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Note

Detection of sympathomimetic central nervous stimulants with special reference to doping

I. Comparative study of a conventional extraction procedure and adsorption chromatography using XAD-2 resin

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In the past, the isolation of sympathomimetic central nervous stimulants (CNS) from alkalinized human urine has been effected by a solvent extraction procedure using either chloroform or diethyl ether. As emulsion problems occur, necessitating centrifugation, and a single extraction step yields low recoveries for some drugs, resulting in the need for greater volumes of solvents, the traditional extraction method can be considered to be slow and expensive.

These problems have now been overcome by using adsorption chromatography with a styrene–divinylbenzene copolymer (XAD-2).

Fujimoto and Wang[1], Weissman *et al.*[2] and Mulé *et al.*[3] extended the application of XAD-2 resin to the extraction of a number of drugs of abuse, including narcotics, sympathomimetics and hypnotics. Several papers from the Association of Official Racing Chemists dealt with the use of XAD-2 resin with special reference to the doping control of racehorses[4].

The effect of varying urinary flow-rate, volume and pH and the elution solvent were studied by Kullberg *et al.*[5]. They obtained a recovery of 97.5 $\pm$ 6.7% for amphetamine using 40% isopropanol in ethyl acetate–1,2-dichloroethane (3:2). The modified Fujimoto method[1], elaborated by Roerig *et al.*[6], resulted in an extraction efficiency of 81.3% for amphetamine at pH 8.1. Hitherto, the application of XAD-2 resin for routine toxicological analysis was followed by thin-layer chromatography[3,6,7].

In order to evaluate the effectiveness of the conventional solvent extraction procedure and the XAD-2 method, a quantitative GC study of several sympathomimetic CNS derivatives was undertaken.

EXPERIMENTAL

Apparatus

All GC experiments were performed with a Varian 1400 gas chromatograph with a flame-ionization detector (FID), connected to a Varian CDS 101 integrator. The glass column (3 m $\times$ 1/8 in. I.D.) was packed with Apiezon L (15%) plus potas-

sium hydroxide (5%) on 80–100-mesh Chromosorb W. The column oven temperature was 160°, the injection port temperature 255° and the detector block temperature 230°. The carrier gas was nitrogen at a flow-rate of 25 ml/min.

Pre-packed XAD-2 resin cartridges containing 2.0 ± 0.1 g of resin were purchased from Brinkmann (Westbury, N.Y., U.S.A.).

The following compounds were investigated: *d,l*-amphetamine sulphate; chlorphentermine hydrochloride; cyclopentamine hydrochloride; dimethylamphetamine hydrochloride; *d,l*-N-ethylamphetamine hydrochloride; fenfluramine; mephentermine sulphate; methoxyphenamine hydrochloride; *d,l*-methylamphetamine hydrochloride; phenmetrazine; phentermine hydrochloride; 1-propylhexedrine hydrochloride and phendimetrazine bitartrate. Stock solutions (250 μg/ml) of these drugs were freshly prepared with doubly distilled water. All solutions were prepared at 20°.

Recovery with the conventional extraction procedure

Urine (10 ml) which contained the drug (25, 12.5 and 6.25 μg/ml) was pipetted into a glass-stoppered tube. All experiments were carried out in quadruplicate for each concentration. After adjusting the pH to 11–12 with a few drops of a 50% sodium hydroxide solution, the urine was extracted three times with 7 ml of chloroform using a mechanical shaker (20 min), centrifuged and the organic phases were separated. The combined chloroform extracts were then dried over anhydrous sodium sulphate, treated with 0.5 ml of an ethereal solution of hydrochloric acid and evaporated to dryness *in vacuo* at 40°.

A 1-ml volume of an internal standard solution (125 μg/ml *d,l*-N-methylamphetamine hydrochloride or mephentermine sulphate in freshly distilled diethylamine–chloroform, 1:100) was added and 2 μl were injected into the gas chromatograph. Standard graphs were obtained using different concentrations (250, 125 and 62.5 μg/ml) of the drug investigated dissolved in the internal standard solution. Each solution was gas chromatographed four times. In all instances the correlation coefficient of the regression equations lies between 0.9997 and 0.9984.

Recovery with the XAD-2 method

In this study, the method described by Kullberg *et al.*[5] was followed. Nevertheless, when using this procedure for GC experiments we found that it was necessary to clean up the pre-packed columns with chloroform (10 ml) followed by doubly distilled water (10 ml) immediately before use. In one series of experiments, urine (20 ml) which contained the drug (6.25 μg/ml) adjusted to pH 9.0 by the addition of 2 ml of ammonium chloride buffer solution (pH 9.5) was passed through the cleaned column, followed by doubly distilled water (20 ml). After aspirating the columns dry, the XAD-2 resin was eluted with 20 ml of isopropanol–ethyl acetate–dichloroethane (25:45:30). The eluates were dried over anhydrous sodium sulphate, treated with 0.5 ml of an ethereal solution of hydrochloric acid and evaporated to dryness *in vacuo*. A 1-ml volume of the internal standard solution was added and 2 μl were gas chromatographed. All experiments were carried out in quadruplicate for each drug.

The XAD-2 resin technique was also tested under the solvent and pH conditions of the conventional procedure. The urine containing the drug was adjusted to

pH 11–12 with a few drops of a 50% sodium hydroxide solution. After cleaning up the XAD-2 resin as described above, the columns were treated with 10 ml of sodium hydroxide solution (pH 12). The drugs were eluted with 20 ml of chloroform. All assays were carried out in quadruplicate for each drug.

RESULTS AND DISCUSSION

The results of the experiments are given in Table I. The recoveries of the compounds studied when using the conventional extraction procedure were 86.9–101.3%. The necessity for three extraction steps was clearly demonstrated for amphetamine, recoveries of $53.6 \pm 4.07\%$, $80.0 \pm 3.05\%$ and $98.4 \pm 3.03\%$ being obtained after one, two and three extractions, respectively, with 7 ml of chloroform. As the adsorption efficiency of amphetamine on XAD-2 is unaffected in the concentration range 0.5–500 μg/ml in urine, as demonstrated by Kullberg *et al.*[5], a relatively high drug concentration (6.25 μg/ml) was chosen for the XAD-2 experiments, resulting in an decrease in the experimental errors (the final residue was re-dissolved in 1 ml of internal standard solution for GC analysis).

TABLE I

COMPARISON OF RECOVERIES OF DRUGS USING CHLOROFORM EXTRACTION AND XAD-2 ADSORPTION CHROMATOGRAPHY

The figures in parentheses are standard deviations.

Compound	*Conventional liquid–liquid extraction*	*XAD-2 procedure*	
		pH 9.5 (isopropanol–ethyl acetate–dichloroethane)	*pH 12 (chloroform)*
Amphetamine	96.1 (5.09)	79.0 (3.20)	83.0 (5.69)
Chlorphentermine	92.3 (6.10)	63.6 (3.88)	88.6 (2.20)
Cyclopentamine	86.9 (3.79)	53.0* (5.08)	53.9* (2.61)
Dimethylamphetamine	101.3 (4.05)	97.3 (2.02)	94.3 (2.10)
Ethylamphetamine	97.0 (3.59)	84.6 (5.12)	90.1 (3.25)
Fenfluramine	90.7 (3.50)	90.9 (3.14)	90.1 (3.71)
Mephentermine	86.7 (4.53)	96.4 (6.79)	76.9 (8.32)
Methoxyphenamine	95.7 (7.09)	61.3 (2.85)	84.4 (4.70)
Methylamphetamine	87.2 (2.17)	59.1 (6.55)	71.6 (1.57)
Phendimetrazine	91.9 (7.04)	—**	—**
Phenmetrazine	95.3 (4.69)	91.5 (5.50)	97.7 (2.90)
Phentermine	93.3 (5.33)	71.5 (4.09)	79.8 (4.41)
Propylhexedrine	96.2 (5.64)	95.1 (3.70)	96.5 (5.32)

* Mean value of 8 assays.

** Unreliable results due to interfering peak.

The results of the XAD-2 method at pH 9.5 using isopropanol–ethyl acetate–dichloroethane (25:45:30) as elution solvent differ widely from those of the conventional method. Moreover, for six of the compounds investigated the recoveries with this procedure are significantly lower.

However, the recoveries with the resin method (pH 12) using chloroform as elution solvent are similar to those with the conventional procedure, except for cyclo-

pentamine (pH 9.5 and 12). The low recovery of this compound could not be attributed to the effects of the flow-rate of urine or elution solvent because for this derivative all experiments were duplicated using different blank urines. Moreover insofar as cyclopentamine can be compared to amphetamine, Kullberg *et al.*[5] showed that the adsorption of amphetamine on to XAD-2 resin was essentially independent of the flow-rate and amount of urine used.

Except for mephentermine, the increased pH resulted in a higher recovery for all compounds. This effect could not be attributed to the change in elution solvent as an additional experiment with phentermine using XAD-2 yielded recoveries of $68.9 \pm 4.56\%$ using chloroform (pH 9.5) and $80.5 \pm 3.83\%$ using isopropanol–ethyl acetate–dichloroethane (pH 12) as the elution solvent.

In conclusion, the results in Table I clearly demonstrate the effectiveness of the XAD-2 resin procedure at pH 12 with chloroform as the elution solvent. Moreover, as this method is also rapid and hence inexpensive, the use of XAD-2 resins could be applied to the detection of sympathomimetic CNS derivatives with special reference to doping analysis.

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