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Note

Separation and measurement of tricyclic antidepressant drugs in plasma by high-performance liquid chromatography

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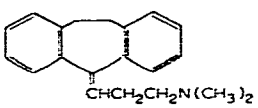
Amitriptyline is one of the most widely used drugs for the treatment of mental depression. The drug is extensively metabolised by animals and man, mainly by 10 (11)-hydroxylation and N-demethylation [1]. One of its metabolites, nortrityline, formed by mono-N-demethylation, also contributes towards the pharmalogical activity and is itself marketed as a therapeutic agent. Studies in patients have indicated that therapeutic effects are better correlated with plasma concentrations of both amitriptyline and nortriptyline [2]. A number of methods have been reported for the measurement of amitriptyline and/or nortriptyline in plasma using various analytical techniques. These include gas chromatography (GC) with specific nitrogen [3—5] flame ionisation [6—8] or electron capture [9] detectors; GC—mass spectrometry in the selected ion monitoring mode [10]; double-radioisotope derivative dilution analysis [11] and radioimmunoassay [12].

High-performance liquid chromatography (HPLC) is proving to be increasingly useful for the routine assay of drugs and their metabolites in biological fluids with sensitivity and specificity equivalent to or superior to corresponding GC methods. The separation of tricyclic antidepressant drugs, including amitriptyline and nortriptyline, by HPLC has been reported [13] using ion-pair partition and adsorption chromatography and a study on the quantitative measurement of these components by HPLC using a silica gel column has been published [14]. The latter method has recently been adapted to the measurement of the drugs in plasma but an unknown metabolite gave some interference with nortriptyline [15].

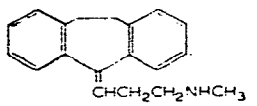
The method described here has been developed for the routine analysis of amitriptyline and/or nortriptyline in plasma.

MATERIALS AND METHODS

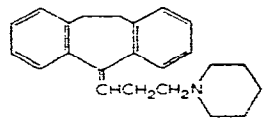
Authentic samples of amitriptyline, nortriptyline, desmethylnortriptyline, 10(11)-hydroxyamitriptyline, 10(11)-hydroxynortriptyline and the internal standard, a piperidine analogue of amitriptyline, were synthesised in these laboratories and used as the hydrochlorides. Standard aqueous solutions of these compounds were stored at 4°. All organic solvents were AnalaR grade.



Amitriptyline



Nortriptyline



Internal standard

HPLC conditions

The HPLC analyses were performed on a Pye Unicam Model 20 LC with a fixed wavelength ultraviolet (UV) detector operating at 254 nm and at a sensitivity of 0.02 a.u.f.s. The Column was a Partisil-10 ODS (25 cm × 4.6 mm I.D.) (Whatman, Maidstone, Great Britain). This column contains a packing material consisting of a C_{18} hydrocarbon bonded to microparticulate silica gel for reversed-phase chromatography. The mobile phase used was 50% (v/v) acetonitrile in potassium dihydrogenphosphate (0.6%, w/v) adjusted to pH 3 with phosphoric acid, at a flow-rate of 2 ml/min.

Calibration and extraction procedure

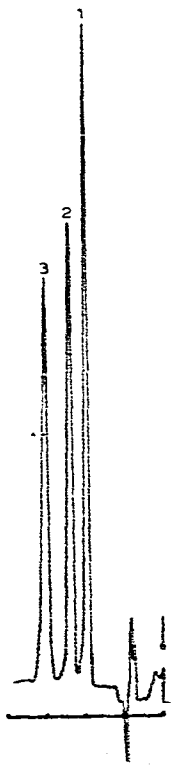
The linear response of the detector was established by constructing calibration curves using standard solutions. Standard solutions containing 40 to 400 ng of both amitriptyline and nortriptyline and 200 ng of the internal standard were injected directly into the chromatograph and peak height ratios measured. The recovery of the internal standard was established by comparing the peak heights after direct injection of a standard solution with those obtained from plasma extracts.

Calibration curves were constructed by adding to blank plasma samples (4 ml), 50 ng/ml of the internal standard and 10, 20, 50 and 100 ng/ml of both amitriptyline and nortriptyline. The plasma samples were made alkaline by adding 0.1 *M*-sodium hydroxide (0.5 ml) and then shaken for 10 min with freshly distilled diethyl ether (15 ml). After centrifugation the ether layer was removed and shaken with 1 *M*-sulphuric acid (2 ml) for 10 min. The mixture was centrifuged, the ether layer discarded and the acid layer neutralised and adjusted to about pH 9.5 with 4 *M* sodium hydroxide in an ice-bath. The mixture was shaken for 10 min with freshly distilled diethyl ether (15 ml), the ether layer was removed and evaporated to dryness under a stream of nitrogen. The residue was dissolved in methanol (25 µl) containing a trace of hydrochloric acid and 20 µl of this solution was injected into the liquid chromatograph.

RESULTS AND DISCUSSION

The chromatogram in Fig. 1 shows the separation of amitriptyline, nortriptyline and the internal standard, the three compounds having retention times of 4.8, 3.9 and 5.9 min, respectively. Under these chromatographic conditions some other possible interfering metabolites, desmethylnortriptyline, 10(11)-hydroxyamitriptyline and 10(11)-hydroxynortriptyline were eluted before nortriptyline and all had retention times of less than 3 min.

A chromatogram of blank plasma extract is shown in Fig. 2a. Most of the endogenous UV-absorbing plasma constituents occurred at retention times less than 3 min and did not interfere with the analysis. It was, however, necessary to incorporate back extraction into acid in the extraction procedure in order



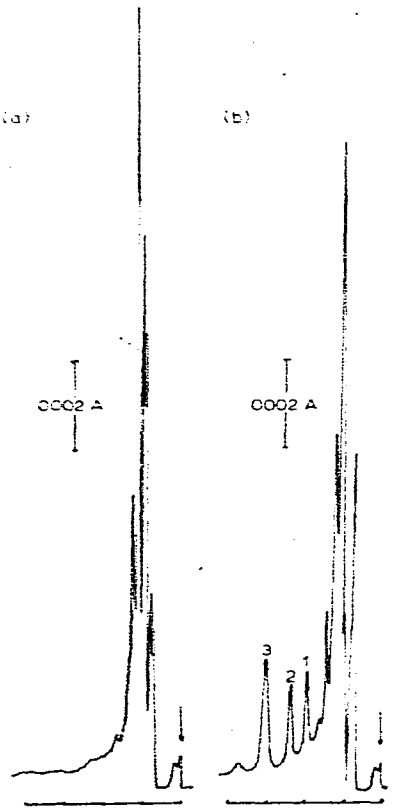


Fig. 1. HPLC separation of standards, nortriptyline, (1), amitriptyline (2) and internal standard (3). Conditions: Column 25 cm × 4.6 mm Partisil-10 ODS; eluent, 50% (v/v) acetonitrile in potassium dihydrogenphosphate (0.6%, w/v) adjusted to pH 3 with phosphoric acid; flow-rate, 2 ml/min.

Fig. 2. Chromatograms of plasma extracts. (a) Blank plasma. (b) Plasma containing 20 ng/ml of nortriptyline (1) and amitriptyline (2) with 50 ng/ml of internal standard (3). Conditions as for Fig. 1.

to remove background interferences. A chromatogram of an extract from plasma containing 20 ng/ml of amitriptyline and nortriptyline is shown in Fig. 2b. The mean recovery of the internal standard from plasma was 94% and the mean relative recoveries of amitriptyline and nortriptyline were 99.8% (n = 8) and 90.8% (n = 8) respectively, calculated by comparing peak height ratios of plasma extracts and standards injected directly.

Calibration curves for both amitriptyline and nortriptyline constructed by plotting peak height ratios relative to internal standard showed a linear relationship over the concentration range 10 to 100 ng/ml. Over this concentration range nortriptyline and amitriptyline could be measured with a precision of ± 4.5% (n = 8) and ± 4.7% (n = 8) respectively.

This method provides an assay for both amitriptyline and nortriptyline with a sensitivity of about 5 ng/ml, comparable to all previously reported methods, and with an analysis time of less than 10 min. Most of the published GC methods report a detection limit of 10—20 ng/ml and one method giving a detection limit of 2 ng/ml involves measurement of total amitriptyline and nortriptyline as a common derivative [8]. Although a better absolute limit of detection can be obtained using GC detectors, comparable sensitivity is achieved during HPLC by the injection and analysis of larger amounts of plasma extracts. Due to the selectivity of the UV detector and the rapid elution of the mainly hydrophilic endogenous plasma components which have a low affinity for the lipophilic stationary phase, there is little background interference.

Subsequent investigations using a variable wavelength UV detector monitoring at 242 nm, corresponding to a λ_{max} for amitriptyline and nortriptyline, have shown that a sensitivity of 2 ng/ml could be easily achieved. This represents a sensitivity superior to most of the presently available methods. Steady-state concentrations of amitriptyline and nortriptyline in patients have been reported to be in the range 10—400 ng/ml [1, 3, 7] and the method reported here provides a reliable routine method for analysis of these components in plasma from patients.

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