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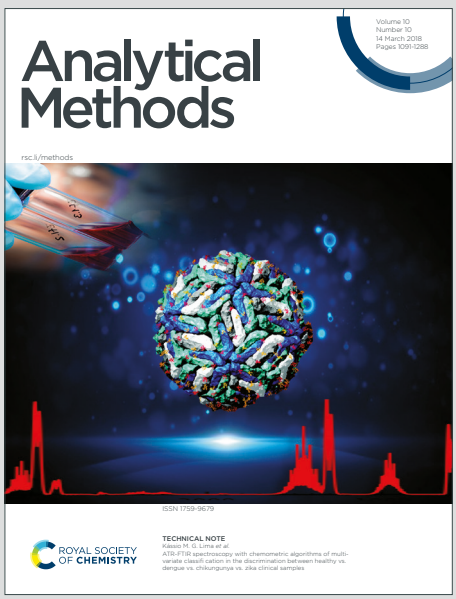

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Analytic Methods

Simple and robust analysis of cefuroxime in human plasma and bone tissues by LC-MS/MS

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In end-stage osteoarthritis, total joint arthroplasty (TJA) represents the definitive therapeutic intervention. Cefuroxime, a second-generation cephalosporin, exhibits a broad spectrum of activity against both Gram-negative and positive microorganisms., making it a cornerstone of surgical antimicrobial prophylaxis (SAP) to mitigate prosthetic joint infection (PJI) risk. However, the escalating demand for revision arthroplasties has paralleled rising implant-associated infections, necessitating target-site pharmacokinetic optimization to ensure effective antibiotic exposure at the bone-implant interface. Therefore, we developed a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) assay for simultaneous quantification of cefuroxime in human plasma and bone tissues. The separation was completed in 7.5 min on a BEH C_{18} column (2.1 × 50 mm, 3.5μm), and the gradient elution was performed in a mobile phase consisting of 0.1% formic acid acetonitrile and 0.1% formic acid water at a flow rate of 0.3 mL/min. The correlation coefficients of calibration curves were all greater than 0.99. The detection accuracy of plasma ranged from 93.11% to 98.60% (89.15-106.2% for bone). The intra- and inter-assay precision for both plasma and bone measurements was within 15% (20% at the lower limit of quantitation, LLOQ). The matrix effects were 2.34% to 2.91% in plasma and 3.13%-5.17% in bone, while extraction recoveries ranged from 99.8% to 102.0% for plasma and 105.0%-107.0% for bone. Upon stability assessment under varying storage conditions, all samples exhibited a difference of less than 15.0%. The method was successfully applied to the determination of cefuroxime in plasma and bone tissues of actual patients.

1. Introduction

Osteoarthritis (OA), classified as a chronic low-grade inflammatory degenerative arthropathy, represents the most prevalent form of non-autoimmune joint disorder, manifesting as progressive degradation of articular cartilage and periarticular connective tissues. It features complex disorders of the entire synovial joint, including structural defects in the articular cartilage, loss of intact subchondral bone, hypertrophy and instability of tendons and ligament, clinically characterized by pain, stiffness and loss of joint function[1]. Generally, the treatment of osteoarthritis includes pain management and joint replacement for end-stage disease[2]. Periprosthetic joint infection (PJI) is a special type of infection associated with joint replacement, the incidence of PJI in primary hip arthroplasty is 0.25%-1.0%[3], and the incidence of PJI in primary knee arthroplasty is 0.4%-2%[4], reinfection rate is 14%[5]. Prosthetic joint infection can be caused by Staphylococcus aureus, Gram-negative bacilli, anaerobic bacteria or a variety of microbial infections[6], while antibiotics have been clearly shown to reduce the incidence of infection in orthopaedic surgery, cefuroxime is a second- generation cephalosporin antibiotic with broad spectrum activity against both Gram-negative and positive microorganisms[7], clinically, cefuroxime (1.5 g) are commonly used as prophylactic antibiotics[8]. Prosthetic joint infection (PJI) occurrence underscores potential limitations in antimicrobial agents' bone penetration capacity[9], characterized by inadequate tissues-to-plasma concentration ratios to achieve effective bactericidal thresholds in osseous compartments[10]. This indicates that information on drug concentrations at the target site of tissues will provide additional value necessary to optimize antibiotic therapy[11, 12].

However, pharmacokinetic studies of cefuroxime in plasma and bone tissue necessitate a validated analytical method for accurate concentration quantification, particularly in bone due to its heterogeneous nature. To date, several analytical methods have been developed for cefuroxime determination, with the majority focusing on plasma exposure[13-17], while others targeting such as breast milk and ocular tissues[7, 18, 19]. Notably, one study described a quantitative method for cefuroxime in bone tissue using ultra-performance convergence chromatography-tandem mass spectrometry (UPC²-MS/MS)[20]. Ultra-performance convergence chromatography-tandem mass spectrometry (UPC²-MS/MS) is not easily obtainable in a typical laboratory, whereas LC-MS/MS is more prevalent and cost-effective. Besides, this method employed meropenem-d6 as an

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internal standard (IS) for cefuroxime, multiple studies have demonstrated that meropenem exhibits poor stability across various matrices and is prone to degradation, when stored at 4°C for 12–24 hours, its concentration-dependent degradation rate can exceed 15%[21], meropenem also remains unstable during long-term storage at –20°C, a phenomenon observed in both plasma samples and stock solutions[22]. Consequently, conventional analytical methods typically incorporate stabilizers (e.g., sodium bicarbonate or specific buffer systems) to ensure its stability during experimental procedures. However, in the present study, no stabilization measures were implemented when using meropenem-d6 as an internal standard (IS), which may directly compromise its quantitative accuracy. Furthermore, meropenem-d6 (a carbapenem antibiotic) and the target analyte cefuroxime (a cephalosporin antibiotic) belong to distinct β-lactam subclasses, their structural differences may lead to the analytical challenge and variability in extraction recovery normalization. Collectively, these factors suggest that meropenem-d6 may not be an optimal IS for the quantitative analysis of cefuroxime. More critically, the method validation process failed to systematically assess matrix effect, further undermining data reliability and limiting its applicability in routine analysis.

Therefore, in the present study, we developed and validated a sensitive and robust liquid chromatography tandem mass spectrometry (LC-MS/MS) method for evaluating systemic prophylactic cefuroxime administration during total knee arthroplasty (TKA), measuring its concentrations in plasma and bone tissue post-administration.

2. Materials and methods

2.1 Materials and reagents

Cefuroxime and cefuroxime-d3(Internal standard, IS) were purchased separately from MedChemExpress (Lot# 29179, New Jersey, United States) and Cayman Chemical (Lot# 0691209-1, Michigan, United States). LC-MS/MS grade formic acid (Lot# 225583), HPLC grade acetonitrile (Lot# F24OBF203) and methanol (Lot#F24OCD203) were obtained from Thermo Fisher Scientific (Shanghai, China). Water was filtered using purification system (Pall, New York, United States). Human plasma was provided by Red Cross Society of Beijing Branch (Lot# 20200727-010, Beijing, China). The blank canine bone powder was purchased from Meikang pharmaceutical company (Anhui, China).

2.2 Instruments and LC-MS/MS Conditions

The analysis was performed using an LC-30AD chromatography system (Shimadzu, Kyoto, Japan) coupled to an API 4000+ triple-quadrupole mass spectrometer (AB SCIEX, Boston, United States. Data acquisition was performed with Analyst 1.6.2 software (AB SCIEX, Boston, United States).

Separation was performed on an XBridge BEH C_{18} Column (2.1×50 mm, 3.5 μm; Waters, Milford, MA, United States) and an XBridge BEH C_{18} Vanguard Cartridge (2.1×5 mm, 3.5 μm) at a column temperature of 40°C. The mobile phase consisted of 0.1% formic acid in acetonitrile (solvent A) and 0.1% formic acid in water (solvent B) used at a flow rate of 0.3 mL/ min, with the injection volume was 1 μL, and the gradient elution was performed as follows: 0-0.5 min (5% A), 0.5-1.5 min (5%-20% A), 1.5-3.5 min (20%-95% A), and 3.5-5.0 min (95%-5% A), 5.0-7.5 min (5% A). The electrospray ionization (ESI) source interface was operated in the negative ionization modes in our study. The following parameters were utilized: ion spray voltage: -4500 V; ion source temperature: 550°C; gas 1: 40 psi; gas 2: 40 psi; curtain gas: 20 psi. The MRM transition was 423.00 → 207.10 m/z for cefuroxime and 426.00 →210.10 m/z for IS. Cefuroxime specific mass spectrometer parameters were as follows: Declustering Potential (DP): -45 V; Entrance Potential (EP): -10 V; Collision Energy (CE): -30 V; Collision Cell Exit Potential (CXP): -12 V. The optimized MS parameters for IS were as follows: DP: -45 V; EP: -10 V; CE: -19 V; CXP: -12 V.

2.3. Preparation of Standard and Quality Control (QC) Samples

Stock solution (4 mg/mL) of the cefuroxime was prepared in 50% methanol and was further diluted with 10% methanol to achieve standard working solutions at concentrations of 2000, 1600, 1000, 500, 200, 100, 40 and 20 μg/mL. The QC stock solutions (low: 60 μg/mL, medium: 500 μg/mL, and high: 1500 μg/mL) were also prepared in the same way. Cefuroxime-d3 (IS) stock solution 1mg/mL was prepared in DMSO, was diluted with 10% methanol to a final concentration of 10 μg/mL. All of these stock solutions were stored at -40°C avoiding light until analysis.

2.4 Sample preparation

Two sample preparation methods were used in our study, specifically for plasma and bone tissues respectively. For plasma, 10 μL working solutions, 50 μL IS solutions and 90 μL blank plasma were mixed, then 200 μL methanol and 200 μL purified water were added, the mixture is vortexed for 2 min each time, used respectively for precipitating proteins and extracting the substances to be analyzed. After centrifugation at 15000rpm for 5 min (at 4°C), the supernatant was diluted 1:1 (v/v) with purified water for sampling analysis. The final concentrations of cefuroxime standard calibration plasma samples were 200, 160,

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100, 50, 20, 10, 4 and 2 μg/mL, respectively. The QC samples were also prepared in the same way by adding 10 μL QC stock solutions, the final concentrations of cefuroxime in the low-, medium-, and high-QC plasma samples were 6 μg/mL, 50 μg/mL, and 150 μg/mL, respectively. For bone tissues, 10 μL working solutions or 10 μL QC stock solutions, 20 μL IS solutions and 50mg blank bone flour were added and the samples were mixed using a vortex mixer for 10 min. Then, 100 μL methanol and 100 μL a mixture containing 60% mobile phase B and 40% mobile phase A were added and the samples were mixed using a vortex mixer for 10 min followed by centrifugation at 15000 rpm for 10 min. The final concentrations of cefuroxime standard calibration bone samples were 400, 320, 200, 100, 40, 20, 8 and 4 μg/g, the final concentrations of cefuroxime in the low-, medium-, and high-QC bone samples were 12, 100 and 300 μg/g, respectively. Finally, 1 μL of supernatant was injected into the LC-MS/MS system.

Frozen human plasma samples were thawed at ambient temperature and adequately vortexed, a total of 100 μL aliquot plasma sample was added with 50 μL IS (10 μg/mL) solution, then samples were prepared the same way as above. Bone tissues samples were fragmented into small pieces, immersed in liquid nitrogen for 10 min (to enhance brittleness for efficient grinding), and then pulverized in a cryogenic grinder pre-cooled to -40°C under the following conditions: 30 seconds per cycle with 15-second intervals, repeated for 2 cycles. Then, the sample extraction procedures were the same as the standard samples.

2.5. Validation of method

According to the latest ICH M10 and FDA guidelines on the Bioanalytical Method Validation, the analytical method was validated for specificity, selectivity, linearity, precision and accuracy, carry-over, matrix effect, extraction recovery, dilution integrity and stability.

2.5.1 Selectivity and specificity

The selectivity and specificity of the method were demonstrated by double blank plasma samples, blank control samples and lower limit of quantitation (LLOQ) samples obtained from different sources, whose chromatograms were evaluated to check for possible interference.

2.5.2 Linearity

Linearity was evaluated by analyzing calibration curves using eight concentration points. Calibration curves were constructed by plotting peak area ratios (analyte/internal standard) versus plasma concentrations. Linear weighted least-squares analysis was performed, and a weighting factor of 1/x2 was used. A coefficient of determination (r^2) $\geqslant$ 0.99 was expected in all calibration curves.

2.5.3 Precision and accuracy

Intra-day precision and accuracy were evaluated in six replicates at four QC levels (LLOQ, low, medium, and high concentrations) within 1 day during the same analytical run. Inter-day precision and accuracy were assessed based on three analysis batches within each concentration level at least two days. The coefficient of variation (CV) was evaluated to determine precision, and accuracy was represented by a percentage of the nominal concentration (%). The precision and accuracy should be within 15% for the low-, medium-, and high-QC levels (20% for the LLOQ).

2.5.4 Carryover

The residual effect was investigated by analyzing the blank sample after the ULOQ sample. The residue in the blank sample after the ULOQ should not exceed 20% of the response of the analyte in the LLOQ sample and 5% of the internal standard response.

2.5.5 Matrix effect

At least six batches of blank matrices from different donors were used, for each batch of matrix, the matrix factor normalized by internal standard was calculated by the ratio of the peak area in the presence of matrix (measured after adding the analyte and internal standard to blank matrix extracts) to the corresponding peak area in the absence of matrix (pure solution of the analyte and internal standard) at low and high concentration quality control (QC) levels. The acceptable precision (CV%) of the internal standard-normalized matrix factors calculated from six batches of matrix cannot exceed 15%.

2.5.6 Extraction recovery

Extraction recovery was determined from the ratio of peak areas from pre-extraction and post-extraction spiked plasma or bone tissues at LQC, MQC, and HQC level of concentrations, which refers to the ability or efficiency of successfully extracting the target compound from the sample.

2.5.7 Dilution integrity

Dilution integrity is the evaluation of the sample dilution process to ensure that it does not affect the accuracy and precision of the concentration of the analyte. Blank plasma was used to dilute the samples at the concentration of higher than the Upper Limit of Quantitation (ULOQ) (4000 μg/mL), with 6 samples for 50-fold dilution factor. Accuracy and precision within±15% were set as acceptance criteria.

2.5.8 Stability reliability

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The stability of cefuroxime was determined by analyzing concentration of LQC and HQC samples stored under different storage conditions, including newly prepared and extracted samples. Freeze-thaw stability was determined after three freeze-thaw cycles (from −80°C to 25°C) on consecutive days. Long-term stability was studied by storing QC samples at −80°C for 30 days, short-term stability was determined by analyzing QC samples stored at 25°C for 6 h and 4°C for 24 h (bone samples stored for 6 h). Post-processing stability was evaluated after 24 h of storage in the autosampler at 15°C. Analyte concentrations were compared with nominal concentration and were considered stable if the accuracy was within ± 15%.

2.6 Clinical application to patient samples

The method was used to analyze the concentration of cefuroxime in plasma and bone tissues. Samples were collected from osteoarthritis patients undergoing joint replacement and using cefuroxime as an infection prophylaxis. The study was approved by the Ethics Committee of Peking University Third Hospital (Beijing, China), and all patients signed informed consent after they were informed. The study population comprised patients aged >18 years undergoing joint replacement surgery who received perioperative prophylactic cefuroxime. Blood samples were collected at 10-30 min and 50-100 min post-dose. Intraoperative bone specimens were obtained during the joint replacement procedure. The primary objective of this study was to determine whether cefuroxime exposure levels exceeded the clinical breakpoint and evaluate target attainment of the current prophylactic regimen, utilizing the newly developed and validated analytical method.

3. Results and discussion

3.1 Method development.

Method was developed by optimizing ionization and fragmentation conditions for analyte and IS. The optimization process was achieved by continuous injection of cefuroxime and cefuroxime-d3 with a syringe pump under a negative ion model. Q1 Full-scan mass spectra exhibited deprotonated precursor ions $[M-H]^-$ as the dominant species at m/z 423.0 for cefuroxime and m/z 426.0 for cefuroxime-d3. To obtain optimal sensitivity and stability, we systematically optimized key MS/MS parameters including GS1, GS2, DP, CE, EP, and CXP for the analyte and IS transitions. The most abundant and stable product ions were observed at m/z 318.5 for cefuroxime, however, to avoid signal saturation at higher concentrations, the m/z 423.0→207.1 transition was selected for cefuroxime quantification. Figure 1 displays the chemical structure of cefuroxime and cefuroxime-d3. As shown in Figure 2, which is the precursor ion spectra and the product ion spectra of cefuroxime and cefuroxime-d3. To obtain better peak shape and higher sensitivity, the chromatographic conditions were optimized by adding 0.1% formic acid in the mobile phase. Adding formic acid to water and acetonitrile phases enhances protonation of cefuroxime and cefuroxime-d3(IS). This is because the primary amino group ($R\text{-}NH_2$) in cefuroxime serves as the key protonation site. Formic acid (pKa ≈ 3.75) ionizes in the mobile phase, supplying H^+ ions that bind to this basic group, forming $[M+H]^+$ ions, then this process improves LC-MS/MS detection sensitivity and stability. The total chromatographic run time was 7.5 min.

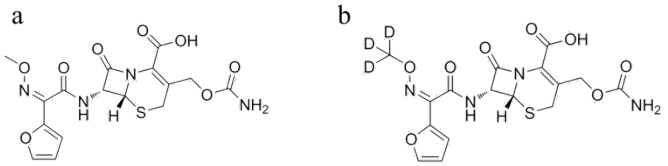

Figure 1. Chemical structure diagrams of cefuroxime(a) and cefuroxime-d3 (b).

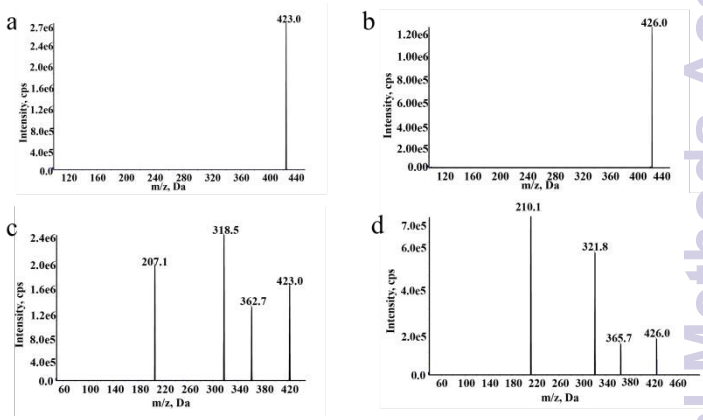

Figure 2. Precursor ion spectra of cefuroxime(a) and cefuroxime-d3(b); the product ion spectra of cefuroxime(c) and cefuroxime-d3(d)

3.2 Method validation

3.2.1 Selectivity and specificity

The typical chromatogram of double blank sample, blank sample, the LLOQ and plasma (bone) sample were demonstrated in Figure 3. The results indicated there was no apparent endogenous component interference for the determination of cefuroxime.

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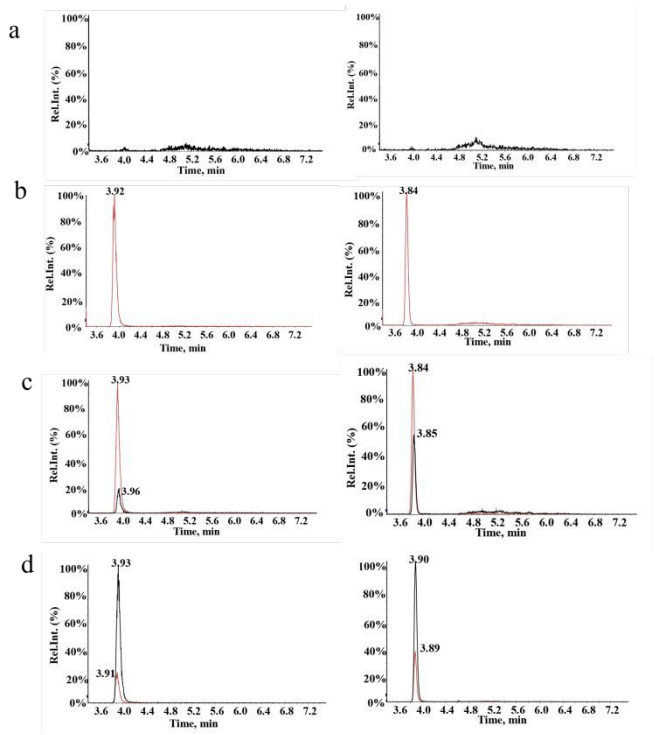

Figure 3. Representative LC-MS/MS chromatograms. (a) double blank sample: plasma(left), bone(right); (b) blank sample: plasma(left), bone(right); (c) LLOQ sample: plasma(left), bone(right); (d) plasma sample from a patient undergoing joint replacement surgery.

3.2.2 Linearity and LLOQ

The standard calibration curve for spiked human plasma containing cefuroxime was linear over the range 2-200 μg/mL (4-400 μg/g for bone sample). The Regression equation and the linearity were y= (0.08±0.001) x ± (0.014±0.0105), r^2= 0.998 for plasma, y= (0.193±0.0026) x ± (0.0503±0.0312), r^2= 0.994 for bone, respectively. Figure. S1 presents standard calibration curves of cefuroxime in human plasma and bone tissue samples. The LLOQ was determined at 2 μg/mL (4 μg/g) according to the requirement.

3.2.3 Precision and accuracy

The accuracy and intra- and inter-day precisions are shown in Table 1, which measured by LLOQ, low, medium, and high QC samples. The accuracy was within acceptance criteria within ±15% (±20% for LLOQ level) The precisions for intra- and inter-day were no more than 15%. Thus, the above values were within the acceptable range, which demonstrated the good stability and repeatability of this described method.

3.2.4 Carryover

The double-blank sample injected after the ULOQ sample showed no apparent carryover effect, which indicated that the residue of the previous sample retained on the analytical instrument could not cause a change in the measured concentration. **(**Figure. S2 and Table. S1)

Table 1. Intraday and interday precision and accuracy of cefuroxime in human plasma and bone.

	QC levels	Nominal concentrations		Measured concentration (mean±SD)		Accuracy (%)		Precision (CV, %)	
		Plasma(μg/mL)	bone(μg/g)	Plasma(μg/mL)	bone(μg/g)	plasma	bone	plasma	bone
Intraday (n=6)	HQC	150.00	300.00	139.67±3.88	272.67±4.27	93.11	90.89	2.78	3.13
	MQC	50.00	100.00	48.60±0.66	98.80±1.66	97.20	98.80	1.37	3.37
	LQC	6.00	12.00	5.91±0.28	12.74±0.35	98.50	106.20	4.82	5.47
	LLOQ	2.00	4.00	1.95±0.07	3.90±0.03	97.50	97.58	3.42	1.57
Interday (3 days, n=6)	HQC	150.00	300.00	141.10±3.30	267.44±5.00	94.07	89.15	2.30	3.74
	MQC	50.00	100.00	48.38±1.95	99.30±1.62	96.70	99.30	4.16	3.26
	LQC	6.00	12.00	5.92±0.29	12.96±0.28	98.60	107.97	4.85	4.35
	LLOQ	2.00	4.00	1.88±0.14	4.20±0.24	94.10	105.11	7.43	11.46

3.2.5 Matrix effect and recovery

The matrix effects were measured by calculating the CV of the internal standard-normalized matrix factors, ranging from 2.34% to 2.91% for plasma (3.13%-5.17% for bone) and the extraction recoveries were between 98.19% and 103.73% (99.90%-113.76% for bone). The results showed that the interfering substances in the biological matrix would not cause the corresponding changes in the tested substance. (Table. S2)

3.2.6 Dilution integrity

Plasma samples were diluted 50-fold at the concentration of 80 μg/mL(n=6), the mean accuracy of dilution quality control was in 102.4%, and the CV was no more than 2.6%.

3.2.7 Stability reliability

The stability of plasma samples was verified at two concentration levels (LQC and HQC) while the short-term stability, long-term stability, and freeze/thaw stability of cefuroxime are presented in Table 2. Considering that the method was optimized using a canine bone matrix, stability assessment was conducted on human bone samples stored under distinct conditions, which shown in Table 3. The analyte concentrations obtained at different stored conditions were benchmarked against the baseline (T0) levels, where T0 represents the concentration measured immediately after sample acquisition. The results showed that the mean value of each concentration was within ±15% of the labeled concentration, therefore, the operation and storage conditions during the preparation, processing and analysis of the sample did not affect the concentration of cefuroxime.

3.3 Method comparison

Table 4 summarizes the analytical methods for cefuroxime, with the primary distinctions lying in the determination of cefuroxime across different biological fluids or tissues. We can observe that, current analytical methods for cefuroxime detection are predominantly focused on plasma[13-17], compared to these existing approaches, our method demonstrates superior performance with faster chromatographic separation, reduced solvent consumption, and improved extraction efficiency for plasma samples. Additional studies targeting drug concentration measurements in breast milk and ocular tissues[7, 18, 19]. Although a previous method utilized ultra-performance convergence chromatography tandem mass spectrometry (UPC²-MS/MS) for cefuroxime quantification in bone tissue with meropenem-d6 as the internal standard[20], however, this approach presents several significant limitations that may compromise its reliability and practical utility. First, the analytical platform itself presents accessibility challenges. UPC²-MS/MS is not readily available in most laboratories, whereas conventional LC-MS/MS systems offer greater prevalence and cost-effectiveness. More critically, the method's choice of internal standard raises substantial concerns. The use of meropenem-d6 (a carbapenem-class antibiotic) for quantifying cefuroxime (a cephalosporin) is problematic due to: well-documented stability issues and structural dissimilarities between the two β-lactam subclasses. Multiple studies have demonstrated meropenem's poor stability across various matrices[21, 22]. Even under refrigerated conditions (4°C), its concentration-dependent degradation can exceed 15% within 12-24 hours[21]. Long-term storage at -20°C fails to prevent degradation, as observed in both plasma samples and stock solutions [22]. While conventional analytical methods typically employ stabilizers (e.g., sodium bicarbonate or buffer systems) to address this issue, the cited study implemented no such stabilization measures for meropenem-d6, potentially compromising quantitative accuracy. Furthermore, the structural disparities between carbapenems and cephalosporins may introduce analytical variability and extraction recovery. These concerns are compounded by the method's failure to systematically evaluate matrix effects during validation, further undermining data reliability and limiting the method's applicability for routine analyses. Collectively, these limitations suggest that meropenem-d6 may not be an optimal internal standard for cefuroxime quantification, and the described UPC²-MS/MS method requires substantial refinement to ensure robust performance. In this study, a single robust method was established for quantifying cefuroxime across distinct biological matrices (plasma and bone tissue) with high specificity. Due to the clinical unavailability of human blank bone matrix, blank canine bone powder was employed as a surrogate matrix. To mitigate potential matrix effects arising from this substitution, a stable isotope-labeled analog of cefuroxime(cefuroxime-d3) was utilized as the internal standard. During the analysis of authentic bone samples, the internal standard signal demonstrated acceptable variability, with a relative standard deviation (RSD) of ⩽15%, thereby validating the robustness of the method

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Table 2. Summary of the stability of cefuroxime in human plasma on different storage conditions (n=3).

Stability conditions		QC levels	Nominal concentrations (μg/mL)	Measured concentration (mean±SD, μg/mL)	Accuracy (%)	Precision (CV, %)
Short term	4℃ 24 h	HQC	150.00	134.67±0.58	89.78	0.43
		LQC	6.00	5.64±0.17	94.00	2.95
	RT 6 h	HQC	150.00	135.33±2.89	90.22	2.13
		LQC	6.00	5.72±0.24	95.44	4.13
Long term	-80℃ 30 days	HQC	150.00	143.33±3.06	95.56	2.13
		LQC	6.00	6.12±0.18	102.06	2.92
Three freeze-thaw cycles (from 25°C to −80°C)		HQC	150.00	137.00±1.00	91.33	0.73
		LQC	6.00	6.05±0.25	100.83	4.09
Treated-samples	Autosampler 24 h	HQC	150.00	141.83±3.37	94.53	2.38
		LQC	6.00	5.85±0.18	97.50	3.12

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Table 3. Summary of the stability of cefuroxime in human bone on different storage conditions (n=3), T0: the concentration measured immediately after sample acquisition.

Stability conditions		Sample ID	T0：sample concentrations (μg/g)	Measured sample Concentration (μg/g)	Accuracy (%)
Short term	4℃ 6 h	1	11.80	11.00	93.22
		2	16.50	15.50	93.40
		3	13.00	13.20	101.54
	RT 6 h	1	11.80	11.60	98.30
		2	16.50	16.40	99.40
		3	13.00	12.20	93.85
Long term	-80℃ 30days	1	11.80	12.30	104.24
		2	16.50	18.40	111.52
		3	13.00	13.50	103.85
Treated-samples	Autosampler 24h	1	11.80	12.00	101.70
		2	16.50	17.10	103.64
		3	13.00	13.40	103.68

3.4 Method complication to patient samples

The validated LC-MS/MS assay was implemented to quantify cefuroxime exposure in plasma and bone samples obtained from 80 osteoarthritis patients undergoing total joint arthroplasty with cefuroxime prophylaxis. The conventional pharmacodynamic index for evaluating cephalosporin dosing is the time during which drug concentrations exceed the minimum inhibitory concentration (MIC) of target pathogens (in this case, the typical microorganism causing PJI, the concentrations of cefuroxime should ideally remain above 8 μg/mL to exert bactericidal effects during perioperative prophylaxis[23]. According to our experiment, the volume of 1 gram of bone powder is approximately 1 mL, thus, in the process of our sample preparation, the correction factor for the conversion from μg/g to μg/mL is 1.0. We matched several blood sampling time points corresponding to bone tissue collection and conducted a preliminary analysis. In Figure 4, the results demonstrated substantially lower drug exposure in bone tissues compared to systemic circulation, with marked interpatient variability, underscoring the necessity for population pharmacokinetic modeling and individualized dosing regimens to optimize target-site pharmacokinetics. Another significant finding is that, under the conventional dosing regimen, we noted that the concentrations of cefuroxime in the cortical and cancellous bones of certain patients were below the target MIC, which suggests whether the dosages of cefuroxime should be increased. This clinical study remains ongoing, subsequent research will recruit more patients to complete the dataset.

Table 4. Analytical methods applied for the determination of cefuroxime.

Reference	Sample matrix	Analytical methods	IS	Analytical run time	Analytical Linear range	Recovery (%)	Matrix effect (CV, %)
[13]	plasma	LC-MS/MS	tazobactam	8 min	0.0525-21.0 μg/mL	89.44-92.3	1.40-4.61
[14]	plasma	LC-MS/MS	cefuroxime-d3	6 min	2.16-216 μg/mL	87.7-112.	2.63-6.60
[15]	plasma/serum	LC-MS/MS	cefuroxime-d3	7 min	1.0-100μg/mL	82.7-103	6.1
[24]	plasma	LC-MS/MS	cefazolin	10 min	0.1-100μg/mL	112.7-122.9	4.0
[7]	breast milk	LC-MS/MS	cefixime	8 min	25–1,000 ng/mL	/	5.3-7.7
[18]	ocular tissues	LC-MS/MS	Tolbutamide	/	12.7–2760 ng/mL	/	/
[19]	ocular tissues	LC-MS/MS	cefuroxime-d3	/	/	/	/
[20]	synovial tissue and bone	UPC2-MS/MS	Meropenem-d6	5 min	1.0–20.7 μg/g	/	/
This study	plasma and bone	LC-MS/MS	cefuroxime-d3	7.5min	2-200 μg/mL（plasma） 4-400 μg/g（bone）	99.80-102.00（plasma） 105.00-107.00 （bone)	2.34 to 2.91 (plasma) 3.13-5.17 (bone)

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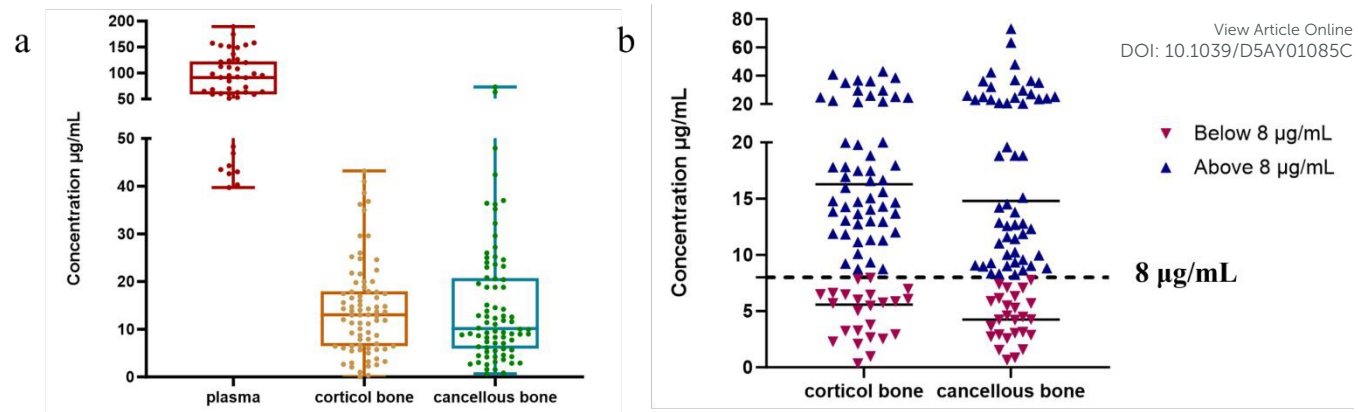

Figure 4. (a) The concentrations of cefuroxime in plasma(n=45), cortical bone(n=78), and cancellous bone(n=80). (b)The box plot depicting the concentrations of cefuroxime measured in cortical bone (78 cases) and cancellous bone (80 cases) is presented. The MIC values (8 μg/mL) are denoted by the dotted lines. Plasma samples were taken 40±20 min (mean (SD)), bone samples were collected 34 ± 18 min after drug infusion.

3.5 Study limitations and future improvements

Although this study successfully developed and validated a robust LC-MS-MS method for the simultaneous quantification of cefuroxime in both plasma and bone tissue, its limitations should also be acknowledged. Due to the inherent complexity of bone tissue matrices, clinically procurable blank human bone matrix is unavailable. This constitutes a methodological limitation in our study, necessitating the use of canine bone powder as a surrogate blank matrix. In total joint arthroplasty (classified as Class I incision surgery), cefuroxime is routinely administered for surgical antimicrobial prophylaxis. For cefuroxime-allergic patients, clindamycin serves as the alternative agent. Although we collected bone tissue samples from two clindamycin-treated patients, the limited sample size precluded comprehensive method validation. Consequently, canine bone powder was selected as the alternative matrix. To evaluate matrix equivalence between canine and human-derived bone powder, parallel calibration curves were established, as demonstrated in Figure. S1, the near-identical slopes (k = 0.223 vs 0.238, RSD = 4.60%) of these curves substantiate the acceptability and analytical accuracy of canine bone powder as a surrogate matrix. To evaluate the repeatability and reliability of the analytical method in actual sample testing, we performed reanalysis on a minimum of 10% of the samples (Table. S3-S4, Figure. S3). The vast majority (86%) of plasma samples demonstrated a reanalysis percentage bias within ±20%. Similarly, approximately 69% of bone tissue samples fell within this ±20% bias limit. Notably, the higher deviations observed in individual bone samples (> 30%) may originate from the inherent heterogeneity of bone tissue, which contrasts with homogeneous matrices like plasma or other biological fluids where drug distribution is more uniform. To enhance clinical dataset accuracy, duplicate measurements will be implemented for future bone sample analysis.

Conclusions

A simple and robust LC-MS/MS method for the quantification of cefuroxime in human plasma and bone tissues was developed. Method validation has been demonstrated by a variety of tests for specificity, selectivity, linearity, precision and accuracy, carry-over, matrix effect, extraction recovery, dilution integrity and stability. This validated method has been successfully implemented in the current clinical study, demonstrating high sensitivity and accuracy that are critical for reliable pharmacokinetic characterization of cefuroxime at the target site.

Author contributions

Qixian Ling: Methodology, Validation, Writing – original draft, Visualization, Data curation; **Yuanyuan Zhang**: Project administration; **Haotian Ma**: Data curation; **Yanan Wang**: Resources, **Zhe Wang**: Resources, Sihan Wang: Resources; **Xin Xiong** and **Libo Zhao**: Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition, Investigation, Resources.

Conflicts of interest

All authors declared no competing interests for this work.

Data availability

The data used in this study were collected and processed independently by the authors and have not been publicly released. However, the authors are willing to share these data

within reasonable bounds to facilitate academic exchange and collaboration. Interested researchers may contact libozhao@bjmu.edu.cn and provide your research background and proposed collaboration plan so we can discuss the feasibility of data sharing.

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Ethical statement

This study was approved by the ethics committee of Peking University Third Hospital (Approval Number: M2024414).

Notes and references

1 Q. Yao, X. Wu, C. Tao, W. Gong, M. Chen, M. Qu, Y. Zhong, T. He, S. Chen and G. Xiao, *Signal Transduct Target Ther*, 2023, **8**, 56.
2 S. Glyn-Jones, A. J. Palmer, R. Agricola, A. J. Price, T. L. Vincent, H. Weinans and A. J. Carr, *Lancet*, 2015, **386**, 376-387.
3 P. H. Gundtoft, A. B. Pedersen, C. Varnum and S. Overgaard, *Clin Orthop Relat Res*, 2017, **475**, 2623-2631.
4 C. K. Koh, I. Zeng, S. Ravi, M. Zhu, K. G. Vince and S. W. Young, *Clin Orthop Relat Res*, 2017, **475**, 2194-2201.
5 P. Izakovicova, O. Borens and A. Trampuz, *EFORT Open Rev*, 2019, **4**, 482-494.
6 E. Beam and D. Osmon, *Infect Dis Clin North Am*, 2018, **32**, 843-859.
7 A. Kul and O. Sagirli, *Biomed Chromatogr*, 2023, **37**, e5744.
8 J. A. Bosco, J. Bookman, J. Slover, E. Edusei and B. Levine, *J Am Acad Orthop Surg*, 2015, **23**, e27-35.
9 B. Spellberg and B. A. Lipsky, *Clin Infect Dis*, 2012, **54**, 393-407.
10 .A. K. Thabit, D. F. Fatani, M. S. Bamakhrama, O. A. Barnawi, L. O. Basudan and S. F. Alhejaili, *Int J Infect Dis*, 2019, **81**, 128-136.
11 C. Joukhadar, M. Frossard, B. X. Mayer, M. Brunner, N. Klein, P. Siostrzonek, H. G. Eichler and M. Müller, *Crit Care Med*, 2001, **29**, 385-391.
12 A. Barbour, S. Schmidt, W. R. Rout, K. Ben-David, O. Burkhardt and H. Derendorf, *Int J Antimicrob Agents*, 2009, **34**, 231-235.
13 X. Hu, M. Huang, J. Liu, J. Chen and J. Shentu, *Adv Pharmacol Sci*, 2014, **2014**, 981624.
14 W. Lu, M. Pan, H. Ke, J. Liang, W. Liang, P. Yu, P. Zhang and Q. Wang, *Front Pharmacol*, 2022, **13**, 1044234.
15 S. K. Neef, A. D. Hinderer, W. Arbash, M. Kinzig, F. Sörgel, C. Wunder, M. Schwab and U. Hofmann, *Clin Chim Acta*, 2024, **559**, 119690.
16 A. Viberg, M. Sandström and B. Jansson, *Rapid Commun Mass Spectrom*, 2004, **18**, 707-710.
17 S. Neugebauer, C. Wichmann, S. Bremer-Streck, S. Hagel and M. Kiehntopf, *Ther Drug Monit*, 2019, **41**, 29-37.
18 B. B. Gabani, V. Kiran, S. Praharaj, N. R. Srinivas and R. Mullangi, *Biomed Chromatogr*, 2020, **34**, e4737.
19 E. M. Del Amo, A. Hammid, M. Tausch, E. Toropainen, A. Sadeghi, A. Valtari, J. Puranen, M. Reinisalo, M. Ruponen, A. Urtti, A. Sauer and P. Honkakoski, *Int J Pharm*, 2022, **613**, 121361.
20 S. Bahmany, A. Holst, M. H. Hoogendoorn, M. Oosterhoff, J. van Oldenrijk, P. K. Bos, E. S. Veltman and B. C. P. Koch, *Journal of Chromatography B*, 2024, **1241**.
21 J. Zander, B. Maier, M. Zoller, G. Döbbeler, L. Frey, D. Teupser and M. Vogeser, *Clin Chem Lab Med*, 2016, **54**, 265-274.
22 S. Rehm and K. M. Rentsch, *J Pharm Biomed Anal*, 2020, **186**, 113289.
23 E. C. o. A. S. T. (EUCAST), 2025.
24 A. Abdulla, S. Bahmany, R. A. Wijma, B. C. H. van der Nagel and B. C. P. Koch, *J Chromatogr B Analyt Technol Biomed Life Sci*, 2017, **1060**, 138-143.

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