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New Chromogenic Spray Reagent for TLC Detection and Identification of Organophosphrous Insecticide Monocrotophos in Biological Material.

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Abstracts

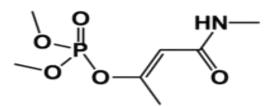
Monocrotophos is a member of Organophosphate insecticide. It is an important insecticide and has a diversified role in agriculture in INDIA. The increasing numbers of human poisoning cases were found to be occurred by the consumption of organophosphate insecticide monocrotophos. So in this paper, we represent a novel Thin Layer Chromatographic spray reagent for the detection and identification of Monocrotophos.

Keywords: Thin-layer Chromatography, Organophosphate insecticide, Monocrotophos, Forensic science, Biological material.

Introduction

During the last few years, Forensic Science Laboratories of Maharashtra, India, detected several human poisoning cases due to consumption of Monocrotophos. Large number of biological samples (postmortem samples) were received for toxicological analysis. Thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) are the cheapest preferred method of choice for a toxicological analysis of pesticides. By observing the results of toxicological chemical analysis the conclusion can be drawn that Monocrotophos played a key role in many poisoning cases. Proposed chromogenic reagent is found to be a selective spray reagent for Monocrotophos in routine analysis by HPTLC. Monocrotophos reacts with this chromogenic reagent and gives intense pink-colored compound. The other organochlorine insecticides, organophosphate insecticides and pyrethroid insecticide did not give any positive reaction with this reagent Biological material constituents (amino acids, peptides, proteins, fats etc.) also do not interfere with this chromogenic reagent.

Monocrotophos Dimethyl (E)-1-methyl-2-(methylcarbamoyl)vinyl phosphate is the organphsphate compound. Toxicology department detected several cases of human poisoning with Monocrotophos in routine forensic toxicological analysis. The insecticides are generally analyzed by thin-layer chromatography (TLC) as a method of choice. The chemical structure of Monocrotophos is shown below it has easily hydrolyzable phosphate ester group.



Thin layer chromatography (TLC) is the method of choice for screening biological sample due to its speed, low cost, and versatility. Several chromogenic reagents are reported such as p-benzoquinone reagent ^[1],potassium iodate-starch^[2],sodium carbonate-chloranil in acetone^[3], mercuric nitrate diphenylcarbazone^[4],palladium



chloride ^[5],vanillin reagent^[6],benzil reagent^[7],methanolic ferric chloride^[8],50% potassium iodate in 1:1ethanolhcl^[9],Cupric acetate^[10] diazotized sulphanilamide or sulphanilic acid ^[11],Chromogenic reagent ^[12] etc.

This study reports a new method for the analytical determination of Monocrotophos in biological samples by TLC. Selective detection of Monocrotophos after TLC is possible by use of reported chromogenic spray reagent. Monocrotophos reacts with the chromogenic reagent and produce an intense pink-colored compound in presence of hydrochloric acid. In continuation of our research work ^[13-16] on the identification and detection of different spraying reagents for the different poisons we developed synthetic poisons, with the hope it gives quick results for forensic toxicology field.

Experimental Materials and Methods

Chemicals and Reagents

All reagents were of analytical-reagent grade commercial formulation of Monocrotophos (UPL Ltd, India) solution was prepared in ethyl acetate (2 mg mL⁻¹).

Chromogenic reagent

2 gm. Stannous chloride (SNCL₂) in 20% of 40 ml Hydrochloric acid (S.D. Fine-Chem Ltd., Mumbai, India).

Both were mixed slowly and with continuous stirring.

Extraction of Monocrotophos from Biological Materials

About 50 g viscera sample [(I) pieces of stomach, small and large intestine with its contents, (II) pieces of liver, spleen, kidney, and lungs] containing history of monocrotophos poisioning was taken. Material was cut into fine pieces and minced carefully, 50 mL ethyl acetate was added and the contents were kept for 2 hours and then the extract was transferred into a steel capsule and evaporated to dryness at room temperature. The residue was re dissolved in 1 mL of ethyl acetate and was used for Thin-Layer chromatography.

Thin-Layer Chromatography

TLC plates (silica gel mfg. by Merck Ltd., Germany) were used the silica gel was mixed with water for four hours with stirring and then was spread on glass plate and kept for two hours at room temperature then it was put an oven in at 110° c then plate was activated and ready to use. Hexane- acetone (9.5:0.5, by volume) mixture was used as solvent system for monocrotophos residues. The samples were spotted on TLC plates with fine capillary tubes along with pure Monocrotophos as the standard. The plates were dried, and the chromatogram was developed in a pre-saturated tank containing the solvent system as mentioned above. After developing the plate, the solvent front (distance travelled by the solvent) was immediately marked and the extra solvent was evaporated (dried) in fume hood. The plate was then sprayed with above mentioned chromogenic reagent and the plate was then kept in oven at 110°c for 10 min. Pink colored spot with white background was clearly visible at *R*F 0.76 as shown in figure 1 RF values and color of spots tallied with commercial sample of standard Monocrotophos.

Results and Discussion

Monocrotophos is an organic compound which reacts with above chromogenic reagent to give intense pinkcolored compound. The color of the spot remains stable for about 6 hours. The limit of detection with this reagent is approximately 6µg. The reagent does not react with the organochlorine insecticides endosulfan, BHC, and DDT, etc and with the organophosphorus insecticides dimethoate, phorate, quinalphos, etc. and with the synthetic pyrethroids like fenvalerate, cypermethrin, and deltamethrin. Visceral constituents (amino acids, peptides, proteins, fats, etc.) do not interfere. The chromogenic reagent is utilized in the proposed method is



cheap and easy to prepare and does not involve any critical reaction condition or tedious sample preparation. Hence, this can be used routinely for the detection of Monocrotophos in biological materials.



Figure 1

TLC showing monocrotophos residues using chromogenic spray reagent: a) blank viscera, b) viscera with monocrotophos poisoning, and c) standard monocrotophos.in shown figure

Conclusions

To the best of our knowledge, chromogenic reagent stannous chloride along with hydrochloric acid was used for the first time for the detection and identification of Monocrotophos in biological post-mortem samples in fatal poisoning cases of Monocrotophos. The proposed reagent is simple, and can be used for routine analysis of Monocrotophos. Work on new spray reagents is in progress, and the authors hope to report it soon when it is done.

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Conflict of Interest The authors declare that they have no conflict of interest.

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