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Simultaneous quantification of cefpodoxime proxetil and clavulanic acid in human plasma by LC–MS using solid phase extraction with application to pharmacokinetic studies

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ABSTRACT

A simple, rapid and selective high performance liquid chromatography–atmospheric pressure chemical ionization-mass spectrometry (HPLC–APCI-MS) method was developed and validated for the simultaneous estimation of cefpodoxime proxetil (CDPX) and clavulanic acid (CA) in human plasma. Extraction of samples was done by solid phase extraction technique (SPE) and chloramphenicol used as internal standard. Chromatographic separation was carried out on a reverse phase Princeton SPHER C_{18} (150 mm × 4 mm i.d., 5 μm) column using mixture of methanol: acetonitrile: 2 mM ammonium acetate (25:25:50, v/v, pH 3.5) at 0.8 mL/min flow rate. Detection was performed on a single quadrupole MS by selected ion monitoring (SIM) mode via APCI source. The calibration curve was linear within the concentration range, 0.04–4.4 μg/mL and 0.1–10.0 μg/mL for CDPX and CA respectively. Pharmacokinetic parameters of tablet (CDPX 200 mg, CA 125 mg) were evaluated. C_{max}, T_{max}, $T_{1/2}$, elimination rate constant (K_{el}), AUC_{0-t}, and $AUC_{0-\infty}$ of tablet were 2.13 ± 0.06 μg/mL, 2 h, 3.05 ± 0.15 h, 0.24 ± 0.37 h^{-1}, 6.81 ± 0.14 μg h/mL and 7.72 ± 0.23 μg h/mL respectively for cefpodoxime (CP), 5.34 ± 0.28 μg/mL, 2 h, 2.73 ± 0.25 h, 0.26 ± 0.31 h^{-1}, 15.37 ± 0.16 μg h/mL and 16.59 ± 0.53 μg h/mL respectively for CA.

1. Introduction

Cefpodoxime proxetil (CDPX), [(RS)-1(isopropoxycarbonyloxy) ethyl (+)-(6*R*, 7*R*)-7-[2-(2-amino-4-thiazolyl)-2-{(*Z*) methoxyimino} acetamido]-3-methoxymethyl-8-Oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylate] is an oral third generation cephalosporin antibiotic, active against many gram positive and negative microorganisms. CDPX has an asymmetric carbon, which is supplied as a racemic mixture of two enantiomers *R* and *S* forms, the *R*- and *S*-isomers of CDPX are reported to exhibit similar biological activity, but differ in their physicochemical and biological properties [1]. Clavulanic acid (CA), [(*Z*) (2*R*, 5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo [3.2.0]-heptane-2-carboxylate] is a beta-lactam structurally related to the Penicillin's [2]. CA covalently binds to a serine residue in the active site of the beta-lactamase, this restructures the CA molecule, creating a much more reactive species that is attacked by another amino acid in the active site, permanently inactivating it, and thus inactivating the enzyme. CA is particularly active against the clinically important plasmid mediated beta-lactamases frequently responsible for transferred drug resistance to β-lactam antibiotics. The combination of CDPX and CA is very effective than individual CDPX, because of inactivation of penicillin resistant beta lactamases by CA. This inhibition restores the antimicrobial activity of beta-lactam antibiotics against lactamase-secreting resistant bacteria.

A number of analytical methods were reported for quantification of CP and CA, both individually and in combination with other drugs [3–10].

Horimoto et al. [11] developed high performance liquid chromatography–atmospheric pressure chemical ionization-mass spectrometry (HPLC–APCI-MS), soft ionization method adding accelerating solvents (chloroform and bromoform) in the negative-ion mode for a heat unstable antibiotic, FC/TA-891 and its active metabolite. By this method, the molecular weights of FC/TA-891 and FCE22101 could be identified clearly and easily. Due to similar chemical–physical properties of β-lactam antibiotics with FC/TA-891, Horimoto et al. [12], analyzed the β-lactam antibiotics by HPLC–APCI-MS in negative mode using bromoform as an ionization accelerating solvent. Results under these conditions showed that bromine adduct ions could be clearly distinguished from other ions, leading to an easy identification of the compounds and it found that APCI-MS with bromoform could be applicable in the separation analysis of β-lactam antibiotics.

Eckers et al. [13] estimated clavam-2-carboxylate (c-2-c), a compound structurally related to CA, by selected reaction monitoring

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(SRM) of m/z 156 giving rise to m/z 114. Applicability of the same method to estimate c-2-c in spiked clavulanate potassium samples were found difficult due to the structural similarity among them leading to clavulanate potassium's interference in ionization during mass and therefore they derivatized CA with imidazole and estimated by LC–MS/MS. In contrast, Yoon et al. [2] later reported a simple analytical method for simultaneous determination of amoxicillin and CA in human plasma using HPLC with ESI mass spectrometry in the negative selected ion-monitoring (SIM) mode at m/z 198 ($(M-H)^-$). In this paper, we present a simple method using HPLC–APCI-MS under the negative-ion mode was applied for the simultaneous quantification of CP and CA in biological samples. The method was optimized, validated and applied to analysis of plasma samples in a pharmacokinetic study involving twelve healthy volunteers.

2. Experimental

2.1. Chemicals and reagents

Working standards of CDPX (99.9%) and CA (99.9%), chloramphenicol (99.9%) (Internal standard, IS) and tablets (Combination of CDPX-200 mg and CA-125 mg) were obtained from 21st Century Pharmaceuticals (Chennai, India). HPLC grade acetonitrile and methanol were from Merck (Mumbai, India), ammonium acetate AR grade obtained from Qualigens fine chemicals (Mumbai, India) and HPLC grade water from Milli-Q RO system (Millipore India, Bangalore, India) were used. Pool of drug free human plasma obtained from healthy volunteers.

2.2. Preparation of calibration standards

Calibration standards were prepared by spiking the working standard solution into a pool of drug free human plasma in order to obtain the following concentrations: 0.04, 0.08, 0.22, 0.44, 0.88, 1.76, 2.64, 3.52, 4.4 μg/mL of cefpodoxime (CP) and 0.1, 0.2, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 μg/mL of CA. These solutions were labeled and stored at −70 °C until analysis.

2.2.1. Preparation of quality control samples

Quality control samples (QCs) at a minimum of three concentrations (one within 3× of the lower limit of quantification (low QC), one in the midrange (middle QC), and one approaching the high end of the range (high QC)) [14] were prepared by spiking the working standard solution into a pool of drug free human plasma in order to obtain following concentration: 0.12, 2.2, and 4.0 μg/mL, respectively, for CP and 0.3, 5.0, and 9.0 μg/mL, respectively, for CA. These solutions were labeled and stored at −70 °C until analysis.

2.3. Extraction of plasma samples

Solid phase extraction (SPE) of plasma samples was performed on Sam prep, C_{18} cartridges (3 mL, 100 mg) (Ranbaxy Fine Chemicals Ltd., New Delhi, India), pretreated with methanol (1000 μL) followed by HPLC grade water (1000 μL). Plasma (500 μL) was diluted with 500 μL of a solution containing IS (200 μg/mL) in acetonitrile and water (1:1) mixture and applied to the extraction column. Each cartridge was rinsed with 900 μL of HPLC grade water, and eluted with 100 μL of mobile phase. A 20 μL volume of the mobile phase eluent was injected into the LC–MS.

2.4. LC–MS analysis

Shimadzu LCMS 2010A (Shimadzu Corporation, Kyoto, Japan) equipped with single quadrupole MS with following configuration LC-10 AD-Vp solvent delivery system (pump), SIL 10 AD-Vp Auto injector, CTO 10 Vp column oven, DGU 14AM de gasser and LC–MS solution data station was used. The separation was performed on a Princeton SPHER (Princeton Chromatography Inc., NJ, USA) C_{18} column (150 mm × 4 mm i.d., 5 μm) using a mixture of methanol:acetonitrile:ammonium acetate (2 mM, pH 3.5) (25:25:50, v/v/v) at a flow rate of 0.8 mL/min, column temperature was maintained to ambient temperature conditions and eluent was introduced into negative APCI-MS. The ion source and desolvation temperatures were set at 400 °C and 250 °C, respectively, capillary voltage was set to 1.3 kV and peak areas of analytes were automatically integrated using LC–MS solution data station.

2.5. Method validation

The method validation of CP and CA in human plasma was performed according to the FDA guidelines [14]. The assay was validated for specificity, linearity, sensitivity, accuracy, precision, extraction recovery, matrix effect, and stability.

2.5.1. Specificity

Specificity of the method assessed by analyzing six different lots of blank human plasma that was either blank alone or it spiked with analytes at LLOQ level. Analytes were extracted using SPE technique described in Section 2.3, and analyzed to determine the potential interferences at the retention window of analytes and IS.

2.5.2. Linearity and sensitivity

Linearity was evaluated using plasma calibration curve standards of CP and CA at concentration range 0.04–4.4 μg/mL and 0.1–10.0 μg/mL, respectively. The IS, concentration was 100 μg/mL in all calibration standards. The calibration curve for CP and CA was obtained through analysis of plasma calibration standards and plot of response factor (peak area ratios of CP and CA to IS) versus the corresponding CP (0.04–4.4 μg/mL) and CA (0.1–10.0 μg/mL) concentrations. The obtained results were fitted into a linear regression equation: $(y) = a + bx$, where 'x' and 'y' were the variables, 'b' was slope of the regression line and 'a' was intercept point of the regression line and the y-axis. The lowest limit of quantification (LLOQ) was set as the lowest amount of analyte in a sample that could be quantitatively determined with acceptable precision and accuracy (i.e., 20% coefficient of variation [CV] and ±20% nominal concentration in these assays, respectively).

2.5.3. Precision and accuracy

The intra-day precision and accuracy was evaluated at three different QC levels in six replicates on the same day and on three different days for inter-day precision and accuracy determination. Acceptable deviation was set within 15% of the nominal concentration for accuracy and within 15% of the CV for precision.

2.5.4. Extraction recovery

The extraction efficiency was measured by comparing the peak area of analyte (A) added into blank plasma and pretreated by SPE with that of analyte (B) spiked in post extracted blank plasma at the same nominal concentrations. The ratio $(A/B \times 100)$ was defined as the extraction efficiency. The extraction recovery of CP and CA were carried out at the three QCs levels (0.12, 2.2, 4.0 μg/mL, for CP, and 0.3, 5.0, 9.0 μg/mL, for CA) in six replicates, and that of IS was determined with the same method.

2.5.5. Ion suppression and matrix effect

Endogenous substances present in biological matrix possibly enhance or suppress analyte ionization to affect the sensitivity, precision or accuracy of the described method. The degree of ion suppression was checked by post column infusion experiment. A standard solution containing analytes and IS was infused through

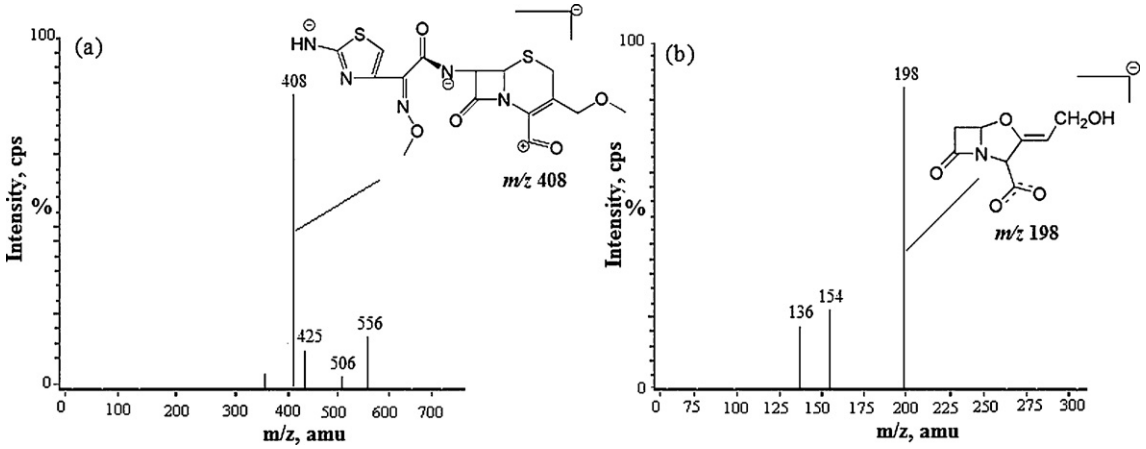

Fig. 1. Chemical structure and mass spectrum of CP (a) and CA (b).

syringe pump at 20 μL/min. Once the baseline stabilizes, an injection of extracted blank plasma was made and SIM chromatograms were acquired for analytes and IS. The matrix effect, due to co-eluting plasma components, was evaluated by spiking six different lots of blank human plasma with the QC solutions [15]. The matrix effect of CP and CA was determined at three QC levels by comparing the peak area ratios of samples prepared in plasma with peak area ratios of samples prepared in acetonitrile:water mixture (1:1), and checked for % CV which should be less than 10% at three QC levels.

2.5.6. Stability

The stability of CP and CA in biological matrix was determined by the analysis of six replicates of QCs ($n = 3$) exposed to various storage conditions. For freeze thaw stability studies, QCs were subjected to freeze thaw (3 cycles). Each sample was stored at −70 °C for 24 h and thawed at room temperature, after which the samples were refrozen for 12–24 h under the same conditions. At the end of each cycle, the samples were processed, analyzed and compared with the freshly prepared QCs. For the short-term and stock solution stability study, QCs were kept at 25 °C for 6 h and samples were processed at different time points and were analyzed and compared with the freshly prepared QCs. To assess the long-term stability, QCs were stored at −70 °C for 3 months, which exceeds the time between sample collection and sample analysis.

3. Clinical design

The Institutional Ethics Committee approved the study protocol. Twelve healthy Indian male subjects with age group of 20–40 years and weight of ±15% from ideal weight for subjects' height and elbow breadth were included in the study. The subjects were certified as healthy subjects by physician based on biochemical, hematological and vital physiological parameters. Subjects were excluded from the study, if one or more, following criteria were present at time of medical screening: allergic to antibiotic, history or clinical data of renal or liver disease, positive test for hepatitis B, HIV, history of alcohol, drug addiction or donated blood within 72 days prior to study. Tablet (CDPX 200 mg and CA 125 mg) was administered with 240 mL of water. Volunteers were fasted 7 h prior to the drug administration. After drug administration standard breakfast and lunch was provided at 3 and 6 h post dose. Blood samples (5 mL) were collected at 0.0 h (pre drug administration) 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0 and 12.0 h of post drug administration through an indwelling cannula into heparinized glass vials, immediately centrifuged at 4000 rpm for 10 min at room temperature (approx. 15–18 °C). Separated plasma samples were stored at −70 °C to allow sufficient numbers of samples to accumulate to perform the analysis as well as to avoid degradation [16,17]. Plasma samples were removed from the deep freezer 15 min prior to sample preparation extracted and analyzed by validated LC–MS method.

3.1. Pharmacokinetic analysis

The plasma concentration-time profile obtained was fed into, PK software (Add-on program to Microsoft Excel) to determine the pharmacokinetic parameters. Maximum concentration C_{max} and the corresponding peak time T_{max} were determined by the inspection of the individual drug plasma concentration–time profiles, elimination rate constant K_{el} was obtained from the least-square fitted terminal log-linear portion of the plasma concentration–time profile, elimination half-life ($T_{1/2}$) was calculated as $0.693/K_{el}$, area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule and area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t} + C_t/K_{el}$ where C_t is the last concentration.

4. Results and discussion

4.1. LC–MS separation and specificity

LC–APCI-MS analysis of CP and CA in negative mode, produced deprotonated molecular ions at m/z 408 ($(M-((CH_3)^-)-2H)^-$) and m/z 198 ($(M-H)^-$) for CP and CA, respectively (Fig. 1) without any evidence of adducts formation. Deprotonated molecular ions from CP and CA were selected as the product ions. The quantification of the CP and CA was performed using SIM mode, due to the high selectivity. SIM mode at m/z 408 for CP, m/z 198 for CA and m/z 321 for IS was selected. The C_{18} column with a mobile phase resulted in chromatographic run time of 10 min with satisfactory separation of CP, CA and IS without using gradient elution. Fig. 2 shows the representative LC–MS chromatogram obtained from the blank plasma and plasma spiked with CP, CA and IS. The analytical run time was 2.03 for IS, 3.87 for CA and 7.7, 8.27 for CP, this is because, CP has an asymmetric carbon and is supplied as a racemic mixture of two enantiomers *R* and *S* forms, due to that CP split into two enantiomer peaks. For assessing the specificity, six replicates of extracted samples at LLOQ level of CP and CA were prepared and analyzed. These six replicates confirming that interference do not affect the quantification at LLOQ level. Utilization of selected product ion for each compound enhanced mass

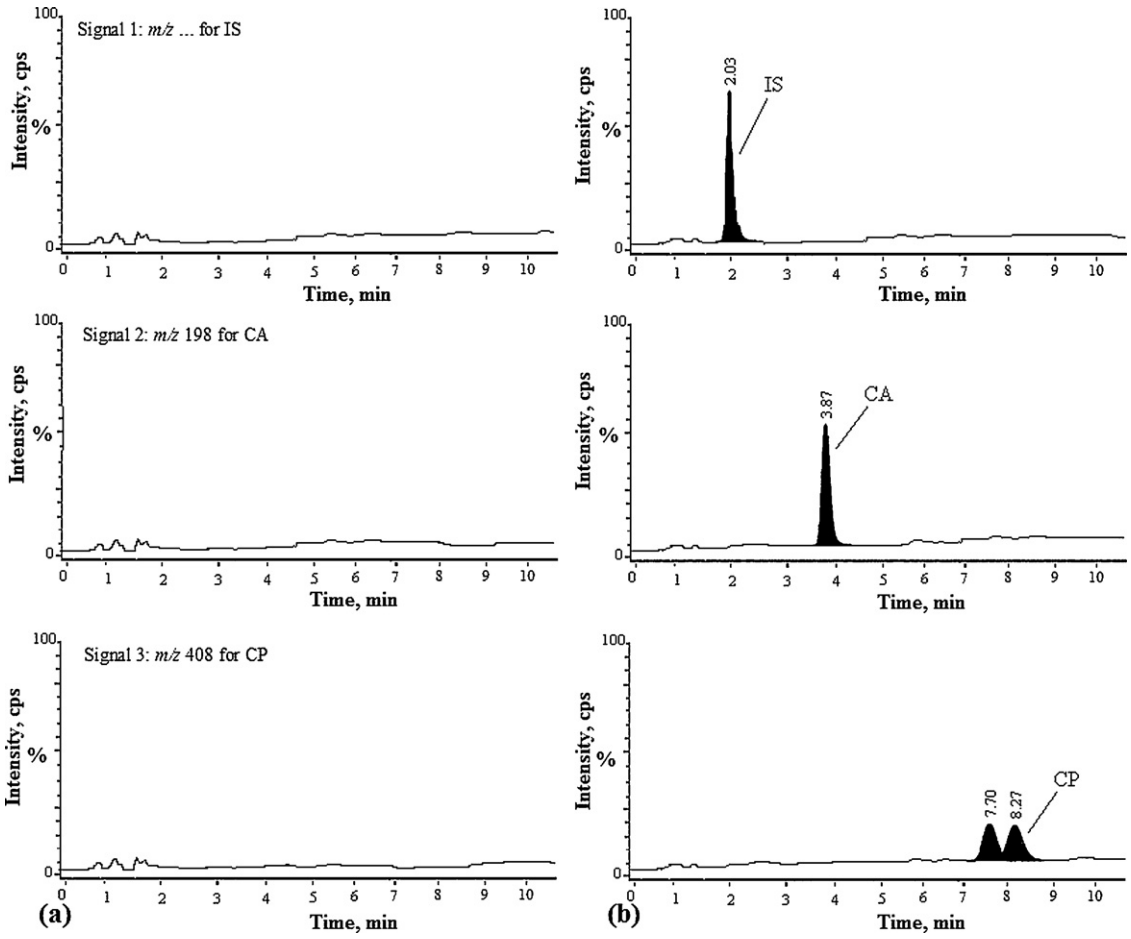

Fig. 2. LC–MS chromatogram from blank plasma (a), plasma spiked with CP, CA and IS (b).

spectrometric selectivity. The product ion of *m*/*z* 408, 198 and 321 were hence concluded to be specific for CP, CA and IS, respectively.

4.2. Recovery, ion suppression and matrix effect

Analytes were extracted from biological samples by SPE using several solvents for their extraction efficiencies such as methanol, acetonitrile, ammonium acetate buffer, mobile phase and HPLC grade water. Mobile phase (ammonium acetate:acetonitrile:methanol, 50:25:25, v/v/v) showed good recovery for both CP and CA. The mean recovery values of CP and CA were ranged from 91.67% to 92.73% and 92.89% to 93.60%, respectively (Table 1). Post column infusion chromatograms in Fig. 3 indicates no ion suppression or enhancement at the retention time of analytes and IS. Assessment of matrix effect constitutes an important and integral part of validation for quantitative LC–MS for supporting pharmacokinetics studies. Table 1 shows the absolute matrix effect values, results found were well within the acceptable limits i.e., 10% as the %CV at three QC levels were ranged from 2.36% to 4.64% for CP and 4.53% to 4.89% for CA. Hence minor suppression of analyte signal due to endogenous matrix interferences did not affect the quantification of analytes.

4.3. Accuracy and precision

Intra-, inter-day precision and accuracy were evaluated with six replicates at three different concentrations for both CP and CA. As shown in Table 2, intra-, inter-day precision values of CP and CA, expressed as % CV, ranged from 2.3% to 3.8% and 2.5–3.8%, respectively, for CP and 1.4–4.3% and 1.6–4.7%, respectively, for CA, where as accuracy values expressed as %nominal, ranged from 90.75% to 92.27% and 83.33–86.75%, respectively, for CP and 92.20–93.33% and 83.67–84.80%, respectively, for CA. The results from intra-, inter-day precision and accuracy indicate that the method reproducibility is acceptable within the same day and on different days.

4.4. Linearity and sensitivity

The standard curve consisting of nine points ranging from 0.04 to 4.4 μg/mL for CP and 0.1 to 10.0 μg/mL for CA were extracted and assayed. A typical regression equation for the calibration curve was: $y = 0.030x + 0.007$ for CP and $y = 0.025x + 0.071$ for CA. Linear calibration curves in plasma were obtained in the indicated CP and CA concentration range, regression coefficient (r^2) was found to be 0.999 for both CP and CA. LLOQ was tested at different levels ranging from 0.01 to 0.1 μg/mL and it was found to be 0.04 μg/mL for CP with an accuracy of 83.33% and 4.7% precision while CA showed an LLOQ of 0.1 μg/mL with 84.74% accuracy and 4.8% precision. The results indicating for LLOQ of CP and CA were with in the acceptable precision and accuracy range.

4.5. Stability study

The stability of CP and CA in biological matrix was evaluated by measuring concentration change in QCs ($n = 3$) under different storage conditions, results from stability studies indicated that both CP and CA were stable in biological matrix at 25 °C for 6 h, at −70 °C

Table 1
Recovery & matrix effect studies of CP and CA ($n = 6$).

Analyte	Quality control standards (μg/mL)	Concentration found (mean, μg/mL)	(%) Recovery	(%) CV	Absolute matrix effect	(%) CV
CP	0.12	0.11	91.67	4.89	93.14	4.64
	2.2	2.04	92.73	2.79	94.96	2.36
	4.0	3.69	92.25	0.68	92.78	4.12
CA	0.3	0.28	93.33	4.05	94.38	4.71
	5.0	4.68	93.60	3.27	94.77	4.89
	9.0	8.36	92.89	1.88	93.54	4.53

Table 2
Accuracy and precision study of CP and CA ($n = 6$).

Analyte	Statistical variables	Intra-batch (μg/mL)			Inter-batch (μg/mL)		
		0.12	2.2	4.0	0.12	2.2	4.0
CP	Mean	0.11	2.03	3.63	0.10	1.88	3.47
	Accuracy (% nominal)	91.67	92.27	90.75	83.33	85.45	86.75
	Precision (%CV)	3.8	1.3	2.3	3.8	3.8	2.5

Analyte	Statistical variables	Intra-batch (μg/mL)			Inter-batch (μg/mL)		
		0.3	5.0	9.0	0.3	5.0	9.0
CA	Mean	0.28	4.61	8.32	0.25	4.53	7.62
	Accuracy (% nominal)	93.33	92.20	92.44	83.67	84.80	84.71
	Precision (%CV)	3.3	4.3	1.4	3.3	4.7	1.6

Table 3
Stability of LC–MS assay method for CP and CA in human plasma ($n = 6$).

Analyte	Stability	Mean			(%) CV			(%) Degradation		
		0.12	2.2	4.0	0.12	2.2	4.0	0.12	2.2	4.0
CP	Freeze-thaw (3 cycles at −70 °C)	0.11	2.01	3.65	3.8	2.5	1.4	8.3	8.6	8.8
	Short term (at 25 °C)	0.10	1.98	3.63	3.8	1.2	2.8	16.7	10.0	9.3
	Long term (at −70 °C)	0.09	1.77	3.21	3.8	3.8	2.3	25.0	19.5	19.8
	Stock solution (at 25 °C)	0.11	2.12	3.86	3.8	2.5	2.9	8.3	3.6	3.5

Analyte	Stability	Mean			(%) CV			(%) Degradation		
		0.3	5.0	9.0	0.3	5.0	9.0	0.3	5.0	9.0
CA	Freeze-thaw (3 cycles at −70 °C)	0.28	4.57	8.28	3.3	4.0	1.6	6.7	8.6	8.0
	Short term (at 25 °C)	0.27	4.49	8.21	3.3	3.3	0.5	10.0	10.2	8.8
	Long term (at −70 °C)	0.23	3.96	7.18	3.3	2.6	23.3	20.8	20.2	23.3
	Stock solution (at 25 °C)	0.29	4.85	8.73	3.3	1.6	1.9	3.3	3.0	3.0

for 3 months and during three freeze thaw cycles. Stock solutions of CP and CA were stable for at least 6 h at 25 °C. Table 3 summarizes the mean concentration ($n = 6$) and percentage degradation of the analytes under all the conditions tested.

4.6. Clinical application

The developed and validated method was successfully applied for the determination of CP and CA in human plasma samples collected from twelve healthy volunteers after oral administration of tablet. Fig. 4 shows the mean plasma concentration–time profile of CP and CA in twelve healthy volunteers who received tablet. The Pharmacokinetic parameters such as C_{max}, T_{max}, $T_{1/2}$, K_{el}, AUC_{0-t} and $AUC_{0-\infty}$ of tablet was 2.13 ± 0.06 μg/mL, 2 h, 3.05 ± 0.15 h, 0.24 ± 0.37 h^{-1}, 6.81 ± 0.14 μg h/mL and 7.72 ± 0.23 μg h/mL, respectively, for CP and 5.34 ± 0.28 μg/mL, 2 h, 2.73 ± 0.25 h, 0.26 ± 0.31 h^{-1}, 15.37 ± 0.16 μg h/mL, 16.59 ± 0.53 μg h/mL, respectively, for CA (Table 4).

4.7. Incurred sample analysis

Assay reproducibility for incurred samples was the defining moment in establishing incurred sample reanalysis (ISR) as a mandatory exercise in demonstrating assay reproducibility using incurred samples. Twelve incurred plasma samples from one volunteer, initially analyzed using an LC–MS assay from

Table 4
Pharmacokinetic parameters of CP and CA.

Parameters	CP	CA
C_{max} (μg/mL)	2.13 ± 0.06	5.34 ± 0.28
T_{max} (h)	2.0 ± 0.0	2.0 ± 0.0
K_{el} ($1\ h^{-1}$)	0.24 ± 0.37	0.26 ± 0.31
$T_{1/2}$ (h)	3.05 ± 0.15	2.73 ± 0.25
AUC_{0-t} (μg h/mL)	6.81 ± 0.14	15.37 ± 0.16
$AUC_{0-\infty}$ (μg h/mL)	7.72 ± 0.23	16.59 ± 0.53

Table 5
Incurred sample analysis for CP and CA by LC–MS ($n = 6$).

Incurred samples (h)	Weeks	CP			CA		
		Original concentration (μg/mL)	ISR concentration (μg/mL)[a]	(%) Difference	Original concentration (μg/mL)	ISR concentration (μg/mL)[a]	(%) Difference
0.0	4	0.0	0.0	0.0	0.0	0.0	0.0
0.5	4	0.50	0.48	−4.2	0.66	0.64	−3.2
1.0	4	0.98	0.96	−2.1	2.85	2.80	−1.9
1.5	4	1.59	1.57	−1.3	3.95	3.88	−1.7
2.0	4	2.13	2.11	−0.9	5.34	5.31	−0.6
2.5	4	1.83	1.82	−0.5	4.06	4.03	−0.8
3.0	4	1.37	1.35	−1.5	3.46	3.38	−2.4
4.0	4	1.12	1.08	−3.7	2.29	2.22	−3.2
5.0	4	0.73	0.72	−1.4	1.83	1.75	−4.3
6.0	4	0.52	0.50	−4.0	1.21	1.16	−4.7
8.0	4	0.45	0.44	−2.3	0.86	0.84	−2.5
10.0	4	0.31	0.30	−3.3	0.57	0.55	−2.9
12.0	4	0.21	0.20	−1.9	0.40	0.39	−3.3

[a] ISR – incurred sample reanalysis.

that twelve samples were randomly selected and were reanalyzed using the same procedure in a different run. The results obtained, as well as their percentage difference, calculated, are presented in Table 5. Results indicated that the percentage difference between initial sample analysis and ISR were with in the 20% limit [18], which showed that the developed method was reproducible.

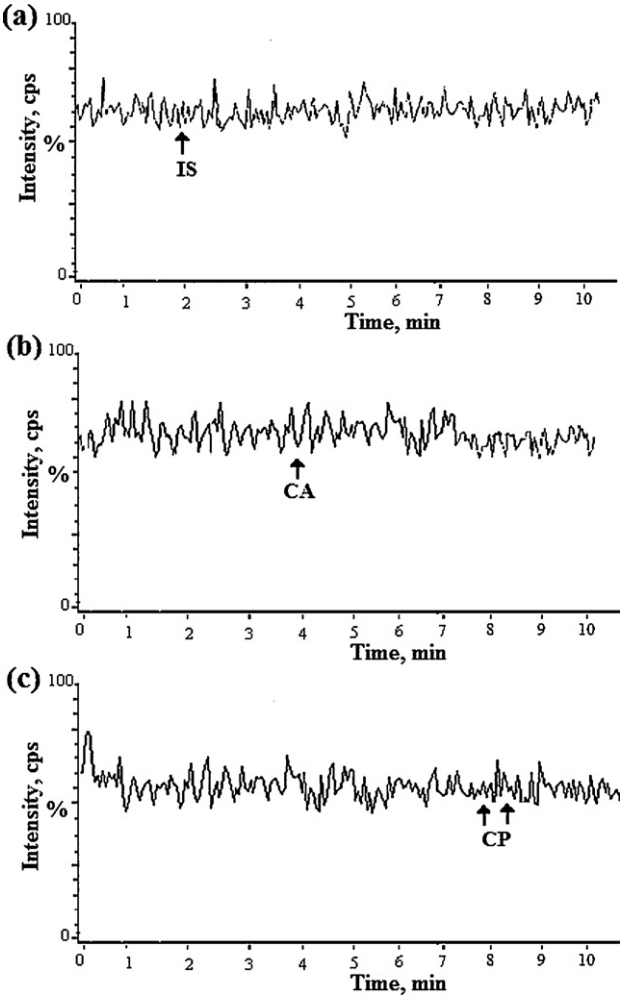

Fig. 3. Post column infusion chromatograms for (a) IS, (b) CA and (c) CP.

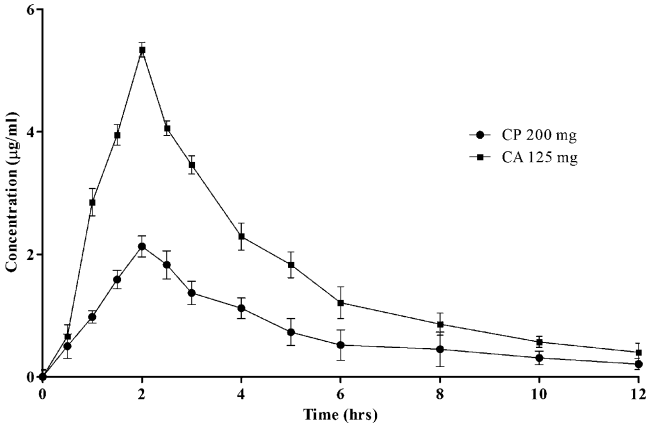

Fig. 4. Plasma concentration–time curve of CP and CA after single oral dose of CP-200/CA-125 mg ($n = 12$), each point represents a mean concentration of 12 volunteers.

5. Conclusion

A rapid, sensitive, reliable and reproducible LC–MS method for the quantification of CP and CA in human plasma has been successfully developed and validated using SPE technique. The sensitivity and simplicity of the method makes it suitable for pharmacokinetic studies. This method has been corroborated to be useful for studying the pharmacokinetics of any combined formulation of CP and CA, simultaneously.

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