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# Liquid chromatography/electrospray tandem mass spectrometry method for the determination of cefuroxime in human plasma: Application to a pharmacokinetic study

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ABSTRACT

A rapid, selective and sensitive high performance liquid chromatography–tandem mass spectrometry method (LC–MS/MS) was developed and validated for the determination and pharmacokinetic investigation of cefuroxime in human plasma. Cefuroxime and the internal standard (IS), cefoxitin, were extracted from plasma samples using solid phase extraction with Oasis HLB cartridges. Chromatographic separation was performed on a LiChrospher® 60 RP Select B column (125 mm × 4 mm i.d., 5 μm particle size) using acetonitrile:5 ± 0.2 mM ammonium acetate solution:glacial acetic acid (70:30:0.020, v/v/v) as the mobile phase at a flow rate of 0.8 mL/min. Detection of cefuroxime and cefoxitin was achieved by tandem mass spectrometry with an electrospray ionization (ESI) interface in negative ion mode. The calibration curves were linear over the range of 81.0–15976.2 ng/mL with the lower limit of quantitation validated at 81.0 ng/mL. The intra- and inter-day precisions were within 7.6%, while the accuracy was within ±6.3% of nominal values. No matrix effect was observed in this method. The validated LC–MS/MS method was successfully applied for the evaluation of pharmacokinetic and bioequivalence parameters of cefuroxime after an oral administration of 500 mg cefuroxime tablet to 36 healthy male volunteers.



## 1. Introduction

Cefuroxime [(6R, 7R)-3-carbamoyloxymethyl-7-[Z-2-methyloxyimino-2-(2-furyl) acetamido]-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid (Fig. 1)], a β-lactum antibacterial, is the first of a α-methoxyiminoacyl substituted second-generation cephalosporin. Due to its low oral bioavailability, cefuroxime is administered orally as a prodrug in the form of cefuroxime axetil [1–3]. Upon administration, this acid-stable lipophilic prodrug undergoes hydrolysis either in the intestinal mucosa, the portal circulation, or in the liver to yield cefuroxime [4]. The oral bioavailability of this ester prodrug is surprisingly high, ranging from 30% to 60% and has a significantly shorter half-life [5,6].

In literature, several analytical methods for monitoring plasma levels of cefuroxime in biological matrix have been reported. Most of these assays were based on the use of microbiological techniques [7–9] or high performance liquid chromatography (HPLC) coupled with UV-detection [10–15]. Among the currently available bio-analytical techniques, liquid chromatography coupled with mass spectrometry (LC–MS) and tandem mass spectrometry (LC–MS/MS) has been emerged as the preeminent analytical tool for quantification of small molecular weight drugs in biological matrix. To the best of our knowledge only one method has been developed and validated for quantification of cefuroxime in human serum or plasma using liquid chromatography tandem mass spectrometry (LC–MS/MS) [16]. The published LC–MS/MS method has relatively long analytical run time (~10 min) that does not meet the requirement of high throughput and speed in biosamples analysis.

The method reported here describes an improved, rapid, selective, robust and high throughput LC–MS/MS assay for quantification of cefuroxime in human plasma using solid phase extraction technique and its application to the bioequivalence study in healthy male volunteers after an oral administration of cefuroxime. The total run time of the method per sample was 1.5 min which was shorter than the reported ones.

## 2. Experimental

### 2.1. Chemicals and materials

Working standards of cefuroxime sodium (92.3%, of cefuroxime) and cefoxitin (99.2%) for use as internal standard (IS) were procured from USP. Ammonium acetate and acetic acid (glacial) were of LC–MS grade and were purchased from FLUKA (Sigma–Aldrich, Steinheim, USA). Acetonitrile and methanol of HPLC grade were

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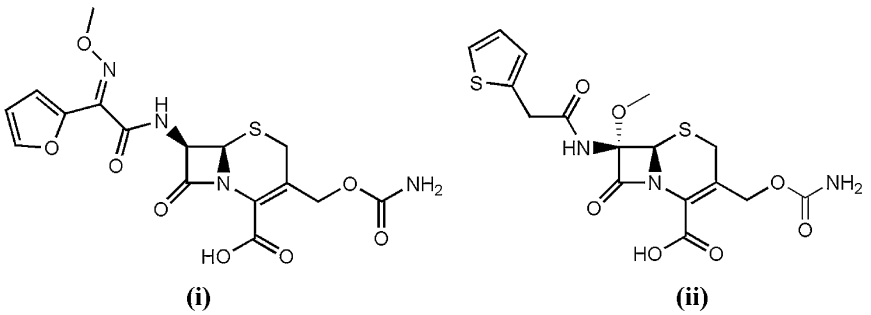


**Fig. 1.** Chemical structure of (i) cefuroxime and (ii) cefoxitin.

obtained from Spectrochem (Mumbai, India) and Qualigens Fine Chemicals (Mumbai, India), respectively. Oasis HLB, 30 mg; 1 $cm^3$, solid phase cartridges were obtained from Waters Corporation (Milford, USA). All aqueous solutions and buffers were prepared using water that was purified using Milli-Q® Gradient A10® (Millipore, Moscheim Cedex, France). Different individual lots of human plasma, used to prepare calibration standards and quality control (QC) samples, were obtained from the clinical unit of Ranbaxy Research Laboratories (Noida, India).

### 2.2. Preparation of stock solution, standard and quality control samples

Stock solution of cefoxitin and two stock solutions of cefuroxime were prepared separately by dissolving the accurately weighed compounds in methanol to give a final concentration of 1 mg/mL. These two stock solutions of cefuroxime were used for calibration standards and QC samples, respectively. The prepared stock solutions were stored in refrigerator between 1 and 10 °C until use. Working solutions of cefuroxime (ranging from 4.05 to 798.81 μg/mL) were prepared by serial dilution of the stock solution in methanol–water (50:50, v/v). A 100 μL aliquot of each working solution was added to blank CPDA plasma to yield spiked calibration standards at eight different concentrations ranging from 81.0 to 15976.2 ng/mL. Quality control (QC) samples at four concentrations (81.7, 240.3, 6008.4 and 12016.8 ng/mL) were prepared in the similar manner as the calibration standards. Spiked calibration standards and quality control samples were stored at around −20 °C until assayed or used for validating the analytical method. The amount of working solution in all spiked samples was kept at 2% of the total sample volume to minimize any systemic errors between the real samples and standards.

The IS working solution (20.0 μg/mL) for routine use was prepared by diluting the cefoxitin stock solution in methanol–water (50:50, v/v) and stored at room temperature.

### 2.3. LC–MS/MS instrumentation and analytical conditions

The liquid chromatographic separation was performed using a Shimadzu scientific instruments (Shimadzu Corporation; Kyoto, Japan) consisted of two LC-20AD delivery pumps, an on-line DGU-20A3 prominence solvent degasser, a SIL-HTc Shimadzu autosampler and a CBM-20A prominence column oven. Liquid chromatographic separations were achieved using LiChrospher® 60 RP Select B column (125 mm × 4 mm i.d., 5 μm particle size) (Merck Scientific, USA). An injection volume of 10 μL was used for each analysis. Mobile phase consisted of acetonitrile:5 ± 0.2 mM ammonium acetate solution:glacial acetic acid (70:30:0.020, v/v/v). The flow rate of the mobile phase was 0.8 mL/min and splitting ratio was set at 5:10. The column and autosampler temperature were maintained at 35 ± 1 °C and 10 ± 1 °C, respectively.

Samples were analyzed with API-3200 triple quadrupole mass spectrometer (MDS Sciex®; Toronto, Canada) equipped with an electrospray ionization source operating in negative polarity. Nitrogen was used as the nebulizer, auxillary, collision and curtain gases. Analytes were detected by tandem mass spectrometry using multiple reaction monitoring (MRM) of precursor–product ion transitions with 200 ms dwell time, at $m/z$ 423.2/207.0 for cefuroxime and $m/z$ 426.4/156.0 for cefoxitin.

The instrument response was optimized by syringe pump infusion of cefuroxime and cefoxitin in mobile phase by constant flow (10 μL/min) into the stream of mobile phase eluting from the LC column. The main working source/gas parameters of the mass spectrometer were optimized and maintained as follows: collision activated dissociation (CAD) gas, 3; curtain gas, 25; gas 1 (nebulizer gas), 40; gas 2 (heater gas), 60; turbo ion spray (IS) voltage, −4500 V; source temperature, 350 °C. The compound parameters like, declustering potential (DP), entrance potential (EP), collision energy (CE), and collision cell exit potential (CXP) were optimized and set at −25, −5, −15 and −3 V, respectively, for cefuroxime and cefoxitin.

Calibration curves were constructed by calculating the analyte to IS peak area ratio ($y$) against analyte concentrations ($x$). Data acquisition and processing were performed using Analyst version 1.4.1 software (MDS Sciex; Toronto, Canada).

### 2.4. Sample preparation

Plasma samples stored at around −20 °C were thawed on the day of extraction at room temperature followed by vortexing to ensure homogeneity.

An eppendorf pipette was used to aliquot 500 μL of spiked plasma samples into polypropylene tubes (13 mm × 50 mm). To each tube then added 50.0 μL of internal standard working solution (20.0 μg/mL, cefoxitin in methanol–water, 50:50, v/v), with the use of multistepper and vortexed for 30 s. To each tube 500 μL of 5 ± 0.2 mM ammonium acetate solution titrated to pH 5.0 ± 0.2 with glacial acetic acid was then added, and vortexed again for 30 s. The tubes were centrifuged at 4000 rpm for 5 min and 900 μL of supernatant was loaded using an eppendorf pipette on SPE cartridge and centrifuged at 1500 rpm for a minute. Before that the Oasis HLB cartridge was conditioned with 1 mL of methanol followed by 1 mL of water. After loading of the samples, the cartridge was washed with 1 mL of water twice and analyte and IS were eluted with 1 mL of methanol twice. The extracted samples were evaporated to dryness using a Zymark TurboVap LV evaporator (Caliper, Hopkinton, MA, USA) and reconstituted with 500 μL of mobile phase. Transfer the sample into autosampler vials using an

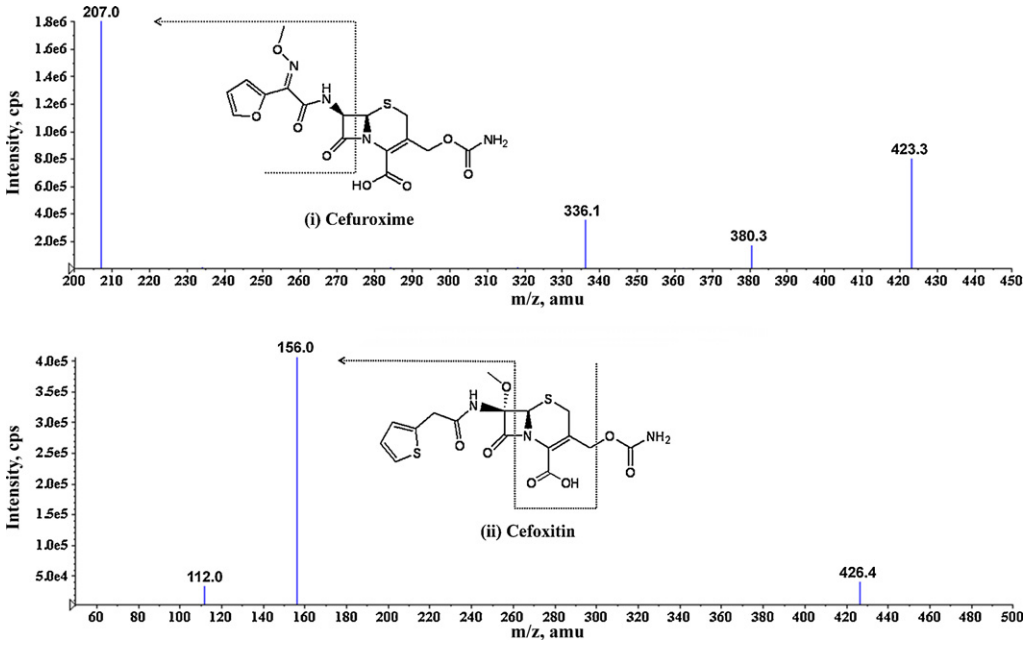


**Fig. 2.** The product ion spectra of cefuroxime and cefoxitin.

eppendorf pipette. 10 μL was injected into the LC–MS/MS system for analysis.

### 2.5. Method validation

A full method validation was performed according to guidelines set by the USFDA [17]. The validation of this procedure was performed in order to evaluate the method in terms of selectivity, sensitivity, linearity of response, accuracy, precision, recovery, matrix effect, and stability of analytes during both short-term sample processing and long-term storage.

#### 2.5.1. Selectivity

The selectivity of the method towards endogenous plasma matrix components, metabolites, and concomitant medications was assessed in 14 lots of blank human plasma (eight CPDA, six $K_2$EDTA, one lipemic and one haemolysed). They were processed and analyzed using the proposed extraction protocol and the set chromatographic conditions of cefuroxime at the LLOQ level.

#### 2.5.2. Linearity and LLOQ

The linearity of the method was determined by analysis of standard plots associated with an eight point standard calibration curve. Calibration curves from accepted three precision and accuracy batches were used to establish linearity. Peak area ratios of analyte/IS obtained from MRM were utilized for the construction of calibration curves; using weighted ($1/x^2$) linear least squares regression of the plasma concentrations and the measured peak area ratios. Back-calculations were made from these curves to determine the concentration of cefuroxime in each calibration standards and the resulting calculated parameters were used to determine concentrations of analyte in quality control or unknown samples. The correlation coefficient $r > 0.98$ was desirable for all the calibration curves. The lowest standard on the calibration curve was to be accepted as the LLOQ, if the analyte response was at least five times more than that of drug free (blank) extracted plasma. In addition, the analyte peak of LLOQ sample should be identifiable, discrete, and reproducible with accuracy within ±20% and a precision ≤20%. The deviation of standards other than LLOQ from the nominal concentration should not be more than ±15.0%.

#### 2.5.3. Accuracy and precision

Intra- and inter-day accuracies expressed as a percentage of deviation from the respective nominal value and the precision of the assay was measured by the percent coefficient of variation (%CV) at concentrations. Intra-day precision and accuracy were assessed by analyzing six replicates of the quality control samples at four levels during a single analytical run. The inter-day precision and accuracy were assessed by analyzing 18 replicates of the quality control samples at each level through three precision and accuracy batches runs on 2 consecutive validation days. The deviation at each concentration level from the nominal concentration was expected to be within ±15.0% except LLOQ QC, for which it should not be more than 20.0%. Similarly, the mean accuracy should not deviate by ±15.0% except for the LLOQ QC where it can be ±20.0% of the nominal concentration.

#### 2.5.4. Recovery

The extraction recoveries for analyte and IS at three QC concentration levels were determined by measuring the mean peak area response of six replicates of extracted quality control samples against the mean peak area response of six replicates each of three neat solutions containing both analyte and IS at concentrations that mimic the final extract for low, middle and high quality control samples.

#### 2.5.5. Matrix effect and matrix factor

Matrix effect was assayed at two concentration levels (low and high quality control concentrations). Six different blank plasma lots, free of any significant interference at the retention time (RT) of analyte and IS, were selected and spiked with the working solution of low and high quality control samples. Spiked samples were processed in duplicate and quantitated against freshly spiked calibration curve. The matrix effect is nullified if the accuracy is within ±15% and precision is ≤15% at the low and high QC concentrations.

The matrix factor (MF) is defined as the peak response in the presence of matrix ions versus the peak response in the absence of matrix ions. Since this method involves terminal drying step as described in Section 2.4, six different blank plasma lots were extracted and reconstituted with reference solution, prepared in mobile phase, containing mixture of IS and cefuroxime at concen-

tration representing 100% extraction of IS and cefuroxime at middle QC concentration.

Matrix factor was determined by measuring the corresponding analyte peak area response in reconstituted matrix samples against the analyte mean peak area response in reference solution. Where as IS normalized matrix factor was determined by comparing the peak area ratio of analyte/IS in reconstituted matrix samples against the mean peak area ratio of analyte/IS in reference solution.

#### 2.5.6. Stability

The stability of cefuroxime in plasma was assessed by analyzing six replicates of QC samples at low and high concentrations of 240.3 and 12016.8 ng/mL during the sample storage and processing procedures. Bench-top stability was assessed after exposure of the plasma samples to room temperature for ~7 h, which exceeds the residence time of the sample processing procedures. The freeze–thaw stability was evaluated after undergoing three freeze (at around −20 °C)–thaw (room temperature) cycles. Long-term stability was assessed after storage of the test samples at around −20 °C for 107 days. The autosampler storage stability was determined by storing the reconstituted QC samples for ~48 h under autosampler condition (maintained at 10 °C) before being analyzed. All stability exercises were performed against freshly spiked calibration standards. The samples were considered stable in plasma at each concentration if the deviation from the mean calculated concentration of stability quality control samples was within ±15%. The working solutions and stock solutions of cefuroxime and the IS were also evaluated for stability at room temperature for 12 h and at refrigerator temperature (between 1 and 10 °C) for 15 days, respectively.

### 2.6. Pharmacokinetic and bioequivalence studies in healthy Volunteers

An open label, balanced, randomized, two-treatment, two-period, two-sequence, single-dose, crossover design was used for the assessment of pharmacokinetics and bioequivalence. Thirty-six healthy adult male volunteers who gave written informed consent took part in this study. After an overnight fast of at least 10 h, all subjects were given a single oral dose of cefuroxime axetil 500 mg tablet of Ranbaxy Laboratories limited and ZINNAT® 500 mg tablet (containing cefuroxime axetil) of GlaxoSmithKline during each period of the study. Blood samples were collected before and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 3, 3.5, 4, 5, 6, 8, 10 and 12 h post-dose in each period. The plasma was immediately separated by centrifugation and stored frozen at around −20 °C until analysis. The pharmacokinetic parameters were calculated by a non-compartmental analysis using WinNonlin Professional software (Version 5.0, Pharsight Corp., Mountain View, CA, USA). The peak plasma concentration ($C_{max}$) and time to reach $C_{max}$ ($T_{max}$) were read directly from the experimental data. The total area under the plasma concentration–time curve from time zero to infinity ($AUC_{0\to\infty}$) and from time zero to the last measurable concentration ($AUC_{0\to t}$) was calculated using the trapezoidal rule-extrapolation method.

## 3. Results and discussion

### 3.1. Optimization of the mass spectrometric condition

Since cefuroxime and cefoxitin have both carboxylic and amino functionality in its chemical structure, the possibility for mass spectrometric detection under positive as well as negative ion mode was first evaluated. The optimal mass spectrometric conditions for the detection of analyte and IS were achieved in negative ion mode with ESI interface. The Q1 full scan spectra were dominated by deprotonated quasimolecular ion $[M-H]^-$ at $m/z$ 423.3 for cefuroxime and 426.4 for cefoxitin with no other additive and fragments ions were observed (data not shown). Fig. 2 shows the product ion spectrum of cefuroxime and cefoxitin. The MS/MS parameters, including the declustering potential (DP), collision cell exit potential (CXP), gases (GS1, GS2, CAD) and collision energy (CE) were optimized to identify the most stable and intense product ion for analyte and IS. The product ion at $m/z$ 207.0 for cefuroxime and at $m/z$ 156.0 for cefoxitin were observed with higher abundance and greater stability. The occurrence of these product ions was typical of all the β-lactum rings and corresponded to the cleavage of cephem nucleus [18,19].

During method development different additives of varying strength were added to the mobile phase, so as to obtain higher abundance of deprotonated parent ion of analyte and IS. Use of ammonium acetate in mobile phase enhances the occurrence of $[M-H]^-$ and eventually results in an improvement in area response for both analyte and IS.

### 3.2. Optimization of chromatographic condition

A number of reversed-phase C18 columns, such as Discovery-C18, Novapack-C18, Zorbax SB-C18 and LiChrospher® 60 RP Select B were tested to obtain optimal response, suitable retention time and good peak shapes for analyte and IS. The LiChrospher® 60 RP Select B column was selected since it provided good peak shape and high intensity with greater signal to noise (S/N) ratio. Later, mobile phase composition was optimized so as to achieve symmetric peak shape, good sensitivity and a shorter run time for the analysis of cefuroxime. Acetonitrile revealed a higher mass spectrometric response and lower background noise than methanol and was chosen for the organic phase. The high organic content shortened the chromatographic cycle time, use of 5 ± 0.2 mM ammonium acetate solution and glacial acetic acid in aqueous phase improves sensitivity for cefuroxime and IS. Thus a mobile phase consisting of acetonitrile:5 ± 0.2 mM ammonium acetate solution:glacial acetic acid (70:30:0.020, v/v/v) was used in the experiment. The retention time for cefuroxime and IS were both 0.81 min as shown in Fig. 3.

### 3.3. Sample preparation optimization

During method development different options were evaluated to optimize sample extraction. Initially, the extraction of analyte was carried out via protein precipitation using common solvents like methanol, acetonitrile, and acetone, but the efforts resulted with poor recoveries (<15%), higher background noise with poor sensitivity. Liquid–liquid extraction technique was also tested to isolate drugs from plasma using methyl tertiary butyl ether, diethyl ether, dichloromethane, and isopropyl alcohol (alone and in combination) as extracting solvents. However, the recovery was inconsistent with frequent clogging of columns. Finally solid phase extraction, using Waters Oasis HLB cartridge, was optimized to extract the analyte and IS from plasma samples. Addition of acidified buffer during sample preparation helped in breaking the drug–protein binding and maximizing their retention on hydrophilic lipophillic balance (HLB) stationary phases. Also, use of water during washing step gave consistent recovery with increased specificity, especially at the LLOQ level with minimized polar matrix interferences. The extraction procedure described here offers a rapid way to isolate analyte and IS from plasma matrix and provides scope for automation.

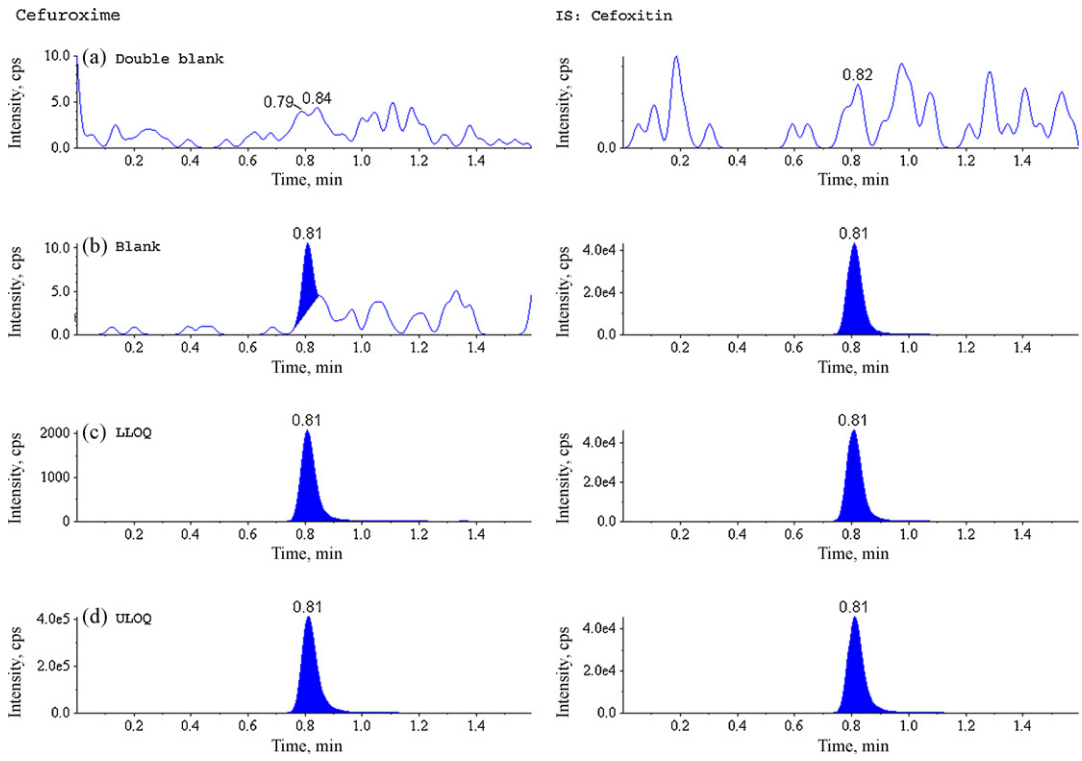


**Fig. 3.** Representative chromatograms in human plasma: (a) double plasma blank; (b) plasma blank with IS; (c) LLOQ, 81.0 ng/mL; and (d) ULOQ 15976.2 ng/mL. Cefuroxime (left panels, a–d) and its IS–cefoxitin (right panels).

### 3.4. Method validation

#### 3.4.1. Selectivity

Selectivity was ascertained in different lots of human plasma by comparing the chromatograms of blank plasma samples with the corresponding spiked LLOQ plasma samples. Fig. 3 shows the typical chromatograms of a double blank, blank spiked with IS, a spiked plasma sample with cefuroxime at LLOQ and ULOQ level. As can be seen no interfering peaks from endogenous compounds were observed at the retention times of the analyte and IS. The chromatograms presented in Fig. 3 indicated that the method was selective.

#### 3.4.2. Linearity and sensitivity

The method was validated using the above criteria and found that plasma calibration standard curve containing cefuroxime was linear over the concentration range of 81.0–15976.2 ng/mL. The intercept with the $y$-axis was not significantly different from zero. A typical regression equation was $y = 2.87 \times 10^{-4}x - 2.67 \times 10^{-3}$ with a correlation coefficient ($r$) of 0.9995, where $y$ represents the peak area ratio of cefuroxime to that of IS and $x$ represents the plasma concentration of cefuroxime. The lower limit of quantification for this assay was 81.0 ng/mL in plasma. With the achieved LLOQ of 81.0 ng/mL, the cefuroxime can be determined in plasma samples until 12 h after a single oral dose of 500 mg cefuroxime, which is sensitive enough to investigate the pharmacokinetic behavior of cefuroxime.

#### 3.4.3. Precision and accuracy

Table 1 summarizes the inter- and intra-day precision and accuracy values for quality control samples. The coefficient of variation values of both intra- and inter-day results were within 7.6% and 6.6%, respectively. Intra- and inter-day accuracies were 93.7–100.9% and 94.9–103.0%, respectively. These results indicate that the present method has good accuracy and precision.

#### 3.4.4. Recovery

At three QC concentration levels 240.3, 6008.4 and 12016.8 ng/mL the percent extraction recoveries (mean ± %SD) of cefuroxime obtained from plasma were 76.4 ± 7.3%, 80.9 ± 6.9% and 78.8 ± 2.3%, respectively. The mean recovery for the internal standard cefoxitin at the concentration employed was 70.0 ± 2.9%. The result indicates that the extraction efficiency for cefuroxime using solid phase extraction was satisfactory, consistent and concentration independent.

#### 3.4.5. Matrix effect and matrix factor

Matrix effect, ion suppression or enhancement, due to the co-eluting endogenous component of sample matrix along with analyte or internal standard may affect the chromatography and

**Table 1**
Accuracy and precision of the method for the determination of cefuroxime in human plasma.

| Nominal concentration (ng/mL) | Intra-day ($n = 6$) | | | | Inter-day ($n = 18$) | | | |
|---|---|---|---|---|---|---|---|---|
| | Average | SD | %Accuracy | CV% | Average | SD | %Accuracy | CV% |
| 81.7 | 82.40 | 4.921 | 100.9 | 6.0 | 84.15 | 4.752 | 103.0 | 5.6 |
| 240.3 | 225.25 | 17.037 | 93.7 | 7.6 | 231.79 | 15.332 | 96.5 | 6.6 |
| 6008.4 | 5719.27 | 130.762 | 95.2 | 2.3 | 5701.61 | 178.761 | 94.9 | 3.1 |
| 12016.8 | 11558.72 | 228.782 | 96.2 | 2.0 | 11746.27 | 402.078 | 97.7 | 3.4 |

**Table 2**
Matrix effect and matrix factor for cefuroxime in six different lots of human plasma.

| Nominal concentration (ng/mL) | Matrix effect | | Matrix factor | |
|---|---|---|---|---|
| | LQC | HQC | | |
| | 240.3 | 12016.8 | Using analyte peak area response | Using analyte/IS peak area ratio |
| Matrix Lot# 1 | 249.6 | 11176.7 | 0.98 | 1.01 |
| | 253.3 | 11264.3 | | |
| Matrix Lot# 2 | 226.1 | 11545.4 | 0.96 | 1.00 |
| | 235.4 | 11321.4 | | |
| Matrix Lot# 3 | 227.3 | 11554.2 | 1.01 | 0.99 |
| | 249.8 | 11056.8 | | |
| Matrix Lot# 4 | 231.1 | 11379.9 | 0.95 | 1.00 |
| | 224.3 | 10302.9 | | |
| Matrix Lot# 5 | 227.7 | 11320.2 | 0.97 | 1.01 |
| | 247.1 | 11401.6 | | |
| Matrix Lot# 6 | 221.7 | 11290.4 | 0.97 | 1.00 |
| | 248.8 | 11412.3 | | |
| Average | 236.85 | 11252.18 | 0.973 | 1.002 |
| SD | 11.914 | 329.945 | 0.0207 | 0.0075 |
| CV% | 5.0 | 2.9 | 2.1 | 0.8 |
| %Accuracy | 98.6 | 93.6 | | |

**Table 3**
Stability data for cefuroxime in human plasma under various conditions ($n = 6$).

| | Nominal concentration (ng/mL) | Average | SD | %Accuracy | CV% |
|---|---|---|---|---|---|
| Three freeze/thaw cycles (−15 °C) | 240.3 | 239.82 | 12.225 | 99.8 | 5.1 |
| | 12016.8 | 11489.38 | 372.846 | 95.6 | 3.2 |
| Bench-top stability 7.1 h | 240.3 | 236.15 | 12.933 | 98.3 | 5.5 |
| | 12016.8 | 11342.47 | 277.615 | 94.4 | 2.4 |
| In-injector stability 48 h | 240.3 | 228.22 | 8.023 | 95.0 | 3.5 |
| | 12016.8 | 11338.42 | 572.452 | 94.4 | 5.1 |
| Long-term stability 107 days | 240.3 | 228.58 | 4.402 | 95.1 | 1.9 |
| | 12016.8 | 11510.18 | 274.015 | 95.8 | 2.4 |

accuracy of quantitation method when developing LC–MS/MS assay. So, to ensure the selectivity of method, matrix effect as well as matrix factor on the presented method was evaluated following the procedures described previously. Results of the exercise were summarized in Table 2. Results obtained therein indicate that no additional variations in plasma concentration due to the use of different plasma lots were observed. Hence the concentration of the analytes obtained from clinical study samples should therefore be considered as reliable.

The matrix ionization suppression or enhancement of analyte and IS was assessed by measuring the matrix factor. The mean absolute matrix factor at the medium concentration from six lots of plasma samples was 0.973 (Table 2). The CVs of absolute MF and IS normalized MF from six lots of plasma samples were ≤2.1%. These results showed that ion suppression or enhancement from the plasma matrix was negligible under the current conditions.

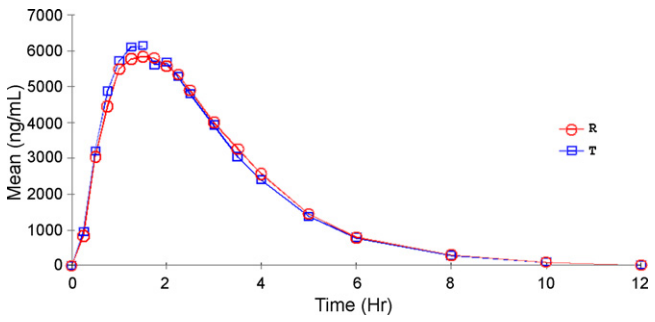


**Fig. 4.** The linear mean plasma concentration versus time profile of cefuroxime in plasma.

### 3.4.6. Stability studies

The stabilities of cefuroxime were investigated at two concentrations of QC samples (low and high concentrations) to cover expected conditions during analysis, storage and processing of all samples, which include the stability data from various stability exercises like in-injector, bench-top, freeze/thaw and long-term stability tests. The stability results summarized in Table 3 showed that cefuroxime spiked into human plasma was stable for at least 7.1 h at room temperature, for at least 48 h in final extract at 10 °C under autosampler storage condition, for 107 days at around −20 °C, and during three freeze–thaw cycles, when stored at around −20 °C and thawed to room temperature. The stock solutions of cefuroxime and IS were stable at refrigerator temperature (between 1 and 10 °C) and the working solutions of cefuroxime and IS were found stable for 12 h at room temperature.

### 3.4.7. Pharmacokinetic and bioequivalence studies in healthy volunteers

This proposed method was successfully applied to a pharmacokinetic study of cefuroxime in 36 healthy adult male

**Table 4**
Pharmacokinetic parameters (mean ± SD) of cefuroxime, after the administration of an oral dose of 500 mg test or reference formulations to healthy Indian male volunteers.

| Parameters | Reference | Test |
|---|---|---|
| $T_{max}$ (h) | 1.715 ± 0.747 | 1.716 ± 0.813 |
| $C_{max}$ (ng/mL) | 6850.717 ± 1889.558 | 6970.772 ± 2131.129 |
| $AUC_{0\to t}$ (μg h/mL) | 21.240 ± 6.346 | 21.144 ± 5.070 |
| $AUC_{0\to\infty}$ (μg h/mL) | 21.491 ± 6.353 | 21.413 ± 5.094 |
| $T_{1/2}$ (h) | 1.3 ± 0.2 | 1.4 ± 1.2 |

volunteers following oral administration of 500 mg of cefuroxime tablet. The mean concentration–time profile of cefuroxime in these volunteers is shown in Fig. 4 and the mean estimated pharmacokinetic parameters derived from the plasma concentration profiles are summarized in Table 4. The bioequivalence parameters almost overlapped between the test and reference samples.

## 4. Conclusions

A rapid, sensitive and selective LC–MS/MS method for the determination of cefuroxime in human plasma was developed and validated. Solid phase extraction methodology was adopted in plasma sample preparation that provides consistent extraction recovery with minimal endogenous interference and matrix effect. An added advantage over the earlier methods was the proposed solid phase extraction procedure was simple, efficient and easy to automate. The applicability of the method was demonstrated in a bioequivalence study of cefuroxime in healthy male volunteers. The results showed that the developed bio-analytical method was a valuable analytic technique for determination of cefuroxime in biological matrix.

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