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Determination of plasma levels of citalopram and its demethylated and deaminated metabolites by gas chromatography and gas chromatography–mass spectrometry

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ABSTRACT

Sensitive and specific methods based on gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) for the determination of levels of citalopram, desmethylcitalopram and didesmethylcitalopram in the plasma of patients treated with citalopram are presented, as well as a GC–MS procedure for the assay of the citalopram propionic acid derivative. After addition of a separate internal standard for each drug, liquid–solvent extraction is used to separate the basic compounds from the acid compounds. The demethylated amines are derivatized with trifluoroacetic anhydride, and the acid metabolite with methyl iodide. GC–MS is performed in the electron impact mode, as mass spectrometry by the (positive-ion) chemical ionization mode (methane and ammonia) appeared to be unsuitable. The limits of quantification were 1 ng/ml for citalopram and desmethylcitalopram and 2 ng/ml for the other metabolites. The correlation coefficients for the calibration curves (range 10–500 ng/ml) were $\geqslant$ 0.999 for all compounds, whether determined by GC or GC–MS.

INTRODUCTION

Citalopram (CIT), a highly selective inhibitor of serotonin reuptake [1], is a bicyclic phthalane derivative with strong antidepressant properties. Its kinetics in man shows a rapid and virtually complete absorption followed by slow elimination [2,3]. Citalopram is metabolized mainly by N-demethylation to form desmethylcitalopram (DCIT) and didesmethylcitalopram (DDCIT). N-Oxidation and deamination [1,4] have also been observed, leading to citalopram N-oxide and the propionic acid (CIT-PROP) derivative (Fig. 1).

The study of citalopram bioavailability requires a highly sensitive analytical method because of the very low plasma levels resulting from therapeutic doses [4,5]. So far, high-performance liquid chromatographic [4,6,7] and thin-layer chromatographic [8] methods are available. To increase sensitivity and specificity, the authors have developed a method that uses gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) in the single-ion monitoring (SIM) mode for the determination of plasma levels of CIT and its demethylated and deaminated metabolites.

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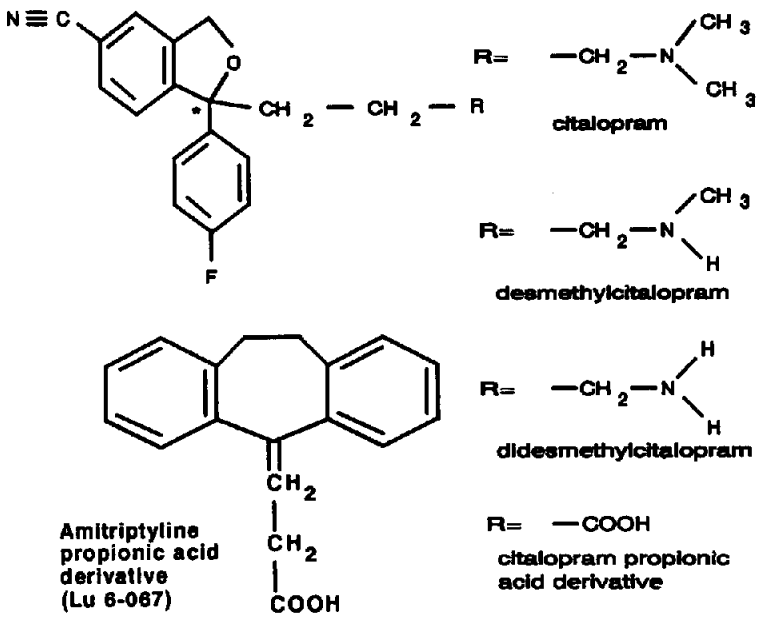

Fig. 1. Chemical formulae of AT-ACID and of citalopram and its metabolites.

EXPERIMENTAL

Standards and reagents

Lundbeck (Copenhagen, Denmark) supplied citalopram hydrobromide (LU 10-171), desmethylcitalopram hydrochloride (LU 11-109), didesmethylcitalopram L-tartrate monohydrate (LU 11-161), citalopram propionic acid metabolite (LU 16-073) (CIT-PROP) and the internal standard for the last, an amitriptyline acid (AT-ACID) derivative (LU 6-067; 3-(10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-yliden)propionic acid) (Fig. 1). Ciba Geigy (Basle, Switzerland) supplied the other internal standards: methylmaprotiline hydrochloride (MMP · HCl), maprotiline hydrochloride (MP · HCl) and N-desmethylmaprotiline (DMP) methane sulphonic acid. Stock solutions of CIT · HBr, DCIT · HCl, DDCIT tartrate and CIT-PROP were made in methanol at a concentration of 1 μg/μl base and stored at −20°C (all concentrations refer to the base). Working solutions were prepared by dilution of the stock solution in 0.01 *M* hydrochloric acid, except for AT-ACID, which was diluted in 0.001 *M* sodium hydroxide to give a concentration of 1 ng/μl for all compounds (stored at −20°C). The working solution of CIT-PROP was diluted in 0.001 *M* sodium hydroxide immediately before use (to avoid adsorption). All reagents were of analytical grade quality. Trifluoroacetic anhydride (TFAA) was purchased from Supelco (Gland, Switzerland) and methyl iodide from Fluka (Buchs, Switzerland).

Extraction and derivatization procedure

CIT and its demethylated metabolites were extracted as follows. To 1 ml of heparinized plasma were added 100 ng of each internal standard [MMP (for CIT), MP (for DCIT), DMP (for DDCIT) in 0.01 *M* hydrochloric acid, and AT-ACID (for CIT-PROP) in 0.001 *M* sodium hydroxide], 50 μl of 1 *M* sodium hydroxide and 6 ml of heptane–isoamyl alcohol (98.5:1.5, v/v). The mixture was shaken for 15 min and centrifuged at 2100 *g* for 6 min at room temperature. The aqueous phase was kept for the extraction of the propionic acid metabolite (stored at −20°C). The organic layer was transferred to another tube containing 1.2 ml of 0.1 *M* hydrochloric acid. The solution was shaken for 15 min, followed by cen-

trifugation at 2100 *g* for 15 min. The organic phase was discarded, and 1 ml of 1 *M* sodium carbonate buffer (pH 9.4) was added to the aqueous phase. The mixture was extracted with 150 μl of toluene–isoamyl alcohol (85:15, v/v) by vortex-mixing for 15 min. The organic layer was then centrifuged at 2100 *g* for 3 min, and the supernatant transferred to a derivatization tube and evaporated to dryness at 50°C under a stream of nitrogen. The residue was reconstituted with 50 μl of toluene and 50 μl of trifluoroacetic anhydride, vortex-mixed and left at 62°C for 1 h. A 1-ml aliquot of 1 *M* sodium carbonate buffer (pH 9.4) was added to the cooled solution and extracted with 70 μl of toluene–isoamyl alcohol (85:15) by vortex-mixing for 15 min. After centrifugation (2100 *g*, 3 min), 3 μl of the organic phase were injected into the GC–MS or GC system.

The CIT-PROP metabolite was extracted as follows. To the aqueous phase from the residual plasma sample obtained after basic extraction were added 150 μl of 1 *M* hydrochloric acid and 1 ml of 0.6% phosphate buffer (pH 3). Before the solution was applied to a 3-ml solid-phase extraction tube (LC-18 Supelclean, Supelco, Baker 10 SPE system vacuum manifold), the tube had to be conditioned. Firstly, it was washed with 2 ml of methanol, followed by 2 ml of acidified water (1% acetic acid, v/v). After loading the sample on the tube, unwanted materials were washed off with 2 ml of 50% methanol in acidified water (1%). CIT-PROP and AT-ACID were then eluted with 2 ml of 90% methanol in acidified water. The methanol was evaporated at 50°C under a stream of nitrogen, 500 μl of acidified water (1% acetic acid, v/v) were added and the resulting solution was extracted with 300 μl of ethyl acetate by vortex-mixing for 15 min. The mixture was centrifuged for 3 min at 2700 *g*, and the organic layer transferred to a derivatization tube. Methylation was achieved by mixing the ethyl acetate layer with 30 μl of methyl iodide and 25 mg of potassium carbonate [9]. After reaction (1 h, 62°C), the supernatant solution was evaporated to dryness under a stream of nitrogen. The residue was again dissolved in 50 μl of toluene–isoamyl alcohol (85:15), and 3 μl of this solution were injected into the GC–MS system.

Gas chromatography and gas chromatography–mass spectrometry

For the gas chromatographic determination of plasma levels of CIT, DCIT and DDCIT, an HP 5890 gas chromatograph (Hewlett-Packard, Meyrin-Geneva, Switzerland) equipped with a splitless capillary system and a nitrogen–phosphorus detector was used as previously described [10]. Splitless injections of 3 μl were made into a fused-silica capillary column (Permabond SE-54-DF-0.25, 25 m × 0.25 mm I.D., 0.27 μm film thickness, Macherey-Nagel, Oensingen, Switzerland) with helium as a carrier gas. The column head pressure was set to 90 kPa, total flow to 60 ml/min, and septum purge to 3 ml/min. Throughout all experiments, GC conditions were: initial temperature 160°C, heating rate 30°C/min, final temperature 260°C and injector temperature 250°C.

The GC–MS analyses were performed on a HP 5890 Series II gas chromatograph linked to a quadripolar Hewlett-Packard HP 5988 A MS system (Palo Alto, CA, USA) operating in the electron impact (EI) mode. The MS conditions were: ionization potential 70 eV, emission 300 μA, ion source temperature 200°C and temperature of GC–MS capillary direct interface 250°C. Mass peaks *m/z* 69, 219 and 502 (perfluorotributylamine) were used for autotuning the mass spectrometer. The GC conditions were as described above.

For positive ion chemical ionization (PICI), the ionization potential was set at 150 eV. The reagent gas was methane or ammonia, introduced directly into the source by external valves. The source pressure was adjusted to give the highest signal (0.07–0.11 kPa).

Recovery studies

Working solutions of each tested drug, containing 50, 200 or 500 ng of the base or acid, and 100 ng of the internal standards were evaporated to dryness at 40°C under a stream of nitrogen. The residue was treated with the derivatization

reagent (TFAA or methyl iodide), as described under *Extraction and derivatization procedure* for GC–MS analysis. The obtained peak areas based on single ions were compared with the peak areas obtained when the whole extraction of the plasma sample and derivatization procedure were carried out.

Calibration curves

Peak-area ratios obtained by analysing plasma samples in duplicate with known concentrations of all drugs and metabolites (10, 20, 50, 100, 200 and 500 ng/ml) were used to construct standard curves. Calibration data were fitted by the least-squares method.

Precision and accuracy

Intra-day variation was determined by analysing sets of seven plasma samples containing the drugs at concentrations of 20, 100 or 200 ng/ml. Inter-day variation was measured by analysing samples with the same concentrations as above over six days.

RESULTS AND DISCUSSION

The simultaneous determination of plasma levels of CIT, DCIT, DDCIT and the deaminated metabolite CIT-PROP in one step was not possible. In the derivatization procedure of CIT-PROP and AT-ACID (internal standard) with

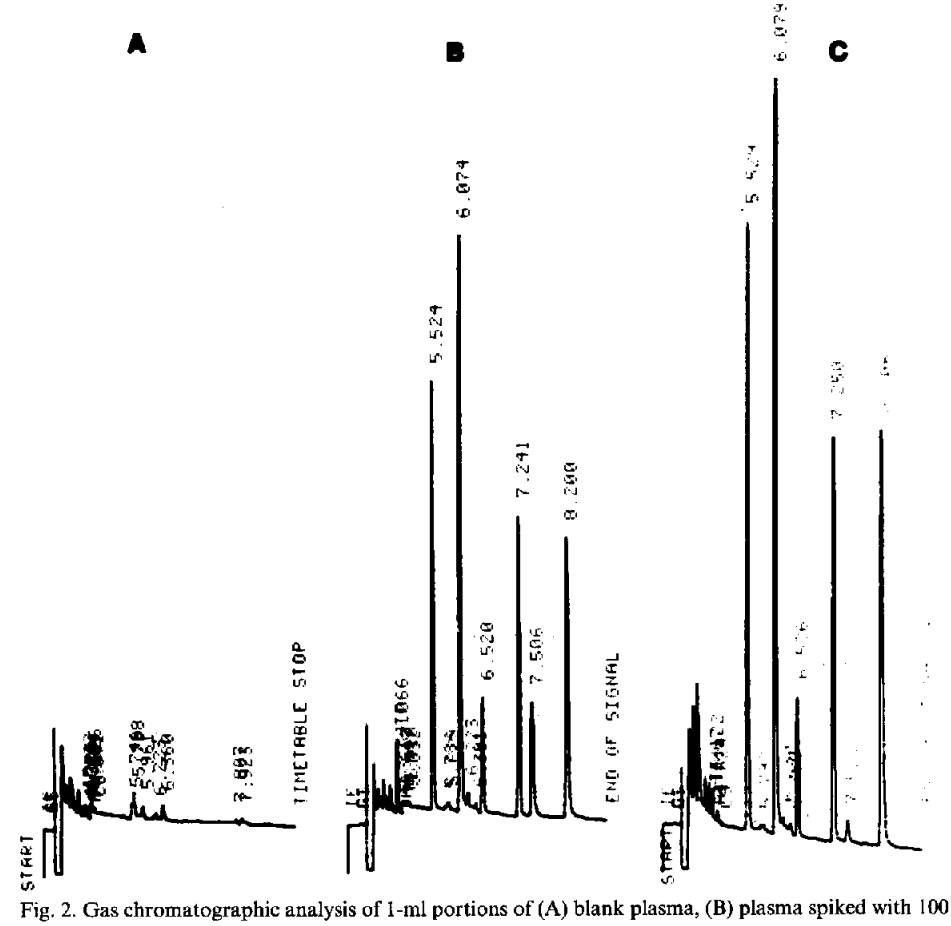

Fig. 2. Gas chromatographic analysis of 1-ml portions of (A) blank plasma, (B) plasma spiked with 100 ng of MMP (retention time, t_R = 5.524 min), CIT (6.074 min), DMP (6.520 min), MP (7.241 min), DDCIT (7.506 min) and DCIT (8.200 min) (native or derivatized compounds) and (C) plasma of a patient treated with citalopram: CIT, 14[illegible]CIT, 109 μg/l; and DDCIT, 15 μg/l.

methyl iodide, DCIT and DDCIT were methylated, resulting in both cases in CIT. Because of high polarity, low volatility and thermal instability of most organic acids, it is essential to convert them into stable volatile derivatives. To achieve a good resolution of DCIT and DDCIT and to avoid peak tailing, they had to be derivatized with TFAA. Fig. 2 presents typical gas chromatograms obtained from blank, spiked and a patient's plasma. They demonstrate the efficiency of the capillary column for separating citalopram and its basic metabolites. The question then arose whether GC was suitable for analysing plasma from a citalopram-treated patient receiving tricyclic drugs as co-medication. Table I lists the relative retention times of native and TFA-derivatized tricyclic drugs, citalopram and their metabolites. In a patient receiving maprotiline as co-medication, imipramine, desipramine and didesmethylimipramine can be used as internal standards (not shown). CIT-PROP cannot be analysed by the present GC–nitrogen–phosphorus detection (NPD) procedure, as the internal standard, AT-ACID, has no nitrogen atom.

In many situations, GC–MS is recommended, as other basic compounds, such as neuroleptics and other antidepressants, may interfere with the assay. EI mass spectra obtained after derivatization of DCIT, DDCIT, MP and DMP with trifluoroacetic anhydride are found to give the highest sensitivity. These findings are in accordance with some, but not all, of the data of Oyehaug and Ostensen [4], who used a lower ionization potential (20 eV) and who restricted MS analysis to the underivatized compounds. In the PICI mode, a considerably less pronounced fragmentation of the intact molecule is observed. There was practically no fragmentation in the case of the TFA derivatives (Table II).

For both GC and GC–MS, the mean overall extraction recoveries for the concentrations 50–500 ng/ml were 80–95% for CIT, 68–92% for DCIT, 26–41% for DDCIT and 45–65% for CIT-PROP (GC–MS), while for the internal standards (100 ng/ml) MMP and MP recovery was 69–77%, 46% for DMP and 71% for AT-ACID (GC–MS). The recovery rates for CIT and DCIT, which have to be considered as the clinically active compounds [1], can be considered to be satisfactory. The correlation coefficients for the calibration curves (linear over the whole range) were $\geqslant 0.999$ and the intercepts were between -0.013 and -0.156 for all compounds (GC and GC–MS). The limits of quantification (signal-to-noise ratio >3) were 1 ng/ml for CIT and DCIT and 2 ng/ml for DDCIT and CIT-PROP. Data concerning the precision and reproducibility of the method are presented in Table III. The EI–SIM recordings of the analysis of a 1-ml plasma sample of a patient treated for five weeks with 60 mg of citalopram are presented in

TABLE I

RELATIVE RETENTION TIMES OF VARIOUS TRICYCLIC DRUGS, OF CITALOPRAM AND THEIR METABOLITES, WITH RESPECT TO AMITRIPTYLINE (t_R = 7.326 min)

TFA = Trifluoroacetic acid derivative.

Compound	Relative retention time
Methadone	0.936
Amitriptyline	1.000
Desmethylnortriptyline	1.000
Nortriptyline	1.023
Imipramine	1.036
Didesmethylimipramine	1.039
Desipramine	1.067
N-Methylmaprotiline	1.150
Desmethylmaprotiline	1.150
Desmethylnortriptyline-TFA	1.155
Maprotiline	1.184
Didesmethylimipramine-TFA	1.195
Citalopram	1.265
Didesmethylcitalopram	1.272
Clomipramine	1.287
Nortriptyline-TFA	1.305
Desmethylcitalopram	1.319
Didesmethylclomipramine	1.319
Desipramine-TFA	1.369
Desmethylmaprotiline-TFA	1.416
Maprotiline-TFA	1.604
Didesmethylclomipramine-TFA	1.605
Didesmethylcitalopram-TFA	1.655
Desmethylclomipramine	1.808
Desmethylcitalopram-TFA	1.841
Desmethylclomipramine-TFA	1.860

TABLE II

GD–MS DATA (IN THE EI, PICI METHANE AND PICI AMMONIA MODES) ON CITALOPRAM, AND ITS METABOLITES AND THEIR DERIVATIVES, AND ON THE INTERNAL STANDARDS

M = Most prominent masses (m/z) and their relative abundance; t_R = typical retention time (see Experimental section); – = poor or no ionization observed.

Compound	t_R (min)	MW	EI mode			
			M 100%	M2 (%)	M3 (%)	M4 (%)
Citalopram	8.35	324	58	324/8.0	238/5.7	–
Desmethylcitalopram	8.72	310	44	238/32.0	57/14.0	310/10.6
Didesmethylcitalopram	8.52	296	238	239/17.8	43/11.0	208/8.6
Desmethylcitalopram-TFA	11.78	406	238	239/17.2	208/5.4	220/5.0
Didesmethylcitalopram-TFA	10.5	392	238	239/16.1	220/5.9	208/5.8
CIT-PROP methyl derivative	8.57	325	238	239/16.8	208/13.7	95/10.1
AT-ACID methyl derivative	6.6	278	205	191/83.0	203/75.0	202/70.2
Methylmaprotiline	7.51	291	58	291/22.9	73/9.2	203/5.5
Maprotiline-TFA	10.33	373	345	191/74.5	346/22.7	218/16.3
Desmethylmaprotiline-TFA	9.09	359	331	191/88.5	332/20.3	191/15.6

Compound	PICI methane mode				PICI ammonia mode			
	M 100%	M2 (%)	M3 (%)	M4 (%)	M 100%	M2 (%)	M3 (%)	M4 (%)
Citalopram	325	326/23.0	353/15.7	58/9.8	325	326/22.6	210/11.7	58/11.0
Desmethylcitalopram	311	312/21.0	339/20.6	291/7.4	311	312/22.8	309/15.8	178/12.1
Didesmethylcitalopram	297	298/20.5	325/18.9	277/10.3	297	164/20.3	298/19.7	295/9.4
Desmethylcitalopram-TFA	–	–	–	–	–	–	–	–
Didesmethylcitalopram-TFA	–	–	–	–	–	–	–	–

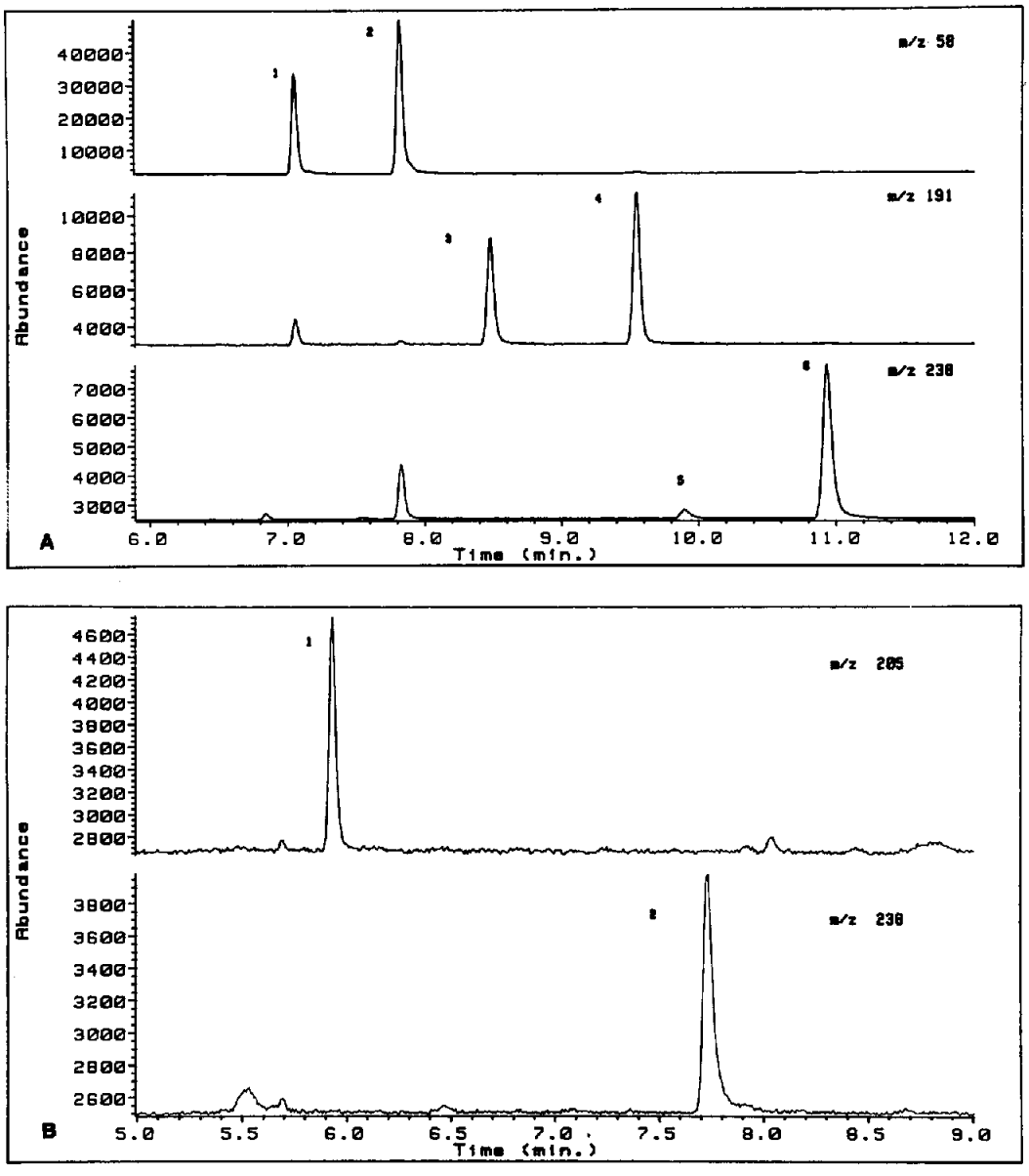

Fig. 3. EI–SIM recordings of a 1-ml plasma extract from a patient treated with 40 mg of citalopram per day for five weeks. (A) 1 = MMP (internal standard); 2 = CIT (124 μg/l); 3 = DMP (internal standard); 4 = MP (internal standard); 5 = DDCIT (11 μg/l); 6 = DCIT (60 μg/l). (B) 1 = AT-ACID (internal standard); 2 = CIT-PROP (78 μg/l).

Fig. 3A and B. The EI–SIM recordings show a low background.

The results suggest that the method is suitable for the analysis of CIT and its active metabolite DCIT in the clinical range. Dufour *et al.* [5] measured steady-state levels of 9–200 μg/l (28–616 nmol/l) and 9–105 μg/l (32–338 nmol/l) for CIT and DCIT, respectively, in patients treated with doses of 30–60 mg of CIT per day.

The present procedure is now being used in a study in which the pharmacogenetics and pharmacokinetics of citalopram are investigated in relation to its clinical profile [11] and in interaction studies [12].

TABLE III

CITALOPRAM ASSAY BY GC AND GC–MS: INTRA-DAY AND INTER-DAY PRECISION

Concentration (ng/ml)		CIT		D-CIT		DD-CIT		CIT-PROP
		GC	GC–MS	GC	GC–MS	GC	GC–MS	GC–MS
Intra-day precision (n = 7)								
20	Mean	20.19	19.79	19.31	19.76	21.21	18.70	21.33
	S.D.	1.10	1.09	1.03	0.85	1.98	1.28	1.12
	C.V. (%)	5.4	5.5	5.4	4.3	9.3	6.8	5.2
100	Mean	98.35	96.93	102.82	103.94	101.57	98.76	111.19
	S.D.	5.57	5.88	3.91	4.97	4.45	2.53	5.14
	C.V. (%)	5.7	6.1	3.8	4.8	4.4	2.6	4.6
200	Mean	204.02	196.53	199.07	196.87	195.32	190.11	215.46
	S.D.	18.83	17.52	8.87	7.24	11.17	14.09	13.40
	C.V. (%)	9.2	8.9	4.5	3.7	5.7	7.4	6.2
Inter-day precision (n = 6)								
20	Mean	20.92	19.87	20.65	20.17	20.68	21.40	20.68
	S.D.	0.74	0.88	0.94	1.23	1.63	2.39	1.36
	C.V. (%)	3.6	4.4	4.5	6.1	7.9	11.2	6.6
100	Mean	100.08	98.30	100.83	98.52	103.42	101.75	101.23
	S.D.	1.53	3.61	1.89	3.49	2.05	3.58	4.47
	C.V. (%)	1.5	3.7	1.9	3.5	2.0	3.5	4.4
200	Mean	201.30	196.02	197.62	203.03	202.25	206.98	196.30
	S.D.	5.14	6.05	4.26	7.15	2.79	4.89	5.30
	C.V. (%)	2.6	3.1	2.2	3.5	1.4	2.4	2.7

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REFERENCES

1 J. Hyttel, *Prog. Neuro-Psychopharmacol. Biol. Psych.*, 6 (1982) 277.
2 P. Kragh-Sørensen, K. Fredricson Overø, O. L. Petersen, K. Jensen and W. Parnas, *Acta Pharmacol. Toxicol.*, 48 (1981) 53.
3 K. Fredricson Overø, *Prog. Neuro-Psychopharmacol. Biol. Psych.*, 6 (1982) 311.
4 E. Oyehaug and E. T. Ostensen, *J. Chromatogr.*, 308 (1984) 199.
5 H. Dufour, M. Bouchacourt, P. Thermoz, A. Viala, Pok Phak Rop, F. Gouezo, A. Durand and H. E. Høpfner Petersen, *Int. Clin. Psychopharmacol.*, 2 (1987) 225.
6 Pok Phak Rop, A. Viala and A. Durand, *J. Chromatogr.*, 338 (1985) 171.
7 Pok Phak Rop, A. Durand and A. Viala, *J. Chromatogr.*, 527 (1990) 226.
8 K. Fredricsen Overø, *J. Chromatogr.*, 224 (1981) 526.
9 W. Dünges and E. Bergheim-Irps, *Anal. Lett.*, 6 (1973) 185.
10 P. Baumann and M. Jonzier-Perey, *Clin. Chim. Acta*, 171 (1988) 211.
11 A. Souche, S. Montaldi, C. Uehlinger, A. Kases, M. J. Reymond, P. Reymond, P. Baumann and H. Dufour, *Encéphale*, 17 (1991) 213.
12 D. Baettig, G. Bondolfi, S. Montaldi, M. Amey and P. Baumann, *Eur. J. Clin. Pharmacol.*, in press.