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Note

Specific spray reagent for the detection of endosulfan by thin-layer chromatography

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Endosulfan (Thiodan) is an organochlorine insecticide widely used for the protection of crops. Owing to its easy availability, it is frequently misused in homicidal and suicidal poisonings, and consequently its specific detection is desirable. A few reagents have been used for its detection in thin-layer chromatography (TLC), *viz.*, alcoholic *o*-tolidine or *o*-dianisidine and irrradiation with UV light[1], ethanolic diphenylamine[2], sodium hydroxide followed by methanolic thymol[3] and cobalt actate–sodium hydroxide followed by potassium iodide–starch[4]. However, these have low sensitivity and none of them is specific. Hence it was desirable to establish a specific and sensitive chromogenic reagent for the detection of endosulfan.

Nickel ammine in an alkaline medium reacts with endosulfan to give greyish black spots. The reagent is specific for the detection of endosulfan; other organochlorine insecticides (*e.g.*, endrin, DDT, BHC, aldrin, dieldrin, heptachlor and toxaphene) failed to give coloured spots. Moreover, organophosphorus and carbamate insecticides and constituents of visceral extracts (amino acids, peptides, proteins, etc.) do not interfere (Fig. 1). The sensitivity of the reagent is *ca.* 1 μg, observed after development.

EXPERIMENTAL AND RESULTS

Reagents

Nickel ammine reagent. Equal volumes of 5% (w/v) aqueous nickel chloride solution and 30% ammonia (sp. gr. 0.89) are mixed.

Sodium hydroxide solution (20%). A 20-g amount of sodium hydroxide is dissolved in 100 ml of distilled water.

Procedure

The standard glass plate was coated with a slurry of silica gel G (ACME) with water (1:2) to a thickness of 0.25 mm and the plate was heated at 110°C for about 1 h. An amount of 1 μg of endosulfan 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) as the solvent up to a height of 10 cm. The plate was removed, dried in air and sprayed with 20% sodium hydroxide solution followed by nickel ammine reagent. Greyish black spots were observed immediately on the TLC plate at R_F values of 0.5 and 0.72.

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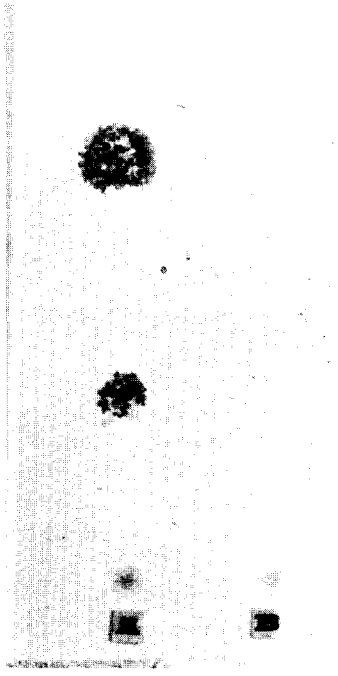

Fig. 1. Chromatogram of (A) a spiked visceral extract and (B) a blank visceral extract.

Recovery experiment

A 1-mg amount of endosulfan was added to 100 g of minced visceral tissue, mixed well 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 thin-layer plate together with 10 μl each of standard technical endosulfan solutions containing known concentrations of 9, 9.5 and 10 mg per 10 ml in ethanol. The plate was then developed as described above and sprayed with 20% sodium hydroxide solution followed by nickel ammine reagent. The intensity of the greyish black spots developed for the visceral extract were compared with those of the known standards and found to agree with the spot of concentration 9.5 mg per 10 ml (average of three experiments). Hence the recovery was *ca.* 95%.

DISCUSSION

Endosulfan, containing cyclic sulphite in its structure, is readily hydrolysed by alkali[5]. The sulphite (characteristic formation from tetravalent sulphur compounds by the action of alkali[6]) in turn reacts with nickel(II) ammine to give greyish black nickel(IV) oxyhydrate $NiO(OH)_2$ (ref. 6):

$$Ni^{2+} + SO_3^{2-} + 2\ OH^- + O_2 \rightarrow SO_4^{2-} + NiO(OH)_2$$

Technical endosulfan gives two black spots at different R_F values, due to the presence of α- and β-isomers[5]. The colour of the spots is stable for more than 24 h.

The specific and sensitive reagent described here can be used routinely for the detection and determination of endosulfan in biological materials in forensic toxicology.

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