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Enantioselective Analysis of Citalopram and Demethylcitalopram in Human and Rat Plasma by Chiral LC-MS/MS: Application to Pharmacokinetics

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ABSTRACT Citalopram (CITA) is available as a racemic mixture and as a pure enantiomer. Its antidepressive action is related to the (+)-(*S*)-CITA and to the metabolite (+)-(*S*)-demethylcitalopram (DCITA). In the present investigation, a method for the analysis of CITA and DCITA enantiomers in human and rat plasma was developed and applied to the study of pharmacokinetics. Plasma samples (1 ml) were extracted at pH 9.0 with toluene:isoamyl alcohol (9:1, v/v). The CITA and DCITA enantiomers were analyzed by LC-MS/MS on a Chiralcel® OD-R column. Recovery was higher than 70% for both enantiomers. The quantification limit was 0.1 ng/ml, and linearity was observed up to 500 ng/ml plasma for each CITA and DCITA enantiomer. The method was applied to the study of the kinetic disposition of CITA administered in a single oral dose of 20 mg to a healthy volunteer and in a single dose of 20 mg/kg (by gavage) to Wistar rats (n = 6 for each time). The results showed a higher proportion of the (−)-(*R*)-CITA in human and rat plasma, with *S/R* AUC ratios for CITA of 0.28 and 0.44, respectively. *S/R* AUC ratios of DCITA were 0.48 for rats and 1.04 for the healthy volunteer. *Chirality 19:793–801, 2007.*

KEY WORDS: citalopram; enantiomers; pharmacokinetics; metabolism; LC-MS/MS

INTRODUCTION

Citalopram (CITA) is a selective serotonin (5-HT) reuptake inhibitor used for the treatment of depression. Because of its poor affinity for histaminergic, muscarinic, and adrenergic receptors, CITA presents a lower incidence of anticholinergic effects compared to conventional antidepressive drugs. The drug is available in the form of a racemic mixture or as a pure enantiomer. Its antidepressive action is related to the (+)-(*S*)-CITA enantiomer and to the metabolite (+)-(*S*)-demethylcitalopram (DCITA). The (−)-(*R*)-CITA enantiomer and its metabolite (−)-(*R*)-DCITA are relatively weak selective inhibitors of serotonin reuptake. In vitro studies suggest that the (+)-(*S*) enantiomer is twice as potent as the racemic mixture as a 5-HT reuptake inhibitor and 100 times more potent than the (−)-(*R*)-CITA antipode.[1–4]

Peak plasma concentration (C_{max}) of CITA is observed within 1–4 h after administration of a single 40 mg dose of the drug to healthy volunteers. Oral bioavailability is high (∼80%). The distribution volume (Vd/F) ranges from 1.2 to 1.6 l, and about 50–80% of the drug and its metabolites are bound to plasma proteins. CYP2C19, CYP2D6, and CYP3A4 are involved in the demethylation of CITA, with preferential metabolism of the (+)-(*S*)-CITA enantiomer. The main metabolites formed are DCITA and didemethylcitalopram (DDCITA).[4,5]

Studies conducted on healthy volunteers who were phenotyped as extensive metabolizers of sparteine and mephenytoin (substrates for CYP2D6 and CYP2C19, respectively) and treated with a single dose of 40 mg showed that the (+)-(*S*)-CITA enantiomer and its metabolites [(+)-(*S*)-DCITA and (+)-(*S*)-DDCITA] were eliminated faster than their antipodes. The elimination half-lives ($t_{1/2\beta}$) for (+)-(*S*)-CITA, (+)-(*S*)-DCITA, and (+)-(*S*)-DDCITA were 31, 42, and 60 h, respectively, whereas half-lives of 47, 65, and 102 h were reported for (−)-(*R*)-CITA, (−)-(*R*)-DCITA, and (−)-(*R*)-DDCITA. The enantiomeric *S/R* ratios relative to the area under the plasma concentration versus time curve ($AUC^{0-\infty}$) for CITA, and its metabolites were less than a unit in healthy volunteers, with ratios of 0.59, 0.74, and 0.48 for CITA, DCITA, and

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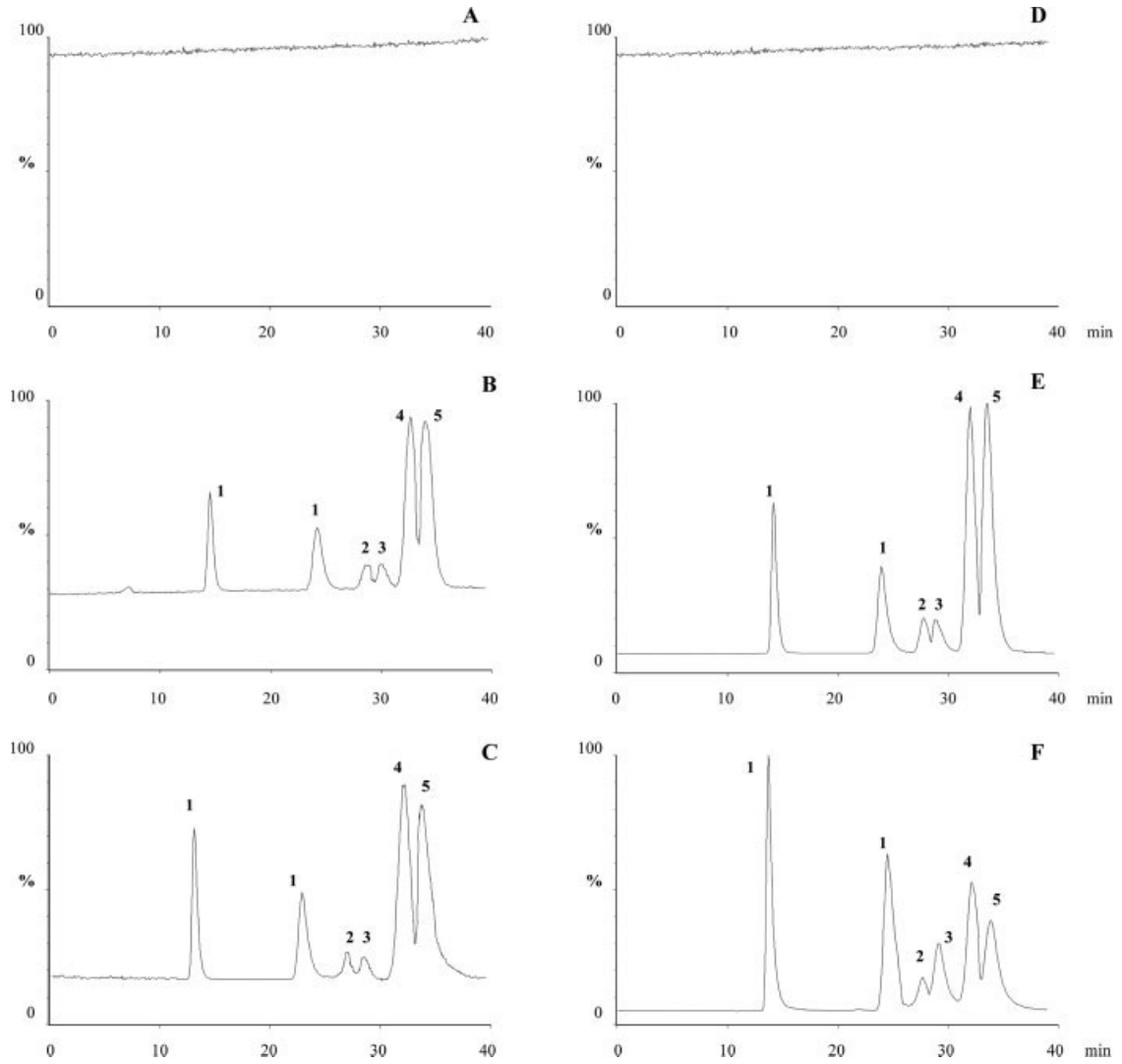

Fig. 1. Chromatograms of (**A**) blank human plasma, (**B**) human plasma sample spiked with *rac*-CITA, *rac*-DCITA, and pindolol, (**C**) human plasma after a single oral dose of 20 mg *rac*-CITA, (**D**) blank rat plasma, (**E**) rat plasma sample spiked with *rac*-CITA, *rac*-DCITA, and pindolol, (**F**) rat plasma after a single oral dose of 20 mg/kg *rac*-CITA. Peaks: (**1**) Pindolol, (**2**) (+)-(*S*)-DCITA, (**3**) (−)-(*R*)-DCITA, (**4**) (−)-(*R*)-CITA, and (**5**) (+)-(*S*)-CITA.

DDCITA, respectively. The higher ratio for DCITA might indicate a smaller influence of stereoselectivity or a lower demethylation rate of (−)-(*R*)-CITA compared with (+)-(*S*)-CITA.[6]

The *S*/*R* plasma concentration ratios of CITA and DCITA were 0.94 and 0.36, respectively, in rats treated with racemic CITA through an osmotic pump (10 mg/kg/day) for 14 days.[7] Kugelberg et al.[8] reported *S*/*R* plasma concentration ratios of 0.94, 0.83, and 0.34 for CITA and of 0.85, 0.37, and 0.36 for DCITA in rats treated with multiple doses of 10, 20, and 100 mg CITA/kg/day for 10 days, respectively, suggesting that a dose increase reduces the *S*/*R* ratio of CITA and DCITA. Kugelberg et al.[9] demonstrated enantioselectivity in the metabolism of CITA in rats receiving subcutaneous administration of a single dose of 20 or 100 mg/kg. The authors reported *S*/*R* plasma concentration ratios of 0.7 and 0.6 for CITA 10 and 20 h after administration of the racemic drug, respectively.

The enantioselectivity of CITA and its metabolites in plasma has been analyzed by HPLC, with UV or fluorescence detection, and by capillary electrophoresis.[2,10–15] The LC-MS and LC-MS/MS systems have only been used for the analysis of CITA and/or its metabolites in plasma and urine as enantiomeric mixture[16–19] or when CITA was administered as the pure enantiomer.[20]

In the present study, we report for the first time the enantioselective analysis of CITA and its metabolite DCITA in plasma of rats and healthy volunteers treated with a single dose of racemic CITA by LC-MS/MS using chiral phase column.

MATERIALS AND METHODS

Standard Solutions and Reagents

The stock solutions of racemic citalopram hydrobromide (CITA, Tocris, Ellisville, MO) and demethylcitalo-

pram hydrochloride (DCITA, H. Lundbeck A/S, Copenhagen, Denmark) were prepared in methanol at a concentration of 1 mg/ml. Each solution was then diluted to concentrations of 80, 320, 800, 1600, and 4000 ng/ml methanol for analysis of rat plasma and to concentrations of 8, 20, 40, 80, and 200 ng/ml methanol for analysis of human plasma. A solution of pindolol (internal standard; Novartis, Switzerland) was prepared at a concentration of 5 μg/ml methanol.

The solvents used for preparation of the solutions, for extraction of CITA and DCITA, and as components of the mobile phase of the HPLC system were purchased from Merck (Darmstadt, Germany) and were of chromatographic grade. Water was purified with the Milli-Q Plus system (Millipore, Bedford, MA).

Chromatographic Analysis

The HPLC system consisted of a Shimadzu chromatograph (Kyoto, Japan) equipped with an LC-10 AD pump and a CTO-10 AS oven. The CITA and DCITA enantiomers were resolved on a chiral Chiralcel® OD-R column (particle size: 10 μm, 250 × 4.6 mm; Chiral Technologies, Exton, PA) using a LiChrospher® 100 RP-8 precolumn (particle size: 5 μm, 4 × 4 mm; Merck). The mobile phase for elution of the enantiomers on the Chiralcel OD-R column consisted of acetonitrile, methanol, and water (30:30:40, v/v/v) containing 0.05% diethylamine. The enantiomers were eluted at a flow rate of 0.5 ml/min. The column was kept at (24 ± 1)°C.

The Quattro Micro triple quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with an electrospray interface (ESI) was used as the mass spectrometry detection system (MS/MS) for the analysis of CITA and DCITA. The analyses were carried out in the positive electrospray mode. The capillary voltage of the ESI was 3.0 kV. The temperatures of the source and desolvation system were kept at 120 and 200°C, respectively. Nitrogen was used as the nebulizer gas at a flow rate of 365 l/h. Argon was used as the collision gas at a pressure of $\sim 2.1 \times 10^{-3}$ mbar. The cone voltage was maintained at 30 V for CITA and internal standard and at 35 V for DCITA. The collision energy was 25 eV for CITA and DCITA and 15 eV for the internal standard.

The conditions for the optimization of MS/MS were determined by direct infusion of standard solution (10 μg/ml) prepared in the mobile phase and introduced with an infusion pump at a flow rate of 20 μl/min. The analyses were carried out in the multiple reaction monitoring mode. Protonated ions $[M + H]^+$ and their respective ion products were monitored at the following transitions: 325.3 > 310.9 for CITA, 311.4 > 108.7 for DCITA, and 249.1 > 172.2 for the internal standard. Data acquisition and sample quantification were performed with the MassLynx version 3.5 program (Micromass).

Sample Preparation

Blank plasma samples were obtained from healthy volunteers (not treated with CITA) recruited from the Blood Center of the local University Hospital. Blank plasma samples of rats were obtained by decapitation of animals not treated with CITA and provided by the Central Animals House of the University of São Paulo (USP), Ribeirão Preto Campus.

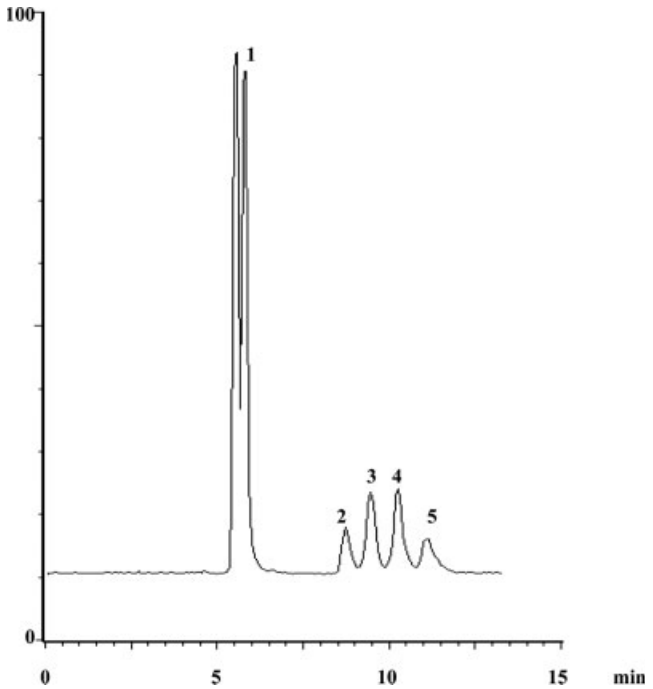

Fig. 2. Chromatograms of CITA and DCITA. Column: Chirobiotic V, mobile phase: methanol:acetic acid:triethylamine (99.9:0.055:0.06, v/v/v). Peaks: (**1**) Pindolol, (**2**) (−)-(*R*)-DCITA, (**3**) (−)-(*R*)-CITA, (**4**) (+)-(*S*)-CITA, and (**5**) (+)-(*S*)-DCITA.

For the analysis of CITA and DCITA, 1-ml aliquots of human or rat plasma were supplemented with 25 μl internal standard, 100 μl of 1 M sodium hydroxide, 200 mg sodium chloride, and 5 ml toluene:isoamyl alcohol (9:1, v/v). The samples were extracted by shaking in a horizontal shaker for 30 min and centrifuged at 2000*g* for 10 min. The organic phases were transferred to conic tubes and evaporated to dryness in a vacuum evaporation system (RCT90 and RC10.22, Jouan AS, St. Herblain, France) at 25°C. The residues were resuspended in 100 μl of the mobile phase and 100 μl hexane, shaken for 30 sec, and centrifuged for 1 min, and 60 μl of the aqueous phase was injected in the chromatographic column. All analytical procedures were carried out under yellow light because of the photosensitivity of CITA and DCITA.

Determination of the Elution Order of the CITA and DCITA Enantiomers

An aliquot (25 μl) of CITA and DCITA solution (4 μg/ml methanol) was evaporated to dryness at room temperature. The residue was resuspended in 100 μl of the mobile phase consisting of acetonitrile, methanol, and water (30:30:40, v/v/v), containing 0.05% diethylamine, and 20 μl of the mixture was submitted to chromatographic analysis on a Chiralcel OD-R column with UV detection at 240 nm.

TABLE 1. Matrix effect for CITA, DCITA, and IS in five different lots of human and rat plasma (mean data)

	Matrix effect (%)				
Nominal concentration (ng/ml)	(−)-*R*-CITA	(+)-*S*-CITA	(+)-*S*-DCITA	(−)-*R*-DCITA	IS
Human plasma					102.5
2.5	97.6	95.0	98.0	105.5	
5.0	94.0	101.0	103.0	101.0	
10.0	99.0	97.0	97.0	97.0	
Rat plasma					107.0
5	101.0	100.4	96.6	100.3	
50	107.0	97.2	106.3	98.3	
500	98.5	97.5	102.0	101.2	

Fractions of the eluate corresponding to the separated enantiomers were collected and the enantiomers were extracted as described earlier. After drying, the residues were submitted to chromatographic analysis as described by Kosel et al.,[13] using a Chirobiotic® V column and a mobile phase consisting of methanol, acetic acid, and triethylamine (99.9:0.055:0.06, v/v/v).

Validation

The calibration curves were constructed from 1 ml samples of drug-free plasma spiked with 25 μl of each diluted standard solution of CITA and DCITA. The linear regression equations and the correlation coefficients were obtained from the peak areas plotted against their respective concentrations (0.1–20 ng/ml human plasma and 0.1–500 ng/ml rat plasma for CITA and DCITA).

Recovery of CITA and DCITA was evaluated by comparing the areas of the peaks obtained after plasma extraction with the areas of the peaks obtained after injection of standard solutions. Standard solutions (25 μl) were evaporated to dryness, the residues obtained were dissolved in 100 μl of the mobile phase and 100 μl hexane, shaken for

TABLE 2. Confidence limits obtained for the analysis of CITA and DCITA enantiomers in human plasma

	(−)-*R*-CITA	(+)-*S*-CITA	(+)-*S*-DCITA	(−)-*R*-DCITA
Recovery (%)				
1 ng/ml	94.0	86.0	93.0	96.0
5 ng/ml	87.5	88.0	72.9	73.1
10 ng/ml	102.0	100.7	82.8	84.8
Linearity (ng/ml)	0.1–20	0.1–20	0.1–20	0.1–20
r	0.99245	0.99049	0.99481	0.99631
LOQ (ng/ml)	0.1	0.1	0.1	0.1
Precision (RSD %, $n = 5$)	7.47	6.43	8.83	10.71
Accuracy (% bias)	12	10	7.00	4.00
Interassay precision ($n = 5$, RSD %)				
1 ng/ml	9.43	6.59	10.17	10.81
10 ng/ml	8.81	14.39	9.43	8.51
Intraassay precision (RSD %)				
1 ng/ml ($n = 10$)	10.47	9.90	10.48	10.91
10 ng/ml ($n = 10$)	8.07	9.41	5.55	3.50
Interassay accuracy (% bias)				
1 ng/ml ($n = 5$)	−2.90	3.20	2.00	3.00
10 ng/ml ($n = 5$)	−1.70	−5.70	0.10	−2.90
Intraassay accuracy (% bias)				
1 ng/ml ($n = 10$)	−4.40	−1.40	0.50	5.20
10 ng/ml ($n = 10$)	4.50	−2.00	7.76	6.34
Stability (*P**)				
Freeze–thaw cycles (−20 to 25°C)				
1 ng/ml	0.2575	0.9825	0.0711	0.0775
10 ng/ml	0.0725	0.1291	0.1671	0.4712
Room temperature for 12 h				
1 ng/ml	0.3275	0.6039	0.3624	0.7318
10 ng/ml	0.2497	0.0950	0.0967	0.0712

*Student *t*-test, $P < 0.05$.

TABLE 3. Confidence limits obtained for the analysis of CITA and DCITA enantiomers in rat plasma

	R-(−)-CITA	*S*-(+)-CITA	*S*-(+)-DCITA	*R*-(−)-DCITA
Recovery (%)				
5 ng/ml	89.4	86.6	85.4	99.1
50 ng/ml	78.1	87.1	80.0	88.3
500 ng/ml	101.5	95.8	71.6	93.9
Linearity (ng/ml)	0.1–500	0.1–500	0.1–500	0.1–500
r	0.99996	0.99845	0.99313	0.99400
LOQ (ng/ml)	0.1	0.1	0.1	0.1
Precision (RSD %, $n = 5$)	7.5	15.8	4.0	15.8
Accuracy (% bias)	12.0	0.1	12.0	0.1
Interassay precision ($n = 5$, RSD %)				
5 ng/ml	11.1	11.3	5.4	5.9
500 ng/ml	11.9	10.9	10.1	9.0
Intraassay precision (RSD %)				
5 ng/ml ($n = 10$)	12.7	12.7	5.2	3.8
500 ng/ml ($n = 10$)	10.3	10.0	7.0	7.8
Interassay accuracy (% bias)				
5 ng/ml ($n = 5$)	2.3	0.7	7.3	7.1
500 ng/ml ($n = 5$)	−1.1	−3.1	−8.2	−6.5
Intraassay accuracy (% bias)				
5 ng/ml ($n = 10$)	1.2	4.3	9.9	7.6
500 ng/ml ($n = 10$)	−1.0	−2.2	−16.8	−10.9
Stability (*P**)				
Freeze–thaw cycles (−20 to 25°C)				
5 ng/ml	0.2238	0.5565	0.8445	0.3273
500 ng/ml	0.1765	0.9702	0.0841	0.0971
Room temperature for 12 h				
5 ng/ml	0.2872	0.9792	0.2605	0.0754
500 ng/ml	0.9177	0.4228	0.0775	0.0901

*Student *t*-test, $P < 0.05$.

30 sec, and centrifuged for 1 min, and 60 μl of the aqueous phase was injected in the chromatographic system.

The quantitation limit (LOQ) was obtained by the analysis in quintuplicate of plasma samples spiked with CITA or DCITA, at concentrations as low as 0.1 ng of each enantiomer per milliliter of plasma. The LOQ was defined as the lowest plasma concentration of each analyte analyzed with an error of 20% or lower.

The linearity was evaluated by the analysis of plasma samples spiked with the increasing analyte concentrations in relation to those employed for the construction of the calibration curve. The method was considered to be linear up to the highest concentration studied, having a linear relationship with the detector response.

Precision and accuracy were evaluated at concentrations of 1 and 10 ng of each enantiomer per milliliter of human plasma and of 5 and 500 ng of each enantiomer per milliliter of rat plasma. For the evaluation of intraassay precision, 10 aliquots of each sample were analyzed using a single calibration curve. For interassay precision, aliquots of the samples were analyzed in duplicate on five consecutive days.

The stability was assured by three freeze (−20°C) and thaw (25°C) cycles. Stability of spiked plasma samples (1 and 10 ng/ml human plasma and 5 and 500 ng/ml rat plasma) was also evaluated after keeping the samples at room temperature (25°C) for 12 h. The results of the stability tests were compared with those obtained for freshly prepared samples by the Student *t*-test ($P < 0.05$).

Clinical and Experimental Protocol

The study was approved by the Ethics Committee of the University Hospital, Faculty of Medicine of Ribeirão Preto, USP. The volunteer received detailed explanations about the procedures and was included in the study after giving written informed consent. After a 12-h fast, the volunteer received a single dose of racemic CITA (Cipramil®, Schering-Plough, Brazil) with 200 ml water. Blood samples (5 ml) were collected through a catheter inserted into the antecubital vein into heparin-containing tubes at times 0, 1, 1.5, 2.5, 4, 6, 8, 12, 48, 72, 96, 120, 144, 168, 192, 216, and 240 h after administration of the drug.

The experiment involving animals was approved by the Ethics Committee on the Use of Animals, USP, Ribeirão Preto Campus. Male Wistar rats weighing 200 ± 20 g were obtained from the Central Animal House, USP, Ribeirão Preto Campus, and allowed to adapt to the Institutional Animal House with the temperature (21–23°C) and humidity (40–60%) controlled room under a 12-h light/dark cycle for 3 days before the beginning of the experiment. Chow and water were available ad libitum.

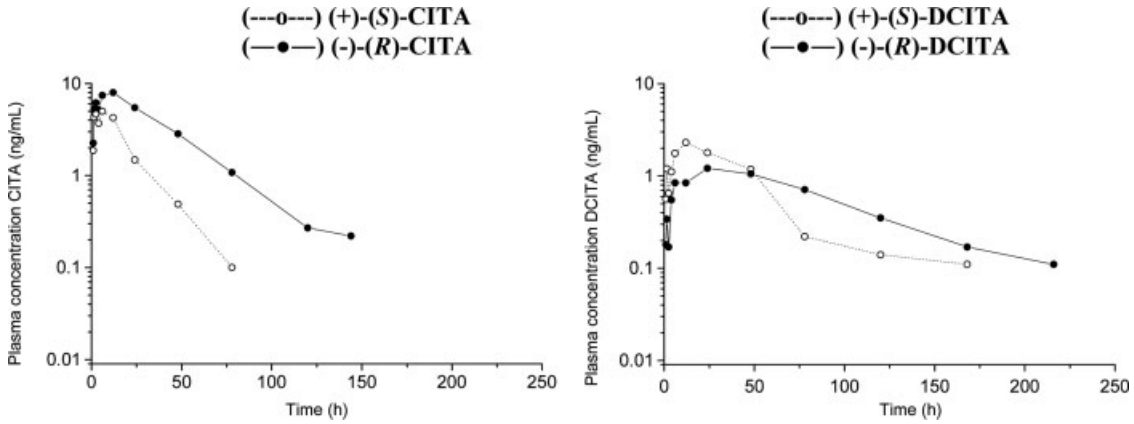

Fig. 3. Plasma concentration versus time curves for CITA and DCITA after oral administration of 20 mg *rac*-CITA to a healthy volunteer.

After a 12-h fast, the animals ($n = 6$ per sampling time) received by gavage a single oral dose of 20 mg racemic CITA/kg dissolved in water. Blood samples (5 ml) were collected by decapitation of the animals at times zero, 15, 30, 45, and 60 min and 1.5, 2, 4, 6, 8, 10, 12, 16, and 20 h after administration of the drug.

Heparin (Liquemine®, 5000 IU, Roche) was used as anticoagulant in all samples, and the plasma obtained after centrifugation was stored at −20°C until the time for analysis.

Pharmacokinetic and Statistical Analyses

The pharmacokinetic parameters were calculated based on the plasma enantiomer concentration versus time curves using the WinNonlin version 4.0 program (Pharsight Corp, Mountain View, CA). The calculations were performed using first-order kinetics, a monocompartmental model and no lag time.

TABLE 4. Enantioselective kinetic disposition of CITA in a healthy volunteer after oral administration of 20 mg *rac*-CITA

Parameter	(−)-*R*-CITA	(+)-*S*-CITA
C_{max} (ng/ml)	6.81	4.89
t_{max} (h)	5.36	3.88
$t_{1/2}$ a (h)	1.30	1.58
K_a (h^{-1})	0.53	0.44
$t_{1/2}$ β (h)	18.47	5.15
β (h^{-1})	0.04	0.13
Vd/f (l/kg)	20.35	20.58
$AUC^{0-\infty}$ (ng h/ml)	222.04	61.24
Cl/f (l/h/kg)	0.76	2.77
$AUC_{(S)}/AUC_{(R)}$	0.28	

C_{max}, maximum plasma concentration; t_{max}, time to reach C_{max}; $t_{1/2}$ a, absorption half-life; K_a, absorption rate constant; $t_{1/2}$ β, elimination half-life; β, elimination rate constant; Vd/f, apparent distribution volume; $AUC^{0-\infty}$, area under the plasma concentration versus time curve; Cl/f, apparent total clearance.

The experimental data were analyzed statistically using the Graphpad Instat® software for the calculation of mean, median, and 95% confidence interval. Data were compared using a nonparametric test for paired data (comparison between enantiomers), with the level of significance set at $P < 0.05$.

RESULTS AND DISCUSSION

Resolution of the CITA and DCITA enantiomers was evaluated on different chiral phase columns (Chiralcel OD-R 250 × 4.6 mm, OB-H 150 × 4.6 mm and OD-H 250 × 4.6 mm, Chiralpak® AS and AD 250 × 4.6 mm, Phenomenex® Chirex 3005 250 × 4.6 mm, and Chiral-AGP® 150 × 4.0 mm). Direct separation of the CITA and DCITA enantiomers was obtained with the Chiralcel OD-R and Chirobiotic V columns. CITA and DCITA enantiomers were resolved on the Chiralcel OD-R column using a mobile phase of acetonitrile, methanol, and water (30:30:40, v/v/v), containing 0.05% diethylamine at a run time of ∼38 min (see Fig. 1). DDCITA enantiomers could not be separated on the Chiralcel OD-R column. Resolution of CITA and DCITA enantiomers on the Chiralcel OD-R column were, respectively, 0.88 and 1.0. On the other hand, the best resolution and shortest analysis time (12 min) were obtained with the Chirobiotic V column using a mobile phase of methanol, acetic acid, and triethylamine

TABLE 5. Enantioselective kinetic disposition of DCITA in a healthy volunteer after oral administration of 20 mg *rac*-CITA

Parameter	(+)-(*S*)-DCITA	(−)-*R*-DCITA
C_{max} (ng/ml)	1.85	1.11
t_{max} (h)	8.25	16.05
$AUC^{0-\infty}$ (ng h/ml)	98.95	95.02
$AUC_{(S)}/AUC_{(R)}$	1.04	

C_{max}, maximum plasma concentration; t_{max}, time to reach Cmax; $AUC^{0-\infty}$, area under the plasma concentration versus time curve.

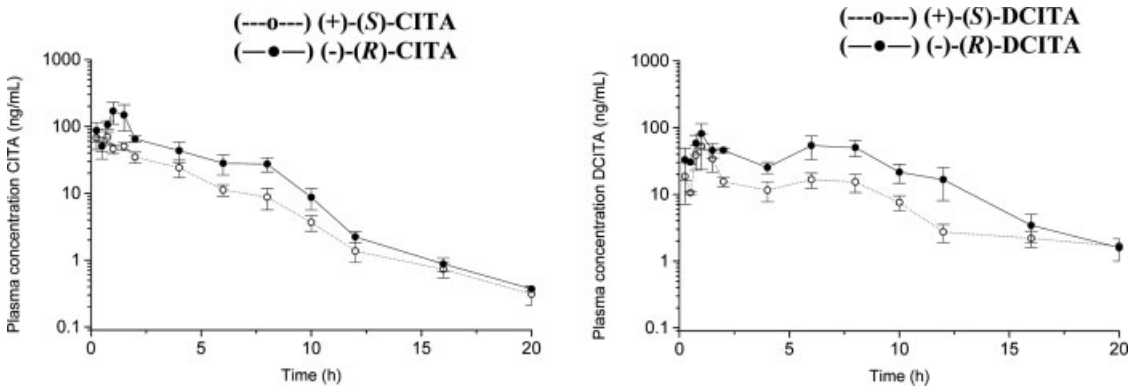

Fig. 4. Plasma concentration versus time curves for CITA and DCITA after oral administration of 20 mg/kg *rac*-CITA to rats.

(99.9:0.06:0.055)[13] (see Fig. 2). However, the sensitivity of ion monitoring with the MS/MS detector was compatible with the application to pharmacokinetics, only under the conditions used for the Chiralcel OD-R column. The results suggest that the presence of triethylamine in the mobile phase of the Chirobiotic V column leads to a significant loss of sensitivity in the MS/MS detector.

The matrix effect was evaluated based on direct comparison of the peak areas of CITA, DCITA, and internal standard (IS) injected directly in mobile phase, and spiked postextraction into extracts originating from five different sources of human and rat plasma. Data showed in Table 1 indicate that the matrix effect for CITA, DCITA, and IS was practically absent.

Analysis of blank plasma samples obtained from healthy volunteers and rats showed no interference of endogenous components with the CITA and DCITA enantiomers (Figs. 1A and 1D). Recovery rates were higher than 70%, irrespective of the concentrations of the CITA and DCITA enantiomers extracted from plasma at pH 9.0 with a mixture of toluene:isoamyl alcohol (9:1, v/v) (Tables 2 and 3). These recovery rates are similar to those reported by Rochat et al.,[10,11] Zheng et al.,[2] and Kosel et al.,[13] ranging from 63 to 98%, who used a mixture of heptane:isoamyl alcohol, and by Carlsson et al.,[21] ranging from 87 to 110%, who used solid-phase extraction.

The CITA and DCITA enantiomers were analyzed with the LC-MS/MS system, in which protonated ions and their respective ion products were monitored at transitions of $325.3 > 310.9$ for CITA, $311.4 > 108.7$ for DCITA, and $249.1 > 172.2$ for the internal standard. These results are compatible with those obtained in studies analyzing enantiomeric mixtures or escitalopram.[16–20]

The method developed using the LC-MS/MS system resulted in a LOQ of 0.1 ng of each CITA and DCITA enantiomer per milliliter of human or rat plasma, demonstrating that this method is more sensitive than those reported in the literature, whose LOQ ranges from 0.5 to 15 and from 0.62 to 11 ng/ml plasma for each CITA and DCITA enantiomer, respectively.[2,10–14,19,21] It should be noted that in the clinical study, concentrations of 0.2 ng (−)-(*R*)-CITA/ml plasma and 0.1 ng/ml plasma of the DCITA enantiomers were observed 144 and 216 h after administration of the drug, respectively (see Fig. 3).

Analysis of precision and accuracy showed coefficients of variation and a percent inaccuracy of less than 15%, indicating that the method is precise and accurate. Evaluation of stability after three freeze–thaw cycles and after storage at room temperature revealed $P \geq 0.05$ (Student *t*-test), thus guaranteeing the stability of samples stored at −20°C (Tables 2 and 3).

The method developed and validated here was applied to the enantioselective analysis of the kinetic disposition of

TABLE 6. Enantioselective kinetic disposition of CITA in rats treated with *rac*-CITA

Parameter	(−)-*R*-CITA	(+)-*S*-CITA
C_{max} (ng/ml)	152.11*	66.02
	157.65 (88.90–226.39)	70.75 (47.52–93.99)
t_{max} (h)	1.06	0.78
	0.93 (0.29–1.56)	0.73 (0.17–1.29)
$t_{1/2}$ a (h)	0.37	0.20
	0.35 (0.03–0.67)	0.22 (0.003–0.44)
K_a (h^{-1})	2.48	4.93
	9.15 (5.02–23.31)	15.46 (4.61–35.53)
$t_{1/2}$ β (h)	2.09	2.38
	2.43 (1.39–3.47)	2.32 (2.07–2.58)
β (h^{-1})	0.34	0.29
	0.32 (0.22–0.41)	0.30 (0.27–0.33)
Vd/f (l/kg)	49.75*	119.90
	56.11 (23.13–89.09)	118.41 (96.48–140.35)
$AUC^{0-\infty}$	622.30*	264.67
(ng h/ml)	724.64 (406.47–1042.80)	290.01 (228.51–351.51)
Cl/f (l/h/kg)	16.11*	37.80
	15.53 (9.93–21.13)	35.47 (29.25–41.69)
$AUC_{(S)}/AUC_{(R)}$	0.44	
	0.44 (0.29–0.59)	

Data are reported as medians and means (95% CI). C_{max}, maximum plasma concentration; t_{max}, time to reach C_{max}; $t_{1/2}$ a, absorption half-life; K_a, absorption rate constant; $t_{1/2}$ β, elimination half-life; β, elimination rate constant; Vd/f, apparent distribution volume; $AUC^{0-\infty}$, area under the plasma concentration versus time curve; Cl/f, apparent total clearance.
*$P < 0.05$, Wilcoxon test.

TABLE 7. Enantioselective kinetic disposition of DCITA in rats treated with *rac*-CITA

Parameter	(+)-*S*-DCITA	(−)-*R*-DCITA
C_{max} (ng/ml)	35.97*	59.66
	47.38 (12.50–82.26)	88.61 (27.12–150.09)
t_{max} (h)	1.27	2.28
	1.48 (0.61–2.36)	2.01 (1.05–2.96)
$AUC^{0-\infty}$	260.87*	505.77
(ng h/ml)	294.55 (126.32–462.78)	696.47 (179.91–1213.00)
$AUC_{(S)}/AUC_{(R)}$	0.48	
	0.45 (0.36–0.54)	

Data are reported as medians and means (95% CI). C_{max}, maximum plasma concentration; t_{max}, time to reach Cmax; $AUC^{0-\infty}$, area under the plasma concentration versus time curve.
*$P < 0.05$, Wilcoxon test.

CITA administered as the racemic mixture in a single dose to a healthy volunteer and to Wistar rats ($n = 6$/sampling time).

Enantioselectivity in the pharmacokinetics of CITA was observed in the clinical study, with the demonstration of a higher proportion of the (−)-(*R*) enantiomer in plasma (see Fig. 3). The $AUC_{S/R}$ ratio for CITA was 0.28 (Table 4), a value compatible with those reported by Carlsson et al.[21] and Herrlin et al.,[22] who obtained *S*/*R* plasma concentration ratio for CITA of 0.59 and $AUC_{S/R}$ ratio of 0.61, respectively. The $AUC_{S/R}$ ratio for the metabolite DCITA was ~1, a finding suggesting the lack of enantioselectivity (Fig. 3 and Table 5). Carlsson et al.[21] and Herrlin et al.[22] reported *S*/*R* plasma concentration ratio for DCITA of 0.82 and $AUC_{S/R}$ ratio of 0.89, respectively.

The pharmacokinetic parameters obtained for CITA in Wistar rats ($n = 6$/sampling time) indicated a higher proportion of the (−)-(*R*) enantiomer in plasma compared to its (+)-(*S*) antipode (see Fig. 4), with significant differences in the parameters C_{max}, $AUC^{0-\infty}$, Cl/f, and Vd/f (Table 6). A higher proportion of the (−)-(*R*)-CITA distomer in plasma was also observed in the clinical study. The $AUC_{S/R}$ ratio for CITA in rats was 0.44. This plasma accumulation was also reported by Wikell et al.[7] and Kugelberg et al.,[8] who obtained *S*/*R* plasma concentration ratios of 0.94, 0.83, and 0.34 for CITA administered to rats in multiple doses of 10, 20, or 100 mg/kg/day for 10 days, respectively. In the two studies, CITA was administered with an osmotic pump, i.e., the absorption process was devoid of presystemic elimination. Kugelberg et al.[9] observed enantioselectivity in the metabolism of CITA in rats subcutaneously injected with a single dose of 20 or 100 mg/kg. The authors reported *S*/*R* plasma concentration ratios of ~0.6 for CITA, 20 h after administration of the drug. The *S*/*R* plasma concentration ratio of DCITA in rats was 0.48 (Table 7), indicating the preferential formation of the (−)-*R* enantiomer (see Fig. 4). The same was reported by Kugelberg et al.[8] and Wikell et al.,[7] who obtained *S*/*R* plasma concentration ratios for DCITA of 0.37 and 0.31, respectively.

In conclusion, the confidence limits of the method for the analysis of the (−)-(*R*)-CITA, (+)-(*S*)-CITA, (−)-(*R*)-DCITA, and (+)-(*R*)-DCITA enantiomers in human and rat plasma are compatible with the application of the method to the clinical study of the kinetic disposition of a single dose of the drug. The clinical and experimental studies demonstrate enantioselectivity in the kinetic disposition of CITA, with a higher proportion of the (−)-(*R*)-CITA distomer in plasma.

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