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Journal of Chromatography B, 703 (1997) 147–158

JOURNAL OF CHROMATOGRAPHY B

Simultaneous determination of imipramine and its metabolite desipramine in human plasma by capillary gas chromatography with mass-selective detection

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Received 12 May 1997; received in revised form 15 July 1997; accepted 25 July 1997

Abstract

An analytical method for the simultaneous determination of imipramine (IMI) and its N-desmethyl metabolite, desipramine (DIMI) in human plasma by capillary gas chromatography–mass selective detection (GC–MS), with D4-imipramine (D4-IMI) and D4-desipramine (D4-DIMI) as internal standards, was developed and validated. After addition of the internal standards, the compounds were extracted from plasma at basic pH into *n*-heptane–isoamyl alcohol (99:1, v/v), back-extracted into acidic aqueous solution and re-extracted at basic pH into toluene. Desipramine and D4-desipramine were converted into their pentafluoropropionyl derivatives. The compounds were determined by gas chromatography using a mass selective detector at m/z 234 for IMI, m/z 238 for D4-IMI, m/z 412 for DIMI and m/z 416 for D4-DIMI. The method was applied to clinical samples. © 1997 Elsevier Science B.V.

Keywords: Imipramine; Desipramine

1. Introduction

Imipramine hydrochloride, the active ingredient of Tofranil, is a tricyclic antidepressant agent.

A review of numerous methods described for the quantitative assay of imipramine (IMI) and its N-demethylated metabolite, desipramine (DIMI), in biological fluids has already been reported [1]. Individual methods using high-performance liquid chromatography (HPLC) [2–8] and gas chromatography (GC) with nitrogen-selective detection [9–13] have also been published. The most sensitive methods were based on gas chromatography–mass spectrometry (GC–MS) [14,15].

Our objective was to develop and validate a sensitive method for the quantitative determination of IMI and DIMI in human plasma samples from bioequivalence studies. This method was derived from the method previously published by Sioufi et al. [16] for the determination of an analog tricyclic drug, clomipramine and its demethylated metabolite.

2. Experimental

2.1. Chemicals and reagents

The chemical structures of IMI, DIMI and the corresponding deuterium-labelled internal standard are shown in Fig. 1. Imipramine hydrochloride and

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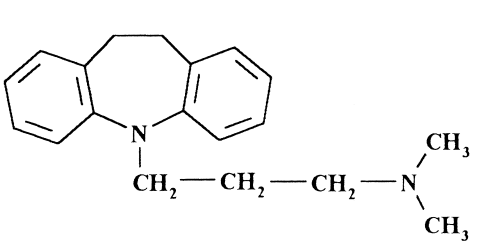
0378-4347/97/$17.00
PII S0378-4347(97)00400-3



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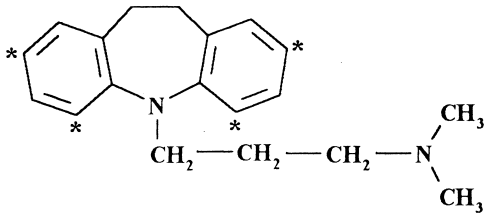

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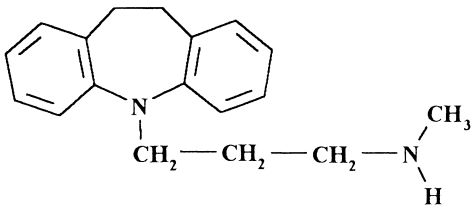
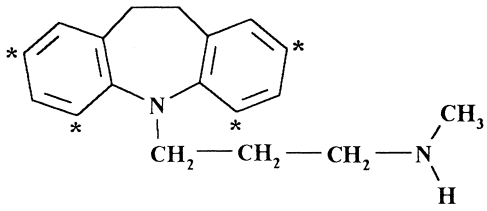


Desipramine



D4-Desipramine

*: indicates positions of deuterium atoms

Fig. 1. Chemical structures of IMI, DIMI and their respective deuterium labelled internal standards.

desipramine hydrochloride were supplied by Novartis Pharma (Basle, Switzerland). The deuterium-labelled internal standards were purchased from Cambridge Isotope Laboratories (Andover, MA, USA).

The solvents and reagents were all of analytical grade: *n*-heptane (Ref. 34873) was obtained from Riedel-de Haën (Seelze, Germany), toluene (Pestipur, SDS) from Solvants Documentation Synthèse (Pépin, France), pentafluoropropionic anhydride (Ref. 77292), pyridine (Ref. 82704) and isoamyl alcohol (Ref. 59090) from Fluka (Saint-Quentin Fallavier, France).

pH 11 alkaline buffer was an aqueous solution containing 2 mol/l sodium carbonate (Ref. S/2920/60) from Fisons (Loughborough, UK).

2.2. Standard solutions

The two stock solutions of IMI and DIMI were prepared by dissolving 1.16 mg and 1.08 mg of substance in 10 ml of 0.01 mol/l hydrochloric acid, respectively.

Appropriate serial dilutions of each stock solution with 0.01 mol/l hydrochloric acid were then made in order to prepare the spiking solutions to be used for calibration samples, at concentrations ranging from 0.580 to 116 ng/ml for IMI and 0.540 to 108 ng/ml for DIMI.

Other stock solutions of IMI and DIMI in 0.01 mol/l hydrochloric acid were prepared from a second weighing (1.16 mg for IMI and 1.09 mg for DIMI) and appropriately diluted to give spiking solutions to be used for validation (accuracy and precision assessment) samples.

The I.S. stock solutions were prepared by dissolving 0.26 mg and 0.24 mg of D4-IMI and D4-DIMI in 10 ml of 0.01 mol/l hydrochloric acid, respectively. Further dilution of the stock solutions with 0.01 mol/l hydrochloric acid resulted in the internal standards spiking solution (1040 ng/ml D4-IMI and 960 ng/ml D4-DIMI).

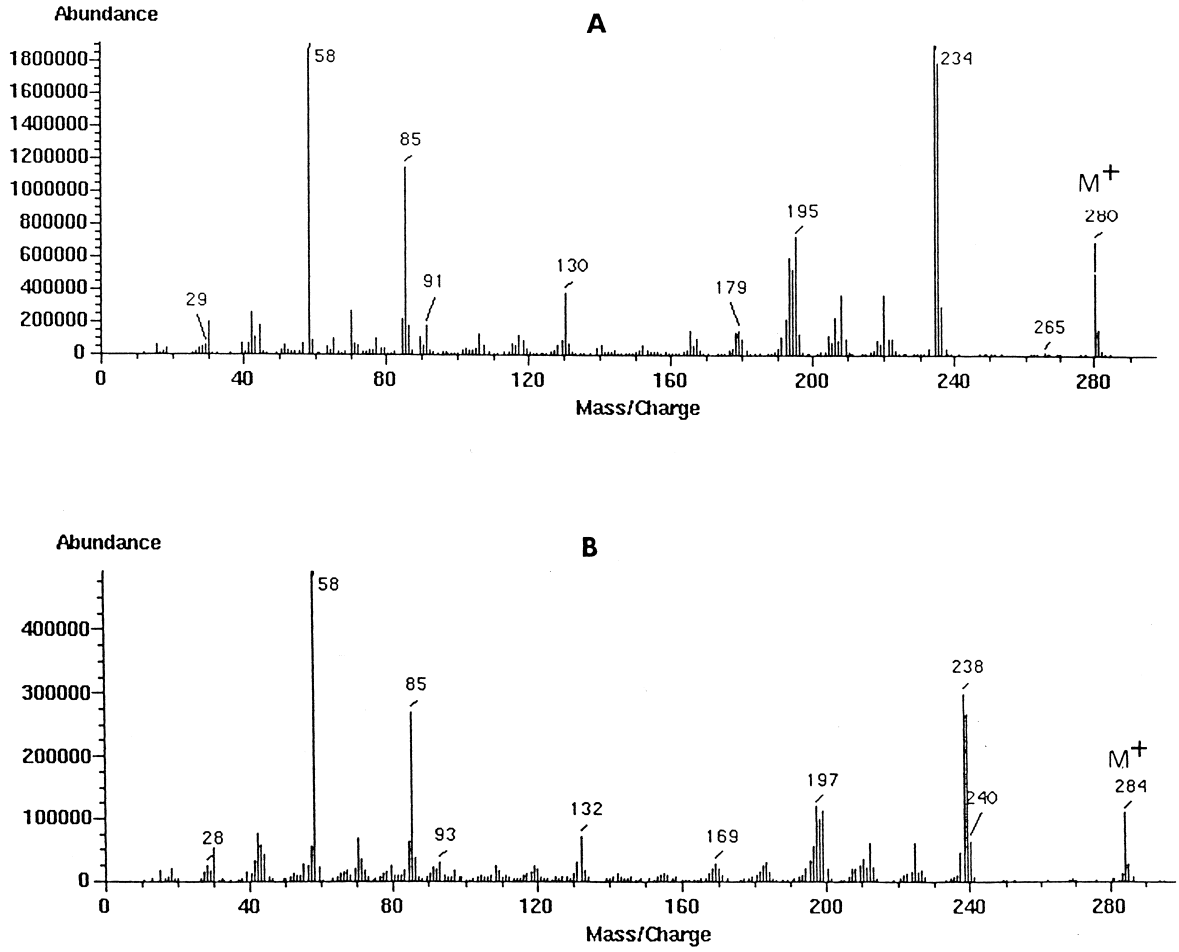


Fig. 2. Electron impact mass spectra of IMI (A) and D4-IMI (B).

While not in use, all the solutions were stored at about +4°C.

2.3. Equipment

A Hewlett-Packard 5890 Series II gas chromatograph, equipped with a capillary inlet system and an HP 7673 automatic sampler was used (Hewlett-Packard, Palo Alto, CA, USA). The column was a 12 m×0.2 mm I.D. fused-silica capillary column coated with cross-linked 5% phenyl methyl silicone with a film thickness of 0.33 μm (Model HP 19091B, Option 101 supplied by Hewlett-Packard). The carrier gas was helium with an inlet pressure of 62 kPa (9 p.s.i.) with a split flow of 50 ml/min and a septum purge of 3.0 ml/min. Sample introduction was performed in the splitless mode at an injection temperature of 250°C with a 2 min splitless-period. The column was initially at 80°C for 0.5 min and the temperature was then raised at a rate of 40°C/min up to 300°C for 3.1 min. A Hewlett-Packard 5970B mass selective detector (MSD) was interfaced with the gas chromatograph, with the capillary column inserted directly into the ion source.

The MSD was calibrated with the Autotune program at the beginning of each day using perfluorotributylamine (PFTBA). The GC–MSD interface was maintained at 280°C. The detector was turned on from 4 to 6 min after injection. The selected ions monitored were *m/z* 234 and *m/z* 238 for IMI and D4-IMI, respectively and *m/z* 412 and *m/z* 416 for

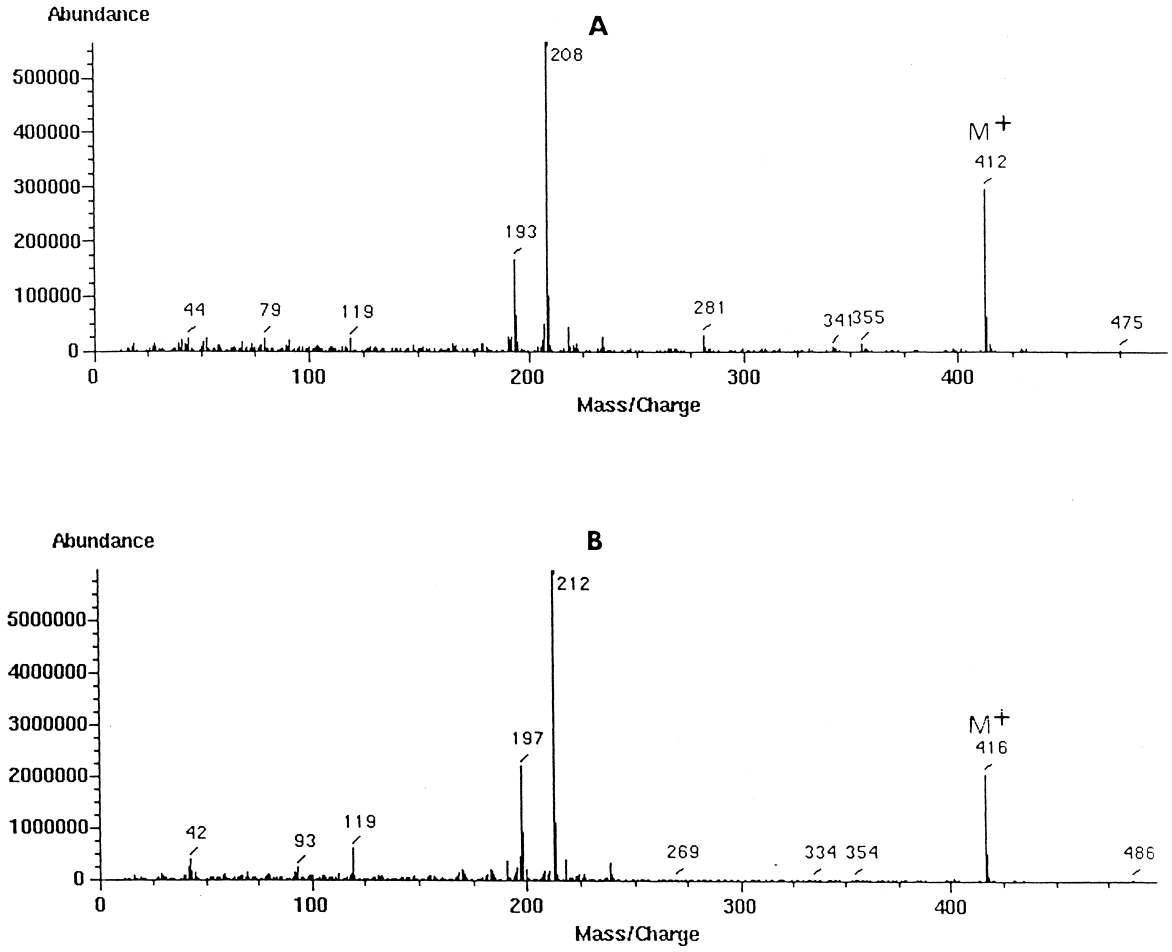


Fig. 3. Electron impact mass spectra of the pentafluoropropionyl derivatives of DIMI (A) and D4-DIMI (B).

pentafluoropropionic derivatives of DIMI and D4-DIMI, respectively.

A HP 59940A MS (HP-UX series) ChemStation was used to control GC and injector instruments and for data acquisition and processing.

2.4. Calibration, validation and clinical samples

For calibration and validation, aliquots of working solutions were added to 1 ml of drug-free human plasma to produce reference samples in the range of concentrations 0.580 to 116 ng/ml for IMI and 0.540 to 108 ng/ml for DIMI. A constant amount of internal standards (20.8 ng D4-IMI and 19.2 ng D4-DIMI/20 μl) was added to each reference sample.

For clinical sample, a constant amount of internal standard (20.8 ng D4-IMI and 19.2 ng D4-DIMI/20 μl) was added to 1 ml of plasma (obtained from blood collected on solid heparin–lithium and centrifuged).

2.5. Extraction from plasma

To 1 ml plasma in an extraction tube were successively added an aliquot of the appropriate standard solution (only for calibration and validation), 20 μl of I.S. solution (20.8 ng D4-IMI and

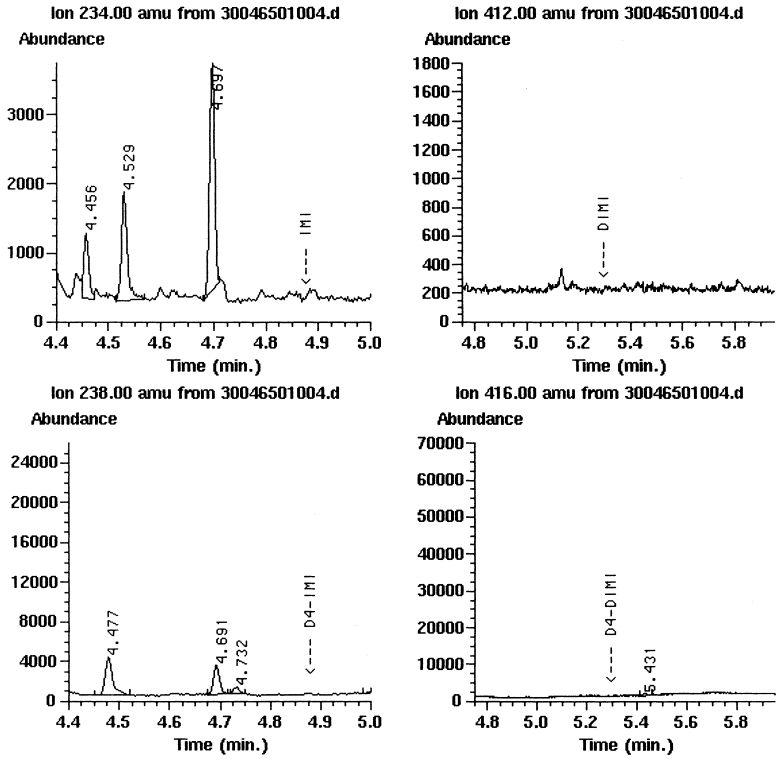


Fig. 4. Examples of selected ion current profiles of an extract of 1 ml drug free human plasma.

19.2 ng D4-DIMI/20 μl), 1 ml of pH 11 alkaline buffer and 4.5 ml of *n*-heptane–isoamyl alcohol (99:1, v/v). The tube was then placed on a rotary mixer for 15 min at a velocity of 300 rpm. Following centrifugation at around 3000 *g* for 10 min at room temperature, the organic layer was transferred into another conical tube, shaken with 500 μl of 0.05 mol/l sulphuric acid for 10 min at 300 rpm and briefly centrifuged (2 min at 2500 *g*). The organic phase was discarded, and 250 μl of 1 mol/l sodium hydroxide and 1.6 ml of toluene were added. The mixture was shaken for 10 min at 300 rpm. After centrifugation (2 min at 2500 *g*), the organic phase was transferred into a 10-ml conical glass tube.

To the organic phase were added 20 μl of pyridine and 100 μl pentafluoropropionyl anhydride. The tube was stoppered and shaken on a Vortex mixer for 15 s. After 1 h at 60°C, 0.5 ml of pH 11 alkaline buffer was added, the mixture was shaken for 5 min at 300 rpm, then centrifuged. The organic phase was transferred into a small conical tube and concentrated to about 40 μl by evaporation under a nitrogen stream at ambient temperature.

After Vortex mixing, the solution was transferred into an insert introduced in an autosampler vial. A 3-μl aliquot was used for the chromatographic analysis.

2.6. Study in humans

The study was conducted in accordance with the World Medical Association's Declaration of Helsinki, Venice and Hong Kong amendments 1983 and 1989, and Good Clinical (Research) Practice (GCP). Written informed consent for each subject was obtained prior to initiating any study procedures.

Eighteen healthy subjects, who had been advised to take no drugs for two weeks prior to the study and none besides imipramine throughout its duration, received 50 mg of imipramine hydrochloride as one Tofranil sugar-coated commercial tablet.

Blood samples were collected before and 1, 2, 3,

4, 6, 8, 10, 13, 24, 32, 48, 56, 72 and 96 h after administration. Plasma was separated by centrifugation and stored at −20°C until analysis.

3. Results and discussion

3.1. Mass spectra

Electron impact mass spectra are shown in Fig. 2 for IMI and D4-IMI and in Fig. 3 for the pentafluoropropionyl derivatives of DIMI and D4-DIMI.

Molecular ions were observed at m/z 280 and m/z 284 for IMI and D4-IMI, respectively.

But the fragment ions at m/z (M−46) which represent a larger abundance than molecular ions were at m/z 234 and 238, respectively. These fragment ions were selected for quantitative measurements in the SIM mode.

Molecular ions were observed at m/z 412 and 416 for DIMI and D4-DIMI pentafluoropropionyl derivatives, respectively. These ions were selected for quantitative measurements in the SIM mode.

3.2. Plasma interferences

Representative selected ion current profiles from extracts of drug-free human plasma and of the same plasma spiked with IMI, DIMI and internal standards are shown in Figs. 4 and 5. IMI and D4-IMI were eluted from the analytical column with retention times of approximately 4.8 min at m/z 234 and 238, respectively. DIMI and D4-DIMI derivatives were eluted from the analytical column with retention times of approximately 5.2 min at m/z 412 and 416, respectively. As shown, the compounds of interest were separated from co-extracted endogenous plasma components. Similar profiles were observed for different plasma pools from volunteers not given any

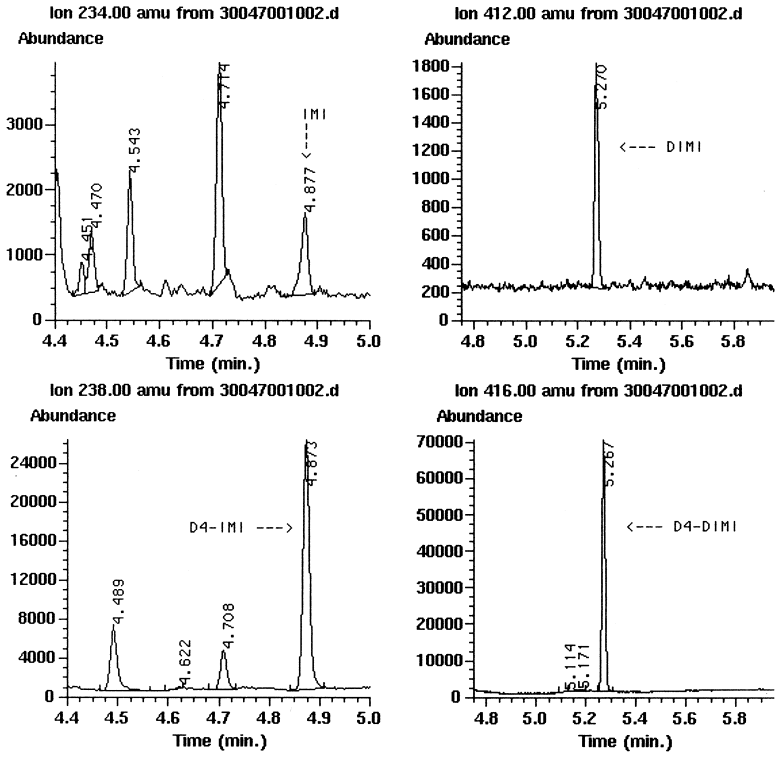


Fig. 5. Examples of selected ion current profiles: an extract of 1 ml plasma spiked with 0.580 ng IMI, 0.540 ng DIMI, 20.8 ng D4-IMI and 19.2 ng of D4-DIMI.

medication. Two analytical columns demonstrated comparable chromatographic characteristics.

3.3. Calibration curves

Daily calibration standard samples at nine different concentrations in single in the range 0.580 to 116 ng/ml for IMI and 0.540 to 108 ng/ml for DIMI were prepared. Calibration curves ($y=mx+b$) were represented by the plots of the peak height ratios (y) of IMI to D4-IMI and of derivatized DIMI to derivatized D4-DIMI versus the concentrations (x) of the calibration samples. They were generated using weighted ($1/x^2$) linear least-squares regression as the mathematical model [17]. For each compound, two calibrations curves corresponding to two different ranges of concentrations were calculated: a low range 0.580 to 11.6 ng/ml for IMI and 0.540 to 10.8 ng/ml for DIMI; a high range 11.6 to 116 ng/ml for IMI and 10.8 to 108 ng/ml for DIMI. Concentrations in validation samples were calculated from the resulting peak height ratios and the regression equations of the calibration curves.

The calibration curve data obtained over six days are shown in Tables 1 and 2 for IMI and Tables 3 and 4 for DIMI. The correlation coefficients were higher than 0.990 for IMI and 0.994 for DIMI. Individual fit of the calibration standards to the curve

Table 1
Statistics, accuracy and precision of calibration curves of IMI (low concentration range)

Curve statistics

Analysis day	Slope	y-Intercept	Correlation coefficient (r)
1	0.06434	0.00739	0.9960
2	0.06062	0.01017	0.9931
3	0.06493	0.00917	0.9982
4	0.06696	0.00406	0.9924
5	0.06516	0.01324	0.9922
6	0.06388	0.01288	0.9905
Mean	0.0643	0.0095	0.9937
S.D.	0.0021	0.0035	0.0028
C.V. (%)[b]	3.3	37	0.3

Accuracy and precision of calibration curves

	Spiked concentrations (ng/ml)					
	0.580	0.580	1.16	2.90	5.80	11.6
Analysis day	Back-calculated concentrations (ng/ml)					
1	0.579	0.568	1.26	2.59	5.87	12.0
2	0.523	0.653	1.08	3.04	5.61	11.9
3	0.618	0.553	1.11	2.97	5.74	11.8
4	0.546	0.574	1.36	2.71	5.66	11.5
5	0.600	0.583	1.11	2.45	6.20	12.6
6	0.567	0.601	1.17	2.67	5.17	13.5
Mean	0.572	0.589	1.18	2.74	5.70	12.2
S.D.	0.035	0.035	0.11	0.23	0.34	0.7
C.V.[b] (%)	6.1	6.0	9.2	8.3	5.9	5.9
Mean recovery[a] (%)	99	102	102	94	99	105
Mean |R.E.|[c] (%)	4.7	4.3	7.0	8.2	4.2	5.7

[a]Recovery: found concentration expressed in % of the nominal concentration.
[b]Coefficient of variation.
[c]|R.E.| (%)=absolute value of R.E. (%): 100×[(back-calculated concentration from the curve)−(nominal concentration)]/(nominal concentration).

Table 2
Statistics, accuracy and precision of calibration curves of IMI (high concentration range).

Curve statistics

Analysis day	Slope	*y*-Intercept	Correlation coefficient (*r*)
1	0.06037	0.09265	0.9972
2	0.06292	0.01848	0.9985
3	0.05952	0.09488	0.9989
4	0.06191	0.06185	0.9990
5	0.06259	0.11614	0.9994
6	0.05738	0.20108	0.9991
Mean	0.0608	0.0975	0.9987
S.D.	0.0021	0.0610	0.0008
C.V.[b] (%)	3.5	63	0.08

Accuracy and precision of calibration curves

	Spiked concentrations (ng/ml)				
	11.6	29.0	58.0	87.0	116
Analysis day	Back-calculated concentrations (ng/ml)				
1	11.4	30.6	57.6	91.5	107
2	11.4	30.9	57.5	87.5	111
3	11.4	30.6	57.8	87.4	111
4	11.5	30.4	56.1	85.2	118
5	11.5	30.0	57.9	84.1	117
6	11.8	28.0	56.3	88.8	120
Mean	11.5	30.1	57.2	87.4	114
S.D.	0.2	1.1	0.8	2.6	5
C.V.[b] (%)	1.3	3.5	1.4	3.0	4.4
Mean recovery[a] (%)	99	104	99	101	98
Mean |R.E.|[c] (%)	1.7	5.0	1.3	2.2	3.7

[a]Recovery: found concentration expressed in % of the nominal concentration.
[b]Coefficient of variation.
[c]|R.E.| (%)=absolute value of R.E. (%): 100×[(back-calculated concentration from the curve)−(nominal concentration)]/(nominal concentration).

was assessed from the relative error (R.E., %): 100× [(back-calculated concentration from the regression line equation)−(nominal concentration)]/(nominal concentration). The maximum of mean R.E. was 8.2% for IMI (Tables 1 and 2), it was 5.0% for DIMI (Tables 3 and 4), indicating a good fit of the regression model over the range of the calibration curves.

3.4. Accuracy and precision

The accuracy and precision were studied from replicate sets of analyte samples of known concentrations at levels corresponding to the lowest, near the lowest, near the middle and the highest concentration values of the calibration range. Accuracy was determined by calculating the mean recovery for the found concentrations as a percent of the nominal concentrations in standard samples. Precision was assessed from the coefficient of variation (C.V., %) of the mean recoveries. The following validation criteria for accuracy and precision were used to assess the method suitability: mean recoveries should be within 85–115% except at the limit of quantitation (LOQ) where it should be within 80–120%; C.V. should not exceed 15%, except at the LOQ where it should not exceed 20% [18].

Table 3
Statistics, accuracy and precision of calibration curves of DIMI (low concentration range)

Curve statistics

Analysis day	Slope	*y*-Intercept	Correlation coefficient (*r*)
1	0.04345	0.00333	0.9999
2	0.04504	0.00106	0.9989
3	0.04197	0.00334	0.9994
4	0.04381	0.00289	0.9940
5	0.04478	0.00215	0.9996
6	0.04381	0.00077	0.9988
Mean	0.0438	0.0023	0.9984
S.D.	0.0011	0.0011	0.0022
C.V. (%)[b]	2.5	50	0.2

Accuracy and precision of calibration curves

	Spiked concentrations (ng/ml)					
	0.540	0.540	1.08	2.70	5.40	10.8
Analysis day	Back-calculated concentrations (ng/ml)					
1	0.548	0.533	1.07	2.72	5.37	10.8
2	0.546	0.522	1.14	2.64	5.32	10.8
3	0.543	0.545	1.04	2.76	5.52	10.6
4	0.538	0.505	1.24	2.71	5.20	10.3
5	0.526	0.555	1.07	2.73	5.44	10.7
6	0.512	0.559	1.11	2.75	5.25	10.8
Mean	0.536	0.537	1.11	2.72	5.35	10.7
S.D.	0.014	0.021	0.07	0.04	0.12	0.2
C.V.[b] (%)	2.6	3.9	6.5	1.6	2.2	1.8
Mean recovery[a] (%)	99	100	103	101	99	99
Mean |R.E.|[c] (%)	1.8	3.0	5.0	1.3	2.0	1.3

[a]Recovery: found concentration expressed in % of the nominal concentration.
[b]Coefficient of variation.
[c]|R.E.| (%)=absolute value of R.E. (%): 100×[(back-calculated concentration from the curve)−(nominal concentration)]/(nominal concentration).

3.4.1. Intra-day measurements

Samples were analysed on the same day. Individual, mean recoveries and corresponding C.V.s are presented in Tables 5 and 6. The mean (n=6) recovery over the low range was: from 91 to 99% over the 0.580 to 11.6 ng/ml IMI concentration range, with the C.V. ranging from 4 to 18%; from 94 to 103% over the 0.545 to 10.9 ng/ml DIMI concentration range, with the C.V. ranging from 3 to 19%. The mean (n=6) recovery over the high range was: from 94 to 98% over the 11.6 to 116 ng/ml IMI concentration range, with the C.V. ranging from 4 to 5%; from 87 to 101% over the 10.9 to 109 ng/ml DIMI concentration range, with the C.V. ranging from 6 to 10%.

3.4.2. Inter-day measurements

Samples were analysed on six different days over a period of ten days using a daily calibration curve. Individual, mean relative recoveries and C.V.s are presented in Tables 5 and 6. The mean (n=6) recovery over the low range was: from 93 to 100% over the 0.580 to 11.6 ng/ml IMI concentration range, with the C.V. ranging from 2 to 8%; from 98 to 102% over the 0.545 to 10.9 ng/ml DIMI concentration range, with the C.V. ranging from 3 to

Table 4
Statistics, accuracy and precision of calibration curves of DIMI (high concentration range)

Curve statistics

Analysis day	Slope	*y*-Intercept	Correlation coefficient (*r*)
1	0.04116	0.03270	0.9986
2	0.03939	0.07077	0.9985
3	0.04005	0.02110	0.9988
4	0.04233	0.00373	0.9982
5	0.04182	0.03019	0.9981
6	0.04114	0.03203	0.9994
Mean	0.0410	0.0318	0.9986
S.D.	0.0011	0.0220	0.0005
C.V.[b] (%)	2.7	69	0.05

Accuracy and precision of calibration curves

	Spiked concentrations (ng/ml)				
	10.8	27.0	54.0	81.0	108
Analysis day	Back-calculated concentrations (ng/ml)				
1	10.7	27.6	54.6	75.9	112
2	10.6	28.0	55.1	82.7	101
3	10.7	28.0	53.4	83.9	103
4	10.6	28.7	51.4	83.1	105
5	10.7	27.0	55.7	84.7	100
6	10.7	27.7	54.3	82.3	104
Mean	10.7	27.8	54.1	82.1	104
S.D.	0.1	0.6	1.5	3.2	4
C.V.[b] (%)	0.5	2.0	2.8	3.8	4.1
Mean recovery[a] (%)	99	103	100	102	97
Mean |R.E.|[c] (%)	1.3	3.2	2.2	3.7	4.8

[a]Recovery: found concentration expressed in % of the nominal concentration.
[b]Coefficient of variation.
[c]|R.E.| (%)=absolute value of R.E. (%): 100×[(back-calculated concentration from the curve)−(nominal concentration)]/(nominal concentration).

7%. The mean (n=6) recovery over the high concentration range studied was: from 92 to 101% over the 11.6 to 116 ng/ml IMI concentration range, with the C.V. ranging from 2 to 6%; from 96 to 101% over the 10.9 to 109 ng/ml DIMI concentration range, with the C.V. ranging from 2 to 6%.

3.5. Limit of quantitation

The LOQ is defined as the lowest concentration on the standard curve that can be measured with acceptable accuracy, precision and variability. As indicated in Section 3.4, the mean recovery should be within 80–120% of the expected value with a C.V. not exceeding 20%. The lowest concentration values of 0.580 ng/ml for IMI and 0.545 ng/ml for DIMI, whose accuracy and precision (Tables 5 and 6) were within the proposed criteria, are quoted as the LOQ. A signal-to-noise ratio of 19 and 28 for IMI and DIMI, respectively, was found for the lowest quantitative amount.

3.6. Stability

3.6.1. Standard solutions

Validation samples, prepared with fresh solutions of IMI and DIMI, were analysed using calibration samples prepared with ''old'' standard solutions stored for 3 and 6 months at +4°C. Using standard solutions stored for 3 months, the mean recoveries

Table 5
Intra-day and inter-day accuracy and precision for IMI in spiked human plasma samples

Measurements	Given (ng/ml)	Mean found (n=6) (ng/ml)	Accuracy Mean recovery[a] (%)	Precision C.V.[b] (%)
Intra-day		Low concentration range		
	0.580	0.530	91	18
	1.16	1.07	93	7
	5.80	5.76	99	5
	11.6	11.4	98	4
		High concentration range		
	11.6	11.0	95	5
	23.2	22.8	98	4
	92.8	90.4	97	5
	116	109	94	5
Inter-day		Low concentration range		
	0.580	0.560	96	5
	1.16	1.07	93	8
	5.80	5.80	100	2
	11.6	11.4	98	6
		High concentration range		
	11.6	10.6	92	2
	23.2	23.5	101	2
	92.8	93.5	101	6
	116	115	99	5

[a]Recovery: found concentration expressed in % of the nominal concentration.
[b]C.V.: coefficient of variation of recovery.

(n=8) for the samples prepared with fresh solutions were: 92% over the 0.580 to 116 ng/ml IMI concentration range with a C.V. of 5%, and 101% over the 0.545 to 109 ng/ml DIMI concentration range with a C.V. of 4%. With standard solutions stored for 6 months, the mean recoveries for the samples prepared with fresh solutions were: 108% over the 0.580 to 116 ng/ml IMI concentration range with a C.V. of 4%, and 107% over the 0.545 to 109 ng/ml DIMI concentration range with a C.V. of 6%. The standard solutions were found to be stable for at least 6 months at about +4°C.

3.6.2. During sample analysis

Validation samples (after extraction and derivatization) left 24 h at room temperature in the tray of the automatic sampler were injected. The mean recoveries (n=8) were: 99% over the 0.580 to 116 ng/ml IMI concentration range with a C.V. of 2%, and 96% over the 0.545 to 109 ng/ml DIMI concentration range with a C.V. of 5%. The derivatized plasma extracts were found to be stable for at least 24 h at room temperature in the tray of the automatic sampler.

3.7. Application

The present method was used to determine the plasma concentrations of imipramine and the metab-

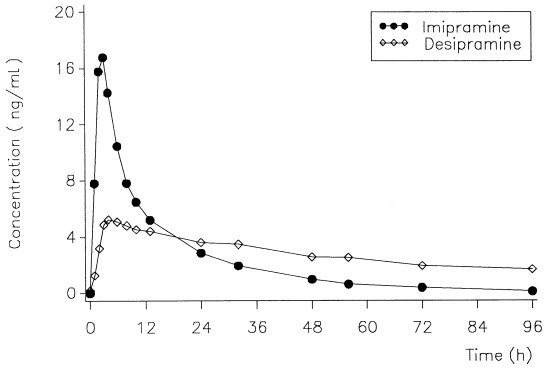


Fig. 6. Mean plasma concentrations of imipramine and desipramine obtained in eighteen healthy subjects after oral administration of one 50-mg Tofranil sugar-coated tablet.

Table 6
Intra-day and inter-day accuracy and precision for DIMI in spiked human plasma samples

Measurements	Given (ng/ml)	Mean found (n=6) (ng/ml)	Accuracy Mean recovery[a] (%)	Precision C.V.[b] (%)
Intra-day		Low concentration range		
	0.545	0.559	103	19
	1.09	1.02	94	3
	5.45	5.25	96	5
	10.9	10.4	96	4
		High concentration range		
	10.9	11.0	101	6
	21.8	20.5	94	8
	87.2	79.7	91	10
	109	95.2	87	9
Inter-day		Low concentration range		
	0.545	0.556	102	7
	1.09	1.10	101	3
	5.45	5.35	98	4
	10.9	10.8	99	5
		High concentration range		
	10.9	10.7	99	6
	21.8	22.1	101	2
	87.2	83.7	96	6
	109	104	96	3

[a]Recovery: found concentration expressed in % of the nominal concentration.
[b]C.V.: coefficient of variation of recovery.

olite, desipramine after oral administration of 50 mg of imipramine hydrochloride as a single Tofranil sugar-coated tablet. Fig. 6 shows the curves of mean plasma concentrations over 96 h of IMI and DIMI obtained in eighteen healthy subjects.

4. Conclusions

The present method has been developed and validated for simultaneously quantifying IMI and DIMI concentrations in human plasma over the range 0.580 to 116 ng/ml and 0.545 to 109 ng/ml, respectively.

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