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Specific gas chromatographic determination of amitriptyline in human urine following therapeutic doses

The tricyclic antidepressant amitriptyline is widely prescribed in the treatment of mental disease. Spectrophotometric methods for the analysis of amitriptyline in blood and urine have been described[1–3], but these do not differentiate the parent drug from its demethylated metabolite, nortriptyline. Other spectrophotometric procedures, involving prior separation of these compounds by thin-layer chromatography[4,5] are prohibitively time-consuming. Further, these methods are insensitive in the concentration range encountered after therapeutic dosage.

HUCKER AND MILLER[6] have reported the gas chromatographic separation of amitriptyline and several related tertiary amines after exhaustive methylation to the corresponding olefinic derivative. The formation of a common product by amitriptyline and nortriptyline mitigates against the application of such a procedure to biological studies.

The present paper describes a gas–liquid chromatographic procedure for the separation of several tricyclic antidepressants and its application to the determination of therapeutic levels of amitriptyline in human urine.

Experimental

Reagents. The following reagents were used: Analar petroleum spirit (40–60°) (Hopkin & Williams Ltd., Chadwell Heath, Essex) purified by re-distillation; 1 *N* sulphuric acid and 2 *N* sodium hydroxide washed with re-distilled petroleum spirit; anhydrous sodium sulphate (Analar). The internal standard was a 0.15 mg% solution of triphenylamine (Koch-Light Laboratories Ltd., Colnbrook, Bucks.) in re-distilled petroleum spirit.

Gas chromatography. A Pye 104 Model 24 dual column gas chromatograph,

equipped with a flame ionisation detector and a 1–mV Honeywell recorder, was used. The column was a 7 ft. × ¼ in. I.D. coiled glass tube, which had been silanised with a 5% solution of dimethyldichlorosilane in benzene over a period of 24 h. Glass wool was silanised in the same solution. After drying at 100°, the column was packed with 1% polyvinyl pyrrolidinone (PVP) (Varian Aerograph, Fife, Scotland) and 3% Versamid 900 (Perkin-Elmer Ltd., Beaconsfield, Bucks.) on 80–100 mesh high-performance Chromosorb W (Perkin-Elmer Ltd.). This packing was prepared as follows: 0.2 g of PVP were dissolved in 200 ml of methanol. 19.8 g of the support material were gradually added to the flask with constant swirling. The solvent was removed under vacuum in a rotary evaporator over a period of 3–4 h. 0.56 g of Versamid 900 were dissolved in 200 ml of a mixture of chloroform and methanol (87:13) and 18.1 g of the PVP-coated support gently added with occasional swirling. Again, the solvent was removed *in vacuo* over a period of 3–4 h at 40°. The prepared column was then packed with the coated support by closing one end with silanised glass-wool and applying a vacuum. After filling, the other end was closed with silanised glass-wool and the packed column conditioned at 245° for 48 h with a nitrogen flow rate of 60 ml/min. This column deteriorates if cooled in the absence of nitrogen, and the maintenance of a constant supply of the carrier gas when not in use is a wise precaution. The instrument settings were as follows: column temp., 215°; injection port temp., max; detector temp., 240°; carrier gas flow rate, 60 ml/min.

Extraction procedure. A 10-ml sample of urine was acidified by the addition of 2.0 ml of 1 *N* sulphuric acid and extracted with 10 ml petroleum spirit by shaking for 10 min in a 30-ml centrifuge tube. After centrifugation for 15 min at 3,000 r.p.m., the organic layer was discarded and the aqueous residue made alkaline (pH 11–12) with 2.0 ml of 2 *N* sodium hydroxide. The extraction was repeated using a further 10 ml of petroleum spirit and after centrifugation, 8 ml of the top organic layer was transferred to a second tube containing approximately 3 g of anhydrous sodium sulphate. On carrying out a second extraction with 10 ml of petroleum spirit, the organic fractions were bulked, thoroughly shaken with the anhydrous sodium sulphate and left to stand for 10 min. The dried extract was transferred to a third tube, the anhydrous sodium sulphate being washed with 5 ml petroleum spirit and the washings added to the extract. Evaporation was carried out under a stream of nitrogen in a 10-ml conical centrifuge tube to which 1.0 ml of the triphenylamine internal standard solution had been added, the tube being immersed in a water bath at 60°. The residue was taken up in 100 μl of dried chloroform and 5 μl of this were injected on to the gas chromatograph.

Results and discussion

Quantitation. A range of standard solutions each containing 15 μg/ml of triphenylamine and from 2 μg/ml to 20 μg/ml of amitriptyline free base were made in petroleum spirit. A standard curve was prepared by injecting 5–μl aliquots of these solutions just prior to measuring test samples. The ratio of the peak height of amitriptyline to triphenylamine was linear over the range 0.01 to 0.1 μg of amitriptyline on injection. The relative retention time of amitriptyline with respect to triphenylamine was 1.80 (Fig. 1).

Recovery studies. Amounts ranging from 0.5 μg to 2.0 μg of amitriptyline as the hydrochloride were added to 10 ml samples of blank urine to examine the efficiency of

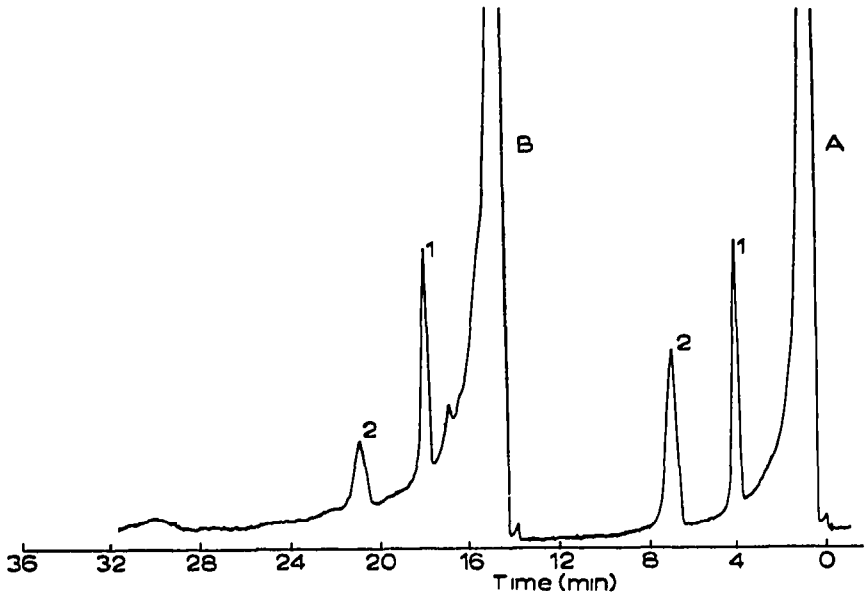

Fig. 1. (A) Chromatogram of a standard amitriptyline solution showing the separation of the internal standard triphenylamine (1) and amitriptyline (2). Attenuation, 10^2 (B) Chromatogram of a urine extract containing amitriptyline (2) with triphenylamine added (1) Attenuation, 10^2.

the extraction procedure. The mean recovery achieved was 90 ± 7%.

Specificity. Amitriptyline was well separated from the other principal tricyclic antidepressants—nortriptyline, imipramine, desipramine, protriptyline and prothiadene (Fig. 2)—though trimipramine (less frequently prescribed) had the same retention time as amitriptyline on this system.

Application. Single oral doses of 50 mg of amitriptyline hydrochloride were

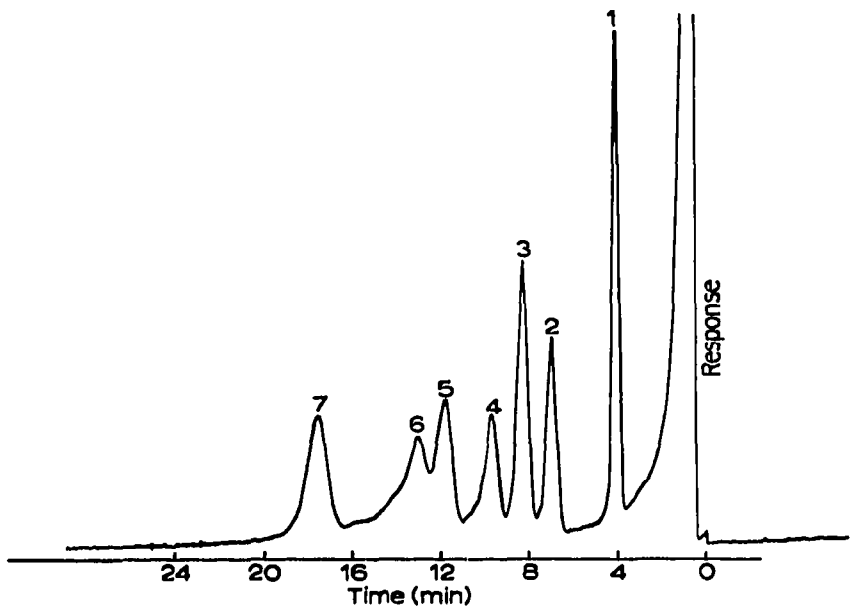

Fig. 2. Separation of triphenylamine (1), amitriptyline (2), imipramine (3), nortriptyline (4), desipramine (5), protriptyline (6), and prothiadene (7).

administered to five subjects in tablet form. Urine samples were collected at regular intervals over a period of 24 h. After measurement of the volume, the samples were stored at 4° prior to analysis.

Results and discussion. The results from five experiments are tabulated in Table I. In the subjects tested, only about 0.15% of the dose ingested appeared in the urine as free amitriptyline during the first 24 h. Amitriptyline could not be detected in the urine after this period. Previous radioactive studies[7,8] have indicated that 10 to 20% of the dose is excreted in the first 24-h urine, as a mixture of amitriptyline, nortriptyline and five other metabolites. No data on the relative amounts of the compounds excreted has been given. Other workers[4,9], using spectrophotometric analysis, have stated that approximately 1.0% of the dose is excreted as unchanged amitriptyline during the first 24 h. HUCKER[10] reported the two major metabolic reactions of amitriptyline in man to be hydroxylation and N-demethylation to nortriptyline. Working with

TABLE I

THE EXCRETION OF AMITRIPTYLINE IN THE URINE OF FIVE SUBJECTS FOLLOWING ORAL DOSES OF 50 mg AMITRIPTYLINE HYDROCHLORIDE

Subject	*Time (h)*	*Volume (ml)*	*Amitriptyline concentration (μg/ml)*	*Excreted amitriptyline (μg)*	*Total excreted in 24 h (μg)*	*% of dose excreted as unchanged amitriptyline*
M1	0	0	0.0	0	74.6	0.17
	4	764	0.028	21.4		
	8	511	0.059	30.2		
	12	113	0.099	11.2		
	16	152	0.029	4.4		
	24	295	0.025	7.4		
M2	0	0	0.0	0	23.0	0.052
	4	742	0.013	9.6		
	8	617	0.009	5.6		
	12	338	0.023	7.8		
	16	240	0.0	0		
	24	505	0.0	0		
M3	0	0	0.0	0	75.5	0.17
	4	1158	0.027	31.3		
	8	418	0.023	9.6		
	12	230	0.060	13.8		
	16	182	0.074	13.5		
	24	302	0.024	7.3		
F1	0	0	0.0	0	89.5	0.20
	4	870	0.035	30.5		
	8	253	0.091	23.0		
	12	263	0.045	11.8		
	16	176	0.063	11.1		
	24	278	0.047	13.1		
F2	0	0	0.0	0	64.4	0.15
	4	497	0.013	6.5		
	8	607	0.073	44.3		
	12	280	0.037	10.4		
	16	112	0.028	3.2		
	24	154	0.0	0		

nortriptyline, AMUNDSON AND MANTHEY[11] found that this drug appeared in very small quantities in the urine, being excreted mainly in the form of hydroxylated derivatives. The present work, in which only a small fraction of the ingested dose of amitriptyline was found in the urine as unchanged drug, was in agreement with these previous findings.

Nortriptyline was not detected in any of the samples analysed, although it should be stated that the gas chromatographic system was approximately ten times less sensitive towards this drug than towards amitriptyline. In cases of amitriptyline overdosage, however, both compounds have been readily detected and measured by this procedure.

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