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Journal of Chromatography A, 704 (1995) 540–543

JOURNAL OF CHROMATOGRAPHY A

Short communication

Thin-layer chromatographic detection of dichlorvos and dimethoate using orcinol

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First received 21 July 1994; revised manuscript received 18 January 1995; accepted 19 January 1995

Abstract

A sensitive and selective thin-layer chromatographic method for the detection of dichlorvos and dimethoate using orcinol is described. The alkali hydrolysis product of dichlorvos or dimethoate with orcinol produces a yellow fluorescent compound. The reagent does not react with other organophosphorus, organochlorine and carbamate insecticides. The constituents of viscera (amino acids, peptides, proteins, etc.) and plant materials do not interfere with the test. The fluorescence detection limits for dichlorvos and dimethoate are ca. 1 μg per spot (1.40 $\mu g/cm^2$) and ca. 15 μg per spot (21 $\mu g/cm^2$), respectively.

1. Introduction

Dichlorvos and dimethoate are organophosphorous insecticides widely used in agriculture for crop protection. Owing to their ready availability they are often misused for homicidal or suicidal purpose. During 1992, the Forensic Laboratories of Maharashtra detected 97 and 459 human poisoning cases with dichlorvos and dimethoate, respectively. The detection of these insecticides in routine forensic work is achieved using thin-layer chromatography (TLC). For instrumental methods, the biological material (vomitus, blood, viscera etc.) needs to be cleaned up before assay in order to remove biological impurities such as amino acids, peptides and proteins. Although these instrumental methods are sensitive, they are expensive and there are limitations to their use in routine forensic work owing to the large number of samples to be analysed. Hence TLC is the procedure of choice owing to its availability, simplicity and rapidity.

A number of chromogenic reagents, such as mercury (II) nitrate–potassium hexacyanaferrate(II) [1], potassium iodate–starch [2], alkaline resorcinol [3], iodine [4,5] and zinc chloride–diphenylamine [6], have been reported for the detection of these insecticides, but none is selective for dichlorvos and dimethoate. In a search for selective and sensitive reagent, orcinol was found to be suitable for detection of dichlorvos and dimethoate.

2. Experimental

All reagents were of analytical-reagent grade and distilled water was used throughout.

Solutions of technical-grade dichlorvos (Ciba-

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0021-9673/95/$09.50
SSDI 0021-9673(95)00091-7

Geigy, Bombay, India, 1 mg/ml, and dimethoate (Rallis, India), 4 mg/ml, were prepared in ethanol. Aqueous solutions of anthranilic acid (1 mg/ml), 2% (w/v) sodium hydroxide and 0.5% (w/v) orcinol (BDH, Poole, UK) were prepared.

2.1. Thin-layer chromatography

TLC plates were prepared as described previously [7]. Known concentrations of dichlorvos or dimethoate together with anthranilic acid were spotted on the plate, which was then developed in a presaturated TLC chamber using two solvent systems, *n*-hexane–acetone–methanol (8:3:0.5) and benzene–ethyl acetate–methanol (9:1:1). After the solvent had migrated ca. 10 cm, the plate was removed and allowed to dry at room temperature. It was sprayed uniformly with 2% sodium hydroxide solution followed by 0.5% orcinol solution, then placed in an oven at 100°C for about 10 min. The plate was removed and allowed to cool to room temperature. A yellow-brown spot for dichlorvos and a yellow spot for dimethoate were observed. The plate was viewed under long-wavelength (365 nm) UV light. Both insecticides appeared as yellow fluorescent spots, whereas the anthranilic acid spot demonstrated a blue fluorescence. The R_F values of dichlorvos and dimethoate with respect to anthranilic acid are given in Table 1.

2.2. Recovery of dichlorovos and dimethoate from biological materials

For the semi-quantitative determination of dichlorovos, 1 mg of insecticide was added to ca. 50 g of minced visceral tissue (stomach, intestine, liver, etc.) and kept for a day. The insecticide was then extracted with diethyl ether, the solvent was evaporated at room temperature and the residue was dissolved in 1 ml of ethanol. A 10-μl volume of this solution was spotted on an activated plate along with 10 μl each of standard solutions containing 8.5, 9.0, 9.5 and 10.0 μg of dichlorovos. The plate was then developed as described above. The intensity of the yellow fluorescent spot produced by the visceral extract was comparable to that of the spot corresponding to 9.0 μg of dichlorvos (average of three experiments). Hence the recovery was ca. 90%. Likewise, the recovery of dimethoate was also found to be ca. 90%.

3. Results and discussion

Dichlorvos and dimethoate are the derivatives of phosphoric and dithiophosphoric acid, respectively, and both are readily hydrolysed in an alkaline medium. Dichlorvos on alkali hydrolysis [8] produce dimethylphosphoric acid (**I**) and dichloroacetaldehyde (**II**). Orcinol (**III**) reacts with dichloroacetaldehyde (**II**) as shown in Fig. 1. Similar reactions of chloral with resorcinol [9] and chloroform with orcinol [10] have been reported.

Dichlorovos and dimethoate gives visible spots at amounts of 4 μg and 90 μg, respectively. Under UV radiation, the detection limit of the reagent is lowered to 1 μg per spot (1.40 μg/cm^2) for dichlorvos and 15 μg per spot (21 μg/cm^2) for dimethoate. The colour of the spots

Table 1
R_F values with respect to anthranilic acid

Insecticide	$R_F \pm$ S.D.[a]		Colour of spot under 365-nm UV radiation
	I[b]	II[b]	
Dichlorvos	2.92 ± 0.04	2.16 ± 0.03	Yellow
Dimethoate	0.68 ± 0.02	0.63 ± 0.03	Yellow
Anthranilic acid	1.00	1.00	Blue

[a] Standard deviations based on ten measurements.
[b] Solvent systems: I = *n*-hexane–acetone– methanol (8:3:0.5) (anthranilic acid $R_F = 0.18$); II = benzene–ethyl acetate–methanol (9:1:1) (anthranilic acid $R_F = 0.33$).

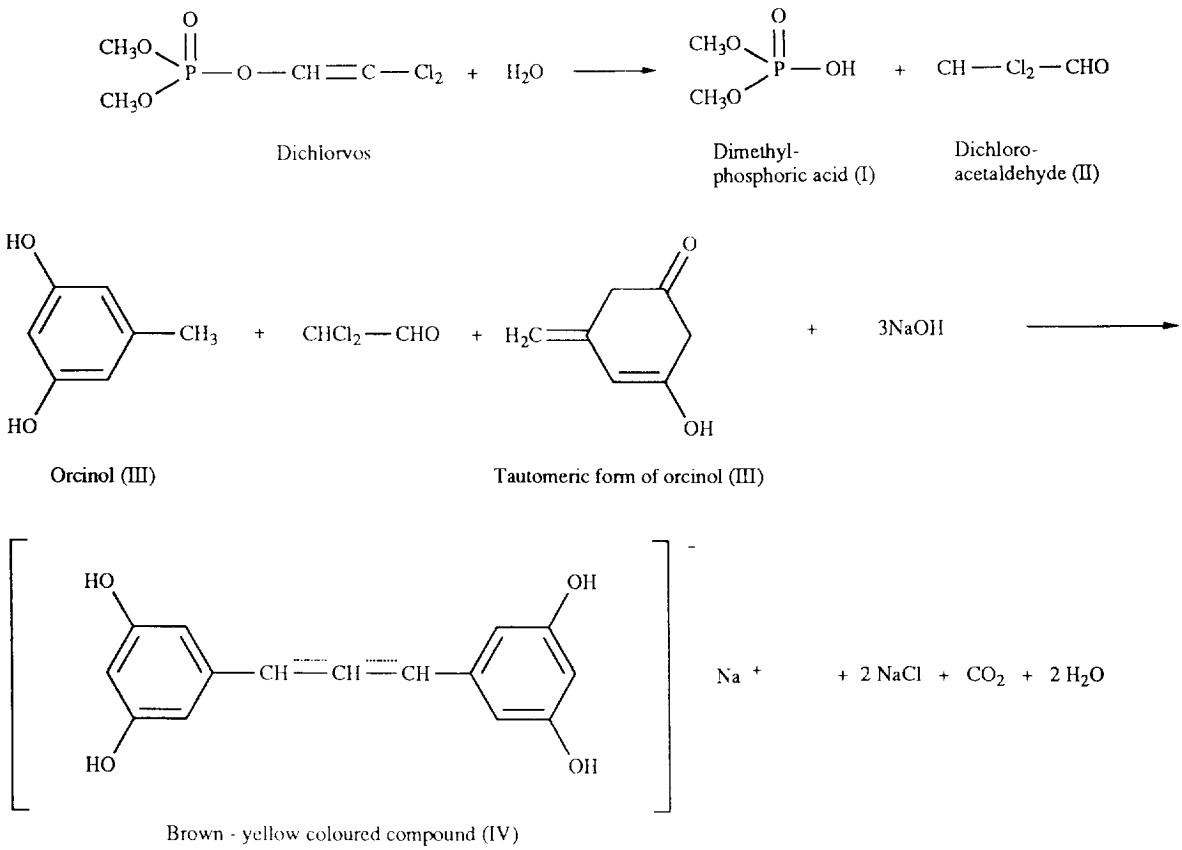

Fig. 1. Formation of coloured compounds.

remains stable for about 2 days. The solvent systems used gives compact spots. It was seen that dichlorvos and dimethoate are not fluorescent. Anthranilic acid does not react with orcinol, hence it was used for obtaining relative R_F values.

Orcinol was found to give yellow fluorescence with chloral hydrate and trichloroacetic acid with detection limits of 25 and 48 μg, respectively. It does not give a colour reaction with other organophosphorus insecticides (malathion, fenthion, phorate, parathion, quinolphos, ekatin and fenitrothion), organochlorine insecticides (endosulphan, DDT and benzene hexachloride) and carbamate insecticides (baygon, carbaryl and carbofuran) as such or after their alkali hydrolysis. The constituents of viscera (amino acids, peptides, proteins, etc.) generally co-extracted with these insecticides and plant materials do not interfere with the test. Hence the proposed reagent, owing to its sensitivity and selectivity, can be useful for detection and semi-quantitative determination of dichlorvos and dimethoate insecticides in biological and vegetable materials.

Acknowledgement

The authors are grateful to Mr. S.O. Baisane, Director, Forensic Science Laboratories, State of Maharashtra, Bombay, for his keen interest and valuable guidance in this work.

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