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Note

Quantitative determination of amitriptyline and nortriptyline in plasma by high-performance liquid chromatography

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Methods for the estimation of the tricyclic antidepressants in plasma have been described employing UV spectrophotometry[1], gas–liquid chromatography[2–4], and mass fragmentography[5,6].

UV methods, while adequate for analysis in cases of overdose, are insufficiently sensitive where plasma levels are required in the therapeutic range. Several GLC methods are available[2–4], but the requirement for a lengthy clean-up procedure and the necessity for derivative formation make these methods too time consuming for emergency use and expensive in terms of operator time for routine therapeutic screening.

Following on the methods developed for determining these compounds[7,8], we wished to produce a method, applicable to both plasma and urine, which was capable of estimating both amitriptyline and nortriptyline on a 2-ml sample of plasma with a rapid extraction procedure. This has now been achieved.

EXPERIMENTAL

Materials

Dichloromethane was obtained from Rathburn Chemicals (Walkerburn, Peebleshire, Great Britain). Ethanol was BDH Aristar grade (BDH, Poole, Great Britain). All other chemicals were BDH Analar grade. Amitriptyline, nortriptyline, and desmethylimipramine were given by Dr. M. Turnbull and Mr. R. Sparks of the Department of Pharmacology, Ninewells Hospital, Dundee, Great Britain.

Methods

All glassware was silanised prior to use with a 2% solution of dimethyldichlorosilane in 1,1,1-trichloroethane.

To 2 ml of plasma in a small conical test tube were added 0.1 ml of 5 *M* NaOH, 2 ml of dichloromethane, and 2 μl of 3.75 mmoles/l desmethylimipramine internal standard. The concentration of the internal standard in the sample was 1.875 μmoles/l (1 μg/ml). The tubes were stoppered and shaken for 1 min. The tubes were centrifuged for 5 min. The aqueous phase was removed by suction. 2 ml of Aristar ethanol were added and the tubes were stoppered and inverted several times to ensure

complete mixing. The tubes were then centrifuged to remove the precipitated protein. The solvent mixture was slowly decanted into a second conical test tube. The extract was dried down under a stream of nitrogen at 60°. The residue was redissolved in 20 μl of dichloromethane with careful washing down the sides of the tube, buzzed on a whirlimixer for 10 sec, and all available solvent was taken up in a 10-μl syringe and injected onto the high-performance liquid chromatograph.

Chromatographic conditions

A Varian Model 8500 high-performance liquid chromatograph was used with a Variscan 635 Series variable-wavelength detector. The column (25 cm × 2 mm I.D.) was packed with 5-μ silica gel (Micropak). The other parameters were: solvent, dichloromethane–propan-2-ol–conc. ammonia (100:2:0.25); flow-rate, 60 ml/h; pressure, 2500 p.s.i.; wavelength, 240 nm; band pass, 2 nm. A 10-mV recorder with a chart speed of 25 cm/h was used.

Calculations

Peak area was calculated according to the formula peak height × half base width. Metabolites were calculated as equivalent to amitriptyline. By experiment it was found that the absorbance of the standards available to us of amitriptyline and nortriptyline, respectively, were in the ratio 3:2; thus the latter were corrected to give a figure equivalent to amitriptyline. Standards were in nmoles/l. Results were calculated by expressing (area of test peak)/(area of desmethylimipramine peak) as a ratio. This ratio was read off a standard graph of concentration *versus* ratio.

RESULTS

Plasma samples

The trace obtained from a blank plasma sample is shown at Fig. 1a. No peaks other than the solvent peak and that of the internal standard were detected.

The traces obtained for specimens from two patients receiving amitriptyline for therapy are shown at Figs. 1b and c. It can be seen that in Fig. 1b there is a very low level of amitriptyline present; nortriptyline was not detected in this patient.

Fig. 1c was obtained from a patient receiving 75 mg/day amitriptyline. The trace shows amitriptyline at a level of 345 moles/l (95 ng/ml) and nortriptyline at a level of 342 nmoles/l (90 ng/ml). The other peaks are thought to be metabolites and are not obtained on plasmas for patients not receiving amitriptyline or nortriptyline. Fig. 2 shows the trace obtained from the plasma of a patient following an overdose of amitriptyline. The levels of amitriptyline and nortriptyline observed in this patient were 1058 nmoles/l (293 ng/ml) and 95 nmoles/l (25 ng/ml), respectively. Of interest are the large metabolite peaks, as yet unidentified.

Precision and recovery

The precision and relative recovery of the method were checked using plasma samples to which amitriptyline and nortriptyline had been added after initially dissolving them in water.

The results, shown in Table I, show an average coefficient of variation of around 10%. They also show that the relative recovery levels are satisfactory.

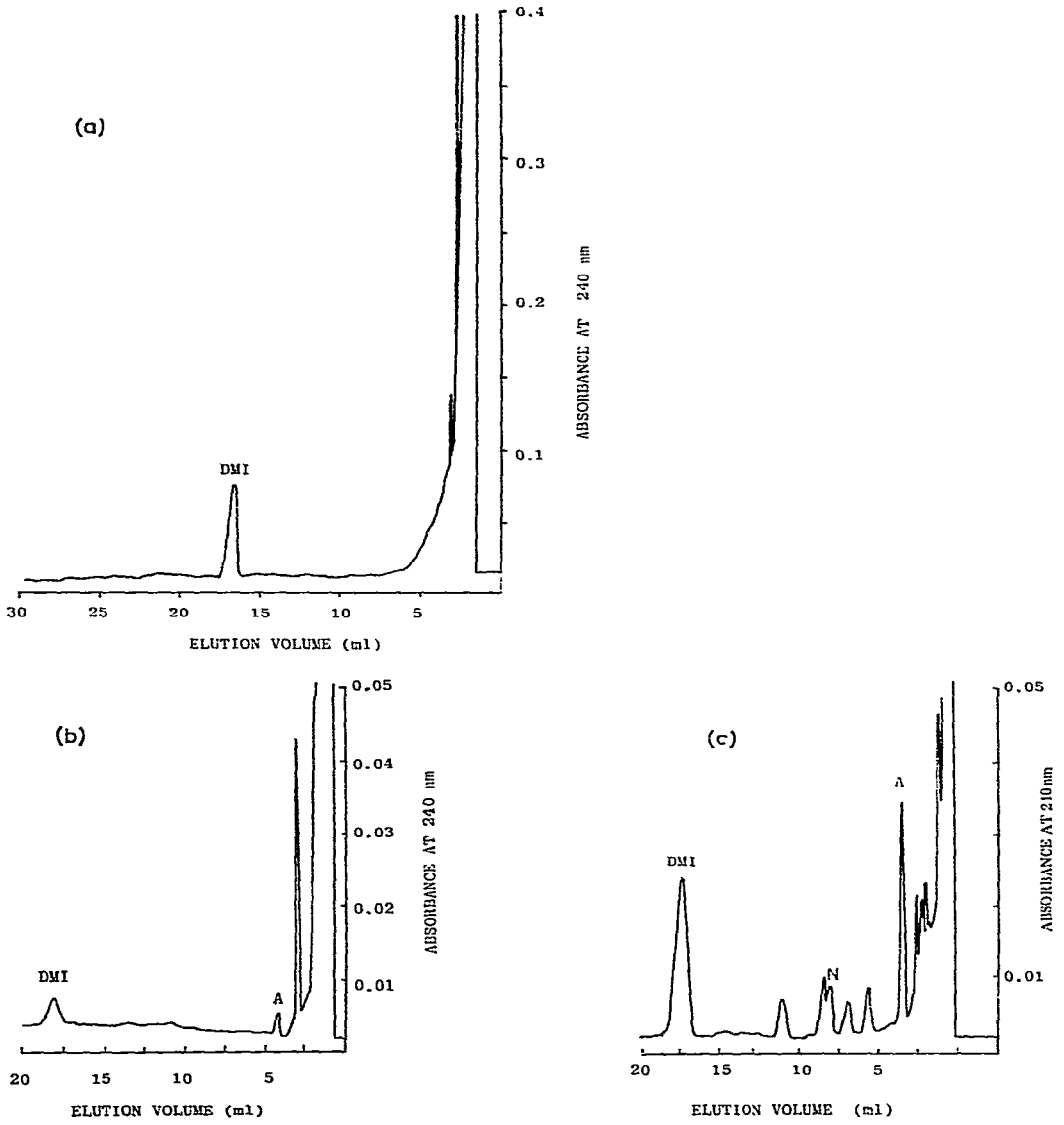

Fig. 1. (a) Trace obtained from plasma of patient receiving no drugs. (b) Trace obtained from a patient receiving 50 mg/day amitriptyline. (c) Trace obtained from patient receiving 75 mg/day amitriptyline. A = Amitriptyline; N = nortriptyline; DMI = desmethylimipramine.

Linearity and sensitivity

The calibration curves for concentration *versus* (peak area of amitriptyline or nortriptyline)/(peak area of desmethylimipramine internal standard) were linear over the range 70 nmoles/l–16 μmoles/l (20–800 ng/ml). The method is capable of detecting levels down to 40 nmoles/l (approx. 10 ng/ml) as is shown in Fig. 1b, where a level of 30 nmoles/l (approx. 8 ng/ml) was determined.

TABLE I

PRECISION AND RECOVERY OF THE METHOD

Compound added	*Amount (nmoles/l)*		*% recovery*	*No. of observations*	*S.D.*	*C.V.*
	Added	*Recovered*				
Amitriptyline	900	958	106	8	101.9	10.6
Amitriptyline	180	202	112	7	17.6	8.7
Nortriptyline	900	860	96	9	99.7	11.6
Nortriptyline	180	179	99	6	14.9	8.3

Studies on urine

The trace obtained from the urine of a patient who had taken an overdose of amitriptyline is shown in Fig. 3. It can be seen that the largest peak is that of amitriptyline. This is not the case following therapeutic doses, where the 10-hydroxy metabolites predominate[8].

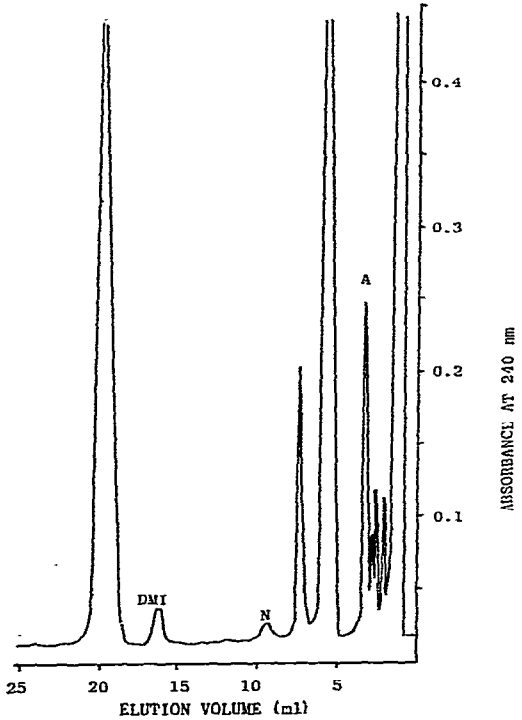

Fig. 2. Trace obtained from the plasma of patient J.P. 6 h after taking an overdose of amitriptyline. For abbreviations, see the legend to Fig. 1.

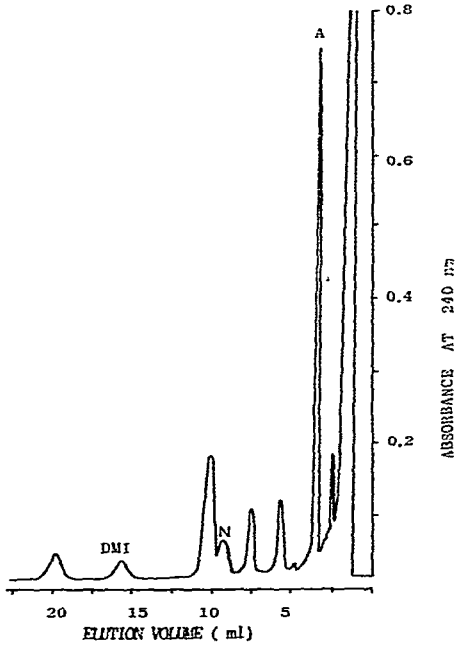

Fig. 3. Trace obtained from the urine of patient J.P. 6 h after taking an overdose of amitriptyline. For abbreviations, see the legend to Fig. 1.

DISCUSSION

The method presented here offers considerable advantages over currently available methods for the study of amitriptyline and nortriptyline metabolism.

The very simple work-up procedure enables the operator to prepare samples for chromatography in 30 min, and the elution time for the internal standard is of the order of 15 min.

We have been able to process some plasma and control samples in one afternoon and provide the wards with levels of both amitriptyline and nortriptyline.

Using a more polar, deactivating solvent, we found that the pharmacologically active metabolites of chlorpromazine, imipramine, and amitriptyline were resolved, as well as the active drug[8]. The solvent used here for the estimation of plasma amitriptyline tends to increase the retention of drugs of tricyclic structure, thus improving resolution. This method could be further adapted to measure the other drugs in plasma either singly or simultaneously.

The current limit on sensitivity is determined by the characteristics of the detector. Using a larger volume of plasma or urine is not helpful as the extra endogenous material eluted with the solvent front obscures the amitriptyline peak.

We are not at present able to put a positive identification on any of the other peaks detected. The relative amounts of these metabolites may be calculated as amitriptyline equivalent in both plasma and urine, which will enable studies of metabolism and excretion to be carried out.

We feel that this method offers a significant improvement at all but the lowest levels of the two drugs over currently available techniques.

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