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Note

Simultaneous determination of therapeutic levels of amitriptyline and nortriptyline in plasma by gas–liquid chromatography

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In recent years, knowledge of the steady-state plasma levels and half-life of tricyclic antidepressants has proved useful in obtaining information on the relationship between the dosage and therapeutic response and in determining a more exact dosage regime. Methods for the determination of amitriptyline and nortriptyline have been published that are more satisfactory in terms of sensitivity and simplicity of the procedure[1–4]; some methods are capable of measuring only amitriptyline[5,6].

The method proposed here permits (a) the impurities that usually disturb the baseline to be avoided by using an extraction procedure and hence the peak areas to be calculated correctly and (b) a good separation of the peaks of some antidepressants from each other and from the peaks that emerge in the initial phase of the chromatogram.

EXPERIMENTAL

Chemicals and reagents

Amitriptyline and nortriptyline were kindly supplied by Merck, Sharpe and Dohme Research Lab. (Rahway, N.J., U.S.A.) and imipramine by Ciba-Geigy (Basle, Switzerland).

All glassware was silanized overnight with 2% dimethyldichlorosilane (BDH, Poole, Great Britain) in benzene. The reagents used in the extraction procedure were of analytical grade. *n*-Heptane was purified by washing 2 l of it with 20% sodium hydroxide solution, then with concentrated sulphuric acid until the yellow colour disappeared and finally with distilled water to neutrality. The *n*-heptane was then refluxed with metallic zinc and concentrated hydrochloric acid for 2 h and distilled after drying over calcium chloride.

Gas chromatography

The analysis was carried out on a Fractovap Series 2200 chromatograph (Carlo Erba) equipped with a flame-ionization detector and an Autolab 6300 digital integrator under the following conditions: column, 170 mm × 3 mm I.D.; liquid phase, 1% poly(vinylpyrrolidinone) (PVP; Varian Aerograph, Fife, Great Britain) and 3% Versamid 900 (Carlo Erba, Milan, Italy); support, Chromosorb W HP, 100–120 mesh (Carlo Erba). This packing was prepared according to Braithwaite and Whatley[1]. The instrument was set at the following conditions: column temperature, 220°;

detector temperature, 290°; injection port temperature, 290°; supporting gas, nitrogen at the flow-rate of 30 ml/min.

Extraction procedure and derivative formation

Samples of 5 ml of plasma were placed in a 50-ml test-tube and, after adding 500 ng of imipramine (free base) as internal standard, 5 ml of 10-fold concentrated borate–potassium chloride buffer (pH 10) and 10 ml of *n*-heptane, the tube was shaken for 2 min in a vortex mixer and centrifuged at 4300 r.p.m. for 10 min. The plasma layer was removed with a Pasteur pipette and transferred into a second test-tube while 0.1 ml of isoamyl alcohol was added to the first test-tube in order to break the emulsion (first extraction). The *n*-heptane layer obtained after centrifuging was transferred into a 10-ml conical flask. The plasma placed in the second tube was re-extracted with a further 10 ml of *n*-heptane (second extraction). The *n*-heptane extracts were pooled and concentrated to a small volume (about 0.5 ml) in a Büchi rotavapor at 40° in a vacuum, transferred into a 10-ml test-tube with a screw cap and extracted twice with 1 ml of 0.1 *N* hydrochloric acid containing 7% of potassium chloride. The acidic extracts were bulked and washed with 1 ml of *n*-heptane and, after adding 1.5 ml of borate buffer, were extracted twice with 1 ml of *n*-heptane. The *n*-heptane recovered was concentrated (0.2 ml) in a 3-ml pear-shaped flask; 10 µl of trifluoroacetic anhydride were added and the flask was maintained at 22° for 6 min. After evaporating the *n*-heptane in a vacuum in a vertical position, the flask was placed in a vacuum desiccator on silica gel and sodium hydroxide pellets for 30 min.

The residue was taken up carefully in 25 µl of carbon disulphide and the solution was injected into the gas chromatograph.

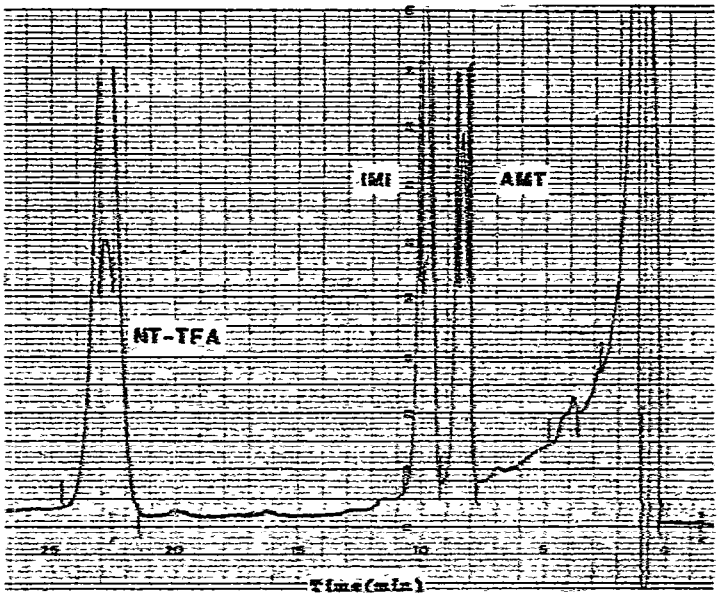

Fig. 1. Gas chromatogram of 25 µl of CS_2 standard solution showing the separation of the internal standard imipramine (IMI) from amitriptyline (AMT) and nortriptyline (N-trifluoroacetyl derivative) (NT-TFA). The amount of each drug was 500 ng as free base.

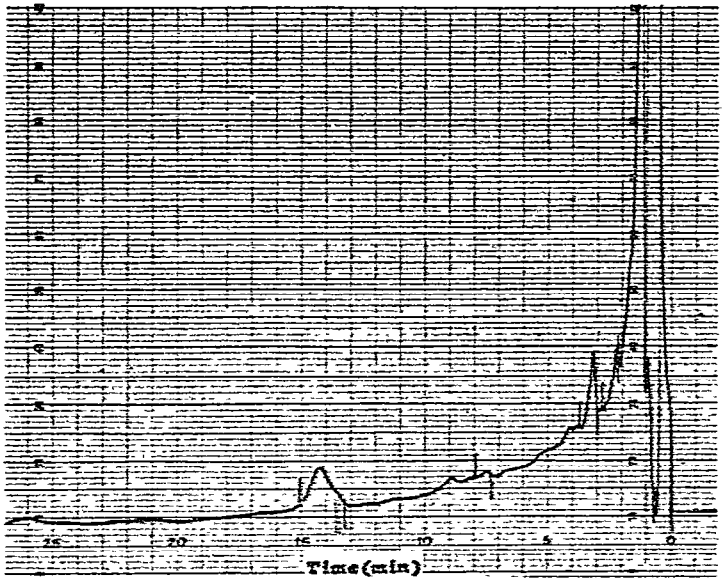

Fig. 2. Gas chromatogram of an extract of 5 ml of human plasma containing no drugs.

RESULTS AND DISCUSSION

Considering the high variability of the recovery of very small amounts of amitriptyline and nortriptyline from the plasma, we thought it was safer to use an internal

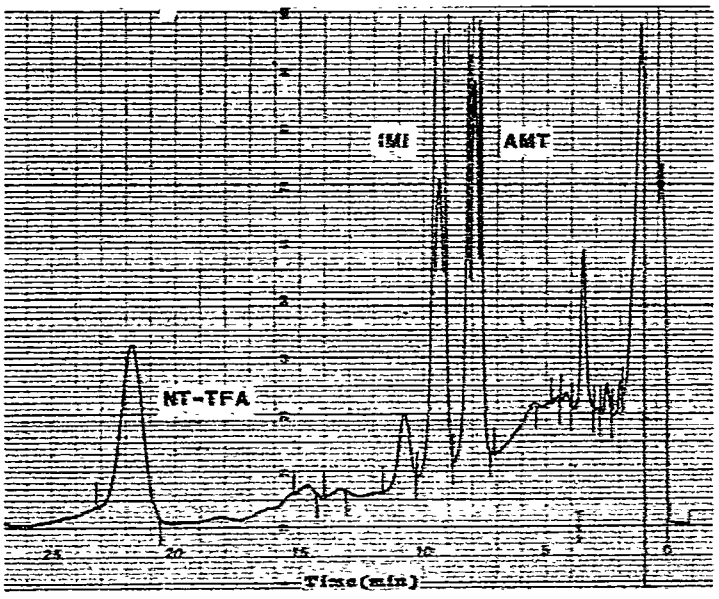

Fig. 3. Gas chromatogram of an extract of 5 ml of plasma from a depressed patient treated with 25 mg of amitriptyline i.m. twice a day, with IMI (500 ng) as internal standard.

TABLE I

GAS CHROMATOGRAPHIC RETENTION TIMES OF AMITRIPTYLINE, NORTRIPTYLINE, IMIPRAMINE AND OTHER DRUGS

Free bases of these compounds were treated with trifluoroacetic anhydride as described in the extraction procedure.

Compound	*Retention time (min)*
Amitriptyline	9
Nortriptyline	23
Imipramine	10
Diazepam	—
Medazepam	15
Flurazepam	—
Trimipramine	8
Sulpiride	—
Orphenadrine	—
Lorazepam	—
Amobarbital	14
Noxiptiline	13.5

standard (Fig. 1). The analyses were performed on samples from depressed females after a preliminary assay before starting the therapeutic treatment (Fig. 2), and blood samples were normally taken 90 min after the administration of the morning dose (Fig. 3). Repeated determinations revealed that plasma levels of amitriptyline ranged from 20 to 250 ng/ml and those of nortriptyline from 5 to 120 ng/ml. The retention times of some compounds structurally related to amitriptyline were also evaluated (Table I).

In conclusion, the method described here allows (a) the selective extraction and the detection of small amounts of some tricyclic antidepressants, and (b) "steady-state" plasma levels of amitriptyline and nortriptyline in patients with poor metabolic activity or treated with a low dosage regime of amitriptyline to be measured.

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