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Colorimetric Determination of Propoxur Residues in Vegetables and Water

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Propoxur /‾ 2-(1-methylethoxy)phenyl methylcarbamate_/ is a non-systemic insecticide which is used on a large scale against a broad spectrum of insects in field crops in India. It has also been used extensively for indoor applications against monquitoes, cockroaches and flies. The analysis for propoxur in vegetables and food grains has therefore become quite important. Propoxur and its metabolites in crops, milk and animal tissue were reported by Miller et al. (1972), Stanley and Thornton (1972) and Stanley et al. (1972), respectively. The trifluoroacetyl derivative (Ueji and Kanazawa, 1973) and 2,4-dinitrophenyl ether derivative (Holden, 1973) of propoxur and its metabolites were determined by gas chromatography with electron-capture detection. Liquid chromatography of propoxur was described by Ishii and Otake (1973). Several colorimetric method using 4-amino-2-nitrobenzenesulfonic acid (Ramaswamy, 1974), 3-nitroaniline-4-sulfonic acid (Peretx, 1964), 4-nitrobenzene-diazonium fluroborate (Miskus et al. 1961), 4,4-diaminodiphenyl sulfone (Appaiah et al. 1983), 4-aminoantipyrine (Piechoka, 1975) and sulphanilic acid (Mukherjee et al. 1975) as coupling reagents for the determination of propoxur have been reported. Thin-layer chromatography (tlc) of dansyl chloride derivative of propoxur (Frei and Lawrence, 1972) and use of several chromogenic spray reagents like diazotized sulphanilic acid (Mukherjee et al. 1975), $AgNO_3$-HNO_3 (Reichling and Fisher, 1975) have also been reported. A tlc-enzyme inhibition method (Ernst et al. 1975) was more sensitive than the other tlc and spectrophotometric method. The methylamine liberated by alkaline hydrolysis of propoxur was titrated with HCl (The Pesticide Manual, 1979).

Although gas and liquid chromatographic methods are ideal for detecting and quantifying propoxur residues their use is often limited by availability and cost of equipment. Tlc-enzymatic methods are very laborious and could not be adopted for rapid routine analysis of large amount of samples. Colorimetric methods have become very popular in the thirdworld countries because of their simplicity and the availability of chromogenic reagents. The advantages of the method described in this paper over other colorimetric methods are that it does not require elaborate clean-up technique, gives high recovery, produces a stable color and is equally sensitive as the method which uses 4,4-diaminodiphenyl sulfone as the chromogenic reagent.

MATERIALS AND METHODS

Perkin Elmer Model 475 spectrophotometer with 1-cm path length silica cells was used for absorbance measurements. Technical grade propoxur (99% pure) obtained from Bayer (India) Ltd., Thana, Maharashtra, India was used for preparing standard solution containing 100 ug/ml of methanol. Alcoholic ammonium hydroxide (1:1) was prepared by diluting 1 volume of ammonium hydroxide (NH_3 24-26%) with 1 volume of absolute ethanol.

Aliquots of the propoxur standard solution containing 25-500 ug (0.25-5.0 ml) were pipetted into 250 ml conical flasks fitted with \$ joints. To each flask 5 ml of methanolic KOH was added and the mixtures were refluxed on a water-bath (100°C) for 30 min. The flasks were connected with air condensers and the mixtures were refluxed on a water bath at 100°C for 1 hr. The flasks were cooled and the contents along with 10 ml of distilled water and 20 ml of 1:1 alcoholic ammonium hydroxide were placed in 50 ml volumetric flasks, and 5 min was allowed for full color development. The absorbance was recorded at 420 nm against a blank (control).

To check the efficiency of the method described above, 100-ml water samples were placed in 2-L separatory funnels and fortified with 50-200 ug of propoxur in 1 ml methanol (0.5-2 ppm). Water samples were extracted 3 times with 100 ml of dichloromethane each time. The dichloromethane extracts were combined and passed through anhydrous sodium sulphate to remove traces of water. The solvent was evaporated in a vacuum evaporator to dryness. The residue was dissolved in 5 ml of methanol and transferred to a 250-ml conical flasks fitted with \$ joints. From this stage onwards the procedure described in the standard curve preparation were followed, and the amount of propoxur recovered from water was calculated by referring to the standard curve. The absorbance was measured against a water blank (control).

One hundred g of each vegetable (cabbage, beans and okra) were placed in a blender jar and fortified with 50-500 ug of propoxur in 1 ml of methanol (0.5-5 ppm) and blended for 5 min at 3000 rpm. The sample was blended 3 times with 200 ml of acetonitrile for 5 min each time at 3000 rpm. The extract was filtered through Whatman No.1 filter paper and the filtrate was transferred to a 2-L separatory funnel. The acetonitrile-hexane partitioning technique described in AOAC (1975) was followed for the extraction of propoxur. The hexane extract thus obtained was evaporated to dryness in a vacuum evaporator, and the residue was dissolved in 5 ml of methanol and transferred to 250 ml conical flask fitted with \$ joints. The flask was connected with air condenser, and the mixture was refluxed for 30 min at 100°C on a water bath. The sample was cooled and transferred to a 1-L, roundbottom distillation flask with the help of 10 ml of methanol and acidified with 3 ml of phosphoric acid. One hundred ml distilled water was added, and the extract was steam distilled until 200-ml of distillate was collected. The distillate was extracted 3 times with 100-ml of diethyl ether. The ether layer was passed through anhydrous sodium sulphate to remove traces of water and evaporated to dryness in a vacuum evaporator. The residue was dissolved in 5 ml of methanol and followed the procedure as outlined under standard curve preparation.

RESULTS AND DISCUSSION

The basic principle underlying this method is treating the phenol obtained after alkaline hydrolysis of propoxur with concentrated nitric and sulphuric acids to get nitrosophenol which in the presence of excess of alcoholic ammonium hydroxide rearranges to form a highly colored quinoid salt. The color is stable for weeks. Beer's law was obeyed over the concentration range tested (0.5 to 10 ug/ml reaction mixture).

To check the adequacy of the method, water and vegetable samples were fortified with known amounts of propoxur and analyzed. Recoveries ranged from 95.1 to 98.0% for water samples (Table 1) and 93.5 to 97.8% for vegetable samples (Table 2). The comparative efficacy of the present method over the method (Appaiah et al. 1983) which uses 4,4-diaminodiphenyl sulfone can be seen from Table 2. It is evident that the proposed method is slightly superior to the 4,4-diaminodiphenyl sulphone method as the per cent recovery is higher than the latter method (88-94%).

Table 1. Recovery of propoxur from fortified water samples

Sample	Amount added (ug)	Amount recovered (ug)	Recovered[a]
Water	50	49.0 ± 0.32*	98.0
	100	97.3 ± 0.54	97.3
	150	144.6 ± 1.12	96.4
	200	190.2 ± 0.77	95.1

a Average of 5 individual determinations

* Standard deviation

The interferences from vegetable samples are avoided by measuring the absorbances against the respective crop controls (blank). Secondly, the interference from pigments and fats are avoided by recovering the phenol by distillation. Further, the sample does not require elaborate cleanup procedure as in the case of GLC, HPLC and TLC-enzymatic method reported earlier.

Table 2. Recovery of propoxur from vegetable samples

Sample	Amount added (ug)	Percent recovery[b] 4,4-diaminodiphenyl sulfone method	Proposed method
Okra	50	93.0	95.5
	100	90.1	94.7
	200	89.8	94.0
	400	88.7	93.7
	500	88.0	93.5
Cabbage	50	94.0	97.6
	100	91.0	97.0
	200	90.0	96.9
	400	89.7	96.5
	500	89.3	96.0
Beans	50	95.0	97.8
	100	91.4	97.5
	200	91.0	97.0
	400	89.9	96.8
	500	89.5	96.7

b Average of 5 individual determinations

Although the minimum detectability of the present method is not as good as GC, HPLC and TLC-enzymatic methods, the method can still be employed for the routine analysis of propoxur residues from vegetables and water. The method could detect as low as 0.5 ppm residues from vegetables (permissible limit 3 ppm) and water.

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