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The enantioselective determination of chlorpheniramine and its major metabolites in human plasma using chiral chromatography on a β-cyclodextrin chiral stationary phase and mass spectrometric detection☆

Karen M. Fried [b], Andrea E. Young [b], Sally Usdin Yasuda [a], Irving W. Wainer [a,*]

[a] *Department of Pharmacology, Georgetown University, Washington, DC, USA*
[b] *Bioanalytical Center, Georgetown University Clinical Research Center, Washington, DC, USA*

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Dedicated to Professor Dr Blaschke on the occasion of his 65th birthday.

Abstract

A sensitive enantioselective high-performance liquid chromatographic (HPLC) method has been developed for the simultaneous determination of plasma concentrations of (−)(*R*)- and (+)(*S*)-chlorpheniramine (CP) and their metabolites, desmethyl-chlorpheniramine (DCP), didesmethyl-chorpheniramine (DDCP) and chlorpheniramine *N*-oxide (CPNO). Enantioselective separations were achieved on a β-cyclodextrin chiral stationary phase (CYCLOBOND I 2000™) with a mobile phase consisting of diethylamine acetate (0.25%, pH 4.4):methanol:acetonitrile {85:7.5:7.5, (v/v/v)} and a flow-rate of 0.5 ml/min. For CP, the enantioselectivity (α) of the separation was 1.12 with a resolution factor (R_s) of 1.17. The method was validated for CP by using mass spectroscopy detection (MSD). Concentrations of each enantiomer could be measured down to 125 pg/ml from a 1-ml plasma sample. Extracted calibration curves were linear from 0.13 to 50.00 ng/ml for each enantiomer. The method was applied to samples from two clinical studies.

Keywords: Chlorpheniramine; Chiral chromatography; Chiral stationary phase; β-Cyclodextrin; LC-MS

☆ This manuscript is dedicated to Professor Gottfried Blauschke in recognition of his great contribution to the application of LC-MS and CE-MS in the bioanalysis of chiral drugs. He is an excellent scientist, a wonderful teacher and a good friend and colleague.

* Corresponding author. Present address: Bioanalytical and Drug Discovery Unit, Gerontology Research Center, National Institute on Aging, National Institutes of Health, 5600 Nathan Shock Drive, Baltimore, MD 21224-6825, USA. Tel.: +1-410-558-8498; fax: +1-410-558-8490.

E-mail address: wainerir@grc.nia.nih.gov (I.W. Wainer).

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1. Introduction

Chlorpheniramine maleate (CP) (Fig. 1) is a histamine H_1-receptor antagonist, which is often found as an ingredient of 'over-the counter' treatments to alleviate symptoms of the common cold and allergic conditions. Its worldwide popularity as an antihistamine is based on effectiveness, low cost (as a generic) and tolerability with little or no side effects. CP is a chiral drug that is marketed as the racemic mixture of (−)(*R*)-CP and (+)(*S*)-CP, although it has long been known that the clinical activity is mainly associated with the (*S*)-enantiomer [1].

A number of achiral and chiral methods have been reported for the analysis of CP in saliva, plasma or urine. These include assays based on high performance liquid chromatography (HPLC) utilizing UV detection [2,3] and pre-column derivatization followed by fluorescence detection [4] or gas chromatography with nitrogen phosphorous detection [5] and mass spectrometric detection [1]. However, enantioselective pharmacokinetic studies of *rac*-CP have been limited due to low therapeutic blood levels, which have made separation and detection of the drug and its metabolites difficult [1,2,6].

As part of a clinical study of the metabolism and disposition of CP, a sensitive and direct analytical method has been developed to determine the plasma concentrations of (*R*)-CP and (*S*)-CP as well as their metabolites, desmethyl-chlorpheniramine (DCP), didesmethyl-chorpheniramine (DDCP) and chlorpheniramine *N*-oxide (CPNO), Fig. 1. The method utilizes a limited volume of human plasma (≤ 1 ml), liquid/liquid extraction, enantioselective separation on a β-cyclodextrin chiral stationary phase (CSP) and mass spectrometric detection using single ion monitoring.

The assay was validated for (*R*)- and (*S*)-CP before being applied to the analysis of samples from two clinical studies. The first study addressed the issue that, previously, most clinical studies were performed using only male subjects. Thus, the pharmacokinetics of (*R*)- and (*S*)-CP were determined in males and females after single and chronic dosing. In addition, although wide inter-patient variability in CP plasma half life ($t_{1/2}$) has been noted [1,6], there is little information regarding the cytochrome P450 (CYP) enzymes involved in the formation of the two main metabolites of CP, DCP and DDCP. One possible CYP involved in the *N*-demethylation of CP is CYP2D6. The participation of this enzyme in the metabolic clearance of CP was addressed by including subjects whose genotype indicated that they were either genetically slow CYP2D6 metabolizers or genetically fast CYP2D6 metabolizers.

2. Experimental

2.1. Chemicals and reagents

(*R*,*S*)-chlorpheniramine maleate (Cat. No. 3025), (+)(*S*)-chlorpheniramine maleate (Cat. No. C4915) and diethylamine (SigmaUltra, Cat. No. D0806) were purchased from Sigma (St. Louis, MO). Small amounts of the metabolites desmethyl-chlorpheniramine, didesmethyl-chlorpheniramine and chlorpheniramine-*N*-oxide, as well as (*R*)-chlorpheniramine maleate, were supplied by Schering-Plough (Kenilworth, NJ). Acetonitrile (J.T. Baker, HPLC grade) and glacial acetic acid (J.T. Baker, HPLC grade) were obtained from Fisher Scientific (Jessup, MD), while methanol (EM Science, HPLC grade) and diethyl ether (Burdick and Jackson, HPLC grade) were purchased through VWR (Bridgeport, NJ). Liquid nitrogen for the mass spectrometer detector was supplied by MG Industries (Jessup, MD). Out-dated pooled drug-free human plasma was received from the Blood Bank at Georgetown University Hospital (Washington, DC).

2.2. Apparatus

The analytical system consisted of a Series 1100 LC/MSD (Agilent Technologies, Palo Alto, CA) equipped with a vacuum de-gasser (G1322A), a binary pump (1312A), an autosampler (G1313A), a thermostated column compartment (G1316A) and a mass selective detector (G1946A) supplied with atmospheric pressure ionization electrospray (API-ES). The system was interfaced to a 250 Mhz Kayak XA computer (Hewlett-Packard,

Palo Alto, CA) using ChemStation software (Hewlett-Packard, rev. A.06.03).

2.3. Chromatographic conditions

The enantioselective separation of (*R*)- and (*S*)-CP and their metabolites was performed on a CYCLOBOND I 2000™column (β-cyclodextrin, 250 × 4.6 mm i.d., 5 μ) supplied by Advanced Separation Technologies (ASTEC, Whippany, NJ). The mobile phase consisted of 0.25% diethylamine (pH adjusted to 4.4 with glacial acetic acid) modified with a pre-mixed 1:1 (v/v) solution of acetonitrile–methanol delivered at a flow-rate of 0.5 ml/min. The buffer to modifier ratio was 85:15, v/v and the column temperature was kept at 15 °C.

The MSD was set for selective ion monitoring (SIM) at 275.1 *m*/*z* for CP, 262 *m*/*z* for DCP, 247 *m*/*z* for DDCP and 307 *m*/*z* for CPNO, with a drying gas flow-rate of 13.0 l/min. The nebulizer pressure was adjusted to 30 psig, with the drying gas temperature at 350 °C. For maximum sensitivity, the fragmentor was set to 70 and the capillary voltage to 2800 V. In order to decrease the risk of contaminating the source of the MSD, the MSD was off-line with the effluent by-passed to the waste for the first 14 min of each run.

The resolution factor (R_s) and enantioselectivity factor (α) were calculated under the above-described conditions.

2.4. Study sample collection

Eight healthy volunteers, ranging in age from 22 to 28 years old, participated in a study (No. 354-92) at the Georgetown University Clinical Research Center. Six of the volunteers (three male and three female) had previously been characterized as extensive metabolizers for drugs metabolized by CYP2D6, while the remaining two volunteers (one male and one female) were poor metabolizers.

The study consisted of a randomized crossover trial, in which each of four study periods was separated by a 3-week washout. For both Phase I and II, a single 8 mg oral dose of *rac*-CP was taken on the morning of day-1 for that phase. However, for Phase II, in addition to the 8-mg dose of *rac*-CP, 50 mg of quinidine sulfate, an inhibitor of CYP2D6 activity, was taken every 6 h for 2 days prior to the study day and continued every 6 h thereafter, until the final blood sample was collected. Phase III and IV followed the same regime, but subjects received 4 mg (+)(*S*)-CP either with (III) or without (IV) quinidine sulfate.

Following administration of the drug, blood samples were collected at fixed times over a period of 60 h. After collection, the blood samples were centrifuged and the resulting plasma samples were stored at −80 °C until time of analysis.

A second study (No. 223-92) concerning chronic oral dosing of *rac*-CP was also completed. Each subject received a single oral dose of placebo, 2 mg *rac*-CP every 6 h or 4 mg *rac*-CP twice daily for 6 days. Blood samples were collected at various intervals on day-1 and day-6 of the study. The study group consisted of both males and females (ten) and all phenotyped for their CYP2D6 metabolic pathway prior to beginning the study. After separation of the plasma from the heparinized blood samples, the plasma was stored at −80 °C until time of analysis.

2.5. Preparation of stock solutions

A concentrated stock solution of *rac*-CP, 1.0 mg/ml as the free base, was prepared in ethanol, placed in a capped polypropylene tube, wrapped in aluminum foil and stored at −20 °C. Spiking solutions for the calibration curve and quality control samples (QCs) were made by serial dilutions with de-ionized water starting with the concentrated stock. The spiking standards were also placed in capped polypropylene tubes, wrapped in aluminum foil and stored at −20 °C.

Aqueous solutions of the metabolites were chromatographed separately to confirm their mass and retention times. In addition, aqueous standards of the separate enantiomers of CP were run to determine their elution order.

2.6. Preparation of calibration curve and quality control standards

Calibration and QC standards were prepared daily by adding 50 μl of a 20-fold concentrated

spiking solution to 950 μl drug-free plasma. The original 8-point calibration curve ranged from 0.05 to 5.00 ng/ml for each enantiomer with QC standards at 0.13 ng/ml as the low quality control (LQC), 1.25 ng/ml as the medium quality control (MQC) and 3.75 ng/ml as the high quality control (HQC) for each enantiomer. A second calibration curve, to accommodate higher concentrations of the drug, was cross-validated. This 9-point curve extended from 0.13 to 50.00 ng/ml for each enantiomer with additional QC standards at 2.50 (LQC), 12.50 (MQC) and 25.00 ng/ml (HQC) for each enantiomer. These QC stands encompassed the actual range of results from the clinical studies.

2.7. Extraction procedure

Sample extraction was performed using a modified version of a previously reported procedure [3]. To 15-ml conical polypropylene capped tubes, 1 ml portions of patient samples or spiked plasma standards (950 μl drug-free plasma plus 50 μl aqueous spiking solution) was added. The resulting solutions were made alkaline by the addition of 50 μl sodium hydroxide (1.0 M), briefly vortex-mixed and 3 ml aliquots of diethyl ether were added. The resulting mixtures were vortex mixed for 1 min, centrifuged at $835 \times g$ (4 °C) for 10 min, placed in a dry ice–acetone bath for 5 min and the non-frozen upper organic layers decanted into clean 15-ml conical polypropylene capped tubes containing 100 μl acetic acid (0.5%). CP was back-extracted from the organic phase into an acidic aqueous layer by following a procedure similar to that described above, i.e. vortex mixing for 1 min, centrifuging at $835 \times g$ (4 °C) for 10 min, freezing the lower aqueous layers before discarding the ether layers. The remaining aqueous solutions were allowed to remain in a fume hood for several hours or until the odor of ether was no longer evident. The solutions were transferred to 200-μl polypropylene autosampler vials and 75 μl aliquots were injected onto the chromatographic system.

2.8. Validation: lower calibration curve

The intra- and inter-day validation studies for precision and accuracy were performed in triplicate with QC standards at 0.13, 1.25 and 3.75 ng/ml for each enantiomer. The analyses were carried out over a period of 5 days for the inter-day validation. Duplicate calibration curves were analyzed daily. The curves were constructed, typically, by plotting the (*R*)-CP or (*S*)-CP peak area against the concentration of the each enantiomer.

Extraction efficiency (% recovery) of *rac*-CP was determined by comparing peak areas for the QC standards to the peak areas resulting from the chromatography of replicate aqueous standards containing the equivalent final concentrations.

Accuracy was determined by comparing the observed concentrations of the QC standards calculated from the calibration curve to their nominal concentrations.

Specificity was demonstrated by screening five different batches of pooled plasma for possible interference caused by endogenous substances.

2.9. Cross validation: extended curve

The intra- and inter-day validation studies for precision and accuracy were performed in triplicate with QC standards at 2.50, 12.50 and 25.00 ng/ml for each enantiomer. The analyses were carried out over a period of 3 days for the inter-day validation. Extended calibration curves were analyzed daily in triplicate.

Accuracy was determined as described above.

3. Results and discussion

3.1. Method development

Previous approaches to the enantioselective HPLC resolution of (*R*)- and (*S*)-CP and the metabolites, involved the use of an ovomucoid CSP [7] and an amylose Tris (3,5-dimethylphenylcarbamate) CSP (the Chiralpak AD CSP) [2,6], both with UV detection. The ovomucoid CSP was not efficient enough to reach the necessary sensitivity, while the Chiralpak AD CSP was used in

an achiral–chiral coupled column method in normal phase mode. In order to achieve sufficient sensitivity, the sample size had to be increased to 3 ml plasma. In addition, CP and DCP could not be separated from each other under the same chromatographic conditions and separate injections with different mobile phases had to be utilized.

β-Cyclodextrin has been used in the enantioselective resolution of *rac*-CP in the form of a chiral additive in HPLC [8] and CE [9,10] or as a chiral stationary phase in capillary GC [1]. While all of these approaches produced some enantioselective separation of *rac*-CP, none were able to achieve sufficient chiral resolution and sensitivity to permit quantification of the plasma levels of (*R*)- and (*S*)-CP and their metabolites after a single dose of *ras*-CP. These results suggested that an enantioselective assay for *rac*-CP could be based upon the use of a β-cyclodextrin CSP and a liquid chromatographic-mass spectrometer (LC-MS) system. This would produce the enantioselectivity and sensitivity required for the clinical assay.

The LC-MS approach was chosen since the previously reported assays using UV detection were not sufficiently sensitive to monitor the anticipated therapeutic levels using 1 ml of plasma [2,3]. The use of benzyl chloroformate as a fluorescent derivatizing agent has been reported to achieve a lower limit of achiral detection of 0.1 ng/ml in whole blood [4]. However, sample preparation was extremely complicated and time-consuming, reducing the applicability of this method to a large clinical study. Another approach to the determination of CP concentration is post-column photolysis followed by electrochemical detection [11,12]. This method produced a slight increase in sensitivity relative to UV detection, but the difference was not enough to detect low levels of CP nor was it selective enough to eliminate interference from endogenous substances in the plasma extract. LC-MS proved to be the best approach for our bioanalytical problem with the optimum detection obtained using single (or selective) ion monitoring at 275.1 m/z for CP, 262 m/z for DCP, 247 m/z for DDCP and 307 m/z for CPNO.

A near baseline separation of (*R*)-CP and (*S*)-CP was achieved on the β-cyclodextrin CSP using a mobile phase composed of sodium acetate modified with acetonitrile, UV detection and high concentrations of aqueous standards. For transition to LC-MS, the buffer was changed to ammonium acetate. Unfortunately, the ammonium ion had a detrimental effect on the column, producing a rapid and irreversible degradation of the observed enantioselectivity. An alternative was the use of aqueous organic base, such as triethylamine (TEA) or diethylamine (DEA), with glacial acetic acid to adjust the pH. DEA was chosen since it was less damaging to the MS and its concentration was optimized to produce the best chromatography at the lowest DEA concentration. To prevent any long-term deterioration of sensitivity due to the use of DEA, the system (without a column) was washed thoroughly with methanol:1% formic acid, 10:90 (v/v).

In the optimization process, acetonitrile was replaced by methanol, since the acetonitrile suppressed ionization and decreased the sensitivity of the method. Substituting methanol for acetonitrile increased the overall sensitivity of the assay, but drastically decreased the observed chiral separation of (*R*)-CP and (*S*)-CP. Using methanol in the mobile phase reduced α from 1.12 to 1.10 and lowered R_s from 1.27 to 0.81.

The optimum mobile phase composition in this system was DEA [0.25%, pH 4.4]:methanol: acetonitrile {85:7.5:7.5, (v/v/v)}. Under these conditions, enantioselective resolutions were obtained for CP, DCP and DDCP in < 20 min (see Table 1 and Figs. 2, 3). The enantiomers of the *N*-oxide were not resolved, as only a single very small peak appeared. As illustrated by Table 1 and Figs. 2 and 3, while the enantioselective resolutions of CP and its de-methylated metabolites were achieved, the compounds were significantly overlapped. This problem was solved with the use of single ion monitoring, since there were no overlaps in the mass spectra of the compounds at the m/z values chosen for the monitoring.

Under the above-described chromatographic conditions, the method was stable and reproducible. During the course of a year, over 1500 plasma extracts, standards and patient samples were analyzed on a single column with little or no change in R_s or α factors.

3.2. Validation

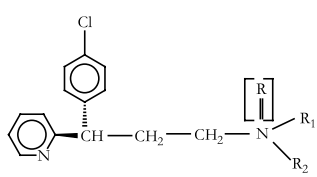
The primary objective of the clinical study was the determination of the plasma pharmacokinetics of (*R*)-CP and (*S*)-CP. Therefore, the method was validated for the quantification of these two compounds in human plasma. In addition, while there were sufficient quantities of the CP for the validation, only small samples of the metabolites were available. Therefore, the CP metabolites were used to establish the specificity of the method and to demonstrate that a qualitative determination of the metabolites could be accomplished, but the quantitative accuracy of the method was not validated for these compounds.

Table 1
Summary of chromatographic parameters for enantiomeric separations

Compound	$k'1$	α	Rs
CP	2.49 ($\pm$0.32)*	1.12 ($\pm$0.02)*	1.17 ($\pm$0.14)*
CPNO	2.29 ($\pm$0.09)*		
DDCP	2.16 (v0.20)*	1.07 ($\pm$0.02)*	0.77 ($\pm$0.17)*
DCP	2.09 ($\pm$0.15)*	1.16 ($\pm$0.01)*	1.66 ($\pm$0.03)*

* $n = 3$.

Chromatographic conditions: column, CYCLOBOND I 2000™ 250 × 4.6 mm i.d.; mobile phase, 0.25% diethylamine acetate (pH 4.4) and acetonitrile–methanol (1:1, v/v), 85:15 (v/v); flow-rate, 0.5 ml/min; column temperature, 15 °C; and detection, see Section 2.2 for details.



	R	R1	R2
		CH_3	CH_3
CPNO	O	CH_3	CH_3
DCP		CH_3	H
DDCP		H	H

Fig. 1. Structure of CP and its metabolites.

3.3. Extraction efficiency (% recovery)

The values for percent of recoveries are shown in Tables 2 and 3. The extraction efficiencies were $\geq$80.2% and 79.5% for (*R*)-CP and (*S*)-CP, respectively, for the extended curve.

3.4. Linearity and detection limits

Calibration curves, where y represents the peak area of CP while x represents the concentration of CP calibration standards in ng/ml, were generated by weighted $(1/x)$ least squares linear regression. The equation used to describe the linear relationship between peak area and (*R*)-CP concentrations from 0.13 through 50.00 ng/ml was $y = (1.9 \times 10^5)\ x + (-2.1 \times 10^4)$. For (*S*)-CP the equation was $y = (1.9 \times 10^5)\ x + (-2.8 \times 10^4)$. Both curves were linear through the full range of 0.13 through 50.00 ng/ml with correlation coefficients (r^2) $\geq$0.991. The above results were based on 14 replicates of a 9-point calibration curve.

The lower limit of quantification (LLOQ) is the concentration of the drug, in the matrix, which could be determined with a percentage accuracy within acceptable limits (80–100%). The LLOQ for both enantiomers of CP was 0.25 ng/ml per enantiomer. In contrast, the lower limit of detection was defined as the concentration of the compound at which the signal versus noise ratio (S/N) was equal to 3. For each enantiomer, the LLOD was equal to 0.13 ng/ml.

3.5. Accuracy and precision

Accuracy and precision of the method were evaluated from triplicate QC standards repeated for 5 days for the lower curve (see Table 2) and triplicate QC standards for the extended curve repeated for 3 days (see Table 3). Accuracy was $\geq$90.4% for *R*-CP or 91.0% for *S*-CP for the extended calibration curve. Precision for both inter- and intra-day QC standards was measured by %CV. For the lower curve, the results were $\leq$6.2 and 11.2 for (*R*)-CP and (*S*)-CP, respectively (see Table 2). The results for the extended curve were $\leq$11.8 and 10.9 for (*R*)-CP and (*S*)-CP, respectively (see Table 3).

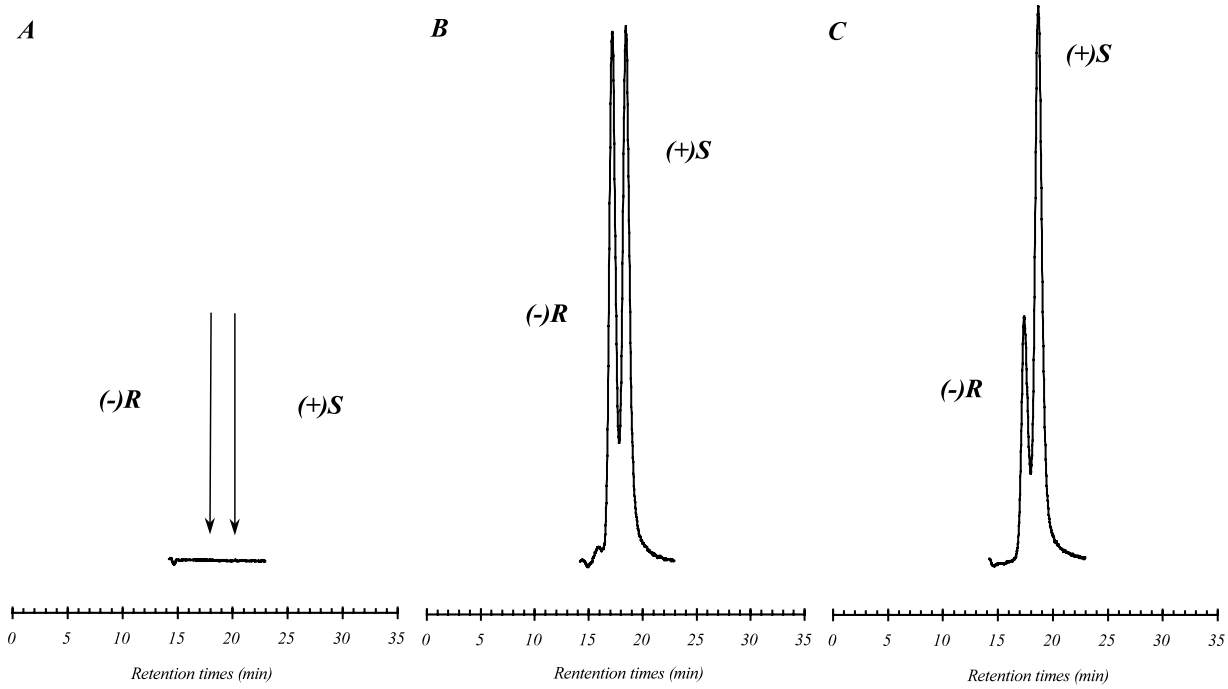


Fig. 2. Representative chromatograms of (A) a pre-dose sample from subject number 008 enrolled in study number 354-92; (B) a spiked plasma sample (3.75 ng/ml per enantiomer); and (C) subject number 008 at 1 h on day-6 of study number 354-92. The scale of the *y*-axis is the same on all three chromatograms. Column, CYCLOBOND I 2000™ 250 × 4.6 mm i.d.; mobile phase, 0.25% diethylamine acetate (pH 4.4) and acetonitrile–methanol (1:1, v/v), 85:15 (v/v); flow-rate, 0.5 ml/min; column temperature, 15 °C; and detection, see Section 2.2 for details.

A representative chromatogram of drug-free plasma extracts is shown in Fig. 2(A). The absence of any interfering peaks at the same retention times of (*R*)- or (*S*)-CP was proof of the specificity of this method for *rac*-CP.

3.6. Application

Clinical samples from the GCRC supported study were analyzed by this method. A set of QCs, in triplicate, was run with each sequence of samples. Initially, it was anticipated that the plasma levels for the separate enantiomers would be in the range of 0.13–5.00 ng/ml. However, it quickly became apparent that many of the plasma levels for the drug would be higher, so the method was adjusted to include a single extended calibration curve covering the full range from 0.13 to 50.00 ng/ml. All calculations for the clinical study were based on the extended calibration curve.

Although we were unable to quantify the metabolites, the MS was programmed to monitor for all three along with CP. In fact, plasma samples from several patients had small peaks at the retention times and with the masses that corresponded to those of the metabolite standards (see Fig. 3).

A plasma concentration–time curve for a volunteer receiving CP without (A) or with (B) quinidine sulfate is presented in Fig. 4(A,B). Fig. 5 represents the results from the chronic dosing study. Fig. 5(A,B) show the time-concentration levels of a volunteer receiving 2 mg *rac*-CP 6 times daily on day-1 and on day-6 of the study. For Fig. 5(C,D), the time-concentration profile shows the same volunteer after receiving 4 mg *rac*-CP twice daily. The results are from day-1 and day-6. The data from the study of the effect of quinidine on the pharmacokinetic parameters of CP indicate that the coadministration of quinidine to fast CYP2D6 metabolizers reduced the oral clearance from 0.54 ± 0.08 to

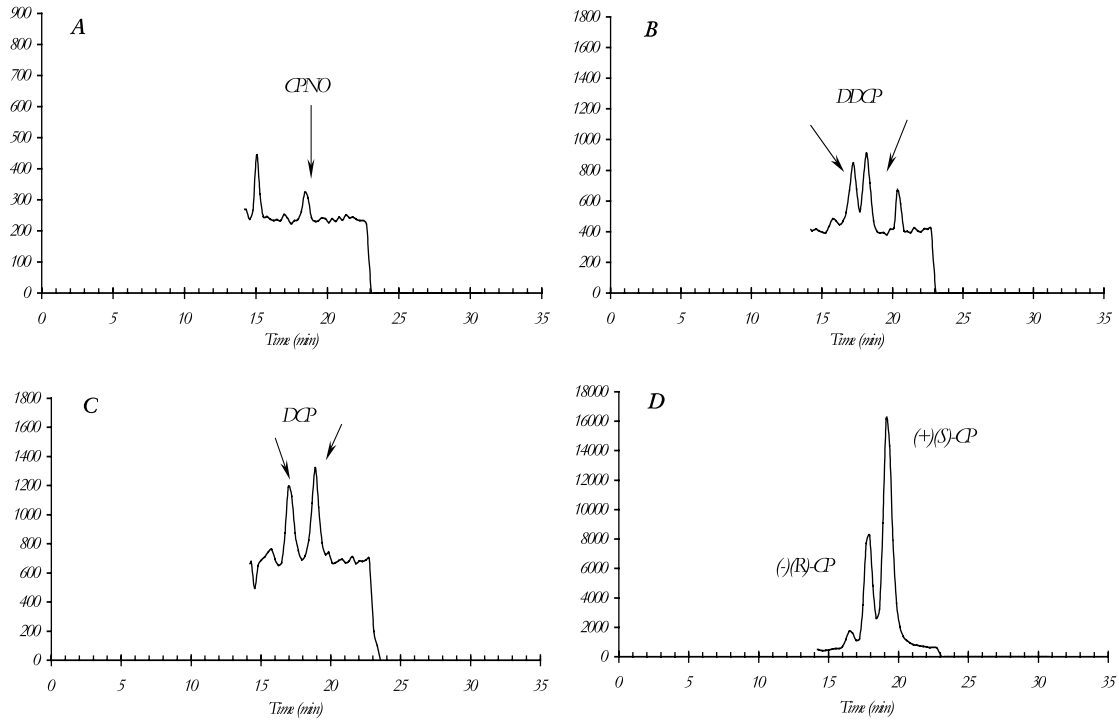


Fig. 3. Representative chromatograms of (A) CPNO, (B) DDCP, (C) DCP and (D) CP from a single subject (No. 008) at 1 h on day-6 of study number 354-92. See Fig. 2 and Section 2.2 for details of chromatographic conditions.

Table 2
Summary of validation statistics for calibration curve ranging from 0.125 to 5.0 ng/ml per enantiomer

	LQC		MQC		HQC	
	R	*S*	*R*	*S*	*R*	*S*
Conc. CP (ng/ml)	0.125	0.125	1.25	1.25	3.75	3.75
Intra-day						
N	3	3	3	3	3	3
Mean	0.13	0.13	1.18	1.16	3.5	3.53
S.D.	0.00	0.00	0.01	0.02	0.25	0.27
% CV	1.89	1.46	3.33	1.5	7.01	7.64
Inter-day						
N	15	15	15	15	14	14
Mean	0.13	0.13	1.2	1.18	3.56	3.54
S.D.	0.01	0.01	0.04	0.04	0.07	0.06
% CV	6.22	11.17	3.62	3.38	2.06	1.62
Accuracy (%)	101.77	104.94	96.15	94.41	95.01	94.45
Recovery (%)	83.60	84.20	80.77	80.42	80.15	79.52

Table 3
Summary of validation statistics for calibration curve ranging from 0.25 to 50 ng/ml per enantiomer

	LLOQ		LQC		MQC		HQC	
	R	*S*	*R*	*S*	*R*	*S*	*R*	*S*
Conc CP (ng/ml)	0.25	0.25	2.5	2.5	12.5	12.5	25.0	25.0
Intra-day								
N	1	1	3	3	3	3	3	3
Mean	0.28	0.27	2.25	2.41	12.11	12.19	26.06	26.51
S.D.			0.03	0.15	0.26	0.02	0.19	0.25
% CV			1.21	6.25	2.17	0.19	0.73	0.94
Inter-day								
N	6	6	9	9	7	7	7	7
Mean	0.24	0.27	2.26	2.27	12.56	12.05	26.25	24.64
S.D.	0.03	0.02	0.27	0.25	0.79	0.61	0.58	2.77
% CV	12.16	6.30	11.75	10.90	6.30	5.05	2.21	11.23
Accuracy (%)	97.67	107.87	90.44	90.98	100.46	96.41	105.00	98.54
Recovery (%)			84.18	84.57	93.68	99.66	99.99	95.76

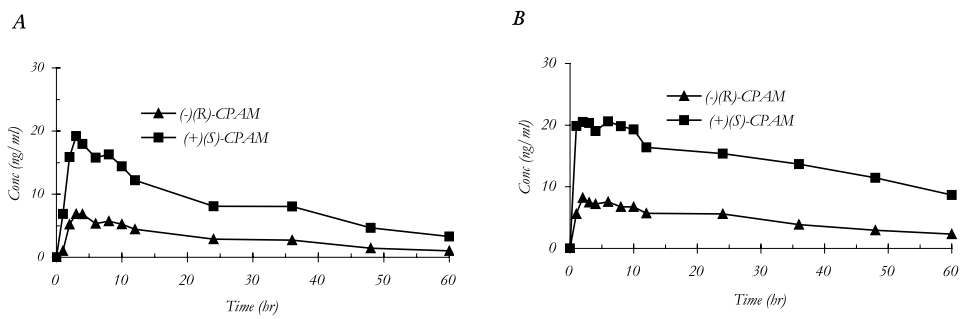


Fig. 4. Representative plasma concentration-time profile of subject number 008 in study number 354-92 during (A) Phase I (without quinidine) and Phase II (with quinidine). Subject received a single 8 mg dose of CP per day for both phases of study; see Section 2.4 for further details of study.

0.29 ± 0.39 l/h/kg ($P < 0.05$) and prolonged the half-life from 18.0 ± 2.0 to 29.3 ± 2.0 h ($P < 0.001$) [13]. The results suggest that the CYP2D6 play a role in CP pharmacokinetics in humans.

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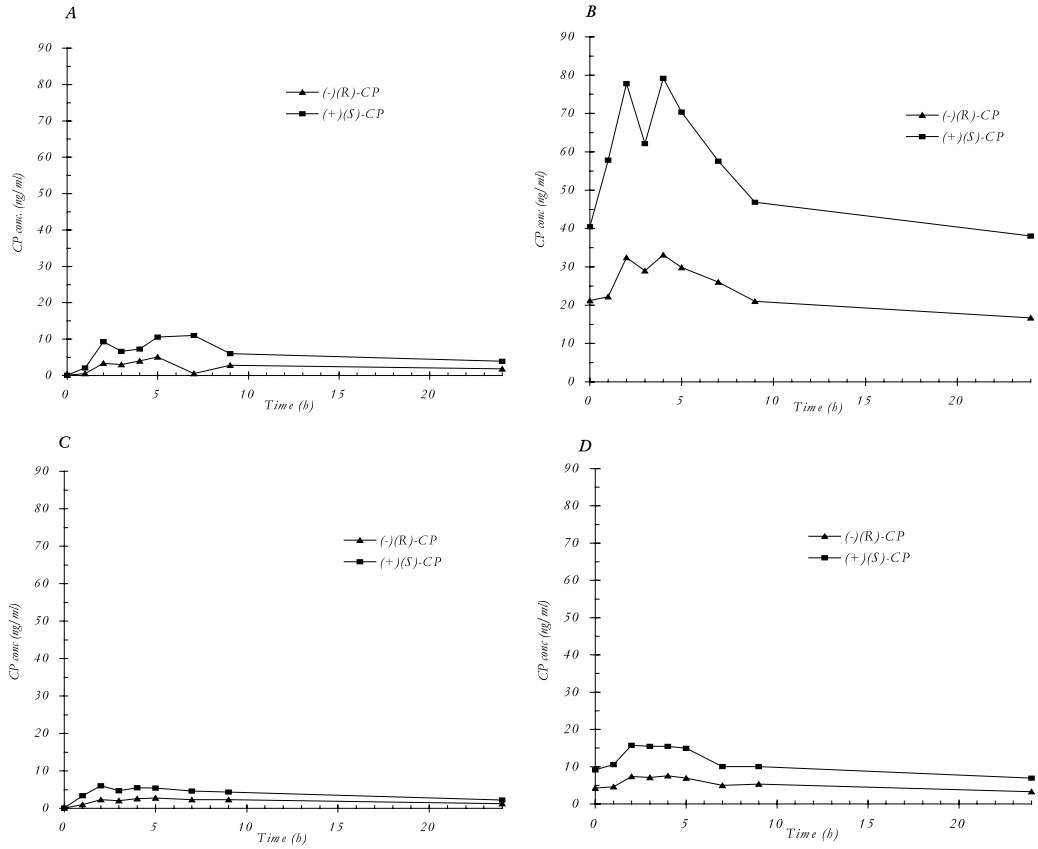

Fig. 5. A plasma concentration–time curve of subject number 004 from study number 223-92 in (A) Phase A1, (B) Phase A6, (C) Phase C1 and (D) Phase C6. Patients in Phase A received 4 mg CP every 12 h, while in Phase C, patients received a 2 mg dose of CP every 6 h. Results are shown for day-1 and day-6 for both dosages; see Section 2.4 for details of study parameters.

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