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Determination of Propoxur and 2-*Iso*propoxyphenol in Blood, Urine and Tissues

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Propoxur /2-isopropoxyphenyl N-methylcarbamate/ is widely used in protecting crops and in the hygienic sector against a number of household pests. For this reason there is often a necessity to determine trace amounts of propoxur and its main metabolite 2-isopropoxyphenol in different biological materials. There are many methods to determine propoxur; actually gas-chromatographic methods are preferred. However, there are some difficulties unless propoxur and its metabolite are determined separately, as during the derivatisation step propoxur is hydrolised to 2-isopropoxyphenol. The methods usually recommended to separate carbamates from phenols /MAGALLONA 1975/: partitioning operations with dilute alkali, column chromatography with selective elution or oxidation with cerium sulfate gave for propoxur and 2-isopropoxyphenol unsatisfactory results.

Therefore a procedure is presented in which propoxur is separated from 2-isopropoxyphenol by thin-layer chromatography and both substances are separately determined by gas chromatography using basically the methods published by COHEN et al. /1970/. Simultaneously with the separation a cleanup of extract occurs on the chromatoplates.

MATERIALS AND METHODS

Reagents: 2,4-dinitro-1-fluoro-benzene /DNFB/, 1 per cent acetone solution; TLC Developing solvent: benzene-acetone /19 + 1v/v/; chromogenic spray reagents:A/ 1.5 N ethanolic KOH solution, B/ 0.1 per cent w/v solution of 2,6-dibromo-p-benzoquinone-4chlorimine in acetone; Phosphate buffer pH 11 /dissolve 8.94 g $Na_2HPO_4 \cdot 12H_2O$ and 0.164 g NaOH and make up with water to 1L/.

Apparatus: Gas chromatograph, Pye 104 equipped with Ni^{63} electron capture detector and glass columns 5'x 1/4" o.d. /for urine often a 18' x 1/4" o.d. column was used/ packed with Chromosorb WHMD 80-100 mesh, coated with 10% DC-200; argon flow rate 60 mL/min, temperatures: inlet 240°, column 230°, detector 260°. For confirmation a 5' x 1/4" o.d. column packed with Chromosorb HMDS 100-120 mesh

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coated with 1,5% OV-17/2% QF1was used.

Preparation of chromatoplates: A 0,25 mm layer of silica gel G is prepared on 20 x 20 cm glass plates. The plates are left in position at room temperature for 15 min and activated by drying at 105° for 30 min, They are then stored in a desiccator until ready for use.

Standard solutions: a/ for GLC: 0.1 ug propoxur in 1 mL acetone; 0.1 ug 2-isopropoxyphenol in 1 mL acetone; b/ for TLC /as reference standards/: 0.5 mg propoxur and 0.5 mg 2-isopropoxyphenol in 1 mL acetone.

Preparation of standard curves: pipet different volumes of standard solutions corresponding to: 0.01, 0.025, 0,05, 0.1, 0.15 ug of propoxur or 2-isopropoxyphenol into glass-stoppered flasks. Add 0.5 mL of 1 per cent acetone solution of DNFB and 10 mL phosphate buffer, stopper the flask and heat on boiling water bath for 30 min. Cool and transfer quantitatively the reacting solution into a 250-mL separatory funnel and extract twice for 3 min with 10 mL portions of hexane. Combine the hexane layers and wash four times with 40 mL portions of redistilled water /to eliminate traces of DNFB/. Pour the extract through a 2 cm layer of sodium sulfate and concentrate on a rotary evaporator at 50° to desired volume /usually 4 mL/. Inject 5 uL of sample into the gas chromatograph.

Preparation of samples.

Blood: Dilute 2.5 mL of heparinized blood with 15 mL water, add 3 mL conc. HCl and heat under air condensor at 95° for 50 min. After the hydrolysis is completed extract the cooled sample in a separatory funnel with two 15 mL portions of dichloromethane for 5 min. Separate the layers by centrifuging. Drain the lower solvent layer through a 2 cm layer of sodium sulfate. Rinse the sulfate with pure solvent and concentrate the combined extracts to 1 mL under a gentle stream of dry nitrogen in temperature not exceeding 50°.

Urine: Dilute a sample of rat urine collected during 12 h with water /1 + 4v/v/. Filter and transfer by pipet 5 mL of diluted urine, add 3 mL of conc.HCl and continue with the procedure as described for blood.

Animal tissues /liver, kidney, brain/: Weigh 0.5 g finelly minced tissue, put into a glass-homogenizer and extract for 2 min with two 10 mL portions of dichloromethane. Drain the combined extracts through sodium sulfate and continue with the procedure as described for blood.

Separation and cleanup.

Spot 0.5 mL of concentrated extracts on the chromatoplate in a 2-cm wide band. For matching 0.02 mL of the standard solution spot on the same plate. Develop the plates in developing solvent /EL-DIB 1970/, dry and spray the standards with reagent A followed by reagent B keeping the analyzed areas covered. Mark the chromatogram with a sharp instrument into identical rectangular areas /about 2x2 cm/ each of which contains a spot of either the unknown or the standard. Elute the scraped out absorbent portions with 3 mL of acetone into glass-stoppered flasks. Add 0.5 mL of DNFB solution and continue with the procedure as described in "preparation of standard curves".

RESULTS AND DISCUSSION

Propoxur and 2-isopropoxyphenol in the range of concentration 0.01-0.15 ug gives linear response to electron-capture detector. For higher concentrations the response was nonlinear.

Recoveries for propoxur and 2-isopropoxyphenol from silica gel chromatoplates were tested. 0.1 - 0.5 ug of both substances were spotted on chromatoplates and treated as described above. The results were presented in table 1.

TABLE 1

Recoveries of propoxur and 2-isopropoxyphenol from TLC-plates

Added /ug/	Percent recoveries x/	
	Propoxur	2-Isopropoxyphenol
0.1	92	98
0.3	91	98
0.5	91	90

x/ Average of three determinations.

Recoveries of propoxur and 2-isopropoxyphenol from fortified blood, urine and tissues /liver, kidney, brain/ were determined. The results were presented in table 2.

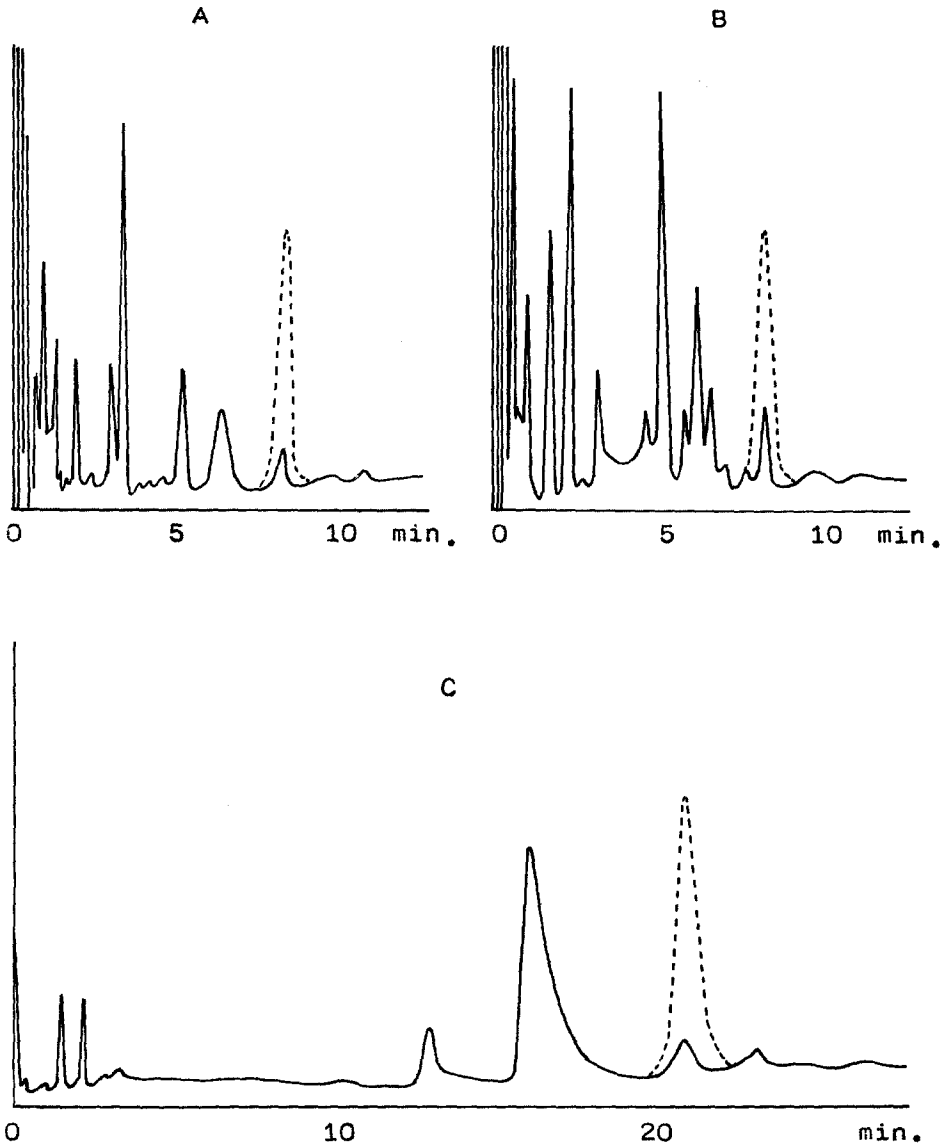

Figure 1. A/ Chromatogram of blood fortified with 0.2 ppm propoxur /column 5´x 1/4"/

B/ Chromatogram of liver fortified with 0.2 ppm 2-isopropoxyphenol /column 5´x 1/4"/

C/ Chromatogram of urine fortified with 0.5 ppm propoxur /column 18´x 1/4"/.

TABLE 2

Recoveries of propoxur and 2-isopropoxyphenol from blood, urine and tissues

Material	Added /ppm/	Average percent recoveries x/	
		Propoxur	2-isopropoxyphenol
blood	0.1	93	89
	0.2	95	92
liver	0.2	89	98
	0.5	95	91
kidney	0.2	101	89
	0.5	89	100
urine	0.2	79	75

x/ Average of three determinations.

The sensivity of the method in the tested materials was 0.05 ppm for propoxur and isopropoxyphenol.

Typical chromatograms of control and fortified blood, tissue and urine are presented in figure 1.

The method described was used with success in a metabolic study in which both substances were determined in rats exposed to propoxur and isopropoxyphenol /results to be published/.

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