Bioanalysis of β-Lactamase Inhibitors on the SCIEX QTRAP® 6500+ System

SCIEX QTRAP® 6500+ System

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Introduction

Antibiotic resistance is an increasing threat to public health, yet since the 1940s antimicrobial agents are a class of pharmaceutical compounds that have had one of the largest positive impacts on human health by reducing illness and death from infectious disease. Due to overuse and misuse, infectious bacteria have adapted and evolved resistance mechanisms that make these drugs less effective.[1] Each year in the US at least 23 000 people die each year as a direct result of infection from bacteria resistant to antibiotics.[1]

One approach to combat antibiotic resistant infections is combination therapy where an antibiotic is administered along with a compound that targets and inhibits a known resistance mechanism. An example is the combination of a β-lactam antibacterial and a β-lactamase inhibitor that protects against enzymatic hydrolysis of the antibiotic compound and restores the activity of the β-lactam in resistant bacteria.

In 2015 the US FDA approved the antibacterial drug Avycaz which is a combination therapy indicated to treat complicated intra-abdominal and urinary tract infections by Gram-negative bacteria. Avycaz is a combination of ceftazidime (a third generation cephalosporin) and the novel compound avibactam which is a β-lactamase inhibitor that increases the susceptibility of Gram-negative bacteria producing extended spectrum β-lactamases and cephalosporinases. While avibactam is structurally distinct from clinically used β-lactamase inhibitors such as sulbactam and tazobactam (Figure 2) in that it does not contain a β-lactam core, it does contain an acidic group which is common among the clinically used β-lactamase inhibitors and means that the bioanalysis of these molecules requires operating the mass spectrometer in negative ion mode. A recent paper[2] on the bioanalysis of ceftazidime and avibactam used a multi-period experiment with the mass spectrometer operating in positive MRM mode for ceftazidime detection (546.9/468.0) and negative MRM mode for avibactam (263.9/95.9) detection. There are significant differences in efficacious dose of the two compounds, the dose of avibactam being 4 fold lower than ceftazidime which necessitated a different quantitative range for each analyte, 0.05-50 µg/mL for ceftazidime and 5 – 5000 ng/mL for avibactam.

The SCIEX QTRAP® 6500+ LC/MS/MS system

Figure 1. The SCIEX QTRAP® 6500+ System featuring the IonDrive™ Turbo V Ion source, QJet® ion guide, HED+ detector and SelexION®+ *differential mobility separation technology* delivers ultra-sensitive detection for bioanalytical quantitation.

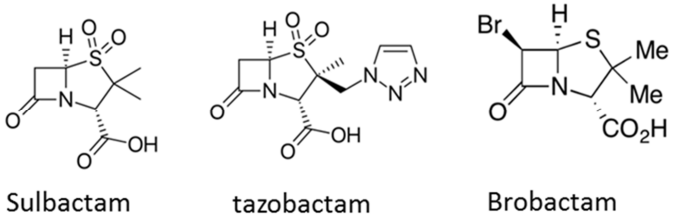

Figure 2. The chemical structure of the **β**-lactamase inhibitors sulbactam, tazobactam and brobactam.

SCIEX provides robust and reliable industry performance in high sensitivity bioanalytical quantitation with the SCIEX 6500 System which includes the IonDrive™ Turbo V Ion source and QJet® ion guide. The SCIEX 6500+ System contains several technologies which expand on the proven performance of the SCIEX 6500 system, one of these; the HED+ detector enhances performance in negative ion

mode. This application note featuring bioanalytical methods for early generation β-lactamase inhibitors sulbactam, tazobactam and brobactam (Figure 2) highlights the bioanalytical performance of the SCIEX 6500+ System in negative ion mode.

Materials and Methods

Sulbactam, tazobactam and brobactam were obtained commercially from Toronto Research Chemicals and Sigma-Aldrich. Rat plasma was purchased from Innovative Research, Inc.

Mass Spectrometry Conditions
A SCIEX QTRAP® 6500+ LC-MS/MS system equipped with the IonDrive™ Turbo V source and the newly designed IonDrive High Energy Detector+ was used in negative Electrospray Ionization (ESI) mode. The optimized MRM conditions for the analytes are summarized in Table 1.

Table 1. MRM transitions for β-lactamase inhibitors analysis

Name	Q1	Q3	DP	CE
Brobactam	277.9	137	-20	-22
	277.9	79	-20	-15
	279.9	139	-20	-22
	279.9	81	-20	-15
Sulbactam	232.0	64	-15	-51
	232.0	43	-15	-40
Tazobactam	299.0	138	-15	-19
	299.0	68	-15	-50

Sample Preparation
Plasma calibration samples were prepared by serial dilution in plasma. Samples were processed as following the steps in Table 2.

Table 2. Sample Preparation

Step 1	A protein precipitation solution (250 µL) of 5:95 100mM ammonium formate pH 9.0: acetonitrile was added to 50 µL of plasma sample and vortexed.
Step 2	Samples were centrifuged to collect the precipitate and the supernatant (225 µL) was then removed to a fresh set of tubes and dried to completeness under N_2.
Step 3	The dried supernatant was reconstituted in 100 uL of water for analysis.

Gradient Conditions
A Shimadzu Prominence HPLC system was used with a gradient elution. The total run time for the method was 3.5 minutes and an injection volume of 1µL was used for all samples.

Table 3. Gradient profile for MRM Quantitation

Column	Phenomenex Kinetex® Biphenyl (50 × 3.0mm, 5µm)	
Mobile Phase A	5mM ammonium acetate in water	
Mobile Phase B	5mM ammonium acetate in methanol	
Flow rate	1.0 mL/min	
Column temperature	45 °C	
Injection volume	1 µL	
Gradient profile	**Time (min)**	**% B**
	0	5
	0.5	5
	1.0	95
	2.0	95
	2.1	5
	3.5	5

Results and Discussion

The method described above was used to analyze a series of rat plasma calibration samples containing concentrations of the β-lactamase inhibitors ranging from 0.3 to 3000 ng/mL. A representative chromatogram for a sample containing 0.3 ng/mL of sulbactam in rat plasma compared to blank plasma is shown in Figure 3. Similar chromatography was obtained for brobactam and tazobactam extracted from rat plasma. The method displayed excellent linearity over the concentration range of 0.3 to 1000 ng/mL (Figure 4).

The statistics of triplicate analysis of the plasma calibration standards are summarized in Table 4. The accuracies range from 88 – 106% over the entire range of Sulbactam in plasma, and the CV% ranges from 0.9 to 11%. Similar results were obtained for the three β-lactamase inhibitors (8 MRMs total) with a summary presented in Table 5.

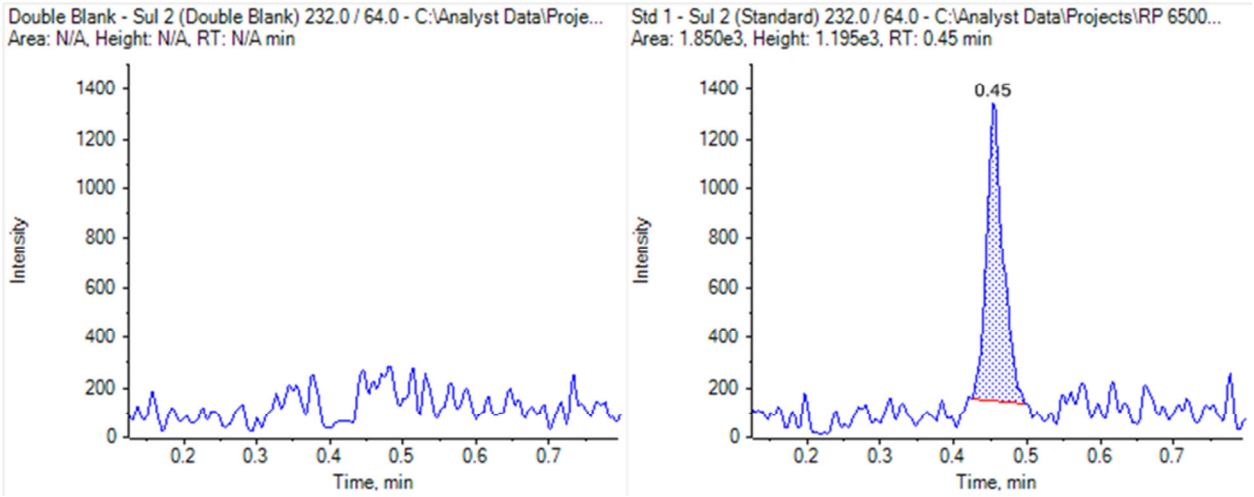

Figure 3. The LC/MS/MS method described herein enabled quantification of beta-lactams in rat plasma as low as 0.3 ng/mL. Blank rat plasma (left) and 0.3 ng/mL Sulbactam in rat plasma (right) are displayed.

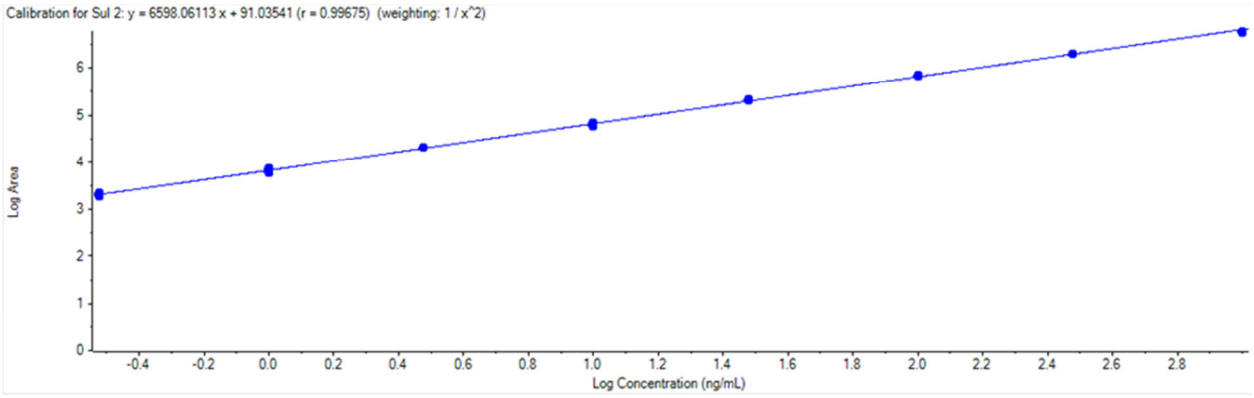

Figure 4. Calibration Curve for Sulbactam in rat plasma, from 0.3 to 1000 ng/mL shown as a log-log plot. The method displayed excellent linearity over the concentration range, with r = 0.99675.

The new HED+ detector in the SCIEX QTRAP 6500+ System offers an improvement in signal intensity for this particular application in negative mode operation. The HED+ detector operates at an increased voltage to attract more negative ions and features a larger surface area to detect more ions. At the lowest calibration level of this application (0.3 ng/mL) the amount of sulbactam and tazobactam from extracted plasma and loaded on the LC-MS/MS system was 0.3 pg.

Conclusions

A sensitive and accurate LC-MS/MS method was developed for a series of β-lactamase inhibitors extracted from rat plasma by protein precipitation. Linearity within a concentration range of 0.30 to 1000 ng/mL was achieved for sulbactam and tazobactam and within 1.0 to 1000 ng/mL for brobactam.

The SCIEX QTRAP 6500+ System builds on the robust and reliable industry performance in high sensitivity bioanalytical quantitation of the SCIEX 6500 System. In addition to the IonDrive™ Turbo V Ion source and QJet® ion guide, the SCIEX QTRAP 6500+ System contains several technology enhancements including: the HED+ detector, Jet Injector SelexION®+, fast polarity switching speeds of 5 msec plus a reduction in audible noise. The technology improvements in the HED+ plus detector in particular enabled sensitive bioanalytical quantitation of three early generation β-lactamase inhibitors in negative mode and will enable analysts to develop sensitive methods for other compounds within this class.

References

1. https://www.cdc.gov/drugresistance/
2. ME Beaudoin, ET Gangl - Bioanalytical method validation for the simultaneous determination of ceftazidime and avibactam in rat plasma. Bioanalysis (2016), 8(2), 111-122.

Table 4. Statistics for the triplicate analysis of sulbactam in rat plasma using the SCIEX QTRAP® 6500+ system.

Actual Concentration	Calculated Concentration (ng/mL)	Accuracy (%)	CV (%)
0.3 ng/mL Sulbactam	0.299	100	9.5
1 ng/mL Sulbactam	1.01	101	10.6
3 ng/mL Sulbactam	3.09	103	1.1
10 ng/mL Sulbactam	9.57	96	6.7
30 ng/mL Sulbactam	31.9	106	0.9
100 ng/mL Sulbactam	105	105	1.5
300 ng/mL Sulbactam	304	101	0.9
1000 ng/mL Sulbactam	884	88	2.2

Table 5. Summary of the linearity and the quantitative ranges obtained for the 8 MRMs analyzed for β-lactamase inhibitors in rat plasma using the SCIEX QTRAP® 6500+ system.

Beta-lactam	MRM	LLOQ (ng/mL)	ULOQ (ng/mL)	r
Brobactam	277.9 / 137.0	1	3000	0.9926
	277.9 / 79.0	1	1000	0.9981
	279.9 / 139.0	1	3000	0.9933
	279.9 / 81.0	1	3000	0.9938
Sulbactam	232.0 / 64.0	0.3	1000	0.9968
	232.0 / 43.0	1	1000	0.9959
Tazobactam	299.0 / 138.0	0.3	300	0.9967
	299.0 / 68.0	0.3	300	0.9966

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