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Note

Detection of sympathomimetic central nervous stimulants with special reference to doping

III. Column extraction with Extrelut®

F. T. DELBEKE and M. DEBACKERE
Laboratorium voor Farmacologie en Toxicologie der Huisdieren, Faculteit Diergeneeskunde, Rijksuniversiteit Gent, Casinoplein 24, B-9000 Gent (Belgium)
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In previous papers[1,2] the recoveries of a series of sympathomimetic central nervous stimulants in human urine were measured using either conventional liquid–liquid extraction with chloroform or resin adsorption chromatography with several XAD resins. Although the comparative drug extractabilities found show very good results with XAD-4 or XAD-2, the less pure extracts made this method less suitable for gas chromatographic (GC) work.

In order to improve the routine doping analysis, column extraction with Extrelut® was performed. As with the XAD resin technique, this method yields extracts which are free from emulsions and hence is less time consuming than conventional liquid–liquid extraction. Moreover, using the Extrelut columns it is not necessary to dry the eluates prior to evaporation.

EXPERIMENTAL

Apparatus

All of the GC experiments were performed with a Varian 1400 gas chromatograph equipped with a flame ionization detector and connected to a Varian CDS 101 integrator. The glass column (3 m × 1/8 in. I.D.) was packed with Apiezon L (15%) plus potassium hydroxide (2.5%) on Chromosorb W (80–100 mesh). For most of the drugs analysed the column oven temperature was 160°. The injection port and detector block temperatures were 250°. The carrier gas was nitrogen at a flow-rate of 25 ml/min.

The GC analysis of the norephedrine derivative was carried out on a 3% OV-7 column at an oven temperature of 145°.

The Extrelut® columns and fillings were purchased from E. Merck (Darmstadt, G.F.R.).

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Compounds

The following compounds were investigated: *d,l*-amphetamine sulphate; benzphetamine hydrochloride; chlorphentermine hydrochloride; cyclopentamine hydrochloride; dimethylamphetamine hydrochloride; ephedrine hydrochloride; *d,l*-N-ethylamphetamine hydrochloride; fencamfamine hydrochloride; fenfluramine hydrochloride; mephentermine sulphate; methoxyphenamine hydrochloride; *d,l*-methylamphetamine hydrochloride; methylephedrine hydrochloride; norephedrine hydrochloride; phenmetrazine hydrochloride; phentermine hydrochloride; *l*-propylhexedrine hydrochloride; phendimetrazine bitartrate and tranylcypromine sulphate. Stock solutions (250 μg/ml) of these drugs were freshly prepared with double distilled water.

All of the analytical work was carried out at 20°.

Elution procedure

A description of the Extrelut columns, general extraction techniques and applications is given elsewhere[3].

In our experiments, human urine, undiluted horse plasma or heparinized horse blood (diluted 1:4) were brought to pH 10.5 and different drug concentrations (4, 2 and 1 μg/ml) were made using these biological fluids.

Twenty millilitres of these biofluids were applied to the column and adsorbed by the support. After 20 min the column was eluted with 40 ml freshly distilled diethyl ether yielding *ca.* 25 ml of eluate within at least 20 min. After treating the eluate with 3 or 4 drops of an ethereal solution of hydrochloric acid and evaporating to dryness *in vacuo* at 40°, 250 μl of an appropriate internal standard solution (125 μg/ml in freshly distilled diethylamine–chloroform, 1:100) were added and 2 μl were injected into the gas chromatograph.

All of the experiments were repeated three times for drug concentrations of 4 μg/ml and 2 μg/ml and four times for the lowest drug concentration. Standard graphs were obtained using different concentrations (320, 160 and 80 μg/ml) of the drug investigated, dissolved in the internal standard solution. Each solution was gas chromatographed (2 μl) four times. In all instances the correlation coefficient of the regression equations lies between 0.9999 and 0.9964.

Owing to the non-linear adsorption of norephedrine, this compound was quantitatively determined as the N-TFA-O-TMS derivative[4,5]. Thus, 100 μl of standard solutions (0.5, 0.25 and 0.125 mg/ml) in methanol containing the internal standard (ephedrine, 0.25 mg/ml) were placed in a Reacti-vial. After addition of 10 μl of a methanolic solution of methyl orange (1 mg/ml) to control the trimethylsilylation potential, the sample was evaporated to dryness under nitrogen and 10 μl of trifluoroacetic acid (TFA) followed by the appropriate amount of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) were added. After reaction at 70° for 1 h, the sample was cooled, 20–25 μl of N-methylbis(trifluoroacetamide) (MBTFA) added and 1–2 μl of the resulting solution were gas chromatographed ($\sigma_R = 0.9957$).

The column eluates of the biological fluids containing norephedrine (6.25, 3.125 and 1.5625 μg/ml) were evaporated *in vacuo* and redissolved in 250 μl of the internal standard solution. An aliquot (100 μl) was analysed as described above. All of the experiments were carried out in triplicate for each concentration.

Finally, it should be noted that all glassware was silanized to reduce drug adsorption[6].

RESULTS AND DISCUSSION

The drug recoveries using Extrelut and elution with diethyl ether are presented in Table I. Except for ephedrine and norephedrine, the recovery rates are very high and could almost be regarded as quantitative. Furthermore, the drug extractabilities are superior both to the conventional extraction procedure[1] (10 ml urine with 3 × 7 ml chloroform) and the XAD adsorption technique[2] (10 ml urine eluted with 20 ml chloroform).

It is well known[7] that there is a considerable degradation of ephedrines when the diethyl ether used as solvent is not completely free from peroxides. Since the elution solvent used here was freshly distilled over Mohr's salt, the lower yield for ephedrine and norephedrine could be attributed to the more polar character of these

TABLE I

DRUG EXTRACTABILITIES USING EXTRELUT

No.	*Compound*	*Human urine*	*Undiluted horse plasma*	*1:4 Diluted horse blood*
1	Amphetamine	94.9 ± 4.99	85.3 ± 4.53	95.2 ± 5.14
2	Benzphetamine*	84.0 ± 6.46	84.6 ± 6.36	88.9 ± 4.74
3	Chlorphentermine	95.5 ± 2.59	84.9 ± 6.66	95.6 ± 2.18
4	Cyclopentamine	98.5 ± 3.47	95.5 ± 3.74	95.8 ± 4.43
5	Dimethylamphetamine	99.7 ± 5.98	95.7 ± 2.48	98.0 ± 2.11
6	Ephedrine	64.8 ± 4.15	63.4 ± 6.44	90.6 ± 6.84
	Ephedrine**	83.7 ± 4.55	—	—
7	Ethylamphetamine	95.9 ± 4.52	93.3 ± 5.76	95.7 ± 3.17
8	Fencamfamine*	87.5 ± 5.29	82.5 ± 6.48***	100.7 ± 7.04
9	Fenfluramine	99.5 ± 2.80	92.5 ± 4.45	96.1 ± 2.18
10	Mephentermine	96.6 ± 4.21	91.0 ± 4.68	99.5 ± 3.58
11	Methoxyphenamine	97.9 ± 4.77	99.7 ± 3.96	98.9 ± 3.56
12	Methylamphetamine	94.5 ± 4.50	93.2 ± 5.14	96.1 ± 3.03
13	Methylephedrine	85.9 ± 4.82	87.0 ± 4.69	95.6 ± 2.37
14	Norephedrine§	39.7 ± 2.33	31.7 ± 4.60	52.9 ± 6.44
	Norephedrine§§	56.5 ± 4.06	45.7 ± 4.81	101.7 ± 2.54
	Norephedrine§§§	79.0 ± 7.27	70.8 ± 3.72	100.9 ± 2.84
	Norephedrine†	63.6 ± 5.62	—	—
15	Phendimetrazine	101.0 ± 6.57	97.5 ± 6.61	—††
16	Phenmetrazine	97.8 ± 4.30	86.4 ± 4.39	94.2 ± 4.01
17	Phentermine	101.0 ± 1.81	87.7 ± 3.17	99.4 ± 4.17
18	Propylhexedrine	94.6 ± 2.04	91.2 ± 3.36	95.0 ± 3.84
19	Tranylcypromine	71.3 ± 5.32	89.7 ± 6.59	74.6 ± 9.24

* Oven temp. 235°.
** Using purified diethyl ether (see text).
*** Diluted 1:1.
§ Eluted with diethyl ether.
§§ Chloroform elution.
§§§ Eluted with dichloromethane–isopropanol (85:15).
† Urine saturated with sodium chloride.
†† Not measured due to interfering peak.

compounds. Although the use of urine saturated with sodium chloride increases the recovery for norephedrine, the results of Table I indicate that fairly good extractabilities are also obtained when chloroform or dichloromethane–isopropanol (85:15) were used as eluting solvents.

However, during this investigation Beckett *et al.*[8] described the degradation of ephedrine during extraction with diethyl ether. To minimize the reaction of ephedrine with aldehydic impurities, their recommended method of purification of diethyl ether was used, resulting in a recovery for ephedrine from human urine of 83.7 ± 4.55 instead of 64.8 ± 4.15 using distilled diethyl ether.

Notwithstanding that the elution time is considerably higher for undiluted plasma than for urine or diluted blood, our results show that it is unnecessary to dilute the plasma as described by Sachs and Kuepper[9]. Therefore the method used here results in a lower detection limit for the determination of central nervous stimulants in plasma. However, due to the higher blood recoveries, diluted blood is preferred instead of plasma for practical purposes.

To demonstrate the purity of the column eluates, 20 ml alkalized horse urine were respectively extracted with 3×10 ml diethyl ether or eluted with 40 ml ether using Extrelut. The extracts were concentrated to 15 μl. Injection of 0.5 μl yields the chromatograms as shown in Figs. 1 and 2 (recorder attenuation, $4 \cdot 10^{-11}$). A routine doping analysis reference chromatogram is presented in Fig. 3.

Owing to the excellent recoveries, the yield of purer concentrates and saving of time, we conclude that adsorption chromatography on Extrelut columns is very suitable for routine doping analysis.

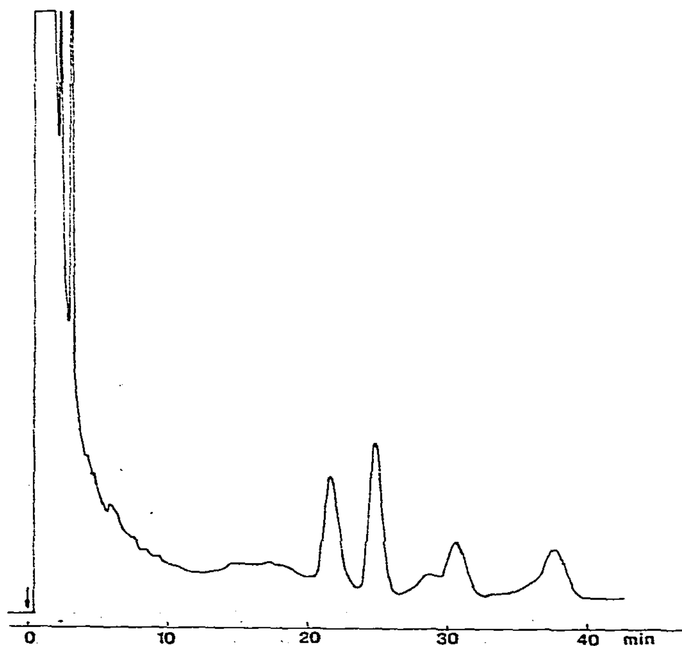

Fig. 1. Gas chromatogram of a horse urine extract after extraction with diethyl ether.

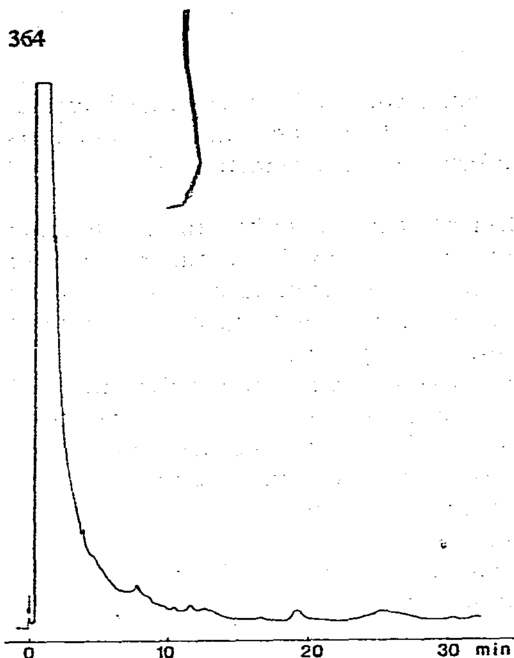

Fig. 2. Gas chromatogram of a horse urine extract after elution with diethyl ether using Extrelut.

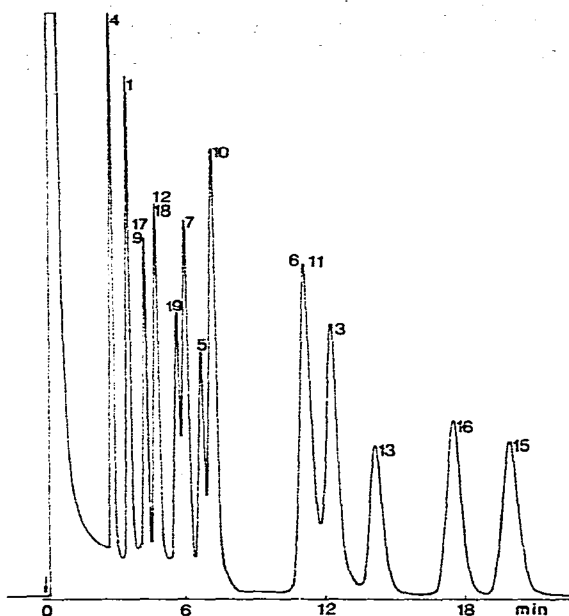

Fig. 3. Reference doping analysis chromatogram. The numbers refer to the compounds given in Table I.

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REFERENCES

1 F. T. Delbeke and M. Debackere, *J. Chromatogr.*, 133 (1977) 214.
2 F. T. Delbeke and M. Debackere, *J. Chromatogr.*, 136 (1977) 385.
3 J. Breiter, R. Helger and H. Lang, *Forensic Sci.*, 7 (1976) 131.
4 M. Donike, *J. Chromatogr.*, 103 (1975) 91.
5 M. Donike, *J. Chromatogr.*, 115 (1975) 591.
6 R. V. Smith, *Int. Lab.*, Nov./Dec. (1976) 71.
7 L. M. Cummins and M. J. Fourier, *Anal. Lett.*, 2 (1969) 403.
8 A. H. Beckett, G. R. Jones and D. A. Hollingsbee, *J. Pharm. Pharmacol.*, 30 (1978) 15.
9 A. Sachs and B. Kuepper, *Diagnostica*, E. Merck, Darmstadt, 1976.