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Note

Separation and quantitative determination of tricyclic antidepressants by high-performance liquid chromatography

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The widespread use of the tricyclic antidepressants in medicine, coupled with reports that there is a relationship between their clinical effect and the plasma levels obtained[1,2], have led to continuing efforts to find rapid and sensitive methods for their identification and quantitative estimation in plasma and urine.

An ultraviolet method for the estimation of amitriptyline was reported by Wallace and Dahl[3] and a fluorimetric method for protriptyline has been published by Moody *et al.*[4]. A method for quantitative analysis using thin-layer chromatography has recently been described[5], but the majority of papers deal with gas chromatographic methods. The disadvantages of gas chromatography for the estimation of this group of compounds in plasma are due to the low concentrations which are found during therapeutic trials, the levels being of the order of 20–200 ng/ml (ref. 2). Sensitivity has been increased by the use of derivatives suitable for electron capture detection[6], the use of linked mass spectrometers, which are not yet widely available[1], or methods which, while employing the flame ionization detector, require lengthy cleanup procedures to eliminate interference from endogenous compounds[2]. High-performance liquid chromatography has been used for the separation of the tricyclic antidepressants by Knox and Jurand[7], but the resolution of the isomeric protriptyline and nortriptyline proved incomplete.

The method described here has the advantage of sensitivity coupled with simplicity of work-up, and provides complete resolution of all three antidepressants.

MATERIALS AND METHODS

1 *N* NaOH was prepared using BDH AnalaR-grade reagent. All solvents were BDH AnalaR grade, and were used without further purification. Amitriptyline·HCl and protriptyline·HCl were a gift from Dr. M. Turnbull (Department of Pharmacology, University of Dundee, Dundee, Great Britain). Protriptyline·HCl was donated by Dr. J. Moody (Department of Psychiatry, Royal Dundee Liff Hospital, Dundee, Great Britain).

Chromatographic conditions

A Varian 8500 chromatograph was used, fitted with a Varian series 635

ultraviolet detector. The column (25 cm × 2 mm I.D.) was made of stainless steel. The packing was Micropak SI10 (Varian): a graded, irregular silica gel with a particle size of 10 μm. The operating conditions were as follows. Temperature, ambient; pressure, 500 p.s.i.; flow-rate, 30 ml/h; chart speed, 1 cm/min; slit width, 1 mm; wavelength, 240 or 290 nm; absorbance range, 0–1.0; solvent, methanol–ammonia (100:1.5); scan speed, 50 nm/min; chart speed (for scans), 5 cm/min.

Extraction procedure

Aqueous solutions were prepared containing 20–200 ng/ml of each of the three compounds. The solutions were mixed in different proportions and made to a final volume of 10 ml. A drop of bromothymol blue indicator was added to the solutions, and 1 *N* NaOH added until alkaline to this indicator. The solutions were then extracted twice with 25 ml light petroleum (b.p. 40–60°), the extracts pooled and filtered through Whatman No. 1 paper. The filtered extracts were evaporated to dryness under a stream of nitrogen at 50°. The residues were taken up in 100-μl aliquots of methanol, and 10-μl samples applied to the column.

RESULTS

Recovery

In specimens without internal standard, a recovery of 89 ± 7% was obtained. In the presence of internal standard, recoveries were 95–105% as calculated by ratios of peak heights.

Linearity

Calibration curves were prepared for amitriptyline and nortriptyline with a constant amount of protriptyline present as internal standard. The curves were linear over the range 0.01–5.5 μg for both compounds.

Identification of peaks

Each of the three peaks were scanned over the wavelength range 330–210 nm.

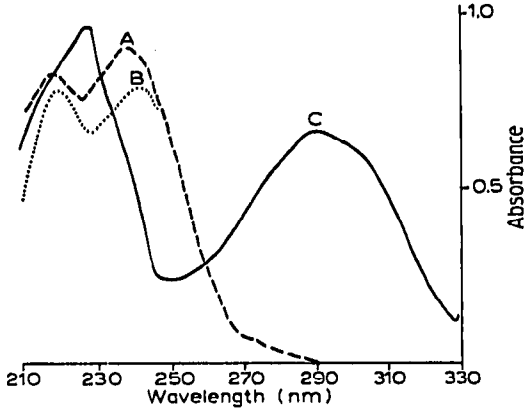

Fig. 1. Spectra of peaks A, B, and C, obtained by stop-flow technique. A = Amitriptyline; B = nortriptyline; C = protriptyline.

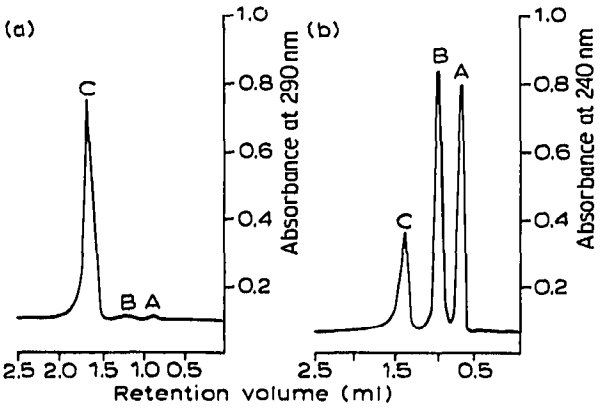

Fig. 2. Chromatograms of equimolar mixtures of amitriptyline, nortriptyline and protriptyline. (a), Detector set at 290 nm; (b) detector set at 240 nm. A = Amitriptyline; B = nortriptyline; C = protriptyline.

The spectra obtained after running extracted specimens are shown in Fig. 1 and correspond with the spectra obtained from the standards dissolved in methanol–ammonia.

Separation

The results obtained from running mixtures of the three compounds are shown in Fig. 2. Figs. 2a and b show the detector set at 290 and 240 nm, respectively. The spectra obtained from the peaks in Fig. 2a are those in Fig. 1. The total amount of each compound applied to the column in this case was 2 μg. The height equivalent to a theoretical plate is 0.5 mm.

Sensitivity

Fig. 3 shows the trace obtained following extraction of an aqueous solution of 10 ng/ml amitriptyline, 2 μg/ml nortriptyline, and 10 ng/ml protriptyline.

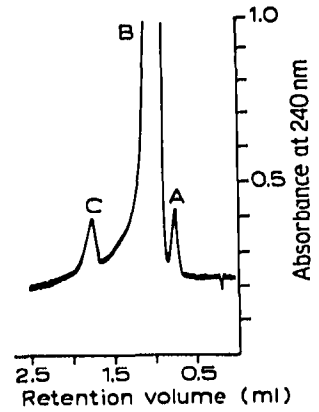

Fig. 3. Chromatogram obtained by extracting 10 ml of a solution containing amitriptyline (10 ng/ml), nortriptyline (2 μg/ml) and protriptyline (10 ng/ml). A = Amitriptyline; B = nortriptyline; C = protriptyline internal standard.

DISCUSSION

The method as described here allows the identification of nortriptyline and amitriptyline at levels approximating to those found in human plasma during treatment. The use of protriptyline as an internal standard allows accurate quantitative determination of the other two compounds. Our initial results on plasma and urine have not so far revealed any contaminating peaks in patients on tricyclic antidepressant therapy alone or in combination with the more common hypnotics.

The method for the determination of protriptyline is less accurate as the wavelength of choice for maximum sensitivity is 290 nm, and at this wavelength almost no absorbance is obtained from the other two compounds.

The choice of solvent for this method was made following our work on thin-layer chromatography of these compounds. We were surprised at the lack of tailing which occurred, and can only surmise that the drugs are eluted as the free bases in the presence of the ammonia. This may well reduce the adsorption by the silica gel. One advantage of methanol–ammonia as a solvent is its optical clarity at these wavelengths.

In conclusion we should like to present this method as a possible tool for the study of therapeutic plasma levels of tricyclic antidepressants, which involves a very simple extraction, rapid elution, and the specificity and sensitivity required for such work.

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