

Figure 2


x10^7
Acetone
1-Propanol
2-Butanol
1-Butanol
Acetal
3-Methylbutanol
Amyl Alcohol
* 3.577
* 5.762
* 6.529
* 7.603
* 7.981
* 8.774
Counts vs. Acquisition Time (min)


x10^6
EIC(43.1)
* 6.392
7.067
Isobutanol
Ethyl Acetate
Counts vs. Acquisition Time (min)


x10^7
EIC(78.0)
7.121
Benzene