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Liquid chromatography/tandem mass spectrometry assay for the simultaneous determination of cefoperazone and sulbactam in plasma and its application to a pharmacokinetic study

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ABSTRACT

A rapid and highly sensitive liquid chromatography-tandem mass spectrometric (LC–MS/MS) method for simultaneous determination of cefoperazone sodium and sulbactam sodium in human plasma was developed. The analytes and internal standard (IS), cefuroxime sodium, were extracted from human plasma via liquid–liquid extraction with ethyl acetate and separated on a Waters Xterra C18 column within 3.5 min. Quantitation was performed on a triple quadrupole mass spectrometer employing electrospray ionization technique, operating in selected reaction monitoring (SRM) and negative ion mode. The precursor to product ion transitions monitored for cefoperazone, sulbactam and IS were m/z 644.1 → 528.0, 232.1 → 140.0, and 423.0 → 362.0, respectively. The assay was validated in the linear range of 0.1–20 μg/mL for cefoperazone and 0.02–4 μg/mL for sulbactam. The intra- and inter-day precisions (CV%) were within 8.39% for each analyte. The recoveries were greater than 87.3% for cefoperazone and 87.2% for sulbactam. Each analyte was found to be stable during all sample storage, preparation and analytical procedures. The method was successfully applied in a pharmacokinetic study of Sulperazon injection in six hospital-acquired pneumonia (HAP) patients.

1. Introduction

Hospital-acquired pneumonia (HAP) remains an important cause of morbidity and mortality despite advances in antimicrobial therapy [1]. Common pathogens of HAP include aerobic gram-negative bacilli, such as *Acinetobacter* spp. [1]. Meanwhile, the increasing prevalence of multidrug-resistant (MDR) *Acinetobacter baumannii* in the clinic has become a frightening reality. Outbreaks of infection with this pathogen have been noted in every inhabited continent in the past decade, with HAP being the most common clinical manifestation [2,3]. Cefoperazone is a third-generation cephalosporin antibiotic with a broad spectrum of activity against most gram-positive and gram-negative bacteria. Sulbactam, a β-lactamase inhibitor, has intrinsic activity against *Acinetobacter* spp. [4,5]. In vitro, the combination of cefoperazone and sulbactam shows a marked degree of synergetic effect against some cefoperazone-resistant organisms [6], especially MDR *A. baumannii* [2,3]. For that reason, cefoperazone-sulbactam is clinically important in the management of MDR *A. baumannii* infections, such as HAP.

Patients who are candidates for treatment with cefoperazone-sulbactam may be physiologically compromised and therefore may not distribute or eliminate these drugs as healthy subjects do. Since the clinical pharmacokinetics of Sulperazon injection, cefoperazone and sulbactam, in HAP patients has not been published to date, we carried out a study to investigate the clinical pharmacokinetics of Sulperazon in HAP patients.

Several high performance liquid chromatography (HPLC) methods for the determination of cefoperazone and sulbactam have been described in the literature [7–11]. One liquid chromatography-mass spectrometric (LC–MS) method applied for whole blood was reported as well [12]. All these methods took a long time to detect cefoperazaon and sulbactam, and the LC–MS method reported in Japan detected these two analytes separately. A rapid and highly sensitive liquid chromatography-tandem mass spectrometric (LC–MS/MS) method for the simultaneous determination of cefoperazone and sulbactam in human plasma was developed. The application of this method was demonstrated in a PK study of six HAP patients after receiving an intravenous administration of 3.0 g Sulperazon injection.

2. Experimental

2.1. Chemicals, materials and reagents

Cefoperazone sodium, sulbactam sodium, and cefuroxime sodium (IS) (Fig. 1) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Lot

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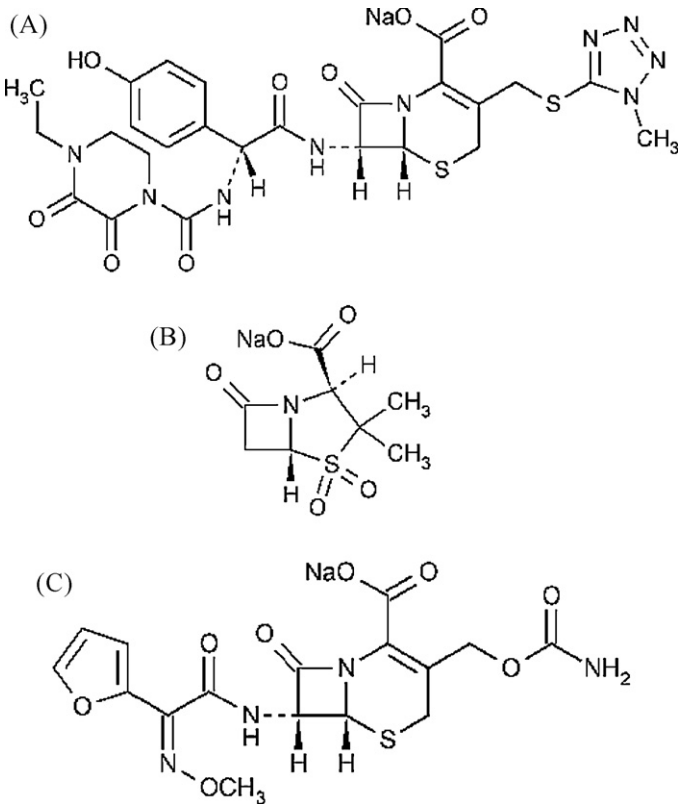

Fig. 1. Structures of two analytes and IS. (A) Cefoperazone sodium; (B) sulbactam sodium; (C) cefuroxime sodium.

number 8213IJ81D, 08391075, and 130493-200704, respectively). Sulperazon injection, 1.5 g/vial, (Lot number 85839755), containing 1000 mg cefoperazone sodium and 500 mg sulbactam sodium, expired in April 2010, was manufactured by Pfizer Pharmaceuticals Ltd. (Liaoning, China). Methanol was of HPLC grade (Sigma-Aldrich Laborchemikalien GmbH, Germany). Others were of AR grade. Ultrapure water used for the LC–MS/MS was from Milli-Q water purification system (Millipore, USA). All concentrations of cefoperazone and sulbactam in this manuscript indicated as sodium salt form.

2.2. Preparation of standards and quality control samples

The standard stock solutions were prepared by dissolving cefoperazone sodium, sulbactam sodium and IS in ammonium formate solution (10 mM, pH 4.5) separately. Quality control (QC) stock solutions were individually prepared from separate weighing in a similar fashion. Combined working solutions of two analytes, required for spiking plasma calibration and QC samples, were subsequently prepared in ammonium formate solution (10 mM, pH 4.5). The concentration of IS working solution was 50 μg/mL. All the solutions were stored at −40 °C until use. Blank plasma, drug free, obtained from health volunteers aged from 20 to 50, was stored in the freezer and allowed to completely thaw before use. The calibration standards and QC samples were prepared by spiking blank plasma with the combined working solution. Calibration standards were made at 0.10, 0.50, 1.00, 2.50, 5.00, 10.0, 20.0 μg/mL for cefoperazone, and 0.02, 0.10, 0.20, 0.50, 1.00, 2.00, 4.00 μg/mL for sulbactam. QCs were prepared at 0.1, 0.3, 3, 18 μg/mL for cefoperazone, and 0.02, 0.06, 0.6, 3.6 μg/mL for sulbactam. The spiked plasma samples were stored at −40 °C until analysis.

2.3. LC–MS/MS instrumentation and conditions

A Thermo Finnigan TSQ Quantum triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source (San Jose, CA, USA), a series 2690 HPLC system (Waters, Milford, MA, USA) consisted of a quat pump, an autosampler and an online degasser were used for LC–MS/MS analysis. The analytes and IS were separated on a Waters Xterra C18 column (5 μm; 2.1 mm × 50 mm) with the mobile phase, methanol-ammonium formate solution (10 mM, pH 4.5) (30:70, v:v), at a flow rate of 0.2 mL/min. The temperature of autosampler was maintained at 4 °C and the injection volume was 5 μL. The total LC run time was 3.5 min. Negative ion ESI was used to form deprotonated molecules. Selected reaction monitoring (SRM) was used to monitor precursor to product ion transition of m/z 644.1 → 528.0 for cefoperazone, 232.1 → 140.0 for sulbactam, and m/z 423.0 → 362.0 for IS. All the parameters of LC and MS were controlled by Finnigan LCQuan software version 2.5.6.

Ion spray voltage was at 4000 V and capillary temperature was at 320 °C. Nitrogen gas was used as sheath gas (48 arbitrary units) and auxiliary gas (12 arbitrary units). Argon gas was used as collision gas at a pressure of approximately 1.5 mTorr. The collision energy was optimized at 11 eV for cefoperazone, 15 eV for sulbactam, and 10 eV for IS, respectively. Dwell time was set at 200 ms for all tested compounds.

2.4. Procedure for sample extraction

An aliquot of 200 μL plasma mixed with 10 μL working solution of IS (50 μg/mL), was added with 50 μL of 0.5 mol/L hydrochloric acid and vortexed until thoroughly mixed. Then the acidified plasma samples were extracted with 1 mL of ethyl acetate, vortexed for 10 min, followed by centrifugation at 7826 × g for 5 min. 0.9 mL of supernatant was transferred into another tube. The extraction procedure was repeated again and the supernatants were combined followed by evaporation to dryness at 40 °C under a gentle stream of nitrogen gas. The residue was reconstituted in 150 μL of mobile phase, and then centrifuged for 5 min at 7826 × g. A portion of the supernatant (5 μL) was injected into the LC–MS/MS system for analysis.

2.5. Method validation

The method was validated for selectivity, sensitivity (lowest limit of quantification, LLOQ), linearity, matrix effect (ME), recovery, precision, accuracy and stability.

Selectivity was assessed by comparing SRM chromatograms of six different sources of blank plasma with those of plasma samples spiked with cefoperazone, sulbactam and IS at the LLOQ to check for any possible interference with the retention time of analytes and IS.

The lowest standard on the calibration curve was defined as the LLOQ.

Six linearity curves containing seven non-zero concentrations were analyzed. Best-fit calibration curves of peak area ratio (cefoperazone versus IS, sulbactam versus IS) versus concentration were plotted. The calibration curve ($y = ax + b$, where y is the peak area ratio and x is the concentration) was obtained by least-squares linear regression analysis with reciprocal of the square of the drug concentration as a weighting factor ($1/x^2$) for cefoperazone and sulbactam. The regression equation for the calibration standards was also used to back-calculate the measured concentration at each QC level.

ME was investigated by comparing the area response of unextracted samples (Ai) to that of aqueous standards (Ar). Unextracted samples were prepared by spiking QC working solutions in six dif-

ferent lots of extracted blank plasma. Matrix effect at four QC levels had been observed by the equations shown below:

$$\mathrm{ME}\,(\%) = \frac{\mathrm{Ai}}{\mathrm{Ar}} \times 100$$

The recoveries of the tested analytes from the extraction procedure were evaluated at each QC level by comparing peak area of extracted samples (spiked before extraction) to the peak area of unextracted samples (QC working solutions spiked in extracted plasma samples).

The intra-day precision and accuracy were evaluated in six replicate analyses for cefoperazone and sulbactam at four QC levels on the same analytical run. Inter-day precision and accuracy were calculated after repeated analysis in six different analytical runs. The precision described the closeness of individual measures when the procedure was applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix; it was determined at each concentration level with the acceptance criteria not exceeding 15% of the coefficient of variation (CV) except for the LLOQ, where it should not exceed 20%. The accuracy was defined as the deviation of the measured concentration over the theoretical concentration; it should be within 15% except for the LLOQ (no more than 20%).

Stability experiments were performed to evaluate the analyte stability in stock solutions and in plasma samples under different conditions. Freeze-thaw stability, 24 h room temperature stability, long-term stability, 24 h post-preparative stability at room temperature and stock solutions stability were assessed using two replicates at each QC level.

2.6. Pharmacokinetic study

The study was conducted at the Institute of Antibiotics, Huashan Hospital, Fudan University. The ethics committee (IRB of Huashan Hospital, Fudan University, Shanghai, China) approved the study protocol. All the patients were informed of the aim and risks involved in the study, and written consent were obtained. Health check up was done by general physical examination, and laboratory testing such as hematology, biochemistry and urine examinations. Six HAP patients with normal renal and hepatic functions were enrolled in the study.

These patients were given Sulperazon injection 3.0 g via 1.5 h intravenous guttae every 8 h. Blood samples were collected from forearm vein at the midpoint of the infusion on the fourth day and 1.5, 2, 2.5, 3.5, 5.5, 7.5 h post-infusion. Blood samples were collected in lithium heparinized tubes, and centrifuged at 3000 × *g* for 10 min. Plasma was separated and stored at −40 °C until analysis. If the concentration of cefoperazone and sulbactam in human plasma were out of the linear range, plasma samples would be diluted with blank plasma (sample: blank, 1:9, v:v) for further analysis.

2.7. Pharmacokinetic analysis

Pharmacokinetic parameters were calculated by Winnonlin 5.2.1 (Pharsight®, USA). Noncompartmental methods were used to estimate pharmacokinetic parameters.

During the analysis, Winnonlin repeated regressions using the last three points with non-zero concentrations, then the last four points, last five, etc. For each regression, an adjusted R^2 was computed: adjusted $R^2 = 1 - [(1 - R^2) \times (n - 1)]/(n - 2)$, where n was the number of data points in the regression and R^2 was the square of the correlation coefficient. The terminal elimination rate constant (λ_Z) was estimated by performing a regression of the natural logarithm of the concentration values in the time points being used in the regression which had the largest adjusted R^2. Maximum plasma concentrations (C_{max}) were observed from the plasma concentration–time curve. Area under the plasma concentration–time curve during the 8-h dosing interval [$AUC_{(0-8)}$] was calculated by linear trapezoidal method. *AUC* from dosing time extrapolated to infinity [$AUC_{(0-\infty)}$] was based on the last observed concentration (obs): $AUC_{(0-8)} + \mathrm{Clast}_{obs}/\lambda_Z$. Terminal half-life ($t_{1/2}$) was calculated by using the equation $t_{1/2} = \ln(2)/\lambda_Z$. Total body clearance ($Cl_{ss}$) was calculated as dose/$AUC_{(0-\infty)}$ or dose/$AUC_{(0-8)ss}$, where $AUC_{(0-8)ss}$ was the *AUC* from 0 to 8 h on day 4 (steady state). Mean residence time extrapolated to infinity (*MRTINF*) was calculated as $\{AUMC_{(0-8)} + 8[AUC_{(0-\infty)} - AUC_{(0-8)}]\}/AUC_{(0-8)} - TI/2$, where $AUMC_{(0-8)}$ was the area under the first moment plasma concentration–time curve calculated by linear trapezoidal method and *TI* represented infusion duration. Volume of distribution at steady state (V_{ss}) was calculated as $MRTINF \times Cl_{ss}$.

3. Results and discussion

3.1. Mass spectrometery

MS/MS parameters were optimized to gain maximum response for sulbactam, cefoperazone and IS simultaneously. Both the positive and negative ion modes were investigated. The response of negative ion was much more sensitive and selective than positive ion for sulbactam and IS, while it was the contrary for cefoperazone. We improved the response of cefoperazone by optimizing electronic parameters such as ion spray voltage, capillary temperature, sheath gas, auxiliary gas and the collision energy.

Tsujikawa et al. [12] detected sulbactam and cefoperazone separately in negative and positive ion mode, using a single quadrupole mass spectrometer. Using a triple quadrupole mass spectrometer of a higher selectivity, the two analytes and IS could be determined simultaneously.

3.2. Chromatographic conditions

The chromatographic conditions, especially the composition of mobile phase, were optimized to achieve a good resolution and symmetric peak shapes for the analytes and the IS, as well as a short analytical time. It was found that a methanol-ammonium formate solution (10 mM, pH 4.5) (30:70, v:v) could achieve this purpose. After careful comparison of several columns, Waters Xterra C18 column (5 μm; 2.1 mm × 50 mm) gave the best chromatogram at a flow rate of 0.2 mL/min. Using these chromatographic conditions, the analytical time was 3.5 min. Tsujikawa et al. [12] used two different columns and various mobile phases to detect cefoperazone and sulbactam separately. Our method seemed much more convenient.

3.3. Sample preparation

A protein precipitation method by acetonitrile was initially used in plasma samples preparation; however the recoveries of the two analytes were below 10%. Liquid–liquid extraction using *N*-hexane-isopropanol (3:1, v:v), ethyl acetate-isopropanol (2:1, v:v), ethyl acetate-isopropanol (3:1, v:v), and ethyl acetate were attempted; the recoveries were all below 10%. We found the recoveries of the two analytes in organic solvent could be enhanced by acidifying the plasma samples, so 50 μL of 0.5 mol/L hydrochloric acid was added to plasma samples before extraction. Since the polarities of the two analytes were different, ethyl acetate was chosen as the extractant to obtain suitable recoveries for both analytes. The recoveries of the two analytes were improved considerably to 87%.

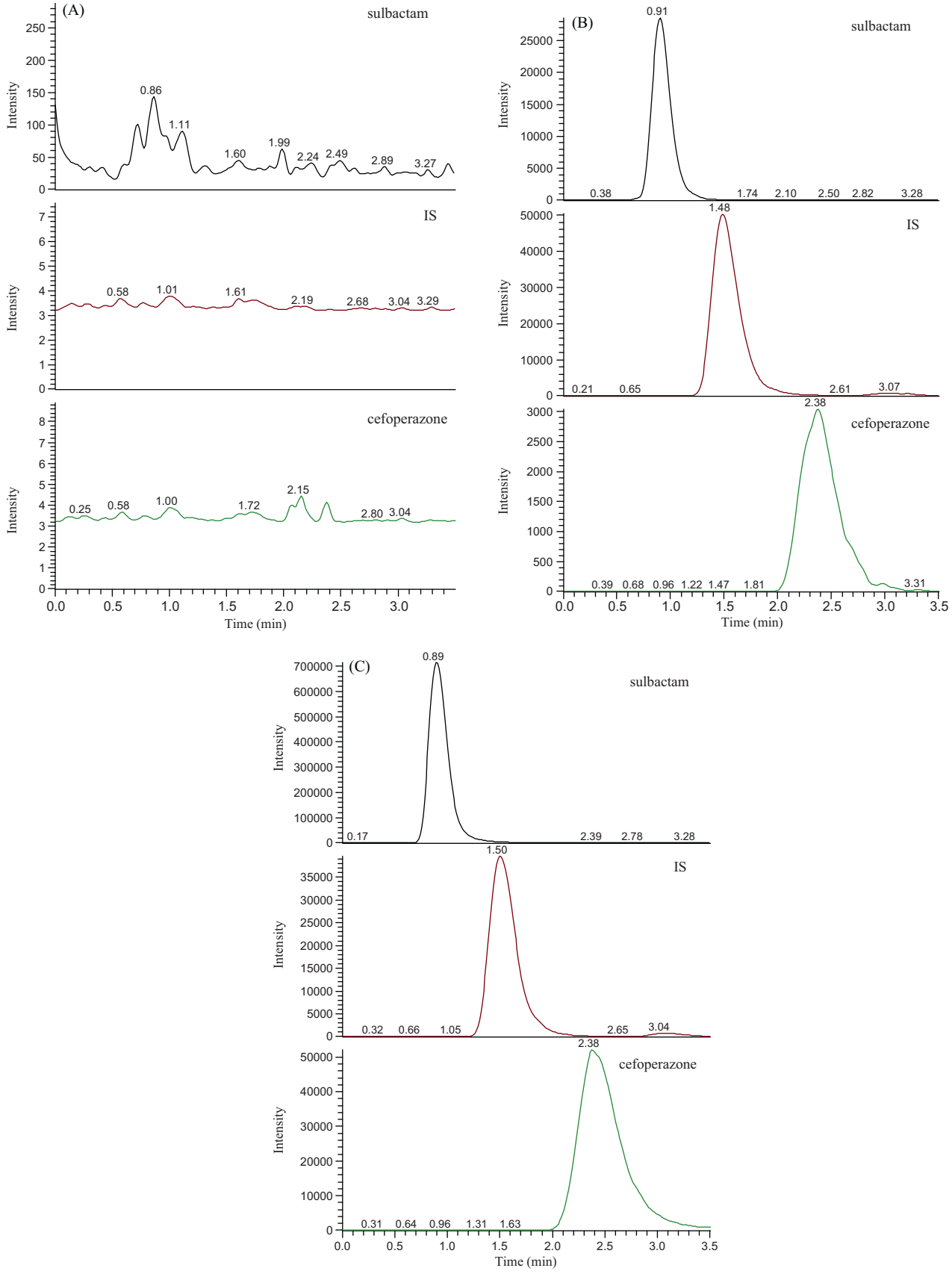

Fig. 2. SRM chromatograms of sulbactam, IS and cefoperazone in human plasma. (A) Blank plasma sample; (B) spiked plasma sample with sulbactam sodium (0.1 μg/mL), IS (2.5 μg/mL) and cefoperazone sodium (0.5 μg/mL); (C) a plasma sample from a HAP patient on the fourth day at 1.5 h post-infusion of Sulperazon injection (3.0 g).

Table 1
Matrix effect of cefoperazone sodium, sulbactam sodium and cefuroxime sodium (IS).

	Injecting conc. (μg/mL)	ME (%)
Cefoperazone sodium ($n = 6$)	0.1	106.5 ± 9.12
	0.3	100.3 ± 2.79
	3	98.1 ± 4.68
	18	100.8 ± 5.43
Sulbactam sodium ($n = 6$)	0.02	122.1 ± 19.68
	0.06	113.1 ± 11.64
	0.6	114.2 ± 10.31
	3.6	111.6 ± 4.85
IS ($n = 6$)	2.5	97.3 ± 4.00

Table 2
Recoveries of cefoperazone sodium, sulbactam sodium from human plasma using ethyl acetate as extracting solvent.

	Injecting conc. (μg/mL)	Recoveries ($n = 6$)
Cefoperazone sodium	0.1	87.3 ± 13.30
	0.3	97.7 ± 8.56
	3	89.8 ± 4.29
	18	90.2 ± 2.52
Sulbactam sodium	0.02	87.2 ± 4.45
	0.06	87.2 ± 2.25
	0.6	91.8 ± 6.10
	3.6	88.1 ± 4.81
IS	2.5	99.47 ± 2.17

3.4. Selectivity and sensitivity (LLOQ)

The liquid–liquid extraction methodology in combination with triple quadrupole mass spectrometry detection resulted in very good selectivity for the analytes and IS. Fig. 2 shows SRM chromatograms of sulbactam, IS and cefoperazone in human plasma. The retention time was 0.90 min for sulbactam, 2.38 min for cefoperazone, and 1.49 min for IS, respectively. No endogenous interference was detected in the blank plasma sample at the retention time of the two analytes and IS, as shown in Fig. 2(A). Fig. 2(B) shows the SRM chromatograms of spiked plasma sample with two analytes (0.5 μg/mL for cefoperazone, 0.1 μg/mL for sulbactam) and IS (2.5 μg/mL). Fig. 2(C) shows the SRM chromatograms of plasma sample obtained from a HAP patient on the fourth day at 1.5 h post-infusion of Sulperazon injection (3.0 g).

The LLOQs of cefoperazone and sulbactam were 0.1 and 0.02 μg/mL, respectively.

3.5. Linearity and matrix effect

The best linear fit and least-squares residuals for the calibration curve were achieved with a $1/x^2$ weighting factor with linear range of 0.1–20 μg/mL for cefoperazone and 0.02–4 μg/mL for sulbactam. For cefoperazone, the linear regression equation for mean of six calibration curves was $y = 0.1517x - 0.00588$, $r^2 = 0.9984$. For sulbactam, the equation was $y = 1.9164x - 0.00235$, $r^2 = 0.9950$. ME was consistent in all the lots and did not affect the quantitative analysis of analytes and IS peak (Table 1).

3.6. Recovery

The recoveries of cefoperazone and sulbactam are shown in Table 2.

3.7. Precision and accuracy

Intra-day and inter-day precision and accuracy for the analysis of cefoperazone and sulbactam in human plasma are presented in Tables 3 and 4. These results suggested that the LC–MS/MS assay was acceptable for the simultaneous quantitative analysis of the two analytes in human plasma.

3.8. Stability

Cefoperazone and sulbactam were found to be stable for 24 h at room temperature in plasma samples, the recoveries were 94.88% and 99.26%, respectively. In extracted plasma samples, the recoveries were 96.44% and 94.42%, respectively. Both analytes were stable under three freeze-thaw cycles with recoveries of 100.02% and 104.61%, respectively. The cefoperazone and sulbactam spiked plasma samples stored at −40 °C were found to be stable for 30 days, but unstable beyond 30 days; the recoveries were greater than 85.74% and 93.67% at 30th day, respectively. Stock solutions of cefoperazone and sulbactam were stable when stored at −40 °C for 3 months; the recoveries were 97.11% and 102.98%.

3.9. Application

Two female and four male patients were enrolled in our study, with age 48 ± 15 (23 to 65), weight 59.7 ± 7.1 kg, and body mass index 21.2 ± 3.2 (mean ± SD).

A noncompartmental model appeared to fit the concentration–time curves. The pharmacokinetic parameters such as C_{max}, $AUC_{(0-8)}$, $t_{1/2}$, Cl_{ss} and V_{ss} were calculated for cefoperazone and sulbactam. The best-fit pharmacokinetic parameters are presented in Table 5. Fig. 3 shows the average plasma concentration–time curves after an intravenous administration of Sulperazon injection to HAP patients.

Both drugs exhibited slower elimination and greater pharmacokinetic variability in these patients, compared with values previously reported in healthy volunteers. Cefoperazone $t_{1/2}$ was found to be 1.9 times longer than that in healthy subjects; V_{ss} for patients was 38.9% greater, and Cl_{ss} was 17.9% lower than those reported in healthy volunteers [11]. Sulbactam $t_{1/2}$ was found to be 1.3 times longer than that in healthy subjects; V_{ss} for patients was 18.1% lower, and Cl_{ss} was 31.6% lower than those reported in healthy volunteers [11].

Table 3
Intra-day accuracy and precision for the analysis of cefoperazone sodium and sulbactam sodium in human plasma.

	Nominal conc. (μg/mL)	Measured conc. (μg/mL)	Accuracy (%)	Precision (%)
Cefoperazone sodium ($n = 6$)	0.1	0.10 ± 0.004	104.5 ± 3.92	3.75
	0.3	0.30 ± 0.005	100.7 ± 1.51	1.50
	3	3.1 ± 0.17	104.0 ± 5.82	5.60
	18	18.7 ± 0.72	103.7 ± 4.00	3.85
Sulbactam sodium ($n = 6$)	0.02	0.018 ± 0.0012	87.5 ± 6.19	7.07
	0.06	0.057 ± 0.0025	94.4 ± 4.12	4.36
	0.6	0.54 ± 0.029	90.1 ± 4.89	5.43
	3.6	3.14 ± 0.015	87.3 ± 0.42	0.48

Table 4
Inter-day accuracy and precision for the analysis of cefoperazone sodium and sulbactam sodium in human plasma.

	Nominal conc. (μg/mL)	Measured conc. (μg/mL)	Accuracy (%)	Precision (%)
Cefoperazone sodium (n = 6)	0.1	0.10 ± 0.007	103.3 ± 6.76	6.55
	0.3	0.30 ± 0.020	100.2 ± 6.72	6.71
	3	3.0 ± 0.26	101.5 ± 8.51	8.39
	18	18.8 ± 0.83	104.4 ± 4.61	4.42
Sulbactam sodium (n = 6)	0.02	0.021 ± 0.0012	106.2 ± 5.80	5.46
	0.06	0.063 ± 0.0039	105.1 ± 6.52	6.21
	0.6	0.64 ± 0.039	105.9 ± 6.46	6.10
	3.6	3.53 ± 0.287	98.2 ± 7.97	8.11

Table 5
Pharmacokinetic parameters of cefoperazone sodium and sulbactam sodium in six HAP patients after an intravenous infusion of Sulperazon injection.

	Cefoperazone sodium	Sulbactam sodium
C_{max} (μg/mL)	155.1 ± 46.66	34.9 ± 12.55
$AUC_{(0-8)}$ (μg h/mL)	586.5 ± 227.69	88.8 ± 32.23
$t_{1/2}$ (h)	3.5 ± 1.11	1.4 ± 0.23
V_{ss} (L)	15.7 ± 2.60	22.6 ± 6.87
Cl_{ss} (mL/min)	62.2 ± 22.60	206.1 ± 69.62

Note – C_{max}: maximum plasma concentrations; $AUC_{(0-8)}$: area under the plasma concentration–time curve during the 8-h dosing interval; $t_{1/2}$: terminal half-life; V_{ss}: volume of distribution at steady state; Cl_{ss}: total body clearance.

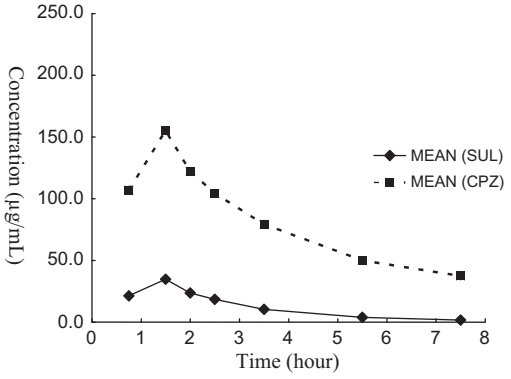

Fig. 3. Average plasma concentration–time curves after an intravenous administration of Sulperazon injection to HAP patients. SUL: sulbactam sodium; CPZ: cefoperazone sodium.

In view of the apparently large intra- and inter-subject variability in physiology and pharmacokinetics, larger-scale studies employing carefully selected patient populations would be needed to delineate the pharmacokinetics of Sulperazon in HAP patients.

4. Conclusions

A simple, specific, rapid and sensitive analytical method for simultaneous determination of cefoperazone sodium and sulbactam sodium in human plasma had been developed and validated. The proposed method is the first LC–MS/MS method for determination of two analytes simultaneously. Simple liquid–liquid extraction procedure and short run time can increase sample throughout, which could be important for processing large sample batches. The method provided excellent specificity and linearity with a lower limit of quantification of 0.1 μg/mL for cefoperazone and 0.02 μg/mL for sulbactam, respectively. The application of this method was demonstrated for the quantitative analysis of two analytes in plasma of HAP patients after intravenous administration of Sulperazon injections. The pharmacokinetic parameters of cefoperazone and sulbactam in HAP patients were also reported for the first time. This information could guide therapy of Sulperazon injection in HAP patients.

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References

[1] American Thoracic Society and the Infectious Diseases Society of America, Am. J. Respir. Crit. Care Med. 171 (2005) 388.
[2] D.E. Karageorgopoulos, M.E. Falagas, Lancet Infect. Dis. 8 (2008) 751.
[3] F. Perez, A.M. Hujer, K.M. Hujer, B.K. Decker, P.N. Rather, R.A. Bonomo, Antimicrob. Agents Chemother. 51 (2007) 3471.
[4] F.D. Wang, M.L. Lin, W.S. Lee, C.Y. Liu, Int. J. Antimicrob. Agents 23 (2004) 590.
[5] J. Brauers, U. Frank, M. Kresken, A.C. Rodloff, H. Seifert, Clin. Microbiol. Infect. 11 (2005) 24.
[6] R.N. Jones, A.L. Barry, R.R. Packer, W.W. Gregory, C. Thornsberry, J. Clin. Microbiol. 25 (1987) 1725.
[7] R.E. Bawdon, D.L. Hemsell, S.P. Guss, Antimicrob. Agents Chemother. 22 (1982) 999.
[8] R.E. Bawdon, P.O. Madsen, Antimicrob. Agents Chemother. 30 (1986) 231.
[9] S.N. Meyyanathan, R. Gopinath, S. Rajan, S. Phanikumar, Y.V. Muralidharan, S. Krishnaveni, N. Suresh, India J. Pharm. Educ. Res. 41 (2007) 256.
[10] T.L Tsou, Y.C. Huang, C.W. Lee, A.R. Lee, H.J. Wang, S.H. Chen, J. Sep. Sci. 30 (2007) 2407.
[11] D.P. Reitberg, T.J. Whall, M. Chung, D. Blickens, H. Swarz, J. Arnold, Antimicrob. Agents Chemother. 32 (1988) 42.
[12] K. Tsujikawa, K. Kuwayama, H. Miyaguchi, T. Kanamori, Y. Iwata, H. Inoue, T. Kishi, J. Forensic Sci. 53 (2008) 226.