High-Throughput Validated Method for the Quantification of Beta-Lactams: Piperacillin/Tazobactam, Cefepime, and Meropenem in Plasma using LC-MS/MS

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Abstract

Introduction: Antibiotics, specifically beta-lactams, remain one of the most important classes of medications due to their high effectiveness, ease of delivery, and minimal side effects. However, one of the greatest concerns with indiscriminant antibiotic use is the development of resistant bacteria, which contribute to 2,000,000 illnesses and 23,000 deaths in the US annually. Coupled with a limited antibiotic development pipeline where new patents have fallen 35% in the last decade and no novel gram-negative agents have reached the market in 45-years, antibiotic resistance is a public health crisis for which innovative immediate solutions are needed to slow progression and preserve existing drugs.

Anti-pseudomonal beta-lactams are the backbone of treatment regimens in the intensive care unit (ICU) due to their favorable spectrums, effectiveness, and safety profiles. These drugs exhibit time-dependent bacterial killing. Yet, even when the drug level is sufficient to treat an infection, it may fall below the "mutant prevention concentration." Selective eradication of susceptible pathogens can leave resistant mutants behind that become dominant. These resistant bacteria can be transferred between patients and lead to difficult to treat infections that require the use of more toxic and less-effective therapies. Achievement of sufficiently high beta-lactam drug levels throughout the dosing interval improves patient outcomes and limits the development of resistance.

Individualized dosing and drug level monitoring has been well established for narrow therapeutic window antibiotics like vancomycin and aminoglycosides. However, use of this approach as an antibiotic stewardship tool for wide therapeutic window drugs like beta-lactams has not yet been broadly adopted. Validated methods for accessing beta-lactams levels in plasma are currently limited. Therefore, we present a method for the quantification of piperacillin/tazobactam, cefepime, and meropenem in plasma using LC-MS/MS.

Methods: A protein crash/dilute-and-shoot method was developed in which plasma samples (50 mcL) was crashed with acetonitrile containing internal standard. Following centrifugation, an aliquot of the supernatant was separated from the precipitated pellet and diluted with clinical laboratory reagent water (CLRW). The resulting diluted sample was analyzed by LC-MS/MS with chromatographic separation achieved using Agilent Select Stream LC system with compounds eluting off the column using a linear gradient with a flow rate of 0.3 mL/min and total run time of 9 minutes. The ability to multiplex samples using the Select Stream allows each sample to be completed in 3 minutes. Eluting compounds were detected using an Agilent 6495 Mass Spectrometer operated in positive (ESI) mode and dynamic multiple reaction monitoring mode. Each analyte of interest was identified by retention time, and Q1/Q3 m/z ion pair ratios using Agilent MassHunter software.

Results: The analytical linear range was 0.5-60 mcg/mL for each analyte. The limit of detection was less than 10% of the LLOQ for each analyte. The average (n=5) linear regression of each analyte demonstrated the following: slope= 1.0±0.1 ; r^2= ≥ 0.990; and y-intercept 95% confidence interval that includes zero. Intra- and inter-assay precision CVs were less than 5% across the analytical range for each analyte. Absence of interference from the top 25 prescribed drugs, concomitant drugs, or common drugs of abuse was observed. Values were unaffected in samples that were grossly lipemic, icteric, or hemolyzed. Minimal ion suppression or enhancement due to the matrix effect was observed. No significant carryover was seen following a sample containing 500 mcg/mL of each analyte. Beta-lactams are well known for their instability in plasma samples due to non-enzymatic hydrolyzation of its -lactam ring. This is consistent with our own observations, values for each analyte decreased significantly at ambient, refrigerate (2-8˚C), and -30˚C temperatures. On the other hand, when stored at −80˚C, no significant trend to lower concentrations was observed in the samples over the course of 35 days. A minimum of 40 patient or blind samples were cross validated with an existing assay or compared to an known spiked value. The results for each analyte were compared using linear regression and demonstrated the following: slope= 1.0±0.1; r^2= ≥ 0.980; and y-intercept 95% confidence interval that included zero.

Conclusion: The method described here provides healthcare professionals a precise and high-throughput LC-MS/MS method to quantitate piperacillin/tazobactam, cefepime, and meropenem

Hardware

Agilent Select Stream LC equipped with Agilent Infinity LC-1290 pumps, column heater, and LC injector
Agilent 6495 Tandem Mass Spectrometer

Mass Spectrometer Parameters

	Precursor Ion	Product Ion 1	Product Ion 2	Product Ion 3
Tazobactam	301.1	168.1	122.0	94.0
Cefepime	481.1	167.1	396.0	86.1
Meropenem	384.2	254.0	68.1	141.1
Piperacillin	518.2	160.1	115.0	143.0
Tazobactam-$^{15}N_3$	304.1	168.0	208.0	-------
Cefepime-d3	484.2	166.9	89.0	-------
Meropenem-d6	390.2	260.0	147.1	-------
Piperacillin-d5	523.2	160.0	148.2	-------

Source Parameters	
Gas Temperature	180 Celsius
Gas Flow	19 L/min
Nebulizer	30 psi
Sheath Gas Temperature	350 Celsius
Sheath Gas Flow	11 L/min
Capillary Voltage	3000 Volts
Nozzle Voltage Voltage	500 Volts

Table 1. Values obtained using an Agilent 6495 Tandem Mass Spectrometer with Jet Stream source

Imprecision

Tazobactam

Intra-day n=20	LLOQ	Level I	Level II	Level III
Mean	0.42	2.25	22.54	37.31
SD	0.01	0.09	0.19	0.49
% CV	2.8	4.0	0.9	1.3

Inter-day n=20	Level I	Level II	Level III
Mean	2.06	21.00	38.53
SD	0.04	0.40	0.62
% CV	1.9	1.9	1.6

Cefepime

Intra-day n=20	LLOQ	Level I	Level II	Level III
Mean	0.47	2.45	24.09	43.06
SD	0.02	0.12	0.73	0.97
% CV	4.0	4.7	3.0	2.2

Inter-day n=20	Level I	Level II	Level III
Mean	2.08	21.32	38.50
SD	0.04	0.40	0.99
% CV	2.0	1.9	2.6

Meropenem

Intra-day n=20	LLOQ	Level I	Level II	Level III
Mean	0.45	2.33	23.08	37.86
SD	0.01	0.10	0.45	0.73
% CV	2.9	4.4	2.0	1.9

Inter-day n=20	Level I	Level II	Level III
Mean	2.29	23.82	43.90
SD	0.06	0.63	1.05
% CV	2.8	2.6	2.4

Piperacillin

Intra-day n=20	LLOQ	Level I	Level II	Level III
Mean	0.43	2.31	22.81	39.66
SD	0.01	0.11	0.46	1.32
% CV	3.2	4.9	2.0	3.3

Inter-day n=20	Level I	Level II	Level III
Mean	2.05	20.92	39.77
SD	0.07	0.55	1.80
% CV	3.6	2.6	4.5

Table 2. Intra-day and Inter-day precision was run over multiple days and unique calibrations

Linearity

Tazobactam	y = 1.0027x + 0.0672	R^2 = 0.9997
Cefepime	y = 0.9967x + 0.2049	R^2 = 0.9976
Meropenem	y = 0.9810x + 0.4378	R^2 = 0.9976
Piperacillin	y = 1.0237x – 0.1873	R^2 = 0.9957

Table 3. 5 spiked samples spanning the linear range were replicated over 5 days. The highest and lowest concentrations were within 10% of the ULOQ and LLOQ.

Limit of Detection (mcg/mL)

Tazobactam	0.02
Cefepime	0.01
Meropenem	0.02
Piperacillin	0.04

Table 4. The limit of detection was defined as the lowest concentration tested that has a peak area that is greater than or equal to the average of 20 blanks + 3 SD.

Chromatography

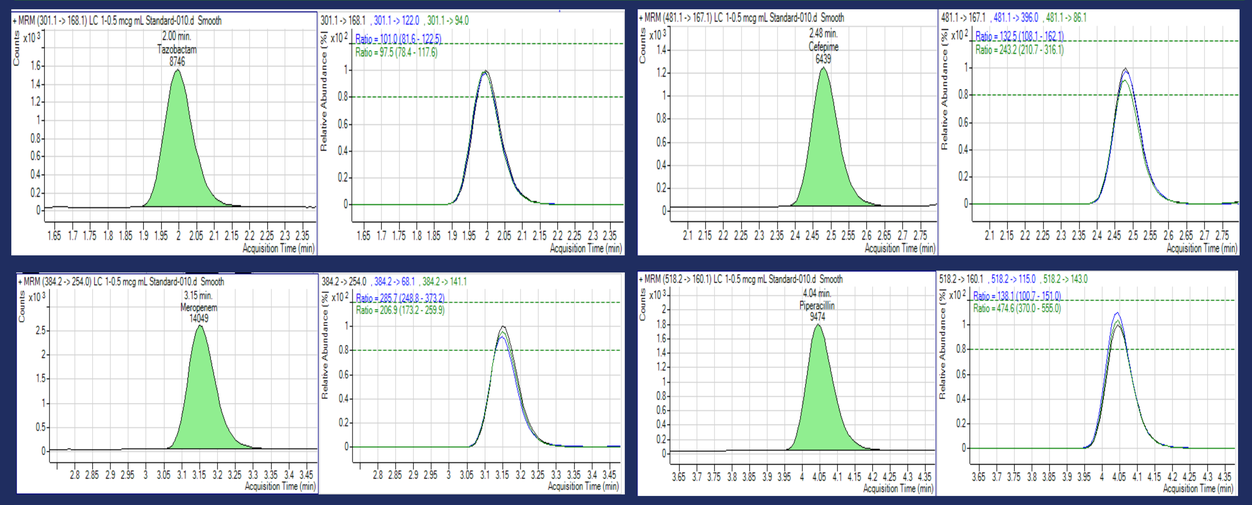

Figure 1. Chromatogram of each analyte, including qualifier transitions, at 0.5 mcg/mL (LLOQ) using MassHunter software.

Accuracy

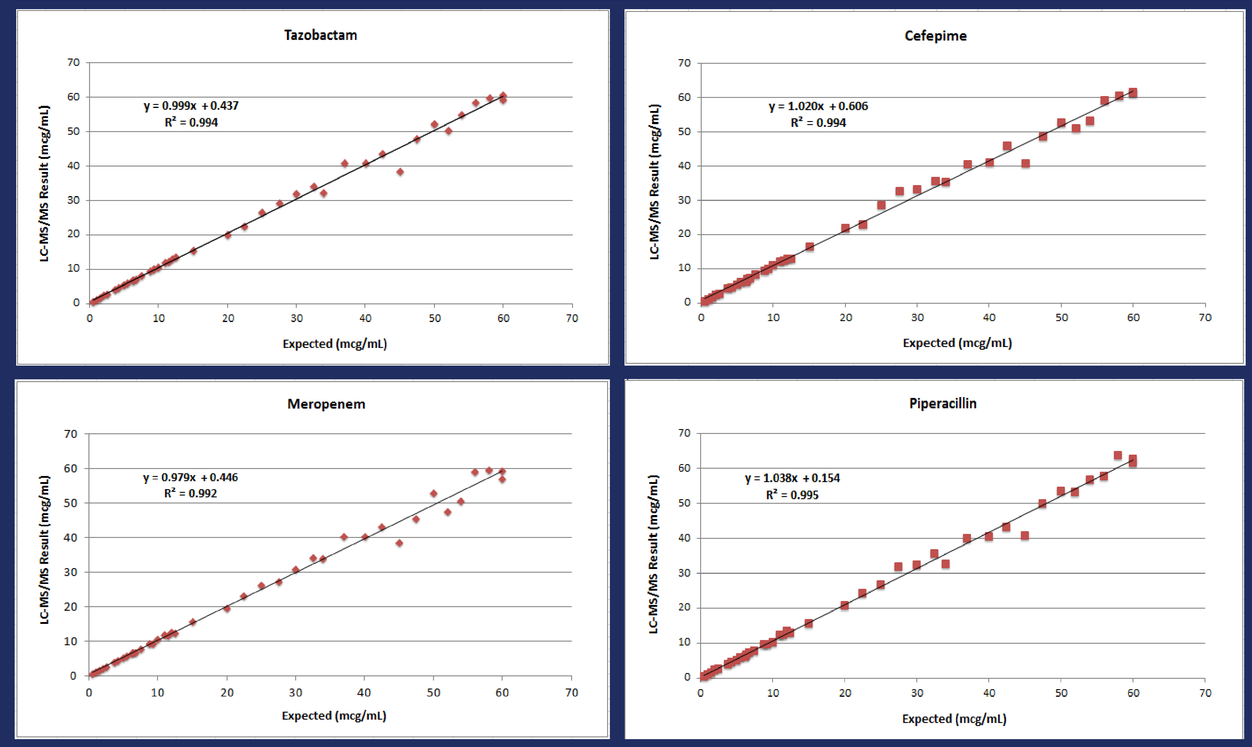

Figure 2. A comparison of 40 blind spiked samples extracted and compared to expected value. A linear regression 1/x was used

Conclusions

The method described here provides healthcare professionals a precise and high throughput LC-MS/MS method to quantitate piperacillin/tazobactam, cefepime, and meropenem in plasma.

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