Journal of Chromatography, 106 (1975) 412–417

CHROM. 8061

Note

Isolation and detection of methylphenidate, phacetoperane and some other sympatomimetic central nervous stimulants with special reference to doping

I. Gas chromatographic detection procedure with electron capture detection for some secondary amines

F. T. DELBEKE and M. DEBACKERE

Laboratorium voor Farmacologie en Toxicologie der Huisdieren, Rijksuniversiteit, Casinoplein 24, B-9000 Gent (Belgium)

(Received September 25th, 1974)

Hitherto, most stimulants derived from phenylisopropylamine used as doping agents have been detected by means of thin-layer chromatography (TLC) combined with gas–liquid chromatography (GLC) on polar or apolar columns using a flame ionization detector (FID)[1]. These amines are extracted from biological fluids at a strongly alkaline pH into an organic solvent such as chloroform[2]. Nevertheless, the detection of the piperidine derivatives methylphenidate (Ritalin, Rilatine) and phacetoperane (Lidepran) in urine was difficult as these compounds are esters and could be hydrolyzed either during the extraction procedure or on the potassium hydroxide-coated column support material.

A method for the detection and identification of methylphenidate in human urine and blood has been described by Schubert[3]. With this method, it was possible to detect unchanged methylphenidate but only after the ingestion of large amounts of the drug. Bernhard *et al.*[4] and Sheppard *et al.*[5], using ^{14}C-labelled methylphenidate in rats and guinea pigs, found by means of a paper chromatographic detection method that only a small part of the methylphenidate is excreted as the unchanged drug.

As both central nervous stimulants are misused on a large scale as doping agents and this laboratory is involved in the control of doping, a rapid, sensitive method was developed for the isolation and detection of the unchanged piperidine derivatives methylphenidate and phacetoperane after the ingestion of small amounts. For these compounds, the overall procedure was carried out in three successive steps: (a) extraction and detection of the drug added to water; (b) extraction and detection of the drug added to urine; and (c) extraction and detection of the drug excreted at several periods after the oral intake by volunteers (7.5 mg of phacetoperane hydrochloride and 20 mg of methylphenidate hydrochloride).

Because for some substances GLC with electron capture detection (ECD) is more sensitive than the FID commonly used in drug analysis, the former was used for the detection of the secondary amines studied here. Therefore, halogenated deriva-

tives were required, and were synthesized by using pentafluoropropionic anhydride (PFPA). This anhydride could react either with the amine group for all compounds or with the hydroxyl group or both for hydrolyzed phacetoperane.

EXPERIMENTAL

Urinary pH

Strong alkalinization (to pH 12) of an aqueous solution of pure methylphenidate hydrochloride and evaporation to dryness yields a white powder with a high decomposition temperature that is insoluble in organic solvents, presumably by hydrolysis and subsequent sodium salt formation. Therefore, during the extraction, the pH of the urine that contains methylphenidate must be held between 8 and 9 so as to prevent hydrolysis and the formation of a water-soluble methylphenidate salt that cannot be extracted into an organic solvent. On the other hand, it is well known[6] that for phacetoperane, migration of the acetyl group to the amine function occurs at pH 6. At higher pH, the hydrolysis of the ester is complete (Fig. 1).

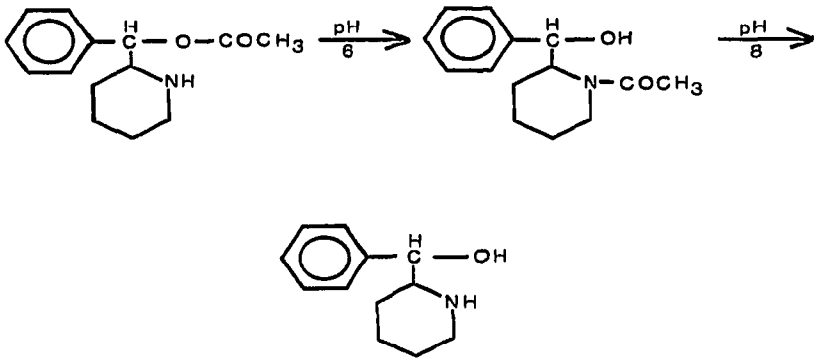

Fig. 1. Hydrolysis of phacetoperane.

As the other secondary amines mentioned are stable towards hydrolysis, the urinary pH can be brought to 12–13 during the extraction.

Extraction

Urine (10 ml) containing the secondary amines is pipetted into a glass-stoppered tube. For methylphenidate, the pH is adjusted to 8–9 with a few millilitres of saturated sodium tetraborate solution. With phacetoperane and the other secondary amines, however, the pH is brought to 12–13 using 50% sodium hydroxide solution (2 drops). The urine is then extracted with 5–6 ml of cyclohexane using a mechanical shaker, centrifuged and the two layers separated using Whatman No. 1 PS paper. After washing the urine on the paper with an additional 2 ml of cyclohexane, the organic solvent is evaporated to dryness *in vacuo* at 40°.

Derivatization with PFPA

Cyclohexane (0.5 ml) is added to the residue together with 20 μl of PFPA and

20 μl of a 5% solution of pyridine in benzene. After a short reaction period (5 min), the cyclohexane phase is washed with 0.1 *N* sodium hydroxide solution (4 ml) so as to decompose the unreacted PFPA, centrifuged for 5 min and 2 μl of the organic solvent are injected into the gas chromatograph. Preliminary results indicated that amounts of 10–20 ng per 2 μl injected could easily be detected.

Supplemental identification for "positive" reactions

A positive methylphenidate reaction could be confirmed by using a supplementary reaction procedure. A further 10 ml of urine are extracted and the residue obtained after evaporating the cyclohexane is re-dissolved in 2–3 ml of cyclohexane and 0.5 ml of a suspension of lithium aluminium hydride in dry diethyl ether is added in order to reduce the methyl ester. After 15 min, the excess of lithium aluminium hydride is decomposed by adding 5 ml of water. The carbinol formed is extracted into the cyclohexane phase (5 min), the two layers are separated using Whatman No. 1 PS paper and the organic solvent is evaporated *in vacuo*, followed by the reaction with PFPA as described above.

Apart from methylphenidate and phacetoperane, all of the secondary amines can easily be detected as the free amines on Apiezon, SE-30 or Carbowax columns. For these compounds, however, the PFPA derivatization procedure could be used to obtain a supplemental conclusive identification of "positive" reactions.

GAS CHROMATOGRAPHIC CONDITIONS

The secondary amine–PFPA derivatives were detected using a Sc^3H ECD detector. The GLC systems used were:

(1) Column A: 2% OV-17 on Gas-Chrom Q, 100–120 mesh, 3 m × $\frac{1}{8}$ in. I.D. Detector temperature, 250°; Sc^3H detector; injector temperature, 250°; carrier gas (nitrogen) flow-rate, 40 ml/min.

(2) Column B: 10% Apiezon L on Gas-Chrom P, 100–120 mesh, 2 m × $\frac{1}{8}$ in. I.D. Detector temperature, 250°; Sc^3H detector; injector temperature, 250°; carrier gas (nitrogen) flow-rate, 40 ml/min.

(3) Column C: 1.5% OV-17–1.95% OV-210 on Chromosorb W, 80–100 mesh, 2 m × $\frac{1}{8}$ in. I.D. Detector temperature, 200°; ^3H detector; injector temperature, 255°; nitrogen inlet pressure, 48 lb./in.^{2}.

The retention times of the PFPA derivatives on different columns and at various oven temperatures are summarized in Table I. A chromatogram of some PFPA secondary amine derivatives is shown in Fig. 2 (column B, 150°). Figs. 3 and 4 show positive reactions for methylphenidate (column A, 180°) and phacetoperane (column B, 180°), respectively.

MASS SPECTRAL IDENTIFICATION

Additional information on these compounds was obtained by using combined GLC-mass spectrometry. A mass spectrum was taken for the PFPA derivatives of methylamphetamine, ethylamphetamine and methylphenidate. The drugs were extracted from water and a PFPA derivative formed as described above. A cyclohexane solution (2 μl) was injected on to a 4% OV-17 column.

TABLE I

RETENTION TIMES (min) OF THE PFPA DERIVATIVES

Compound	*Column A*			*Column B*		*Column C*
	140°	*150°*	*160°*	*150°*	*180°*	*175°*
Phenylisopropylmethylamine(N-methyl-amphetamine)	5.1			7.5		
2-Ethylamino-1-phenylpropane(N-ethyl-amphetamine)	5.6			9.0		
2-Cyclohexyl-N-methylpropylamine (cyclexedrine)	3.4			6.6		
3-Methyl-2-phenylmorpholine (phenmetrazine)	15.2			19.6		
α-Phenyl-2-piperidineacetic acid methyl ester (methylphenidate)			32.6		21.7	10.1
Methylphenidate, reduced		16.5			8.2	39.2
α-Phenyl-2-piperidine-methanolacetate (phacetoperane)			8.0		6.0	3.1

Mass spectrum of methylamphetamine–PFPA derivative

The amide formed in the reaction between PFPA and methylamphetamine has a molecular weight of 295. The mass spectrum shows ion abundance peaks at 204, 162, 119, 118, 91, 77 and 65. It is noteworthy that the spectrum does not show significant amounts of the parent molecular ion at mass 295. However, the strong peaks at 204 and 91 provide with sufficient information to confirm the reaction product

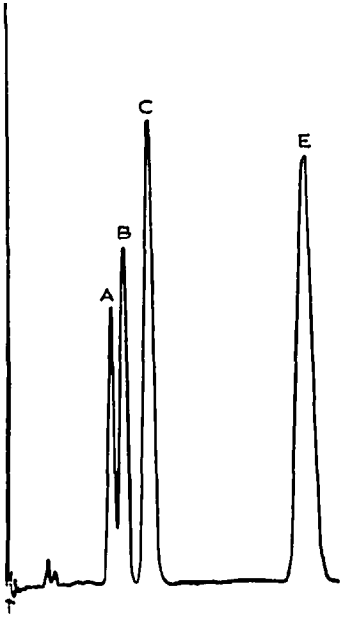

Fig. 2. Gas chromatogram of PFPA derivatives of secondary amines. A, Cyclexedrine; B, N-methyl-amphetamine; C, N-ethylamphetamine; D, phenmetrazine.

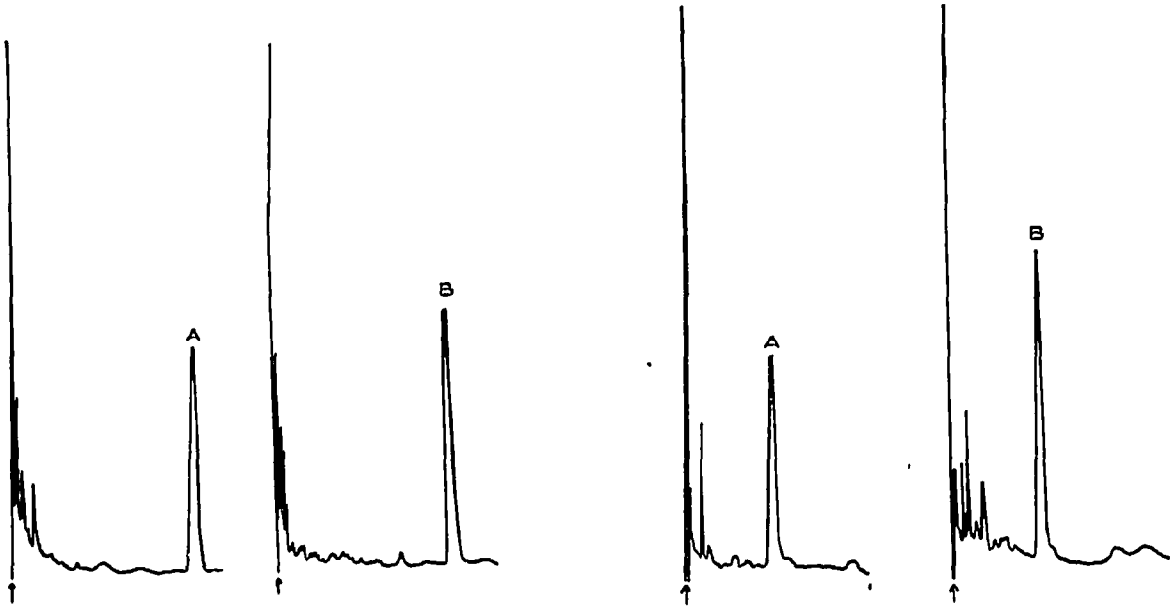

Fig. 3. Gas chromatogram of methylphenidate–PFPA derivative. A, Reference; B, extract from the urine of a person who had teken methylphenidate.

Fig. 4. Gas chromatogram of phacetoperane–PFPA derivative. A, Reference; B, extract from the urine of a person who had taken phacetoperane.

formed (Fig. 5). The peaks at mass 77 and 91 are due to $[C_6H_5]^+$ and $[C_6H_5–CH_2]^+$, respectively. The presence of an abundant ion of mass 65 results from the removal of C_2H_2 of the tropylium ion (91 —26). Loss of the CF_3CF_2 group could yield the ion abundance peak at 119.

Mass spectrum of ethylamphetamine–PFPA derivative

The spectrum of this compound contains substantial peaks at masses 218, 189, 174, 118, 91, 77 and 65. As for the analogous methylamphetamine derivative, no molecular ion peak is observed. The peaks at 218 and 91 result from the removal of $C_6H_5–CH_2$ while the presence of the abundant ions of masses 77 and 65 has been discussed above. The loss of the ethyl and methyl groups in the fragment with mass 218 could yield the peaks observed at 189 and 174.

Mass spectrum of methylphenidate–PFPA derivative

Substantial peaks at masses 232, 176, 147, 119 and 56 are found. No molecular ion peak is found in the spectrum of methylphenidate–PFPA derivative (Fig. 6). The

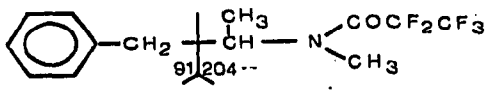

Fig. 5. Cleavage of methylamphetamine–PFPA derivative.

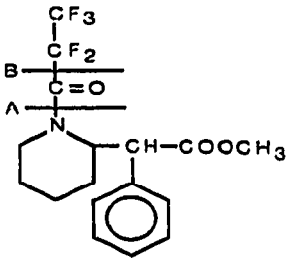

Fig. 6. Cleavage of methylphenidate–PFPA derivative.

peaks at masses 119 (cleavage B), 232 and 147 (cleavage A) should confirm the derivative formed. The peaks at 176 and 56 could result from a breakdown of the piperidine moiety.

CONCLUSION

A rapid, specific and very sensitive method for the extraction and detection of central nervous stimulating secondary phenylisopropylamines used as doping agents in sport has been developed. Hitherto, some of these drugs (methylphenidate and phacetoperane) could not been detected by the usual analytical methods, probably because extraction at too high a pH induced hydrolysis of the ester with formation of a water-soluble salt in the case of methylphenidate and also because the detection methods used were not sensitive enough to detect the small amounts of unchanged drug excreted.

This paper is simply a preliminary report with a description of the method used. Further studies on the GLC detection limits, mass spectra, metabolism and quantitative and qualitative excretion patterns of several stimulating phenylisopropylamines derived from piperidine are in progress and will be published later.

ACKNOWLEDGEMENTS

The authors thank Dr. M. T. Rosseel and Mr. N. Desmet for technical assistance.

REFERENCES

1 A. H. Beckett, G. T. Tucker and A. C. Moffat, *J. Pharm. Pharmacol.*, 19 (1967) 273.
2 M. Debackere, to be published.
3 B. Schubert, *Acta Chem. Scand.*, 24 (1970) 433.
4 K. Bernhard, V. Buehler and M. H. Bickel, *Helv. Chim. Acta*, 42 (1959) 802.
5 H. Sheppard, W. H. Tsien, W. Rodegker and A. J. Plummer, *Toxicol. Appl. Pharmacol.*, 2 (1960) 353.
6 Specia, *Technical Note 8228 R.P.*