Simultaneous determination of different classes of β-lactam antibiotics in human plasma

Using the SCIEX QTRAP 4500 system

S. Uhlen[1], C. Marzullo[1], A. Morla[2]
[1]Hospital of Mulhouse, Mulhouse, France; [2]SCIEX, France

β-Lactam antibiotics are among the most widely used class of drugs for the treatment of bacterial infections in humans. Most β-lactam antibiotics work by inhibiting cell wall biosynthesis in the bacterial organism. Bacteria often develop resistance to these antibiotics by synthesizing a β-lactamase, an enzyme that attacks the β-lactam ring common to this class of antibiotic. To overcome this resistance, β-lactam antibiotics can be given with β-lactamase inhibitors such as clavulanic acid. The antibacterial characteristics the drugs display are dependent on both the concentration of drug in relation to the minimum inhibitory concentration (MIC) and the time that this exposure is maintained. Therefore, monitoring their concentrations in plasma is of high importance.[1]

The commonality of the structures of the β-lactam class of antibiotics can be seen in Figure 1. This class includes among others, penicillin derivatives (penams), cephalosporins (cephems) and penems.

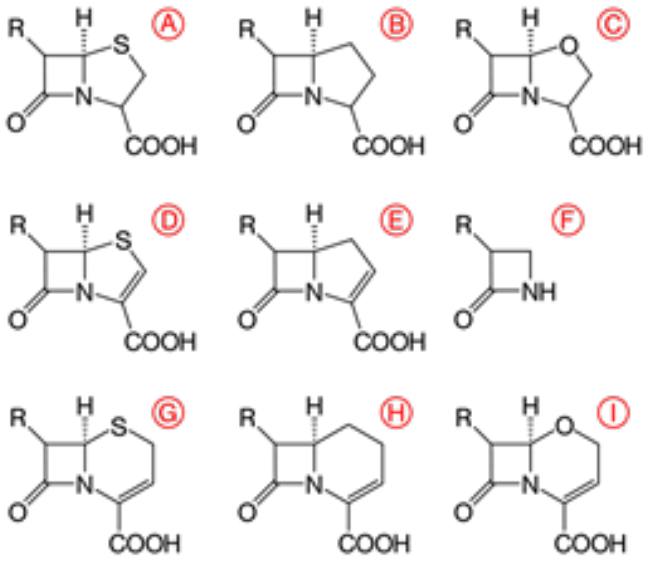

Figure 1. The β-lactam core structures. (A) A penam, (B) a carbapenam, (C) an oxapenam, (D) a penem, (E) a carbapenem, (F) a monobactam, (G) a cephem, (H) a carbacephem, and (I) an oxacephem are shown.

In this technical note, a fast LC-MS/MS method with a simple sample preparation on the QTRAP 4500 system is described for the quantitative analysis of nine β-lactams antibiotics, amoxicillin (AMO), cloxacillin (CLO), piperacillin (PIP), cephazolin (CEP), cefotaxime (CEFO), ceftazidime (CEFT) and cefepime (CEFE), imipenem (IMI) and meropenem (MER).

Key features of the QTRAP 4500 system for antibiotic quantification

- Protein precipitation for fast, simple sample preparation
- Fast analysis using the QTRAP 4500 system
- Robust sample introduction using the Turbo V ion source with Curtain Gas interface for efficient ionization and system robustness
- QJet ion guide for efficient ion capture and focusing from instrument orifice
- Curved LINAC collision cell permits greatly reduced pause and dwell times without a loss in sensitivity, allowing multi-target analyses
- Efficient data processing using SCIEX OS software

Methods

Sample preparation: 100 µL of each sample, calibrator or QC was spiked with 10 µL of internal standard mix at 100 µg/mL. Protein precipitation was carried out by the addition of 200 µL of methanol containing 0.1% formic acid. Following vortex mixing, the samples were centrifuged for 10 minutes before the supernatant was transferred to autosampler vials for injection.

Liquid chromatography: Chromatographic separation was accomplished using a Phenomenex Kinetex Biphenyl column (100 x 2.1 mm, 2.6 µm). Water with 0.1% formic acid (A) and methanol with 0.1% formic acid (B) were used as mobile phase solvents. 1 µL of prepared samples was injected into the UHPLC system. A fast gradient elution method was employed with a total run time of 8 minutes. An example chromatogram is shown in Figure 2.

Mass spectrometry: MS/MS detection was performed using the SCIEX QTRAP 4500 system equipped with Turbo V ion source using electrospray ionization, operating in positive mode. Multiple reaction monitoring (MRM) mode was employed, using two specific transitions of each analyte. The primary transition was used for quantification while the secondary transition was used for confirmation through the generation of ion ratios. Dwell and cycle times were optimized using the Scheduled MRM algorithm. Table 1 shows the summarized results of optimized MRM transitions and parameters of the nine β-Lactam antibiotics and their respective isotope-labeled internal standards. For imipenem, meropenem-d6 was used as the internal standard

Data processing: Data were acquired using Analyst software and processed using SCIEX OS software.

Results

Figure 3 shows examples of chromatograms obtained from the lowest calibrator sample. As can be seen, the polar compound imipenem eluted early on the chromatography used, and the resolved split peak is due to a mixture of two rotational Z and E formamidinium isomers in equilibrium.[2]

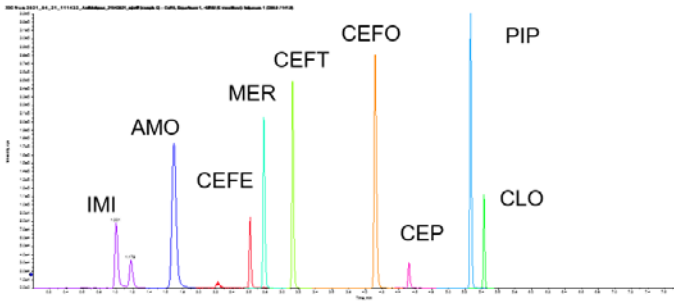

Figure 2. Chromatogram showing the elution profile of all analytes.

Table 1. Summary of MRM parameters.

Compound ID	RT (min)	Precursor Ion (m/z)	Quantifier Ion (m/z)	Qualifier Ion (m/z)
IMI	1.1	299.9	141.9	97.9
*AMO**	1.7	367.1	114.9	209.2
AMO-d4	1.7	370.1	113.9	-
*CEFE**	2.6	482.1	397.0	126.0
CEFE-d3	2.6	484.1	125.0	-
MER	2.8	384.0	141.1	114.1
MER-d6	2.8	390.0	147.1	-
*CEFT**	3.1	547.9	469.1	168.0
CEFT-d5	3.1	551.9	167.0	-
*CEFO***	4.2	457.9	126.8	325.9
*CEFO-d3**	4.2	459.9	125.8	-
*CEP**	4.6	455.9	324.0	156.9
`CEP-13C2_15N	4.6	457.9	326.0	-
*PIP**	5.2	519.0	143.9	115.9
PIP-d5	5.2	523.0	147.9	-
*CLO**	5.4	437.1	278.1	161.1
CLO-13C4	5.4	440.1	281.1	-

Calibration curves over the concentration ranges between 1 and 150 µg/mL for all analytes except imipenem (0.05 to 7.5 µg/mL) and meropenem (0.1 to 15 µg/mL) are reported in Figure 4. Curves were generated using a 1/x weighted linear regression of the peak-area ratios of the antibiotic to corresponding internal standard. Regression coefficients (r^2) of all calibration curves were greater than 0.99 with back-calculated concentrations of the calibration samples within ±15% (±20% at LLOQ) of the

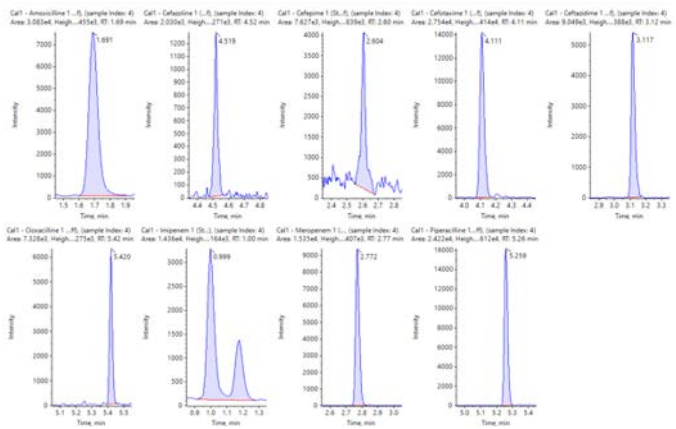

Figure 3. Examples of chromatograms obtained for the lowest calibrator (1, 0.1 or 0.05 µg/mL depending the analyte).

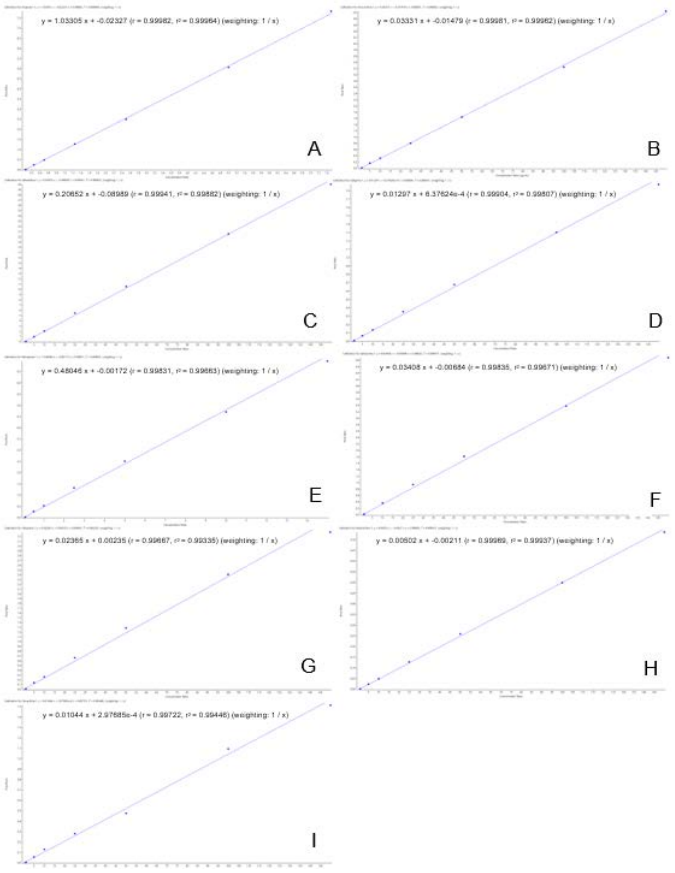

Figure 4. Calibration curves for the nine β-lactam antibiotics. Imipenem (A), amoxicilline (B), ceftazidime (C), cefepime (D), meropenem (E), cefotaxime (F), cefazoline (G), piperacilline (H) and cloxacilline (I)

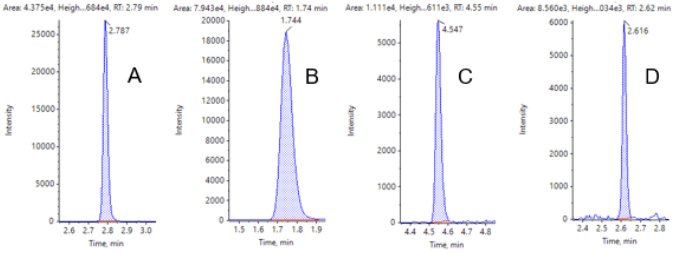

Figure 5. Plasma samples analyzed using method. (A) Meropenem at 0.185 µg/mL, (B) amoxicillin at 1.563 µg/mL, (C) cephazolin at 2.418 µg/mL, and (D) cefepime at 0.906 µg/mL are shown.

nominal concentrations. The recorded accuracy and precision were within EMA guidelines (European Medicines Agency, Science Medicines Health, 2011).

The application of this multiplexed targeted assay for nine β-lactams antibiotics has been demonstrated using plasma samples from the Hospital of Mulhouse Antibiotic Therapeutic Drug Monitoring (TDM) program previously analyzed for these antibiotics by alternative methods.[1] Examples of chromatograms at concentrations recorded are shown in Figure 5.

Conclusions

Presented here are the results from the development of a simple, sensitive and robust assay utilizing the SCIEX QTRAP 4500 system coupled with fast UHPLC chromatography for the analysis of nine β-lactam antibiotics in human plasma. The method demonstrated the following advantages:

- Simple protein precipitation sample preparation
- High sensitivity, specificity and linearity
- Short, 8 minute analytical run time

References

1. Guilhaumou R et al., (2019) Optimization of the treatment with beta-lactam antibiotics in critically ill patients—guidelines from the French Society of Pharmacology and Therapeutics (Société Française de Pharmacologie et Thérapeutique—SFPT) and the French Society of Anaesthesia and Intensive Care Medicine (Société Française d'Anesthésie et Réanimation—SFAR), *Critical Care* **23** 104.
2. Ratcliffe R et al. (1989) Studies on the Structures of Imipenem, Dehydropeptidase I, Hydrolyzed Imipenem, and Related Analogues, *J Org Chem* **54** 653-660.

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
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