Simple and accurate quantitative analysis of cefiderocol and ceftobiprole in human plasma using liquid chromatography-isotope dilution tandem mass spectrometry: interest for their therapeutic drug monitoring and pharmacokinetic studies

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1 **Simple and accurate quantitative analysis of cefiderocol and ceftobiprole in human**
2 **plasma using liquid chromatography-isotope dilution tandem mass spectrometry:**
3 **interest for their therapeutic drug monitoring and pharmacokinetic studies.**

4

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36 **Abstract**

37 Objectives: Cefiderocol and ceftobiprole are new generation cephalosporin antibiotics that
38 exhibit high inter-individual plasma concentration variability that potentially impact their
39 efficacy or toxicity. The aim of this study was to develop and validate a selective, simple and
40 fast UPLC-MS/MS method for simultaneous quantification of cefiderocol and ceftobiprole in
41 human plasma to enable their therapeutic drug monitoring and support PK and PK/PD studies,
42 in particular in critically ill patients.

43 Methods: After a simple and fast single-step protein precipitation, cefiderocol and
44 ceftobiprole were separated on a Waters Acquity UPLC BEH C18 column by linear gradient
45 elution; with subsequent detection by Shimadzu MS 8060 triple quadrupole tandem mass
46 spectrometer in a positive ionization mode.

47 Results: Analysis time was 5 minutes per run. The analytical performance of the method in
48 terms of specificity, sensitivity, linearity, precision, accuracy, matrix effect, extraction
49 recovery, limit of quantification, dilution integrity and stability of analytes under different
50 conditions met all criteria for a bioanalytical method for the quantification of drugs. The
51 calibration curves were linear over the range of 1-200 mg/L for cefiderocol and 0.5-100 mg/L
52 for ceftobiprole with a linear regression coefficient above 0.995 for both.

53 Conclusion: A simple, fast, and selective liquid chromatography-tandem mass spectrometry
54 method was developed and validated for the simultaneous quantification of cefiderocol and
55 ceftobiprole. This new method was successfully applied to the measurement of plasma
56 concentration of cefiderocol and ceftobiprole in critically ill patients and showed good
57 performance for their therapeutic monitoring and optimizing antibiotic therapy.

58 Keywords: liquid chromatography, mass spectrometry, cefiderocol, ceftobiprole,
59 cephalosporin, therapeutic drug monitoring.

60 **1. Introduction**

61 Ceftobiprole, a fifth-generation parenteral cephalosporin, has shown antimicrobial activity
62 against a large range of bacteria involved in pneumonia, including Gram-positive bacteria
63 (GPB), such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis*,
64 and *Streptococcus pneumoniae*, and Gram-negative bacteria (GNB), such as *Pseudomonas*
65 *aeruginosa* and *Haemophilus influenza* [1]. Hospital-acquired pneumonia (HAP) and
66 community-acquired pneumonia (CAP) are among the most common infections treated in the
67 hospital setting [2, 3]. Two randomized, double-blind, phase III clinical trials have
68 demonstrated the efficacy of ceftobiprole in patients with HAP or CAP [4, 5]. Ceftobiprole
69 exhibits high inter-individual pharmacokinetic (PK) variability [6]. Moreover, an exposure-
70 efficacy relationship [7] and an exposure-toxicity relationship [8] have been demonstrated.
71 The PK variability and the pharmacokinetics/pharmacodynamics (PK/PD) relationship of
72 ceftobiprole suggest the potential interest of its routine therapeutic drug monitoring (TDM).
73 Cefiderocol (S-649266) is a newly *Food and drug administration* (FDA) and *European*
74 *Medicines Agency* (EMA) approved, first in its class, parenterally administered siderophore
75 cephalosporin with a novel mechanism to penetrate the outer cell membrane of GNB,
76 including multidrug-resistant strains [9]. The emergence of carbapenem resistance in many
77 GNB, involved in a variety of serious infections including pneumonia, urinary tract infections,
78 intra-abdominal infections and bloodstream infections, is an urgent threat to global public
79 health [10-13], and the presence of multi-drug resistance complicates the management of
80 these infections due to the limited treatment options available [14, 15]. Data from global
81 surveillance studies for cefiderocol have shown potent *in vitro* activity against a wide range of
82 GNB, including carbapenem-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa*,
83 Enterobacteriaceae and *Stenotrophomonas maltophilia* strains [16]. The noninferiority and
84 tolerability of cefiderocol versus imipenem-cilastatin was demonstrated in a randomized,

85 double blind, phase II study for the treatment of patients with complicated urinary tract
86 infections due to multidrug-resistant GNB [17]. The PK profile of cefiderocol has been
87 described in phase I and II single- and multiple-dose clinical studies [18-20]. PK and safety
88 have also been described in subjects with renal impairment [19, 20]. However, the population
89 PK and PK/PD properties of cefiderocol in ill patients, and specifically in critically ill patients
90 are currently limited [21]. This is particularly important because there are, in this population,
91 several co-morbidities and modifications of the physiological state which can modify the PK
92 and PK/PD profiles of most beta-lactam antibiotics used in clinical practice [22-26].
93 The aim of this study was to develop and validate a selective, simple and fast UPLC-MS/MS
94 method for simultaneous quantification of cefiderocol and ceftobiprole in human plasma to
95 enable their TDM and support PK and PK/PD studies.

96 ## 2. Materials and methods
97
98 ### 2.1. Chemical and reagents

99 Cefiderocol and [2H_{12}]-cefiderocol powders were provided by Shionogi & Co, Ltd (Osaka,
100 Japan) while ceftobiprole (BAL9141, the active form) and [2H_4]-ceftobiprole powders were
101 provided by Basilea Pharmaceutica Ltd (Basel, Switzerland). The chemical structures and
102 mass spectrum of cefiderocol and ceftobiprole are shown in **Figure 1** (mass spectrum of
103 [2H_{12}]-cefiderocol and [2H_4]-ceftobiprole are shown in **Supplemental Figure 1**). Methanol
104 was obtained from Merck (Darmstadt, Germany). Formic acid and ammonium acetate were
105 obtained from Sigma-Aldrich (Munich, Germany). Sulphosalicylic acid dihydrate (SSA) and
106 acetonitrile were obtained from VWR (Fontenay-sous-Bois, France). All reagents used were
107 of the highest available analytical grades. Liquid chromatography–MS/MS grade water (ultra-
108 pure water) was purchased from a water distribution hypergrade system Purelab Flex®

109 (ELGA®), and drug-free plasma from healthy donors was supplied by the French Blood
110 Establishment (Paris, France).

111 2.2. Preparation of stock solutions, standards and quality control samples

112 Individual stock solutions of cefiderocol and ceftobiprole were prepared at 1,000 mg/L.
113 Cefiderocol was prepared in ultra-pure water while ceftobiprole was prepared in a solution of
114 methanol-hydrochloric acid 1N (95:5, v/v). Working solutions of cefiderocol and ceftobiprole,
115 obtained by diluting the stock solution with methanol, were used to spike drug-free plasma to
116 prepare independent calibration standards and quality control (QC) samples. Calibration
117 ranges covered in the current method are: 1-200 mg/L (1-5-10-25-50-100-200) for cefiderocol
118 and 0.5-100 mg/L (0.5-1-5-10-25-50-100) for ceftobiprole. The QC samples were tested at
119 four different concentrations: high QC (HQC: 80% of upper limit of quantification [ULOQ]),
120 medium QC (MQC: 50% of selected range), low QC (LQC: 2 times the lower limit of
121 quantification [LLOQ]) and QC at LLOQ. A solution of mix (ISmix) of [$^2H_{12}$]-cefiderocol
122 and [$^2H_4$]-ceftobiprole, used as internal standard, at 10 mg/L for both, was prepared in ultra-
123 pure water. Stock solutions, working solutions, calibration standards, ISmix and QC samples
124 were stored at -80°C.

125 2.3. Instruments and analytical conditions

126 Chromatography was performed on a Nexera X2 system (Shimadzu, Japan) with an
127 autosampler temperature at 8°C. Acquity UPLC BEH C18 column (4.6 x 50 mm, 3.5 µm
128 particle size) was used for chromatographic separation and column temperature was
129 maintained at 45°C. Separation of cefiderocol and ceftobiprole was achieved over 5 min using
130 two mobile phases: (A) water-formic acid (100:0.1, v/v)-ammonium acetate 2 mM and (B)
131 acetonitrile-formic acid (100:0.1, v/v). A binary pump delivered the mobile phases at a flow
132 rate of 0.5 mL/min using a linear gradient elution. The UPLC system was coupled to a triple

133 quadripole mass spectrometer: MS 8060 (Shimadzu, Japan). Quantifications were achieved in
134 Multiple Reactions Monitoring (MRM) mode and electrospray ionization (ESI) was operated
135 in positive mode. The interface temperature, the desolvatation line (DL) temperature and the
136 heat block temperature were set at 300°C, 250°C and 400°C, respectively, with a drying gas
137 flow of 10.0 L/min and a nebulizing gas flow of 3.0 L/min. The interface voltage was set at
138 4.0 kV. Argon was used as collision gas. Chromatographic data acquisition; peak integration
139 and quantification were performed using LabSolutions Insight LC-MS 3.2 SP1 software.

140 ### 2.4. Samples pre-treatment

141 Sample preparation was performed by protein precipitation: 10 µL of 30% sulphosalicylic
142 acid and 50 µL of ISmix were added to 100 µL of human plasma, calibrator or QC samples.
143 The mixture was vortexed for 1 min and centrifuged for 10 min at 18,900 g at room
144 temperature. Subsequently, 5 µL of the clear supernatant was transferred to an autosampler
145 vial containing 200 µL of mobile phase A. 1 µL was injected into the LC-MS/MS system.

146 ### 2.5. Method validation

147 The validation was performed according to *European Medicines Agency* (EMA) guideline for
148 the validation of bioanalytical methods [27]. Parameters included were selectivity, carry-over,
149 linearity, accuracy and precision, lower limit of quantification, matrix effect, stability in
150 human plasma and dilution integrity.

151 #### 2.5.1. Selectivity

152 The analytical method should be able to differentiate the analyte(s) of interest from
153 endogenous components in the matrix or other components in the sample such as other drugs.
154 To perform this, fifteen different sources of plasma samples were tested. A selective method
155 should not have interference of more than 20% of the LLOQ for the analytes and 5% for the
156 ISs.

157 2.5.2. Carry-over

158 Carry-over was assessed by injecting drug-free plasma samples after a high concentration 159 calibrator. Carry-over in the drug-free plasma samples following the high concentration 160 calibrator should not be greater than 20% of the LLOQ.

161 2.5.3. Linearity

162 Calibration curve was acquired by plotting the peak area ratio of the concentration of 163 cefiderocol and ceftobiprole standards to the area of their respective isotopic IS over the range 164 from 1 to 200 mg/L for cefiderocol and 0.5 to 100 mg/L for ceftobiprole. Curves were 165 assayed by least square weighted (1/x). Linearity was defined by a linear regression 166 coefficient $(R)^2 \geq 0.995$.

167 2.5.4. Precision and accuracy

168 The intra-day precision and accuracy were evaluated using six different replicates, extracted 169 in the same day, at the four QC levels (LLOQ, LQC, MQC and HQC). The inter-day 170 precision and accuracy were determined by repeating each QC levels six times a day for three 171 consecutive days (n = 18 replicates). The concentration of each QC levels was determined 172 using calibration standards prepared on the same day. The precision was calculated as the 173 coefficient of variation (CV, %) within a single run (intra-day assay) and between different 174 runs (inter-day assay), and the accuracy as the percentage ratio of the measured and nominal 175 concentration (mean of measured/nominal × 100). The acceptance limits were CV <15% for 176 precision and within ± 15% of the nominal concentration for accuracy (ranged from 85- 177 115%), except for the LLOQ at which deviation of 20% is acceptable.

178 2.5.5. Lower limit of quantification

179 The signal of the analyte at the LLOQ should be at least 5 times higher than that of a drug-
180 free plasma sample. LLOQ should be measured with a variation not exceeding 20% of the
181 nominal expected value and a CV <20%. The LLOQs of cefiderocol and ceftobiprole were
182 selected as the lower concentration covered by the selected range.
183
184 2.5.6. Matrix effect and extraction recovery

185 Matrix effect (ME) and extraction recovery (ER) were assessed at two QC levels (LQC and
186 HQC) in quintuplicate with five different sources of plasma. The approach involves
187 determination of ratio of peak areas of analytes in three different sets [28]. One consisting of
188 analyte standards in methanol (set A), one prepared in drug-free matrix extracts and spiked
189 after extraction (set B), and one prepared in drug-free matrix from the same sources but
190 spiked before extraction (set C). ME and ER were calculated by the following equations: ME
191 (%) = $B/A \times 100$ and ER (%) = $C/B \times 100$. Normalized ME was obtained by dividing the
192 values reported for the analytes by those of the IS. A value above or below 100% for the ME
193 indicates an ionization enhancement or suppression respectively. The CV obtained for the IS-
194 normalized ME should be less than 15%.

195 2.5.7. Stability

196 The stability in human plasma of cefiderocol and ceftobiprole in different storage conditions
197 was performed at LQC, MQC and HQC. During each day tested, we used freshly prepared
198 calibrators and each QC levels were analysed on three different replicates. The value was
199 estimated by comparing measured concentration before storage to after storage, and reported
200 as the percentage ratio of mean measured concentration to nominal concentration. The short
201 term stability at room temperature (bench-top stability) was evaluated by measuring each QC
202 level, stored at 25°C, every hour for 8 consecutive hours. The short term stability in the fridge
203 was determined after 24h at 4-8°C and the short term stability in the freezer was determined

204 after 1 week at -25°C. The long term stability was determined after 2 month storage at -80°C.
205 The freeze and thaw stability was estimated after three complete freeze and thaw cycles of -
206 80°C to 25°C. Stability of extracts kept onboard the autosampler at 8°C during 24h was also
207 tested. Cefiderocol and ceftobiprole was considered to be stable in plasma, during this
208 different storage conditions, when measured concentration within ± 15% of the nominal
209 concentration.

210 2.5.8. Dilution integrity

211 The dilution integrity was examined to ascertain that an unknown sample with concentration
212 exceeding the upper limit of compound calibration range, could be diluted with drug-free
213 matrix without influencing the accuracy and precision of the measurement. To achieve this, a
214 sample was prepared in a two-fold higher concentration than of the ULOQ (400 mg/L for
215 cefiderocol and 200 mg/L for ceftobiprole) followed by dilution (1:3) in drug-free plasma
216 before extraction. Diluted sample was tested for accuracy and precision: the acceptance limits
217 were CV <15% for precision and within ± 15% of the nominal concentration for accuracy.
218 Samples were analyzed in quintuplicate.

219 2.6. Clinical application

220 This UPLC-MS/MS quantification method was applied to measurement of cefiderocol and
221 ceftobiprole in plasma of critically ill patients hospitalized in ICU at Pitié-Salpêtrière hospital
222 (Paris, France) to perform TDM and PK studies. Ten patients with compassionate use of
223 cefiderocol and twenty five patients treated with ceftobiprole were included in the study.
224 Cefiderocol and ceftobiprole were administrated by intravenous infusion over 3-hours and
225 over 2-hours, respectively, and two successive blood samples were collected into lithium
226 heparin tubes at steady state for both: one prior to the start of the infusion (C_{trough}) and one 15
227 ± 10 min after the end of the infusion (C_{max}). Plasma samples were prepared by centrifuging

228 collected blood samples for 5 min at 4,500 *g* at room temperature. All plasma samples were
229 frozen at -80°C until analysis, and were process and analysed as previously described. French
230 regulations on non-interventional observational studies do not require patient's consent when
231 analyzing data obtained from routine care. Approval for data collection was obtained from the
232 Commission Nationale de l'Informatique et des Libertés (n°1491960v0).

233

234 **3. Results**

235

236 3.1. Optimization of LC-MS/MS conditions

237 Electrospray positive mode yielded a better spectrometer response than the negative mode. To
238 achieve symmetrical peak shapes, good resolution and a short chromatographic run time, a
239 mobile phase consisting of (A) water-formic acid (100:0.1, v/v)-ammonium acetate 2 mM and
240 (B) acetonitrile-formic acid (100:0.1, v/v) was used in the experiments using gradient elution:
241 the gradient was initiated at 3% B, then increasing linearly to 51.3% B in 2 min, after which it
242 was directly changed to 100% B, where it was maintained for 1 min, before returning to the
243 initial condition for the last 2 min, resulting in a total run time of 5 min. Mass spectrometry
244 parameters for the LC-MS/MS determination of cefiderocol, ceftobiprole and their respective
245 IS are shown in **Table 1**.

246 3.2. Method validation

247 3.2.1. Selectivity and carry-over

248 Fifteen different sources of heparin-plasma samples without analytes but containing the
249 following anti-infective drugs: levofloxacin, ciprofloxacin, moxifloxacin, rifampicin,
250 ceftazidime, cefepime, amoxicillin, voriconazole, posaconazole, fluconazole and
251 isavuvonazole were tested. These anti-infective drugs were tested due to their relatively
252 common use in ICU and for their possible concomitant administration in our cohort of
253 patients. No interference with endogenous compounds or tested anti-infective drugs was

254 observed above 20% of the LLOQ of cefiderocol or ceftobiprole and above 5% of their
255 respective IS. Furthermore, these same fifteen heparin-plasma samples were spiked at the
256 LLOQ of cefiderocol and ceftobiprole and were tested for accuracy and precision No
257 significant interference was found in spiked plasma samples compared to non-spiked samples.
258 The accuracy (% true) and precision (% CV) of the spiked samples were: cefiderocol (112;
259 10.7) and ceftobiprole (109; 11.2) indicating an absence of significant interference that could
260 influence the accuracy and precision of the measurement.
261 The carry-over observed was less than 20% of the LLOQ for both drugs [cefiderocol (8.4%);
262 ceftobiprole (1.8%)]. Furthermore, no carry over was observed for the IS used. The MRM-
263 chromatogram of drug-free plasma sample of ceftobiprole and cefiderocol are shown in
264 **Figure 2A4 and 2B4** respectively.

265 ### 3.2.2. Linearity

266 Calibration curve were linear with linear regression coefficient $(R)^2$ ≥0.995 for both analytes
267 (0.998 and 0.999 for cefiderocol and ceftobiprole respectively). All calibrators, analyzed on
268 seven different days, were measured with an accuracy ranged from 87-111% and coefficient
269 of variation less than 10.2%. The highest calibration point was defined as ULOQ. The MRM-
270 chromatogram of ceftobiprole and cefiderocol at their ULOQ are shown in **Figure 2A2 and**
271 **2B2** respectively. The linear regression equations of pooled data obtained over seven distinct
272 days were: $y = 0.20360x - 0.08026$ and $y = 0.72831x - 0.07829$ for cefiderocol and
273 ceftobiprole respectively.

274 ### 3.2.3. Accuracy and precision

275 Intra- and inter-day precision and accuracy outcomes of QC samples are shown in **Table 2**.
276 The intra- and inter-day coefficients of variation ranged from 2.6% to 5.3% and from 4.5% to
277 10.4% respectively, for both analyte at all tested concentrations (LQC, MQC and HQC).

278 Likewise, the intra- and inter-day accuracy ranged from 91-108% and from 92-102%
279 respectively, for both analyte at all tested concentrations (LQC, MQC and HQC).

280 3.2.4. Lower limit of quantification

281 The LLOQ was established at 0.5 mg/L for ceftobiprole and at 1 mg/L for cefiderocol (**Table**
282 **2**). The MRM-chromatogram of ceftobiprole and cefiderocol at their LLOQ are shown in
283 **Figure 2A1 and 2B1** respectively.

284 3.2.5. Matrix effect and extraction recovery

285 **Table 3** presents the matrix effect (absolute and IS-normalized) and extraction recovery data
286 at low and high concentrations using heparin-plasma samples containing different anti-
287 infective drugs (see list in selectivity section). For cefiderocol, a significant ion enhancement
288 was observed, but this matrix effect was compensated by the use of its isotope-labeled IS. The
289 CV of the IS-normalized matrix effect was less than 15% for both analyte. The absolute
290 extraction recovery for both analyte ranged from 90 to 103%, with in all instances CVs below
291 10%.

292 3.2.6. Stability

293 Analyte stability was determined at five different storage conditions, using three
294 concentrations of QC (LQC, MQC, HQC), as percent ratio of mean measured concentration to
295 nominal concentration. Results are summarized in **Table 4**. The long term stability proved
296 that both analytes are stable in plasma for at least 2 months at -80°C and after three cycles of
297 freeze and thaw. The short term stability proved that both analytes are stable for 8h at room
298 temperature; 24h at 4°C; for at least1 week at -25°C and for 24h in the autosampler after
299 extraction. In addition, the stock, working and ISmix solutions have also been stable at -80°C
300 for at least 2 months.

301 3.2.7. Dilution integrity

302 The accuracy (% true) and precision (% CV) of the diluted sample were: cefiderocol (94; 5.7)
303 and ceftobiprole (106; 7.2).

304 3.3. Clinical application

305 This validated UPLC-MS/MS method was successfully applied to the measurement of plasma
306 concentration of cefiderocol and ceftobiprole in critically ill patients. For cefiderocol, the
307 usual dosing was 2g TID [three times a day] (6/10). Three patients with renal impairment
308 were treated with 0.75g BID [twice a day], 1g TID and 1.5g TID regimen, respectively, and
309 one patient with renal hyperfiltration was treated with 2g QID [four times a day] regimen. In
310 all patients, cefiderocol was infused over 3-hours. Concerning the ceftobiprole, all patients
311 were treated with 500mg TID regimen infused over 2-hours. Both analytes were easily
312 detected and measured in patients' plasma. The results are represented in **Figure 3**. Moreover,
313 as shown in **Figure 2A5 and 2B5**, no interferences were observed between ceftobiprole or
314 cefiderocol and endogenous compounds or others drugs given to participating ICU patients.

315

316 **4. Discussion**

317 We present here a liquid chromatography-isotope dilution tandem mass spectrometry method
318 for the simultaneous measurement of cefiderocol and ceftobiprole. Based on the high
319 recovery, relatively low intra- and inter-day CVs, and good linearity, the present method is
320 suitable for detection and quantification of these two antibiotics in human plasma. To the best
321 of our knowledge, very few analytical methods intended for routine cefiderocol or
322 ceftobiprole clinical application have yet been published. Lima *et al.* reported an HPLC-UV
323 method for measurement of ceftobiprole in human plasma with concentration range of 1-80
324 mg/L [8]. Likewise, Mernissi *et al.* and Zimmer *et al.* reported an HPLC-UV method for

325 routine therapeutic drug monitoring of cefiderocol with concentration range of 2.5-100 mg/L
326 and 4-160 mg/L, respectively [29, 30]. Although HPLC-UV has the advantage of being cost-
327 effective and easily accessible to most laboratories, HPLC-MS/MS method operating the
328 multiple reaction monitoring has become one the top choices for high speed selective and
329 sensitive analysis of compounds including drugs [31]. HPLC-MS/MS has been employed for
330 cefiderocol and ceftobiprole PK studies in healthy volunteers [18, 32, 33] but in these reports,
331 information about the analytical methods is lacking making their reproducibility difficult. Our
332 method is the first method allowing the simultaneous determination of cefiderocol and
333 ceftobiprole by mass spectrometric detection (analysed using their respective isotopic internal
334 standard) and designed to perform their therapeutic monitoring in a routine setting.
335 The specificity of our method was acceptable since we did not observed interference with
336 endogenous compounds or drugs given to patients hospitalized in ICU. Likewise, the
337 sensitivity was sufficient with a first calibration point (1 mg/L for cefiderocol and 0.5 mg/L
338 for ceftobiprole) well below the mean trough concentration measured in clinical studies and in
339 our patients. This new method uses a wide concentration range which makes it suitable for the
340 measurements of concentration for future pharmacokinetic studies. In comparison to other
341 methods intended for routine clinical application, the current one has an extended lower and
342 upper limit of quantification for both antibiotics. Given the potentially high plasma
343 concentration variability, this could be interesting in case of very low concentration at C_{min}
344 and very high concentration at C_{max}. In addition, compared to the previously reported HPLC-
345 UV methods for which the run times analysis varies from 12-15 min [8, 29, 30], our method is
346 rapid with fast sample preparation and run times (5 min) and requires only a small volume of
347 plasma (100µL), which could reduce the time required for quantification of large number of
348 samples and the blood volume collected from the patients.
349 Concerning stability, we found that cefiderocol and ceftobiprole samples should be dispatched

350 to the laboratory without delay due to their poor stability at room temperature (<24h). This is
351 an important information for the management of the pre-analytical stage and to manage
352 shipping of samples coming from other hospitals. Likewise, both antibiotics were stable for at
353 least 2 months at -80°C in plasma. This is particularly important for clinical research
354 protocols, where samples may need to be stored for a long time before they can be assayed.
355 Our stability results for cefiderocol were consistent with those reported by Mernissi *et al.*
356 [29], but significantly differs from those reported by Zimmer *et al.* [30] in particular for
357 bench-top and fridge stability.
358 Concerning the clinical application, conducted in critically ill patients, our results suggest that
359 there is significant inter-individual plasma concentration variability; this argues to consider
360 cefiderocol and ceftobiprole as candidates for TDM in this patient population. A PK/PD
361 studies should be performed to explore if this exposure variability can influence their efficacy
362 or toxicity and to define their therapeutic targets concentration.
363
364 **5. Conclusion**
365 We have developed and validated a rapid, sensitive, selective, accurate, precise and reliable
366 UPLC-MS/MS method for the simultaneous quantification of cefiderocol and ceftobiprole in
367 human plasma. This method was successfully applied to their therapeutic monitoring and will
368 allow us to carry out PK/PD studies in critically ill patients.
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374

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379 review and editing. **D.S**: investigation. **P.R**: investigation. **O.P**: investigation, writing-review
380 and editing. **N.T**: investigation, writing-review and editing. **G.N**: investigation, writing-
381 review and editing. **H.J**: investigation, writing-review and editing. **CE.L**: investigation,
382 writing-review and editing. **C.FB**: investigation, writing-review and editing. **N.Z**: supervision,
383 conceptualization, investigation, writing-review and editing. All authors have accepted
384 responsibility for the entire content of this manuscript and approved its submission.

385 **Competing interests**: **A.B** has received fees from Shionogi, the manufacturer of cefiderocol.
386 **CE.L** has received grant and travel fees from Correvio, the manufacturer of ceftobiprole.
387 Other authors state no conflict of interest.

388 **Informed consent**: French regulations on non-interventional observational studies do not
389 require patient's consent when analyzing data obtained from routine care.

390 **Ethical approval**: Approval for data collection was obtained from the Commission Nationale
391 de l'Informatique et des Libertés (n°1491960v0).

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399 **References**

400 1. Morosini MI, Diez-Aguilar M, Canton R. Mechanisms of action and antimicrobial activity of
401 ceftobiprole. Rev Esp Quimioter. 2019;32 Suppl 3:3-10.
402 2. Torres A, Ferrer M, Badia JR. Treatment guidelines and outcomes of hospital-acquired and
403 ventilator-associated pneumonia. Clin Infect Dis. 2010;51 Suppl 1:S48-53.
404 3. Welte T, Torres A, Nathwani D. Clinical and economic burden of community-acquired
405 pneumonia among adults in Europe. Thorax. 2012;67(1):71-9.
406 4. Awad SS, Rodriguez AH, Chuang YC, Marjanek Z, Pareigis AJ, Reis G, et al. A phase 3
407 randomized double-blind comparison of ceftobiprole medocaril versus ceftazidime plus linezolid for
408 the treatment of hospital-acquired pneumonia. Clin Infect Dis. 2014;59(1):51-61.
409 5. Nicholson SC, Welte T, File TM, Jr., Strauss RS, Michiels B, Kaul P, et al. A randomised, double-
410 blind trial comparing ceftobiprole medocaril with ceftriaxone with or without linezolid for the
411 treatment of patients with community-acquired pneumonia requiring hospitalisation. Int J
412 Antimicrob Agents. 2012;39(3):240-6.
413 6. Torres A, Mouton JW, Pea F. Pharmacokinetics and Dosing of Ceftobiprole Medocaril for the
414 Treatment of Hospital- and Community-Acquired Pneumonia in Different Patient Populations. Clin
415 Pharmacokinet. 2016;55(12):1507-20.
416 7. Muller AE, Punt N, Mouton JW. Exposure to ceftobiprole is associated with microbiological
417 eradication and clinical cure in patients with nosocomial pneumonia. Antimicrob Agents Chemother.
418 2014;58(5):2512-9.
419 8. Lima B, Bodeau S, Quinton MC, Leven C, Lemaire-Hurtel AS, Bennis Y. Validation and
420 Application of an HPLC-DAD Method for Routine Therapeutic Drug Monitoring of Ceftobiprole.
421 Antimicrob Agents Chemother. 2019;63(7).
422 9. Andrei S, Droc G, Stefan G. FDA approved antibacterial drugs: 2018-2019. Discoveries
423 (Craiova). 2019;7(4):e102.
424 10. Antibiotic Resistance Threats in the United States, 2019.

425 http://dx.doi.org/10.15620/cdc:82532.
426 11. Meletis G, Exindari M, Vavatsi N, Sofianou D, Diza E. Mechanisms responsible for the
427 emergence of carbapenem resistance in Pseudomonas aeruginosa. Hippokratia. 2012;16(4):303-7.
428 12. Tillotson GS. Trojan Horse Antibiotics-A Novel Way to Circumvent Gram-Negative Bacterial
429 Resistance? Infect Dis (Auckl). 2016;9:45-52.
430 13. Nordmann P, Poirel L. Epidemiology and Diagnostics of Carbapenem Resistance in Gram-
431 negative Bacteria. Clin Infect Dis. 2019;69(Suppl 7):S521-S8.
432 14. Morrill HJ, Pogue JM, Kaye KS, LaPlante KL. Treatment Options for Carbapenem-Resistant
433 Enterobacteriaceae Infections. Open Forum Infect Dis. 2015;2(2):ofv050.
434 15. Doi Y. Treatment Options for Carbapenem-resistant Gram-negative Bacterial Infections. Clin
435 Infect Dis. 2019;69(Suppl 7):S565-S75.
436 16. Hackel MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF. In Vitro Activity of the
437 Siderophore Cephalosporin, Cefiderocol, against a Recent Collection of Clinically Relevant Gram-
438 Negative Bacilli from North America and Europe, Including Carbapenem-Nonsusceptible Isolates
439 (SIDERO-WT-2014 Study). Antimicrob Agents Chemother. 2017;61(9).
440 17. Portsmouth S, van Veenhuyzen D, Echols R, Machida M, Ferreira JCA, Ariyasu M, et al.
441 Cefiderocol versus imipenem-cilastatin for the treatment of complicated urinary tract infections
442 caused by Gram-negative uropathogens: a phase 2, randomised, double-blind, non-inferiority trial.
443 Lancet Infect Dis. 2018;18(12):1319-28.
444 18. Saisho Y, Katsube T, White S, Fukase H, Shimada J. Pharmacokinetics, Safety, and Tolerability
445 of Cefiderocol, a Novel Siderophore Cephalosporin for Gram-Negative Bacteria, in Healthy Subjects.
446 Antimicrob Agents Chemother. 2018;62(3).
447 19. Kawaguchi N, Katsube T, Echols R, Wajima T. Population Pharmacokinetic Analysis of
448 Cefiderocol, a Parenteral Siderophore Cephalosporin, in Healthy Subjects, Subjects with Various
449 Degrees of Renal Function, and Patients with Complicated Urinary Tract Infection or Acute
450 Uncomplicated Pyelonephritis. Antimicrob Agents Chemother. 2018;62(2).

451 20. Katsube T, Echols R, Arjona Ferreira JC, Krenz HK, Berg JK, Galloway C. Cefiderocol, a
452 Siderophore Cephalosporin for Gram-Negative Bacterial Infections: Pharmacokinetics and Safety in
453 Subjects With Renal Impairment. J Clin Pharmacol. 2017;57(5):584-91.
454 21. Kawaguchi N, Katsube T, Echols R, Wajima T. Population Pharmacokinetic and
455 Pharmacokinetic/Pharmacodynamic Analyses of Cefiderocol, a Parenteral Siderophore
456 Cephalosporin, in Patients with Pneumonia, Bloodstream Infection/Sepsis, or Complicated Urinary
457 Tract Infection. Antimicrob Agents Chemother. 2021;65(3).
458 22. Neuner EA, Gallagher JC. Pharmacodynamic and pharmacokinetic considerations in the
459 treatment of critically Ill patients infected with carbapenem-resistant Enterobacteriaceae. Virulence.
460 2017;8(4):440-52.
461 23. Parker SL, Abdul-Aziz MH, Roberts JA. The role of antibiotic pharmacokinetic studies
462 performed post-licensing. Int J Antimicrob Agents. 2020;56(6):106165.
463 24. Abdul-Aziz MH, Lipman J, Akova M, Bassetti M, De Waele JJ, Dimopoulos G, et al. Is
464 prolonged infusion of piperacillin/tazobactam and meropenem in critically ill patients associated with
465 improved pharmacokinetic/pharmacodynamic and patient outcomes? An observation from the
466 Defining Antibiotic Levels in Intensive care unit patients (DALI) cohort. J Antimicrob Chemother.
467 2016;71(1):196-207.
468 25. Scharf C, Liebchen U, Paal M, Taubert M, Vogeser M, Irlbeck M, et al. The higher the better?
469 Defining the optimal beta-lactam target for critically ill patients to reach infection resolution and
470 improve outcome. J Intensive Care. 2020;8(1):86.
471 26. Gatti M, Pea F. Pharmacokinetic/pharmacodynamic target attainment in critically ill renal
472 patients on antimicrobial usage: focus on novel beta-lactams and beta lactams/beta-lactamase
473 inhibitors. Expert Rev Clin Pharmacol. 2021.
474 27. Bioanalytical method validation. European Medicines Agency. 2018.
475 https://www.ema.europa.eu/en/bioanalytical-method-validation.
476 28. Matuszewski BK, Constanzer ML, Chavez-Eng CM. Strategies for the assessment of matrix
477 effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem. 2003;75(13):3019-
478 30.
479 29. Mernissi T, Bodeau S, Andre C, Zahr N, Mary A, Dupont H, et al. An HPLC assay for the
480 therapeutic drug monitoring of cefiderocol in critically ill patients. J Antimicrob Chemother. 2021.
481 30. Zimmer J, Rohr AC, Kluge S, Faller J, Frey OR, Wichmann D, et al. Validation and Application of
482 an HPLC-UV Method for Routine Therapeutic Drug Monitoring of Cefiderocol. Antibiotics (Basel).
483 2021;10(3).
484 31. Pitt JJ. Principles and applications of liquid chromatography-mass spectrometry in clinical
485 biochemistry. Clin Biochem Rev. 2009;30(1):19-34.
486 32. Miyazaki S, Katsube T, Shen H, Tomek C, Narukawa Y. Metabolism, Excretion, and
487 Pharmacokinetics of [(14) C]-Cefiderocol (S-649266), a Siderophore Cephalosporin, in Healthy
488 Subjects Following Intravenous Administration. J Clin Pharmacol. 2019;59(7):958-67.
489 33. Schmitt-Hoffmann A, Nyman L, Roos B, Schleimer M, Sauer J, Nashed N, et al. Multiple-dose
490 pharmacokinetics and safety of a novel broad-spectrum cephalosporin (BAL5788) in healthy
491 volunteers. Antimicrob Agents Chemother. 2004;48(7):2576-80.

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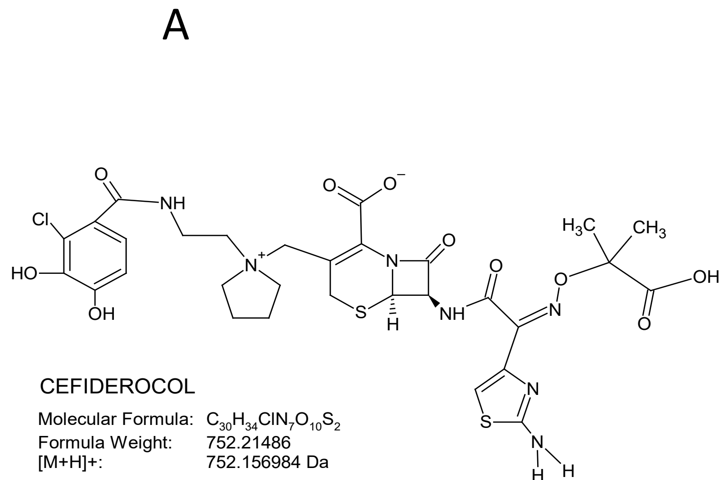
A



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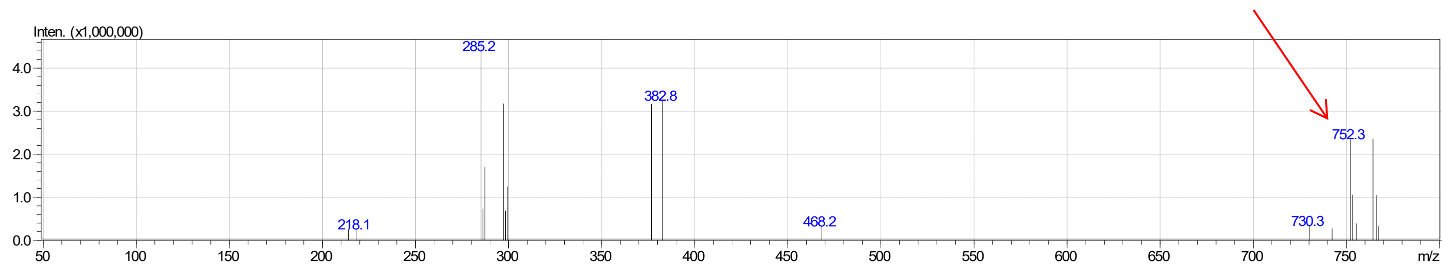

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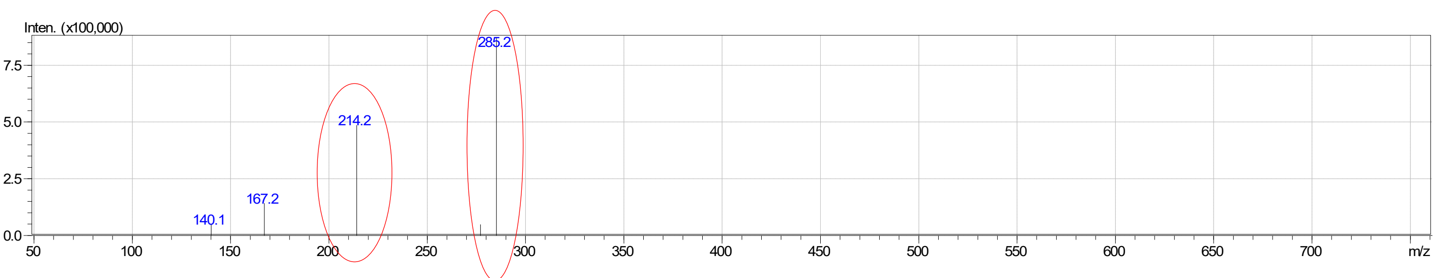

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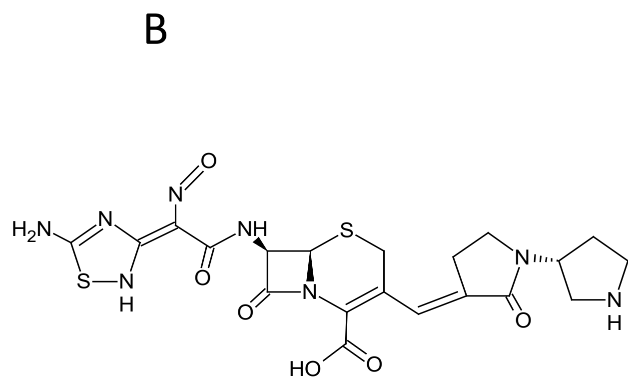

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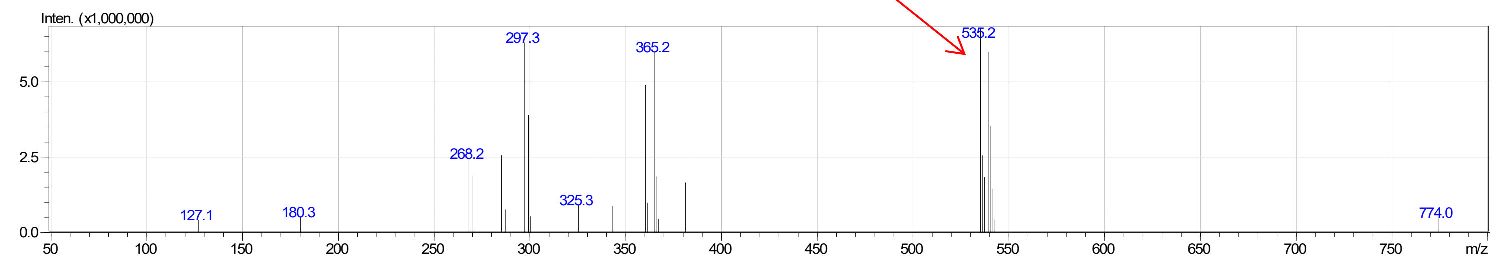

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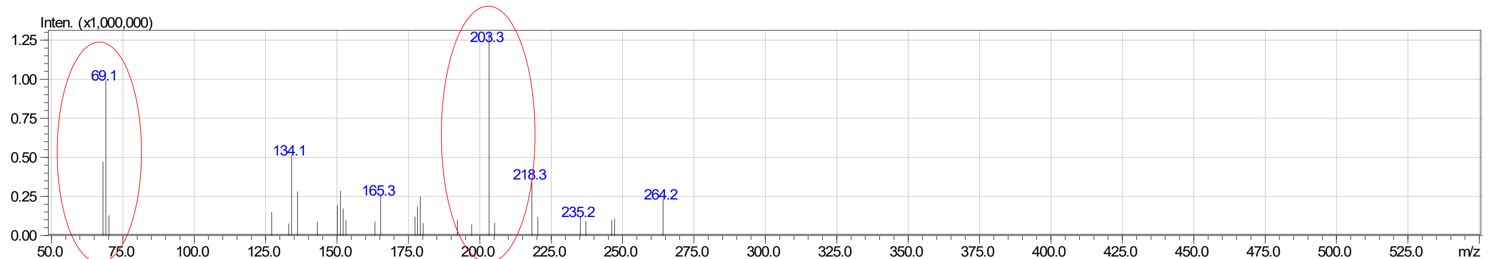
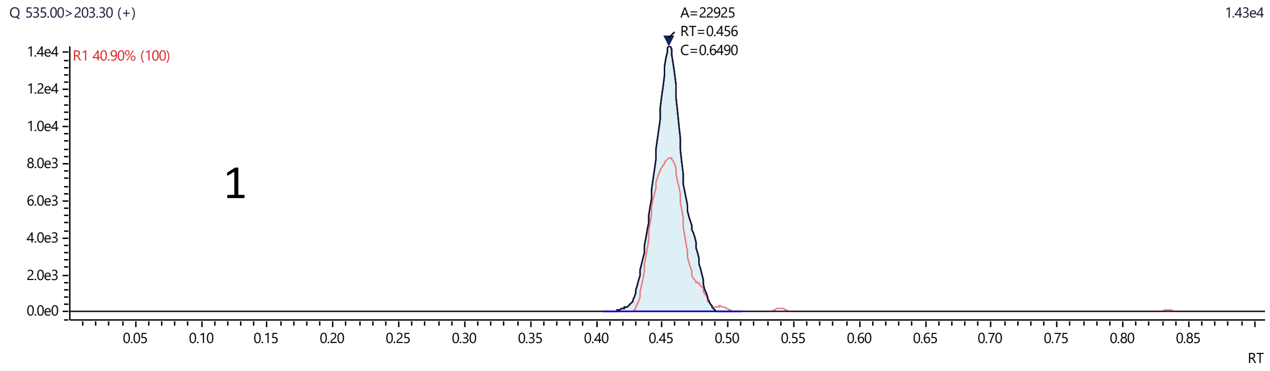
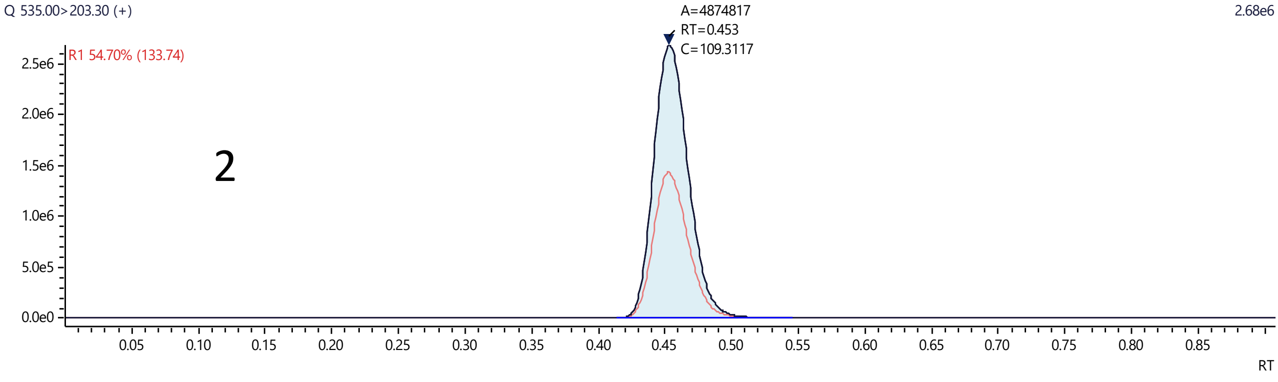
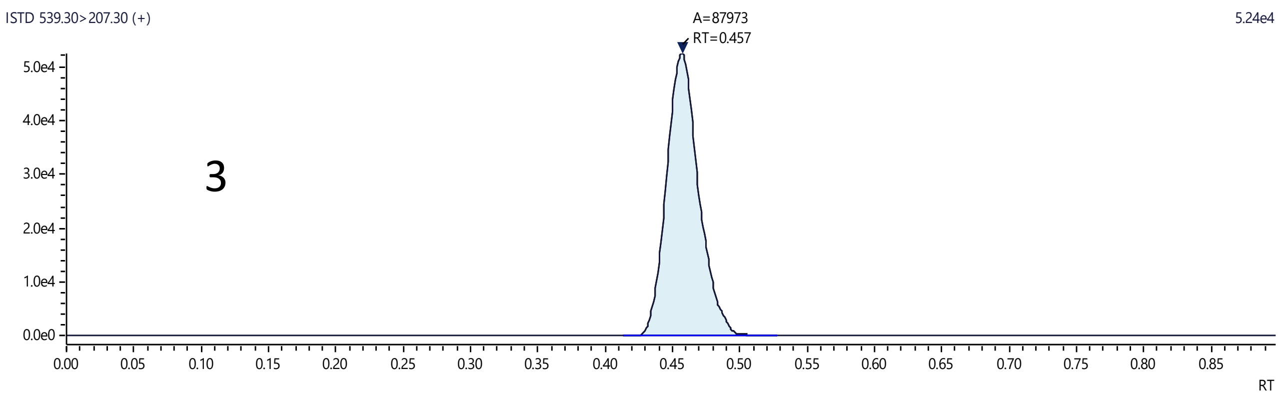


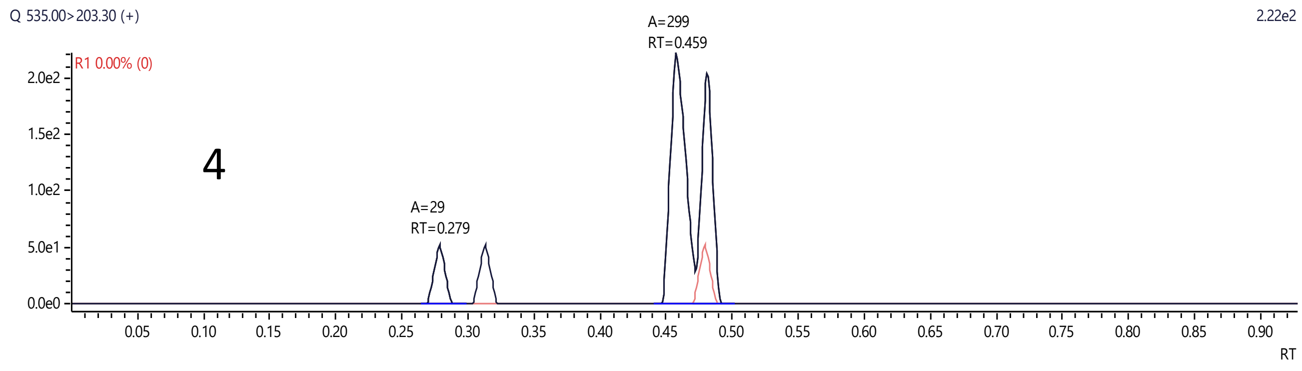
Figure 1. Chemical structures and mass spectrum of (A) cefiderocol and (B) ceftobiprole

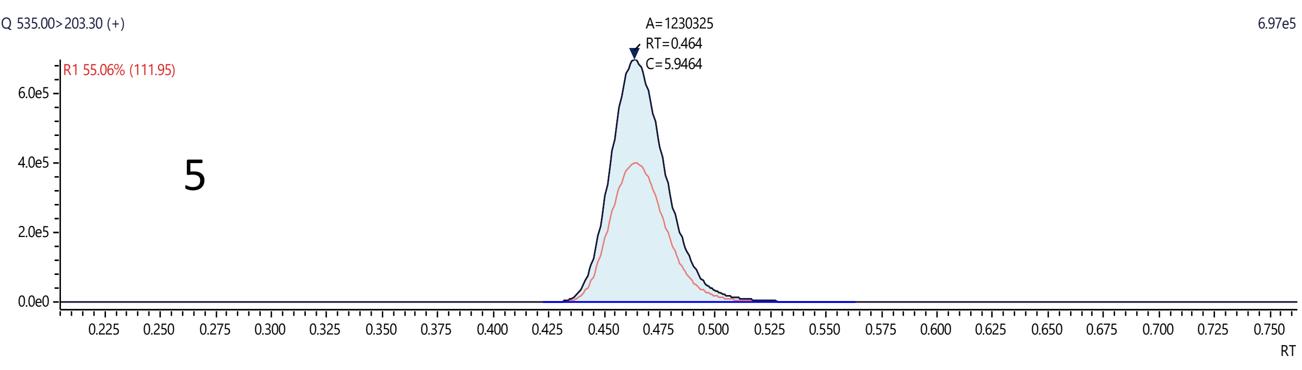
A Ceftobiprole



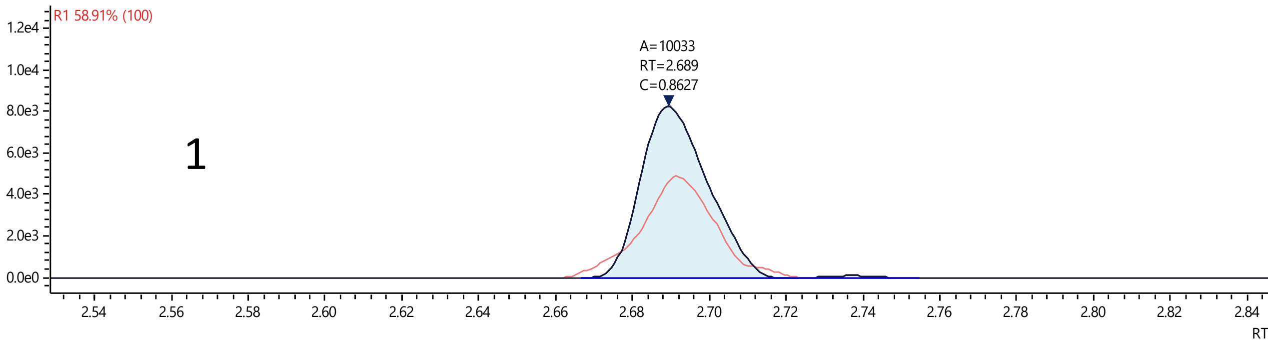


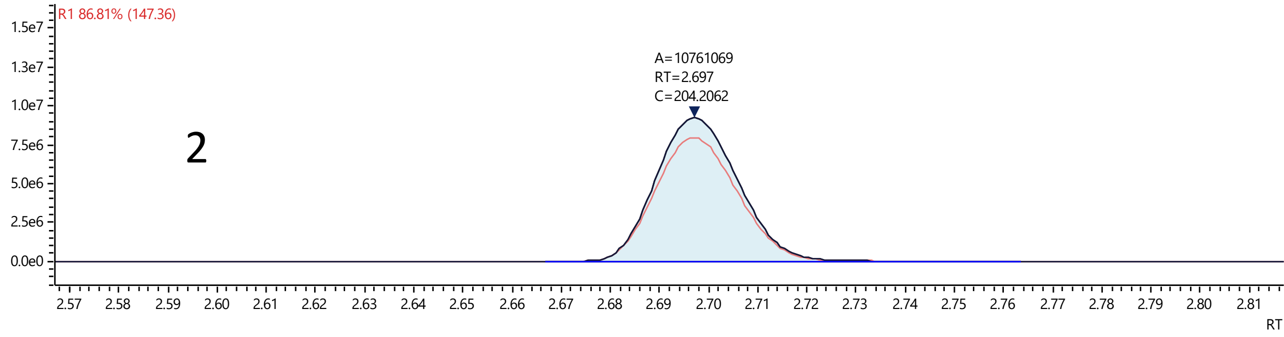


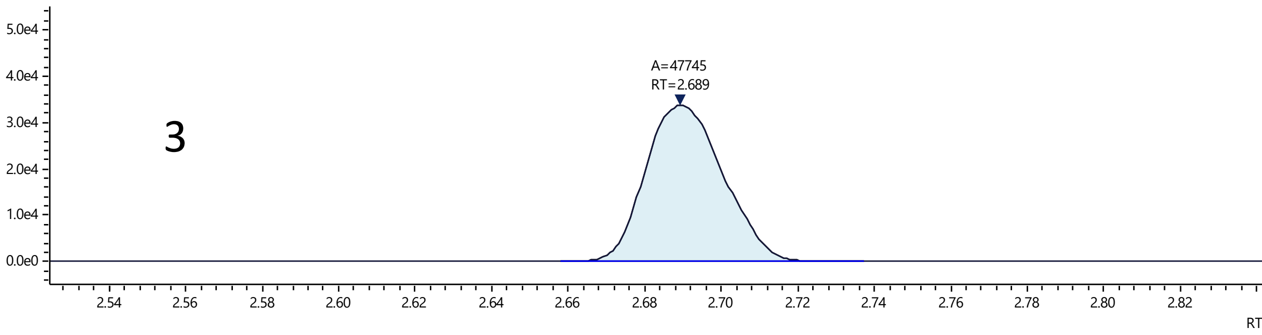


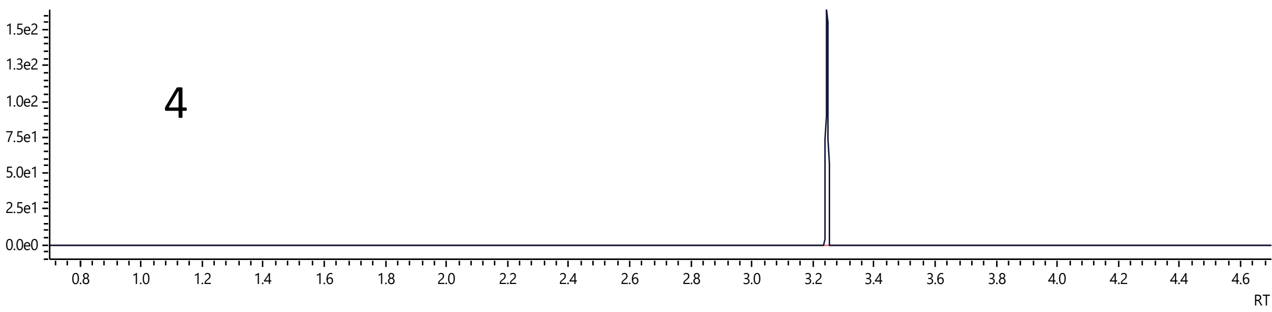


B Cefiderocol









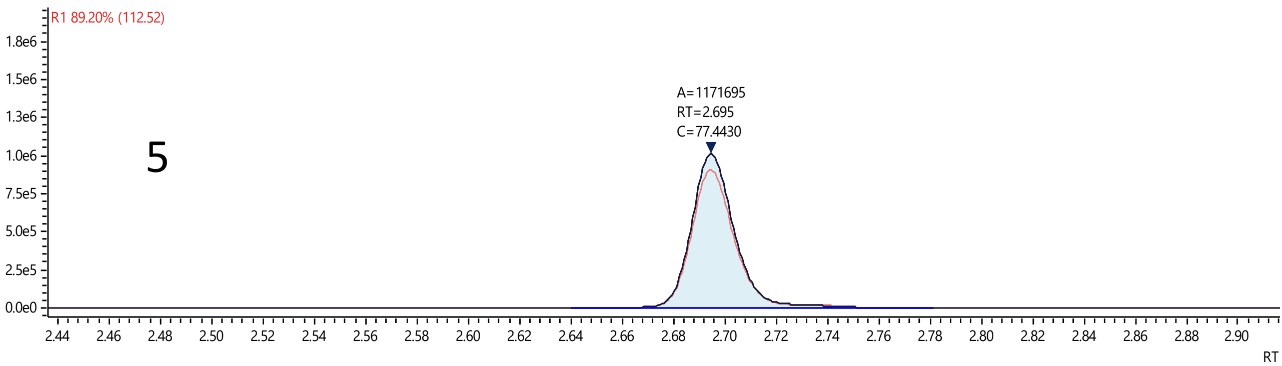
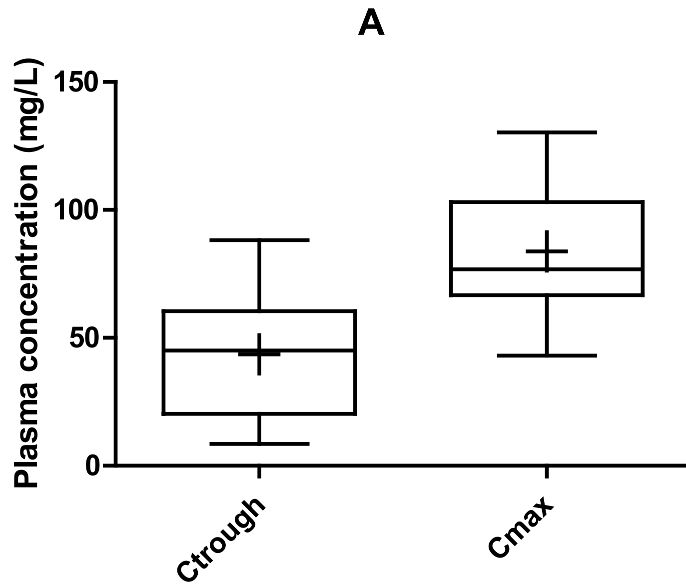


Figure 2. MRM ion-chromatograms of (A1) blank human plasma spiked with ceftobiprole at 0.5 mg/L (LLOQ), (A2) blank human plasma spiked with ceftobiprole at 100 mg/L (ULOQ), (A3) d4-ceftobiprole (IS of ceftobiprole) at 10 mg/L, (A4) blank human plasma without ceftobiprole, (A5) real patient sample treated with ceftobiprole at Ctrough, (B1) blank human plasma spiked with cefiderocol at 1 mg/L (LLOQ), (B2) blank human plasma spiked with cefiderocol at 200 mg/L (ULOQ), (B3) d12-cefiderocol (IS of cefiderocol) at 10 mg/L, (B4) blank human plasma without cefiderocol, (B5) real patient sample treated with cefiderocol at 15min after the end of infusion.



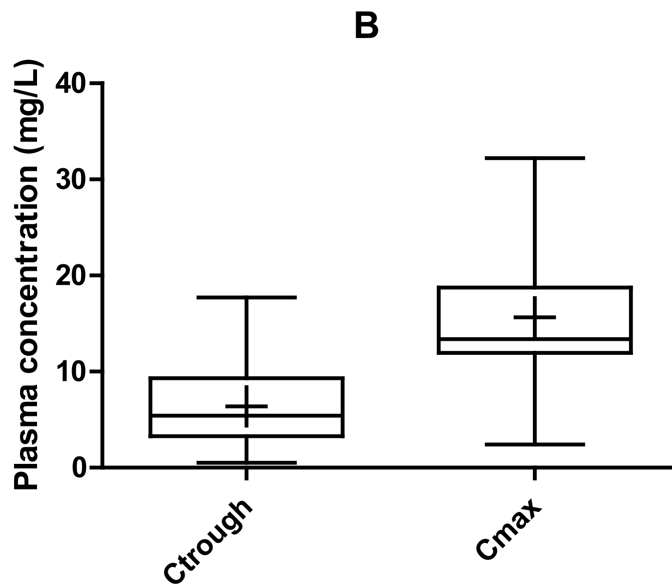
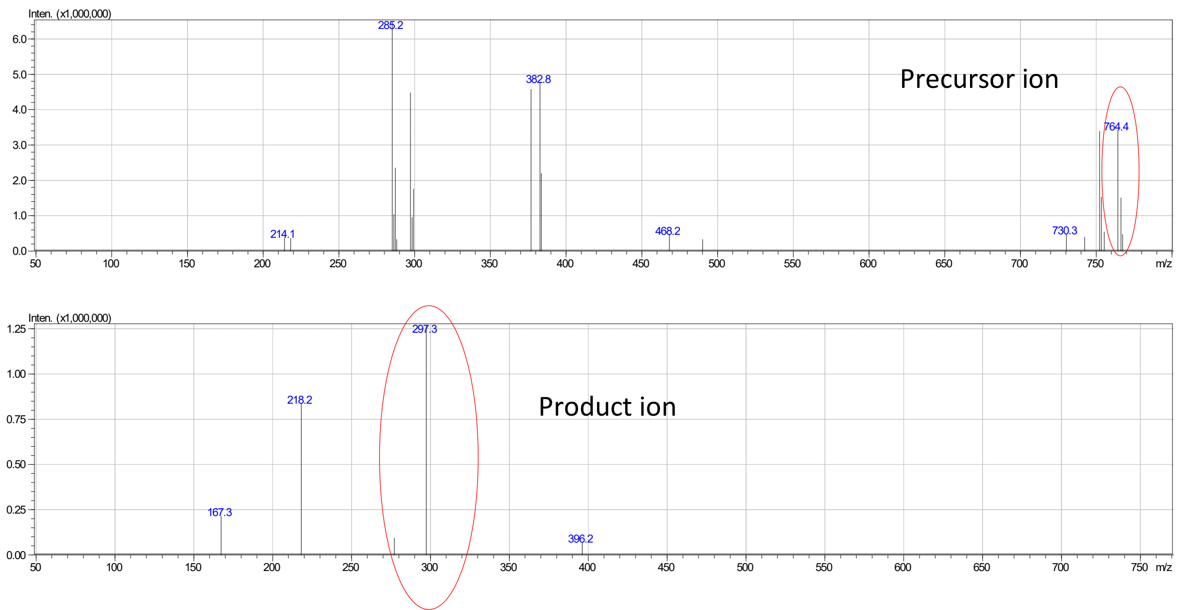
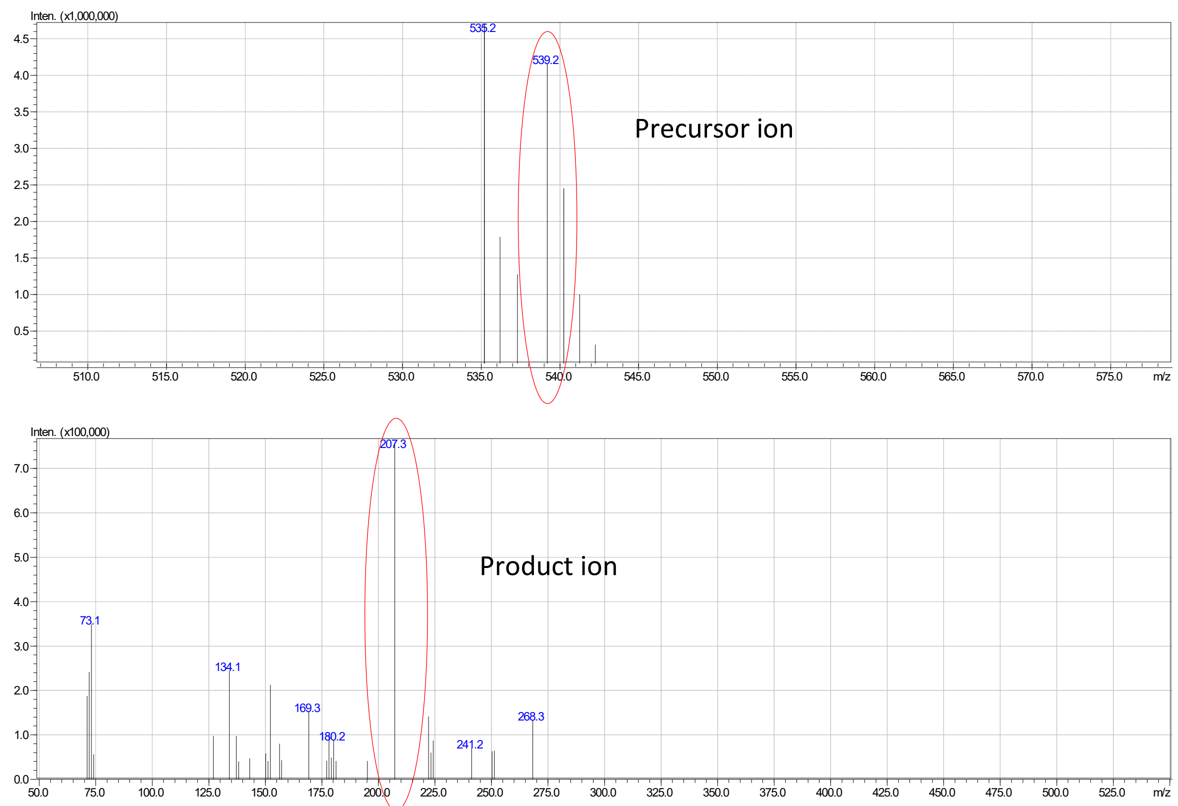


Figure 3. Observed (A) cefiderocol (n = 10) and (B) ceftobiprol (n = 25) plasma concentration at steady-state Ctrough (the median time of blood collection was 2 [1-5] days after start treatment) and at Cmax (samples were collected at 15 ± 10 min after the end of infusion). The box plot show the interquartile and extreme range, the mean (+) and median (horizontal line) values for each group.

A. [$^2H_{12}$]-cefiderocol



B. [$^2H_4$]-ceftobiprole



Supplementary Figure 1. Mass spectrum of (A) [$^2H_{12}$]-cefiderocol and (B) [$^2H_4$]-ceftobiprole

Table 1. Analyte quantification characteristics

Analyte	Retention Time (min)	Precursor (*m/z*)	Product (*m/z*)	Dwell Time (msec)	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Cefiderocol	2,69	752,10	285,20 (Quantifier ion)	1,0	-34,0	-20,0	-18,0
		752,10	214,20 (Qualifier ion)	1,0	-34,0	-52,0	-21,0
Ceftobiprol	0,46	535,00	203,30 (Quantifier ion)	1,6	-24,0	-31,0	-19,0
		535,00	69,10 (Qualifier ion)	1,6	-24,0	-32,0	-27,0
[$2H_{12}$]-Cefiderocol	2,69	764,10	297,30	1,0	-36,0	-21,0	-19,0
[$2H_4$]-Ceftobiprol	0,46	539,30	207,30	1,6	-24,0	-31,0	-20,0

CE: collision energy

Table 2. Summarized results of the precision and accuracy experiments.

QC levels	NC (mg/L)	Intra-day				Inter-day			
		n	Mean measured concentration (mg/L)	CV (%)	Accuracy (%)	n	Mean measured concentration (mg/L)	CV (%)	Accuracy (%)
Cefiderocol									
LLOQ	1	6	1,18	7,8	118	18	1,15	14,7	115
LQC	2,5	6	2,62	5,3	105	18	2,38	7,2	95
MQC	25	6	22,8	3,3	91	18	23,3	7,1	92
HQC	160	6	173	2,6	108	18	163	4,5	102
Ceftobiprol									
LLOQ	0,5	6	0,58	12,5	116	18	0,54	15,2	108
LQC	1	6	0,95	4,7	95	18	0,97	10,2	97
MQC	10	6	9,4	4,2	94	18	9,9	7,7	99
HQC	80	6	77,5	3,6	97	18	79,2	6,4	99

NC: nominal concentration, n: number of replicate, CV: coefficient of variation

Table 3. Absolute matrix effect (ME), IS-normalized ME and absolute extraction recovery (ER) at low and high concentration with their corresponding CVs (n = 5).

Analyte	Low concentration			High concentration		
	ME% (CV%)	IS-normalized ME% (CV%)	ER% (CV%)	ME% (CV%)	IS-normalized ME% (CV%)	ER% (CV%)
Cefiderocol	145 (4,6)	105 (7,0)	90 (9,6)	178 (7,6)	104 (9,2)	99 (6,1)
Ceftobiprol	102 (4,1)	107 (6,5)	93 (6,0)	96 (1,4)	102 (2,8)	103 (8,2)

n: number of replicates

Table 4. Stability under different storage conditions (n = 3).

Storage condition	Cefiderocol			Ceftobiprol		
	Nominal Concentration (mg/L)	Mean Measured (mg/L) (CV%)	Accuracy (%)	Nominal Concentration (mg/L)	Mean Measured (mg/L) (CV%)	Accuracy (%)
Bench-top stability: 25°C up to 8h						
LQC	2,5	2,25 (11,7)	90	1	0,89 (8,7)	89
MQC	25	21,5 (4,5)	86	10	9,5 (5,4)	95
HQC	160	160 (2,6)	100	80	72,2 (5,7)	90
Fridge stability: 4-8°C up to 24h						
LQC	2,5	2,28 (4,3)	91	1	1,12 (8,4)	110
MQC	25	25,5 (3,5)	102	10	9,8 (6,2)	98
HQC	160	155 (3,0)	97	80	73,6 (3,6)	92
Autosampler extract stability: 8°C up to 24h						
LQC	2,5	2,43 (4,5)	97	1	1,05 (13,3)	105
MQC	25	25,3 (8,3)	101	10	9,9 (3,0)	99
HQC	160	170 (6,5)	106	80	73,6 (7,3)	92
Freeze-thaw stability: 3 cycles of -80°C to 25°C						
LQC	2,5	2,55 (12,2)	102	1	1,04 (12,5)	104
MQC	25	23,5 (3,4)	94	10	10,0 (9,0)	100
HQC	160	157 (5,7)	98	80	77,6 (5,1)	97
Freezer stability: -25°C up to 1 week						
LQC	2,5	2,2 (4,8)	89	1	1,14 (13,6)	111
MQC	25	24,3 (3,9)	97	10	10,9 (3,6)	109
HQC	160	150 (1,9)	94	80	72,2 (3,2)	90
Long term stability: -80°C up to 2 months						
LQC	2,5	2,23 (1,2)	89	1	0,95 (7,0)	95
MQC	25	25,1 (1,8)	100	10	9,9 (1,5)	99
HQC	160	157 (3,3)	98	80	83,2 (7,1)	104

n: number of replicates