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Note

Method for the determination of 2,4-dichlorophenoxyacetic acid residues in urine

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Studies with humans who have ingested 2,4-dichlorophenoxyacetic acid (2,4-D) have indicated that the herbicide is first absorbed into the blood plasma, whereupon it is excreted into the urine at a rate dependent upon the concentration in the blood[1–3].

Several analytical procedures have been described for the extraction and analysis of 2,4-D in human urine, but these involve either the use of toxic benzene for the extraction[1,4], or sophisticated analytical procedures not available in most laboratories[5].

Macroreticular resins, especially XAD-2, have been used to remove drug related compounds from urine, blood, and stomach contents prior to analysis[6–8]. XAD-2 resins have also proved very useful for absorbing phenoxyacetic herbicides from air[9] and aqueous solutions[10–13].

On the Canadian prairies approximately $5 \cdot 10^6$ kg of 2,4-D formulations are applied annually for weed control. The present studies were conducted as part of a continuing investigation to monitor the possible presence of 2,4-D in the urine of sprayer operatives, resulting from constant exposure to the spray drift. Recovery of 2,4-D acid from urine samples fortified at the 0.1-ppm level was determined after absorption and subsequent elution from Amberlite XAD-2 resin. The 2,4-D was analysed gas chromatographically as the methyl ester, and confirmed by transesterification to the *n*-butyl derivative.

EXPERIMENTAL

Urine samples and fortification

Specimens were taken from 5 males, ranging in age from 25 to 55 years, by having them collect their overnight urine on awakening in the morning. Aliquots of 100 ml were used as controls, while further 100-ml portions were fortified with 40 μl of a solution of 2,4-D acid containing 0.25 mg/ml of methanol so that the urine samples were 0.1 ppm with respect to 2,4-D. The specimens were stored under refrigeration at 4° and extracted and analysed within 24 h of collection and fortification.

Extraction

To a glass chromatography column (40 × 1.4 cm I.D.) fitted with a stop-cock was added a small plug of glass wool and 5 g Amberlite XAD-2 resin (Mallinckrodt,

St. Louis, Mo., U.S.A.). The resin was washed consecutively, at a rate of 5 ml/min, with 50 ml distilled water, 50 ml 0.1 *M* sodium bicarbonate in 80% aqueous acetonitrile, and finally 50 ml distilled water.

Urine samples were first basified with 5 ml 20% aqueous sodium hydroxide for 2 min and then acidified using 10 ml concentrated hydrochloric acid before percolation through the XAD-2 resin with a flow-rate of approximately 5 ml/min. Following percolation, the resin was washed with distilled water until the pH of the eluate was neutral. The eluates were discarded. The column was percolated with 50 ml 0.1 *M* sodium bicarbonate in 80% aqueous acetonitrile at a flow-rate of 2 ml/min. This eluate was acidified with 5 ml concentrated hydrochloric acid and extracted twice with 50 ml volumes of diethyl ether. The aqueous phase was discarded while the combined ether extracts were evaporated on a rotary evaporator at 35°. Traces of water were removed from the flask by azeotropic distillation under reduced pressure following the addition of equal volumes of benzene and methanol. The residue was quantitatively transferred, using ether, to a 50-ml glass tube and methylated using 2 ml of a solution of diazomethane[1]. After evaporation almost to dryness to remove excess reagent and ether, the volume was adjusted to 50 ml with *n*-hexane and 5-μl aliquots examined gas chromatographically for the presence of 2,4-D methyl ester.

Confirmation of 2,4-D as the butyl ester

Any 2,4-D considered to be present as the methyl ester in the above *n*-hexane extracts was confirmed by trans-butylation to the *n*-butyl ester. This was carried out by adding 2 ml of *n*-butyl alcohol to 20 ml of the hexane extract in a 50-ml glass tube. Following removal of the hexane under reduced pressure using the rotary evaporator, 4 drops of concentrated sulphuric acid were added to the tube which was then immersed in an oil-bath at 100° for 1 h. On cooling, 25 ml of water and 10 ml of *n*-hexane were added, the tube stoppered and vigorously shaken. A 5-μl aliquot of the *n*-hexane layer was examined gas chromatographically for the presence of the *n*-butyl ester of 2,4-D.

Gas chromatographic analysis

A Hewlett-Packard Model 5713A gas chromatograph was used in these studies which was equipped with on-column injection facilities and a ^{63}Ni electron-capture detector operated at 300°. The glass column (1.5 m × 4 mm I.D.) was packed with 100–120 mesh Ultra-Bond 20 M (RFR, Hope, R.I., U.S.A.). Carrier gas was argon containing 5% methane at a flow-rate of 40 ml/min. With a column temperature of 150°, 2,4-D methyl ester had a retention time of 3.5 min, while the *n*-butyl ester eluted at 165° with a retention time of 4.0 min.

Standards of the methyl ester were prepared (in *n*-hexane) by methylation of pure 2,4-D, while those of the *n*-butyl ester, also in *n*-hexane, were prepared from the pure ester (K & K Labs., Jamaica, N.Y., U.S.A.). The concentrations of the esters present in the samples were calculated by comparing the sample peak height with those of the appropriate standards.

RESULTS AND DISCUSSION

Because there is no evidence to show that ingested 2,4-D esters are excreted

as the free acid, the urine samples in these studies were first basified. This would have the effect of hydrolysing any ester present to the acid form[14].

The XAD-2 resin was successful in removing the 2,4-D from the fortified urine specimens, while allowing much of the yellow colour to pass through the column. Aqueous acetonitrile containing sodium bicarbonate was used to elute the 2,4-D from the resin since this mixture has proved satisfactory for the separation of chlorinated phenoxyacetic acids and phenols on Amberlite XAD-2 columns during analysis by reversed-phase high-pressure liquid chromatography[13]. It was also noted that whereas acidified urine formed emulsions on shaking with diethyl ether, these did not occur with acidified eluting solvent following passage through the urine-percolated resin.

The recoveries of 2,4-D from the 5 fortified urine specimens were excellent (Table I), with a mean recovery and standard deviation of 94 $\pm$ 6%. All chromatograms derived from untreated urine samples showed the absence of any interfering substances with the same retention time as 2,4-D methyl ester. The chromatograms A and B (Fig. 1), derived from the 41 year old donor are typical. Although specimens were usually fortified and analysed within 24 h, separate experiments confirmed that

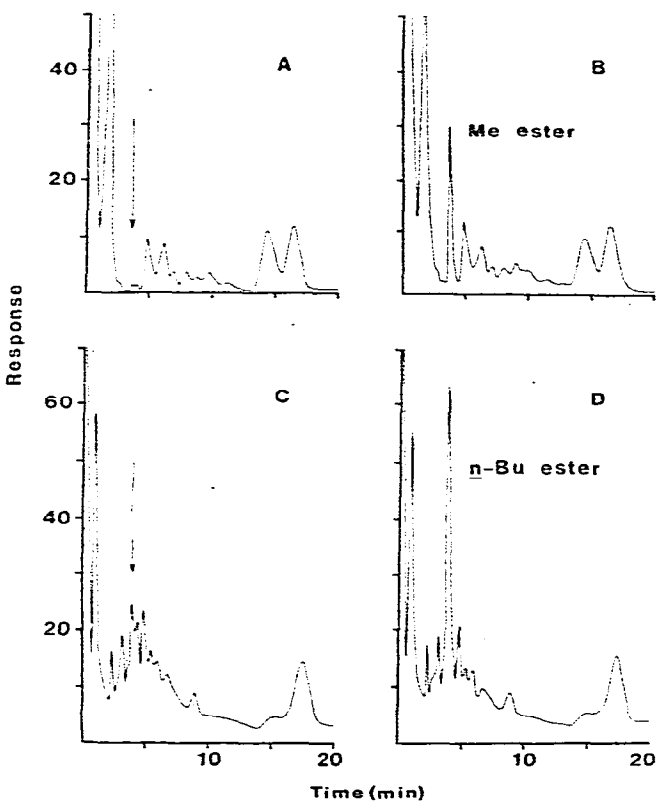

Fig. 1. Gas chromatograms derived from 5-μl injections of (A) methylated control urine extract, arrow indicates retention time of 2,4-D methyl ester; (B) methylated extract containing 0.1 ppm 2,4-D; (C) trans-butylated extract (A), arrow indicates retention time of 2,4-D *n*-butyl ester and (D) trans-butylated extract (B).

TABLE I

RECOVERY OF 2,4-D FROM FORTIFIED HUMAN URINE SAMPLES

Donor age (years)	*Amount added (ppm)*	*Amount recovered (ppm)*	*Recovery (%)*
25	0.1	0.093	93
27	0.1	0.095	95
30	0.1	0.099	99
41	0.1	0.100	100
55	0.1	0.085	85
		Average	94 ± 6

using this procedure over 90% of the 2,4-D could be recovered from urine, fortified at the 0.1-ppm level, and refrigerated at 4° for 5 days prior to analysis.

The butylation of herbicidal acids for gas chromatographic analysis using *n*-butanol and concentrated acid has been described[15,16]. These esters being less volatile than methyl esters have longer retention times and may thus be used for confirmatory purposes. By treating the methyl ester extracts as described trans-butylation was effected in yields of 85–90%. A slight drawback was that the trans-butylated extracts derived from the methylated control samples showed some interference with the same retention time as the *n*-butyl ester of 2,4-D (Fig. 1, chromatograms C and D). However, although quantitative analyses based on the trans-butylated extracts would be difficult the chromatograms were certainly adequate for the confirmation of 2,4-D in urine at the 0.1-ppm level.

This procedure using XAD-2 resin is rapid, requires no complex clean-up, and appears satisfactory for the extraction and analysis (as the methyl ester) of 2,4-D at the 0.1-ppm level in urine from males over an age range, while allowing confirmation by conversion of the methyl ester in the original analytical extract to the *n*-butyl ester.

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