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Note

Thin-layer chromatographic detection of endosulfan and phosphamidon by use of cobalt acetate and *o*-tolidine

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Endosulfan and phosphamidon, which are organochlorine and organophosphorus insecticides, respectively, are widely used in agriculture. They are frequently misused in homicidal and suicidal poisoning cases and their selective characterization is therefore necessary. Reagents reported for their detection by thin-layer chromatography (TLC) include alcoholic *o*-tolidine or *o*-dianisidine and irradiation with UV light [1], ethanolic diphenylamine [2], sodium hydroxide followed by methanolic thymol [3] for the detection of organochlorine insecticides in general and cobalt acetate–sodium hydroxide followed by potassium iodide–starch [4] for endosulfan and phosphamidon and 20% sodium hydroxide followed by 5% (w/v) aqueous nickel chloride 30% ammonia (50:50, v/v) [5] for endosulfan specifically.

In this paper we report the use of 5% sodium hydroxide solution cobalt acetate solution followed by 1% *o*-tolidine in acetic acid, yielding intense blue colour. The reagent is selective for endosulfan and phosphamidon. Other organochlorine insecticides, such as endrin, aldrin, dialdrin, DDT and BHC, organophosphorus insecticides, such as malathion, parathion, dimethoate, quinalphos, phorate and fenitrothion, and carbamate insecticides, such as baygon, carbaryl and carbofuran, do not give coloured spot. Moreover, constituents of viscera (amino acids, peptides, proteins, etc.), which are generally co-extracted with the insecticides, do not interfere. The limit of detection for 1 g of biological material is *ca.* 10 μg for both insecticides. This reagent is more sensitive than ethanolic diphenylamine [2] (sensitivity *ca.* 50 μg) and *o*-tolidine or *o*-dianisidine and irradiation with UV light [1] (sensitivity *ca.* 40 μg).

EXPERIMENTAL

Reagents

All reagents were of analytical-reagent grade. Distilled water was used throughout. Technical endosulfan and phosphamidon were supplied by All India Medical Corporation (Bombay, India) and Hindusthan Ciba-Geigy (Bombay, India), respectively.

Aqueous hydroxide solution (5%, w/v) and aqueous cobalt acetate solution (5%, w/v) were prepared. A 1% (w/v) solution of *o*-tolidine was prepared by dissolving 1 g of *o*-tolidine in 100 ml of 10% (v/v) acetic acid.

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Procedure

A standard glass TLC plate was coated with a slurry of silica gel G (Acme Synthetic Chemicals, Bombay, India) in water (1:2) to a thickness of 0.25 mm. The plate was activated at 110°C for about 1 h. A 10-μl volume of a standard solution of endosulfan and phosphamidon in ethanol (1 mg/ml) was spotted on the plate, which was then developed in a previously saturated TLC chamber using *n*-hexane–acetone (4:1). After the solvent had eluted 10 cm up the plate, the latter was removed from the chamber, dried in air and sprayed with 5% sodium hydroxide solution followed by 5% cobalt acetate solution. After 5 min yellowish brown spots of tervalent cobalt appeared (the sensitivity at this stage for obtaining the yellowish brown spots is *ca.* 150 μg). On spraying with 1% *o*-tolidine in acetic acid the spots turned intense blue due to the quinoidal oxidation product of *o*-tolidine.

Recovery experiment

A 1-mg amount each of endosulfan and phosphamidon, *i.e.*, a 1-ml portion of a solution of 10 mg in 10 ml of ethanol, was added separately to 10 g of minced (in an aqueous solution) visceral tissue (pieces of stomach, intestine, liver, spleen and kidney, all separately), mixed well and kept for 1 day. Both insecticides were then extracted with ethyl acetate, the solvent was evaporated at room temperature and the residue was dissolved in 1 ml of ethanol. A 10-μl volume of the solution was spotted on an activated TLC plate together with 10 μl each of standard technical endosulfan and phosphanidon solutions containing known concentrations of 8.5, 9, 9.5 and 10 mg per 10 ml in ethanol. The plate was then developed as described above and sprayed with 5% sodium hydroxide solution followed by 5% cobalt acetate solution. After 5 min and then spraying with 1% *o*-tolidine reagent, the intensities of the blue spots developed for the visceral extract were compared with those of the known standards. The intensities for endosulfan and phosphamidon were found to agree with those for spots of concentration 9.5 and 9 mg per 10 ml, respectively (average of three experiments). Hence the recovery for endosulfan was *ca.* 95% and for phosphamidon *ca.* 90%.

RESULTS AND DISCUSSION

The intense blue spots are stable for about 30 min in acidic medium and then slowly fade. Endosulfan gave two spots at R_F 0.5 and 0.7, due to the α- and β-isomers [6], whereas phosphamidon gave one spot at R_F 0.12.

Endosulfan, containing cyclic sulphite in its structure, is readily hydrolysed by alkali [6]. The sulphite (characteristic formation from tetravalent sulphur compounds by the action of alkali [7]) in turn reacts with cobalt(II) in the presence of atmospheric oxygen to give oxidized yellowish brown cobalt(III), which further oxidizes the *o*-tolidine to give the blue quinoidal oxidation product of *o*-tolidine [8]. Analogue to the formation of black Ni(IV) [7], the formation of brown Co(III) via $(CoOH)_2SO_3$ can be postulated as

$$2\,Co^{2+} + 6\,OH^- + SO_2 + O_2 \rightarrow 2\,CoO(OH) + SO_4^{2-} + 2\,H_2O$$

or

$$2\,Co^{2+} + 4\,OH^- + SO_3^{2-} + O_2 \rightarrow 2\,CoO(OH) + SO_4^{2-} + H_2O$$

Similarly, in alkaline medium, phosphamidon, which contains a 2-chloro-2-diethylcarbamoyl group reacts suitably with cobalt(II) to give oxidized yellowish brown cobalt(III), which in turn oxidizes *o*-tolidine. Benzidine and *o*-dianisidine react in the same way, giving a blue complex with the above insecticides. As benzidine is carcinogenic the use of *o*-tolidine is preferred.

The proposed reagent can be routinely used for the identification and semiquantitative determination of endosulfan and phosphamidon in biological material in forensic toxicological work.

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REFERENCES

1 I. Kawashira and Y. Hosagai, *Shokuhin Eiseigaku Zaashi*, 5 (1964) 54.
2 A. Coutselinis, P. K. Kentarchoa and D. Bonkis, *Forensic Sci.*, 8 (1976) 251.
3 H. Thielemann, *Z. Chem.*, 18 (1978) 147.
4 V. B. Patil, H. N. Katkar and B. M. Shinde, *Indian J. Criminol. Criminalist.*, 3 (1983) 40.
5 V. B. Patil, M. T. Sevalkar and S. V. Padalikar, *J. Chromatogr.*, 396 (1987) 441.
6 *The Merck Index*, Merck, Rahway, NJ, 9th ed., 1977, p. 409.
7 F. Feigl, *Spot Tests in Organic Analysis*, Elsevier, Amsterdam, Oxford, New York, 7th ed., 1975, p. 87.
8 F. Feigl, *Spot Tests in Inorganic Analysis*, Elsevier, Amsterdam, Oxford, New York, 6th ed., 1972, p. 447.