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SIMPLIFIED METHOD FOR MONITORING TRICYCLIC ANTIDEPRESSANT THERAPY USING GAS—LIQUID CHROMATOGRAPHY WITH NITROGEN DETECTION

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SUMMARY

A simplified gas chromatographic method for the rapid measurement of tricyclic antidepressant drugs in plasma using a nitrogen-sensitive detector is described. All drugs are extracted and chromatographed under identical conditions. Tertiary amines are separated from their secondary amine metabolites, which are determined simultaneously without the need for derivatisation. The lower limit of accurate determination for most drugs is 10 μg/l.

The method has been applied to the routine measurement of amitriptyline and nortriptyline in plasma from patients receiving antidepressant treatment. Large and important interindividual differences in plasma concentrations in the patients investigated have been found, and the significance of these results is discussed.

INTRODUCTION

Many studies have shown that patients receiving similar doses of tricyclic antidepressant drugs exhibit a wide (10—20-fold) interindividual variation in steady-state plasma concentrations achieved [1—6]. Relationships between these concentrations and therapeutic response have been proposed [4, 5, 7—13] and there is also an increased incidence of toxic side-effects associated with high plasma concentrations [14—16]. It is important therefore, that rapid and sensitive methods should be available for the routine measurement of therapeutic concentrations of the commonly used tricyclic antidepressants.

Over recent years, a large number of gas chromatographic methods have been reported, most of which have concentrated on the most popular antidepressant, amitriptyline, and its demethylated metabolite, nortriptyline. The earliest methods used flame-ionization detection [17—19], but were relatively insensitive, requiring large volumes (5—10 ml) of plasma.

Electron-capture detection has been found to greatly increase sensitivity, and has provided reliable methods for nortriptyline [20—22]. However, polyfluorinated derivatives must be prepared, which is extremely time consuming.

Further, since tertiary amine antidepressants do not derivatise without prior demethylation, the technique has only a limited routine application.

A significant advance in methodology has been brought about by the introduction of "nitrogen-specific" detectors [23—31]. Some of these methods still involve a derivatisation procedure [23, 24, 26, 28] making them unsatisfactory for routine use. Several of these methods have specified special pre-treatment of glassware [23—26] and large samples (>4 ml for duplicate analyses) are still required by some assays [25, 26, 31] making them suitable only for research purposes.

Sensitivity and selectivity have been dramatically improved by the use of mass fragmentography [32, 33], but this expensive and highly specialised equipment is not available in the average laboratory. Immunological assays are still in the early stages of development, and problems of antibody cross-reactivity with other drugs and metabolites remain to be solved [34].

We describe here an improved method using gas—liquid chromatography (GLC) with nitrogen detection which has been in regular routine use for over a year for the measurement of plasma antidepressant concentrations in patients receiving medication with amitriptyline, nortriptyline, imipramine and clomipramine.

EXPERIMENTAL

Reagents

Reagents used were: absolute ethanol (GPR grade); borate buffer, pH 10 (Fisons, Loughborough, Great Britain); *n*-butyl acetate (Fisons; AnalaR grade); *n*-hexane (Fisons; Distol grade); 4 *M* NaOH (AnalaR) prepared freshly each week and 0.1 *M* H_2SO_4.

Glassware

Extraction tubes are glass with ground glass stoppers and are rinsed with ethanol just prior to use to reduce drug adsorption onto glass surfaces. The micro tubes (10 cm × 6 mm I.D.) are glass freeze-dry ampoules (FBG-Trident, Bristol, Great Britain). These are discarded after use.

Internal standard

Maprotiline hydrochloride, 2 μg/ml free base equivalent in aqueous solution was prepared from a concentrated acidic stock solution stored at 4°. For the determination of maprotiline an aqueous solution of nortriptyline hydrochloride (1 μg/ml) was used as an internal standard.

Standards

Plasma standards were prepared by spiking fresh bovine plasma to concentrations within the range 50—500 μg/l. These were stored deep frozen in 2-ml aliquots until required.

Extraction procedure

The plasma samples (1 ml) were added to buffer (1 ml) containing internal standard (150 μl) in a 10-ml stoppered glass tube. The drugs were extracted

into hexane (5 ml) by gentle mixing for 20 min. Extraction of drugs and internal standards was found to be maximal and constant after 15 min. Following centrifugation, the organic phase was transferred to a clean 10-ml glass tube containing sulphuric acid (1 ml) using a pasteur pipette rinsed in ethanol. After mixing for 10 min, the lower acid layer was transferred to a clean micro tube, then sodium hydroxide (100 μl) and butyl acetate (100 μl) were added. The tube was vortex mixed for 30 sec and centrifuged for 3 min. Aliquots (3—5 μl) of the upper organic phase were injected onto the chromatograph.

Chromatography

The gas chromatograph used was a Perkin-Elmer Model F33 equipped with an alkali flame nitrogen—phosphorus detector. The column (2 m × 2 mm I.D.) was of silanised glass packed with 3% SP 2250 on Supelcoport (80—100 mesh; Supelco, Bellefonte, Pa., U.S.A.). The column temperature was 250° and the carrier gas was argon at an inlet pressure of $3 \cdot 10^5$ N/m^2. The injection port temperature was 300°, with hydrogen pressure at $8 \cdot 10^4$ N/m^2 and an air pressure of $6 \cdot 10^4$ N/m^2. The detector bead setting ranged from 500 to 650 depending on the age of the bead.

Quantitation

A range of plasma standards were extracted simultaneously with the samples. The drug concentrations in the samples were calculated by comparison of peak height ratio to the internal standard. All standards and samples were run in duplicate, and repeated if the duplicates differed by more than ± 5%.

Blood samples

Blood samples (10 ml heparinised) were obtained from patients undergoing tricyclic antidepressant therapy. The plasma was separated and stored at 4° prior to analysis.

RESULTS

The absolute and relative retention times of a range of tricyclic antidepressants and related compounds are shown in Table I. As can be seen from this table, all the commonly prescribed antidepressant agents can be eluted on the system described. A number of tricyclic antidepressants (e.g., amitriptyline, imipramine, clomipramine and doxepin) produce active metabolites which can be resolved from their respective parent compounds.

Fig. 1 shows the resolution of imipramine and related compounds from the internal standard, maprotiline. Fig. 2 shows the resolution of amitriptyline and related compounds from maprotiline.

The sensitivity of the detector to the different compounds can be directly compared since each peak represents 10 ng on injection (Figs. 1 and 2).

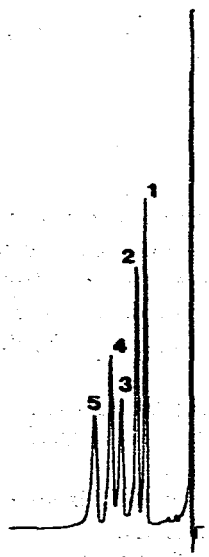
Typical chromatograms obtained from the analysis of samples from patients receiving medication with amitriptyline and imipramine are shown in Fig. 3.

For the plasma standards, a good linear correlation was obtained between peak height ratio and concentration over the range 50—500 μg/l. Where plasma concentrations were expected to exceed this limit, smaller samples of plasma (0.2—0.5 ml) were used.

TABLE I

ABSOLUTE AND RETENTION TIME RELATIVE TO MAPROTILINE FOR TRICYCLIC ANTIDEPRESSANTS AND RELATED DRUGS

Drug	Retention time (min)	Retention relative to maprotiline
Butriptyline	3.2	0.56
Trimipramine	3.4	0.60
Amitriptyline	3.5	0.61
Imipramine	3.6	0.63
Doxepin	3.8	0.67
Zimelidine	3.9	0.68
Nortriptyline	4.0	0.70
Mianserin	4.1	0.72
Nomifensine	4.1	0.72
Desmethyltrimipramine	4.3	0.75
Protriptyline	4.4	0.77
Desmethyl imipramine	4.4	0.77
Desmethyl doxepin	4.6	0.81
Norzimelidine	4.8	0.84
Maprotiline	5.7	1.0
Clomipramine	6.5	1.14
Dothiepin	6.6	1.16
Desmethyl clomipramine	7.6	1.33
Northiaden	8.0	1.40
Dibenzepin	10.0	1.75



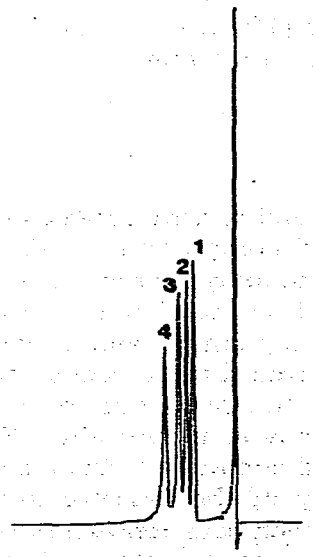


Fig. 1. The separation of imipramine (1), desipramine (2), maprotiline (3), clomipramine (4) and desmethyl clomipramine (5). Each peak represents 10 ng on injection.

Fig. 2. The separation of amitriptyline (1), nortriptyline (2), protriptyline (3) and maprotiline (4). Each peak represents 10 ng on injection.

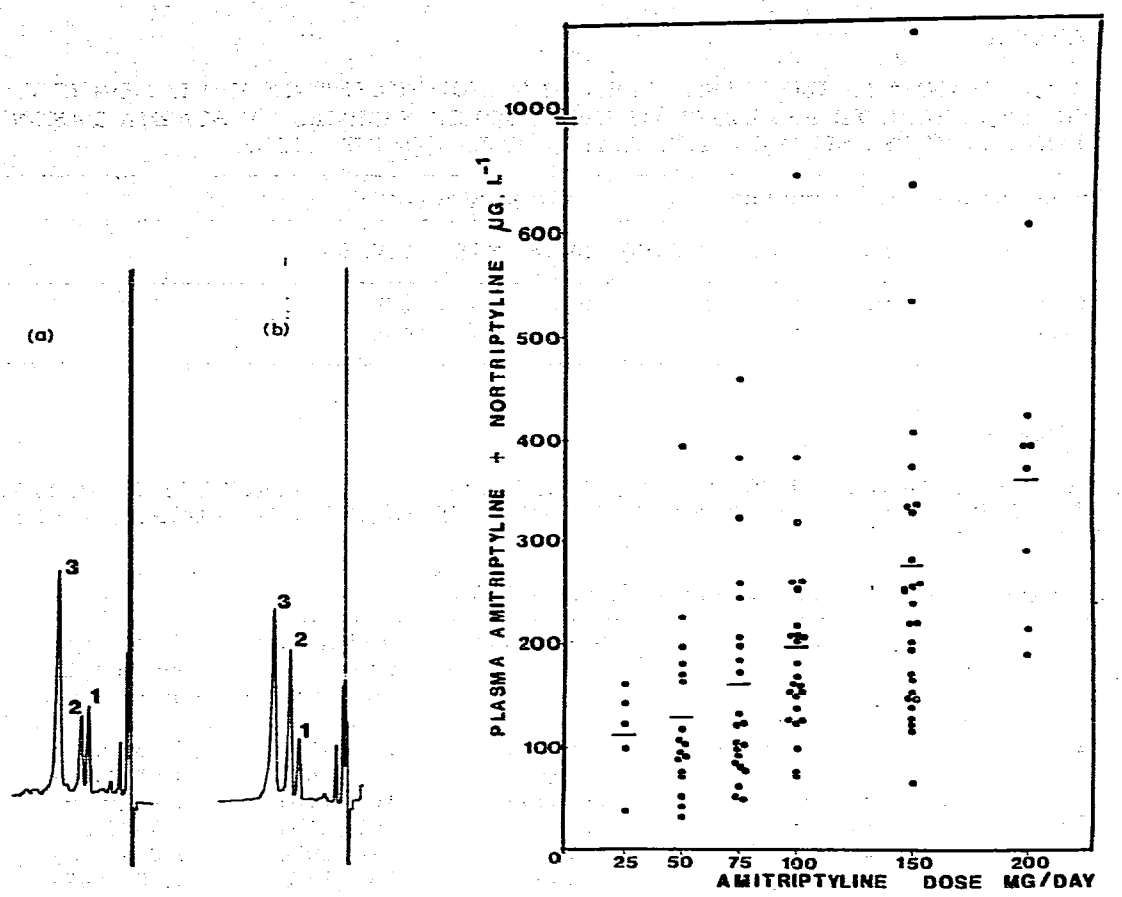


Fig. 3. (a) Trace obtained from the analysis of a plasma sample from a patient receiving amitriptyline, containing 45 μg/l amitriptyline (1) and 56 μg/l nortriptyline (2) with 3000 μg/l maprotiline internal standard (3). (b) Trace obtained from the analysis of a plasma sample from a patient receiving imipramine, containing 27 μg/l imipramine (1), and 112 μg/l desipramine (2) with 300 μg/l maprotiline internal standard (3).

Fig. 4. Relationship between daily dose and plasma concentration of amitriptyline plus nortriptyline in 103 patients receiving amitriptyline medication.

The reproducibility of the method was assessed by repeated analysis of pooled plasma samples taken from patients undergoing amitriptyiline medication. Table II shows the mean, standard deviation (S.D.), and coefficient of variation (C.V.) obtained from 20 replicate analyses of two different pooled samples. In all cases, a C.V. of less than 5% was achieved.

No interference with the assay by either endogenous plasma constituents or other commonly prescribed psychotropic drugs has yet been encountered.

Fig. 4 shows the distribution of amitriptyline plus nortriptyline plasma levels over a range of different doses obtained in the routine analysis of samples from 103 patients receiving amitriptyline treatment. It can be seen from Fig. 4 that mean plasma concentrations tended to increase with dosage.

TABLE II

MEAN, STANDARD DEVIATION (S.D.) AND COEFFICIENT OF VARIATION (C.V.) FOR 20 REPLICATE ANALYSES OF TWO POOLED SAMPLES OF PLASMA TAKEN FROM PATIENTS RECEIVING TREATMENT WITH AMITRIPTYLINE

Pooled plasma	Amitriptyline (μg/l)			Nortriptyline (μg/l)		
	Mean	S.D.	C.V.(%)	Mean	S.D.	C.V.(%)
"High" value	295	5.4	1.8	200	5.7	2.9
"Low" value	75	3.0	4.0	57	2.6	4.5

TABLE III

MEAN PLASMA AMITRIPTYLINE, NORTRIPTYLINE AND AMITRIPTYLINE—NORTRIPTYLINE RATIO IN 103 PATIENTS RECEIVING DIFFERENT DAILY DOSES OF AMITRIPTYLINE

Dose (mg/day)	*n*	Plasma conc. (μ/l), mean (S.D.)			
		Amitriptyline	Nortriptyline	Amitrityline plus nortriptyline	Amitriptyline/ nortriptyline, mean (S.D.)
25	5	48 (18)	65 (37)	113 (48)	0.88 (0.41)
50	17	74 (53)	57 (45)	130 (88)	1.65 (1.39)
75	22	84 (58)	81 (61)	162 (113)	1.15 (0.49)
100	24	57 (57)	95 (81)	197 (123)	1.53 (1.33)
150	27	132 (80)	145 (146)	276 (205)	1.25 (0.85)
200	8	173 (104)	173 (47)	359 (132)	1.00 (0.47)
All doses (mean) 103.6	103	105 (71)	103 (98)	209 (154)	1.32 (1.01)

However, large interindividual differences in plasma levels were seen in patients prescribed similar doses. Table III shows the mean and S.D. plasma amitriptyline plus nortriptyline concentrations for different daily doses.

The relationship between amitriptyline and nortriptyline concentrations in individual patients is shown in Fig. 5. Individual ratios of amitriptyline to nortriptyline plasma concentrations ranged between 0.3 and 6.6 (mean 1.3, S.D. 1.0). However, there was a highly significant correlation ($r = 0.63$, $p < 0.0001$) between amitriptyline and nortriptyline plasma concentrations as shown in Fig. 5.

DISCUSSION

Most of the commonly used antidepressant drugs and, where applicable, their demethylated metabolites, can be measured accurately in plasma at therapeutic concentrations by the procedure described above. Analytical time has been saved by eliminating the need for derivative formation prior to chromatography and by introducing a final micro-phase extraction step in

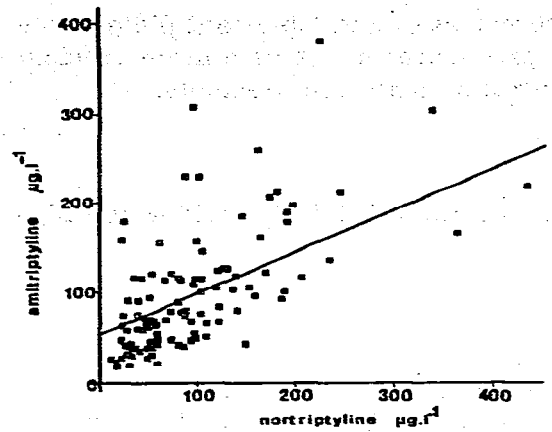


Fig. 5. Relationship between plasma amitriptyline (y) and nortriptyline (x) in 103 individual patients. The point representing a patient with a plasma AT and NT concentration of 311 and 758 µg/l, respectively, is not shown, but has been included in the regression analysis: $y = 0.46\,x + 56.2$ ($r = 0.63$, $p < 0.0001$).

place of the more usual procedure of extract concentration. Other than rinsing with ethanol, no special pre-treatment of glassware is required and the use of disposable tubes for the final extraction stage reduces the risk of contamination from recycled glassware.

The method has also been successfully applied to the measurement of antidepressant plasma concentrations in cases of overdosage. In such cases only 1 ml of plasma is required for duplicate analyses. This has been carried out for all the drugs shown in Table I, with the exception of zimelidine and its active metabolite norzimelidine. These measurements have been found to be useful in the diagnosis of suspected antidepressant poisoning in both adults and children.

The major application of the technique in this laboratory has been the routine analyses of drug plasma concentrations in patients receiving tricyclic antidepressant medication. This is demonstrated in the results obtained from 103 patients receiving treatment with amitriptyline which are shown in Fig. 4 and Table III. As can be seen in Fig. 4, extremely large interindividual differences in "steady-state" drug plasma concentrations are obtained in the patients receiving routine treatment with amitriptyline. Although mean plasma levels of amitriptyline and nortriptyline tended to increase with prescribed daily dose, much overlap is apparent. The plasma concentration ratio of amitriptyline to nortriptyline in individual patients ranged from 0.3 to 6.6 (mean 1.3, S.D. 1.0) overall, however, there was a highly significant correlation ($r = 0.63$, $p < 0.0001$) between amitriptyline and nortriptyline concentrations (Fig. 5). In view of the reported relationship between plasma concentrations of tricyclic antidepressants and their clinical effects [4, 5, 7—16] plasma concentrations achieved by patients during routine treatment may be extremely important. From the results shown in Fig. 4 for amitriptyiine medication, it is reasonable to argue that a large proportion of patients may never achieve therapeutically effective drug levels, whereas others may have levels that

are likely to cause serious toxicity. Measurement of antidepressant plasma concentrations using rapid and simple assay procedures might be a more efficient way of tailoring drug dosage to suit individual patients' requirements.

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