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Analytical evaluation for the quantitation of fifteen antibiotics in plasma by liquid chromatography coupled to high-resolution, accurate-mass measurements for clinical research

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Keywords

Antibiotics, betalactamines, quinolones, high resolution, accurate mass, Q Exactive Focus, Orbitrap

Application benefits

- Different classes of antibiotics in one method
- Short method based on full scan acquisition and quick method development
- Minimal sample preparation

Goal

Development and analytical evaluation of a method for the analysis of betalactam antibiotics in plasma using a high-resolution, accurate-mass Orbitrap mass spectrometer.

Introduction

The quantitation of antibiotics in plasma can be of interest for clinical research. The main challenge of antibiotics lies in the number of different compounds with different chemistries that need to be quantitated. This type of analysis has often been performed by high-performance liquid chromatography (HPLC) coupled to UV detection, but the approach sometimes requires long sample treatments, and in most cases only a few compounds are analyzed. The use of a high-resolution, accurate-mass (HRAM) instrument for this type of analysis is beneficial to increase the number of searched compounds in a single run while having good specificity for the analytes even with a simple sample preparation.

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A method based on the use of the Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap™ benchtop HRAM mass spectrometer was developed for the analysis of 15 antibiotics in plasma. Straightforward sample preparation based on protein precipitation followed by dilution was performed before analysis.

Experimental

Target analytes

A panel of 15 antibiotics was analyzed. The chemical structures of the compounds are presented in Figure 1. For quantitation purposes, seven internal standards were added: cefepime-D3, cefotaxime-D3, ciprofloxacin-D8, clindamycin-D3, meropenem-D6, levofloxacin-D8, and piperacillin-D5.

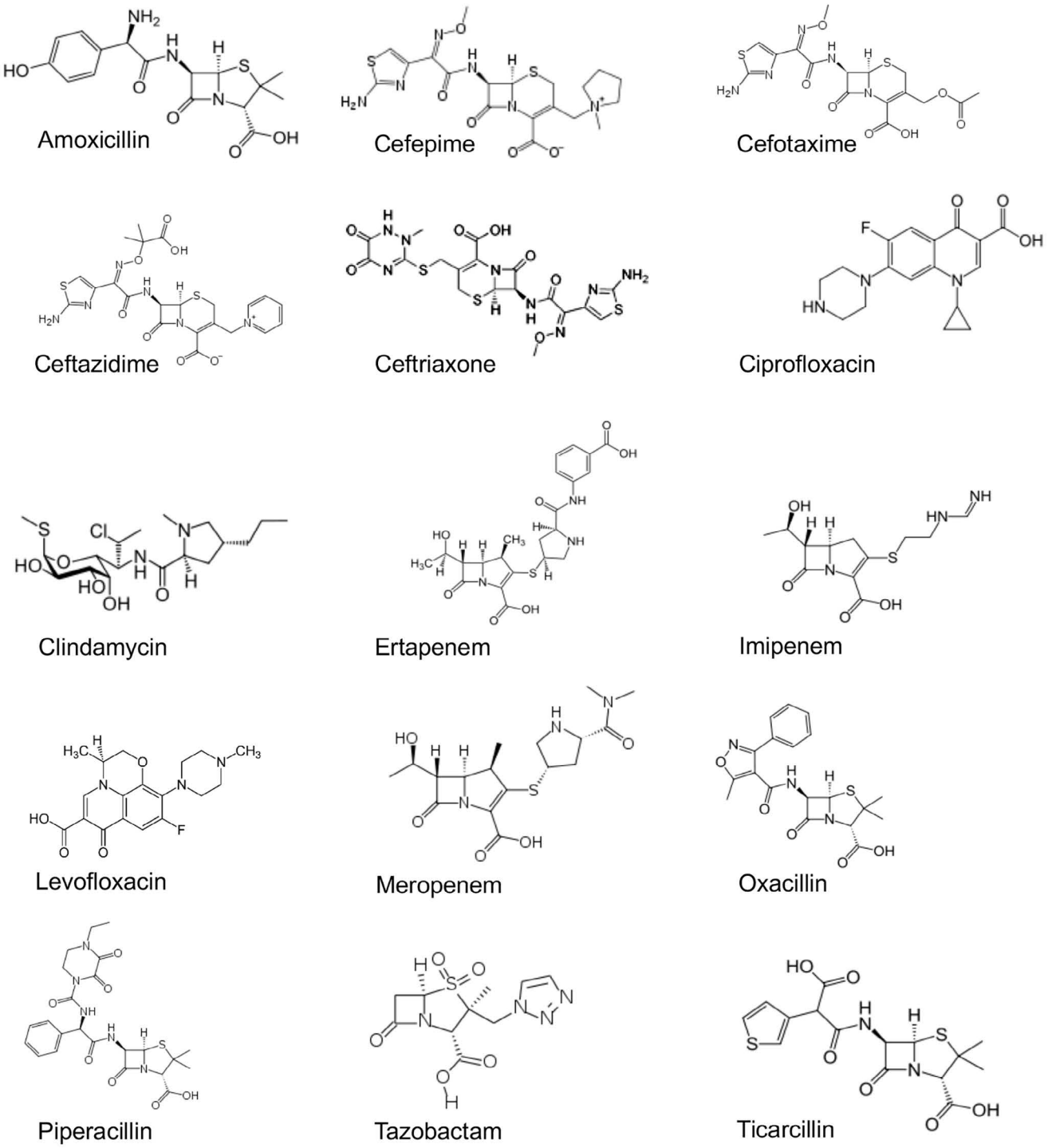

Figure 1. Chemical structures of the studied antibiotics

Calibration standards and control samples

Calibration solutions were prepared by spiking plasma (from Etablissement Français du Sang) with a mixture of standards in methanol/water 50/50 (v/v) solution. Seven calibration levels were used from 0.5 mg/L to 32 mg/L. A different set of standard solutions was used to prepare quality controls at four different levels.

Sample preparation

100 µL of plasma sample were mixed with 10 µL of an internal standard solution in methanol/water 50/50 v/v. After vortex mixing, 200 µL of methanol/formic acid 99.9/0.1 (v/v) were added to the sample. The mixture was vortex mixed for 15 s and centrifuged at 10,000×g for 10 min at 4 °C. The supernatant was diluted 1:4 using a mixture of water/formic acid (99.9/0.1; v/v) before injection (5 µL) for chromatographic analysis.

Liquid chromatography

A chromatographic method of nine minutes was used for the analysis of the antibiotics using a Thermo Scientific™ UltiMate™ 3000RS system consisting of an HPG pump, a column oven, and an autosampler. The separation was performed on a Thermo Scientific™ Accucore™ C18 100 × 2.1 mm (2.6 µm) column thermostatted at 40 °C. Mobile phases consisted of 2 mM ammonium formate with 0.1% of formic acid in water for phase A, and 0.1% of formic acid in acetonitrile for phase B. The mobile phase was set to 2% B for 1.5 minutes, increased linearly to 50% B in 3.5 minutes, then increased to 100% B in 1 minute. It remained at 100% for 1.5 minutes, while equilibration to 2% B lasted 1.5 minutes. The flow rate was set to 0.5 mL/min.

Mass spectrometry

Compounds were detected on a Q Exactive Focus benchtop quadrupole-Orbitrap mass spectrometer equipped with a Thermo Scientific™ Ion Max source and a HESI-II probe. Data were acquired in positive mode in full scan covering a mass range from *m/z* 120 to *m/z* 650 at a resolution of 35,000 (FWHM) at *m/z* 200. The chromatograms were then obtained by extracting the signal of the $[M+H]^+$ or the $[M+2H]^{2+}$ mass-to-charge ratio ions with a mass accuracy window of 5 ppm. The chemical formulas and accurate masses for the studied compounds are presented in Table 1.

Method evaluation

Linearity was evaluated by collecting calibration curve data on each of six days of analysis (n=6). The limit of detection (LOD) was determined as the lowest concentration that can be detected but not quantified, using a signal-to-noise ratio of 3. The lower limit of quantification (LOQ) was defined as the lowest concentration within the studied calibration range yielding intra- and inter-day precision and an accuracy less than 20% each. The calibration parameters as well as the LOD and LOQ are presented in Table 2.

Intra- and inter-assay accuracy and precision were obtained for the analysis of four quality control concentrations: LOQ (0.5 mg/L), low range (1.5 mg/L), middle range (4 mg/L) and upper concentration range (25 mg/L). Intra-assay precision and accuracy were obtained for the four control levels analyzed in replicates of n=6. Precision was calculated as percentage coefficient of variation (%CV) and accuracy as the percent bias from expected values. Inter-assay precision and accuracy were obtained in the same way but for the quality controls prepared and analyzed on six different days. The results of this study are presented in Table 3.

Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ 4.1 software. The parent mass was used for the quantitation with resulting chromatograms extracted and reconstructed with a mass accuracy of 5 ppm.

Table 1. Compound chemical formulas and accurate masses of the studied adducts

Molecule	Antibiotic Class	Chemical Formula	Adduct	Exact Mass (*m/z*)
Amoxicillin	Penicillins	$C_{16}H_{19}N_3O_5S$	$[M+H]^+$	366.11182
Cefepime	Cephalosporines	$C_{19}H_{24}N_6O_5S_2$	$[M+2H]^{2+}$	241.06976
Cefepime D3	Not applicable	$C_{19}H_{21}D_3N_6O_5S_2$	$[M+2H]^{2+}$	242.57918
Cefotaxime	Cephalosporines	$C_{16}H_{17}N_5O_7S_2$	$[M+H]^+$	456.06422
Cefotaxime D3	Not applicable	$C_{16}H_{14}D_3N_5O_7S_2$	$[M+H]^+$	460.09032
Ceftazidime	Cephalosporines	$C_{22}H_{22}N_6O_7S_2$	$[M+2H]^{2+}$	274.05685
Ceftazidime D5	Not applicable	$C_{22}H_{17}D_5N_6O_7S_2$	$[M+2H]^{2+}$	276.57254
Ceftriaxone	Cephalosporines	$C_{18}H_{18}N_8O_7S_3$	$[M+H]^+$	555.05333
Ciprofloxacin	Quinolones	$C_{17}H_{18}FN_3O_3$	$[M+H]^+$	332.14050
Ciprofloxacin D8	Not applicable	$C_{17}H_{10}D_8FN_3O_3$	$[M+H]^+$	340.19071
Clindamycin	Lincosamides	$C_{18}H_{33}ClN_2O_5S$	$[M+H]^+$	425.18715
Clindamycin D3	Not applicable	$C_{18}H_{30}D_3ClN_2O_5S$	$[M+H]^+$	428.20597
Ertapenem	Carbapenems	$C_{22}H_{25}N_3O_7S$	$[M+H]^+$	476.14860
Imipenem	Carbapenems	$C_{12}H_{17}N_3O_4S$	$[M+H]^+$	300.10125
Levofloxacin	Quinolones	$C_{18}H_{20}FN_3O_4$	$[M+H]^+$	362.15106
Levofloxacin D8	Not applicable	$C_{18}H_{12}D_8FN_3O_4$	$[M+H]^+$	370.20127
Meropenem	Carbapenems	$C_{17}H_{25}N_3O_5S$	$[M+H]^+$	384.15877
Meropenem D6	Not applicable	$C_{17}H_{19}D_6N_3O_5S$	$[M+H]^+$	390.19642
Oxacillin	Penicillins	$C_{19}H_{21}N_3O_5S$	$[M+H]^+$	402.11182
Piperacillin	Penicillins	$C_{23}H_{27}N_5O_7S$	$[M+H]^+$	518.17040
Piperacillin D5	Not applicable	$C_{23}H_{22}D_5N_5O_7S$	$[M+H]^+$	523.20177
Tazobactam	Others	$C_{10}H_{12}N_4O_5S$	$[M+H]^+$	301.06012
Ticarcillin	Penicillins	$C^{15}H_{16}N_2O_6S_2$	$[M+H]^+$	385.05225

Table 2. Calibration parameters, LOD, and LOQ for the target compounds

Molecule	Retention Time	Internal Standard	Slope	Intercept	LOQ (mg/L)	LOD (mg/L)
Amoxicillin	1.65	Cefepime D3	1.00 ± 0.12	-0.095 ± 0.090	0.5	0.06
Cefepime	2.15	Cefepime D3	0.98 ± 0.07	-0.135 ± 0.040	0.5	0.06
Cefotaxime	3.84	Cefotaxime D3	1.24 ± 0.09	-0.029 ± 0.004	0.5	0.06
Ceftazidime	3.42	Cefepime D3	0.56 ± 0.02	-0.077 ± 0.029	0.5	0.06
Ceftriaxone	3.69	Ciprofloxacin D8	0.23 ± 0.02	-0.004 ± 0.003	0.5	0.10
Ciprofloxacin	3.84	Ciprofloxacin D8	11.17 ± 1.06	-0.320 ± 0.600	0.5	0.02
Clindamycin	4.53	Clindamycin D3	4.85 ± 0.67	0.127 ± 0.258	0.5	0.03
Ertapenem	3.89	Meropenem D6	0.25 ± 0.03	-0.006 ± 0.009	0.5	0.06
Imipenem	0.98	Meropenem D6	0.10 ± 0.01	-0.002 ± 0.010	0.5	0.10
Levofloxacin	3.80	Levofloxacin D8	0.72 ± 0.07	0.002 ± 0.005	0.5	0.02
Meropenem	3.45	Meropenem D6	0.95 ± 0.10	-0.008 ± 0.015	0.5	0.03
Oxacillin	5.55	Piperacillin D5	0.24 ± 0.03	-0.004 ± 0.014	0.5	0.03
Piperacillin	5.00	Piperacillin D5	0.79 ± 0.07	-0.003 ± 0.009	0.5	0.02
Tazobactam	1.57	Cefepime D3	5.89 ± 0.29	-0.072 ± 0.132	0.5	0.06
Ticarcillin	4.55	Piperacillin D5	0.24 ± 0.03	-0.008 ± 0.011	0.5	0.03

Table 3. Intra-day and inter-day precision and accuracy for the studied compounds

Molecule	Control 1								Control 2							
	QC Lower		QC Low		QC Medium		QC High		QC Lower		QC Low		QC Medium		QC High	
	Bias %	CV%	Bias %	CV%	Bias %	CV%	Bias %	CV%	Bias %	CV%	Bias %	CV%	Bias %	CV%	Bias %	CV%
Amoxicillin	3.8	12.2	5.3	6.5	1.2	3.9	1.8	6.9	1.0	12.7	3.8	7.5	1.8	6.9	2.4	5.3
Cefepime	7.7	3.9	11.8	0.8	8.2	2.7	0.6	10.6	6.0	9.2	4.4	3.9	0.6	10.6	0.5	7.3
Cefotaxime	3.5	3.7	10.2	7.1	6.3	3.8	3.0	3.5	10.8	6.4	6.0	6.4	3.0	3.5	6.8	10
Ceftazidime	3.5	3.7	0.5	3.2	2.1	2.8	4.4	6.4	10.8	6.0	1.5	7.7	4.4	6.4	0.7	6.2
Ceftriaxone	10.9	4.7	6.1	9.6	14.1	8.6	1.3	10	11.3	7.5	10.5	6.8	1.3	10.0	2.2	12.8
Ciprofloxacin	4.5	1.2	0.8	6.6	4.1	3.1	0.8	7.3	1.8	9.5	2.2	4.5	0.8	7.3	0.2	10.9
Clindamycin	3.4	5.5	7.5	8.4	7.1	3.4	1.2	4.4	7.7	2.3	2.8	6.6	1.2	4.4	5.6	8.2
Ertapenem	4.4	3.3	7.3	6.1	9.1	2.3	0.2	6.6	1.1	3.5	5.1	4.4	0.2	6.6	0.1	5.2
Imipenem	2.8	6.5	0.9	3.3	12.8	7.3	4.6	8.4	0.9	7.2	3.9	6.7	4.6	8.4	7.2	10.2
Levofloxacin	4.9	1.6	2.0	1.6	3.4	2.6	3.6	3.5	1.4	6.4	4.1	6.6	3.6	3.5	1.3	5.0
Meropenem	6.3	1.9	6.6	2.9	4.7	2.5	6.9	8.9	1.3	7.9	2.7	8.9	6.9	8.9	0.3	6.3
Oxacillin	5.8	5.1	2.9	6.7	5.0	0.1	7.4	7.4	2.3	9.4	2.0	9.8	7.4	7.4	0.2	8.3
Piperacillin	2.0	1.7	0.7	0.9	3.0	3.0	3.9	6.8	0.1	3.4	0.7	0.9	3.9	6.8	0.3	7.8
Tazobactam	1.6	3.9	1.4	5.1	4.5	1.4	1.0	8.1	1.4	9.7	1.7	8.4	1.0	8.1	4.3	11.1
Ticarcillin	1.4	5.4	8.4	1.5	8.4	4.9	1.1	7.6	3.6	5.2	0.6	8.9	1.1	7.6	1.8	8.4

Results and discussion

Sample preparation required a dilution prior to injection since some of the compounds of the panel are quite polar and do not give a good chromatographic shape with high organic content in the sample. The method proved to be linear for all the analytes from the LOQ (0.5 mg/L) to a concentration of 32 mg/L. Table 2 presents the calibration results as well as the internal standards used for each compound, the slope, and intercept statistics for a six-day study. Representative chromatograms at the LOQ and reconstructed with a mass accuracy of 5 ppm are reported in Figure 2. Imipenem is a compound prone to tautomerism, which explains the existence of a double peak in the chromatogram.

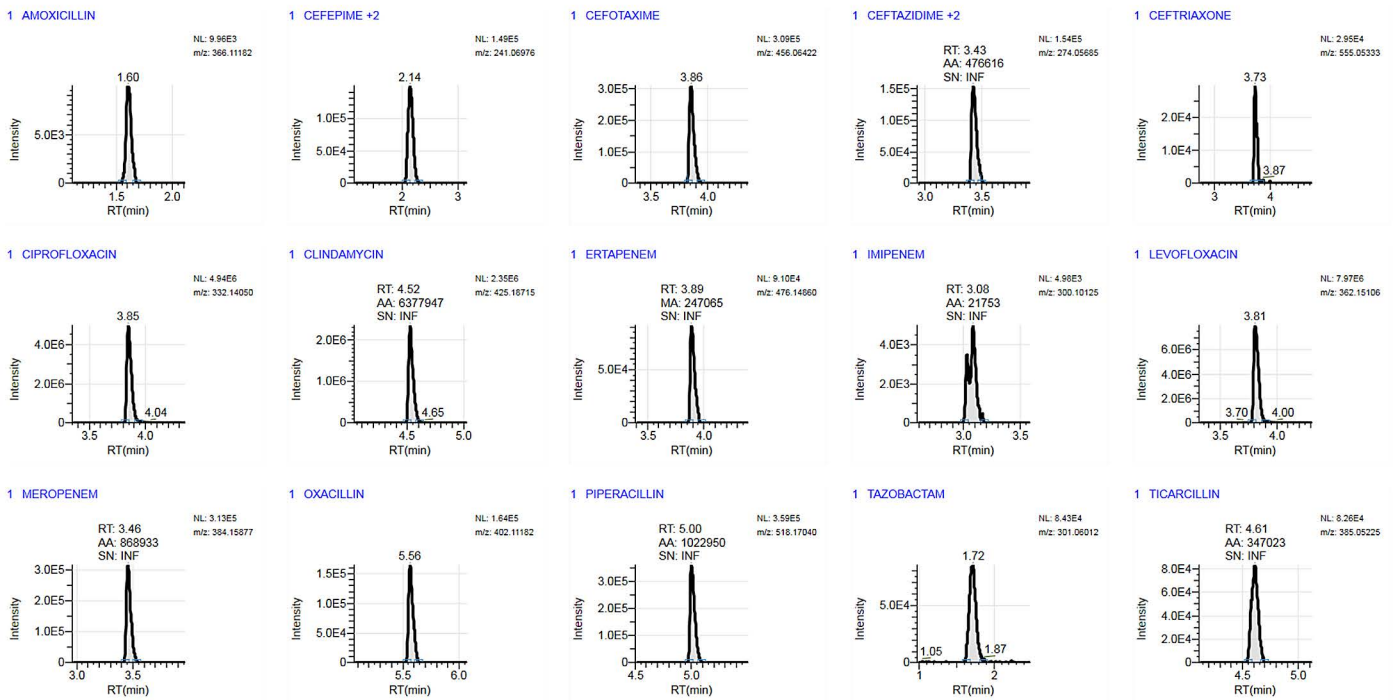

Figure 2. Extracted chromatograms at a precision of 5 ppm for the LOQ of the studied compounds (0.5 mg/L)

Intra- and inter-assay accuracy was below 20% for the LOQ and below 15% for the other quality controls. As for precision, the %CV was less than 20% for the LOQ and less than 15% for the other quality controls. These results demonstrate a good accuracy and precision for the four quality controls and all the studied compounds. The complete results of this evaluation are found in Table 3.

Conclusions

A research method for the quantification of 15 antibiotics in plasma using an UltiMate 3000 LC system coupled to an HRAM Orbitrap mass spectrometer was implemented and analytically evaluated. The sample preparation was convenient for this analysis, and the sensitivity was high enough even considering an important dilution factor used during sample preparation. Good accuracy and precision were obtained for the studied range of concentrations.

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