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Note

Gas—liquid chromatographic determination of phentermine in human plasma following oral administration to healthy subjects

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Phentermine is a phenylethylamine derivative (Fig. 1). It has appetite depressant properties. When bound to a cationic resin, it is commercially available under the trade name Ionamin®.

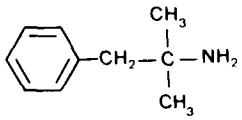

Fig. 1. Chemical structure of phentermine.

Separation and quantitation of biologically active amines by gas chromatographic methods have usually been carried out by derivatization followed by electron-capture [1, 2] or flame-ionization [3—5] detection. Most of these procedures involve chromatography of the trifluoracetamide derivatives of these amines using columns such as 3% OV-17, 3% SE 30, 2% SP-2510-DA and 1% SP-1240-DA [5]. Although these procedures are satisfactory for many amines of biological significance, they have proved unsatisfactory when applied to the assay of phentermine. O'Brien et al. [6] reported that volatilization, adsorption onto glass, as well as incomplete extraction and derivatization combine to produce a decrease in reliability for the determination of plasma phentermine, especially at the low blood concentrations attained when therapeutic doses are administered. These workers introduced procedural modifications to overcome these difficulties. However, in our laboratory reproduction of this method, particularly at low plasma concentrations of phentermine, proved to be difficult.

In this paper we describe a simple, sensitive and selective gas—liquid chro-

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matographic method for the determination of therapeutic levels of phentermine in human blood plasma, using nitrogen—phosphorus detection.

EXPERIMENTAL

Reagents and solvents

Amantadine hydrochloride was obtained from Sigma, 1 *M* sodium hydroxide (concentrated volumetric solution), 0.1 *M* hydrochloric acid (concentrated volumetric solution), diethylamine (AnalaR), and toluene (AnalaR) from BDH and phentermine hydrochloride (pharmaceutical grade) from Loftus-Bryan Chemicals (Ireland).

For the preparation of plasma standards, dried human plasma from The Blood Transfusion Service Board (Ireland) was dissolved in deionised water obtained by reverse osmosis (Barnstead nanopure system). The blank plasma obtained was examined for the presence of endogenous components which might interfere with phentermine or amantadine in the assay system. The reconstituted plasma was stored at 4°C and used within two weeks of preparation.

Standards

Phentermine · HCl (12.5 mg, equivalent to 10.00 mg phentermine base) was dissolved in 100 mg deionized water to yield the stock standard solution (100 μg/ml).

A second stock solution was prepared by 1:10 dilution of the 100 μg/ml solution, to yield stock solution A (10 μg/ml). The working standard solutions containing 200, 500, 750, 1000, 1250 and 1500 ng/ml phentermine were obtained by serial dilution of stock solution A with water. Spiked plasma standards were prepared by addition of 100 μl of the working standard solutions to 1 ml of plasma, to provide plasma standards containing 20, 50, 75, 100, 125 and 150 ng/ml phentermine.

Amantadine · HCl (5 mg) was dissolved in 100 ml deionised water to yield an internal standard (I.S.) stock solution (50 μg/ml). This was further diluted to give a solution of 5 μg/ml, of which 100 μl were used to spike both plasma standards and test samples.

Instrumentation

A Perkin-Elmer Sigma 2000 gas chromatograph equipped with a nitrogen—phosphorus detector was used. The column employed was 2 m × 4 mm I.D., glass packed with 10% Apiezon-L + 2% potassium hydroxide on 80—100 mesh, acid-washed, Chromosorb W (Perkin-Elmer).

The following operational parameters were found to be optimal: injector temperature 170°C and detector temperature 250°C. The initial oven temperature was 155°C for 7.5 min during which phentermine and amantadine were eluted and the temperature was then increased at the rate of 30°C/min to 200°C and remained at 200°C for 2 min to expedite late-eluting peaks before the system was ready for the next injection. Nitrogen at a flow-rate of 30 ml/min was used as carrier gas. Air and hydrogen were supplied to the detector at a flow-rate of 160 ml/min and 2 ml/min, respectively. Detector output

was set at 10 pA and attenuation was 1 or 2 at range 1. A Perkin-Elmer Model 024 chart recorder was used to monitor the signals, with a chart speed of 0.5 cm/min.

Procedure

Plasma (1 ml) spiked with 100 μl phentermine standard and 100 μl internal standard (5 μg/ml amantadine · HCl) was mixed with 1 ml of 1 *M* sodium hydroxide solution in a glass-stoppered tube. After the addition of 5 ml toluene (containing 0.05%, v/v, diethyl amine) the drugs were extracted by rotating the tubes gently for 15 min on a mechanical rotator, followed by centriguation for 10 min at 700 *g* at 5°C. The organic phase was transferred into a glass culture tube and 1 ml of 0.1 *M* hydrochloric acid was added. The tube was vortexed for 1 min to form the hydrochloride salt and the acidic phase transferred into a small (10 × 75 mm) glass culture tube. Sodium hydroxide (1 *M*, 0.5 ml) was added and the tube again vortexed for 10 sec, after which 100 μl toluene were added and the tube was vortexed again for 45 sec. The toluene layer was removed and 2 μl of this solution were injected into the gas chromatograph.

Plasma samples obtained from volunteers, following ingestion of phentermine, were treated in the same way except that phentermine standards were not added.

Plasma levels of phentermine

The described method has been successfully applied to the measurement of phentermine in plasma samples from a single-dose pharmacokinetic study. Six healthy male volunteers, aged between 18 and 40 years and within 10% of their ideal body weights, participated in this study. Each subject gave written informed consent to participate in the study, the protocol for which was approved by the Institutional Review Board. Subjects were excluded if there was any abnormality in physical or laboratory findings, a history of any previous allergic conditions, a history of medication within two weeks of enrollment or a history of drug abuse. Alcohol was forbidden for the duration of each study period. Subjects received an oral dose of phentermine as a capsule containing 30 mg of the active drug Ionamin, supplied by Pennwalt (Ireland). All doses were administered with a glass of water after an overnight fast. Blood (10 ml) was collected in a lithium heparin vacutainer from an antecubital vein at the following times: pre-dose and 1, 2, 3, 4, 6, 8, 12, 18 and 24 h after dosing. After each blood sampling the plasma was immediately separated and stored at −20°C until required for analysis.

Calibration and calculation

Evaluation of the assay was carried out using five-point calibration standards in duplicate at a concentration range of 20—125 ng/ml phentermine in plasma. The calibration curves were obtained by linear regression of the peak height ratios of phentermine/I.S., versus concentrations of phentermine. These calibration curves were then used to interpolate the concentrations of phentermine in plasma from the measured peak height ratio of phentermine/I.S.

RESULTS

Separation

Fig. 2A and B shows typical chromatograms of phentermine as determined in extracts of human plasma. The mean retention times of phentermine and amantadine were 3.1 and 5.9 min, respectively.

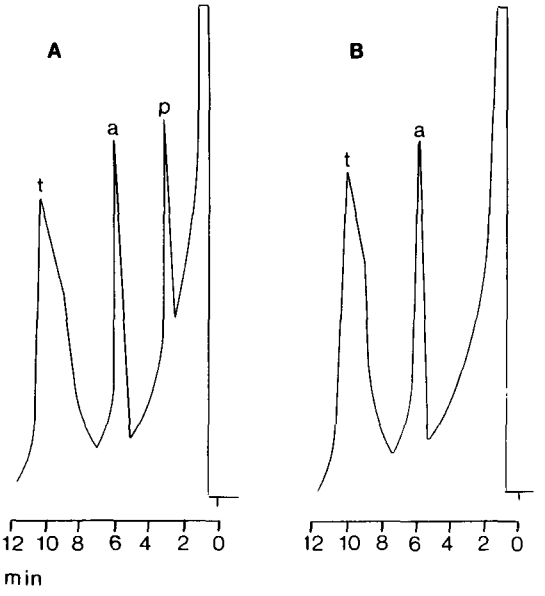

Fig. 2. Chromatograms of (A) a human plasma sample 4 h after a single dose of 30 mg of phentermine; (B) a human pre-dose plasma sample spiked with internal standard, amantadine · HCl. Peaks: p = phentermine; a = amantadine; t = peak due to temperature programming.

Limit of detection and quantitation

Under optimal conditions the limit of detection using a 1-ml plasma sample and 2-μl injection volume was found to be 5 ng/ml where the signal-to-noise ratio was greater than 3:1. The limit of quantitation was taken as 20 ng/ml, where the signal-to-noise ratio was greater than 10:1.

Selectivity

A number of drug-free plasma samples from different human subjects and aliquots of pooled plasma were tested for the presence of interfering endogenous compounds using the outlined extraction procedure. No interference was observed. However, during development of the method an interfering peak was observed which had the same retention time as phentermine (equivalent sometimes to 75 ng/ml phentermine). A systematic study showed that use of plastic pipette tips was responsible for this interference. Subsequently, during all stages of the extraction procedure, glass tubes, pipettes and capillary tubes were used.

Linearity

A measure of linearity is outlined under intra-assay reproducibility (Table I).

TABLE I

METHOD PRECISION AND REPRODUCIBILITY

Concentration added (ng/ml)	Mean concentration found ± S.D. (n = 4, ng/ml)	Coefficient of variation (%)	Difference between added and found concentration (%)
*Intra-assay variation**			
20.0	21.7 ± 0.7	3.36	8.5
50.0	48.6 ± 1.5	3.00	2.8
75.0	75.6 ± 2.6	3.39	0.8
100.0	99.1 ± 2.3	2.36	0.9
125.0	122.5 ± 4.4	3.61	2.0
Inter-assay variation			
20.0	21.8 ± 1.3	5.97	9.0
50.0	52.0 ± 3.1	5.90	4.0
75.0	70.7 ± 1.3	1.89	5.7
100.0	93.7 ± 8.8	9.42	6.3
125.0	130.6 ± 4.0	3.08	4.5

*Regression equation for intra-assay was $Y = 0.024X + 0.039$.

The correlation coefficient was 0.999 and the intercept did not differ significantly from zero.

Precision and reproducibility

The data presented in Table I demonstrate the precision and accuracy of this assay. Intra-assay variability was determined at five concentrations in quadruplicate, at 20, 50, 75, 100 and 125 ng/ml of plasma. Inter-assay variability was determined singly at the same five concentrations in four replicate runs. The precision of the method (mean percent coefficient of variation) and the accuracy (difference between added and found concentrations) for the values of recovered determined standards, when calculated as unknowns against the linear regression line, were acceptable (less than 10% variation from amount added) over the concentration range investigated.

Recovery

Recovery was calculated by comparing the peak height of phentermine after its extraction from plasma with the peak height of a series of unextracted reference standards. In the concentration range 50—150 ng/ml, the mean overall percentage recovery was 94.5 ± 5.7%.

DISCUSSION

This paper describes a method for the measurement of phentermine in human plasma, which has several advantages over previously described procedures [6, 7]. By using a 10% Apiezon L + 2% potassium hydroxide column, the requirement for derivatization of the amine was eliminated. The clean-up and concentration steps, which utilized back-extraction rather than solvent evaporation, improved the overall recovery of phentermine, since losses due to the volatility of the amine were avoided. In addition, the use of

TABLE II

PLASMA PHENTERMINE LEVELS (n = 2)

Time (h)	Plasma phentermine level (ng/ml)						Mean ± S.D.
	Subject						
	1	2	3	4	5	6	
Pre dose	N.D.*	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Post dose							
1	36.0	46.3	36.7	50.0	42.5	32.5	40.7 ± 6.7
2	54.8	71.3	60.0	64.2	101.3	48.8	66.7 ± 18.6
3	72.3	75.0	100.5	160.4	118.8	118.8	107.6 ± 32.8
4	91.8	83.8	105.0	165.8	121.3	185.0	125.5 ± 41.2
6	109.6	102.5	161.7	114.6	122.5	196.3	134.5 ± 36.7
8	113.1	87.5	126.7	162.0	258.8	150.0	149.7 ± 59.7
12	119.9	95.0	123.4	132.2	120.0	111.3	117.0 ± 12.7
18	109.3	82.5	121.7	133.4	110.0	95.0	108.7 ± 18.2
24	128.1	72.8	111.7	236.0	81.3	105.0	122.7 ± 59.0

*N.D. = not detected, below limit of quantitation (< 20 ng/ml).

nitrogen—phosphorus rather than flame-ionization detection significantly improved the sensitivity and selectivity of the gas chromatographic procedure.

O'Brien et al. [6] described a gas chromatographic procedure for the measurement of phentermine in blood at levels to be expected following administration of therapeutic doses of the drug. This method was later employed by Hinsvark et al. [7] who investigated the selective bioavailability of phentermine in man, following oral administration of both resin-bound phentermine and the soluble hydrochloride salt. In this study plasma levels of 70 and 90 ng/ml were observed following administration of 0.375 mg/kg phentermine resinate and hydrochloride, respectively. Following the resinate preparation peak plasma levels were somewhat delayed, occurring at 8—9 h after the oral dose, as compared to 2—3 h following the hydrochloride. The results of the present study (see Table II) confirm these findings, with peak plasma levels being observed 8 h after a 30-mg single oral dose of phentermine resinate (Ionamin).

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REFERENCES

1 E. Anggard and A. Hackey, Acta Chem. Scand., 23 (a) (1969) 310.
2 R.B. Bruce and W.R. Maynard, J. Anal. Chem., 41 (1969) 977.
3 W.J.A. VandenHeuvel, W.L. Gardiner and E.C. Horning, J. Anal. Chem., 36 (1964) 1550.
4 G. Brugaard and K.E. Rasmussen, J. Chromatogr., 147 (1978) 476.
5 R.D. Budd and W.J. Leung, J. Chromatogr., 179 (1979) 355.
6 J.E. O'Brien, W. Zazulak, V. Abbey and O. Hinsvark, J. Chromatogr. Sci., 10 (1972) 336.
7 O.N. Hinsvark, A.P. Truant, D.J. Jenden and J.A. Steinborn, J. Pharmacok. Biopharm., 1 (1973) 319.