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Triple quadrupole LC/MS method for the simultaneous quantitative measurement of cefiderocol and meropenem in serum†

Alaa Riezk, *[a] Vasin Vasikasin,[a] Richard C. Wilson,[a] Timothy M. Rawson,[a] James G. McLeod,[a] Rishi Dhillon,[b] Jamie Duckers,[b] Anthony E. G. Cass [c] and Alison H. Holmes[a]

Background: therapeutic drug monitoring is a crucial aspect of the management of hospitalized patients. The correct dosage of antibiotics is imperative to ensure their adequate exposure specially in critically ill patients. The aim of this study is to establish and validate a robust and fast liquid chromatography-tandem mass spectrometry (LC/MS) method for the simultaneous quantification of two important antibiotics in critically ill patients, cefiderocol and meropenem in human plasma. *Methods*: sample clean-up was performed by protein precipitation using acetonitrile. Reverse phase chromatography was performed using triple quadrupole LC/MS. The mobile phase was consisted of 55% methanol in water +0.1% formic acid, with flow rate of 0.4 ml min^{-1}. Antibiotics stability was assessed at different temperatures. Serum protein binding was assessed using ultrafiltration devices. *Results*: chromatographic separation was achieved within 1.5 minutes for all analytes. Validation has demonstrated the method to be linear over the range 0.0025–50 mg L^{-1} for cefiderocol and 0.00028–50 mg L^{-1} for meropenem, with accuracy of 94–101% and highly sensitive, with LLOQ ≈ 0.02 mg L^{-1} and 0.003 mg L^{-1} for cefiderocol and meropenem, respectively. Both cefiderocol and meropenem showed a good stability at room temperature over 6 h, and at (4 °C) over 24 h. Cefiderocol and meropenem demonstrated a protein binding of 49–60% and 98%, respectively in human plasma. *Conclusion*: the developed method is simple, rapid, accurate and clinically applicable for the quantification of cefiderocol and meropenem.

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Introduction

Carbapenems are prescribed in almost two-thirds of all critically ill patients with evidence of bacterial infection.[1] Of the carbapenem class of beta-lactam antibiotics, meropenem is the most widely prescribed[2] and is the commonest agent used in the treatment of hospital acquired infections.[3] In critically ill patients, we observe wide variations in beta-lactam pharmacokinetics and plasma concentration. Failure to achieve optimal plasma concentrations is associated with worse clinical outcomes for critically ill patients.[4] Therefore, meropenem is one of the most important beta-lactams needing therapeutic drug monitoring (TDM).[5] The use of TDM-guided dose optimisation of meropenem in critically ill patients improves clinical outcomes with recent data demonstrating trends toward lower mortality.[6]

Carbapenem resistance, especially carbapenemase-producing carbapenem-resistant Enterobacterales (CP-CRE) is increasing in incidence globally.[7–9] Meropenem is ineffective for CP-CRE. CP-CRE is associated with mortality rates as high as 50%, posing a significant global threat to modern medicine.[10] CP-CRE have been categorised by the World Health Organisation as critical priority pathogens for discovery, research, and development of new antibiotics.[11]

Cefiderocol is a siderophore cephalosporin with a novel side chain that facilitates entry into cells. It has been demonstrated to retain activity against many carbapenem resistant Gram-negative bacteria and has been newly approved in many countries. Since its approval, cefiderocol has rapidly become the treatment of choice for metallo-beta-lactamase producing CP-CRE and an alternative option for other groups of CP-CRE.[12] However, pharmacokinetic and pharmacodynamic (PK/PD) data is still limited.[13] Individualized dosage may be necessary, especially for critically ill patients to promote clinical efficacy and prolong its effectiveness.[14]

A sensitive and reliable method for the simultaneous quantification of cefiderocol and meropenem in short time and using small serum volume is required to determine the higher-

[a]*Centre for Antimicrobial Optimisation, Imperial College London, Hammersmith Hospital, Hammersmith Campus, Du Cane Road, Acton, London, W12 0NN, UK. E-mail: a.riezk@imperial.ac.uk; alaa.riezk@yahoo.com; Tel: +44 (0)20 3313 2732*

[b]*Public Health Wales Microbiology, University Hospital of Wales, Heath Park Cardiff, UK*

[c]*Department of Chemistry, Molecular Sciences Research Hub, Imperial College, London, UK*

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or under-dosing of the at-risk patients particularly in the case of parallel administration of different antimicrobials and to perform individual adjustments of treatment algorithms. Another practical advantage of simultaneous quantification is improving laboratory workflow to save resources.

Plasma protein binding of antibiotics has important role on their both pharmacokinetics and pharmacodynamics.[15] This study aimed to develop a fast, sensitive, and clinically applicable triple quadrupole liquid chromatography mass spectrometry (TQ LC/MS) method to simultaneously measure both total and free serum concentrations of the two important antibiotics in critically ill patients, cefiderocol and meropenem. To our knowledge, only one study is published on the analysis of cefiderocol concentrations in human serum using LC/MS, and this is the first study to measure simultaneously two important antibiotics in critically ill patients, cefiderocol and meropenem.

Materials and methods

Chemicals and reagents

Cefiderocol (CAS-no – 1225208-94-5, purity: 95.1%), meropenem (CAS-no – 1217976-95-8, purity: 99.4%), [2H8]-cefiderocol (CAS-no – 1225208-94-5, purity: 95.5%), [2H6]-meropenem (CAS-no – 1217976-95-8, purity: 98.2%) were purchased from Alsachim (Illkirch, France) (Fig. 1). LC/MS grade methanol, formic acid, water, and acetonitrile were purchased from Sigma (St. Louis, MO, USA).

Instrumentation and analytical conditions

Chromatography and mass-spectrometric detection were performed using a 1290 Infinity II LC equipped with a pump coupled to an Ultivo TQ LC/MS. Chromatographic separation was performed on a reversed-phase analytical column ZORBAX RR Eclipse Plus C18, 95 Å, 4.6 × 50 mm, 3.5 μm. Data were acquired and analysed using MassHunter software version 10 (Agilent, Santa Clara, CA, USA).

Chromatography used a mobile phase consisting of 55% of methanol in water +0.1% formic acid. Chromatographic separation was performed at a column temperature of 25 °C using a 0.4 ml min^{-1} flow rate, with injection of 2 μl and a 5.5 minute run time. Electrospray Ionisation (ESI) was used for the detection of cefiderocol and meropenem with multiple reaction monitoring (MRM) in positive mode both qualitative and quantitative data were collected.

Triple quadrupole detector transitions were used for quantification and qualification, as follows: *m/z* 384[M + 1]; → *m/z* 68; 141.1 (for meropenem); *m/z* 389.5 [M +1]; → *m/z* 68; 260.1 (for meropenem IS); *m/z* 752.2 [M +1]; → *m/z* 285; 214.1 (for cefiderocol); *m/z* 760.3 [M +1]; → *m/z* 213.9; 292.9 (for cefiderocol IS).

Source parameters were optimised using the Agilent Source optimizer program with the following results: capillary voltage = 4500 V, gas flow = 9 L min^{-1}, gas temperature = 260 °C, nebulizer = 30 psi, sheath, sheath gas flow = 12 L min^{-1} and gas temperature = 300 °C.

Sample and calibration preparation

Blank serum samples were obtained as a part of a clinical trial evaluating PK/PD of antibiotics in patients.

Serum samples were kept at −80 °C for further analysis. Blank serum samples were subsequently spiked with 12 different 3-fold serial dilution concentrations (0.00028–50 mg L^{-1}) of meropenem and 10 different 3-fold dilution concentrations (0.0025–50 mg L^{-1}) of cefiderocol. Internal standard (stored at −80 °C, prepared weekly) was added to each calibrator and sample at 2.5 mg L^{-1}. Fifty microliter of serum was added to 150 μL of

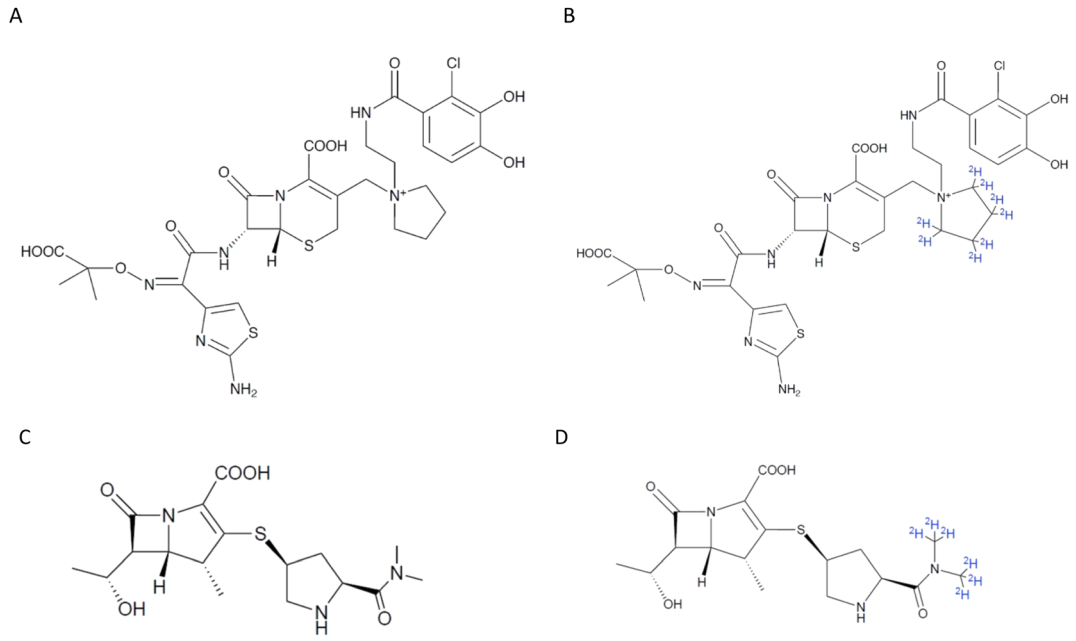

Fig. 1 Chemical structures of standard compounds. (A) cefiderocol, (B) cefiderocol, IS, (C) meropenem and (D) meropenem, IS.

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acetonitrile, vortexed, and allowed to equilibrate for 10 minutes. After which, precipitated proteins were separated by centrifugation for 10 minutes at 14 000 × *g*. The supernatant was collected and analysed onto the LC/MS system.

Quality control specimens were prepared independently of standards in serum at three concentration levels (cefiderocol: Qc1; high; 50 mg L^{-1}; Qc2; medium; 0.617 mg L^{-1}, and Qc3; low; 0.0025 mg L^{-1}; meropenem: Qc1; high; 50 mg L^{-1}; Qc2; medium; 0.205 mg L^{-1}, and Qc3; low; 0.00028 mg L^{-1}). Aliquots of Qc specimen were stored at −80 °C. Signal-to-noise ratio (S/N)—the ratio of the analyte signal to the noise measured on a blank was measured using MassHunter software. This software allows noise to be auto-integrated, measuring the baseline at a pre-fixed time interval near the analyte peak.

Stability experiments

Quality controls in serum samples (Qc1, Qc2, and Qc3) were stored at room temperature (RT) for 6 hours, after which, these sample were analysed, and the data extrapolated to time zero were compared to evaluate the short-term stability of the antibiotic-containing serum samples. Similarly, the stability of samples in the autosampler was assessed at 4 °C. The long-term stability of analytes was assessed after freezing the serum samples at −80 °C for 3 months.

The freeze–thaw stability of analytes was evaluated over three cycles within 3 days. In each cycle, Qc1, Qc2 and Qc3 were kept frozen at −80 °C and thawed at RT. When completely thawed, the samples were refrozen for 24 hours at −80 °C.

The stability was expressed as follows:

$$\mathrm{St}\% = C_0/C_t \times 100$$

where C_0 is the initial concentration, determined without introducing any extra pauses in the analysis process, and C_t is the concentration obtained when analysis is carried out with a pause of duration t in the analysis.

Method validation and quality characterization

The analytical method was validated according the International Conference on Harmonization guidelines.[16] A triplicate run of calibration standards was carried out every day for three consecutive days and triplicates of quality controls samples (at low Qc1, medium Qc2, and high Qc3 concentrations), were performed to evaluate the robustness of the developed method.

Matrix effects

Standard solutions were prepared at appropriate concentrations depending on the expected drug level in three different sets. In the first set, standards of cefiderocol and meropenem and their internal standards (IS) were prepared in the mobile phase. A second set was made in blank serum samples. The matrix effect was evaluated by comparing the peak area measurements obtained from the standard solutions:[17]

$$\mathrm{ME}\ (\%) = B/A \times 100$$

A: the peak areas obtained from set 1, *B*: the peak areas obtained from set 2 or 3. A value of 100% indicates that there is no absolute matrix effect.

Linearity

To assess linearity of the developed method, nine calibration standards were run for each analyte in triplicate over 3 consecutive days.

Selectivity, precision and accuracy

The lower limit of quantification (LLOQ) is the lowest concentration of analyte in a sample which can be quantified reliably, with an acceptable accuracy and precision.

Selectivity was assessed by analysing six different blank serum samples and confirmed by the absence of peaks at the respective retention times. Precision (inter and intra-day reproducibility) of the developed method was evaluated in terms of relative standard deviation (RSD) for the analysis of Qc1, Qc2 and Qc3 samples in triplicate in the same day for intra-day precision. Inter-day precision was determined by the analysis of triplicate QC samples on six consecutive days. Accuracy of the method was determined by the percentage agreement between the measured and spiked concentration of the Qc samples.

$$\text{Accuracy (percentage)} = (\text{measured/known spiked}) \times 100$$

Carry-over

Carry-over of analytes and IS was assessed by analysing blank samples after the highest standard samples concentration. Then, standard samples at the lower limit of quantification were also analysed after the blank samples. The experiment was repeated three times. Carry-over in the blank samples should not be greater than 20% of the analyte's response at the lower limit of quantification and 5% of the response for the IS.

Pharmacokinetic study

Serum samples from 2 separate clinical studies evaluating serum level of cefiderocol and meropenem were included in this study as an example of the application of this method to clinical samples.

The cefiderocol study was approved by the Cardiff University Biobank Ethics Committee (ref. 18/WA/0089). Samples were provided through the Cardiff University Biobank, Application Number CUB-2104-14-0027. Participants of the cefiderocol study received cefiderocol 2 g intravenously every 8 hours. Written informed patient consent was taken from each participant.

The meropenem study was approved by the London-Bromley Research Ethics Committee (ref. 16/LO/2179) and registered on **https://www.clinicaltrials.gov** (NCT03033394). Participants of the meropenem study received meropenem 1 g intravenously every 8 hours. Written informed patient consent was taken from each participant.

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Serum protein binding

A method to quantify cefiderocol and meropenem fractions bound and unbound to serum proteins was developed by using Centrifree Ultrafiltration Device (Millipore, Bedford, MA). The influence of temperature, pH and relative centrifugation forces (RCF) on the protein binding was investigated. Accordingly, the optimised condition for a higher recover was when unbound cefiderocol was quantified by pipetting 0.5 mL of serum at pH of 7.4 using 0.1 M sodium phosphate buffer, centrifuging at 1500*g* for 10 minutes at 25 °C. The filtrate was then analysed by TQ LC/MS as described before.

Results and discussion

The aim of the project was to develop a reliable and rapid analytical method for the quantification of cefiderocol and meropenem in human serum to provide reliable pharmacokinetic data after drug administration, including towards the end of a dosage interval when serum concentrations are low.

Different combinations and gradients of mobile phases (consisting of water and acetonitrile or methanol) and reverse-phase uHPLC columns were assessed. Other parameters, such as column oven temperature and injection volume, were evaluated to obtain the simultaneous measurements of the concentrations of the analytes with a short retention time and high response. Optimised conditions consisted of a mobile phase of 55% methanol in water + 0.1% formic acid, 25 °C column temperature, 2 μL injection and a flow rate of 0.4 ml min^{-1}. This achieved the highest MS response and produced quite sharp peaks at retention times at 1.2 minutes for cefiderocol and cefiderocol IS, and 1.1 minutes for meropenem and meropenem IS (Fig. 1S†). The observed chromatographic data were used in quantification.

Sample pre-treatment was optimised. Deproteination processes were analysed using different volumes of acetonitrile and samples. The selected process was 50 μl of sample and 150 μl of acetonitrile with recovery greater than 97%. Several steps of optimisation led to a developed method with isocratic elution, short retention time, small sample volume and a greatly improved LLOQ. LLOQ ≈ 0.02 mg L^{-1} and 0.003 mg L^{-1} for cefiderocol and meropenem, respectively.

The relative detector response (peak area), when plotted *vs.* injected relative concentration to IS was found to be linear over the concentration range of 0.0025–50 mg L^{-1} for cefiderocol and 0.00028–50 mg L^{-1} for meropenem with a correlation coefficient ($R^2 = 1$), Fig. 2S.†

Matrix effect recovery, accuracy and carry-over

The recovery was 96–105% for cefiderocol, 97–106% for cefiderocol, IS, 99–105% for meropenem and 94–103% for meropenem, IS in serum samples, indicating that the matrix effect did not significantly interfere with the calibration, as the internal standard corrected for any matrix effects.[18]

Accuracy and precision were tested in three different concentrations (Qc1, Qc2 and Qc3, Tables 1 and 2). Precision within-intra-day and inter-day run ranged from 0.4 to 1.1% for both cefiderocol and meropenem in serum samples. While within intra-day and inter-day run accuracies ranged from 94% to 101%. Carry-over for was considered acceptable for all analytes and IS with less than 4%.

Table 1 Inter- and intra-day variability, precision, and accuracy of the method for cefiderocol ($n = 6$ for inter-day assay and $n = 3$ for intra-day assay)

	Spiking level, mg L^{-1}	RSD %	Accuracy %
Intra-day			
Qc1	50	0.9	101
Qc2	0.617	0.4	94
Qc3	0.0025	0.8	96
Inter-day			
Qc1	50	1	95
Qc2	0.617	0.5	98
Qc3	0.0025	1.1	97

Table 2 Inter- and intra-day variability, precision, and accuracy of the method for meropenem ($n = 6$ for intra-day assay and $n = 3$ for inter-day assay)

	Spiking level, mg L^{-1}	RSD%	Accuracy%
Intra-day			
Qc1	50	0.7	100
Qc2	0.205	0.5	96
Qc3	0.00028	0.7	94
Inter-day			
Qc1	50	1.1	101
Qc2	0.205	0.4	95
Qc3	0.00028	0.6	99

Stability study

For meropenem and cefiderocol, long-term stability studies showed no significant degradation in Qc samples (in serum) stored at −80 °C for at least 3 months. Three times of freeze–thaw cycles did cause any significant effect on drugs stability (97–101%). Both drugs showed good stability within the range of 95 to 102% while kept at room temperature (RT) (23 ± 2 °C) for 6 h, and when kept in the autosampler (4 °C) over 24 h, remaining in the range of 99 to 102% from the original concentration.

Application to clinical samples

The method has been used in two clinical studies measuring total and free serum cefiderocol and meropenem concentrations in patients. Table 3 shows antibiotic concentrations obtained from patients treated with cefiderocol or meropenem. The observed antibiotic concentrations ranged from <0.02–91.65 mg L^{-1} for cefiderocol and 0.64–22.4 mg L^{-1} for meropenem. The geometric mean of fraction of unbound cefiderocol in serum (fu) was 0.55 (CV = 0.088). The protein binding was higher than a previous study in healthy subjects with different renal functions which reported fu at 0.35–0.47,[19] but

Table 3 Therapeutic monitoring of cefiderocol and meropenem (total and free antibiotic concentrations) on patient serum samples. The values are means ± SDs ($n = 3$)

Specimens	Antibiotics	Total ± SD, mg L^{-1}	Free ± SD, mg L^{-1}	f_u
Sample 1	Cefiderocol	<0.02	<0.02	—
Sample 2	Cefiderocol	77.68 ± 0.4	42.73 ± 0.2	0.55
Sample 3	Cefiderocol	77.28 ± 0.5	37.10 ± 0.3	0.48
Sample 4	Cefiderocol	91.12 ± 0.6	51.03 ± 0.5	0.56
Sample 5	Cefiderocol	91.65 ± 0.7	52.24 ± 0.6	0.57
Sample 6	Cefiderocol	67.26 ± 0.4	40.36 ± 0.4	0.60
Sample 7	Cefiderocol	68.39 ± 0.4	41.03 ± 0.4	0.60
Sample 8	Cefiderocol	39.16 ± 0.3	19.19 2	0.49
Sample 9	Meropenem	32.70 ± 0.4	31.78 3	0.97
Sample 10	Meropenem	1.33 ± 0.05	1.30 ± 0.04	0.98
Sample 11	Meropenem	0.65 ± 0.05	0.64 ± 0.05	0.98
Sample 12	Meropenem	22.40 ± 0.2	21.90 ± 0.2	0.99

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comparable to a study in spiked human serum which reported fu at 0.58.[20] Higher fu may be explained by the higher prevalence of hypoalbuminemia in patients than in healthy subjects.[21] The geometric mean of meropenem fu was 0.98 (CV = 0.0035), which was comparable to the manufacturer' information at 0.98.[22]

Conclusion

The method presented here has been successfully used in clinical studies measuring total and free serum cefiderocol and meropenem concentrations. It is rapid, accurate, has low LLOQ, and has a simple sample preparation. This may help in optimising treatment to improve patient outcomes whilst minimising the risk of development of antibiotic resistance.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Data availability statement

Data and materials are available from the authors on reasonable request.

Author contributions

Conceptualization: AR; RCW, TMR, VV, JGM, RD, JD, AEGC, AHH. Formal Analysis: AR. Investigation: AR; RCW, TMR, VV, AEGC, AHH. Methodology: AR. Project administration: AHH. Validation: AR, VV. Visualization: AR; RCW, TMR, VV, AEGC, AHH. Writing – original draft: VV, AR. Writing – review & editing: AR; RCW, TMR, VV, JGM, RD, JD, AEGC, AHH.

Conflicts of interest

Timothy M. Rawson has received honoraria from Sandoz (2020), bioMérieux (2021/2022) and Roche Diagnostics (2021). Alison H. Holmes is a Co-Supervisor of a PhD fellowship (James G. McLeod), funded through a collaborative programme involving industry partnerships. Industry partner: Shionogi. All other authors have no potential conflicts of interest to declare.

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