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QUANTITATIVE ASSAY OF MAPROTILINE IN BIOLOGICAL FLUIDS BY GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

A specific and sensitive gas–liquid chromatographic method has been developed for the assay of maprotiline in biological fluids. Maprotiline is isolated from the biological sample by base-specific extraction followed by conversion into the heptafluorobutyramide. The derivative is determined quantitatively by gas–liquid chromatography with an electron capture detector, nortriptyline being used as the internal standard. Amounts below 10 ng per biological sample can be measured.

INTRODUCTION

Maprotiline {Ludiomil®; 1-(3-methylaminopropyl)dibenzo[*b*,*e*]bicyclo[2,2,2]-octadiene; CIBA 34,276-Ba} is an antidepressive agent that has been used therapeutically for several years[1]. The method described in this paper is based on the gas–liquid chromatographic (GLC) assay of nortriptyline, using maprotiline as the internal standard, developed by Borgå and Garle[2], and on the double radioisotope derivative (DRID) assay of maprotiline described by Riess[3].

In order to permit the quantitative assay of maprotiline in biological fluids such as whole blood, plasma or urine by laboratories that are not equipped with the instrumentation necessary for the DRID method, a sensitive and simple analytical method had to be developed. The method presented here permits concentrations of maprotiline in blood, plasma and urine to be measured down to the low nanograms per millilitre level, and is thus suitable for pharmacokinetic studies in man.

DEVELOPMENT OF THE METHOD

Derivative formation

Maprotiline and nortriptyline, which serves as the internal standard, can easily be converted into their heptafluorobutyramides[2]. In contrast to the trifluoroacetamides, which have also been prepared, these derivatives yield electron capture detector (ECD) signals that are sufficiently sensitive for quantitative assay. The molar detector

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response of heptafluorobutyric (HFB)-maprotiline is about 70% of that of HFB-nortriptyline.

A suitable method of preparing the HFB derivatives consists in heating the free bases of maprotiline and nortriptyline in acetonitrile–*n*-heptane–heptafluorobutyric anhydride (1:93:6) at 70–75° for 90 min. The kinetics of this reaction are illustrated in Fig. 1.

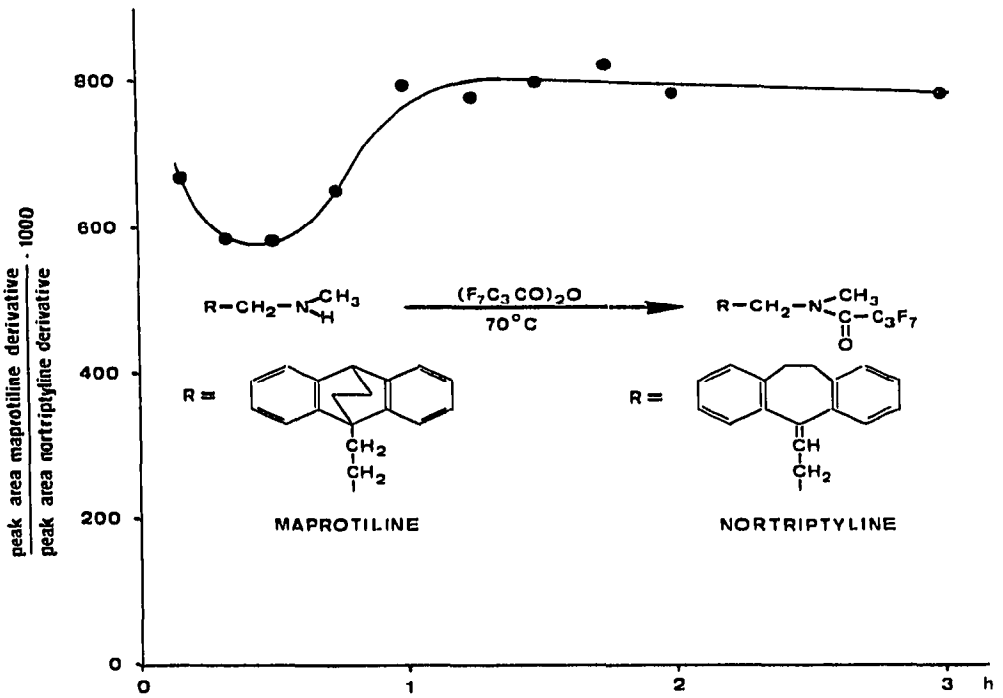

Fig. 1. Kinetics of the formation of the HFB-amides of 100 ng of maprotiline and nortriptyline in 100 µl of acetonitrile–*n*-heptane–heptafluorobutyric anhydride (1:93:6) at 70°, shown as the peak area ratio of the HFB-derivatives of maprotiline and nortriptyline.

Extraction from biological material

For the extraction of maprotiline from water, urine, plasma or blood, *n*-heptane–isopropanol (99:1) was found to be suitable[3]. This solvent shows little tendency to form emulsions with the biological material. As can be seen in the partition curves in Fig. 2, the pH of the aqueous phase must be greater than 9 in order to extract the amines in high yield. To avoid the formation of emulsions with blood or plasma, a concentrated borate buffer of pH 10 was chosen[3]. This buffer must be replaced with sodium hydroxide solution when urine is to be extracted.

The amines can be re-extracted from the solvent with 0.1 *N* sulphuric acid. In the extraction steps, when the amines are to be extracted from an alkaline aqueous phase into a solvent, certain precautions must be taken in order to prevent uncontrollable losses. The polycyclic amines have a tendency to adhere to acidic glass surfaces. If the tubes are wetted with aqueous alkali prior to the transfer of the amine-contain-

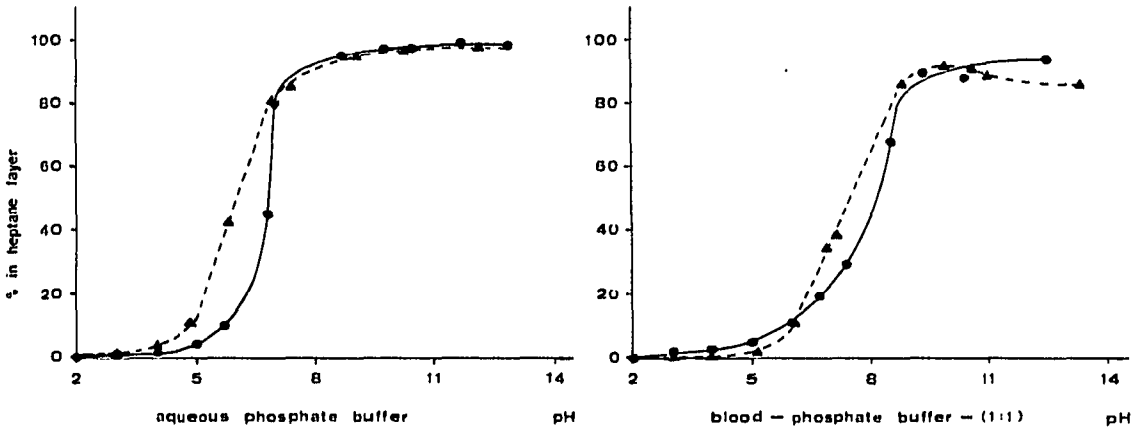

Fig. 2. Partition of 500 ng of [^{3}H]maprotiline (●) and [^{14}C]nortriptyline (▲) (internal standard) between 10 ml of *n*-heptane and 6 ml of aqueous phase, with or without blood, after mechanical shaking at 150 rpm for 30 min. The pH values were measured after equilibration and the yields were determined by liquid scintillation counting[3].

ing aqueous solution, these losses can be prevented. Further, in the alkaline extraction steps, it is advisable to use tubes with a capacity of at least twice the volume of liquid to be shaken.

Internal standard

In order to correct the variable overall yields of the extraction and derivation processes, it is necessary to add an internal standard to the original sample prior to extraction. Ideally, this compound should fulfil the following requirements:

(1) its partition behaviour at the pH values chosen should be the same as, or at least proportional to, that of maprotiline;

(2) the proportion of the derivatives of maprotiline and of the internal standard formed upon reaction with heptafluorobutyric anhydride should reach and remain at a steady state for a reasonable period of time (*e.g.*, 1 h);

(3) the derivatives of maprotiline and of the internal standard must be separated completely on the gas chromatogram;

(4) the specific detector responses of the two derivatives should be close to each other.

A compound that largely meets these requirements is nortriptyline. The physical and chemical behaviour of the internal standard is shown in Figs. 1 and 2.

EXPERIMENTAL

Reagents

All of the chemicals used were of analytical-reagent grade and were specially tested for purity in blank runs.

Extraction and derivative formation

Step 1. A 20-ml ground-glass stoppered centrifuge tube is wetted with 1 ml of

borate buffer of pH 10 (24.74 g of orthoboric acid, 29.82 g of potassium chloride, 14.05 g of sodium hydroxide plus water to 1000 ml) or, if the biological fluid is urine, with 1 ml of 1 *N* sodium hydroxide solution. A 1-ml volume of the biological fluid, diluted if necessary, containing not more than 200 ng of maprotiline plus 0.4 ml of a 250 mg/ml solution of nortriptyline in water (amount of nortriptyline added = 100 ng) plus 7 ml of *n*-heptane–isopropanol (99:1), is added and shaken for 30 min at 150 rpm, then centrifuged.

Step 2. A maximum aliquot of the solvent phase from step 1 is transferred by pipette into a 10-ml ground-glass stoppered centrifuge tube, 1.3 ml of 0.1 *N* sulphuric acid are added and the mixture is shaken for 15 min at 150 rpm, then centrifuged if necessary. The solvent layer is sucked off completely and discarded.

Step 3. A 20-ml ground-glass stoppered centrifuge tube is wetted with 0.5 ml of 1 *N* sodium hydroxide solution. A maximum aliquot of the sulphuric acid extract from step 2 is transferred carefully by pipette into the sodium hydroxide solution, 5.5 ml of *n*-hexane are added and the mixture is shaken for 30 minutes at 150 rpm.

Step 4. A maximum of the *n*-hexane extract from step 3 is evaporated in a gentle stream of nitrogen at room temperature, not to dryness, because the free amines are volatile, but until a volume of about 50 μl remains. To the concentrated *n*-hexane extract 100 μl of a freshly prepared mixture of acetonitrile, *n*-heptane and heptafluorobutyric anhydride (1:93:6) are added. The reaction tube is well stoppered and immersed in a water-bath at 70–75° for 90 min. After cooling, the reaction mixture is evaporated in a gentle stream of nitrogen at room temperature until dry and odourless. The residue is dissolved in 1.3 ml of *n*-hexane and shaken with 0.5 ml of 1 *N* sodium hydroxide solution on a high-speed mixer for 20 sec. A maximum aliquot of the *n*-hexane phase is evaporated to dryness in a gentle stream of nitrogen at room temperature. The residue is dissolved in 200 μl of *n*-hexane and 3 μl of the resulting solution are injected into the gas chromatograph.

Gas–liquid chromatography

GLC analysis was carried out on a Perkin-Elmer Model 900 A gas chromatograph equipped with a non-linear ^{63}Ni ECD, which was operated at a pulse rate of 20 kHz and a temperature of 300°. The column used was 4 ft. × 3 mm I.D., filled with 3% JXR (methylsilicone) on Gas-Chrom Q. The carrier gas was nitrogen at a flow-rate of 45 ml/min. Before it entered the detector, nitrogen at the rate of 40 ml/min was added to the effluent gas. The temperatures were 250° at the injection port, 230° in the column oven and 280° in the manifold. The peak areas were integrated with an electronic integrator (Infotronics CRS 208 E) equipped with an angular baseline corrector.

Three typical gas chromatograms obtained with extracts from human whole blood are shown in Fig. 3.

QUANTITATIVE EVALUATION OF GAS CHROMATOGRAMS

As a non-linear ECD was used and the relative extractability of maprotiline differed from one biological fluid to another, several precautions had to be taken in order to overcome the factors that rendered quantitative analysis difficult.

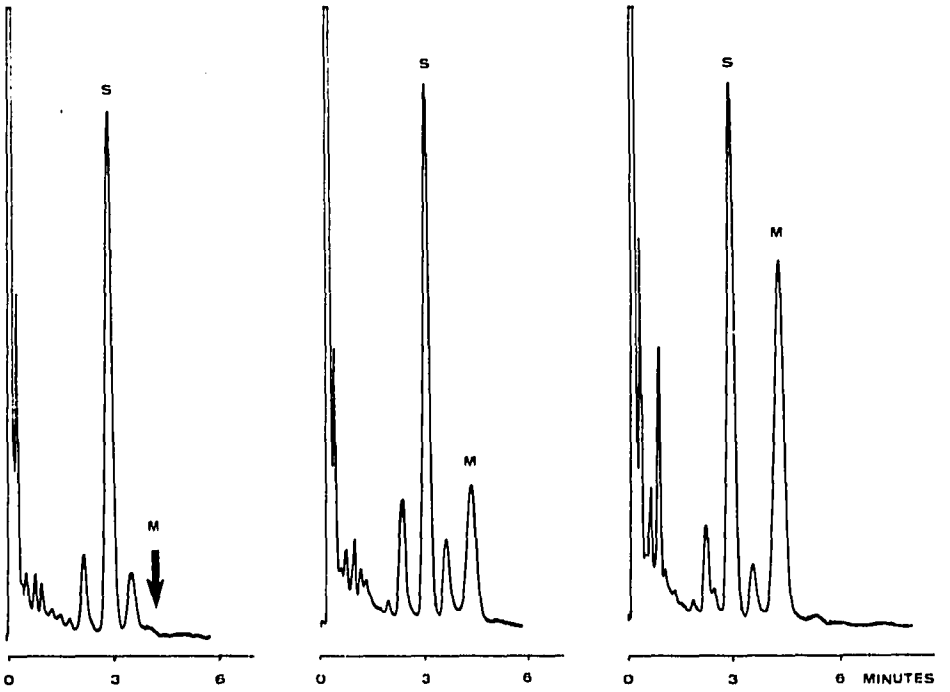

Fig. 3. Gas chromatograms obtained with extracts from 0.5–1 ml of whole blood samples containing 0, 50 or 200 ng of maprotiline and 100 ng of nortriptyline (internal standard). The amounts injected were 0.375 and 1.5 ng of derivatized maprotiline (M) and 0.75 ng of derivatized nortriptyline (S).

Calibration graph for extraction and derivative formation

When a constant amount of internal standard (100 ng of nortriptyline) is used, the ratio of the amounts of the derivatives from maprotiline and from nortriptyline is a function not only of the amount of maprotiline originally present in the analysis, but also of the type of biological fluid in which it is dissolved. It therefore appeared advisable to add, in each series of analyses, a number of samples of the same type of biological fluid containing known amounts of maprotiline. Thus an overall calibration graph was obtained that could be used to evaluate the analyses of unknown concentrations. Typical calibration graphs obtained from different biological fluids are shown in Fig. 4.

In order to make allowance for the non-linearity of the ECD, it is necessary to choose the volume to be injected into the gas chromatograph so that the area of the internal standard peak is kept within certain limits (*e.g.*, $\pm$ 20%).

Reproducibility of the method

The results of test analyses on water, urine, plasma and human whole blood samples containing known concentrations of maprotiline ranging from 12.5 to 200 ng/ml are shown in Table I.

In addition, clinical samples of human whole blood were analyzed by means of the GLC method and also by means of the DRID method described by Riess[3]. The results obtained with the two methods were virtually identical (Table II).

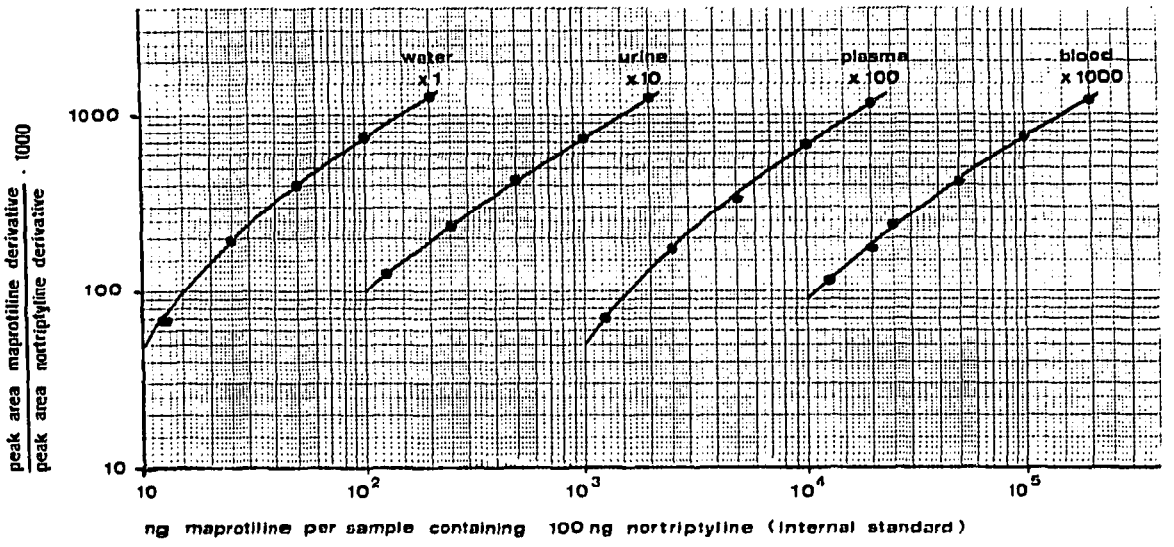

Fig. 4. Calibration graphs for four different starting fluids containing 12.5–200 ng of maprotiline and 100 ng of nortriptyline (internal standard). The graph obtained from water is drawn on the original scale. For ease of legibility, the other graphs are shifted on the abscissa by a factor of 10–1000, as indicated.

TABLE I

TEST ANALYSES TO ASSESS THE REPRODUCIBILITY OF THE METHOD

Four independent analyses per concentration and per starting fluid, which was 1 ml per sample. Results are concentrations of maprotiline found (ng/ml) in the different samples.

Sample	*Concentration of maprotiline present (ng/ml)*					
	0	*12.5*	*25*	*50*	*100*	*200*
Water	0.0	12.6	26.2	50	104	210
	−0.9	13.0	26.0	50	100	209
	−0.7	11.9	25.0	53	97	208
	1.5	12.2	24.8	47	96	190
Mean ± S.D.	0.0 ± 1.1	12.4 ± 0.5	25.5 ± 0.7	50.0 ± 2.4	99.3 ± 3.6	204.3 ± 9.5
Urine	1.1	12.5	25.1	49	101	197
	0.0	11.3	24.5	49	97	201
	−0.6	14.6	26.0	51	101	206
	−0.5	11.6	25.1	51	103	200
Mean ± S.D.	0.0 ± 0.8	12.5 ± 1.5	25.2 ± 0.6	50.0 ± 1.2	100.5 ± 2.5	201.0 ± 3.7
Plasma	0.4	12.7	25.0	51	105	212
	−1.6	12.5	23.8	50	99	209
	−0.2	11.9	27.5	48	96	197
	1.2	10.0	25.4	49	104	195
Mean ± S.D.	−0.1 ± 1.2	11.8 ± 1.2	25.4 ± 1.5	49.5 ± 1.3	101.0 ± 4.2	203.3 ± 8.5
Whole blood	−0.2	12.4	23.9	46	93	195
	0.2	12.5	25.0	48	96	200
	−0.2	12.1	25.5	46	106	202
	−0.4	11.6	24.0	51	100	195
Mean ± S.D.	−0.15 ± 0.25	12.2 ± 0.4	24.6 ± 0.8	47.8 ± 2.4	98.8 ± 5.6	198.0 ± 1.8

TABLE II

ANALYSES OF HUMAN WHOLE BLOOD OBTAINED FROM A CLINICAL STUDY

The GLC analysis was carried out with 0.5–1 ml of blood. The DRID analysis was carried out with 3 ml of blood.

Sample No.	*Amount of maprotiline found in blood (ng/ml)*	
	GC method	*DRID method*[3]
1	0	<5
2	322	321
3	225	243
4	279	291
5	0	<5
6	183	190
7	239	256
8	185	190
9	87	86
10	210	204
11	327	326
12	433	435

ACKNOWLEDGEMENTS

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