

RESEARCH ARTICLE

A Specific Chromogenic Reagent for the Detection of Profenofos in Biological Samples by HPTLC

Lanjewar RB¹ Chutke NL² and Lanjewar MR³

¹Dharampeth M.P.Deo Memorial Science College, Nagpur 440033 India

²Forensic Science Laboratory, State of Maharashtra, Vidyanagri, Kalina, Mumbai-400 098 (India).

Department of Chemistry, R.T.M Nagpur University, Nagpur 440033 M.S.

Corresponding author email: rb_lanjewar@rediffmail.com

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ABSTRACT

In the existing study, an efforts has been made to evaluate and determine organophosphorus pesticides in biological samples by High Performance Liquid Chromatography. A new specific sensitive chromogenic reagent has been developed for the detection of "Profenofos", an organophosphorus insecticide / acaricide. Profenofos on heating with sodium hydroxide undergoes hydrolysis forming 2-methyl-4-bromo-6-chlorophenol (II). This hydrolysed product reacts with o-Toluidine in presence of potassium ferricyanide and forms pink- violet complex on HPTLC. This reagent is specific for profenofos other organophosphorous insecticide failed to give a colour spot The sensitivity of the reagent is 5µg.

Key Words: Accelerated Solvent Extractor (ASE-200), HPTLC, Profenofos, Potassium Ferricyanide, o-Toluidine.

INTRODUCTION

Vegetables are important ingredient of our food having a high nutritional value. Vegetables, like, okra, egg plant, spinach, cauliflower, tomato, pumpkin, carrots, turnips etc. are produced in the country for local consumption as well as for export purposes. For better production and aesthetic value, farmers are using a large amount of insecticides during the entire period of growth of vegetables, even at fruiting stage and sometimes farmers also ignored the recommended waiting period between the harvest and last spray (Agnihotri, 1999; Kumar, 2003). Owing to this and other injudicious practice related to pesticide usage, pesticides become inner part of vegetable, in the shape of residues, which could be used by consumers thus creating health hazards. Profenofos is an organophosphorus insecticide used to control the insects (Tomlin, 1997) particularly Lepidoptera and mites on cotton, maize, sugarbeet, soyabeans, potatoes, vegetables, tobacco, and other crops. It is a non-systemic insecticide and acaricide with contact of stomach action. It shows different type of insecticidal activity and ability to inhibit the enzyme acetylcholinesterase (Leader, 1982). Poisoning by organophosphorus insecticide is very common in India and hence in Forensic toxicology it has become necessary to identify organophosphorus insecticide as a group and further identify the individual.

A number of reagents, such as mercuric nitrate followed by diphenyl carbazone (Joglekar, 1968), mercuric nitrate followed by potassium ferrocyanide (Katkar, 1976) Nessler's or Tollens reagent (Kawale, 1976) alkaline resorcinol (Geiger, 1976) etc has been reported for the detection of organophosphorus insecticides by thin layer chromatography.

The above reported reagents were used for detection of organophosphorus insecticides from biological samples but cannot give positive reaction with profenofos. Therefore in this paper efforts were made to develop a new chromogenic reagent for the detection of profenofos by HPTLC. In this paper reagent consisting of sodium hydroxide, potassium ferricyanide and o-Toluidine used for detection of profenofos.

MATERIALS AND METHODS

Chemical and Reagents: Sodium hydroxide pellets, potassium ferricyanide, O-toluidine, ethanol, chloroform, Acetone used were of analytical grade (Merck). Profenofos, malathion, propoxur, thiodan, cypermethrin standards were available in our Forensic Science Laboratory. Accelerated Solvent Extractor ASE-200 was used for extraction of profenofos from biological samples.

An aqueous solution of NaOH (5 % *m/v*) Potassiumferricyanide (0.5 % *m/v*) and (0.25%*m/v*) solution of o-Toluidine in acetone were prepared.

A standard solution of profenofos (1mgml⁻¹) was prepared in ethanol. Similarly separate (1mg mL⁻¹) standards of malathion, propoxur, thiodan, cypermethrin were also prepared in ethanol.

Extraction Procedure: Profenofos was extracted from biological samples, using Accelerated Solvent Extractor, ASE-200(Dionex).

Method: It is an automated system for extracting organic compounds from variety of solid and semi-solid samples. If the sample contains water then diatomaceous earth is added to absorb the water content and get a solid or semi-solid sample for extraction. The ASE-200 accelerates the traditional extraction process by using solvent at elevated temperature. Pressure is applied to 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 20gm of visceral sample such as stomach, intestine, liver, spleen, kidney having history of consumption of profenofos insecticide cut into fine

pieces, along 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 1000Psi pressure in two cycles. The extract obtained were transferred into a steel capsule and evaporated to dryness at room temperature. The residues were dissolved in 2ml of ethanol and processed further by HPTLC.

High Performance Thin Layer Chromatography

Chromatography was performed on 20cm X 20cm silica gel 60F₂₅₄ HPTLC glass plate (Merck), A Camag (Switzerland), Linomat IV Applicator was used to apply 2, 4, 6 & 10µl Standard solution of Profenofos (10µl) in ethanol equivalent to 10µg along with extracts of viscera having history of death due to consumption of Profenofos, blank viscera, malathion (organophosphorous), propoxur (carbamate), thiodan (organochloro), cypermethrin (pyrethroid insecticide) were also applied on HPTLC plate.

The plate was then developed in pre-saturated 24cm×8cm×22.5cm Camag twin-trough TLC chamber to a distance of 10cm using Hexane:Acetone 8:2 (v/v) as mobile phase. The plate was removed from the chamber, dried in air and sprayed with 5% sodium hydroxide solution followed by 0.5% potassium ferricyanide solution and 0.25% o-toluidine solution in acetone, by using 50 ml borosil glass sprayer. Successively pink-violet spots were developed at Rf 0.45 for standard profenofos and viscera having history of death due to consumption of profenofos. (Fig. no. 1). Approximately 20 to 30 min were required to completed analysis of biological samples.

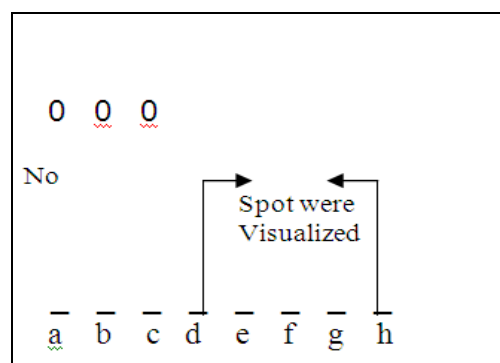


Fig.1. Thin Layer Chromatography of profenofos ,biological samples and Other insecticides. **a**-profenofos standard, **b**-Ether extract of stomach/intestine **c**-Ether extract of Liver ,Spleen, Kidney, **d**-Blank viscera, **e**-Malathion, **f**-Propoxur, **g**-Thiodan, **h**-Cypermethrin. Mobile phase hexane acetone 8:2 (v/v).

RESULTS AND DISCUSSIONS

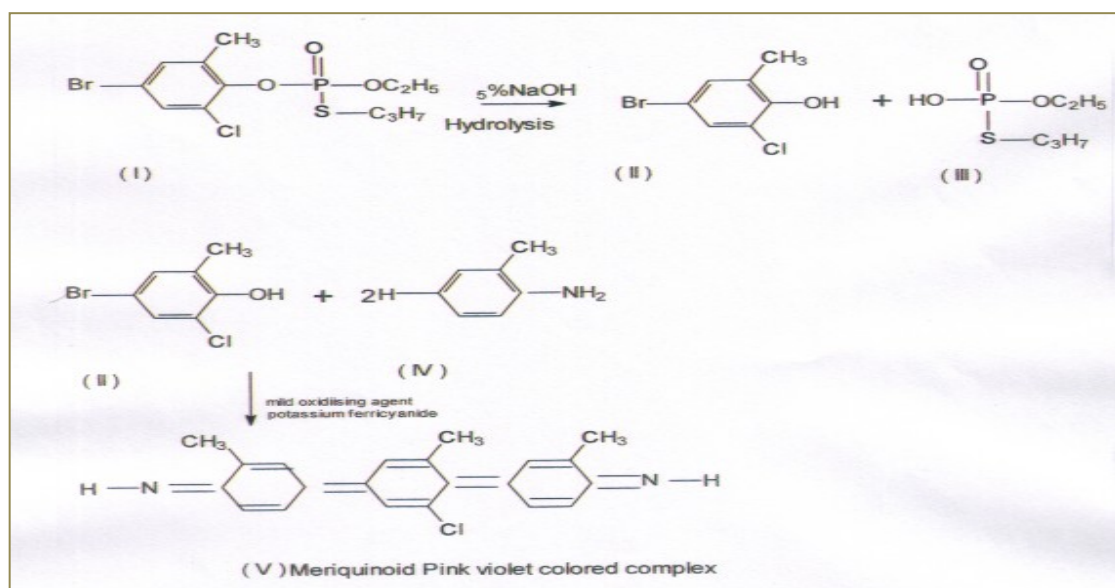


Fig. 2: Probable reaction of Profenofos with o-toluidine

Profenofos (I) undergo hydrolysis with sodium hydroxide to give 2-methyl-4-bromo-6-chlorophenol (II) and phosphorothionic acid-o-ethyl-s-propyl ester (III). 2-methyl-4-bromo-6-chlorophenol (II) couples with two molecules of o-toluidine(IV) in presence of mild oxidizing agent, potassium ferricyanide to give pink-violet coloured complex (V). Most probably it is an intermediate meriquinoid complex (Tomlin, 1997). The probable reaction is shown in Fig. 2.

The reagent is highly sensitive and specific for detection of profenofos from biological samples. Under the condition used the R_f of standard profenofos exactly matches with R_f of viscera having history of consumption of profenofos. No spots were observed for blank viscera and insecticide such as malathion, propoxur, thiodan, cypermethrin. Biological impurities such as aminoacids, peptides, and proteins do not interfere in the test. This reagent is therefore sensitive and specific for profenofos. The detection limit of the reagent is $5\mu\text{g}$. The colour is stable for several days. This method is very useful for the detection of profenofos in micro quantities in biological samples.

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