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Liquid chromatography–positive ion electrospray mass spectrometry method for the quantification of citalopram in human plasma

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Abstract

A rapid, sensitive and novel narrow-bore liquid chromatography–mass spectrometric method was developed and fully validated for the quantification of citalopram in human plasma. The analyte and internal standard (imipramine) were extracted by liquid–liquid extraction with a mixture of hexane–heptane–isopropanol (88:10:2, v/v/v). The use of a Hypersil BDS C_8 micro-bore column (250 mm × 2.1 mm i.d.; 3.5 μm particle size), results in substantial reduction in solvent consumption. The mobile phase consisted of 10 mM ammonium formate–formic acid (pH 4.5) and acetonitrile (30:70, v/v), pumped at a flow rate of 0.15 ml min^{-1}. The analytes were detected after positive electrospray ionization using the selected ion-monitoring mode of the species at *m*/*z* 325 for citalopram and *m*/*z* 281 for imipramine. The method had a chromatographic run time of 10.0 min and a linear calibration curve over the range 0.50–250 ng ml^{-1} ($r^2 > 0.996$). The limit of quantitation was 0.50 ng ml^{-1}. Accuracy and precision were below the acceptance limits of 15%.
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Keywords: Citalopram; Imipramine; High-performance liquid chromatography; Electrospray ionization; Mass spectrometry

1. Introduction

Citalopram (Fig. 1) is a bicyclic benzofurazan derivative that belongs to a class of antidepressants, which preferably increase 5-hydroxytryptamine (5-HT, serotonine) transmission by inhibiting 5-HT uptake. It is administered as a racemic mixture of the (−)-*R*- and (+)-*S*-enantiomers, with the latter having the most potent and selective effect on 5-HT uptake [1,2]. In vitro and in vivo studies in animals suggest that citalopram is a highly selective serotonin reuptake inhibitor (SSRI), having no, or very little, affinity for noradrenaline or dopamine uptake sites, and does not inhibit monoamine oxidase [3]. Bioavailability after oral administration is approximately 80%, with peak plasma levels occurring 2–4 h post-dose [4]. Steady-state plasma levels are achieved in patients in 1–2 weeks. At a daily dose of 40 mg, the average plasma concentration is about 83 ng ml^{-1} with a range from 30 to 200 ng ml^{-1} [5]. The elimination of citalopram is largely mediated via oxidative metabolism in the liver mainly to a primary metabolite *N*-demethylcitalopram, which is then converted to *N*-didemethylcitalopram, citalopram-*N*-oxide, and a deaminated propionic acid derivative [6]. Based on potency relative to citalopram, these metabolites are not considered to have any clinically relevant antidepressant effect [7], while unchanged citalopram is the predominant compound in human plasma [5].

A number of analytical applications have been developed for quantitation or screening purposes of citalopram alone or with other antidepressants [8]. Thus, citalopram has been quantified by reversed-phase liquid chromatographic proce-

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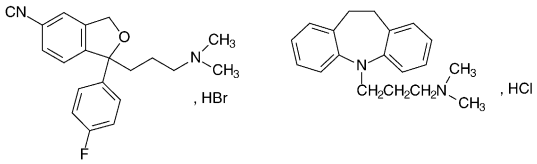

Fig. 1. Chemical structures of citalopram hydrobromide (I) and the internal standard, imipramine hydrochloride (II).

dures coupled with UV [9–14], photodiode array [15–17] and fluorescence [18–24] detection. Micellar electrokinetic capillary chromatography coupled to diode array detection has been recently applied to the determination of citalopram with other antidepressants in biological fluids [25]. Capillary electrophoresis [26] and chiral capillary electrophoresis [27] methods have also been reported and shown to be low cost and selective approaches for the bioanalytical determination of citalopram with limits of quantitation 16.5 and 11.2 ng ml^{-1}, respectively. Gas chromatographic–mass spectrometric [28] and liquid chromatographic–mass spectrometric screening procedures have been reported for the detection of several antidepressants in biofluids and hair samples [29,30]. A GC–MS procedure reported in literature for the determination of citalopram and other selective serotonin reuptake inhibitors in human plasma proved to be the more selective with a limit of quantitation (LOQ) 2 ng ml^{-1} for citalopram [31]. It requires though excessive derivatization procedure prior to the gas chromatographic analysis of the drug.

Although the liquid chromatographic determination of citalopram has been referenced in the literature using conventional chromatographic columns, the application of a narrow-bore liquid chromatographic procedure coupled to mass spectrometric detection has not been described. In recent years, the combination of high-performance liquid chromatography and mass spectrometry (LC–MS) has had a significant influence on drug analysis [32–36]. Moreover, there has been a general trend towards the miniaturization of separation techniques especially with configurations involving electrospray mass spectrometry [37].

The focus of the present study was to develop and validate a rapid, sensitive, selective and environmentally friendly narrow-bore liquid chromatographic procedure coupled to a single quadrupole mass spectrometric detector with a positive electrospray ionization (ESI) interface for the determination of citalopram in human plasma. Narrow-bore columns can be used with common HPLC pumps and operate at optimum flow rates (0.02–0.30 ml min^{-1}) offering the advantage of the lower solvents consumption over the conventional chromatographic methods. These columns could be a better approach for ESI sources than conventional columns because a smaller split or no split ratio is required. While mass spectrometric detection enhances the selectivity and sensitivity of the proposed methodology. The analytical procedure described in this manuscript provides a useful insight into the quantitation of citalopram in human plasma and could be further applied as a routine method to support a wide range of pharmacokinetic studies.

2. Experimental

2.1. Chemicals and reagents

Citalopram hydrobromide, of pharmaceutical purity grade was provided by Neuland Laboratories Limited (Hyderabad, India). The internal standard (IS), imipramine hydrochloride, of pharmaceutical purity grade was kindly provided by Aventis SA (Cedex, France). Water was deionized and further purified by means of a Milli-Q Plus water purification system, Millipore SA (Molsheim, France) and was filtered through a 0.22 μm filter prior to liquid chromatographic procedure. Solvents were of HPLC grade and were purchased from Merck (Darmstadt, Germany). Ammonium formate and sodium hydroxide (analytical reagent grade) were purchased from Sigma–Aldrich (Seelze, Germany) and Panreac Quimica SA (Barcelona, Spain), respectively.

2.2. Liquid chromatographic and mass spectrometric conditions

The HPLC system included a SpectraSeries model P100 isocratic pump, SP ThermoSeparation Products, and a Rheodyne model 7725i injector with a 10 μl loop. The analytical column was a Hypersil BDS C_8 column (250 mm × 2.1 mm i.d.; 3.5 μm particle size), Thermo-hypersil, USA. The mobile phase consisted of 70% acetonitrile in 10 mM ammonium formate–formic acid (pH 4.5) and pumped isocratically at a flow rate of 0.15 ml min^{-1}. The HPLC column temperature was ambient.

Mass spectrometry was performed using a Finnigan AQA mass spectrometer (ThermoQuest, Manchester, UK) equipped with an electrospray ionization (ESI) source operating in the positive ion mode. The ESI probe temperature was set at 200 °C, the capillary voltage was set at 3.5 kV and the source block voltage (AQA_{max}) was set at 20 V for both citalopram and imipramine. Mass spectrometric detection of the analytes was performed in selected ion-monitoring (SIM) mode, citalopram and imipramine were monitored at their base peaks at m/z = 325.0 and 281.3 ($[M + H]^+$), respectively. A Nitrox-N_2 model UHPLCMS12E nitrogen generator, domnick hunter (Gateshead, UK) was used to provide highly pure nitrogen that utilized as sheath and nebulizing gas. Data acquisition and analyses were performed using the Xcalibur (version 1.2) IBM data system running under Windows NT (version 4.0) on an IBM Pentium III computer.

2.3. Stock and working standard solutions

Appropriate amount of citalopram hydrobromide was diluted in acetonitrile–water (50:50, v/v) to prepare a stock standard solution equivalent to 100 μg ml^{-1} of citalopram as free

base. Stock standard solution of imipramine (internal standard) 100 μg ml^{-1}, was prepared by dissolving appropriate amounts of imipramine hydrochloride in acetonitrile–water (50:50, v/v). All stock standard solutions were stored in the dark and under freeze (−20 °C), and were found to be stable for several weeks.

A series of working standard solutions of citalopram, were prepared by the appropriate dilution of the above mentioned stock standard solution in acetonitrile–water (50:50, v/v), to reach concentration ranges of 5.0–2500 ng ml^{-1} for citalopram. A working standard solution of imipramine, 100 ng ml^{-1}, was also prepared in acetonitrile–water (50:50, v/v). The working standard solutions were freshly prepared every week and stored in the dark and under refrigeration.

2.4. Calibration standards and quality control samples

Drug-free human plasma was screened prior to use to ensure that it was free of endogenous interference at the retention time of the analyte. A 50 μl aliquot of the appropriate working standard solution of citalopram along with a 50 μl aliquot of the working standard solution of imipramine were added to a 500 μl aliquot of human plasma. Calibration standard solutions were prepared freshly every day over the concentration range of 0.50, 0.75, 1.0, 1.5, 2.0, 4.0, 10, 20, 40, 100 and 250 ng ml^{-1} for citalopram. The upper concentration level of the calibration curve was set at 250 ng ml^{-1} close to the highest steady-state plasma concentration of citalopram, while the lower concentration level was set at the lower limit of quantitation (LLOQ) of the proposed method. In each calibration sample, 10.0 ng ml^{-1} of the internal standard, imipramine, was added. Quality control samples were prepared in human plasma at three concentration levels (10, 40 and 250 ng ml^{-1}) and additional levels at the lower limit of quantitation (0.50 ng ml^{-1}) and at concentration exceeding the upper limit of quantitation (ULOQ; 1000 ng ml^{-1}). The latter was diluted 10-fold in drug-free human plasma prior to sample processing so as to demonstrate the ability to dilute samples above the ULOQ. All QC solutions were stored at −20 °C. Calibration standard solutions and quality control samples were prepared from separate stock solutions prepared with separate weighing of the analyte.

2.5. Sample preparation

The frozen drug-free human plasma was thawed under room temperature and centrifuged at 2000 × *g* for 15 min at 4 °C to settle solids. A 50 μl aliquot of the IS working standard solution was added to a 500 μl aliquot of plasma sample. The pH value of the plasma sample was adjusted to 12.0 by the addition of a 200 μl aliquot of 0.10 M sodium hydroxide. After a slight vortex, 4 ml of a mixture of hexane–heptane–isopropanol (88:10:2, v/v/v) were added. The samples were mixed for 10 min with an orbital shaker, and centrifuged at 800 × *g* for 10 min. The organic phase was evaporated to dryness at room temperature under a gentle stream of nitrogen. Dry extracts were reconstituted in 200 μl of the mobile phase and 10 μl were injected into the LC–MS system.

2.6. Validation procedures

Eleven plasma calibration standards were prepared in duplicate and analyzed in three separate analytical runs. Calibration curves were calculated based on the measurement of the ratio of peak area signal of citalopram to that of the internal standard. Least-squares linear regression was used to fit the measured signal versus the theoretical concentration. It was also found that a weighting factor of 1/(concentration)^2 gives residuals that are normally distributed over the full concentration range and also decreases the bias from the upper points, and the error at the lower end of the calibration curve.

Quality control samples at four concentration levels (0.50, 10, 40, 250 and 1000 ng ml^{-1}) were analyzed to assess accuracy and precision of the proposed methodology. Six replicates were analyzed in each of three analytical runs. The accuracy was expressed by the relative percentage error, E_r (%), and the precision by relative standard deviation (R.S.D., %). The intra- and inter-day accuracy was required to be within ±20% at the LLOQ level and within ±15% for other concentrations. The precision was required to be less than 20% at the LLOQ level and less than 15% at other concentrations.

Ion suppression effect was also evaluated by analyzing six samples of drug-free human plasma. These samples were processed according to the sample preparation procedure (liquid–liquid extraction). The organic layer was evaporated to dryness under a gentle stream of nitrogen. Dry extracts were reconstituted in the mobile phase adding appropriate aliquot (50 μl) of the 1000 ng ml^{-1} working standard solution of citalopram that represent 100% recovery. Ion suppression was determined by comparing the analytical response of this sample with that of the unextracted standard.

The recovery of the liquid–liquid extraction procedure was evaluated at 250 ng ml^{-1} for citalopram and at 10 ng ml^{-1} for the internal standard. It was determined by comparing the peak areas obtained from the plasma calibration samples after standard analysis, to the peak areas, obtained from extracts of blank plasma samples reconstituted with working standard solutions as described earlier.

Stability of citalopram in human plasma was evaluated by assaying spiked plasma samples containing 100 ng ml^{-1} of citalopram. The spiked plasma samples were analyzed after storage at ambient temperature for 6 h, at 6 °C for 40 days, at −20 °C for 75 days and after three complete freeze–thaw cycles at −20 °C. Peak area measurements obtained from the analysis of the stored samples were compared to the peak measurements that were obtained from the analysis of freshly prepared plasma samples. The analyte was considered stable in the biological matrix when 80–120% of the initial concentration was found.

3. Results and discussion

3.1. Optimization of MS detection and chromatographic conditions

In order to achieve the quantitative determination of citalopram, the electrospray ionization interface parameters need to be optimized for maximum abundance of the molecular ions of the compounds. Acquisition parameters were determined by direct infusion into the mass spectrometer of a 1000 ng ml^{-1} solution (in mobile phase) of each one of the compounds, citalopram and imipramine, respectively, at a flow rate of 20 μl min^{-1}. Variable mass spectrometric conditions (ESI probe temperature; source block voltage, AQA_{max}; capillary voltage) have been investigated. Citalopram and imipramine were monitored at their base peaks at m/z = 325.0 and 281.3 ($[M + H]^+$), respectively. Fig. 2 presents the mass spectrum of citalopram obtained under different source block voltage conditions; AQA_{max} was ranged from 20 to 40 V. It was found that the highest ion intensity for the molecular ion of citalopram was achieved when the source block voltage including the entrance and exit cones, AQA_{max}, was set at 20.0 V. An increase in AQA_{max} voltage to above 25.0 V increases the fragmentation of the compound and thus decreases significantly the abundance of the molecular ion. Moreover, the choice of dominant mass peak at m/z = 325.0 ($[M + H]^+$) for the mass spectrometric detection of citalopram was crucial for the selectivity of the method at this m/z none of its major metabolites (*N*-demethylcitalopram, MW = 310.3; *N*-didemethylcitalopram, MV = 296.3) exhibit a mass signal. Fig. 3 represents a mass spectrum of the IS, imipramine obtained under the optimized MS conditions described in Section 2.

The pH of the aqueous phase of the liquid chromatographic eluent influences both the chromatographic elution of the compounds and the formation of the $[M + H]^+$ molecular ions and is strongly related to their degree of ionization. The pK_a values of the analyte and IS were calculated

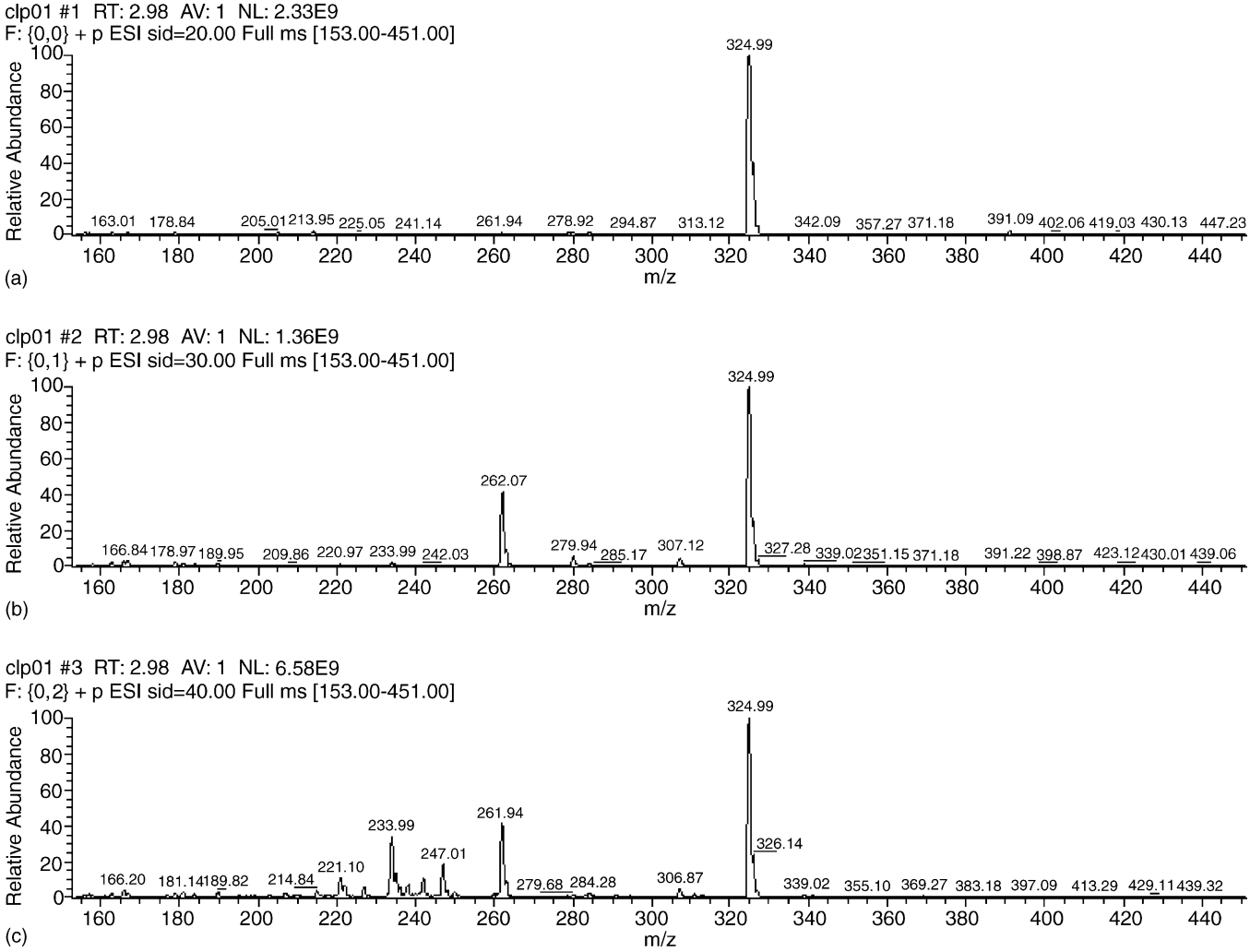

Fig. 2. Mass spectra of a 1000 ng ml^{-1} citalopram standard solution in a mixture of 70% acetonitrile in 10 mM ammonium formate–formic acid (pH 4.5). MS conditions: positive ESI mode; ESI probe temperature 200 °C; capillary voltage 3.5 kV; source block voltage AQA_{max}: (a) 20 V, (b) 30 V and (c) 40 V; AQA flow rate 20 μl min^{-1}.

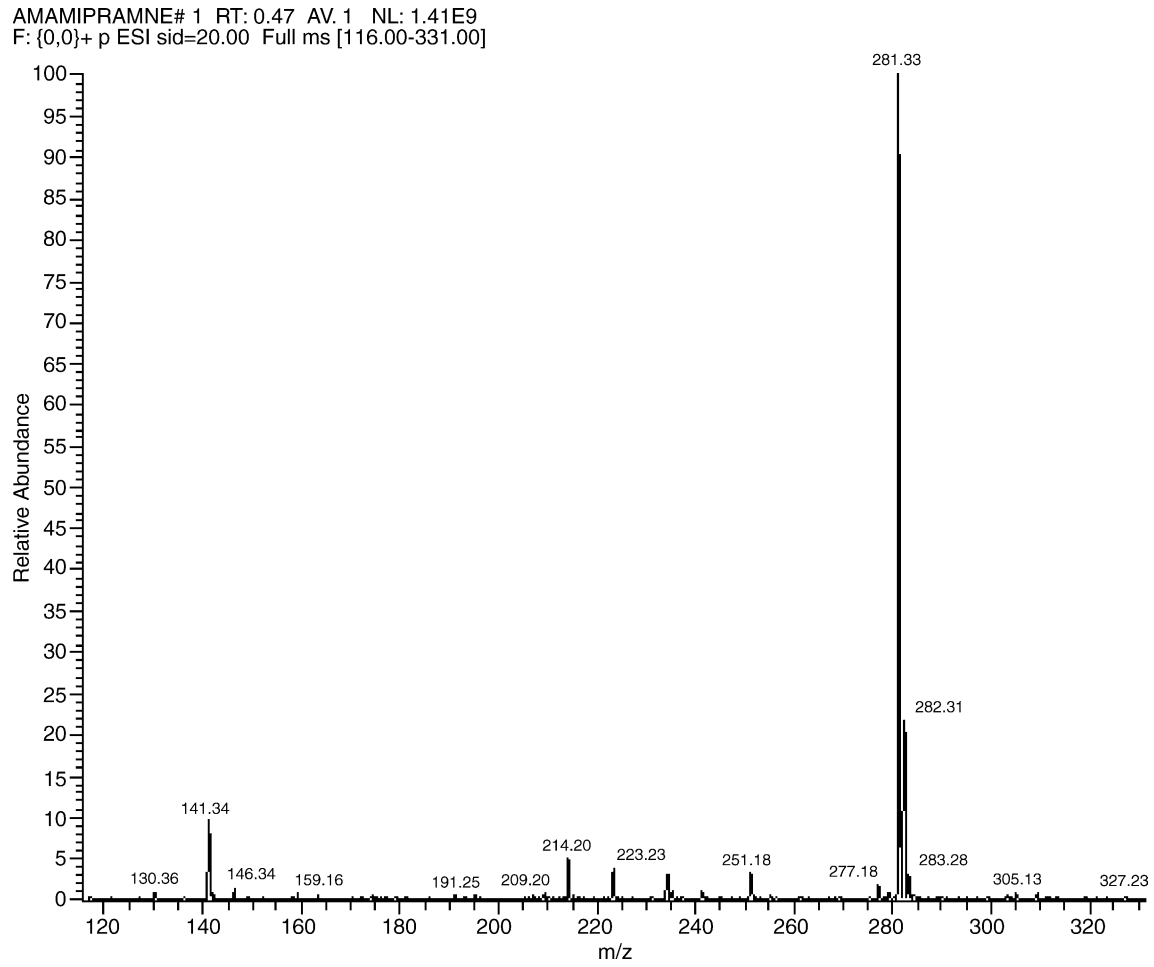

Fig. 3. Mass spectra of a 1000 ng ml^{-1} imipramine standard solution in a mixture of 70% acetonitrile in 10 mM ammonium formate–formic acid (pH 4.5). MS conditions: positive ESI mode; ESI probe temperature 200 °C; capillary voltage 3.5 kV; source block voltage AQA_{max} 20 V; AQA flow rate 20 μl min^{-1}.

using the ACD/ChemSketch, pK_a predictor version 3.0 software. As both citalopram and imipramine are basic compounds with pK_a values 9.59 and 9.66, respectively, the use of slightly acidic solutions favours ionization of the analytes by protonation of their basic sites. Therefore, it was found that, positive ionization of the compounds in the electrospray ion source increases in acidic eluents. Additionally, a decrease of pH from 6.0 to 4.5 causes concomitant decrease in the retention times of both compounds and exceptional improvement in the chromatographic peak shapes. Hence, an eluent consisting of acetonitrile–10 mM ammonium formate–formic acid (pH 4.5) (30:70, v/v) appeared to be most appropriate for the LC–MS analysis of citalopram from both mass spectrometric and chromatographic point of view. Furthermore, the chromatographic elution of the analyte and the IS could be obtained in less than 10.0 min. A mass fragmentogram obtained from the analysis of a plasma sample spiked with 100 ng ml^{-1} of citalopram and 10.0 ng ml^{-1} of the internal standard is displayed in Fig. 4 and illustrates the selectivity of the proposed chromatographic procedure. Under the current chromatographic conditions, citalopram and imipramine were eluted at 5.70 and 6.61 min, respectively.

3.2. Statistical analysis of data

Calibration standards were analyzed in duplicate in three analytical runs to determine the citalopram concentration in the range 0.50–250 ng ml^{-1} with a coefficient of determination better than 0.996 in all runs. Linear fits with a weighting factor 1/(concentration)^2 appeared to be most appropriate to describe the signal versus concentration curves. Analytical parameters of the calibration equations for the determination of citalopram are presented in Table 1. In order to evaluate further the linearity of the proposed method, duplicate measurements were used for the preparation of the five calibration curves over a period of 4 weeks. The average regression equations are also presented in Table 1, along with the R.S.D. values of the slopes and intercepts.

A Student's *t*-test was performed to determine whether the experimental intercepts (α) of the above mentioned regression equations were significantly different from the

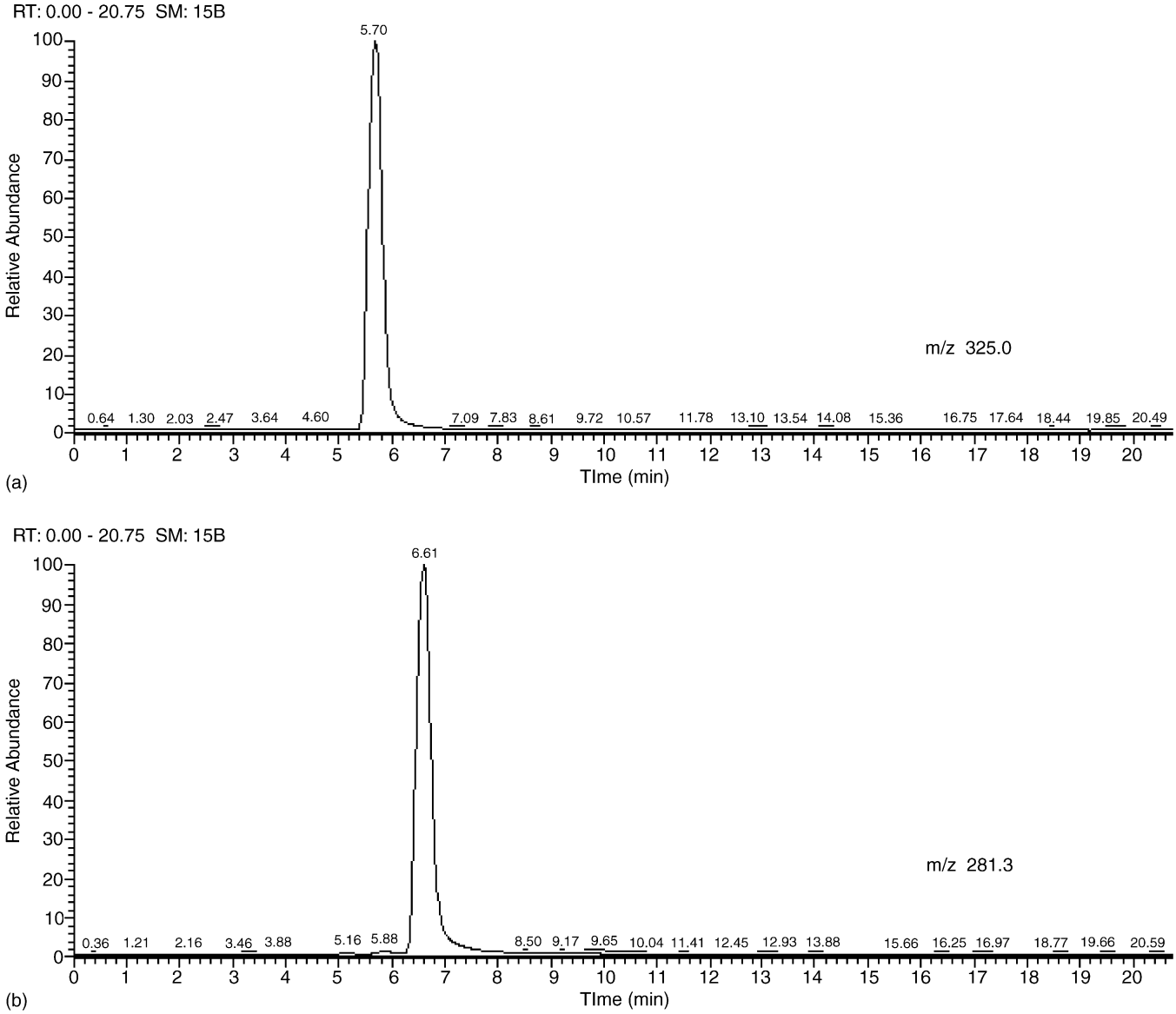

Fig. 4. Mass fragmentogram obtained from the analysis of mixed standard solutions containing 100 ng ml^{-1} citalopram (a) and 10 ng ml^{-1} imipramine (b). Chromatographic conditions: reversed-phase HPLC on a semi-micro BDS C_8 column; mobile phase: 10 mM ammonium formate–formic acid (pH 4.5) and acetonitrile (30:70, v/v); flow rate 0.15 ml min^{-1}.

Table 1
Analytical parameters of the calibration equations for the determination of citalopram by liquid chromatography–electrospray ionization mass spectrometry

Concentration range (ng ml^{-1})	Regression equation[a]	r[b]	S.D.[c]		S_r[d]	α/S_α[e]
			Slope	Intercept		
Mean of five calibration curves over a period of 4 weeks						
0.50–250	$R_{Ctl} = 2.201 \times C_{Ctl} + 0.19$	>0.996	0.290	0.11	<0.14	<1.70

[a] Ratio of the peak area of citalopram to that of the internal standard, R_{Ctl}, vs. the corresponding concentration; $C_{Ctl.}$ is the concentration of citalopram.
[b] Correlation coefficient.
[c] Standard deviation of slope and intercept.
[d] Standard error of the estimate.
[e] Theoretical value of t at $P = 0.025$ level of significance, for $f = n - 2 = 9$ d.f., 2.26.

Table 2
Accuracy and precision of intra- and inter-assay analysis for the determination of citalopram in human plasma by liquid chromatography–electrospray ionization mass spectrometry (n = 3 days, six replicates per day)

	Concentration (ng ml^{-1})				
	0.50	10.0	40.0	250	1000
Day 1 (mean ± S.D.)	0.51 ± 0.07	10.3 ± 1.2	39.6 ± 3.5	248.9 ± 5.8	962.2 ± 9.0
Day 2 (mean ± S.D.)	0.50 ± 0.08	10.7 ± 1.9	37.6 ± 2.2	247.1 ± 7.6	953.3 ± 6.4
Day 3 (mean ± S.D.)	0.54 ± 0.09	11.1 ± 1.5	37.8 ± 2.2	245.5 ± 6.8	953.5 ± 4.6
Overall mean	0.52	10.7	38.3	247.1	956.3
Intra-assay R.S.D. (%)[a]	15.0	14.5	7.0	2.7	0.7
Inter-assay R.S.D. (%)[a,b]	N.V.	N.V.	N.V.	N.V.	1.8
E_r (%)[c]	4.0	7.0	−4.2	−1.2	−4.4
Overall mean recovery (%)	104.0	107.0	95.8	98.8	96.0

[a] Percentage relative standard deviation; intra- and inter-assay R.S.D. were calculated by ANOVA.
[b] N.V.: no significant additional variation was observed as a result of performing the assay on different days.
[c] Relative percentage error = (overall mean assayed concentration − added concentration)/(added concentration) × 100.

theoretical zero value. The test is based on the calculation of the quantities $t = \alpha/S_\alpha$, where α is the intercept of the regression equations and S_α is the standard deviation of α, and their comparison with tabulated data of the t-distribution. The calculated t-values are also presented in Table 1, these values do not exceed the 95% criterion of t_P = 2.26 for f = 9 d.f., which denotes that the intercept of all regression lines is not significantly different from zero.

One-way analysis of variance (ANOVA) with a grouping variable of day was used for the evaluation of intra- and inter-assay precision. Results presented in Table 2 indicate an intra-assay R.S.D. (%) ranging from 0.7 to 15.0% for citalopram. Moreover, no significant additional variation was observed as a result of performing the assay on different days. The overall accuracy was assessed by the relative percentage error, E_r (%), which was ranged from −1.2 to 7.0% for citalopram.

The limit of detection for citalopram was determined according to the definitions of ICH Topic Q2B [38]. Therefore, it was calculated using the equations $y - \alpha = 3.3 \times S_\alpha$ and $y - \alpha = b \times$ LOD (where b is the slope and S_α is the standard deviation of the intercept of the regression line) and it was found to be at the level of 0.16 ng ml^{-1}. The limit of quantitation for citalopram was also determined according to the definitions of ICH Topic Q2B [38]. Thus, it was calculated using the equations $y - \alpha = 10 \times S_\alpha$ and $y - \alpha = b \times$ LOQ (where b is the slope and S_α is the standard deviation of the intercept of the regression line). The limit of quantitation was found to be at the level of 0.50 ng ml^{-1}. In Fig. 5 is presented a representative mass fragmentogram

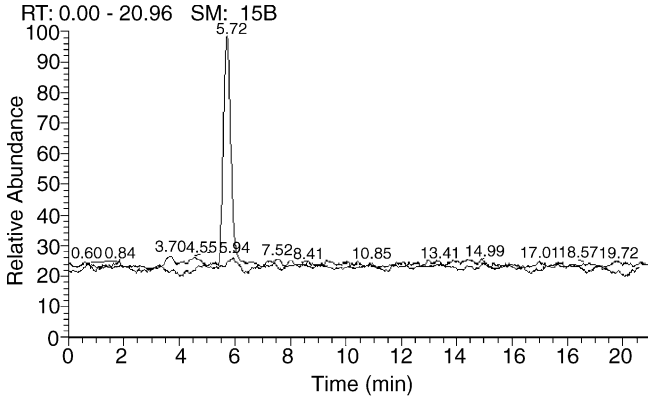

Fig. 5. Representative mass fragmentograms of a calibration plasma sample at the LLOQ level, 0.50 ng ml^{-1} of citalopram overlaid with a chromatogram of an extracted blank plasma sample; the retention time of citalopram is 5.72 min. Chromatographic conditions: reversed-phase HPLC on a semi-micro BDS C_8 column; mobile phase: 10 mM ammonium formate–formic acid (pH 4.5) and acetonitrile (30:70, v/v); flow rate 0.15 ml min^{-1}.

obtained from the analysis of a sample spiked at the LLOQ level of citalopram along with a chromatogram obtained from the analysis of a blank plasma sample.

Moreover, the selectivity towards endogenous plasma compounds was tested with six different lots of analyte free human plasma by analyzing blanks (non-spiked plasma) and samples spiked separately with 0.50 ng ml^{-1} of citalopram (LLOQ level). Thus, chromatograms of six batches of drug-free plasma contained no co-eluting peaks >20% of the

Table 3
Stability data for citalopram in human plasma under various storage conditions

Storage conditions	Time	Concentration (ng ml^{-1})		Deviation (%)[a]	R.S.D. (%, n = 3)[b]
		Initial	Found		
Ambient temperature	6 h	100.1	99.45	−0.65	4.9
6 °C	40 days	98.9	106.0	7.20	6.3
−20 °C	75 days	99.3	104.7	5.40	5.1
Freeze–thaw cycles (−20 °C)	3 cycles	100.2	99.4	−0.77	3.6

[a] Percentage of mean deviation from t = 0.
[b] Percentage relative standard deviation.

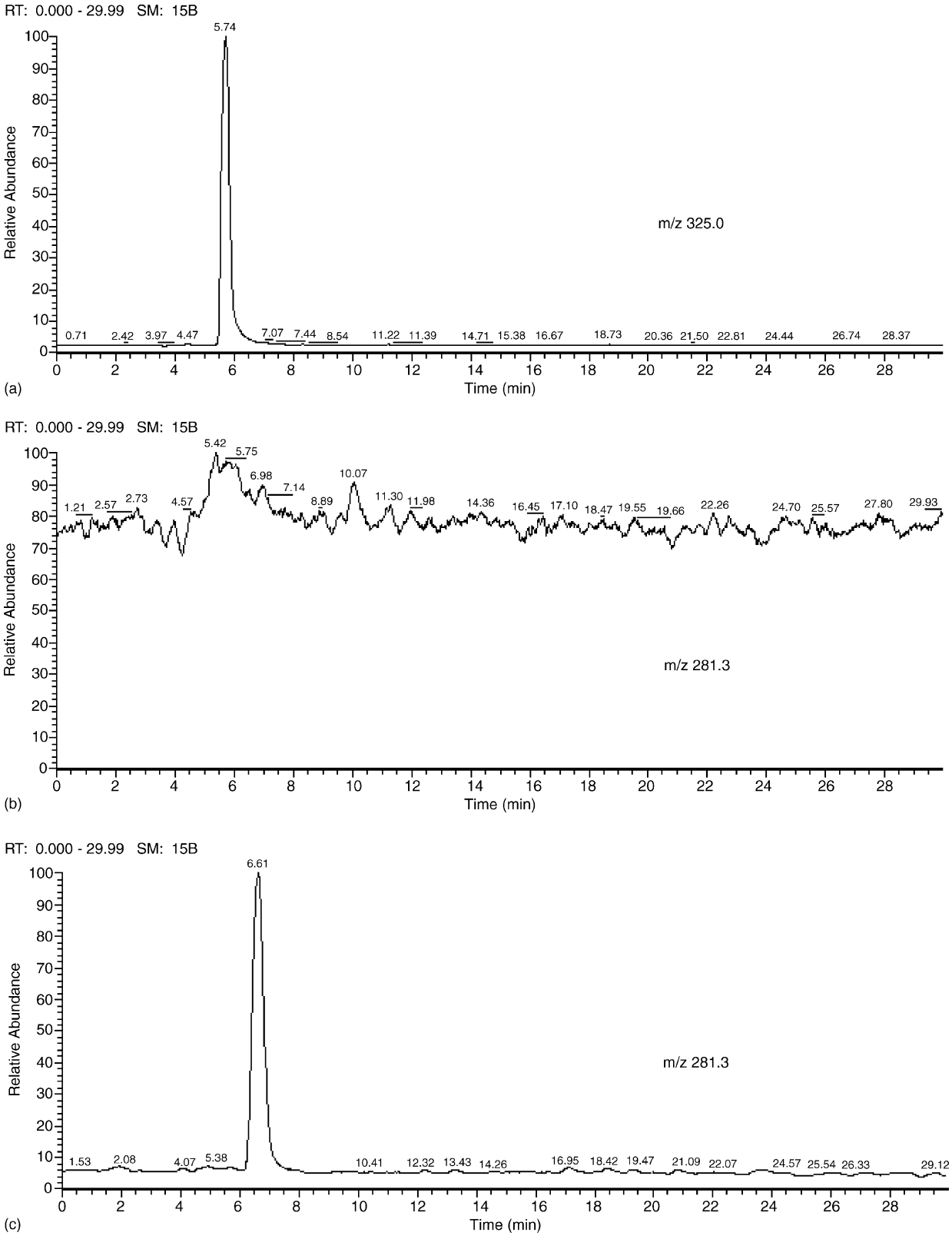

Fig. 6. Representative mass fragmentogram obtained from the analysis of a clinical sample containing citalopram (a), without imipramine (IS) addition (b) and with imipramine (IS) addition (c). Citalopram and imipramine is eluted at 5.70 and 6.61 min, respectively. Chromatographic conditions: reversed-phase HPLC on a semi-micro BDS C_8 column; mobile phase: 10 mM ammonium formate–formic acid (pH 4.5) and acetonitrile (30:70, v/v); flow rate 0.15 ml min^{-1}.

citalopram area at the LLOQ level, and no co-eluting peaks >5% of the area of the internal standard imipramine.

A thorough investigation was conducted in order to choose the optimum solvent mixture for the liquid–liquid extraction procedure. A number of organic solvents such as hexane and heptane were tested for the extraction procedure leading to poor recoveries (less than 60.0%) for both of the analyte and IS. It was found that the addition of a 2% of isopropanol in a mixture of hexane and heptane led to almost complete extraction recoveries of the compounds. Thus, a mixture of hexane–heptane–isopropanol (88:10:2, v/v/v) was chosen as the optimum solvent mixture for the extraction procedure. Under the optimum extraction conditions, overall recovery (liquid–liquid extraction plus ion suppression) of plasma samples spiked with 250.0 ng ml^{-1} for citalopram and at 10 ng ml^{-1} for IS was found to be 94.2 ± 4.4 and 89.1 ± 5.2% for citalopram and IS, respectively.

Ion suppression recovery of drug-free plasma samples spiked after the sample preparation with 100.0 ng ml^{-1} of citalopram was found to be 97.9 ± 2.3%.

The stability results presented in Table 3 indicate that the analyte can be considered stable under the various conditions investigated, since its concentrations deviate by no more than 7.2% relative to the reference for any of the tests and no degradation products were observed. Calibration plasma samples containing citalopram may therefore be kept for up to 75 days at −20 °C, 40 days at 6 °C and for 6 h at ambient temperature without any significant degradation.

3.3. Sample analysis

The method was successfully applied to the analysis of citalopram in a plasma sample obtained from a 53-year-old female psychiatric patient, with a body weight of 65 kg. The patient was administered with 20 mg of citalopram daily (10:00 p.m.) for a long-term treatment. The patient was also receiving 10 mg bisoprolol fumarate daily for antihypertensive treatment and occasionally 1.5 mg of bromazepam. The plasma sample was analyzed one day after storage at −20 °C. Three aliquots (500 μl) of this plasma sample were analyzed according to the sample preparation procedure (IS was added) in order to calculate the concentration of citalopram. Citalopram plasma concentration was found to be 90.8 ± 3.8 (n = 3). Fig. 6 illustrates a typical MS fragmentogram obtained from the analysis of a real plasma sample without IS addition so as to demonstrate that there is no interference at the retention time of imipramine.

4. Conclusions

The proposed liquid chromatographic–mass spectrometric method enables a rapid, accurate and selective assay for the determination of citalopram in human plasma with a run time lower than 10.0 min. The method consists of a simple liquid–liquid extraction for sample pretreatment and an environmentally friendly chromatographic procedure using a narrow-bore column. Mass spectrometric detection increases the sensitivity and selectivity of the proposed method. Recovery and precision studies successfully quantified citalopram in spiked plasma samples. The method had a LLOQ of 0.50 ng ml^{-1} and proved to be superior in sensitivity and speed of analysis with the analytical methods reported previously. Thus, the proposed method is suitable to support a wide range of pharmacokinetic studies.

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