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Note

Isolation of drugs with macroreticular resins

Determination of phentermine in blood

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Recently, macroreticular resins have been used in order to increase the concentration of organic compounds from aqueous solutions[1,2]. Amberlite XAD-4 was suggested for the isolation of drugs from blood without prior precipitation[3]. The method has the advantages of preventing losses of substances due to the precipitation of proteins and reducing the time of analysis.

A combination of this isolation method with GC detection by means of a nitrogen detector permits an exact and easy determination of phentermine (α,α-dimethylphenethylamine) in blood. This investigation was carried out in order to obtain information on the relationship between the concentration and time of excretion of drugs. The procedure and the GC conditions were according to O'Brien *et al.*[4] and Beckett *et al.*[5], although, owing to the high specificity of the nitrogen detector, no extra derivatization step was needed.

EXPERIMENTAL

The GC analysis was performed with a Perkin-Elmer F 20/B chromatograph equipped with a nitrogen detector and a 2-m stainless-steel column filled with 10% Apiezon L–10% potassium hydroxide on Chromosorb G AW DMCS, 80–100 mesh, at 200°, attenuation 8 and range 10. The temperature of the injection port was 240°. Amberlite XAD-4 (50 Å mean pore width) was carefully cleaned by solvent extraction in order to remove low-molecular-weight organic substances, soaked in water and stored at 4°. All of the solvents used were of analytical-reagent grade (E. Merck, Darmstadt, G.F.R.).

Method

About 30 ml of blood were homogenized with a Turrax mixer, 0.1 ml of dimethylamphetamine in methanol (*e.g.*, 2.7 mg per 100 ml) as internal standard and 0.5 ml of 25 % ammonia solution were added and mixed with about 5 g of wet Amberlite XAD-4 resin on a Vortex mixer for 10–15 min. The polymer was removed by suction through a coarse sintered-glass funnel and cleaned by washing several times with water in order to remove the protein residues.

After the addition of sodium sulphate for drying purposes, the drug was eluted

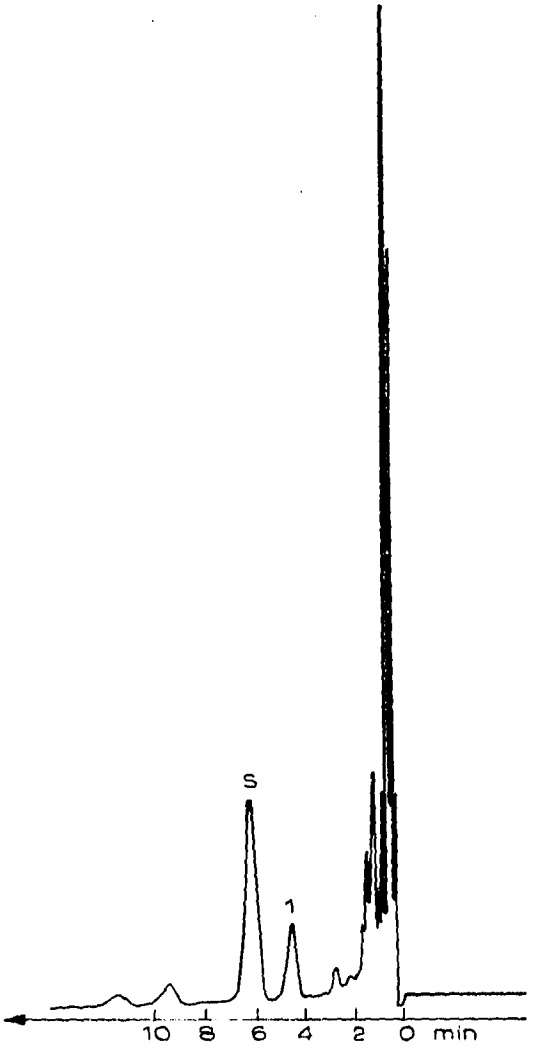

Fig. 1. Gas chromatogram of a blood extract analyzed according to the method described. The blood contained 40 ng/ml of phentermine. S = internal standard; 1 = phentermine.

with 50 ml of chloroform–isopropanol (4:1) by means of the Vortex mixer. The solvent was filtered off and evaporated after addition of 1 ml of 0.1 *N* tartaric acid; the resulting aqueous solution was transferred to a PTFE-stoppered centrifuge tube and extracted with light petroleum (b.p. 50°; E. Merck) on the Vortex mixer. After discarding the solvent, the aqueous solution was saturated with solid ammonium sulphate, made alkaline with ammonia and extracted with 0.1 ml of light petroleum (b.p. 50°) again, 1 μl of the supernatant liquid being used for the GC analysis. For ease of handling, it is advisable to sample the solvent first with a 20-μl cappillary and to use this to fill the syringe.

Gas chromatography

The best results were obtained with Apiezon L–potassium hydroxide or Carbowax 20M–potassium hydroxide[5]. After five injections, the column temperature was

TABLE I

RECOVERY OF PHENTERMINE FROM SPIKED BLOOD SAMPLES

*Amount added (μg)**	*Amount found (μg)***	*Recovery (%)*
1	0.95	95 ± 7
5	4.7	94 ± 8
10	9.2	92 ± 7
100	93	93 ± 5

* Amount added to 30 ml of blood and *ca.* 5 g of Amberlite XAD-4.
** Mean of four determinations.

increased to 240° for 20 min in order to elute more polar substances that have relatively long retention times. Fig. 1 shows a typical gas chromatogram. The quantitative determination was made by plotting the quotient of the peak heights or areas of phentermine and the internal standard measured by an integrator against concentration.

RESULTS

The extraction of phentermine was studied by determining its recovery from spiked blood samples within the range 1–10 μg. The range of extractability extends to 100 μg, although high recoveries can, in this instance, be obtained only by the addition of *ca.* 0.5 ml of 25 % ammonia solution to the blood sample.

Table I shows the results obtained by the Amberlite XAD extraction method with dimethylamphetamine as external standard.

The extraction of phentermine by this method is quantitative. Neither the internal standard nor phentermine could be detected after re-extraction of the blood with diethyl ether. Careful purification of the resin is required in order to ob-

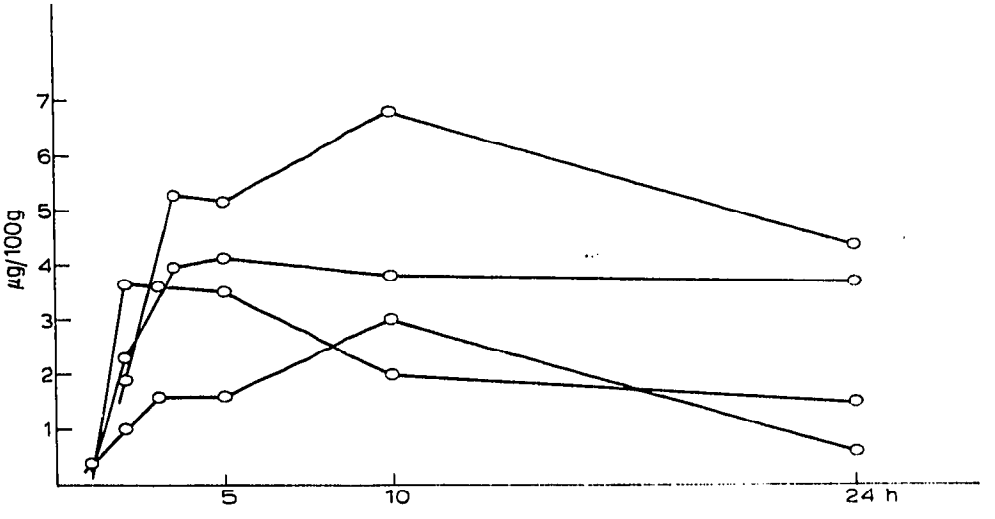

Fig. 2. Blood levels of phentermine in four male patients after oral administration of 30 mg of phentermine in different retard preparations.

tain a low background in GC. Both laboratory and analytical grades of resins contain a number of unidentified organic substances that can be removed by extraction with diethyl ether, ethyl acetate or ethanol. Losses due to evaporation of lowboiling basic substances are prevented by the use of dilute tartaric acid. When using a small but sufficient extraction volume, the concentration of the drug is in an easily accessible range for the nitrogen detector. It is important that the solvent extraction should be performed from the minimum aqueous volume possible (salt-saturated solution).

An increase in the limit of detection could be achieved only by the use of substance-specific methods (mass spectroscopy) together with highly purified solvents. Approximately 2 ng/ml of phentermine in blood was determined as the limit of detection. The blood levels were obtained from four male patients after oral administration of 30 mg of phentermine in different retard preparations (resinate, different gelatine capsules) (Fig. 2).

Owing to variations in physiological conditions, wide deviations of the concentrations must be assumed, but in each case the phentermine taken could be detected even after 24 h.

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