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# A fast, sensitive, and high throughput method for the determination of cefuroxime lysine in dog plasma by UPLC–MS/MS

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ABSTRACT

In order to investigate the preclinical pharmacokinetics of cefuroxime lysine, a fast, sensitive and high throughput UPLC–ESI–MS/MS method has been developed and validated for the quantitative determination of cefuroxime in dog plasma. Cefuroxime and IS phenacetin were extracted from plasma samples by PPT or LLE procedure, and then separated on an ACQUITY UPLC™ BEH $C_{18}$ column with an isocratic elution of acetonitrile–0.1% formic acid in 10 mM ammonium acetate (40:60, v/v). MRM using the fragmentation transitions of $m/z$ 442 → 364 and 180 → 110 in positive ESI mode was performed to quantify cefuroxime and IS, respectively. The calibration curves were linear over the concentration range of 2–400 μg/ml for PPT and 0.01–5 μg/ml for LLE. The LLOQ was 0.01 μg/ml. The intra- and inter-day precisions in all samples were no more than 8.1%, while the accuracy was within ±6.2% of nominal values. The method was successfully applied to the evaluation of pharmacokinetic parameters of cefuroxime lysine in beagle dogs.



## 1. Introduction

Cefuroxime, the second-generation cephalosporine antibiotic agent manufactured by GlaxoSmithKline, has low cost and favourable spectrum of action compared with the third- and fourth-generation cephalosporins [1–3]. It has been wildly used in clinics for the treatment of patients with infections of soft tissue, respiratory tract, urinary tract, bone and joint tissues, etc. [4–6]. Another new cefuroxime salt, named cefuroxime lysine, with the merits of extremely higher water solubility and less irritation to the veins compared with cefuroxime sodium, has been investigated. Both cefuroxime sodium and cefuroxime lysine are changed to cefuroxime in vivo which is highly active against most Gram-negative aerobic bacteria. As a new drug, there is limited pharmacokinetic information available regarding its absorption, distribution, metabolism and excretion following intravenous administration to animals.

Earlier publications have described methods for cefuroxime determination in biological matrix (such as plasma, urine, tissue, skin, etc.) using HPLC-UV, microdialysis, micellar electrokinetic capillary chromatography, and liquid chromatography–tandem mass spectrometry (LC–MS/MS), etc. [7–16]. The techniques for pretreatment procedures included solid phase extraction, liquid–liquid extraction, column-switching technique and molybdophosphoric acid, etc. [17–20]. Some limitations existed in these methods including long run time, large volumes of biological samples, complicated sample processing procedures or inadequate sensitivity. The lowest values of the lower limits of quantitation (LLOQ) for all UV detection techniques are 100 ng/ml and 25 ng/ml for mass spectrometry [15,16].

In order to investigate the pharmacokinetic profiles of cefuroxime lysine in healthy beagle dogs after low, middle and high dosage, it is necessary to develop a rapid, sensitivity and robust method for the high-throughput determination of cefuroxime in plasma. Different from traditional HPLC methods, the application of UPLC method coupled with tandem mass could achieve those requirements [21–23]. To the best of our knowledge, there is still no method reported for the determination of cefuroxime in dog biological matrix using UPLC–MS/MS-based method. This paper describes a relatively simple and sensitive UPLC–MS/MS method with an electrospray ionization (ESI) source in multiple reaction monitoring (MRM) mode for the determination of cefuroxime in dog plasma. It was demonstrated that the high sample throughput with respect to high sensitivity and wide linear concentration

*Abbreviations:* UPLC–ESI–MS/MS, ultra-performance liquid chromatography –electrospray ionization tandem mass spectrometric; PPT, protein precipitation; LLE, liquid–liquid extraction; MRM, multiple reaction monitoring; ESI, electrospray ionization; LLOQ, lower limit of quantification; LLOD, lower limit of detection; IS, internal standard; QC, quality control; SD, standard deviation.

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**Table 1**
Demographic data of subjects for group L, M and H (mean ± SD).

| Items | L ($n$ = 6) | M ($n$ = 6) | H ($n$ = 6) | Male ($n$ = 9) | Female ($n$ = 9) |
|---|---|---|---|---|---|
| Age (m) | 11.17 ± 0.41 | 11.17 ± 1.60 | 11.67 ± 2.58 | 11.33 ± 1.80 | 11.33 ± 1.66 |
| Weight (kg) | 13.23 ± 0.87 | 13.81 ± 1.55 | 11.28 ± 0.64 | 13.09 ± 1.38 | 12.46 ± 1.66 |

range were achieved using highly efficient UPLC–MS/MS system by two sample procedures, which will be adaptive to the pharmacokinetics, bioavailability, metabolism and tissue distribution studies of cefuroxime lysine, in order to facilitate the further research and development of cefuroxime lysine.

## 2. Materials and methods

### 2.1. Chemicals and materials

The reference standards of cefuroxime (purity, 91.6%) and phenacetin (purity, 99%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). Cefuroxime lysine injection (0.25 g, batch no. 20101201) was supplied by Shandong Luoxin Pharmacy Stock Co., Ltd. (Shandong Province, PR China). Drug-free dog plasma for the preparation of calibration standards was obtained from the experimental animal research and development center of Guangzhou Institute of Pharmaceutical Industry (Guangzhou, China) and stored at −80 °C prior to use. Methanol, acetonitrile, ammonium acetate and formic acid of chromatographic grade were purchased from Fisher Scientific (NJ, USA). All other reagents were of analytical grade. Water was purified by redistillation and filtered through a 0.22 μm membrane filter before use.

### 2.2. Animals

Eighteen beagle dogs (gender in half, obtained from the experimental animal research and development center of Guangzhou Institute of Pharmaceutical Industry, Guangzhou, China) were randomly divided into three groups by intravenous administration of 27 (L), 54 (M), 108 (H) mg/kg cefuroxime lysine. The demographic data of subjects in the three dose groups are shown in Table 1 without significant differences ($p > 0.05$) found in the age and weight for different doses and genders. Animal study was carried out in accordance with the Guidelines for Animal Experimentation of Shenyang Pharmaceutical University (Shenyang, China) and the protocol was approved by the Animal Ethics Committee of the institute.

### 2.3. Apparatus

The UPLC–ESI–MS/MS system consisted of an ACQUITY™ Ultra Performance Liquid Chromatography system and a triple quadrupole mass spectrometer (Waters Corp., Milford, MA, USA). The data acquisition and sample quantification were operated using MassLynx™ NT 4.1 software with QuanLynx™ program (Waters Corp., Milford, MA, USA). An ACQUITY UPLC™ bridged ethyl hybrid (BEH) $C_{18}$ column (50 mm × 2.1 mm, 1.7 μm; Waters) and a SecurityGuard $C_{18}$ guard column (4 mm × 3.0 mm, i.d., Phenomenex, Torrance, CA, USA) were used in this study. The conditions for the MS/MS detection are listed in Table 2.

### 2.4. Chromatographic conditions

The chromatographic separation of cefuroxime and IS was achieved on a BEH $C_{18}$ column protected by a Security Guard $C_{18}$ guard column. Analysis was completed with an isocratic elution of acetonitrile–0.1% formic acid in 10 mM ammonium acetate (40:60, v/v) within 1.5 min at a flow rate of 0.2 ml/min. The temperature of column and autosampler were maintained at 25 °C and 4 °C, respectively.

**Table 2**
UPLC–ESI–MS/MS conditions.

| Items | Parameters |
|---|---|
| Polarity | $ESI^+$ (V mode) |
| Capillary voltage | 2.8 kV |
| Cone voltage | 14 V |
| Scan time pre transition | 0.2 s |
| Source temperature | 110 °C |
| Desolvation temperature | 450 °C |
| Desolvation gas flow | 500 l/h |
| Cone gas flow | 30 l/h |
| MS/MS range | 100–900 $m/z$ |

### 2.5. Preparation of standard solutions

Stock solutions of cefuroxime (8 mg/ml) and IS (100 μg/ml) were separately prepared in methanol:water (50:50, v/v) mixture. The stock solution of cefuroxime was further diluted with water to obtain the working solutions so as to prepare the calibration standards. The IS working solution of 1.05 μg/ml and 12.5 ng/ml were prepared by dilution of the stock standard solution with methanol. All solutions were kept at 4 °C and were brought to room temperature before use.

Calibration standard samples of cefuroxime (2, 5, 10, 20, 80, 400 μg/ml for PPT and 0.01, 0.03, 0.1, 0.4, 2, 5 μg/ml for LLE) were prepared by spiking blank plasma (200 μl) with working standards at different concentrations. The QC samples were prepared with blank plasma at low, medium and high concentration levels of 4, 32 and 360 μg/ml for PPT and 0.03, 0.2 and 4 μg/ml for LLE, which were used in developing the analytical method and during the pharmacokinetic study.

### 2.6. Sample preparation

#### 2.6.1. Protein precipitation

An aliquot of 200 μl plasma sample was transferred to an Eppendorf micro tube, and vortex-mixed with 100 μl IS, 100 μl water for 30 s, then 600 μl acetonitrile was added and vortexed for 1 min. After centrifugation at 15,000 rpm for 10 min, a 50 μl aliquot of the supernatant was added with 800 μl water, and then vortex-mixed for 30 s, 10 μl of the mixture was injected onto the UPLC–MS/MS system for analysis.

#### 2.6.2. Liquid–liquid extraction

To an aliquot of 200 μl plasma sample in a 10 ml glass tube, 100 μl 0.2 mol/l hydrochloric acid and 100 μl IS were added and vortex-mixed for 30 s, then the mixture was extracted with 3 ml ethyl acetate by vortex-mixing for 1 min. After centrifugation at 3500 rpm for 10 min, the organic layer was quantitatively transferred to another glass tube and evaporated to dryness using evaporator at 40 °C. The residue was reconstituted in 200 μl of acetonitrile–water (40:60, v/v), and then vortex-mixed for 30 s. After centrifugation at 15,000 rpm for 10 min, 10 μl of the supernatant was injected onto the UPLC–MS/MS system for analysis.

### 2.7. Validation of the method

This method was validated according to the US-FDA document and other related guidelines with respect to specificity, linearity, precision and accuracy, recovery, matrix effect and stability [24–27]. Selectivity was assessed by comparing the chromatograms of six different sources of blank plasma processed by PPT or LLE method. The linearity was evaluated by weighted ($1/x^2$) linear regression analysis of cefuroxime/IS peak area ratio versus the spiked concentrations based on three independent six-point calibration curves [28,29]. The lower limit of detection (LLOD) and the lower limit of quantification (LLOQ) were determined as the concentrations with a signal-to-noise ratio of 3 and 10, respectively, with acceptable accuracy within 20% deviation and precision between 80% and 120%. Precision and accuracy were carried out in six replicates at three QC levels on the same day and three batches on three consecutive validation days. While the extraction recovery for cefuroxime was determined by comparing the responses from blood samples spiked before extraction with those from blood samples spiked after extraction at three QC levels. The matrix effect was evaluated by comparing the peak areas of the post-extracted blank plasma spiked with cefuroxime working solutions with those of corresponding standard solutions. Stability tests of the analyte were assessed using triplicate of spiked samples at three QC levels under different conditions: 4 h at room temperature, three freeze–thaw cycles, stored at −80 °C for a month and reconstituted extract at 4 °C for 12 h.

### 2.8. Pharmacokinetic studies

After a 12 h fast, 18 beagle dogs were randomly divided into three groups and given a single intravenous infusion dosage of 27, 54, and 108 mg/kg cefuroxime lysine. The blood samples (1 ml) were withdrawn via the foreleg vein into 1.5 ml heparinized tubes at −30 (to serve as a control), −25, −20, −15, 0 (the end of infusion), 15, 30 min, 1, 1.5, 2, 4, 6, 8, 10, 12 h after the infusion, respectively. The plasma was immediately separated by centrifugation at 15,000 rpm for 10 min and stored at −80 °C until analysis. The pharmacokinetic parameters of cefuroxime lysine were calculated by WinNonlin 5.2 (Pharsight Corporation, Mountain View, CA, USA). Noncompartmental analysis was used to determine and compare standard pharmacokinetic parameters of cefuroxime lysine in dogs. The data are presented as mean ± SD.

## 3. Results and discussion

### 3.1. Optimization of chromatographic and mass conditions

The separation and ionization of cefuroxime and IS were affected by the composition of mobile phase. Therefore, the selection of mobile phase is important for improving peak shape, detection sensitivity and shortening run time. When the ratio of organic phase increased, there were double peaks for cefuroxime, while the addition of formic acid made it become single, sharp and symmetrical. 0.2% formic acid added in the mobile phase was found to decrease the response of cefuroxime by 3 times, finally, addition of 0.1% formic acid was indicated to be with enough significant sensitivity of both analytes. Gradient elution was also investigated, but the results indicated that it showed shortcomings of lower response, and longer equilibrium time. On the other hand, the ionization of cefuroxime and IS was increased by adding ammonium acetate in the mobile phase. Both analytes and IS were found to have the highest response and best peak shapes in the mobile phase containing 10 mmol/l ammonium acetate compared with 5 mmol/l or 20 mmol/l ammonium acetate. This is the shortest analysis time (1.5 min) reported so far for the determination of cefuroxime, to which both the fast UPLC and the selective MRM contributed. This method meets the requirement for high sample throughput in bioanalysis.

To optimize ESI conditions for detection of cefuroxime, cefuroxime was dissolved in water and infused into the mass spectrometer to carry out quadrupole full scans in positive or negative ion detection mode. Different from the articles reported [14,16], the ion $[M+NH_4]^+$ at $m/z$ 442 was the main peak with the highest signal intensity in positive ion mode.

Cefuroxime has the chemicophysical properties of freely soluble in water and buffered solutions, and during the experiment we found that there was a little carryover influence on the low concentration samples determination for its residual in the system, three times of both weak and strong needle wash solution (100:300 μl) were performed, which was proved to be valid with no carryover to be detected after determination.

### 3.2. Selection of internal standard

Several compounds were tested as internal standards including cefuroxime axetil, cefalexin, benazepil and phenacetin under the present experiment conditions. Under the present chromatographic and mass spectrometry conditions, cefuroxime axetil had lower mass response, and the extraction recovery of cefalexin processed by PPT or LLE method was less than 24%, so they are not the appropriate IS. Finally, phenacetin was selected as the IS because its chromatographic behaviour, MS response and extraction efficiency were similar to those of cefuroxime. Full-scan product ion spectra of $[M+NH_4]^+$ ion of cefuroxime and $[M+H]^+$ ion of phenacetin and their fragmentation pathways are shown in Fig. 1. The transitions $m/z$ 442 → 364 and 180 → 110 in positive electrospray ionization were chosen for quantitation of cefuroxime and IS.

### 3.3. Sample preparation

Cefuroxime is a highly polar compound, with good solubility in water and buffered solutions. After intravenous administration, the cefuroxime plasma concentration was high enough to reach about 200 μg/ml, while the LLOQ was determined to be 0.01 μg/ml. The wide concentration range required two calibration curves by its corresponding sample pretreatments. Two kinds of extraction procedures (including PPT and LLE) were evaluated during the method development. Initially, we tried protein precipitation (PPT) method with methanol or acetonitrile for high concentrations, and liquid–liquid extraction (LLE) method with ether, methyl tert-butyl ether, ethyl acetate or chloroform for low concentrations. Finally, acetonitrile was selected as the precipitant for PPT and the extracts were injected without evaporation and reconstitution, which could simplify the operating process and meet the analysis requirements. Ethyl acetate was selected for LLE and the addition of hydrochloric acid was beneficial to enhance the extraction recovery of cefuroxime in plasma. From the results of preliminary experiment, the plasma samples taken before the sampling point of 6 h were processed by PPT, and the left were dealt with LLE. If the observed plasma concentration of cefuroxime was less than 2 μg/ml after PPT, then the same plasma sample should be processed by LLE.

### 3.4. Method validation

#### 3.4.1. Specificity

The retention times for cefuroxime and IS were 0.85 and 1.26 min, respectively. No significant interference or ion suppression from endogenous substances was observed at the retention time of cefuroxime and IS. Fig. 2 shows the representative LC–MS/MS MRM chromatograms obtained from the analysis of blank plasma, plasma spiked with cefuroxime at 2 μg/ml and

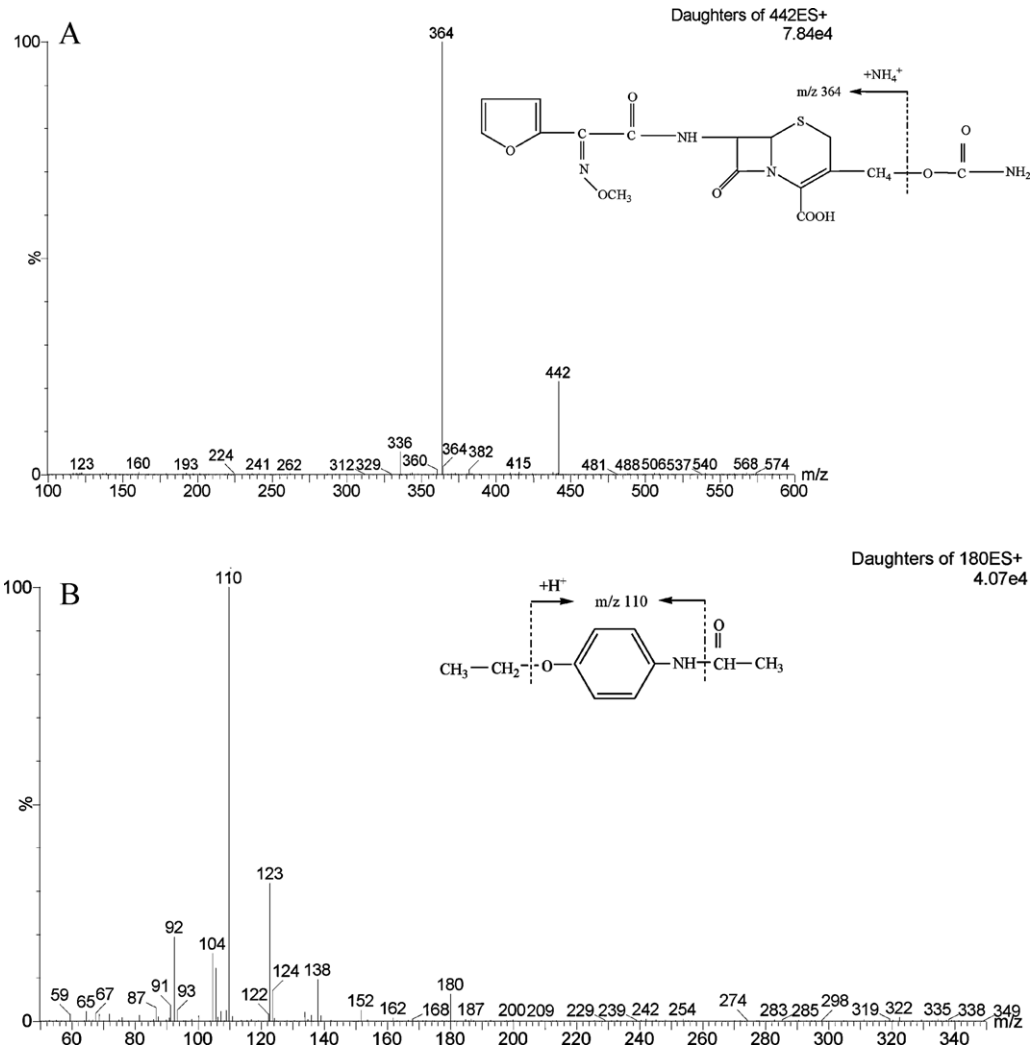


**Fig. 1.** Full-scan product ion spectra of cefuroxime (A) and phenacetin (B).

0.01 μg/ml, and dog plasma samples obtained at 0.5 h and 12 h, which were processed by PPT (A1–A3) and LLE (B1–B3) method. With the help of UPLC system and appropriate mobile phase, a total analysis time of less than 1.5 min was achieved which could put high-throughput analysis to practice.

### 3.4.2. Linearity and sensitivity

Calibration curves were linear over the concentration range of 2–400 μg/ml for PPT and 0.01–5 μg/ml for LLE, with the corresponding linear regression equation of $y = 0.029x + 0.005$ ($n = 3$, $r = 0.9975$) and $y = 2.478x + 0.044$ ($n = 3$, $r = 0.9953$), respectively, where $y$ was the peak area ratio of the analyte to IS and $x$ was the concentration of the analyte. Application of weighted least squares regression instead of ordinary linear regression can achieve more realistic results and LLOQ with small data sets [30].

The LLOQ and LLOD for cefuroxime in dog plasma were 0.01 μg/ml and 0.005 μg/ml within the acceptable limits, respectively. As the lowest LLOQ compared with reports, it is sensitive enough to be applied to investigate the pharmacokinetic profiles of cefuroxime lysine in young beagle dogs, so as to speculate the possible relationship between dosage and pharmacological actions.

### 3.4.3. Precision and accuracy

The precision and accuracy for cefuroxime were evaluated by a one-way analysis of variance (ANOVA) at three levels of QC samples prepared by PPT and LLE procedures, respectively (Table 3). The intra- and inter-day precisions were measured to be within 6.0% and 8.1%, and the accuracy was within −6.2 to 5.0%, respectively, indicating the acceptable accuracy and precision of the method developed.

### 3.4.4. Extraction recovery and matrix effect

The PPT recoveries of cefuroxime at concentrations of 4, 32 and 360 μg/ml were determined to be 91.7 ± 7.8%, 86.4 ± 10.1%, and 82.4 ± 4.7%, while LLE recoveries of cefuroxime at three QC levels were 76.2 ± 6.9% (0.03 μg/ml), 70.9 ± 10.3% (0.2 μg/ml) and 87.0 ± 11.5% (4 μg/ml), respectively. The recoveries of IS were 93.2 ± 8.8% and 84.3± 3.5% for PPT and LLE, respectively, which could meet the requirements of analysis.

The PPT matrix effect evaluated for cefuroxime was 110.8, 113.9, and 94.7% (with the R.S.D. % of 6.3, 8.3, 8.3%, respectively) at the three QC concentration levels, along with the values of 78.6, 83.8, and 108.8 (with the R.S.D. % of 10.2, 10.7, 13.0%, respectively) for LLE. In addition, the matrix effect for IS was 115%, and 108% for PPT and LLE. These results indicated that the endogenous substances showing no significant effect on the ionization for both cefuroxime and IS in both methods.

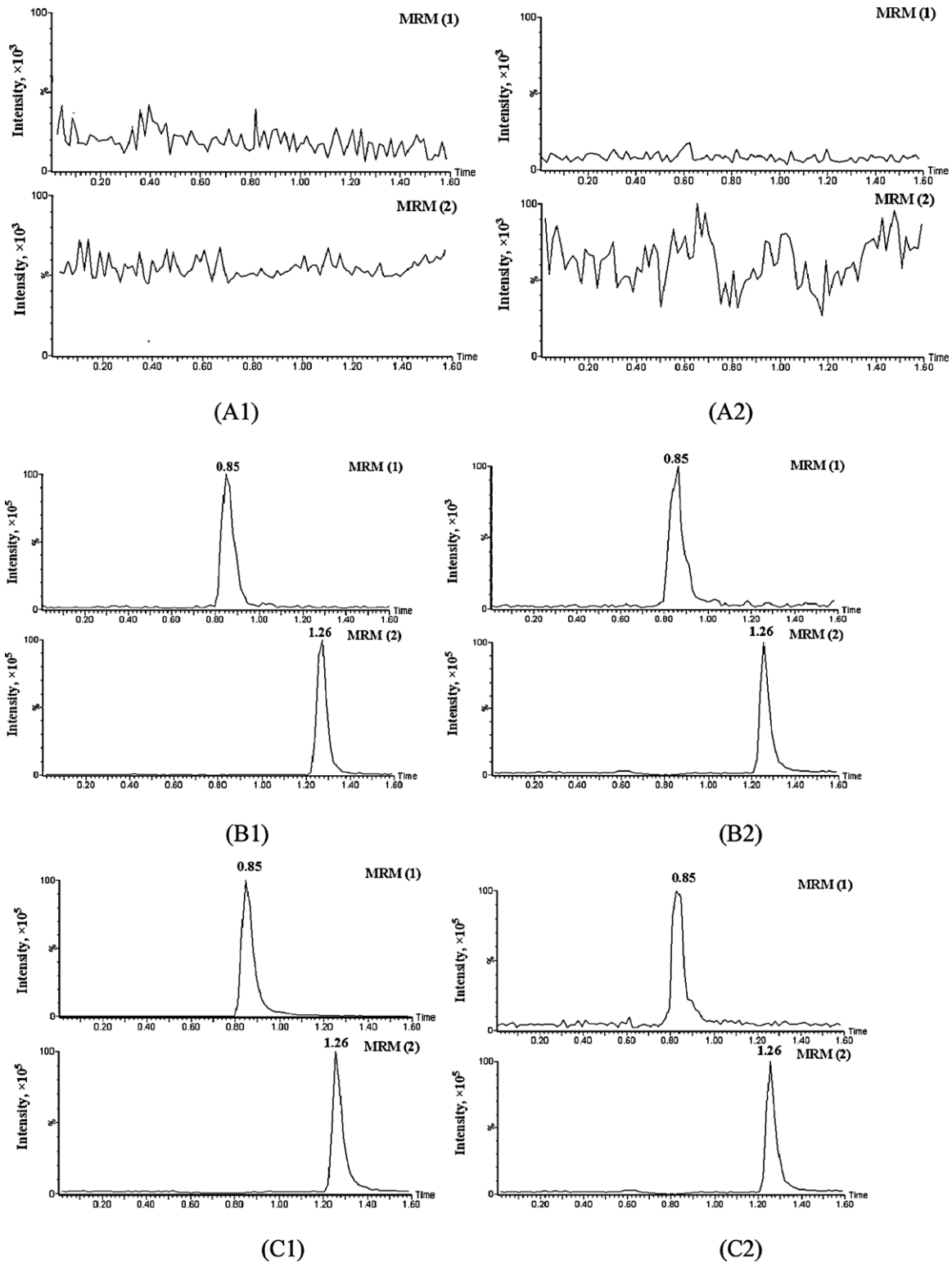


**Fig. 2.** Representative MRM chromatograms of cefuroxime (0.85 min, MRM 1) and IS (1.26 min, MRM 2) in dog plasma prepared by PPT (A1, B1, C1) and LLE (A2, B2, C2). (A1, A2) Chromatograms of blank plasma. Chromatograms of cefuroxime and IS at 2 μg/ml, 1.05 μg/ml (B1) and 10 ng/ml, 12.5 ng/ml. Representative chromatograms of dog plasma sample at 0.5 h (C1) and 12 h (C2).

### 3.4.5. Stability

Table 4 summarizes the results of stability experiments under various conditions as mentioned above. The results indicated that cefuroxime was stable in plasma samples kept at −80 °C for 30 days, or three freeze–thaw cycles at −80 °C, in prepared sample at 4 °C for 12 h, and 4 h at room temperature during the analysis process.

### 3.5. Method application

This method has been applied to study the pharmacokinetics of intravenous cefuroxime lysine using beagle dog as the animal model. The mean cefuroxime concentrations in plasma versus time curves for three dose groups are shown in Fig. 3. And the

**Table 3**
Summary of inter- and intra-precision and accuracy data for assays of cefuroxime lysine ($n = 6$).

| Procedure | Added C (μg/ml) | Found C(μg/ml) | Intra-run R.S.D. (%) | Inter-run R.S.D. (%) | Relative error (%) |
|---|---|---|---|---|---|
| PPT | 4 | 3.753 ± 0.22 | 5.8 | 4.6 | −6.2 |
| | 32 | 32.13 ± 1.36 | 4.2 | 4. 9 | 0.4 |
| | 360 | 378.1 ± 5.19 | 1.4 | 4.0 | 5.0 |
| LLE | 0.03 | 0.0295 ± 0.002 | 6.0 | 8.1 | −1.7 |
| | 0.2 | 0.1793 ± 0.002 | 1.1 | 7.0 | −10.3 |
| | 4 | 3.799 ± 0.218 | 5.8 | 4.8 | −5.0 |

**Table 4**
Summary of stability of cefuroxime under various storage conditions ($n = 6$).

| Methods | QC | Freezing for 30 d | | Freeze/thaw ($n = 3$) | | 12 h, 4 °C | | 4 h, 25 °C | |
|---|---|---|---|---|---|---|---|---|---|
| | μg/ml | Mean | R.S.D. | Mean | R.S.D. | Mean | R.S.D. | Mean | R.S.D. |
| PPT | 4 | 3.871 | 6.4 | 3.917 | 8.3 | 3.601 | 4.3 | 3.828 | 3.7 |
| | 32 | 31.80 | 4.8 | 33.02 | 2.1 | 30.17 | 2.9 | 31.84 | 2.5 |
| | 360 | 374.1 | 1.2 | 383.4 | 2.8 | 342.6 | 2.9 | 369.1 | 2.6 |
| LLE | 0.03 | 0.028 | 7.2 | 0.030 | 4.9 | 0.030 | 9.4 | 0.029 | 8.3 |
| | 0.2 | 0.179 | 3.1 | 0.191 | 9.4 | 0.180 | 4.2 | 0.182 | 4.4 |
| | 4 | 3.905 | 4.3 | 3.765 | 7.2 | 3.534 | 2.1 | 3.719 | 3.3 |

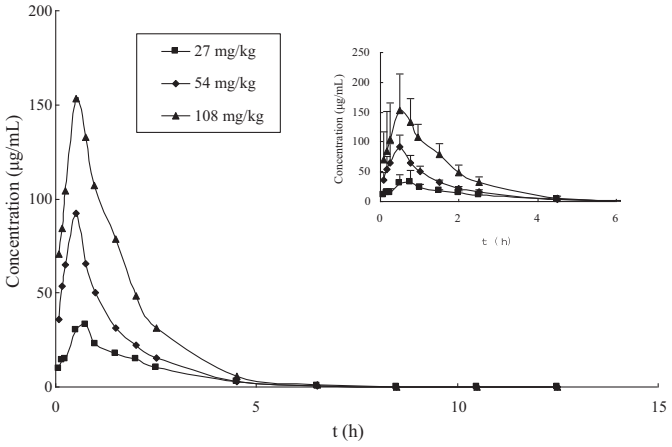


**Fig. 3.** Geometric mean plasma concentrations versus time linear curves for cefuroxime lysine after administration of 27, 54, 108 mg/kg to Beagle dogs ($n = 6$). Inset shows initial 5 h profile expanded.

corresponding noncompartmental pharmacokinetic parameters of cefuroxime lysine are listed in Table 5. The results indicated that the plasma concentrations increased rapidly during intravenous infusion, and reached the mean $C_{max}$ concentrations of 40.37, 92.46 and 175.7 μg/ml at the end of infusion for three dose groups, respectively. After then the plasma concentration decreased rapidly with

**Table 5**
Noncompartmental pharmacokinetic parameters of cefuroxime lysine infused over 0.5 h in Beagle dogs (mean ± SD, $n = 6$).

| Parameters[a] | 27 mg/kg | 54 mg/kg | 108 mg/kg |
|---|---|---|---|
| $T_{max}$ (h) | 0.79 ± 0.60 | 0.50 ± 0.00 | 0.54 ± 0.10 |
| $C_{max}$ (μg/ml) | 40.37 ± 15.80 | 92.46 ± 19.34 | 175.66 ± 48.65 |
| $t_{1/2}$ (h) | 1.46 ± 0.57 | 0.91 ± 0.10 | 1.08 ± 0.35 |
| $V$ (ml/kg) | 445.9 ± 54.74 | 395.2 ± 66.64 | 404.4 ± 115.2 |
| Cl (ml/h/kg) | 319.3 ± 31.44 | 324.40 ± 44.70 | 335.9 ± 75.38 |
| $AUC_{0-t}$ (μg h/ml) | 63.06 ± 6.34 | 125.3 ± 17.97 | 248.4 ± 55.68 |

[a] $T_{max}$, time to reach the maximum plasma concentration; $C_{max}$, peak plasma concentration; $t_{1/2}$, terminal elimination half life; $V$, apparent volume of distribution; Cl, plasma clearance; $AUC_{0-t}$, area under the plasma concentration–time curve from time 0 to $t$.

an elimination half time ($t_{1/2}$) between 0.84 h and 2.32 h and a plasma clearance (Cl) of 263.5–410.5 ml/h/kg. On the other hand, the individual values of peak plasma concentration ($C_{max}$) and area under curve (AUC) were prone to increase in proportion to the dose with the linear regression of $C_{max} = 1.65\text{Dose} - 1.23$ ($r^2 = 0.7729$) and $AUC_{0-\infty} = 2.29\text{Dose} + 1.50$ ($r^2 = 0.8517$) without significant differences for plasma clearance (Cl), which indicated that the linear pharmacokinetic properties of cefuroxime lysine were observed in the given dosage groups (Fig. 4). However, the parameters of $T_{max}$, $t_{1/2}$ were apparently independent of dose.

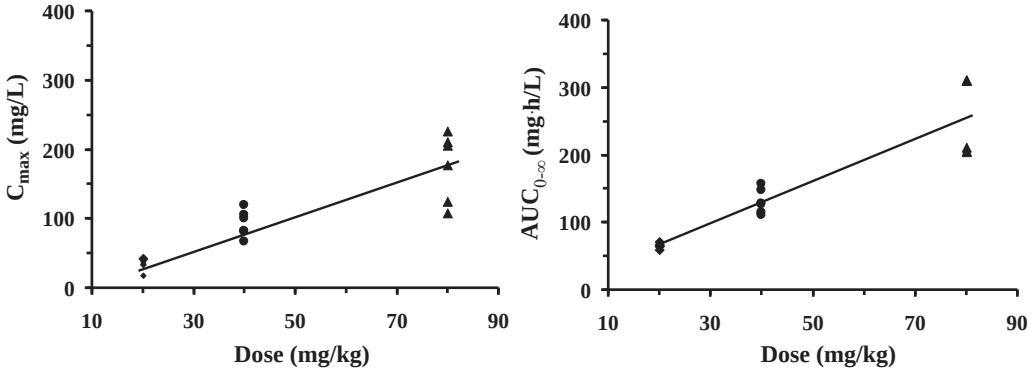


**Fig. 4.** Relationship between $C_{max}$ and $AUC_{0-\infty}$ of cefuroxime lysine and dose.

## 4. Conclusion

A fast, sensitive and robust UPLC–MS/MS assay for the quantification of cefuroxime in small volumes of dog plasma was developed and validated. Two pretreatment methods of PPT and LLE showed perfect reproducibility. The linear range of 0.01–400 μg/ml was wide enough to monitor the low and high-dosage pharmacokinetic investigation of cefuroxime lysine in beagle dogs, and the LLOQ of 0.01 μg/ml was low enough to monitor at least five-lives of the analyte concentration with perfect intra- and inter-assay reproducibility. It was shown that this validated method is suitable for the analysis of cefuroxime in dog plasma samples collected for pharmacokinetic study.

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