Liquid Chromatographic Method for the Simultaneous Determination of Imipenem and Sulbactam in Mouse Plasma

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Abstract

The first analytical method is developed and validated for the simultaneous determination of imipenem and sulbactam in mouse plasma. Sample treatment is based on plasma stabilization with 4-(2-hydroxyethyl)piperazine-ethanesulfonic acid (HEPES) (0.5 mol/L; pH 7.0)–water–ethylene glycol (2:1:1, v/v/v), precipitation of plasma proteins with acetonitrile, centrifugation, evaporation, and reconstitution with borate buffer. Analytical determination is carried out by high-performance liquid chromatography with diode-array detection. Chromatographic separation is achieved within 11 min on a C_{18} column by gradient elution with borate buffer (0.1 mol/L, pH 7.2) and methanol. Imipenem and sulbactam are monitored at 295 and 230 nm, respectively. The overall interday accuracy is in the range of 95% to 100% and from 98% and 101% for imipenem and sulbactam, respectively. Interday precision is below 8% and 4% for imipenem and sulbactam, respectively. Limits of quantitation of imipenem and sulbactam are 0.05 and 1.0 µg/mL, respectively. The mean extraction recoveries are 94.5% and 94.2% for imipenem and sulbactam, respectively. The described method allows an accurate, simple, and rapid identification and quantitation of imipenem and sulbactam in mouse plasma. This method is applied to the analysis of imipenem and sulbactam in mouse plasma after drug administration.

Introduction

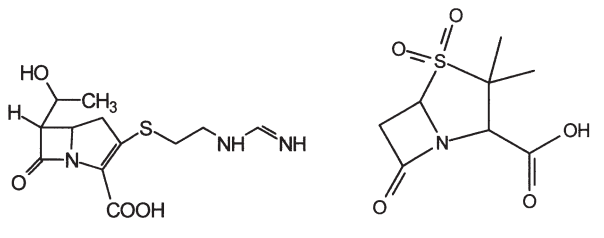
At present, the combination of imipenem and sulbactam is being evaluated for the treatment of *Acinetobacter baumannii* infections. Their effectiveness against *Acinetobacter baumannii* has been described not only in in vitro (1–3) studies but also in in vivo studies (3,4). *Acinetobacter baumannii* is a microorganism that has emerged, worldwide, as an important pathogen because of its marked multiresistance (4–6) and its high mortality rate. Clinical illness associated with Acinetobacter baumanii include: pneumonia, meningitis, endocarditis, peritonitis, skin and soft tissue infections, urinary tract infections, and bloodstream infections (4). Currently, the search for new therapeutic alternatives for the treatment of these infections has become a serious challenge to clinical researchers. Imipenem, a β-lactam antiobiotic, is one of the drugs most often used against *A. baumannii* (Figure 1). Sulbactam is a penicillanic acid sulphone with β-lactamase inhibitory properties (Figure 1). When imipenem is combined with sulbactam, the resistant rates against imipenem-resistant *A. baumannii* have been reported to be significantly reduced (1).

Despite the interest on this drug combination, no analytical method has been reported yet for their simultaneous determination. A method for their simultaneous determination is clearly more recommended than the individual determination of each substance, mainly because of the low sample amount available in preclinical studies and also to reduce analysis time. The aim of this work was to provide to clinical researchers an analytical method as simple and inexpensive as possible to properly evaluate this promising drug combination.

Experimental

Chemicals and reagents

Imipenem was purchased from Merck, Sharp & Dohme (Madrid, Spain), and sulbactam was purchased from Pfizer



Imipenem Sulbactam

Figure 1. Chemical structures of imipenem and sulbactam.

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(Orsay, France). High-performance liquid chromatography (HPLC)-grade acetonitrile and methanol were purchased from Romil (Waterbach, Cambridge, UK). 4-(2-Hydroxyethyl)piperazine-ethanesulfonic acid (HEPES) was purchased from Sigma-Aldrich (Steinheim, Germany). Boric acid, sodium hydroxide, and ethylene glycol were of analytical grade and purchased from Merck (Darmstadt, Germany). Deionized water was obtained from a Milli-Q Plus water purification system (Millipore, Milford, MA).

Stock solutions and calibration standards

Imipenem solutions were prepared in a stabilizing solution. The stabilizing solution was HEPES (0.5 mol/L; pH 7.0)–water–ethylene glycol (2:1:1, v/v/v). Sulbactam stock standard solutions were prepared in deionized water. Working solutions of imipenem and sulbactam were prepared from stock standard solutions by diluting the appropriate aliquot in the stabilizing solution. Plasma calibration standards at seven concentration levels were prepared in the concentration range of 0.5–100 µg/mL for imipenem and 2–150 µg/mL for sulbactam. Calibration standards were prepared in triplicate by spiking drug-free plasma with working solutions.

Plasma sample treatment

Two hundred microliters of stabilized plasma samples (100 µL of plasma and 100 µL of stabilizing solution) were transferred to 1.5-mL Eppendorf tubes; 200 µL of acetonitrile was added (7), and the tubes were vortex-mixed for 2 min and centrifuged at 1000 × *g* for 5 min. The liquid phase was transferred to another tube and evaporated to dryness under a nitrogen stream. Finally, the residue was dissolved in 100 µL of borate buffer and injected into the HPLC system.

HPLC system and chromatographic conditions

Chromatography was performed using a LaChrom instrument (Merck-Hitachi, Barcelona, Spain) equipped with a quaternary L-7100 pump and a L-7455 diode-array detection system. The injector was a Rheodyne manual injection valve Model 7725i, equipped with a 20-µL sample loop. Separations were carried out on a LiChrospher 100 C_{18} column (250 × 4-mm i.d., 5 µm) (Merck, Darmstadt, Germany) protected by a LiChrospher 100 C_{18} guard column (4 × 4-mm i.d., 5 µm) (Merck, Darmstadt, Germany). The system was controlled by an interface module and personal computer. Chromatograms were processed by an HPLC-System Manager HSM D-7000 (Merck-Hitachi).

Imipenem and sulbactam were separated by gradient elution with methanol and borate buffer aqueous solution (0.1 mol/L; pH 7.2 ± 0.1) and measured at 295 and 230 nm, respectively. Borate buffer was prepared daily from a 0.1 mol/L boric acid aqueous solution by adjusting the pH to 7.2 with sodium hydroxide solution (1 mol/L). Before use, the borate buffer solution was vacuum filtered through a 0.45-µm membrane filter. Both solutions were degassed by sonication prior to use. The elution program was 100% of borate buffer during the first 3 min and then the proportion of borate buffer was linearly decreased to 70% in 10 min. Chromatography was performed at room temperature at a flow rate of 1.0 mL/min.

Validation procedures

The method has been validated following the general guidelines for validation of analytical methods: International Conference on Harmonization Q2B (8) and Food and Drug Administration (9). A specific guideline for validation of HPLC methods was also followed (10).

Selectivity

Selectivity was assessed by measuring six blank samples. Additionally, the selectivity was assessed by comparing the UV spectra of imipenem and sulbactam chromatographic

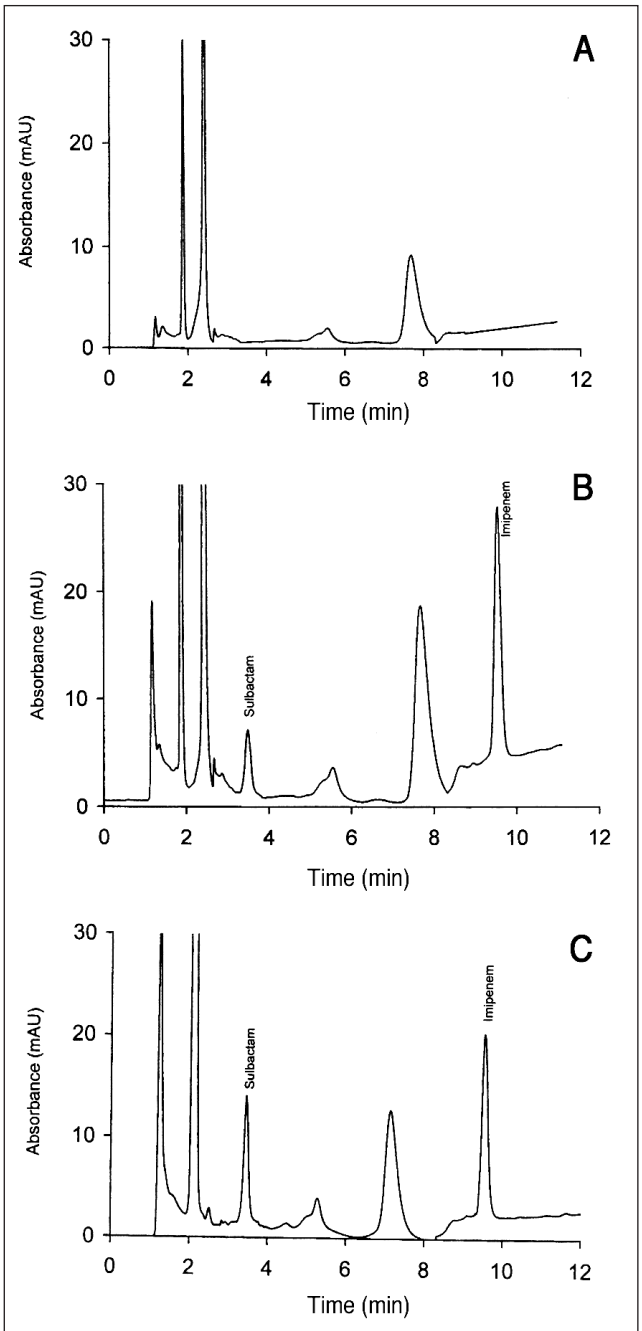

Figure 2. Chromatograms of treated samples of blank mouse plasma (A), mouse plasma spiked with 10 µg/mL of each compound (B), and mouse plasma at 60 min after drug administration (C).

peaks in spiked plasma samples at the peak upslope, apex, and downslope with the UV spectra of the drugs in working solution standards.

Accuracy and Precision

In order to calculate the accuracy and precision of the method, blank plasma samples were spiked with imipenem at concentrations of 1, 15, 30, 60, and 90 µg/mL and with sulbactam at concentrations of 5, 30, 60, 90, and 120 µg/mL. Five samples of each concentration level were measured. The accuracy of the method was determined by comparing the means of the measured concentrations in spiked plasma with the corresponding nominal concentrations. The precision at each concentration level was expressed as the coefficient of variation (%CV) of those measurements. To study the intraday accuracy and precision, the described spiked samples were prepared and consecutively measured. This procedure was repeated on three different days to test the interday accuracy and precision.

Recovery, calibration curves, and limits of detection and quantitation

The recovery values of the method were calculated by comparing the peak areas from extracted spiked plasma with that of standard solutions at the same concentration level.

The calibration line was constructed by plotting the peak area against the concentration. Linearity was assessed by linear regression analysis. Limits of detection (LOD) were calculated as the concentrations of analyte that generated a signal three times higher than the baseline noise. Limits of quantitation (LOQ) were calculated as the concentrations that generated a signal 10 times higher than the baseline noise.

Drug stability

Drug stability, before and after plasma treatment, was tested by comparing concentration values obtained along the study with those obtained at the beginning of the run. Drug-free plasma samples were spiked in triplicate at concentration levels of 1 and 60 µg/mL for imipenem and 5 and 90 µg/mL for sulbactam, and they were diluted 1:1 (v/v) in stabilizing solution. To study the stability of the drugs before plasma treatment, samples were stored at –20°C and analyzed 0, 2, 4, and 6 days after being spiked. To study the postpreparative stability, reconstituted extracts were stored at room temperature and analyzed 0, 2, 4, and 6 h after sample treatment.

Results and Discussion

Chromatography

Under the chromatographic conditions previously described, retention times of imipenem and sulbactam were 3.46 ± 0.02 and 9.53 ± 0.02 min, respectively. No interferences from endogenous plasma components or reagents were found. Peak purity of the analytes was corroborated by comparing the UV spectra of imipenem and sulbactam peaks in plasma samples with the spectra of the drugs in the working solution standards. The UV spectra were compared at the peak upslope, apex, and downslope. Figure 2 shows chromatograms obtained from blank plasma (A), plasma spiked with 10 µg/mL of both imipenem and sulbactam (B), and a plasma sample obtained at 60 min after drug administration of a weight-adjusted dose of 120 mg/kg of imipenem and 240 mg/kg of sulbactam (C).

Method validation

The intra- and interday accuracy and precision and recovery data are summarized in Table I. The average intra- and interday precision were 2.88% and 3.78% for imipenem and 1.94% and 2.68% for sulbactam, respectively. The mean percents of recovery were 94.5% and 95.2% for imipenem and sulbactam,

Table I. Accuracy, Precision, and Recovery of Imipenem and Sulbactum in Plasma (*n* = 5)

Spiked concentration (µg/mL)	Intraday		Interday		
	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)	Recovery (%)
Imipenem					
1	92.0	6.5	95.0	7.4	93.6
15	98.2	3.4	97.0	4.3	92.1
30	98.9	2.2	97.7	3.6	95.4
60	99.3	1.4	98.6	2.1	95.0
90	100.3	0.9	100.4	1.5	96.3
Sulbactam					
5	101.8	2.8	101.4	3.4	89.5
30	97.5	1.8	98.1	2.2	93.2
60	98.8	2.5	99.7	2.4	96.2
90	101.3	1.3	98.3	1.9	96.5
120	99.8	1.3	99.2	3.5	95.4

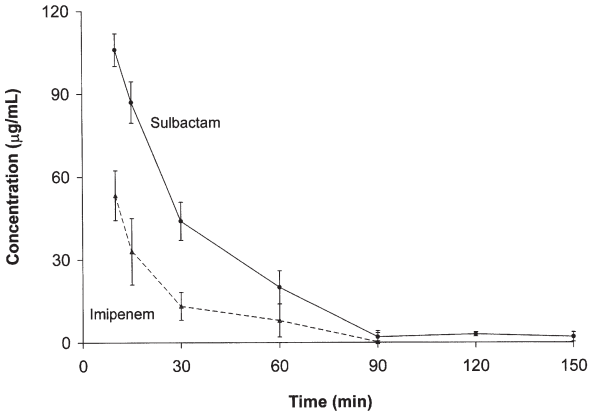

Figure 3. Plasma concentration-time profile of imipenem and sulbactam after drug administration.

respectively.

The calibration curves were linear over the concentration range studied, with correlation coefficients of 0.9993 for imipenem and 0.998 for sulbactam. LODs were 0.02 µg/mL for imipenem and 0.5 µg/mL for sulbactam. LOQs were 0.05 µg/mL for imipenem and 1.0 µg/mL for sulbactam.

Sulbactam remains stable in plasma before and after treatment. Concentrations of sulbactam found before and after treatment were in the range of 95.2–98.0% of the initial concentrations. Imipenem is not as stable as sulbactam in plasma samples, especially after sample treatment. Concentrations of imipenem before treatment were in the range from 93.2% to 96.9% of the initial concentrations. The concentration of imipenem decreased from 95.2–97.3%, observed during the first 4 h after treatment, and decreased further to 89% at 6 h after treatment.

Application of the method

The method was applied to the simultaneous determination of imipenem and sulbactam in plasma from C57BL/6 mice, weighing between 16–20 g (BK Universal Ltd., Barcelona, Spain) after an individualized intramuscular weight-adjusted dose of 120 mg/kg of imipenem and 240 mg/kg of sulbactam. Blood samples were collected at 10, 15, 30, 60, 90, 120, and 150 min after drug administration. After collection, plasma was separated from blood by centrifugation at 1000 × *g* for 10 min and diluted 1:1 (v/v) in the stabilizing solution. Then the treatment with acetonitrile, described in the plasma sample treatment section was applied. Three animals were used for each time point. A similar rapid elimination of both compounds was observed after drug administration (Figure 3).

Conclusion

The described method allows a simple, accurate, and precise determination of imipenem and sulbactam in mouse plasma. This method is, to our knowledge, the first analytical method for their simultaneous determination. Analysis are carried out on widely available equipment (HPLC with diode-array detection), so the method can be used in most clinical laboratories. The method was found to be suitable for pharmacokinetic studies.

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