A validated LC-MSMS method for the simultaneous quantification of meropenem and vaborbactam in human plasma and renal replacement therapy effluent and its application to a pharmacokinetic study

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Abstract

A simple method for the simultaneous quantification of meropenem and the recently approved β -lactamase inhibitor, vaborbactam, in human plasma and renal replacement therapy effluent (RRTE) was developed and validated. This antibiotic combination protects a primary β -lactam, meropenem, with a new β -lactamase inhibitor, and expands the limited options for treatment of multidrug-resistant Gram-negative infections. Meropenem, vaborbactam, and the internal standards [$^2\text{H}_6$]-meropenem and sulbactam in plasma and RRTE were processed using acetonitrile followed by a chromatographic separation on a Poroshell HPH-C18 column with a gradient elution of the mobile phases and monitored using mass spectrometry detection. The calibration range was 0.05 to 100 $\mu\text{g mL}^{-1}$ for both meropenem and vaborbactam. The intra-day and inter-day precision and accuracy were less than 15% for both meropenem and vaborbactam and the recovery from plasma was 96% for both meropenem and vaborbactam and the recovery from RRTE was 93% and 103% for meropenem and vaborbactam, respectively. This methodology was successfully applied to an ex vivo characterisation study of the effects of renal replacement therapy modalities on the pharmacokinetics of meropenem and vaborbactam (Antimicrob Agents Chemother 62(10), 2018).

Keywords Meropenem · Vaborbactam · Chromatography · Mass spectrometry · Antibiotic

Introduction

The research and development of novel antibiotics to treat carbapenem-resistant Gram-negative bacteria have been identified by the World Health Organisation's (WHO) as being a critical priority [1]. Meropenem-vaborbactam is a combination

carbapenem and β -lactamase inhibitor antibiotic approved by the US Food and Drug Administration in late 2017 for treatment of complicated urinary tract infections [2] and by the European Medicines Agency in 2018 for complicated urinary tract infections, complicated intra-abdominal infections, and for hospital-acquired pneumonia, including ventilator-associated pneumonia [3]. This antibiotic protects a primary β -lactam, meropenem, with a new β -lactamase inhibitor, and expands the limited options for treatment of multidrug-resistant Gram-negative infections [4]. It is available under the trade-name Vabomere®, previously named Carbavance®. It is not uncommon for patients with infections from carbapenemase-resistant pathogens to develop severe renal impairment that necessitates the use of renal replacement therapy [5, 6]. There is accumulating evidence that this medical intervention can necessitate dosing adjustment of antibiotics due to altered and variable extracorporeal clearances [6–8]. As neither meropenem nor vaborbactam is metabolized, with both primarily excreted unchanged in the urine [9], no methods for the analysis of metabolites are required.

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Methodology to quantify drugs in biological fluids is important to support pre-clinical and clinical studies. Chromatographic methods have been reported for meropenem in plasma [10–23] and serum [24–27] using UV [12, 16, 26], diode array [15, 20, 28, 29], and mass spectrometry [10, 11, 13, 14, 17–19, 21–25, 27, 30] detection. To the best of our knowledge, there are currently no analytical methods reporting the quantification of the combination of meropenem-vaborbactam in biological fluids and no methods for the quantification of newly developed β -lactamase inhibitor, vaborbactam.

We have developed a method suitable for the reliable and sensitive quantification of meropenem and vaborbactam in human plasma and renal replacement therapy fluid effluent (RRTE) using LC-MS/MS. The advantages of this method include a small sample volume (10 μ L) with a simple protein precipitation sample treatment. This methodology has been applied to an ex vivo characterisation study of the effects of renal replacement therapy modalities on the pharmacokinetics of meropenem and vaborbactam [6].

Experimental

Materials

Meropenem and vaborbactam were supplied as a pharmaceutical combination product (Carbavance®) by Rempex Pharmaceuticals (San Diego, USA) containing 1 g of meropenem and 1 g of vaborbactam, [$^2\text{H}_6$]-meropenem was supplied by AlsaChim (Illkirch, France) and sulbactam was supplied by Sigma-Aldrich (St Louis, USA). The chemical structures for these compounds are found in Fig. 1. Acetonitrile was HPLC-gradient grade (Merck, Darmstadt, Germany) and formic acid (Fisher Scientific, Victoria, Australia) was analytical grade. Ultrapure water was obtained using a Permutit system (resistivity at 25 °C greater than 18 ΩMcm). Drug-free human plasma was obtained from Innovative Research (Michigan, USA). Compound sodium lactate IV solution was obtained from Baxter (Old Toongabbie, Australia). For drug-free RRTE, ultrafiltered drug-free plasma was combined with compound sodium lactate solution (10/90% v/v).

Chromatographic conditions

A Shimadzu 8030+ triple quadrupole mass spectrometer (MS) detector was used in this analysis. An electro-spray ionisation (ESI) source interface, switching between both positive ion (for meropenem and [$^2\text{H}_6$]-meropenem) and negative ion mode (for vaborbactam and sulbactam), was used for the selected reaction monitoring (SRM) LC-MS/MS analysis. Nitrogen was used as the nebulizing gas, with interface setting

consisting of the nebulizing gas flow of 3 L min^{-1} , a desolvation line temperature of 250 °C, heat block temperature 400 °C, and a drying gas flow of 10 L min^{-1} . The collision gas was argon. Meropenem and [$^2\text{H}_6$]-meropenem were monitored in positive mode electrospray. Vaborbactam and sulbactam were monitored by negative mode electrospray. MS conditions for meropenem, [$^2\text{H}_6$]-meropenem, vaborbactam, and sulbactam are reported in Table 1.

The liquid chromatographic analysis was performed using a Shimadzu Nexera2 LC equipped with dual pumps and an autosampler compartment set to 5 °C. The analytical column was a Poroshell HPH-C18 2.1 $\times$ 50 mm, 2.7 μm column (Agilent, USA) operated at room temperature. Mobile phase A was 0.1% formic acid in water (v/v), and mobile phase B was 100% acetonitrile with 0.1% formic acid (v/v). The mobile phase B was delivered with gradient from 5 to 50% at a flow rate of 0.4 mL min^{-1} for 5.5 min run-time and produced a back pressure of approximately 3300 psi.

Preparation of the calibration standards and quality control samples

Meropenem-vaborbactam is co-formulated to contain equal amounts of meropenem and vaborbactam. Aqueous standards were prepared from the co-formulated standard with a concentration of 50, 100, and 1000 mg mL^{-1} for meropenem and vaborbactam. [$^2\text{H}_6$]-meropenem and sulbactam were used as internal standards. [$^2\text{H}_6$]-meropenem was prepared to contain 100 $\mu\text{g mL}^{-1}$ in water and sulbactam was prepared to contain 1550 $\mu\text{g mL}^{-1}$ in water. These internal standard stock solutions were combined to prepare a working solution containing 2 $\mu\text{g mL}^{-1}$ of [$^2\text{H}_6$]-meropenem and 50 $\mu\text{g mL}^{-1}$ of sulbactam. All aqueous stock solutions were stored at or below -20 °C.

On the day of assay, calibration standards were prepared by serial dilution with water to contain both meropenem and vaborbactam in concentrations ranging from 0.05 to 100 $\mu\text{g mL}^{-1}$. Each calibration range contained eleven (11) calibration standards at nominal concentrations of 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, and 100 $\mu\text{g mL}^{-1}$. Calibration standard solutions were prepared by combining these solutions with an equal volume of drug-free matrix (plasma or RRTE). The calibration standards were processed alongside the study and quality control samples.

Quality control samples were prepared from an aqueous stock solutions prepared to contain 100, 500, and 2000 mg mL^{-1} of meropenem and vaborbactam and stored at -80 °C. Stock solutions were diluted with drug-free matrix (plasma or RRTE). Quality control samples were prepared to contain both meropenem and vaborbactam at concentrations of 0.3, 1.5, 10, and 80 $\mu\text{g mL}^{-1}$, with the dilution of drug-free matrix by aqueous stock maintained below 5%. Quality control samples were stored at -80 °C alongside the study samples.

Fig. 1 Structures of meropenem (a), [$^2\text{H}_6$]-meropenem (b), vaborbactam (c), and sulbactam (d)

Sample preparation for analysis

An aliquot (10 μL) of study sample (plasma or RRTE) was combined with 10 μL of water (to correspond to the dilution during preparation of the calibration standards). All samples were briefly vortexed followed by the addition of 10 μL of the internal standard working solution (containing 2 $\mu\text{g mL}^{-1}$ of [$^2\text{H}_6$]-meropenem and 50 $\mu\text{g mL}^{-1}$ of sulbactam), except for a drug-free matrix sample which received 10 μL of water. Samples were again vortexed briefly and 40 μL of acetonitrile was added to all samples. Samples were vortexed for 3 s and then centrifuged for 5 min at 4000g to remove precipitated proteins. The resulting supernatant was injected into the LC-MS/MS (sample injection volume of 0.2 μL).

Data analysis

For both meropenem and vaborbactam, the concentration of each study sample and quality control sample was obtained using data from the calibration line prepared from calibration standards within each batch. A quadratic regression based on peak area ratio (analyte/internal standard peak area response) against concentration (x) with a $1/x^2$ weighting was used for the mathematical basis of calibration line calculations.

Method validation

The bioanalytical method validation was performed with reference to the guidelines provided by the US FDA [31] and

Table 1 Mass spectrometry settings

Analyte	Meropenem ^a	Vaborbactam ^b	[$^2\text{H}_6$]-meropenem ^a	Sulbactam ^b
Precursor ion	384	296	390	232
Product ion 1 (quantifying ion)	68	234	147	140
Product ion 2 (qualifier ion)	141	278	NA	NA
Dwell time (ms)	100	100	100	100
Q1 (V)	−19	14	−15	25
CE (V)	−45	21	−20	14
Q3 (V)	−27	24	−15	24

^a Precursor and product ions measured as $[\text{MH}^+]$

^b Precursor and products ion measured as $[\text{MH}^-]$

EMA [32]. The validation was assessed for limit of detection (LOD), lower limit of quantification (LLOQ), linearity, inter- and intra-day precision and accuracy, selectivity, recovery, matrix effects, and stability.

Linearity, LOD, LLOQ, accuracy, and precision

Linearity was assessed using data from calibration curves on six occasions across a calibration range of 0.05 to 100 $\mu\text{g mL}^{-1}$ for both meropenem and vaborbactam in plasma, with the LLOQ tested at 0.05 $\mu\text{g mL}^{-1}$ ($n = 5$) with a pre-established acceptance criteria of $\pm 20\%$ precision and accuracy. The LOD was defined as being the lowest peak distinguishable from the background noise, and calculated as greater than three-times the noise of the blank matrix sample. An assessment of accuracy and precision was performed from data obtained from the analysis of quality control samples at all four concentrations for meropenem and vaborbactam, which were assayed alongside freshly prepared calibration lines. Accuracy (percent relative error, %RE) and precision (percent coefficient of variation, %CV) was calculated against nominal quality control sample concentrations with a pre-established acceptance criteria of $\pm 15\%$ precision and accuracy.

Recovery, matrix effects, and selectivity

Three different sets of samples were prepared: one prepared in drug-free matrix spiked before extraction (set A), one

prepared in drug-free matrix and spiked after extraction (set B), and one prepared in water (set C). The extraction recovery was determined by the responses of set A relative to set B. The matrix effects were calculated as described by Matsuzewski [33] by comparing the results of set B to set C with a pre-established acceptance criteria of $\pm 15\%$ precision.

Selectivity testing was assessed to determine the influence of the presence of components of plasma and RRTE on the quantification of meropenem and vaborbactam. This was evaluated by testing different sources of drug-free plasma that contained different types of anticoagulant (including lithium heparin, ethylenediaminetetraacetic acid, and sodium citrate). Testing was performed by spiking drug-free matrix at the lower limit of quantification and calculating the precision of the resulting concentrations with a pre-established acceptance criteria of $\pm 15\%$ precision and accuracy.

Stability studies

The stability of meropenem and vaborbactam was assessed in frozen storage ($-80\text{ }^{\circ}\text{C}$) and across three freeze-thaw cycles (from $-80\text{ }^{\circ}\text{C}$ to ambient temperature). This testing was performed using quality control samples prepared at four concentrations and comparing the results to nominal concentrations with a pre-established acceptance criteria of $\pm 15\%$ precision and accuracy. Stability of aqueous stock solutions stored at $-20\text{ }^{\circ}\text{C}$ was assessed by comparing the area of meropenem and vaborbactam in stored solutions to those obtained in freshly prepared solutions with a pre-established acceptance criteria of $\pm 5\%$ accuracy.

Fig. 2 LC-MSMS chromatograms for meropenem (MRP) and vaborbactam (VBT) at the lower limit of quantification (0.05 $\mu\text{g mL}^{-1}$) and internal standards [$^2\text{H}_6$]-meropenem (d6-MRP) and sulbactam (SBT) at the working concentrations in plasma (a) and renal replacement therapy effluent (b)

Table 2 Calibration analysis of meropenem and vaborbactam in plasma and renal replacement therapy effluent (RRTE) across concentrations from 0.05 to 100 $\mu\text{g mL}^{-1}$

Meropenem	Vaborbactam					
	Matrix	Slope	Intercept (mean $\pm$ SD)	Correlation coefficient (mean $\pm$ SD)	Maximum deviation* (%)	Maximum deviation* (%)
		linear term (mean $\pm$ SD)	quadratic term (mean $\pm$ SD)	linear term (mean $\pm$ SD)	quadratic term (mean $\pm$ SD)	
Plasma ($n = 6$)		1.40 ± 0.187	-0.000585 ± 0.000991	0.00793 ± 0.0108	0.9959 ± 0.0019	$\pm 15.0\%$
RRTE ($n = 6$)		1.88 ± 1.42	-0.000995 ± 0.000985	0.0101 ± 0.0100	0.9955 ± 0.0022	$\pm 13.0\%$
					Plasma ($n = 4$)	1.14 ± 0.270
					RRTE ($n = 6$)	1.28 ± 0.178
						0.00284 ± 0.0200
						0.00715 ± 0.0174
						0.9962 ± 0.0010
						$\pm 13.1\%$
						0.9948 ± 0.0015
						$\pm 11.5\%$

*Reported maximum deviation from nominal (%) across all standard curves and all concentration levels

SD standard deviation, RRTE renal replacement therapy effluent

Table 3 Intra- and inter-assay precision and accuracy for meropenem and vaborbactam in plasma and RRTE

Meropenem	Vaborbactam									
	Plasma					RRTE				
	Mean ($\mu\text{g mL}^{-1}$)	Precision (%)	Accuracy (%)	Mean ($\mu\text{g mL}^{-1}$)	Precision (%)	Accuracy (%)	Mean ($\mu\text{g mL}^{-1}$)	Precision (%)	Accuracy (%)	Precision (%)
Intra- ($n = 5$)	0.054	13.6	108	0.054	6.0	108	0.053	3.1	106	5.9
	1.55	9.3	104	1.46	6.3	97.6	1.64	3.0	110	3.2
	10.4	3.7	104	9.7	3.6	97.0	11.5	5.4	115	4.8
	71.6	3.9	89.5	73.1	2.6	91.4	77.1	4.3	96.4	6.8
Inter- ($n = 9$)	1.45 ^a	5.3	96.7	1.54 ^b	7.0	97.0	1.53 ^a	8.3	102	4.7
	9.90	6.8	99.0	10.3	5.7	103	10.2	7.7	102	4.8
	78.2	6.8	97.8	81.3	4.8	102	77.5	8.0	96.9	4.4

RRTE renal replacement therapy effluent

^a $n = 16$ ^b $n = 9$

Table 4 Matrix effect and recovery studies for meropenem and vaborbactam

Analyte		Meropenem				Vaborbactam			
Matrix		Plasma		RRTE		Plasma		RRTE	
Study	Nominal concentration ($\mu\text{g mL}^{-1}$)	Mean	Precision (%)	Mean	Precision (%)	Mean	Precision (%)	Mean	Precision (%)
Matrix factor ($\text{MF}_{\text{normalised}}$) ($n = 6$)	20	1.07	7.4	1.08	11.2	1.01	3.2	1.01	5.6
	50	1.08	8.1	0.95	12.7	1.00	7.2	1.02	4.8
	100	1.08	12.9	1.08	8.3	1.04	3.3	1.03	3.4
Recovery (%) ($n = 3$)	1.5	105	3.0	86.7	3.1	87.4	6.3	107	7.7
	10	92.4	9.3	110	9.0	90.4	12.1	103	6.5
	80	90.1	1.9	81.6	12.9	116.3	6.9	98.0	3.5

RRTE renal replacement therapy effluent

Ex vivo study of the effects of renal replacement therapy modalities

This methodology has been applied to the analysis of plasma and RRTE samples from an ex vivo study investigating the effects of renal replacement therapy modalities on the pharmacokinetics of meropenem and vaborbactam [6]. In this study, two concentrations of meropenem and vaborbactam (5 and $50 \mu\text{g mL}^{-1}$) were combined with drug-free human whole blood (maximum dilution of blood with aqueous standard was 0.1%). Whole blood was circulated through a Prismaflex CRRT System (Gambro Lundia, Sweden) and renal replacement fluid introduced into the circuit by pre- or post-filter dilution. Serial blood samples (1 mL) were collected at 30 , 60 , 120 , and 180 min . A sample of RRTE was removed from the effluent sampling port (1 mL). Blood samples were centrifuged (5 min at $2000g$) and both the resultant plasma and effluent samples were stored (within 15 min of collection) at -80°C until assay.

Results and discussion

Mass spectrometry

This method employs rapid ionisation switching between positive and negative modes to allow simultaneous monitoring of all analytes. Fragmentation patterns were manually selected with subsequent auto-optimization using pre-programmed routines to identify alternative-fragmentation patterns and to optimise voltages. The protonated meropenem fragmentation pattern is due to the cleavage of the C-S bond to yield the ion at 141 and losses of hydrogen cyanide (HCN), but-3-enitrile (CHN) and CO to yield the ion at 68 [34]. There are no reports describing the fragmentation pattern of vaborbactam. The fragmentation observed in this study to form an ion at 234 may be due to an α -cleavage for the loss of the carboxylic acid and the formation of an ion at 278 may be due to the loss of a hydroxyl ion. The stable labelled internal standard, [$^2\text{H}_6$]-meropenem, was selected as an internal standard for the analysis of meropenem. Sulbactam was selected as an internal standard for vaborbactam due to its structural similarity and

Table 5 Stability studies for meropenem and vaborbactam in plasma

Stability study	Nominal concentration ($\mu\text{g mL}^{-1}$)	Meropenem			Vaborbactam		
		Mean ($\mu\text{g mL}^{-1}$)	Accuracy (%)	Precision (%)	Mean ($\mu\text{g mL}^{-1}$)	Accuracy (%)	Precision (%)
Freeze-thaw ^a ($n = 3$)	0.3	0.292	97.2	3.5	0.319	106	12.3
	1.5	1.56	104	10.3	1.26	84.0	8.9
	10	9.85	98.5	4.3	9.04	90.4	2.7
	80	69.5	86.9	3.2	62.2	96.3	3.8
Long term ^b ($n = 3$)	1.5	1.45	96.6	5.2	1.33	88.3	1.7
	10	10.2	102	0.9	8.88	88.8	4.6
	80	76.0	94.9	9.2	82.8	96.5	4.8

^a Three freeze-thaw cycles

^b Stored at -80°C for 2 months

Fig. 3 Mean plasma concentrations ($\mu\text{g mL}^{-1}$) of meropenem (**a**) and vaborbactam (**b**) from an ex vivo study of the extent of adsorption of meropenem and vaborbactam (using plasma concentrations of $5 \mu\text{g mL}^{-1}$ (triangle) and $50 \mu\text{g mL}^{-1}$ (circle)) on a renal replacement therapy circuit. Errors bars represent the standard deviation

its similar behaviour during sample preparation and analysis [35], which compensated for variation in extraction efficiency and ionisation effects observed for vaborbactam. The identification of analyte peaks were confirmed using ion ratios calculated using the quantifying and qualifier ions (see Table 1), set at 95% for meropenem and 45% for vaborbactam, with the criteria for acceptance that all ion ratios fall within $\pm 20\%$.

Chromatography

LC-MS/MS chromatograms of samples extracted from plasma and RRTE at the LLOQ are shown in Fig. 2. The methodology was found to be suitable for both the proteaceous plasma and high salt concentration of RRTE. The retention time of meropenem and [$^2\text{H}_6$]-meropenem was 1.6 min, vaborbactam eluted at 1.7 min, and sulbactam at 1.3 min. The total run time for each sample was 5.5 min, allowing time for the column to re-equilibrate from the cycle of the mobile phase gradient. A needle-wash of 0.1% formic acid was used and no autosampler carry-over was observed during the validation or study batch analysis.

This was confirmed by using a run-order in which after each injection of a calibration standard of $100 \mu\text{g mL}^{-1}$, a drug-free and internal standard-free extracted matrix sample was injected.

Validation

The method was assessed across the validation concentration range of 0.05 to $100 \mu\text{g mL}^{-1}$ for both meropenem and vaborbactam in plasma and from 0.05 to $100 \mu\text{g mL}^{-1}$ for both meropenem and vaborbactam in RRTE. The concentration ranges selected for meropenem and vaborbactam were based on data from published clinical studies [7, 9, 36–38]. A quadratic regression with a $1/\text{concentration}^2$ weighting best described the response across the calibration range. The slope (linear and quadratic terms), intercept, mean correlation coefficient (r^2), and percent maximum deviation from the nominal concentration for the calibration standards were calculated from testing performed on different occasions for both meropenem and vaborbactam and the results (mean $\pm$ standard deviation) are reported in Table 2. The limit of detection for

meropenem in both plasma and RRTE was $0.01 \mu\text{g mL}^{-1}$, respectively, and for vaborbactam in both plasma and RRTE was $0.02 \mu\text{g mL}^{-1}$, respectively. Precision and accuracy was assessed on one occasion as an intra-assay assessment ($n = 5$; performed by one analyst on one instrument) and on eight occasions for plasma and on four occasions for RRTE as an inter-day assay assessment ($n = 16$ for plasma, $n = 9$ for RRTE; performed by one analyst on one instrument). The results are reported in Table 3, with all results meeting acceptance criteria. The lower limit of quantification results met acceptance criteria for meropenem and vaborbactam in both matrices (Table 3).

The matrix factor study found there was no unacceptable enhancement or suppression signal response for plasma or RRTE. The results are reported in Table 4. The mean recovery across the three concentrations tested for the protein precipitation of plasma was $95.7 \pm 7.7\%$ for meropenem and $95.7 \pm 7.0\%$ for vaborbactam. The mean recovery across the three concentrations tested for the processing of RRTE was $92.9 \pm 15.3\%$ for meropenem and $102.6 \pm 4.4\%$ for vaborbactam. Recovery results for both analytes and at each concentration for plasma and RRTE are reported in Table 4.

The results of the selectivity testing found that when introducing variability in terms of different sources of plasma and types of anticoagulant at the LLOQ, the method was able to achieve an accuracy of 110% and 113% and precision of 8.5% and 14.8% for meropenem in plasma and RRTE, respectively. At the LLOQ, the method was able to achieve an accuracy of 94.8% and 98.9% and precision of 7.7% and 11.7% for vaborbactam in plasma and RRTE, respectively. Furthermore, there was no evidence of interference from the matrix, on the basis of there being no observable matrix enhancement or suppression or observable peak at the retention time of the peaks of interest.

Stability testing performed for three freeze-thaw cycles and long-term storage of 2 months found both meropenem and vaborbactam in plasma were stable in these conditions. The results are reported in Table 5. Stock solutions tested after storage of 1.5 months at -80°C showed acceptable deviations from freshly prepared solutions of -0.2% for meropenem and $+4.4\%$ for vaborbactam. Storage of processed samples on an autosampler at 4°C for 1 day found acceptable deviation of -7.6% for meropenem and 3.3% for vaborbactam, thus allowing re-injection of samples if an instrumental failure was to occur.

Application to an ex vivo study of renal replacement therapy modalities

This methodology has been successfully applied to an ex vivo study investigating the effect of renal replacement therapy on the pharmacokinetics of meropenem and vaborbactam [6]. Mean plasma concentrations of

meropenem and vaborbactam collected over 3 h with a blood flow rate of 200 mL min^{-1} circulated through a Prismaflex CRRT System are presented in Fig. 3 ($n = 3$). Renal replacement therapy effluent concentrations, with two blood flow rates (100 or 200 mL min^{-1}), across a range of filtration flow rates (1 to 4 mL min^{-1}) and tested pre- and post-dilution, were measured ranging from 0.053 to $33.4 \mu\text{g mL}^{-1}$ for meropenem and 0.052 to $35.6 \mu\text{g mL}^{-1}$ of vaborbactam. Quality control samples tested within batches of study samples were prepared at 0.3 , 1.5 , 10 , and $80 \mu\text{g mL}^{-1}$ of meropenem and vaborbactam and the results for all quality control samples were within 13.6% precision and -93.5 to 106% accuracy.

Conclusion

A simple and reliable method for the simultaneous quantification of meropenem and vaborbactam in human plasma and renal replacement therapy fluid effluent (RRTE) was developed and validated. The method used a small sample volume ($10 \mu\text{L}$) and employed a single-step extraction followed by chromatographic separation and mass spectrometry detection. This methodology has been applied to an ex vivo characterisation study of the effects of renal replacement therapy modalities on the pharmacokinetics of meropenem and vaborbactam [6]. The method is also suitable for a clinical pharmacokinetic study of co-administered meropenem and vaborbactam in humans.

Authors' contributions and authorship S. L. Parker: method design, data analysis, interpretation of results, and writing of manuscript; S. Pandey: method design, data analysis, interpretation of results, and writing of manuscript; F. B. Sime: ex vivo study protocol design and writing of manuscript; J. A. Roberts: interpretation of results and writing of manuscript; S. C. Wallis: method design, data interpretation, and writing of manuscript.

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Compliance with ethical standards

The study was performed in accordance with the ethical standards, with ethical approval obtained for the use of drug-free human blood from the Royal Brisbane & Women's Hospital Human Research Ethics Committee HREC/15/QRBW/249.

Conflict of interest The authors declare that they have no conflict of interest.

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