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Note

Simultaneous determination of trimipramine and its demethylated metabolites in plasma by gas chromatography–mass spectrometry

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Trimipramine, (dimethylamino-3-methyl-2-propyl)dihydro-10,11-[5*H*]-dibenz[*b*,*f*]azepine (Surmontil®, Specia, Paris, France), is a tricyclic antidepressant used in the treatment of depression occurring with anxiety and neurosis. It is characterized by a central anticholinergic activity leading to sedative properties. In clinical investigations, it exhibits an antidepressant activity similar to those of amitriptyline and imipramine [1,2].

In order to study the bioavailability of trimipramine, a highly sensitive analytical method is required because of the very low plasma levels following therapeutic doses. Methods have been described that use gas chromatography [3–5] and liquid chromatography [6,7], but the specificity does not seem sufficient and sensitive enough to assay both trimipramine and its two major demethylated metabolites (Fig. 1).

This paper describes a selective and sensitive method involving derivatization followed by gas chromatography–mass spectrometry (GC–MS). This assay was developed to allow a further study of the pharmacokinetics of trimipramine and its metabolites in human volunteers.

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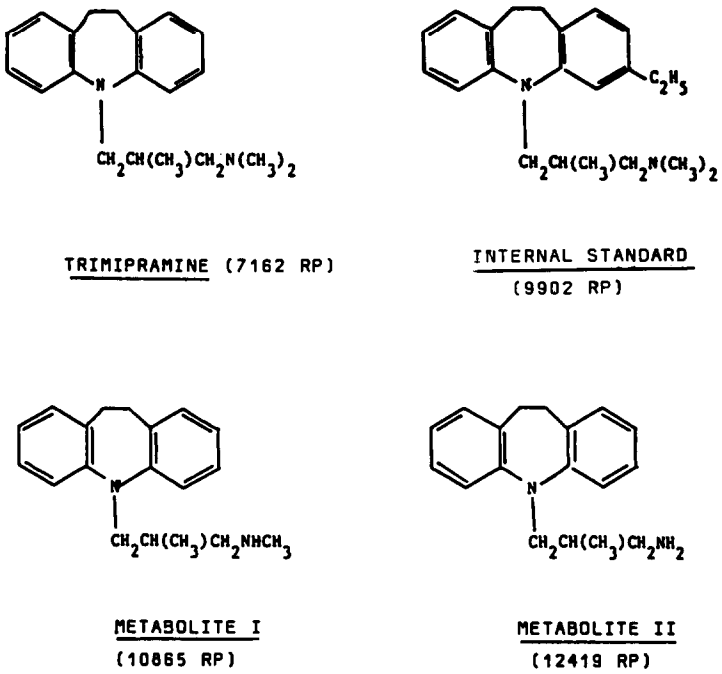

Fig. 1. Chemical structures of trimipramine, metabolite I, metabolite II and internal standard.

EXPERIMENTAL

Reagents and materials

Trimipramine, its metabolites I and II and the internal standard were gifts from Specia. All reagents were of analytical-reagent grade. Heptane, methanol and ethyl acetate were solvents RS (Carlo Erba, Milan, Italy) and hexane was solvent RH (Rathburn, Walkerburn, U.K.). Trifluoroacetic anhydride was purchased from Pierce (Rockford, IL, U.S.A.).

Standard solutions of trimipramine, its metabolites and the internal standard (I.S.) were prepared in methanol. Working solutions (0.05–5 μg/ml) were freshly prepared before analysis, by dilution in methanol.

Gas chromatography–mass spectrometry

The determinations were carried out on a quadrupolar GC–MS system (Hewlett-Packard, 5970 A) in the electron impact (EI) mode. The column (25 m $\times$ 0.25 mm I.D.) used was an SE 30 fused-silica capillary (Hewlett-Packard, U.S.A.). Helium was used as carrier gas. The column was maintained at 80°C for 1 min followed by a 30°C/min programme to 270°C. The temperatures of the injection port and GC–MS interface were both 270°C.

Extraction and derivatization procedure

To a 15-ml polypropylene centrifuge tube were added 2 ml of plasma, 100 ng of I.S. (20 μl of a 5 μg/ml solution), 200 μl of a 2 *M* sodium hydroxide solution and

6 ml of isoamyl alcohol–heptane (1.5:98.5). The mixture was shaken mechanically for 20 min and centrifuged for 10 min at 1600 *g*. The organic phase was transferred to another centrifuge tube containing 2 ml of 0.05 *M* sulphuric acid. The tube was shaken for 20 min and centrifuged for 10 min. The upper organic layer was discarded, and 800 μl of 2 *M* sodium hydroxide solution were added to the acid phase. The final organic extraction was done by addition of 800 μl of isoamyl alcohol–hexane (1.5:98.5) to the tube. This mixture was shaken for 20 min and centrifuged for 10 min. The organic layer was transferred to an Eppendorf microcentrifuge tube and then evaporated to dryness in a stream of nitrogen at ambient temperature.

The residue was taken up in 40 μl of ethyl acetate and 100 μl of trifluoroacetic anhydride, and left for 10 min at 55°C. The cold solution was evaporated to dryness under nitrogen and the residue redissolved in 20 μl of methanol; 3 μl of this solution were injected into the gas chromatograph.

RESULTS AND DISCUSSION

Evaluation of the method

Precision. The repeatability was determined by analysing within-day seven identically spiked plasma samples containing 5 and 50 ng/ml trimipramine and 2.5 and 25 ng/ml metabolites I and II (Table I). The variability of the assay, as evidenced by the coefficient of variation (C.V.) for peak-area ratios, was always less than 10%. The day-to-day variation of samples analysed on seven different days over a period of two months was 2.33–6.22% (Table I).

Limit of detection. No interfering peaks from endogenous compounds were observed in the control plasma with the same retention time as trimipramine, its metabolites or the internal standard. The limit of detection under the experimental conditions used was 1 ng/ml when a 2-ml plasma sample was analysed.

TABLE I

INTRA- AND INTER-DAY VARIATIONS IN THE MEASUREMENT OF TRIMIPRAMINE AND ITS MONODEMETHYLATED METABOLITE

Compound	Amount added (ng/ml)	Amount recovered (mean ± S.D.) (ng/ml)	C.V. (%)
Intra-day variation (n = 7)			
Trimipramine	5	4.90 ± 0.18	9.80
	50	52.10 ± 1.23	6.26
Metabolite I	2.5	2.60 ± 0.09	9.72
	25	24.90 ± 0.60	6.37
Inter-day variation (n = 7)			
Trimipramine	5	4.96 ± 0.12	6.22
	50	50.40 ± 0.45	2.33
Metabolite I	2.5	2.42 ± 0.06	5.98
	25	25.30 ± 0.54	5.69

TABLE II

REPRODUCIBILITY AT THE DETECTION LIMIT FOR TRIMIPRAMINE AND ITS DEMETHYLATED METABOLITES

In each case the amount added was 1 ng/ml.

	Amounts found (ng/ml)		
	Trimipramine	Metabolite I	Metabolite II
	0.99	0.98	1.15
	1.13	1.00	1.04
	0.89	0.98	0.99
	1.14	0.97	0.85
	1.12	1.07	1.03
	1.16	0.89	0.98
	1.02	1.15	1.12
Mean ± S.D.	1.06 ± 0.04	1.01 ± 0.032	1.02 ± 0.037
C.V.(%)	9.10	8.20	9.67

Repeated determinations of seven plasma samples at this concentration yielded C.V. values of 9.10% for trimipramine, 8.20% for metabolite I and 9.67% for metabolite II (Table II).

Linearity. Typical plots of the peak-area ratio of drug to I.S. versus concentrations for the plasma standards were linear over the range 0–100 ng/ml trimipramine and metabolites I and II in plasma: $y=0.0441x+0.0181$, $r=0.9975$ for trimipramine; $y=0.0999x+0.1018$, $r=0.9969$ for metabolite I; $y=0.1021x-0.095$, $r=0.9942$ for metabolite II where y is the peak-area ratio of substance to I.S., x is the concentration of substance and r is the correlation coefficient.

The stability of trimipramine and its two metabolites in plasma stored at $-20°C$ was evaluated by analysing pooled plasma samples against fresh standards. There was no loss in potency after several months of storage.

Multiple-ion monitoring and specificity. Mass spectra of trimipramine, the I.S. and the trifluoroacetyl derivatives of the two metabolites are shown in Fig. 2. Under the operating conditions described above, selected-ion monitoring of the plasma extracts was recorded. Ions at m/z 249 for trimipramine, at m/z 208 for metabolites I and II and at m/z 277 for the I.S. were chosen.

The mass fragmentogram in Fig. 3 represents the plasma extract of a subject receiving orally 100 mg of trimipramine and shows that a good separation of the compounds was obtained. The retention times are 9.62, 10.38, 10.62 and 11.40 min for trimipramine, I.S., metabolite II and metabolite I, respectively.

Application to human volunteer samples

The method was applied to human plasma from healthy subjects in order to establish the pharmacokinetics of trimipramine and its metabolites, the relative bioavailability of two formulations and the influence of the time of administration on the pharmacokinetic parameters.

A pilot study was conducted with a volunteer. Blood samples were collected for

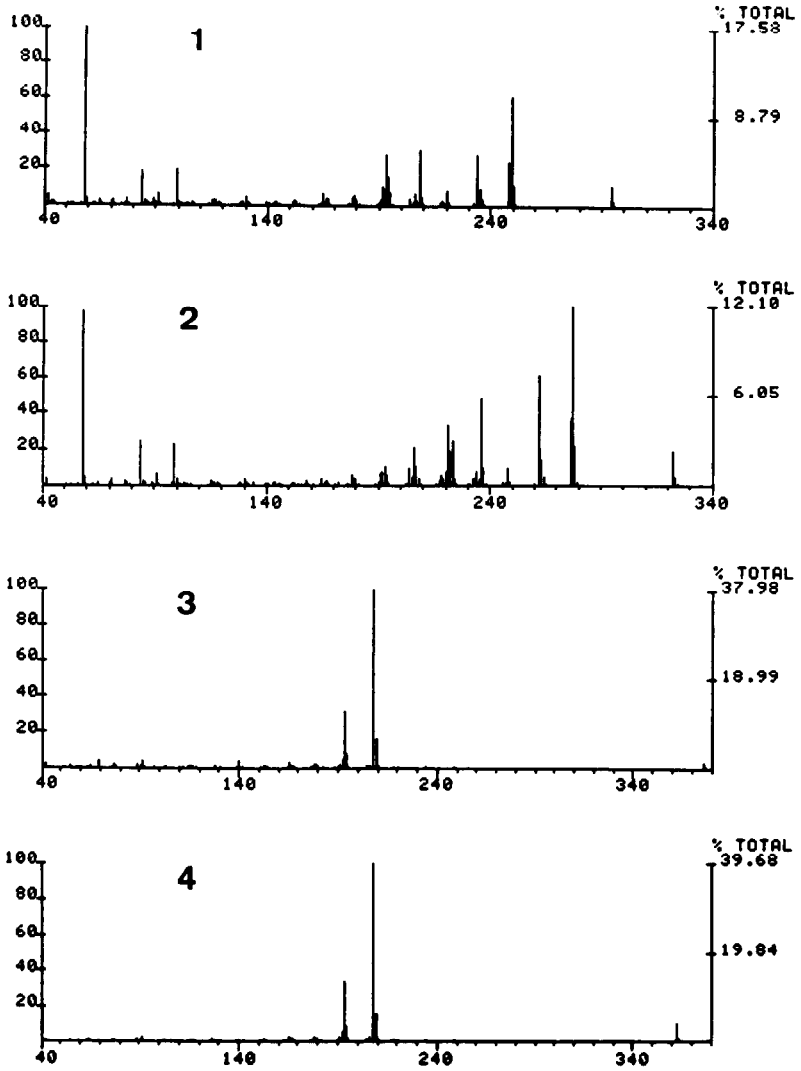

Fig. 2. EI mass spectra of trimipramine (1), internal standard (2) and N-trifluoroacetyl derivatives of metabolites I (3) and II (4).

24 h after oral administration. Each sample was immediately centrifuged, and the plasma was removed and analysed as described above. Results of the analysis were plotted as concentration–time curves (Fig. 4).

The four curves illustrate the kinetics after administration of 100 mg of trimipramine: day 1, morning administration of oral solution (formulation A); day 8, evening administration of oral solution (formulation B); day 15, morning administration of tablet (formulation C); day 22, evening administration of tablet (formulation D).

The method is applicable to the determination of unchanged drug in plasma and to simultaneous measurement of the two metabolites (the concentration of

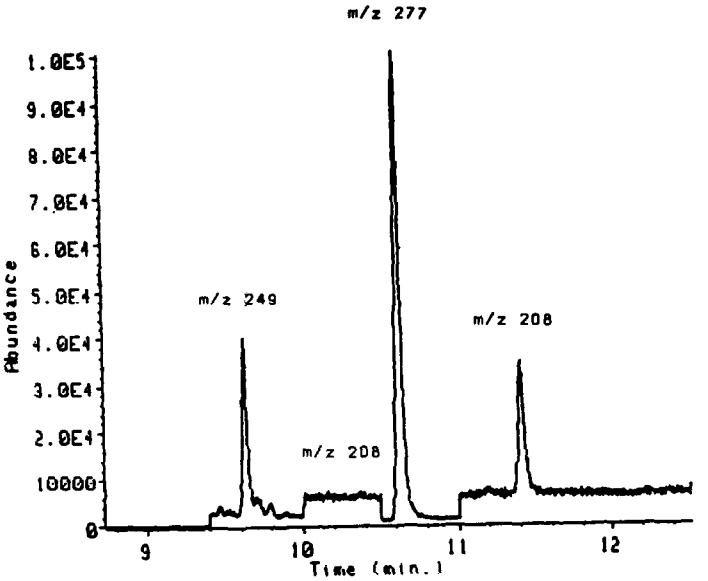

Fig. 3. Mass fragmentogram of a plasma extract.

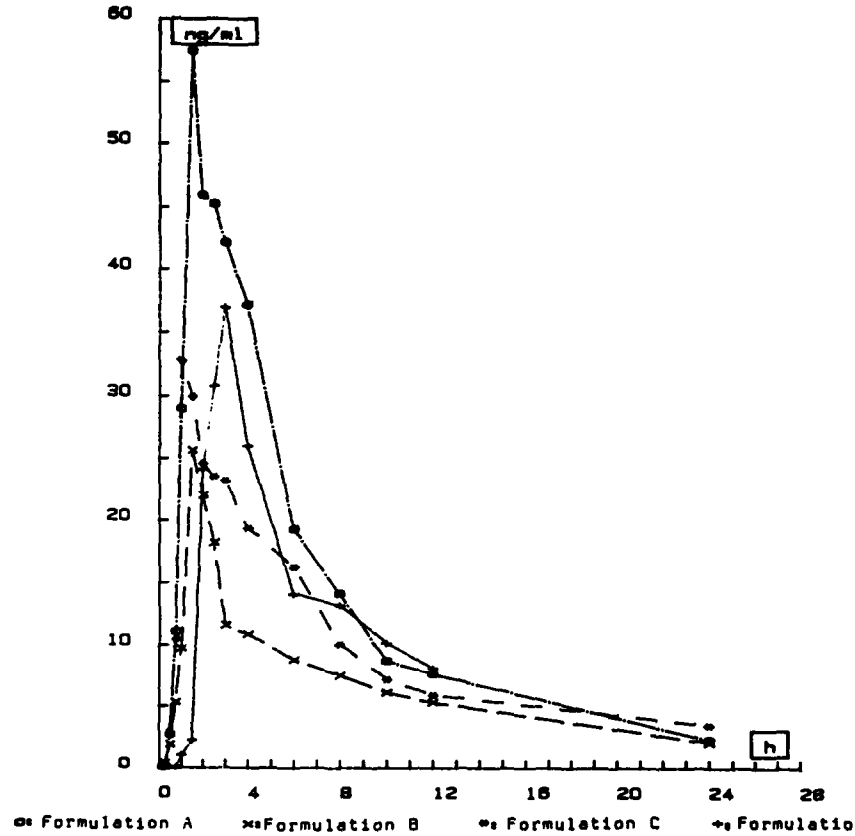

Fig. 4. Plasma levels of trimipramine after a single oral 100-mg dose: (○) Formulation A, oral solution (day); (×) formulation B, oral solution (night); (*) formulation C, tablet (day); (+) formulation D, tablet (night).

metabolite I was not high enough after a single dose for formal kinetic analysis and the level of metabolite II was lower than 1 ng/ml, the limit of detection of the method). A previous similar study involving the simultaneous assay of another antidepressant drug, metapramine, and its major demethylated metabolites [8] has shown that the monodemethylated levels were higher and reached 41% of unchanged drug.

Fig. 4 shows that formulation A was more rapidly absorbed than the other formulations (C_{max} = 57.4 ng/ml; T_{max} = 1 h). The peak plasma level of trimipramine (37 ng/ml) was only reached 3 h after administration of a tablet in the evening (formulation D). There was a large difference in absorption between the morning and evening oral solution administrations ($AUC_{0\rightarrow\infty}$ = 343.9 h ng/ml and 180.6 h ng/ml, respectively). The difference was not so marked for the tablet formulation ($AUC_{0\rightarrow\infty}$ = 302.2 h ng/ml and 264.5 h ng/ml).

CONCLUSION

This method is faster than previously published techniques because only one analytical run is needed for all the compounds of interest, the unchanged drug and the two demethylated metabolites. It is sufficiently reliable and selective to allow further studies, first in human volunteers and then in patients in order to establish correlations between dosage levels and effects.

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