New Chromogenic Spray Reeagent for Detection of Acephate from Biological Material

U. K. Kulkarni, K. V. Kulkarni, D. V. Mane, R. K. Pardeshi

Abstract: High performance thin layer chromatography has found wide recognition in many fields and in sensitivity of detection offers particular advantage to the toxicologist, which has increased, 10 to 100 times as compared to the chemical method. It has become an important analytical tool since it can separate complex mixture in a relatively short time. In existing study, an effort has been taken to determine organ phosphorus insecticide Acephate by using high performance thin layer chromatography. A new specific Sensitive chromogenic reagent 0.1% solution of ferric chloride in 80% ethanol and 1 % Sulfosalicylic acid in 80% ethanol has been developed for detection of Acephate an organophosphorus insecticide with solvent system petroleum ether: methanol (95: 5).

Keyword: chromatography, chromogenic, Acephate, ferric chloride, ethanol, Sulfosalicylic, organophosphorus.

I. INTRODCTION

Generally Acephate (metamedophos) is widely used as organosphosphorus insecticide. The international union of pure and applied chemistry (IUPAC) chemical name for Acephate is O,S-Dimethyl acetylphosphoramidothioate. it is white crystalline transparence solid , has a strong odor similar to mercaptan, which smells like sulphur[1,2] . Molecular weight 186-16 g/mol. Solubility (water) 79-83.5 g/100ml. Acephate is a general use pesticide registered for use on food crops, agriculture seed and nonbearing plants, ant mounds and horticultural nursery[4].

II. ACEPHATE STRUCTURE

There are various types of insecticide and pesticiede but owing to their easy availability these pesticide are used in the criminal poisoning cases. Pesticide and insecticides are extensively used in agriculture and household remidies for the control of insects and pests. Due to their easy availability inadvertent knowledge and quick action these pesticides are being largely used for suicidal and homicidal purpose.[3] In such cases medical officers preserve proper biological sample for process of toxicology case work. In such situation toxicologist play a vital role in identification and detection of the toxic substances In medico legal autopsy cases.Organ phosphorus compounds soon became active constituents of several household formulation of for killing bugs and rats. Organ phosphorous poisonings is very common in India (especially in rural area).

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This poisoning has claimed the largest number of victim in last fifteen years in Maharashtra state and hence in the forensic toxicology it has became necessary to identify organophosphorus insecticide as a group. In literature, a number of reagent such as mercuric nitrate followed by biphenyl carbazone (Joglekar1968)[5] mercuric nitrate followed by potassium ferrocynide (Katkar 1976)[6] Nesslers or tollens reagent (Kawale 1976)[7] alkaline resorcinol (Geiger,1976) NaOH and orthotoludine followed by potassium ferricynide (Lanjewar)[8] etc has been reported for the detection of organ phosphorus insect ides by thin layer chromatography. In existing paper we develop a new chromogenic spray reagent and new solvent system for the detection and identification of Acephate by HPTLC. The reagent consisting of 0.1% solution of ferric chloride in 80% ethanol and 1% Sulfosalicylic acid in 80% ethanol.
Solvent system-(petroleum ether: methanol 95:5)

III. MATERIAL AND METHOD

Chemical and Reagent:

Acetone, methanol, ethanol, petroleum ether, chloroform, sulphosalisilic acid, ferric chloride used were of analytical grade (Merck) Acephate, Endosulfan, melathion ,propoxur,Deltamithirin,Diazepam standard were available in our laboratory. Accelerated solvent extractor (ASE 200) was used for extraction of Acephate from biological sample distilled water were used throughout..

Spray reagent:

1) 0.1% solution of ferric chloride in 80% ethanol

2) 1% solution of Sulfosalicylic acid in 80% ethanol

Standard solution:

Standard solution of Acephate (1 mg/ml) was prepared in methanol. Similarly separate standard of Endosulfan, deltamethrin, propoxur, malathion, Phosphomidon Diazepam were also prepared in methanol.

Extraction procedure: Acephate was extracted from biological sample using accelerated solvent extractor ASE 200 (Dionex)

Method: It is an automated system for extracting organic compounds from variety of solid and semisolid samples. If the sample contains water then diatomaceous earth is added to absorb the water content and get a solid or semisolid sample for extraction. The ASE 200 accelerates the traditional extraction process by using solvent at elevated temperature and pressure is applied in the sample extraction cell to maintain the heated solvent in a liquid state during the extraction. After heating the extract is flushed into the collection vials and is ready for analysis.

Approximately 20 gm of visceral sample such as stomach, intestine, liver, and spleen, kidney cut into fine pieces along

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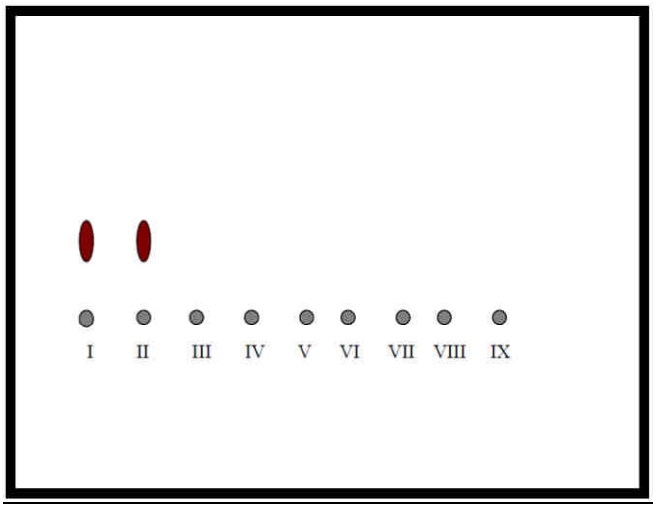
with liquid and blank viscera were mixed with diatomaceous earth and transferred into the extraction cell. The extracts were collected in a clean collection vial. Diethyl ether was used for extraction at 50□ C at 100 PSI pressure in two cycles. The extract obtained were transformed into steel capsule and evaporated to dryness at room temperature. The residue were dissolved at 2 ml of ethanol and processed further by HPTLC.

High Performance Thin Layer Chromatographic Method:

Chromatography was performed on 20 cm X 20 cm silica gel 60 F HPTLC glass plate (Merck). A camag (Switzerland) ,linomat, IV applicator was used to apply 2,4,6 and 10 µl standard solution of AcephaTE (10 µl) in ethanol equivalent to 10 µg along with extract of viscera, propoxur (carbmate) Deltamethrin (pyrethroid) Melathion, Phosphomidon (organophosphorus) Endosulfan (Organochloro insecticide) were also applied on HPTLC plate. The plate was then developed in a saturated 24 cm X 8 cm X 22.5 cm Camang twin through TLC chamber to a distance of 10 cm using petroleum ether, methanol (95:5) v/v as mobile phase. The plate was removed from the chamber, dried in air and sprayed with 1% solution of ferric chloride in 80% ethanol and 1% solution of Sulfosalicylic acid in 80% of ethanol. Successively white spot on (pink background) were developed at Rf 0.43 for standard Acephate and viscera having history of death due to Acephate



HPTLC Chromatogram obtained from:
I) Standard Acephate II) Acehate from Visceral extract. III) Blank Viscera IV) Dimethoate V) Phosphomidon (Organophosphorous insectiside) VI) Encosulfan (Organochloro insecticide) VII) Propoxur (Carbamate insceticide) VIII) Cypermethrin (Pyrethroid insecticide) IX) Diazepam (Drug) .

HPTLC Chromatogram obtained from:
I) Standard Acephate II) Acehate from Visceral extract. III) Blank Viscera IV) Melathion V) Phosphomidon (Organophosphorous insectiside) VI) Endosulfan (Organochloro insecticide) VII) Propoxur (Carbamate insceticide) VIII) Deltamethrin (Pyrethroid insecticide) IX) Diazepam (Drug) .

IV. RESULT AND DISCUSSION

The new developed spray reagent is highly sensitive and specific for the detection of Acephate from biological material also the solvent system used in this work is different. No spots were observed for propoxur(carbamate) Deltamethrin (pyrethroid) Malathion (organophosphorus) Endosulfan (Organochloro insecticide) and Diazepam (drug).Biological impurities such as amino acid,peptides and proteins do not interfere in this spray. This reagent is therefore sensitive and specific for Acephate.

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