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SIMULTANEOUS DETERMINATION OF METAPRAMINE AND ITS DEMETHYLATED METABOLITES IN PLASMA BY GAS CHROMATOGRAPHY—MASS SPECTROMETRY

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SUMMARY

A capillary column gas chromatography—mass fragmentographic method for metapramine and its three major demethylated metabolites is described. Compounds are extracted from plasma using a double-extraction procedure and transformed into N-trifluoroacetyl derivatives. The detection is performed by monitoring specific ions for metapramine and for its metabolites with a mass detector. In spite of extensive metabolism in the liver and rapid elimination of metapramine, plasma concentrations of both metapramine and its metabolites can be simultaneously followed over 24 h after a single 150-mg oral dose, because of the sensitivity and selectivity of the method. This method has been successfully applied to the analysis of samples obtained from patients who were at steady state with metapramine and to a pharmacokinetic study in a healthy volunteer.

INTRODUCTION

Metapramine (5-methyl-10-methylamino-10,11-dihydrodibenzo(*b*,*f*)-azepine: Timaxel®, Specia, Paris, France) is a new antidepressant drug belonging to the class of tricyclic compounds. Chemically, this substance

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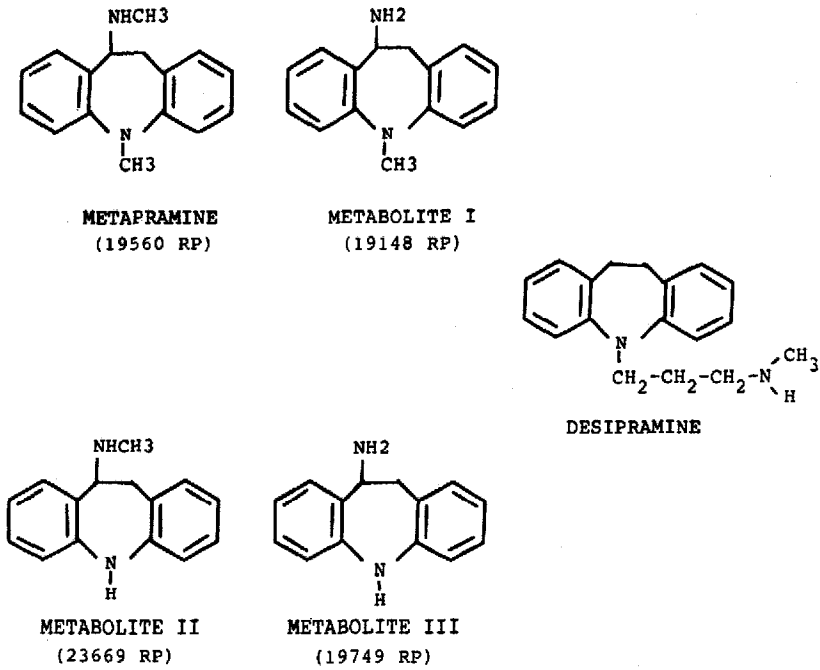

Fig. 1. Structure of metapramine, its metabolites and desipramine (internal standard).

differs from imipramine derivatives in that it contains only a methyl group on the azepine nitrogen atom and a methylamino group at the C-10 position, as shown in Fig. 1. Although its pharmacology is virtually identical with that of the tricyclic antidepressants, metapramine differs biochemically from them because of a specific action on the noradrenergic system [1]. Earlier studies have shown that metapramine has to characteristics which considerably affect pharmacokinetic studies. Its distribution in the human body is very rapid [2, 3] and it undergoes extensive metabolism in the liver [4], so that only low concentrations are found in plasma.

Several authors have described methods for the determination of metapramine in biological fluids, including gas chromatography, thin-layer chromatography and spectrofluorimetry [2, 5], but detection limits were insufficient for the pharmacokinetic investigation of metapramine following therapeutic doses.

Recently, one high-performance liquid chromatographic method was published involving dansylation and fluorescence detection [6], but this has not allowed complete separation of the two monodemethylated metabolites. Another method based on gas chromatography, with a nitrogen–phosphorus detector, was used to compare the bioavailability of three oral forms of metapramine in human volunteers [7]. However, neither of these methods was sensitive or specific enough to allow simultaneous analysis of metapramine and its major postulated metabolites presented in Fig. 1.

In this paper, we describe a selective and sensitive method involving derivatization followed by gas chromatography–mass spectrometry (GC–MS). This assay was developed to allow further study of the pharmacokinetics of metapramine and its metabolites in human volunteers and in patients with

hepatic diseases and studies of dose—effect correlation in mouse and in man.

EXPERIMENTAL

Reagents and materials

Metapramine (19560 RP), its major metabolites I (19148 RP), II (23669 RP) and III (19749 RP), and the internal standard desipramine were gifts from Specia. All reagents were of analytical-reagent grade purity. Hexane, heptane and methanol were solvents RS (Carlo Erba, Milan, Italy). Trifluoroacetic anhydride was purchased from Pierce (Rockford, IL, U.S.A.). Standard solutions of metapramine, its metabolites and desipramine were prepared in methanol. Working solutions (varying from 0.05 to 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, 5985 B) in the electron-impact (EI) mode. The column (25 m × 0.25 mm I.D.) used was an SE 30 fused-silica capillary. Samples were introduced using a Ros capillary injector [8]. Helium was used as the carrier gas. The column was maintained at 120°C for 1 min followed by a 30°C/min programme to 225°C. The temperatures of the injection port, GC—MS interface and ion source were 280, 250 and 200°C, respectively.

Extraction and derivatization procedure

To a 20-ml glass-stoppered centrifuge tube were added 2 ml of plasma, 400 ng of desipramine (internal standard), 200 μl of 2 *M* sodium hydroxide solution and 10 ml of isoamylic 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 organic layer was discarded and 200 μl of 2 *M* sodium hydroxide solution were added to the acid phase. The mixture was extracted with 800 μl of isoamylic alcohol—hexane (1.5:98.5) by shaking for 20 min and centrifuging 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 75 μl of trifluoroacetic anhydride and left for 10 min at 55°C, such conditions giving more quantitative and reproductive results. The solution was evaporated to dryness under nitrogen and the residue redissolved in 30 μl of methanol; 3 μl of this solution was injected into the chromatograph.

RESULTS AND DISCUSSION

Evaluation of the method

Linearity. Samples of plasma standards (2 ml) containing 1—10 ng/ml each of metabolites I and III, 1—50 ng/ml metabolite II and 1—100 ng/ml metapramine were analysed. The relationship between the concentration of each compound and its peak area compared to desipramine (internal standard) was found to be linear over the concentration range.

TABLE I

ACCURACY OF THE METHOD

Compound	Concentration added (ng/ml)	Concentration found (mean ± S.D., n = 7) (ng/ml)	Coefficient of variation (%)
Metapramine	1	1.16 ± 0.11	9.42
(19560 RP)	10	9.76 ± 0.69	7.15
	50	49.98 ± 1.62	3.24
	100	101.28 ± 4.05	4.00
Metabolite II	5	4.98 ± 0.41	8.22
(23669 RP)	10	9.98 ± 0.87	8.71
	50	50.71 ± 0.81	1.60
Metabolite I	5	4.76 ± 0.35	7.56
(19148 RP)	10	10.30 ± 0.35	3.42
Metabolite III	5	4.79 ± 0.38	8.05
(19749 RP)	10	10.52 ± 0.40	3.79

Limit of detection. No interfering peaks were present in the control plasma with the same retention times as metapramine, 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.

Precision. The repeatability was determined by analysing 2-ml plasma samples (n = 7) containing 5 and 10 ng/ml I and III; 5, 10 and 50 ng/ml II; 1, 10, 50 and 100 ng/ml metapramine (Table I). The measurement of compounds in plasma was chosen over this particular concentration range as a result of a preliminary assay performed in plasma samples from a patient receiving 300 mg of metapramine.

The reproducibility was determined by analysing two series of ten replicates containing 10 and 100 ng/ml metapramine. The coefficients of variation were 8.63 and 3.95% at the 10 and 100 ng/ml levels, respectively.

Multiple-ion monitoring and specificity. Mass spectra of the trifluoroacetyl derivatives of metapramine, its metabolites and desipramine were recorded. These spectra are shown in Fig. 2.

Under the operating conditions described above, selected-ion monitoring of the plasma extracts was recorded. Ions at m/z 192 and 289 for metabolites II and III, at m/z 192 and 320 for metabolite I, at m/z 192, 207 and 334 for metapramine and at m/z 208 and 362 for desipramine were chosen, respectively.

The mass fragmentogram in Fig. 3 represents the plasma extract of a patient receiving 300 mg of metapramine and shows that a good separation of the compounds was obtained. Peaks of the correct intensity for the ions of each compound were obtained, at the correct retention times. No interference was observed in the different plasma samples.

Pharmacokinetic studies

The approach to quantitation of metapramine and its major metabolites was performed on plasma samples from four patients receiving daily therapeutic

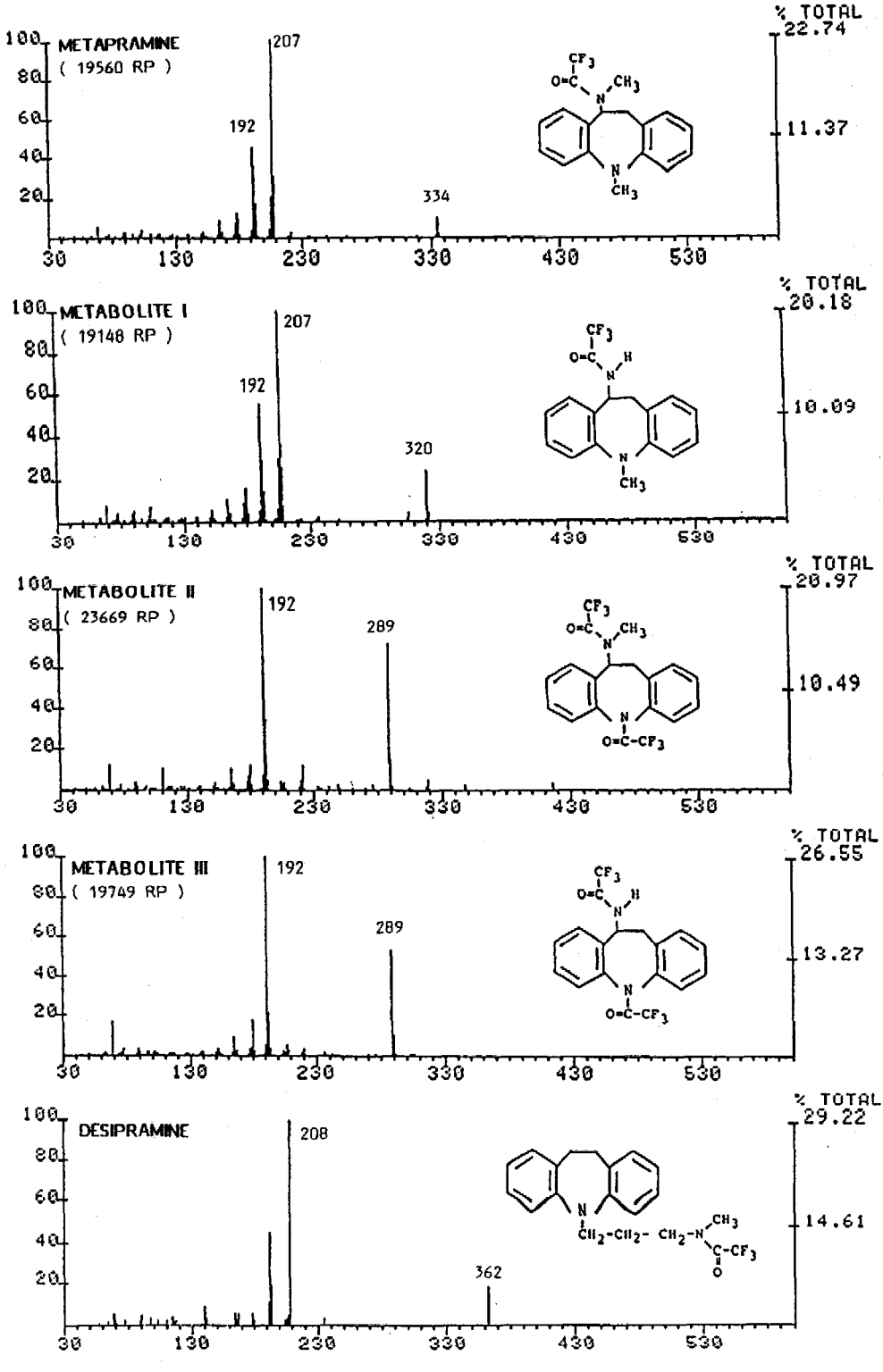

Fig. 2. Electron-impact mass spectra of N-trifluoroacetyl derivatives of metapramine, its metabolites and desipramine.

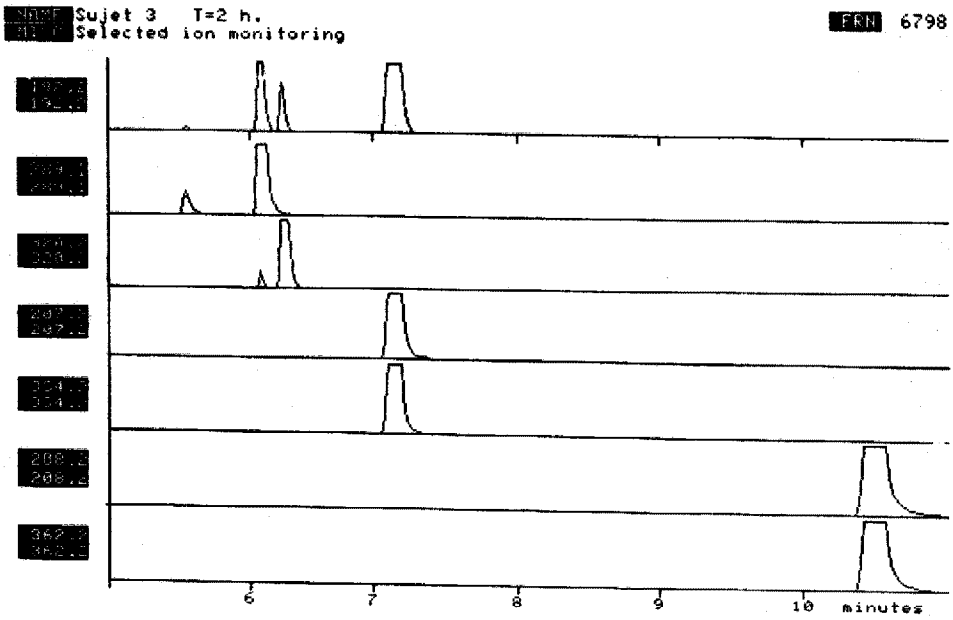

Fig. 3. Selected-ion monitoring records for plasma extract from a patient receiving 300 mg of metapramine. Retention times: 5.6 min (III), 6.1 min (II), 6.3 min (I), 7.2 min (metapramine), 10.6 min (desipramine).

doses (150—300 mg) of metapramine. Blood samples were collected at specified times after administration of the oral dose (Table II). It was possible to demonstrate the presence of metapramine and its metabolites in measurable concentrations, although there was marked inter-individual variability. The monodemethylated metabolite II seemed to be the major one in each blood sample, whatever the administered dose.

Moreover, metapramine and its metabolites were analysed by this method in plasma samples from a volunteer receiving a 150-mg oral dose. This is the daily dosage currently used in depressive illness. Plasma concentrations (fifteen

TABLE II

ASSAYS OF PATIENT SAMPLES

Time of sampling after administration (h)	Daily dose (mg)	Morning single administration (mg)	Metapramine (ng/ml)	Metabolite II (ng/ml)	Metabolite I (ng/ml)	Metabolite III (ng/ml)
Patient C.A.E.						
1.5	150	100	60.81	8.83	4.17	—
2.5			53.31	6.06	1.91	—
3.5			51.44	6.10	3.65	—
Patient V.I.A.						
1.5	200	150	232.25	25.39	19.64	1.28
2.5			228.80	31.00	27.62	3.00
3.5			151.64	28.10	19.92	4.16
Patient E.Q.U.						
2.0	300	100	318.36	63.35	40.49	1.78
Patient L.A.C.						
2.0	300	100	109.86	56.19	16.39	1.55

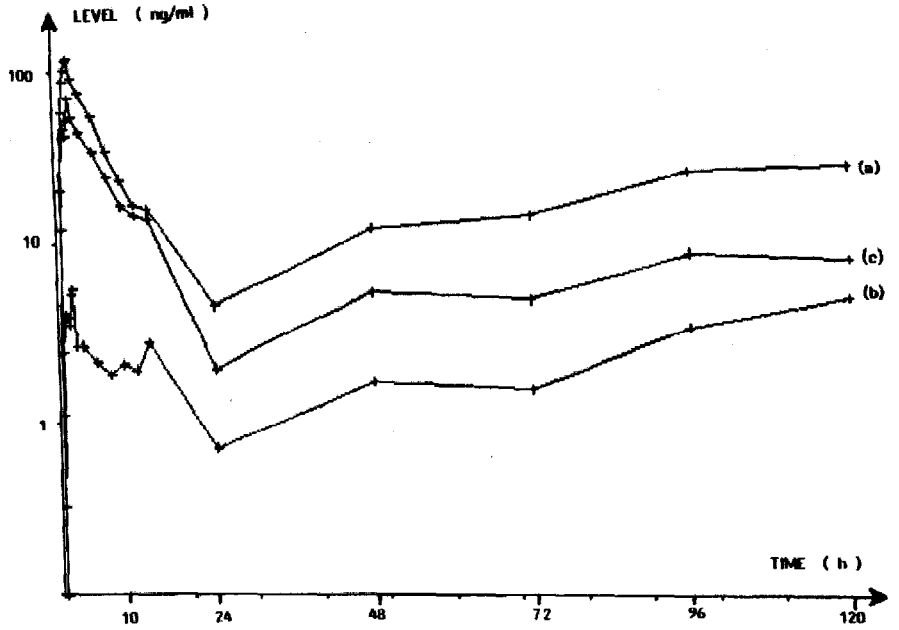

Fig. 4. Plasma levels of metapramine (a), metabolite I (b) and metabolite II (c) after a single oral 150-mg dose (day 1) and a 3 × 50 mg dose (day 2—6) of metapramine were given to a volunteer.

samples) were followed for 24 h (day 1) after the single 150-mg oral dose. Over the following five days, a 3 × 50 mg daily oral dose was administered and plasma samples were collected before the morning 50-mg dose. The blood samples were 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 peak plasma level of metapramine (123 ng/ml) was reached at 2.5 h after administration. Within the first 24 h, the time course of metapramine concentration seemed in good agreement with the reported pharmacokinetic results [6, 7].

The major metabolite was the monodemethylated metabolite II, which reached significant levels, i.e. a 70.3-ng/ml level after 2.5 h.

Plasma profiles of metapramine and of its two monodemethylated metabolites were approximately identical, but the concentrations of the latter were lower than that of metapramine. However, the didemethylated metabolite III appeared only 1 h after administration and the 1 ng/ml level was never reached over the six-day study.

After chronic administration (day 2—6), the minimum plasma levels were found for metapramine and metabolites I and II, varying from 12.05 to 26.88 ng/ml, from 1.74 to 4.94 ng/ml and from 5.36 to 8.17 ng/ml, respectively.

To conclude, this sufficiently sensitive and selective method allows further studies to gain a better understanding of the pharmacological properties of metapramine and possibly of its metabolites with dose—effect correlation studies.

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REFERENCES

1 C. Garrett, B. Scatton and L. Julou, Psychol. Med., 9 (1977) 293.
2 A. Viala, J.P. Cano, A. Durand and S. Monjanel, J. Chromatogr., 168 (1979) 195.
3 Y.C. Sumirtapura, Y. Leroux, A. Raj Komar, J.P. Cano and J. Gaillot, Eur. J. Clin. Pharmacol., 25 (1983) 673.
4 B. Decouvelaere, B. Terlain and A. Bieder, Therapie, 37 (1982) 249.
5 A. Viala, J.P. Cano, A. Durand, T. Erlenmaier and R.M. Garreau, Anal. Chem., 49 (1977) 2354.
6 J.P. Sommadossi, M. Lemar, J. Necciari, Y. Sumirtapura, J.P. Cano and J. Gaillot, J. Chromatogr., 228 (1982) 205.
7 A.M. Bougerolle, J.L. Chabard, G. Dordain, J. Gaillot, J.J. Piron and J.A. Berger, Therapie, 39 (1984) 619.
8 A. Ros, J. Gas Chromatogr., 3 (1965) 252.