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A NEW SPRAY REAGENT FOR SELECTIVE DETECTION OF DICHLORVOS BY THIN-LAYER CHROMATOGRAPHY

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Summary—The misuse of dichlorvos (DDVP), an organophosphorus insecticide, results in many instances of poisoning This paper describes a new spray reagent for selective detection of dichlorvos in biological materials by thin-layer chromatography Dichlorvos in presence of moisture breaks down to dichloroacetaldehyde which in turn reacts with phenylhydrazine hydrochloride to give a yellowish red colour In acidic media the colour is intensified and consequently the sensitivity of detection increases The reagent is selective for dichlorvos, other organophosphorus insecticides failed to give a coloured spot Moreover organochlorine, carbamate and synthetic pyrethroid insecticides or even constituents of visceral extracts (amino acids, peptides, proteins etc) do not interfere The limit of detection is *ca* 10 μg

Dichlorvos (DDVP), an organophosphorus insecticide, is a systemic insecticide and acaricide, used to control sucking, chewing and boring insects and spider mites on a very wide range of crops. Unfortunately, its ready access has resulted in its increased misuse in homicidal and suicidal poisoning. Hence the need has therefore arisen for a rapid and reliable method for the detection and determination of dichlorvos in biological materials.

Very few reagents have been described in the literature for the detection of dichlorvos by thin-layer chromatography. Most common spray reagents are bromine-fluorescein-silver nitrate,[1] zinc-chloride-diphenylamine,[2-3] alcoholic *o*-tolidine or *o*-dianisidine and irradiation with UV light,[4] and 3,3′,5,5′tetramethylbenzidine,[5] for detection of chlorine containing organophosphorus insecticides and organochloro insecticides in general and ethanolic resorcinol containing 5% of sodium hydroxide for dichlorvos and trichlorfon.

In this paper we proposed a new spray reagent *viz.* 1% w/v aqueous phenylhydrazine hydrochloride followed by 10% v/v hydrochloric acid for detection of dichlorvos, by TLC. The reagent is selective for dichlorvos, other organophosphorus insecticides failed to give a coloured spot. Moreover organochlorine, synthetic pyrethroids and carbamate insecticides, and coextractives from visceral material (amino acids, peptides proteins, *etc.*) do not interfere. The limit of detection of the reagent is *ca* 10 μg per spot, after development.

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EXPERIMENTAL

Reagents

All reagents used were of analytical reagent grade. Distilled water was used throughout.

Silica gel G, particle size 15 μm with 13% $CaSO_4$ binder (Merck, Darmstadt, FRG) was employed to prepared TLC plates.

A dichlorvos stock solution (1 mg/ml) was prepared by dissolving 13.15 mg of, 76% technical grade dichlorvos (Hindustan Ciba-geigy Bombay, India) in 10 ml of ethanol

Aqueous phenylhydrazine hydrochloride reagent (1% w/v) was prepared by dissolving 1 g of phenylhydrazine hydrochloride (Qualigen, Bombay, India) in 100 ml distilled water and filter. Hydrochloric acid (10% v/v) was prepared by diluting 10 ml of con. HCl (32%) to 100 ml with distilled water

Extraction of dichlorvos from biological material

A portion of *ca* 100 g each of various types of viscerial tissue (stomach, intestine, liver, spleen and kidney) containing dichlorvos was

individually minced in an aqueous solution. Each sample was extracted in a separating funnel with 150 ml of diethyl ether, shaking the funnel for 2–3 min. The ether extract was transferred into an evaporating dish. The aqueous phase was re-extracted with 50 ml of diethyl ether (2–3 times). The extract was combined and the solvent was evaporated at room temperature. The residue was dissolved in 1–2 ml ethanol. A known volume (10 μl) of the solution was dropped onto an activated TLC plate together with the standard solution of dichlorvos insecticide. The plate was then developed as described under TLC procedure and sprayed with 1% w/v aqueous phenylhydrazine hydrochloride reagent followed by 10% v/v hydrochloric acid solution.

TLC procedure

A standard glass TLC plate was coated with a slurry of silica gel G in water (1 + 2) to a thickness of 0.25 mm The plate was activated at 110°C for about 1 h. Volume of 10 μl of standard solution of dichlorvos in ethanol (1 mg/ml) was dropped on the plate, which was then developed in a previously saturated TLC chamber using *n*-hexane:ethyl acetate:methanol (14 + 3 + 3) as the solvent. After the solvent had eluted a distance of 10 cm up the plate, the latter was removed from the chamber dried in air and sprayed with 1% w/v phenylhydrazin hydrochloride reagent, after 5 min yellowish red spot appeared. On spraying with 10% v/v hydrochloric acid, an intense red coloured spot develops at $R_F = 0.5$.

RESULTS AND DISCUSSION

Recovery experiment

A 100 mg amount of dichlorvos was added to 100 g of minced visceral tissue, mixed well and kept for a day. The insecticide was then extracted with diethyl ether as described under *Extraction of dichlorvos* and the solvent was evaporated at room temperature. The residue was then dissolved in 100 ml of ethanol. A 10 μl volume of this solution was dropped onto an activated thin-layer plate together with 10 μl each of standard technical dichlorvos solutions containing known concentrations of 80, 90, 100 and 110 mg of dichlorvos per 100 ml of ethanol. The plate was then developed as described under the previous section and sprayed with 1% w/v phenylhydrazine hydrochloride reagent, followed by 10% v/v hydrochloric acid solution. The intensity of the yellowish red spot developed for the spiked visceral extract were compared with those obtained for the known standards and was found to correspond to the spot representing a concentration of 90 mg per 100 ml (average of three experiments). Hence the recovery was *ca* 90%.

This reagent is selective for dichlorvos Other organophosphorus insecticides, such as phosphamidon, monocrotophos, malathion, parathion, dimethoate, quinalphos, phorate, fenthion, fenitrothion, and phosalone; organochlorine insecticides such as endrin, aldrin, dialdrin, endosulfan, DDT and BHC; carbamate insecticides such as propoxur, carbaryl, carbofuran, and synthetic pyrethroid insecticides, such as fenvalerate, cypermethrin and

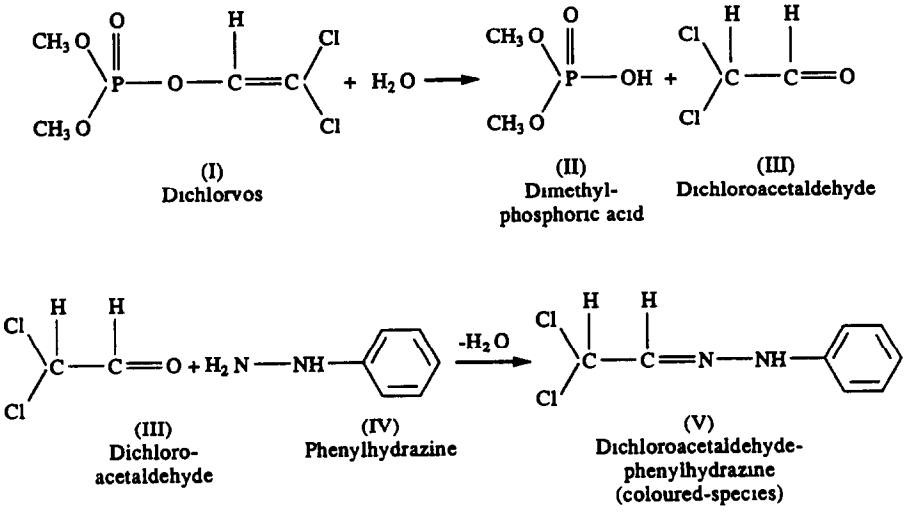

Fig 1 Proposed reaction for formation of coloured species.

deltamethrin do not give coloured spot. Moreover constituents of viscera (amino acid, peptides, proteins, *etc.*) which are generally co-extracted with the insecticides, do not interfere. The sensitivity of the reagent is *ca* 10 μg per spot observed after development.

Dichlorvos in the presence of moisture, breaks down to acidic products, dimethylphosphoric acid and dichloroacetaldehyde,[7] which further catalyse the decomposition. The dichloroacetaldehyde thus formed further reacts with phenylhydrazine to give yellowish red coloured dichloroacetaldehyde phenylhydrazine complex, as shown in the following scheme (Fig. 1). The colour of the spot is stable for a couple of days.

The reagent described here is sensitive and specific for dichlorvos and hence can be used routinely for the detection and determination of carbaryl in biological and non-biological materials in forensic toxicology.

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