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Abstract

Critically ill patients are often affected by several pathophysiological conditions requiring antibiotic administration and, frequently, extracorporeal therapy that significantly alter the normal pharmacokinetics of drugs. Therapeutic drug monitoring (TDM) may assist to establish the correct antibiotic dosage, but a TDM service is usually available only for some aminoglycosides and glycopeptides. The aim of this study is the validation of an HPLC-UV method for the simultaneous quantification of meropenem, vancomycin, piperacillin and tazobactam in human plasma samples.

The analytes were extracted from 250 µL of human plasma by the addition of acetonitrile for protein precipitation. After evaporation to dryness of the solvent, samples were reconstituted with 250 µL of mobile phase, and 100 µL were injected in HPLC. Chromatographic analysis was performed using a Kinetex C18 column and an UV/Vis detector set at 220 and 298 nm. The mobile phase was a mixture of phosphate buffer 0.1 M pH 3.15 and methanol in gradient, delivered at 1 mL/min.

The method was validated over clinical concentration ranges. For all the analytes, the lower limit of quantification was 1 µg/mL, and the calibration curves were linear between 1 and 100 µg/mL, with coefficients of ≥0.999. Intra-day precision was <4%, while inter-day precision was <7% for each analyte.

The applicability of the method has been evaluated by analysing plasma samples collected from 4 critically ill patients undergoing continuous renal replacement therapy. Moreover, the analysis of vancomycin with VANC Flex® confirmed a good correlation between the results of HPLC-UV and commercially available kits usually used by TDM service.

The method we developed only requires a small volume of plasma and uses a common sample preparation protocol, stationary phase and elution conditions for all analytes. This method offers the additional advantages of a rapid preparation and analysis time, and a simple and rather inexpensive instrumentation, features that make this method an easy implementation for a general TDM laboratory.

Keywords: HPLC-UV; vancomycin; meropenem; piperacillin /tazobactam; Therapeutic Drug Monitoring; Intensive Care Unit

Abbreviations:

Cefotaxime (CTX); Coefficient of variation (CV); Continuous Renal Replacement Therapy (CRRT); Extracorporeal Membrane Oxygenation (ECMO); High concentration quality control (HQC); High performance liquid chromatography with ultraviolet detection (HPLC-UV); Intensive Care Unit (ICU); Limit of detection (LOD); Limit of quantitation (LOQ); Liquid chromatography tandem mass spectrometry (LC-MS/MS); Low concentration quality control (LQC); Medium concentration quality control (MQC); Meropenem (MER); Multidrug resistance (MDR); Multiple Organ Failure (MOF); Particle-Enhanced Turbidimetric Inhibition Immunoassay (PETINIA); Piperacillin (PIP); Quality control (QC); Standard deviations (SD); Tazobactam (TAZ); Therapeutic Drug Monitoring (TDM); Vancomycin (VAN).

Highlights

- Critically ill patients often require extracorporeal therapies
- Antimicrobial pharmacokinetics is impaired in critically ill patients
- A validated HPLC-UV analytical method for most used antimicrobial is described
- The method is suitable for Therapeutic Drug Monitoring or experimental trials

1. Introduction

Sepsis and septic shock are the leading causes of morbidity and mortality in critically ill patients worldwide [1]. In Intensive Care Unit (ICU), antimicrobial drugs are usually administered based on the results of the studies on healthy volunteers but, on the other hand, critically ill patients are characterised by marked homeostatic disturbance and an altered organ function that leads to Multiple Organ Failure (MOF) [2]. Furthermore, they can be treated with extracorporeal therapies (e.g. Extracorporeal Membrane Oxygenation (ECMO), Continuous Renal Replacement Therapy (CRRT)) to support the failed organs [3]. Such pathophysiological changes and extracorporeal therapies significantly affect the normal antimicrobial pharmacokinetics and the pharmacodynamics profile, resulting in drug exposure that is markedly different from that expected after antibiotic dosing regimens derived from studies on healthy volunteers or on non-critically ill patients [4-6].

Recently, the ICU microbial epidemiology showed an increased prevalence of bacteria multidrug resistance (MDR), requiring higher antibiotic concentrations for killing bacteria successfully or right antimicrobial plasma concentration to prevent increased MDR [7]. For these reasons, the Therapeutic Drug Monitoring (TDM) would be the best tool to target the right dose in critical illness [8-10].

A useful assay method for routine TDM should be rapid, sensible and it should require a cheap and easy-to-use instrumentation. As regards aminoglycosides and glycopeptides, immunochemical assays are commercially available and used worldwide for TDM to ensure appropriate exposure and minimize their toxicity. Unfortunately, similar assay methods are not routinely available for other antibiotic classes.

The aim of this study is to validate a high-performance liquid chromatography with ultraviolet detection (HPLC-UV) method for the *in vivo* quantification, of meropenem (MER), vancomycin (VAN) and piperacillin/tazobactam (PIP/TAZ) plasma levels. The reason of our choice is related to the high frequency of these antimicrobial use as monotherapy or in association.

A number of HPLC-UV or liquid chromatography tandem mass spectrometry (LC-MS/MS) methods for the quantification of these antibiotics, singularly or in combination, has been recently described in the literature [11-18]. However, there is no HPLC-UV method described for the simultaneous quantification of the analytes we are interested in. In addition, the simultaneous quantification of both piperacillin and tazobactam offers

the advantage of monitoring both the antibacterial agent and the β-lactamases inhibitor, in order to confirm the optimal ratio between their plasma concentrations, especially in patients requiring extracorporeal procedures, which could remove the two molecules with different efficiencies [19, 20].

The choice of developing a chromatographic method based on HPLC-UV assures its applicability in the majority of hospital clinical laboratories, thanks to a quick, easy and economic preparation of the samples and a minimal basic equipment requirement.

2. Materials and methods

2.1. Chemicals and reagents

United States Pharmacopeia reference standards of VAN, MER, PIP, TAZ, and cefotaxime (CTX) and other chemicals were purchased from Sigma-Aldrich (Milan, Italy). Organic solvents (HPLC grade) were obtained from Carlo Erba (Milan, Italy).

Blank plasma was obtained from patients undergoing plasmapheresis at the Molinette Hospital of Turin, Italy. Ultrapure water used for the buffers was obtained from a Milli-Q Plus Purification System (Merck Millipore, Vimodrone Milan, Italy). All the buffers were passed through a 0.22-µm membrane filter before use.

2.2. Instrumentation

The method was developed and validated on a HP Agilent 1100 HPLC (GMI, Ramsey, MN, USA), equipped with a quaternary pump, on-line degasser and autosampler, coupled with a Diode Array Detector. HPLC control, data acquisition and data processing were carried out using HP ChemStation software running on a Windows XP equipped computer.

2.3. Chromatographic conditions

Chromatographic separations were performed at 30 °C on a reverse phase Kinetex 5 µ C18 100 Å, 150x4,6 mm (Phenomenex, Castelmaggiore, Bologna, Italy), equipped with a C18 4.0 × 3.0 mm SecurityGuard cartridge. The

volume of injection was 100 µL, and the mobile phase was a mixture of phosphate buffer 0.1 M pH 3.15 (solution A) and methanol (solution B) in gradient, delivered at a flow rate of 1 ml/min. The program of gradient is: from 0 to 16 min linear gradient from 4% B to 25% B, from 16 to 20 min linear gradient from 25% B to 50% B, from 20 to 22 min 50% B in isocratic, from 22 to 25 min linear gradient from 50% B to 4% B, from 25 to 27min 4% B in isocratic. The analytes were detected by UV absorbance at the wavelengths of 220 nm (for VAN, PIP, TAZ, and CTX), and 298 nm (for MER and CTX).

2.4. Preparation of solutions, standards, and quality control samples

Stock standard solutions for each analyte and internal standard (CTX) were prepared by dissolving 10 mg of standard in 5 mL of ultrapure water to get a 2.0 mg/mL.

Plasma calibration standards at 1, 2, 5, 10, 25, 50 and 100 µg/mL concentration of each component, and plasma quality control (QC) samples at 3 (low concentration, LQC), 30 (medium concentration, MQC), and 75 (high concentration, HQC) µg/mL were prepared in batches of 10 mL by adding appropriate volumes of each stock solution to blank plasma.

All the solutions were stored as 300-600 µL aliquots in polypropylene Eppendorf tubes at −80 ◦C and thawed immediately before the use.

Solvent A (0.1 M phosphate buffer solution) was prepared by dissolving 13.61 g of potassium dihydrogen phosphate to 1000.0 mL of ultrapure water and adjusting the pH to 3.15 with concentrate phosphoric acid. The solution was filtered through a 0.45 µm nylon filter membrane (Sigma-Aldrich) prior to use.

2.5. Sample extraction

Calibration curve standards and quality controls stored at −80 ◦C were thawed at room temperature and vortexed. A 250 µL aliquot of each sample was transferred to an Eppendorf tube, then 10 µL of internal standard (final concentration 80 µg/mL) and 100 µL of saline solution were added. Adding saline solution before protein precipitation increase the recovery of analytes. The mixture was vortexed, and 500 µL of acetonitrile were added to precipitate the proteins. The sample was vortexed for 30 s and then centrifuged for 10 min at 1,000 g. The supernatant was transferred into a clean glass tube and dried under a stream of nitrogen

at room temperature. The residuals were reconstituted with 250 µL of a mixture of phosphate buffer 0.1 M pH 3.15 and methanol (96:4, v/v), vortexed for 30 s, and transferred into autosampler microvials for injection.

2.6. Validation of the method

The method has been validated following the guidelines developed by European Medicines Agency and Food and Drug Administration for bioanalytical method validation [21, 22].

2.6.1. Calibration curve and limit of quantification

Calibration curves were obtained by plotting the ratios of nominal concentration of the standard to that of internal standard versus the ratios of the standard peak area to the internal standard peak area. Curves were calculated by least-squares linear regression from seven different standard concentrations ranging from 1 to 100 µg/mL (1, 2, 5, 10, 25, 50 and 100 µg/mL), with each point consisting of eight independent measurements. The percentage deviation from nominal concentration was back-calculated at each standard concentration with ≤15% as acceptance criterion for inclusion in the calibration curve.

The linearity of the method was confirmed by Student's t-test with a confidence interval of 95%, showing that the slopes of linear calibration curves were statistically different from zero, the intercepts were not statistically different from zero and the correlation coefficients were not statistically different from 1.

The limit of quantitation (LOQ) was defined as the lowest drug plasma concentration, which can be quantified with an accuracy and precision of 20%, as calculated from chromatograms for six independent samples. In our method, LOQ for each analyte was set at the lowest standard calibration concentration (1 µg/mL).

The limit of detection (LOD) was estimated at a signal-to-noise ratio of 3:1.

2.6.2. Accuracy and precision

An intra-day study (repeatability) was performed by analyzing the three QC concentrations 6 times during the same day, while an inter-day study (reproducibility) was performed by analyzing the three QC concentrations once-a-day on 7 separate days. Mean measured concentrations and their standard deviations (SD) were calculated.

Accuracy was reported by calculating the bias expressed as ((measured concentration−nominal concentration)/(nominal concentration))×100, and precision by the coefficient of variation (CV) expressed as (SD/mean)×100.

Acceptance criteria for accuracy were: bias within ±15% for HQC and MQC and within ±20% for the LQC.

Acceptance criteria for precision were: CV within ±15% for HQC and MQC and within ±20% for the LQC.

2.6.3. Recovery and specificity

The recovery was determined at three concentrations (LQC, MQC and HQC) for VAN, MER, PIP, and TAZ, and 80 µg/mL for CTX, run in six replicates each. The absolute recoveries (%) were calculated by comparing the peak areas of each analyte in spiked plasma samples, extracted as described, with those of spiked aqueous solutions at the same concentration levels not subjected to the extraction procedure.

Specificity was measured by analyzing six different blank plasma samples and comparing their chromatograms with the corresponding spiked plasma samples.

2.6.4. Stability

As regards the stability study, storage conditions and periods were chosen in order to simulate those at the moment of blood collection and plasma separation (20 °C and 4 °C), during long-term storage of stock solutions and plasma samples (-80 °C), after two freeze-thaw cycles, at the bench during processing, and in the autosampler awaiting analysis.

Stability of stock solutions of VAN, MER, PIP, TAZ, and CTX at 2 mg/mL was determined in triplicate after 1 month of storage at −80 ◦C.

Three concentrations (LQC, MQC and HQC) for VAN, MER, PIP, and TAZ, were tested in triplicate for stability after storage at the following conditions: (a) in plasma at 20 and 4 °C (1 day of storage), (b) in plasma at -80 °C (1 day, 1 week, 1 month of storage), (c) in plasma after two freeze-thaw cycles (24 h at -80 °C), and (d) in extracts at 4 °C (1 day of storage) or -20 and -80 °C (1 week of storage).

Stability of different samples was determined by comparing peak areas between stored samples and freshly prepared solutions. The results were expressed as percentage of drug remaining.

2.7. Applicability of the method

We evaluated the applicability of the method by analyzing plasma samples collected from 4 critically ill patients undergoing CRRT at the ICU of San Bortolo Hospitals in Vicenza. All the patients suffered from bacterial infections and were treated with continuous infusion of antibiotics for which our method has been developed (2 patients treated with the combination of PIP/TAZ, 1 treated with VAN, and 1 treated with MER). At different time points during CRRT, blood samples were collected, in K_2EDTA tube tubes, and immediately centrifuged at 4000xg for 10 min at 4 °C. Plasma were stored at −20 °C until analysis. A total of 14 plasma samples are taken for the analysis of PIP/TAZ, 4 for VAN, and 6 for MER.

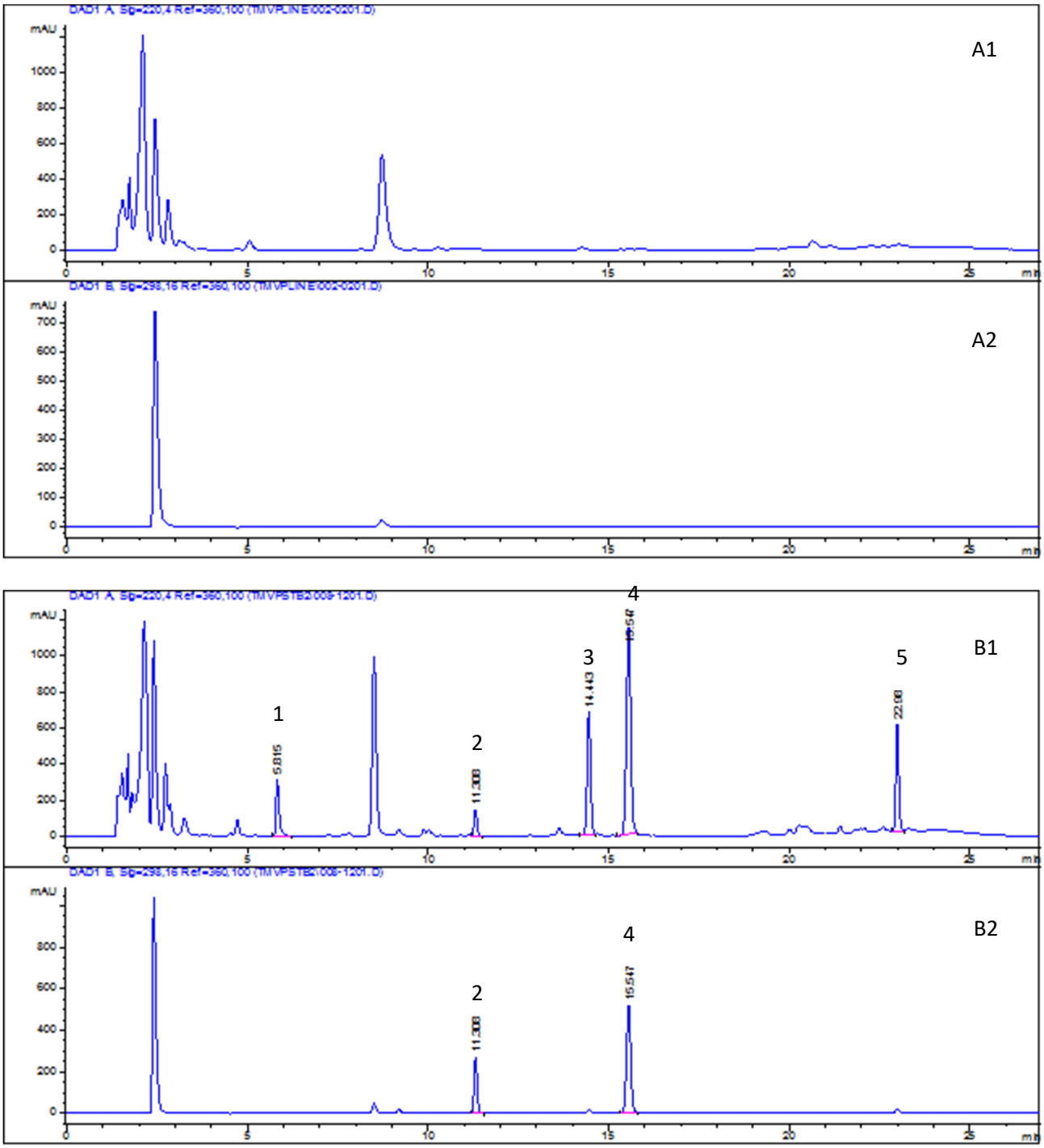
Total VAN plasma concentrations were measured also with VANC Flex® (Siemens Healthcare Diagnostics, Frimley, Camberley, UK), a Particle-Enhanced Turbidimetric Inibition Immunoassay (PETINIA) with a detection range of 0,8-50.0 mg/L. Intra- and inter-run CV, evaluated by paired analysis of 10 different runs, were 2.9% and 3.9%, respectively.

3. Results

3.1 Chromatography

The method enables the simultaneous quantification of VAN, MER, PIP and TAZ in plasma samples, after a common extraction procedure and chromatographic protocol. The retention times of TAZ, MER, VAN, PIP, and CTX (internal standard) were 5.8, 11.3, 14.4, 23.0, and 15.5 min, respectively and the total run time of analysis was 30 min (Fig. 1).

Fig. 1. HPLC chromatograms of (A) a blank patient plasma at λ=220 nm (A1) and λ=298 nm (A2) and (B) a plasma spiked with 30 μg/mL of each antibiotic and 80 μg/mL of the internal standard at λ=220 nm (B1) and λ=298 nm (B2). 1, TAZ; 2, MER; 3, VAN; 4, IS; 5, PIP.



3.2 Calibration curve and limit of quantification

The calibration curves were constructed in the range of 1.0–100 µg/mL because the lowest concentration of standard (1.0 µg/mL, LOQ) is sufficiently lower than expected plasma trough concentration in patients, while the highest concentration of standard (100 µg/mL) is expected to be higher than C_{max} reached in most cases during antibiotic treatment [23-26]. In the event that a concentration above the upper limit of quantification

is expected, sample from patient can be diluted with blank drug free plasma before the extraction procedure, in order to bring the concentration in the range of the calibration curve.

The method was found to be linear over the range of 1.0–100 μg/mL for all the analytes, with an $r^2 \geq 0.999$ ($p=0.001$). Mean±SD slopes were 0.293±0.028, 0.216±0.033, 0.202±0.022, and 0.113±0.022 for VAN, MER, PIP and TAZ, respectively. The good linearity was also confirmed by the percentage deviation of standards from their nominal concentration: for all analytes, in fact, the mean absolute percentage deviation of standards was <6% for higher concentration, and <8% for the LOQ (Table 1). The signal to noise ratio at the LOQ for all analytes was greater than 20:1.

The LODs were 0.08, 0.08, 0.05 and 0.13 for VAN, MER, PIP and TAZ, respectively.

Table 1. Percentage deviation from mean back-calculated concentration and nominal concentration in the calibration curves

Nominal concentration (μg/mL)	Measured concentration (μg/mL)±SD (n= 8)							
	VAN		MER		PIP		TAZ	
		CV%		CV%		CV%		CV%
100	99.00 ± 1.59	1,61	99.54 ± 2.28	2,30	99.92 ± 1.23	1,24	100.28 ± 1.07	1,07
50	51.31 ± 1.43	2,79	51.52 ± 2.46	4,77	50.78 ± 1.64	3,24	50.04 ± 2.90	5,79
25	25.80 ± 0.74	2,86	25.93 ± 1.39	5,34	24.94 ± 1.24	4,96	25.04 ± 1.68	4,35
10	10.21 ± 0.31	3,08	10.29 ± 0.46	4,49	10.19 ± 0.37	3,60	10.14 ± 0.56	5,52
5	5.10 ± 0.14	2,72	5.14 ± 0.15	2,94	5.02 ± 0.04	0,83	5.03 ± 0.16	3,25
2	2.07 ± 0.07	3,56	2.01 ± 0.10	4,91	2.05 ± 0.05	2,45	2.00 ± 0.06	2,80
1	1.02 ± 0.03	2,84	1.04 ± 0.05	4,38	1.00 ± 0.04	3,73	1.02 ± 0.07	7,22

3.3. Accuracy and precision

Acceptance criteria for accuracy and precision were met in all QCs. Intra-day accuracy ranged from −2.00 to 4.29% and inter-day accuracy ranged from −1.38 to 4.15% for all analytes. Intra-day precision was <4%, while inter-day precision was <7% for each analyte. Results are summarized in Table 2.

Table 2. Intra-day and inter-day accuracy (Bias%) and precision (CV%) of VAN, MER, PIP and TAZ.

Nominal concentration (µg/mL)	Intra-day (n= 6)			Inter-day (n= 7)		
	Mean measured concentration (µg/mL) ±S.D.	Bias %	CV%	Mean measured concentration (µg/mL) ±S.D.	Bias %	CV%
VAN						
3	3.13 ± 0.10	4.29	3.14	3.12 ± 0.15	4.15	4.86
30	30.65 ± 0.95	2.17	3.10	31.14 ± 1.23	3.81	3.94
75	75.11 ± 2.54	0.14	3.38	76.67 ± 2.23	2.23	2.90
MER						
3	3.01 ± 0.04	0.33	1.41	3.00 ± 0.12	-0.15	4.12
30	30.52 ± 0.80	1.74	2.61	30.47 ± 1.66	1.56	5.44
75	73.99 ± 2.23	-1.35	3.01	73.27 ± 4.50	-2.30	6.14
PIP						
3	2.94 ± 0.07	-2.00	2.41	2,96 ± 0.12	-1.38	3.99
30	30.20 ± 0.80	0.67	2.65	30.44 ± 1.25	1.46	4.09
75	75.78 ± 2.29	1.04	3.02	76.61 ± 3.47	2.15	4.53
TAZ						
3	2.98 ± 0.10	-0.75	3.44	2.99 ± 0.14	-0.28	4.55
30	30.29 ± 0.75	0.98	2.46	30.35 ± 1.40	1.16	4.60
75	75.29 ± 2.99	0.38	3.97	75.56 ± 3.85	0.75	5.09

3.4 Recovery and specificity

The recovery was >70% for TAZ, >80% for VAN, and about 100% for PIP, MER and CTX (Table 3).

At the wavelengths of 220 nm and 298 nm, no interfering peaks are present in the six different blank drug-free plasma samples tested at the retention times corresponding to analytes, so demonstrating the specificity of the method. Furthermore, peak purity of the analytes was demonstrated by comparing the UV-Vis spectra of peaks in plasma samples, recorded with the diode-array detector, with the spectra of the drugs in the working solution standards.

Table 3. Recovery of VAN, MER, PIP, TAZ and CTX.

Compound	Concentration	Recovery %	SD
VAN	3	90.36	1.06
	30	89.31	4.48

	75	88.96	5.62
MER	3	97.60	3.30
	30	100.00	2.59
	75	98.33	4.50
PIP	3	99.00	2.99
	30	99.89	7.14
	75	97.75	6.20
TAZ	3	73.59	2.95
	30	74.80	4.95
	75	73.71	5.11
CTX	80	98.30	3.90

3.5 Stability

Stock solutions were stable for at least 1 month at −80 ◦C with a degradation less than 3%.

Table 4 summarizes the percentage of drug remaining after storing spiked plasma samples at different conditions. At 20 °C, VAN and TAZ are stable for at least 1 day, whereas MER and PIP showed a degradation higher than 10%. All the compounds are stable for at least 1 day when stored at 4 °C and 2 months at -80 °C and no degradation was observed after two freeze-thaw cycles.

After plasma extraction, all the compounds are stable for at least 1 day at 4 °C and 1 week at -20 or -80 °C.

Taking into account the stability, treatment of plasma samples at room temperature had to be as short as possible. Plasma samples from patients had to be stored at 4 °C and frozen at −80 ◦C as quickly as possible. Extracted samples can be stored frozen for at least 1 week before the HPLC analysis.

Table 4. Stability of VAN, MER, PIP and TAZ in plasma and extract at different storage conditions (percentage of nominal concentration).

Parameter	Level	VAN	MER	PIP	TAZ
1 day at 20°C	LQC	99.91	88.10	72.94	98.81
	MQC	100.11	83.96	74.02	89.78
	HQC	105.10	87.17	81.58	94.60
1 day at 4°C	LQC	99.41	99.08	98.89	97.66
	MQC	104.51	101.61	101.58	99.19
	HQC	105.17	101.57	104.20	100.43
1 day at -80°C	LQC	99.42	101.66	99.11	99.81
	MQC	99.11	105.29	103.69	102.50
	HQC	101.99	102.20	104.09	99.88
1 week at -80°C	LQC	99.32	99.87	99.33	99.52
	MQC	101.08	103.17	102.90	97.24
	HQC	99.12	102.52	101.85	100.96
2 months at -80°C	LQC	102.02	100.18	99.67	98.98
	MQC	98.97	99.73	101.95	100.36
	HQC	100.54	101.55	98.89	99.57
After 2 freeze-thaw cycles	LQC	95.90	101.18	99.74	99.98
	MQC	98.58	104.69	104.02	99.09
	HQC	100.17	101.80	103.49	103.05
Extract 1 day at 4°C	LQC	98.15	97.55	97.57	98.71
	MQC	99.89	99.42	98.62	97.57
	HQC	97.23	98.76	97.31	98.18

Extract 1 week at -20°C	LQC	99.11	102.16	100.25	99.52
	MQC	100.26	101.07	99.45	101.40
	HQC	101.34	99.73	102.14	102.53
Extract 1 week at -80°C	LQC	100.58	99.69	101.03	100.99
	MQC	101.49	102.56	100.78	98.34
	HQC	102.11	101.23	99.67	101.25

3.6 Applicability of the method

The described method is currently employed in ongoing clinical study that investigates the effects of CRRT on pharmacokinetic profile of these antimicrobial drugs. Table 5 shows the plasma level of analytes in the first 4 enrolled patients.

For the VAN, the only analyte for which some commercial kits are available, the total plasma concentration has been evaluated with VANC Flex® also, and the results are strictly comparable to those obtained by HPLC.

Table 5. Plasma concentration of analytes in 4 patients.

	Piperacillin µg/mL	Tazobactam µg/mL
Patient 1 (a)		
day 1	15.60	1.57
day 2	46.89	3.41
day 3	21.59	3.36
day 4	29.17	3.88
day 5	68.86	9.19
day 6	55.71	6.89
Patient 2 (a)		
day 1 - 1h CRRT	84.23	16.51
day 1 - 6h CRRT	56.77	8.63
day 1 - 12h CRRT	56.43	9.34
day 1 - 24h CRRT	65.47	7.73
day 3 - 1h CRRT	64.45	7.08
day 3 - 6h CRRT	81.14	11.44
day 3 - 12h CRRT	81.54	11.19
day 3 - 24h CRRT	64.87	9.24

	Vancomycin µg/mL	
Patient 3 (b)	**HPLC**	**VANC Flex®**
day 1	22.15	21.25
day 2	33.76	32.48
day 3	28.18	27.13
day 4	31.34	30.11

	Meropenem µg/mL
Patient 4 (c)	
day 1 - 1h CRRT	21.58
day 1 - 6h CRRT	18.65
day 1 - 12h CRRT	17.40
day 2 - 1h CRRT	23.09
day 2 - 6h CRRT	19.76
day 2 - 12h CRRT	11.85

(a): 4.5g x 4/die, extended infusion; (b): 2 g/die, continuous infusion; (c): 2g x 3/die, extended infusion

4. Discussion

Last Surviving Sepsis Campaign guidelines [27] defined that source control, antibiotics, pharmacological and mechanical supports for vital functions remain the mainstay therapy for septic patients. In the last decade,

several studies have focused on the understanding of the pharmacokinetic or pharmacodynamic changes of antibiotics in critically ill patients and during extracorporeal therapy [28-30].

In septic patients, successful therapy includes the timely initiation of the antimicrobial therapy and right antimicrobial plasma concentration according to their pharmacodynamic profile and MIC of bacteria against which they should act. However, antibiotic dosing regimens are actually derived from healthy volunteers in pre-market studies, so it might lead to suboptimal target in critically ill patients. For these reasons, the availability of a simple, rapid and economic method for the quantification of these drugs in plasma samples is strongly advisable.

In this paper we described a validated method that allow the simultaneous determination of VAN, MER, PIP, TAZ in the same blood sample, with the advantage of a common sample preparation and analytical protocol. The method was found to be linear from 1.0 to 100 µg/mL for all the analytes, a range covering the usual plasma concentration of clinical interest.

The analysis of plasma samples from 4 critically ill patients undergoing CRRT has proved that this method is fast and reliable, and could be useful for routine TDM in critically ill patients. Moreover, the analysis of VAN with VANC Flex® confirmed a good correlation between the results of HPLC-UV and commercially available kits usually used by TDM service.

5. Conclusion

The described HPLC-UV method allows the simultaneous determination of antibiotics commonly used in ICU, with a rapid preparation and analysis time, and a simple and rather inexpensive instrumentation, features that make this method an easy implementation for a general TDM laboratory. The small volume of sample required for analysis also makes this method suitable for pharmacokinetic studies in elderly, critically ill or pediatric patients.

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