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Short Communication

Determination of trimipramine and its demethylated and hydroxylated metabolites in plasma by gas chromatography–mass spectrometry.

C.B. Eap *, L. Koeb, P. Baumann

Unité de Biochimie et Psychopharmacologie Clinique, Département Universitaire de Psychiatrie Adulte (DUPA), Site de Cery, CH-1008 Prilly-Lausanne, Switzerland

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Abstract

A gas chromatographic–mass spectrometric (GC–MS) method has been developed, for the determination of trimipramine (TRI), desmethyltrimipramine (DTRI), didesmethyltrimipramine (DDTRI), 2-hydroxytrimipramine (2-OH-TRI) and 2-hydroxydesmethyltrimipramine (2-OH-DTRI). The method includes two derivatization steps with trifluoroacetic acid anhydride and N-methyl-N-(*tert.*-butyldimethyl silyl)trifluoroacetamide and the use of an SE-54 capillary silica column. The limits of quantitation were found to be 2 ng/ml for DTRI and 4 ng/ml for all other substances. Besides, methods have been optimized for the hydrolysis of the glucuronic acid conjugated metabolites. This specific detection method is useful, as polymedication is a usual practice in clinical situations, and its sensitivity allows its use for single-dose pharmacokinetic studies.

1. Introduction

Trimipramine (TRI), a tricyclic psychotropic drug, is commonly used for its antidepressive and anxiolytic activity [1] owing to its structural resemblance to both imipramine and levomepromazine. It is extensively biotransformed in the organism (see Fig. 1), and its main metabolites are desmethyltrimipramine (DTRI), didesmethyltrimipramine (DDTRI), 2-hydroxytrimipramine (2-OH-TRI) and 2-hydroxydesmethyltrimipramine (2-OH-DTRI) [2,3].

Several HPLC and GC methods have been described for the assay of trimipramine and its metabolites in blood [2–8]. However, as polymedication is a usual practice in clinical situations, a highly specific and sensitive detection method is most often needed. Until recently, published methods using gas chromatography [2] or gas chromatography–mass spectrometry (GC–MS) [9] only allowed the measurement of the concentrations of trimipramine and its demethylated metabolites. Very recently, a gas chromatographic method with nitrogen-selective detection for the determination of TRI, DTRI and 2-OH-TRI has been published [10]. Owing to the increased interest in the hydroxylated metabolites of psychotropic drugs [7,11], a GC–MS method has been developed allowing the determination of TRI, DTRI, DDTRI, 2-OH-TRI and 2-OH-DTRI.

* Corresponding author.

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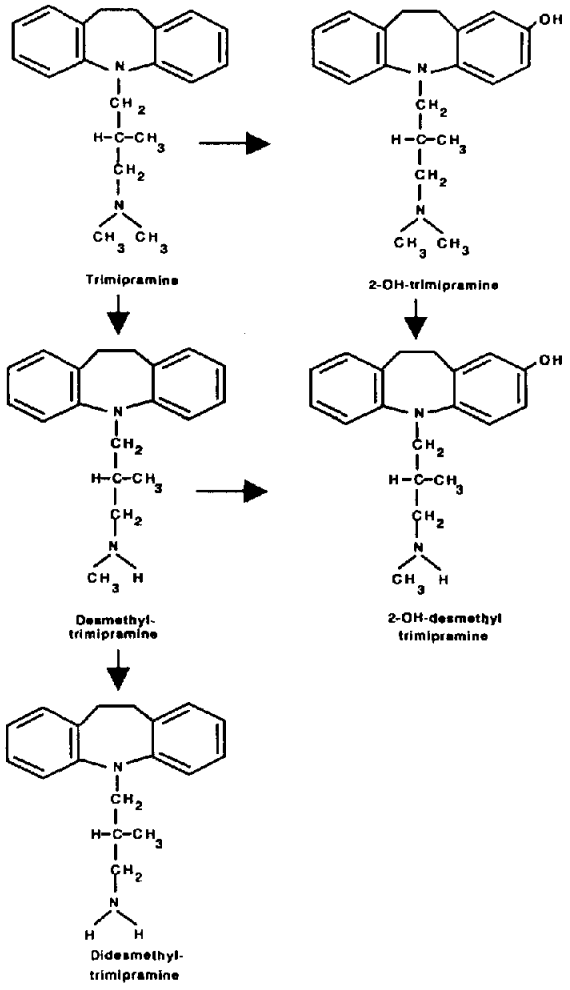

Fig. 1. Metabolic pathway of trimipramine in humans.

2. Experimental

2.1. Reagents

Trimipramine maleate (TRI), desmethyltrimipramine maleate (DTRI), didesmethyltrimipramine maleate (DDTRI), 2-hydroxytrimipramine fumarate (2-OH-TRI) and 2-hydroxydesmethyltrimipramine fumarate (2-OH-DTRI) were obtained from Rhône-Poulenc (Vitry-sur-Seine, France). Clomipramine hydrochloride (CMI), desmethylclomipramine hydrochloride (DCMI), didesmethylclomipramine hydrochloride (DDCMI) 8-hydroxy-clomipramine base (8-OH-CMI) and 8-hydroxydesmethylclomipramine hydrochloride (8-OH-DCMI) were supplied by Ciba-Geigy (Basel, Switzerland).

N - Methyl - N - (tert - butyldimethylsilyl)trifluoroacetamide (MTBSTFA) and trifluoroacetic acid anhydride (TFAA) were from Pierce (Rockford, Il, USA). *Helix pomatia* β-glucuronidase was from Sigma (Catalogue Number G-7770, 91 000 units per ml, St Louis, Mo, USA).

Stock solutions of TRI, DTRI, DDTRI, CMI, DCMI, and DDCMI were prepared with a concentration of 1 mg base/ml in 0.1 *M* HCl and those of 2-OH TRI, 2-OH DTRI, 8-OH CMI and 8-OH DCMI with a concentration of 0.5 mg/ml in methanol. Working solutions of TRI, DTRI, DDTRI, CMI, DCMI and DCMI were made with a concentration of 1 ng/μl in 0.01 *M* HCl, and those of 2-OH TRI and 2-OH DTRI, 8-OH CMI and 8-OH DCMI with a concentration of 10, 5 and 20 ng/μl in 0.01 *M* HCl, respectively. All other reagents were of analytical or HPLC grade.

2.2. Instrumentation and chromatographic conditions

Analyses were performed on a Hewlett-Packard HP 5890 series II gas chromatograph equipped with a splitless capillary system and linked to a quadrupole HP 5988 A mass spectrometer (MS) system operating in the electron impact mode (EI). The MS conditions were: ionization potential 70 eV, emission 300 μA, ion source temperature 200°C, and GC–MS capillary direct interface 250°. Splitless injections of 3 μl were made into a fused-silica SE-54 capillary column (Macherey-Nagel, Oensingen, Switzerland), 12 m $\times$ 0.25 mm I.D., 0.25 μm film thickness, with helium as the carrier gas. The column head-pressure was set to 20 kPa, total flow to 60 ml/min, and septum purge to 3 ml/min. GC conditions were: initial temperature 160°C, heating rate 30°C, final temperature 260°C, and injector temperature 250°C. Analyses were performed in the selected-ion monitoring mode for the ions at *m/z* 208 (DTRI and DDTRI), 242 (DCMI AND DDCMI), 249 (TRI), 268 (CMI), 338 (2-OH DTRI), 372 (8-OH DCMI), 379 (2-OH TRI) and 399 (8-OH

CMI). To improve the sensitivity, only four ions were recorded simultaneously (first at m/z 208, 242, 249, 268 and then at m/z 338, 372, 379, 399).

2.3. Extraction conditions

A 0.5-ml volume of heparinized plasma was incubated with 0.5 ml of 0.2 *M* acetate buffer pH 5.0, 50 μl 4 % sodium azide, and 250 μl *H. pomatia* glucuronidase at 37° C for 24 h. A 500-μl volume of 1 *M* carbonate buffer pH 9.3, 50 μl 2 *M* NaOH, 100 ng of CMI, DCMI and DDCMI (internal standards for TRI, DTRI and DDTRI respectively), 250 ng of 8-OH-CMI (internal standard for 2-OH-TRI) and 1000 ng of 8-OH-DCMI (internal standard for 2-OH-DTRI) and 6 ml of *n*-heptane–ethylacetate (80:20, v/v) were added to the plasma/enzymes mixture. Extraction was performed on a rotary shaker for 15 min. After centrifugation (8 min, 3400 *g*) the organic layer was transferred to another tube containing 1.2 ml of 0.1 *M* HCl. After 15 min shaking and centrifugation (see above), the aqueous phase was transferred to another tube containing 1 ml of 1 *M* carbonate buffer pH 9.3 and 200 μl of toluene–isoamylalcohol (85:15, v/v). After 15 min shaking and 2 min centrifugation, the solvent was transferred to a conical tube and evaporated to dryness under a stream of nitrogen at 40°C.

2.4. Derivatization conditions

The residue was dissolved in 100 μl of toluene and 50 μl of TFAA (for the derivatization of the demethylated metabolites) and left for 1 h at 62° C. One ml of carbonate buffer and 100 μl of toluene–isoamylalcohol were then added, the mixture was shaken for 15 min and centrifuged (3400 *g*) for 2 min. The solvent was transferred to another tube and evaporated. The residue was dissolved in 50 μl pyridine and 50 μl MTBSTFA (for the derivatization of the hydroxylated metabolites) and left for 1 h at 62°C. The mixture was then evaporated to dryness, reconstituted in 100 μl toluene, and 3 μl were injected into the GG–MS system.

3. Results and discussion

For the assays of trimipramine and its metabolites in blood, an HPLC method has been developed previously in our laboratory [7]. Owing to polymedication of psychiatric patients, a more specific detection method is often needed. Trimipramine and its N-demethylated and hydroxylated metabolites were first analysed in our laboratory by GC–MS, without any derivatization steps [5]. However, adsorption of the hydroxy-metabolites on the column resulted in a low sensitivity (limits of quantitation of 10 and 40 ng/ml for 2-OH-TRI and 2-OH-DTRI respectively). Several preliminary experiments (data not shown) were necessary to optimize the derivatization procedures. A good derivatization was obtained for the demethylated but not for the hydroxylated metabolites with TFAA. A good derivatization was also obtained for all metabolites with pentafluoropropionic acid anhydride, but the esters of the hydroxy-metabolites were found to be unstable. A good stability of the latter compounds was obtained with MTBSTFA (at least three weeks at − 20°C, or 72 h at room temperature), but it was not possible to derivatize the demethylated metabolites with this latter reagent. It was then decided to perform a double derivatization step (TFAA for DTRI, DDTRI, 2-OH-DTRI, DCMI, DDCMI and 8-OH-DCMI, and MTBSTFA for 2-OH-TRI, 2-OH-DTRI, 8-OH-CMI and 8-OH-DCMI). Figs. 2 and 3 show the EI mass spectra of trimipramine and clomipramine, of the trifluoroacetyl derivatives of DTRI, DDTRI, DCMI and DDCMI, of the *tert.*-butyldimethylsilyl derivatives of 2-OH-TRI and 8-OH-CMI, and of the trifluoroacetyl-*tert.*-butyldimethylsilyl derivatives of 2-OH-DTRI and 8-OH-DCMI.

It is noteworthy to add that, in preliminary experiments, high amounts of glucuronidase (see Experimental) were found to be necessary to obtain complete hydrolysis of the glucuronic acid-conjugated metabolites (*ca.* 95% of the total plasma hydroxy metabolites are conjugated, see also ref. 3). It was also noted (data not shown) that the enzymes purified from *H. pomatia* were more efficient than those from *E. coli* (Boehring-

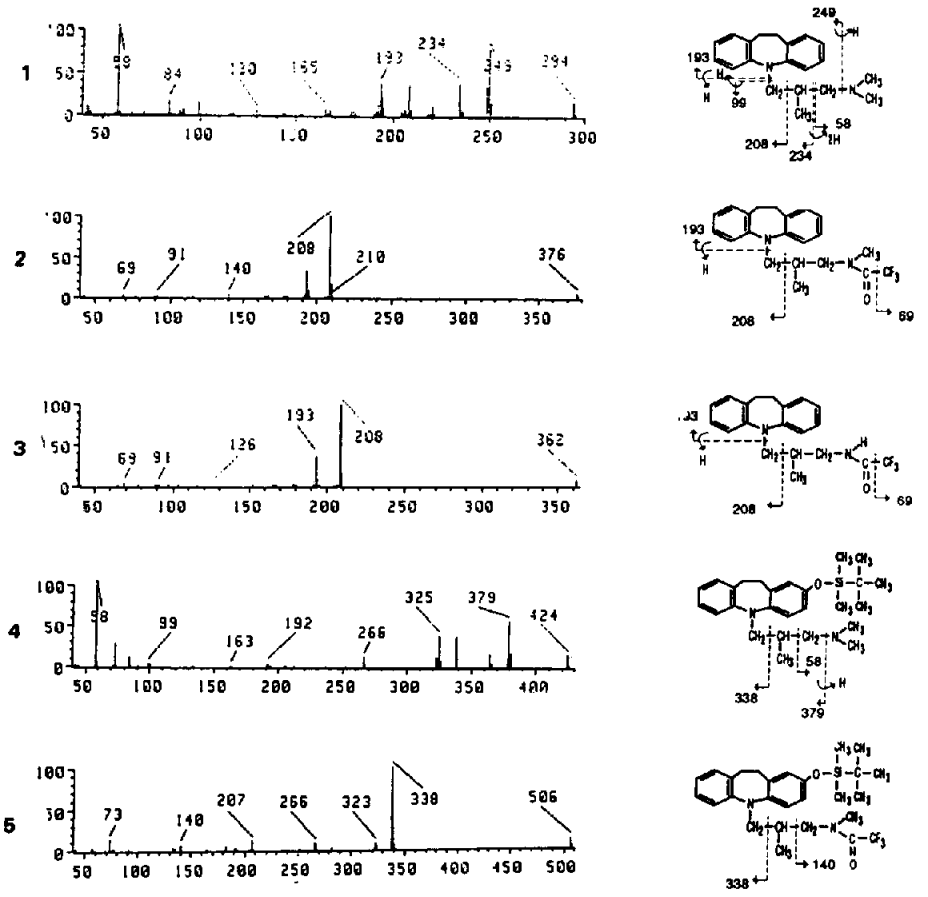

Fig. 2. EI mass spectra of trimipramine (1, $M^+ = 294$), of the trifluoroacetyl derivatives of desmethyltrimipramine (2, $M^+ = 376$) and didesmethyltrimipramine (3, $M^+ = 362$) of the *tert.*-butyldimethylsilyl derivative of 2-hydroxytrimipramine (4, $M^+ = 424$) and of the trifluoroacetyl-*tert.*-butyldimethylsilyl derivatives of 2-hydroxydesmethyltrimipramine (5, $M^+ = 506$). Examples of the probable fragmentation modes are given.

er, Catalogue No. 27580, Rotkreuz, Switzerland), that the *H. pomatia* enzyme preparation from Sigma (Catalogue No. G-2887) had an efficiency similar to that from Boehringer (Catalogue No. 127698), and better results were obtained at 37°C than at 51°C, a temperature stated in some publications.

3.1. Evaluation of the methods

Table 1 shows a summary of the statistical data on the analysis of trimipramine and its metabolites. In summary, coefficients of correlation obtained for the calibration curves (10–1000 ng) were consistently higher than 0.998, and the variability of the assays, as assessed by the coefficients of variation (C.V.) measured at two concentrations for each substance, were always less than 15%, both for the intra-day ($n = 6$) and inter-day ($n = 5$) experiments. The limits of quantitation, defined as the concentration for which the mean value of five determinations is within ± 20% of the actual value and the coefficient of variation less than 20%, were found to be 2 ng/ml for DTRI and 4 ng/ml for all other substances. Although it was not precisely quantified, the limit of detection was found to be at

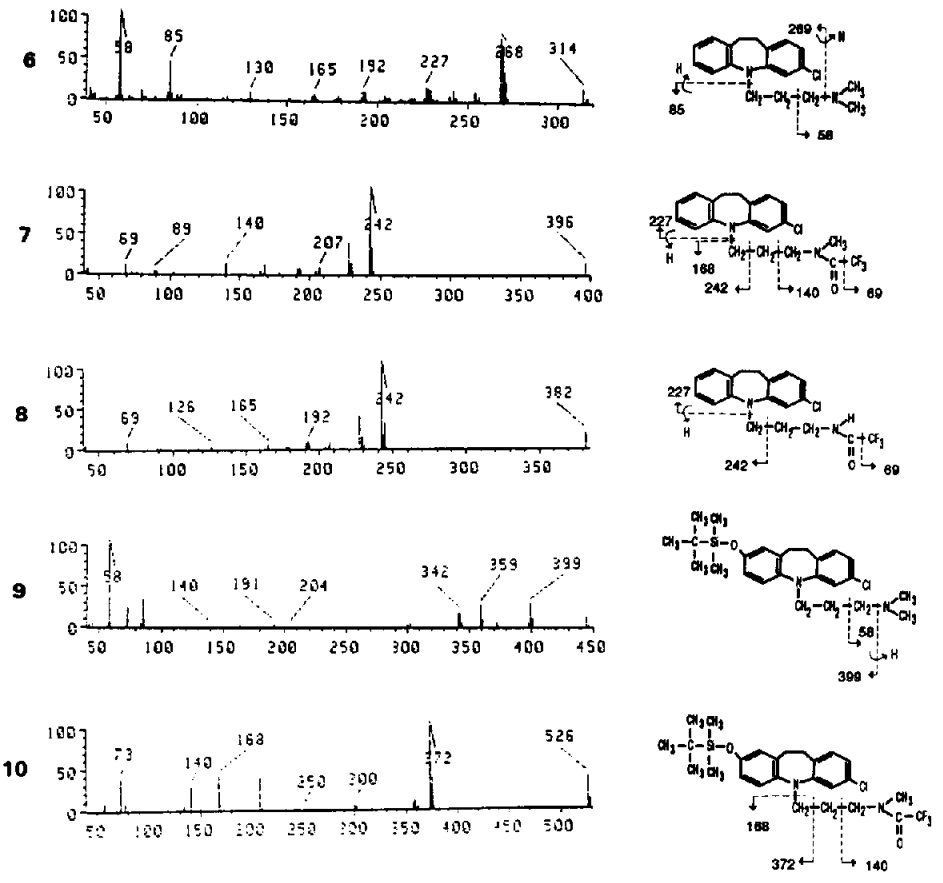

Fig. 3. EI mass spectra of clomipramine (6, $M^+ = 314$), of the trifluoroacetyl derivatives of desmethylclomipramine (7, $M^+ = 396$) and didesmethylclomipramine (8, $M^+ = 382$), of the *tert.*-butyldimethylsilyl derivative of 8-hydroxyclomipramine (9, $M^+ = 444$), and of the trifluoroacetyl-*tert.*-butyldimethylsilyl derivative of 8-hydroxydesmethylclomipramine (10, $M^+ = 526$). Examples of the probable fragmentation modes are given.

least 1 ng/ml for all substances. As pure standards of the derivatized metabolites are not available, recovery was calculated by dividing mean areas ($n = 7$) obtained after the complete extraction and derivatization procedure of a plasma containing 100 ng/ml of TRI, DTRI, DDTRI and 500 ng/ml of 2-OH-TRI and 2-OH-DTRI by mean areas obtained after direct derivatization of the same quantities of the pure standards. Recovery was found to be satisfactory for all compounds (56% for 2-OH-DTRI, 78% for DTRI, 92% for 2-OH-TRI and 93 % for TRI) except for DDTRI (35%), but the analysis of this latter substance can still be performed with a satisfactory precision. Finally, the stability of trimipramine and its metabolites was evaluated by analysing spiked plasmas stored at − 20°C for different periods of time. No loss was noted after a three-month storage.

3.2. *Analysis of human samples*

Fig. 4 shows an example of a chromatogram obtained for the plasma analysis of a patient receiving a daily dose of 200 mg of trimipramine. The measured concentrations of TRI, DTRI, 2-OH-TRI and 2-OH-DTRI were 294, 62, 816 and 1436 ng/ml, respectively (a very low level of DDTRI, near the quantitation limit, was measured).

Table 1
Statistical Data Concerning the Analysis of Trimipramine and of its Metabolites

Drug[a]	TRI	DTRI	DDTRI	2-OH-TRI	2-OH-DTRI
Calibration					
Range (ng/ml)	10–1000	10–1000	10–1000	10–1000	1–1000
Slope	1.39	2.24	2.43	1.7	1.13
Intercept	− 0.0643	− 0.0565	− 0.127	− 0.101	− 0.0225
r	0.999	1	0.999	0.999	0.998
Recovery in %, (concentrations used)(n = 6)					
Within-day variation (*n* = 6)	93 (100 ng/ml)	78(100 ng/ml)	35 (100 ng/ml)	92 (500 ng/ml)	56 (500 ng ml)
Theoretical values (ng/ml)	20	20	20	100	100
Measured values (ng/ml): mean ± S.D.(C.V. in %)	18.3 ± 1.9(10.4)	19.3 ± 2.1(10.9)	18.8 ± 2.4(12.8)	103.3 ± 5.1(4.9)	96.4 ± 9.1(9.4)
Theoretical values (ng/ml)	100	100	100	500	500
Measured values (ng/ml): mean ± S.D.(C.V. in %)	96.6 ± 8.5(8.8)	111.1 ± 10.0(9.0)	95.8 ± 4.7(4.9)	497 ± 18.4(3.7)	532 ± 41(7.7)
Day-to-day variation (n = 5)					
Theoretical values (ng/ml)	20	20	20	100	100
Measured values (ng/ml): mean ± S.D.(C.V. in %)	20.9 ± 3.0(14.4)	20.9 ± 2.3(11.0)	19.6 ± 1(5.1)	101.7 ± 7(6.9)	97.7 ± 7.8(8.0)
Theoretical values (ng/ml)	100	100	100	500	500
Measured values (ng/ml): mean ± S.D.(C.V. in %)	104.6 ± 6(5.7)	107.5 ± 6(5.6)	98.9 ± 3(3.0)	512.8 ± 24(4.7)	511.3 ± 16(3.1)
Limit of quantitation (n = 5)					
Theoretical values (ng/ml)	4	2	4	4	4
Measured values (ng/ml): mean ± S.D.(C.V. in %)	3.90 ± 0.30(7.6)	1.99 ± 0.11(5.5)	4.04 ± 0.52(12.9)	3.65 ± 0.34(9.3)	4.31 ± 0.44(10.2)

Trimipramine (TRI), desmethyltrimipramine (DTRI), didesmethyltrimipramine (DDTRI)
2-hydroxytrimipramine (2-OH-TRI) and 2-hydroxydesmethyltrimipramine (2-OH-DTRI).

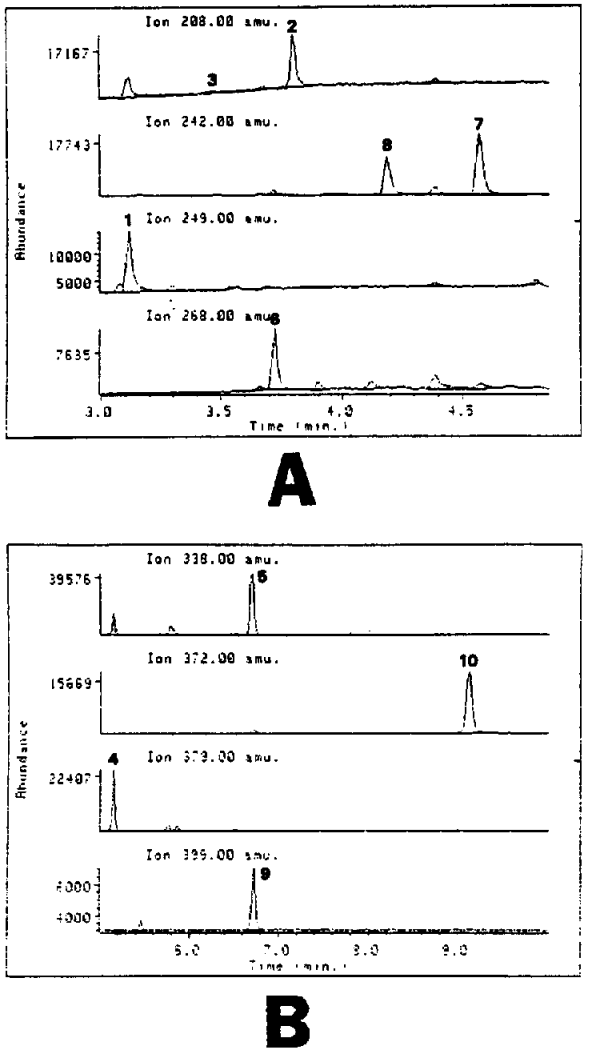

Fig. 4. SIM tracing of a 0.5-ml plasma analysis of a patient treated with a daily dose of 200 mg trimipramine. (A) Trimipramine (1, 3.11 min), desmethyltrimipramine (2, 3.81 min), didesmethyltrimipramine (3, 3.47 min), clomipramine (6, 3.73 min), desmethylclomipramine (7, 4.59 min) and didesmethylclomipramine (8, 4.20 min). (B) 2-Hydroxytrimipramine (4, 5.17 min), 2-hydroxydesmethyltrimipramine (5, 6.73 min), 8-hydroxyclomipramine (9, 6.76 min) and 8-hydroxydesmethylclomipramine (10, 9.18 min).

4. Conclusions

This method, both sensitive and selective, allows the quantification of trimipramine and its demethylated and hydroxylated metabolites in plasmas or serums. It can be used for single-dose pharmacokinetic studies and offers a good possibility for the analysis of blood samples drawn from psychiatric patients who are often comedicated with many different drugs, which may result in interferring or overlapping peaks when such samples are analysed by HPLC.

5. Acknowledgements

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6. References

[1] E.C. Settle and F.J. Ayd, *J. Clin. Psychiatry*, 41 (1980) 266–274.
[2] D.R. Abernethy, D.J. Greenblatt and R.I. Shader, *Clin. Pharmacol. Ther.*, 35 (1984) 348–353.
[3] R.F. Suckow and T.B. Cooper, *J. Pharm. Sci.*, 73 (1984) 1745–1748.
[4] G. Caillé, J.G. Besner, Y. Lacasse and M. Vézina, *Biopharm. Drug Dispos.*, 1 (1980) 187–194.
[5] C.B. Eap, S. Laurian, A. Souche, L. Koeb, P. Reymond, T. Buclin and P. Baumann, *Neuropsychobiology*, 25 (1992) 214–220.
[6] P.P. Rop, T. Conquy, F. Gouezo, A. Viala and F. Grimaldi, *J. Chromatogr.*, 375 (1986) 339–347.
[7] C.B. Eap, L. Koeb, E. Holsboer-Trachsler and P. Baumann, *Ther. Drug Monit.*, 14 (1992) 380–385.
[8] A.A. Gulaid, G.A. Jahn, C. Maslen and M.J. Dennis, *J. Chromatogr.*, 566 (1991) 228–233.
[9] A.M. Bougerolle, J.L. Chabard, N. Jbilou, H. Bargnoux, J. Petit, J.A. Berger and G. Dordain, *J. Chromatogr.*, 434 (1988) 232–238.
[10] N. Jourdil, B. Pinteur, F. Vincent, C. Marka and G. Bessard, *J. Chromatogr.*, 613 (1993) 59–65.
[11] R.C. Young, *Psychopharmacol. Bull.*, 27 (1991) 521–532.