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GAS CHROMATOGRAPHIC ANALYSIS OF THERAPEUTIC CONCENTRATIONS OF MAPROTILINE IN SERUM, USING FLAME-IONIZATION DETECTION

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SUMMARY

For the measurement of the tetracyclic antidepressant maprotiline in human serum, a gas chromatographic method with flame-ionization detection has been developed. The assay specifications obtained are as follows: a precision (C.V.) of 3.5—6.4%, and a relative recovery of 97—109% using amitriptyline as internal standard. The sensitivity of the assay from serum was 40 nmol/l. The applicability of the method has been shown by measuring steady-state serum levels of five inpatients. The steady-state serum levels of maprotiline given at a daily dosage of 75 mg varied from 272 to 570 nmol/l.

INTRODUCTION

Numerous studies have shown that patients receiving the same doses of tricyclic antidepressant drugs exhibit a wide (10—30-fold) inter-individual variation in steady-state plasma or serum concentrations [1—3]. The correlation between the plasma levels of some tricyclic antidepressants and their therapeutic effects found in some [4—7] but not in all [8,9] studies suggests that measurement of antidepressant plasma concentration may provide valuable information for improving clinical management of depressed patients.

Maprotiline is a new antidepressant drug. Its structure differs from conventional tricyclic antidepressants in that it has a hexagonal central ring in the molecule with an ethylene bridge (Fig. 1). Its pharmacological effects have been reviewed recently by Pinder et al. [10]. In the review article of Scoggins et al. [11] there are 138 references describing various methods for the measurement of antidepressant concentration in biological fluids. In only one of these was there an assay technique for maprotiline [12]. In the method described, maprotiline was converted into the heptafluorobutyramide derivative after

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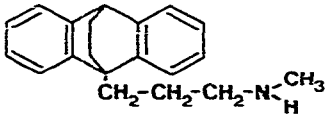
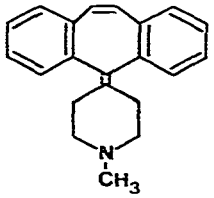
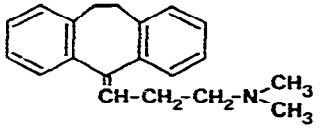


MAPROTILINE (M.W. 277.4)



CYPROHEPTADINE (M.W. 287.4)



AMITRIPTYLINE (M.W. 277.4)

Fig. 1. Structures of maprotiline, cyproheptadine and amitriptyline. Amitriptyline and cyproheptadine were used as internal standards.

separation from biological fluid. The derivative was determined quantitatively by gas—liquid chromatography using an electron-capture detector. Riess [13] used the double radio-isotope derivative technique for the assay of maprotiline.

While mass fragmentographic and gas chromatographic determinations using a nitrogen detector are optimal for the determination of many drugs at low concentrations, the equipment is not available in all laboratories. The method described here is designed for those laboratories that do not have access to more complex and expensive equipment, such as gas—liquid chromatography with a nitrogen detector or high-performance liquid chromatography. Thus we report here a relatively simple gas chromatographic procedure for direct determination of underivatized maprotiline in human serum using flame-ionization detection.

MATERIALS AND METHODS

Reagents

The following reagents were used. Petroleum ether (b.p. 40—60°C, redistilled), isoamyl alcohol and toluene were obtained from E. Merck (Darmstadt, G.F.R.). *n*-Heptane was from J.T. Baker (Deventer, The Netherlands), and diethylamine from Fluka (Buchs, Switzerland). All reagents were of analytical grade. The antidepressant drugs used for calibration were donated by the manufacturers and were of Ph.Nord. grade.

Glassware

All extraction tubes were acid-washed and finally rinsed with distilled water in a dish-washing machine. The small conical test-tubes in the final step were of a disposable type made from new Pasteur pipettes. The extraction tubes were equipped with PTFE-lined screw-caps.

Internal standards

For determination of maprotiline, an aqueous solution of amitriptyline hydrochloride was used as an internal standard. For determination of other

antidepressants, cyproheptadine was used as internal standard.

Amitriptyline hydrochloride was purchased from Oy Star Ab (Tampere, Finland). A stock internal standard, 1 m*M*, was prepared in distilled water; it was shown to be stable for at least a month at +4°C. A working internal standard, 30 μM, was prepared by diluting the stock solution in water.

EXTRACTION PROCEDURE

To 4 ml of serum, 0.1 ml of 30 μM amitriptyline standard was added in a 15-ml PTFE-lined screw-capped tube. The solution was made alkaline with 0.2 ml of 2 *M* sodium hydroxide solution and extracted with 7 ml of *n*-hexane—isoamyl alcohol (100:3) by a rotation mixer for 15 min, followed by centrifugation for 5 min at 1500 *g*. The organic phase was transferred to a clean 10-ml screw-capped tube, then 2 ml of 0.1 *M* hydrochloric acid solution was added and the solution mixed for 15 min. After centrifugation of 5 min at 1500 *g*, the acid phase was transferred to a 5-ml conical test-tube. The aqueous phase was made alkaline with 0.2 ml of 2 *M* sodium hydroxide solution and mixed with 0.6 ml of *n*-hexane—isoamyl alcohol. After phase separation, the organic phase was transferred to a small tube made from a Pasteur pipette and evaporated to dryness using a KOH-containing vacuum desiccator. The residue was redissolved in 10 μl of *n*-heptane—toluene—isoamyl alcohol—diethylamine (80:20:1.5:1), and 1 μl was injected into the gas chromatograph.

Chromatography

The gas chromatograph used was a Varian Model 2100 with a flame-ionization detector. The glass column, 1.80 m × 2 mm I.D., was silanized and packed with 1.4% Carbowax 20M plus 1.4% KOH on Gas-Chrom Q (60—80 mesh). The column temperature was 210°C. The temperatures in the injector and detector were 250°C. The flow-rate for the carrier gas (nitrogen) was set at 30 ml/min; the hydrogen flow-rate was 30 ml/min; the air flow-rate was 300 ml/min.

The peak areas of the drugs were recorded using a Hewlett-Packard integrator 3380S, which also printed out retention times.

Quantitation

Standard samples containing known amounts of the substances to be determined were included in each determination series. The concentrations in the unknown samples were calculated by comparing peak areas to those of the internal standards. All standards and samples were run in duplicate.

Blood samples

Blood samples were obtained from patients undergoing chronic maprotiline therapy. The samples were drawn between 7 and 8 a.m., and maprotiline (75 mg) was given between 7 and 8 p.m. The sera were stored at +4°C until analyzed. The analyses were performed within 24 h.

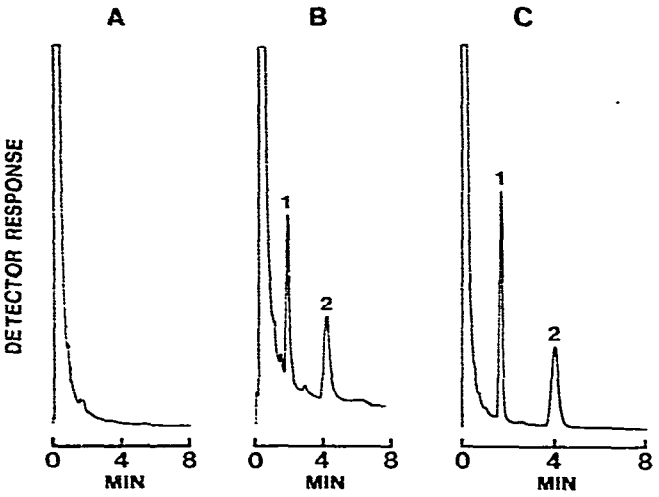


Fig. 2. (A) Chromatogram obtained from an extract of 4 ml of blank serum. (B) Chromatogram obtained from an extract of 4 ml of human serum containing amitriptyline (1) as internal standard and maprotiline (2). (C) The same as (B), but extraction made from water. Amitriptyline and maprotiline peaks correspond to 750 n*M* and 500 n*M*, respectively.

RESULTS

In Fig. 2, chromatograms obtained from an extract of 4 ml of blank human serum (A) are shown, as well as extracts from serum (B) and water (C), spiked with internal standard (amitriptyline) and maprotiline 500 n*M*.

There was a linear correlation between maprotiline serum concentrations and the area ratio between the drug and internal standard over the range measured, 100—1500 n*M*. The calibration curve for maprotiline had a slope of 0.714, an ordinate intercept of —0.048, and a correlation coefficient of 0.999. The lower limit of sensitivity of the assay was 40 n*M* of serum.

Within-run precision (Table I) was determined on eight replicate extractions of 100, 500 and 1000 n*M* maprotiline in serum. The precision of determination [expressed as coefficient of variation (C.V.) %] was between 3.5 and 6.4. In the between-run precision studies, C.V. % were 5.8 and 4.8, respectively, in the pools 500 n*M* and 1000 n*M*.

In the accuracy studies, the recovery of maprotiline was determined. The absolute recovery of maprotiline from serum was measured in the following way. Known amounts of maprotiline were added to blank serum samples, and the extraction procedure was performed without internal standard, which was not added until just before the last evaporation. As shown in Table II, absolute analytical recoveries of the drug ranged from 55 to 71%. Table II shows that the relative recoveries at therapeutic concentrations varied between 97 and 109%. These results confirm that amitriptyline is a suitable internal standard for maprotiline determination in this assay system.

The mean morning serum concentration of five inpatients receiving maprotiline 75 mg on the previous evening was 107 μg/l (385 n*M*). There were inter-individual variations from 75 to 158 μg/l (272—570 n*M*) (Table III).

In Table IV and Fig. 3 we have shown that the method described can be used

TABLE I

PRECISION OF ASSAY OF MAPROTILINE IN HUMAN SERUM

	100 n*M*	500 n*M*	1000 n*M*
Within-run			
n	8	8	8
$\bar{x}$	109	484	1007
S.D.	3.8	22.9	64.0
C.V. (%)	3.5	4.7	6.4
Between-run		Pool A	Pool B
n		6	6
$\bar{x}$		480	986
S.D.		28.1	47.6
C.V. (%)		5.8	4.8

TABLE II

ACCURACY OF THE ASSAY OF MAPROTILINE

n	Amount added (n*M*)	Relative recovery (%)	Absolute recovery (%)
8	100	109	55
8	500	97	70
8	1000	101	71

TABLE III

PLASMA LEVELS OF MAPROTILINE IN FIVE PATIENTS

Patient			Concentration		Maprotiline dose (mg)	Other drugs	Dose
No.	Sex	Age	n*M*	μg/l			
1	F	28	337	93	75	Thioridazine hydrochloride	2× 25 mg + 1× 50 mg
2	M	61	570	158	75	Thioridazine hydrochloride	3× 100 mg
3	F	71	389	108	75	—	
4	F	53	356	99	75	Thioridazine hydrochloride	1× 100 mg
5	M	63	272	75	75	Melperone hydrochloride	2× 25 mg + 1× 100 mg
						Oxazepam	2× 15 mg
						Lactulose	1× 15 mg

TABLE IV

RELATIVE RETENTION TIMES OF SOME ANTIDEPRESSANTS

Drug	Relative to cyproheptadine	Relative to amitriptyline
Trimipramine	0.45	0.99
Amitriptyline	0.46	1.00
Imipramine	0.54	1.22
Mianserine	0.68	1.52
Nortriptyline	0.69	1.53
Desipramine	0.81	1.83
Protriptyline	0.92	2.06
Maprotiline	1.07	2.20
Clomipramine	1.13	2.52

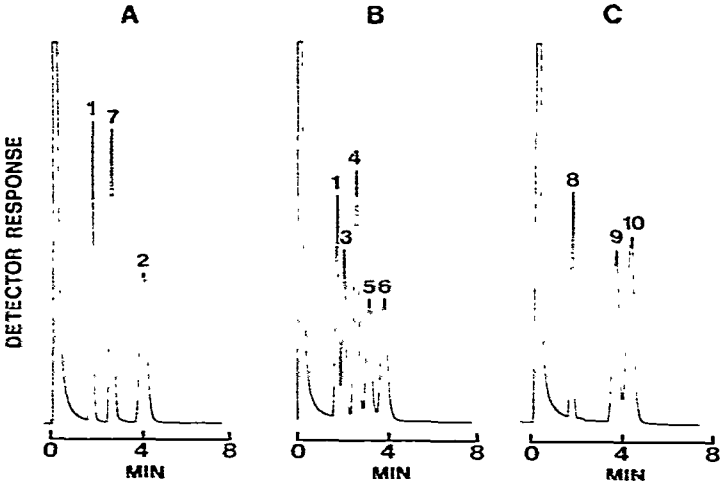


Fig. 3. (A) Chromatogram obtained from the extract of amitriptyline (1), mianserine (7), and maprotiline (2) in aqueous solution. (B) Chromatogram from aqueous extract of amitriptyline (1), imipramine (3), nortriptyline (4), desipramine (5) and cyproheptadine (6). (C) Chromatogram from an extract of trimipramine (8), protriptyline (9) and clomipramine (10) in aqueous solution.

for identification of the main antidepressants. Well-resolved symmetrical peaks were obtained. In the columns packed with supports with Carbowax + KOH as liquid phase it is typical that even after the stabilization of the column the retention times will eventually become shorter. Instead of this the relative retention times remained stabile. Because cyproheptadine is structurally related to tricyclic antidepressants (Fig. 1) and is a compound rarely administered to psychiatric patients, we selected it for the internal standard of the assay.

DISCUSSION

There are two reasons why we consider the determination of maprotiline in the serum of patients to be valuable. (1) There is a growing awareness among

clinical pharmacologists and clinical chemists that the serum levels of antidepressant medication may be important in controlling clinical efficacy, monitoring compliance and preventing side-effects [14]. (2) The potential risk of accidental and suicidal maprotiline overdoses seems to be similar to that of tricyclic antidepressants [10].

In the procedure presented, accuracy and precision values are sufficient for most clinical purposes and are comparable to previously reported values, using gas–liquid chromatography, of tricyclic antidepressants [11]. In the determination of standard curves, extractions made from serum and water gave an identical peak area ratio relationship. We therefore used aqueous standards in day-to-day precision studies.

The relative retention time between amitriptyline as an internal standard and maprotiline was 2.2. Even 40 n*M* maprotiline could be detected. Thus the method has a sensitivity required for the determination of the serum levels of maprotiline in man, both in therapeutic and in toxic concentrations.

Concentrations measured in the morning from the serum of inpatients after chronic medication of maprotiline given at bedtime agree well with those obtained by Riess using a double radio-isotope derivative technique [13]. In his study, the mean steady-state concentrations for daily dose levels of 50, 100 and 150 mg were 67, 143 and 216 μg/l, respectively. Twofold inter-individual variations in the steady-state serum levels of maprotiline found in the present study are smaller than those described earlier [3] for other antidepressants, but the small number of patients in our material might explain this discrepancy.

The main advantage of our method is the possibility for simultaneous identification and quantitation of the major antidepressants. As can be seen from Table IV, all the commonly prescribed antidepressants can be eluted in the system described. On the other hand, mianserine and nortriptyline, as well as trimipramine and amitriptyline, had the same retention times. Probably not in treatment, but theoretically, these compounds could be combined in an overdose situation. Drugs used by our patients (thioridazine, melperone, oxazepam) did not interfere with maprotiline.

There are some important aspects of the technique which should be taken into consideration: to eliminate interfering peaks in chromatograms glassware has to be thoroughly cleaned, solvents have to be very pure, and plastic sealings have to be tested before analyses are carried out.

To summarize — due to the increasing therapeutic value of maprotiline as an antidepressant, the measurement of its serum concentrations has been regarded as important, and a new, sensitive, specific method for this purpose has been described.

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