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DETECTION OF SYMPATHOMIMETIC CENTRAL NERVOUS STIMULANTS WITH SPECIAL REFERENCE TO DOPING

II. COMPARATIVE STUDY OF TWO ADSORPTION CHROMATOGRAPHY METHODS USING DIFFERENT XAD RESINS

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SUMMARY

Recoveries of a series of sympathomimetic central nervous stimulants in human urine are measured using either adsorption chromatography on self-filled columns (method A) or with a special resin method suitable for racehorse urine (method B). The Amberlite resins used are XAD-2, XAD-4, XAD-7 and XAD-8 and elution is performed using chloroform.

The reported comparative drug extractabilities indicate that in most instances the recoveries follow the sequence XAD-4 > XAD-2 ≈ XAD-8 ≫ XAD-7 using method A. Based on the recovery and purity of the extracts obtained, XAD-8 is preferred for gas chromatographic analysis while XAD-4 is very suitable for thin-layer chromatographic screening work.

Comparing the two methods, equally good or better results were obtained with method A for all of the resins studied except XAD-7. Finally, it was found that the effect of refrigerated storage of the resins on the drug extractabilities for central nervous stimulants could be neglected.

INTRODUCTION

In a previous paper[1], the recoveries of a series of sympathomimetic central nervous stimulants (CNS) in human urine were measured using either conventional liquid–liquid extraction with chloroform or resin adsorption chromatography on prepacked columns filled with XAD-2. The comparative drug extractabilities found between chloroform extraction and adsorption chromatography indicated that in most instances the drugs were extracted almost equally well by the rapid XAD-2 technique using chloroform as elution solvent.

As a result of this work, a comparative study was undertaken in order to optimize the recoveries using different XAD resins and the method already described[1] (method A).

On the other hand, owing to the frequently high viscosity of alkalinized horse urine, the direct passage of such samples through a column is not recommended. Therefore, large volumes of both diluted and undiluted racehorse urine adjusted to an appropriate pH were extracted by shaking with XAD-2 resin[2–5], and the resin washed and transferred into a column for the elution step. The drug recoveries from human urine using this method (method B) were also determined in this work, using XAD-2, XAD-4, XAD-7 and XAD-8 resins.

Further, the effect of refrigerated storage of the different XAD resins on the drug extractabilities of some CNS compounds using both methods was investigated.

EXPERIMENTAL

Apparatus

All gas chromatography (GC) experiments were performed with a Varian 1400 FID gas chromatograph connected to a Varian CDS 101 integrator. The glass column (3 m × 1/8 in. I.D.) was packed with Apiezon L (15%) and potassium hydroxide (5%) on 80–100-mesh Chromosorb W. The operating conditions were: column oven temperature, 160°; injection port temperature, 255°; detector block temperature, 230°; and carrier gas (nitrogen) flow-rate, 25 ml/min.

Sample reservoirs and empty chromatography columns were purchased from Brinkmann (Westbury, N.Y., U.S.A.).

Amberlite XAD resins (300–1000 μm) were purchased from Serva Feinbiochemica (Heidelberg, G.F.R.). The pore sizes and surface areas of the resins were: XAD-2, 90 Å and 330 m^2/g; XAD-4, 50 Å and 750 m^2/g; XAD-7, 80 Å and 150 m^2/g; and XAD-8, 250 Å and 140 m^2/g.

A polyester screen (80 mesh) was kindly supplied by Mr. G. H. Johnston, Lynn & Johnston Labs. (Lachine, Canada).

Compounds

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, phendimetrazine bitartrate, phenmetrazine, phentermine hydrochloride and *d,l*-propylhexedrine hydrochloride. Stock solutions (250 μg/ml) of these drugs were freshly prepared with double-distilled water. All analytical work was carried out at 20°

Column preparation and conditioning of Amberlite resins

The chromatographic columns used in method A were filled with 2.0 ± 0.1 g of resin. The bottom of the column contained a piece of 80-mesh polyester screen while the top of the resin bed was covered with a small plug of cotton-wool. The resin was washed with the following solvents: 10 ml of chloroform, 10 ml of methanol and 2 × 10 ml of double-distilled water. Immediately before use, the columns were treated with 10 ml of 0.01 *N* sodium hydroxide solution.

Using method B, 2.0 ± 0.1 g of resin were rinsed in an erlenmeyer flask (50 ml) with the same solvents, except 0.01 N sodium hydroxide solution, and the sequence described in method A.

RESULTS AND DISCUSSION

Recovery of method A

The method developed by Kullberg *et al.*[6] and modified as mentioned in a previous paper[1] was used (urinary pH, 11–12; elution solvent, chloroform). The urinary drug concentration and standard solutions were as described earlier[1]. All experiments were replicated six times for each drug. The adsorption and elution of the compounds were performed under conditions of free gravitational flow. The recoveries of this procedure for XAD-2, XAD-4, XAD-7 and XAD-8 are given in Table I.

TABLE I

COMPARATIVE DRUG EXTRACTABILITIES (%) USING DIFFERENT XAD RESINS (METHOD A)

The figures in parentheses are standard deviations.

Drug	*XAD-2*	*XAD-4*	*XAD-7*	*XAD-8*
Amphetamine	72.8 (3.70)	79.6 (3.15)	63.4 (5.28)	78.6 (3.72)
Chlorphentermine	78.6 (8.10)	92.4 (1.98)	76.2 (6.19)	86.3 (2.04)
Cyclopentamine	68.9 (5.66)	70.2 (4.61)	11.6 (2.02)	43.1 (4.02)
Dimethylamphetamine	60.3 (6.01)	81.4 (7.16)	36.5 (3.85)	53.4 (2.89)
Ethylamphetamine	80.3 (5.03)	92.7 (2.10)	53.8 (6.18)	76.9 (2.76)
Fenfluramine	75.7 (2.41)	67.5 (1.31)	31.7 (3.56)	77.9 (5.55)
Mephentermine	51.1 (7.68)	69.3 (1.96)	34.5 (2.03)	77.1 (4.40)
Methoxyphenamine	68.4 (2.52)	70.4 (4.61)	12.5 (1.79)	62.7 (5.79)
Methylamphetamine	75.1 (5.47)	82.3 (2.86)	47.3 (2.01)	90.2 (7.33)
Phendimetrazine	91.8 (9.32)	93.6 (5.56)	99.8 (8.11)	92.5 (8.16)
Phenmetrazine	85.8 (8.00)	89.4 (2.10)	88.9 (3.77)	70.5 (6.97)
Phentermine	81.7 (2.71)	96.1 (8.32)	69.3 (5.52)	81.4 (2.78)
Propylhexedrine	68.9 (0.76)	42.7 (6.45)	16.1 (2.98)	46.2 (6.93)

The results in Table I indicate that the drug extractabilities on XAD resins follow the sequence XAD-4 > XAD-2 ≈ XAD-8 ≫ XAD-7. Nevertheless, as a result of the great adsorption of urinary impurities on XAD-4 and/or the incomplete removal of the styrene monomers in the column cleaning procedure, this resin is not recommended for use in the GC of very concentrated urinary extracts. Moreover, the use of the purer Servachrom XAD-4 resin did not improve the results.

As mentioned by Machata *et al.*[7], the pore size of XAD-4 seems to be optimal for the extraction of drugs. Nevertheless, we believe that in addition to the lower pore size, the large surface area also plays an important role in the very good results obtained with XAD-4.

Although the recoveries of cyclopentamine, dimethylamphetamine and propylhexedrine on XAD-8 are poor, this resin is to be preferred to XAD-2 for the analysis of drugs in concentrated urinary extracts by GC owing to the very pure chromatograms obtained. For screening purposes using thin-layer chromatography, however, adsorption chromatography on XAD-4 could be used without difficulty.

Further, it is noteworthy that in most instances the extractabilities using XAD-2 in this work were lower than the corresponding recoveries obtained with the prepacked XAD-2 resin cartridges[1]. It was demonstrated by Kullberg and co-workers[6,8]

that in contrast to morphine and phenobarbital, the extractability of amphetamine on XAD-2 resin was independent of the urinary flow-rate. Nevertheless, the lower recoveries with the procedure used compared with the pre-packed column method[1] could be due to the greater urinary and elution solvent flow-rates resulting from the replacement of the cotton-wool plug at the bottom of the column with an 80-mesh screen. Moreover, it should be noted that the dependence of amphetamine recovery on urinary flow-rate seems to be rather controversial[6,8,9].

Recovery of method B

As already mentioned, the passage of undiluted horse urine through XAD-2 columns causes some difficulties owing to the high viscosity. This problem was overcome by shaking 100 ml of buffered urine (pH 9.5) with 5 g of XAD-2 resin, pouring the resin through a glass column and eluting with 25 ml of ethyl acetate–dichloromethane (60:40)[2]. Other workers[5] used four 5-ml fractions (aqueous drug solutions), which were shaken with the same amount of resin.

To compare the two methods (A and B), 2.0 ± 0.1 g of rinsed resin were shaken with 20 ml of spiked human urine (pH 12–13) for 15 min. After decanting the urine, the resin was poured through the column with small volumes of 0.001 *N* sodium hydroxide solution. The columns were sucked dry and eluted with 20 ml of chloroform Subsequent stages, urinary drug concentration and standard solutions were as described earlier[1].

The recoveries of this method for the resins used are given in Table II, and are the mean values of six determinations.

The recoveries in Table II do not obey the general sequence found with method A. For use in doping analysis with method B, XAD-8 and XAD-2 are to be preferred to the other resins.

On comparing the drug extractabilities for the two methods, it should be mentioned that the lower recoveries with XAD-7 in method A are substantially higher

TABLE II

COMPARATIVE DRUG EXTRACTABILITIES (%) USING DIFFERENT XAD RESINS (METHOD B)

The figures in parentheses are standard deviations.

Drug	*XAD-2*	*XAD-4*	*XAD-7*	*XAD-8*
Amphetamine	71.2 (4.20)	51.1 (2.96)	72.1 (4.17)	45.3 (2.72)
Chlorphentermine	84.8 (1.58)	70.7 (3.94)	85.6 (2.96)	87.0 (3.53)
Cyclopentamine	57.5 (3.36)	39.8 (3.80)	13.2 (1.41)	44.1 (6.09)
Dimethylamphetamine	65.8 (5.90)	— *	29.1 (5.95)	49.8 (5.90)
Ethylamphetamine	86.1 (5.21)	80.7 (5.35)	65.6 (3.30)	84.4 (3.20)
Fenfluramine	66.6 (4.12)	69.9 (3.56)	71.2 (8.72)	80.9 (6.69)
Mephentermine	51.5 (4.99)	74.7 (6.46)	34.0 (4.08)	57.9 (4.82)
Methoxyphenamine	57.5 (5.47)	56.4 (3.39)	21.3 (3.73)	53.8 (5.76)
Methylamphetamine	76.2 (6.31)	68.4 (3.22)	52.5 (5.00)	86.3 (5.64)
Phendimetrazine	82.9 (8.48)	97.0 (4.37)	99.0 (6.37)	90.2 (5.39)
Phenmetrazine	76.2 (4.75)	96.0 (3.80)	63.5 (4.32)	54.6 (2.36)
Phentermine	59.4 (6.17)	75.4 (3.89)	60.4 (4.12)	68.9 (1.63)
Propylhexedrine	28.9 (4.46)	43.0 (7.22)	11.0 (0.74)	55.9 (2.30)

* Not measured owing to interfering peak.

TABLE III

EFFECT OF REFRIGERATED STORAGE OF XAD RESINS ON THE DRUG EXTRACTABILITY (%) USING METHOD A

The figures in parentheses are the recoveries (%) without refrigerated storage of the resin.

Resin	*Amphetamine*	*Cyclopentamine*	*Fenfluramine*	*Methoxyphenamine*	*Methylamphetamine*	*Phendimetrazine*	*Phenmetrazine*
XAD-2	77.9 ± 4.63	70.8 ± 3.62	73.9 ± 4.46	91.0 ± 3.60	78.9 ± 0.34	97.8 ± 3.02	96.6 ± 3.20
	(72.8 ± 3.70)	(68.9 ± 5.66)	(75.7 ± 2.41)	(68.4 ± 2.52)	(75.1 ± 5.47)	(91.8 ± 9.32)	(85.8 ± 8.00)
XAD-4	78.1 ± 2 12	65 2 ± 2.85	66.0 ± 3.72	66.4 ± 2.93	80.2 ± 4.47	87.3 ± 5.06	93.3 ± 2.09
	(79.6 ± 3.15)	(70.2 ± 4.61)	(67.5 ± 1.31)	(70.4 ± 4.61)	(82.3 ± 2.86)	(93.6 ± 5.56)	(89.4 ± 2.10)
XAD-7	72.8 ± 5.02	17.9 ± 2.03	72.9 ± 6.34	36.3 ± 3.83	69.1 ± 5.44	—*	90.3 ± 5.08
	(63.4 ± 5.28)	(11.6 ± 2.02)	(31.7 ± 3.56)	(12.5 ± 1.79)	(47.3 ± 2.01)		(88.9 ± 3.77)
XAD-8	65.1 ± 2.82	55.5 ± 5.96	77.8 ± 3.53	74.4 ± 3.57	100.1 ± 5.86	91.5 ± 3.38	59.6 ± 2.30
	(78.6 ± 3.72)	(43.1 ± 4.02)	(77.9 ± 5.55)	(62.7 ± 5.79)	(90.2 ± 7.33)	(92.5 ± 8.16)	(70.5 ± 6.97)

* Not measured.

TABLE IV

EFFECT OF REFRIGERATED STORAGE OF XAD RESINS ON THE DRUG EXTRACTABILITY (%) USING METHOD B

The figures in parentheses are the recoveries (%) without refrigerated storage of the resin.

Resin	*Amphetamine*	*Cyclopentamine*	*Fenfluramine*	*Methoxyphenamine*	*Methylamphetamine*	*Phendimetrazine*	*Phenmetrazine*
XAD-2	80.8 ± 1.82	53.6 ± 3.70	61.5 ± 4.81	55.1 ± 4.72	63.7 ± 4.91	81.8 ± 1.76	76.3 ± 3.14
	(71.2 ± 4.20)	(57.5 ± 3.36)	(66.6 ± 4.12)	(57.5 ± 5.47)	(76.2 ± 6.31)	(82.9 ± 8.48)	(76.2 ± 4.75)
XAD-2	50.6 ± 3.09	43.2 ± 4.41	65.3 ± 1.31	54.9 ± 3.38	63.4 ± 4.50	99.6 ± 2.79	83.6 ± 0.77
	(51.1 ± 2.96)	(39.8 ± 3.80)	(69.9 ± 3.56)	(56.4 ± 3.39)	(68.4 ± 3.22)	(97.0 ± 4.37)	(96.0 ± 3.80)
XAD-7	58.0 ± 4.70	16.4 ± 3.10	71.4 ± 1.70	27.5 ± 3.99	54.9 ± 2.06	101.1 ± 5.00	63.7 ± 4.86
	(72.1 ± 4.17)	(13.2 ± 1.41)	(71.2 ± 8.72)	(21.3 ± 3.73)	(52.5 ± 5.00)	(99.0 ± 6.37)	(63.5 ± 4.32)
XAD-8	50.1 ± 5.15	47.4 ± 3.96	72.6 ± 2.25	53.9 ± 4.23	98.1 ± 3.46	76.1 ± 3.34	42.8 ± 1.67
	(45.3 ± 2.72)	(44.1 ± 6.09)	(80.9 ± 6.69)	(53.8 ± 5.76)	(86.3 ± 5.64)	(90.2 ± 5.39)	(54.6 ± 2.36)

in some instances when method B is used. In a liquid chromatographic separation study of phenols using XAD-7 resin, it was noticed by Fritz and Willis[10] that this resin had been chemically altered under alkaline conditions. Hence the low recoveries using XAD-7 (method A) could be attributed to the lower absorptive capacity of the resin owing to partial hydrolysis of the ester groups during the washing step with 0.01 *N* sodium hydroxide solution. Indeed, the results in Table II demonstrate the better recoveries with XAD-7 in method B without preliminary washing with sodium hydroxide solution.

On the other hand, generally similar (XAD-2) or even better results (XAD-4, XAD-8) are obtained with method A. The low values obtained in method B could not be improved by increasing the shaking time; in an additional experiment with propylhexedrine using XAD-8 resin, the recoveries were 35.3 $\pm$ 4.62, 51.2 $\pm$ 4.72, 55.9 $\pm$ 2.48, 47.9 $\pm$ 8.04 and 51.2 $\pm$ 8.05% for shaking times of 5, 10, 15, 30 and 60 min, respectively. Nevertheless, it is possible that an enhancement of the resin to urine ratio could increase the recoveries for method B.

Effect of refrigerated storage of XAD resin on the drug extractability

Bastos *et al.*[11] mentioned that the refrigerated storage of XAD-2 under distilled water for 7–14 days increased the recoveries of morphine and phenobarbital by 20% and 12.6%, respectively.

The effect of refrigerated storage on the recovery of some CNS stimulants was studied here using different XAD resins and methods A and B, 2.0 $\pm$ 0.1-g portions of the XAD resins being washed and stored for 7 days under distilled water at 4°. The results of these experiments (mean values of four determinations) compared with those found with the normal procedure are given in Table III (method A) and Table IV (method B).

Taking into account the standard deviations, the drug extractabilities in Tables III and IV clearly show, with the exception of XAD-7 (method A), that in contrast to morphine and phenobarbital the effect of refrigerated storage of the resins on the recovery of CNS stimulants is negligible for both methods.

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