Clinica Chimica Acta 553 (2024) 117689

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Dried blood spot analysis for the quantification of vancomycin and creatinine using liquid chromatography – tandem mass spectrometry: Method development and validation

Soma Bahmany [a,*], Moska Hassanzai [a], Robert B. Flint [a,b], Hein A.W. van Onzenoort [c], Brenda C.M. de Winter [a,d], Birgit C.P. Koch [a,d]

[a] Department of Hospital Pharmacy, Erasmus University Medical Center, Rotterdam, the Netherlands
[b] Department of Pediatric and Neonatal Intensive Care, Division of Neonatology, Erasmus University Medical Center, Rotterdam, the Netherlands
[c] Department of Pharmacy, Radboud University Medical Center, Radboud Institute for Health Sciences, Nijmegen, the Netherlands
[d] CATOR: Center for Antimicrobial Treatment Optimization Rotterdam, the Netherlands

ARTICLE INFO

Keywords:
Antibiotics
Dried blood spot
Tandem mass spectrometry
Ultra-high performance liquid chromatography
Therapeutic drug monitoring
Vancomycin
OPAT

ABSTRACT

Background: Vancomycin is a widely used antibiotic for the treatment of gram-positive bacterial infections, especially for methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Due to a small therapeutic range and large inter-patient variability, therapeutic drug monitoring (TDM) of vancomycin is required to minimize toxicity and maximize treatment efficacy. Venous blood sampling is mostly applied for TDM of vancomycin, although this widely used sampling method is more invasive compared to less painful alternatives, such as the dried blood spot (DBS) method, which can be performed at home.

Method: We developed an UPLC-MS/MS method for the quantification of vancomycin and creatinine in DBS. A fast sample preparation and short analysis run time of 5.2 min were applied, which makes this method highly suitable for clinical settings. Validation was performed according to international (FDA and EMA) guidelines.

Results: The validated concentration range was found linear for creatinine from 41.8 μmol/L to 722 μmol/L and for vancomycin from 3.8 mg/L to 76.6 mg/L ($r^2 > 0.990$) and the inaccuracies, imprecisions, hematocrit effects, and recoveries were < 15 % for both compounds. No significant carryover effect was observed.

Conclusion: Hence, we successfully validated a quantification method for the simultaneous determination of creatinine and vancomycin in DBS.

1. Introduction

Vancomycin is a widely used antibiotic for the treatment of gram-positive bacterial infections, especially in case of methicillin-resistant *Staphylococcus aureus* (MRSA) infections. As the target exposure for efficacy of vancomycin is close to the exposure related to toxic side effects, such as nephrotoxicity [1–3] and ototoxicity [4], vancomycin is considered to have a small therapeutic window. Strikingly, failure of vancomycin treatment in patients with MRSA [5–8] has increased and many patients do not achieve the target therapeutic concentrations of vancomycin [9–18] due to the large inter-patient variability. For this reason, the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists, as well as the Japanese Society of Chemotherapy and the Japanese Society of Therapeutic Drug Monitoring, recommend to perform therapeutic drug monitoring (TDM) for vancomycin to minimize toxicities and maximize treatment efficacy [19,20]. Furthermore, as vancomycin is cleared by the kidney and therefore correlated to creatinine clearance [21], quantification methods for the simultaneous determination of creatinine and vancomycin levels are very useful for the optimization of the therapy.

Mostly, venous blood sampling is applied in clinical settings for the quantification of vancomycin and creatinine for TDM purposes. However, venous blood sampling has several disadvantages. First, patients need to visit the hospital or phlebotomy facilities to perform their blood sampling, which results in more clinical visits compared to patients who can perform their blood sampling at home. Second, a trained health care performer needs to perform the venous blood sampling. Third, it is more

* Corresponding author at: Department of Hospital Pharmacy, Erasmus MC, Postal box 2040, 3000 CA Rotterdam, the Netherlands.
E-mail address: s.bahmany@erasmusmc.nl (S. Bahmany).

https://doi.org/10.1016/j.cca.2023.117689
Received 14 July 2023; Received in revised form 30 November 2023; Accepted 2 December 2023
Available online 3 December 2023

invasive and painful compared to more upcoming alternative methods of blood sampling, such as the dried blood spot (DBS) method [22]. Briefly, DBS is a more patient friendly, less invasive and less painful alternative which could be performed at home without visiting the hospital. Blood collection is performed by using a simple finger prick by the patient him/herself, who collects the blood drops on a specific paper and sends this by mail to the laboratory [22].

Despite the benefits of DBS, there are several challenges related to the clinical use of DBS which should be addressed [37]. These challenges include DBS sampling and DBS homogeneity. Before performing DBS, it is highly recommended to provide the patient with the right instructions of DBS sampling to reduce sampling mistakes. Second, when DBS samples arrive at the hospital laboratory, sample spots must be evaluated before punching out the spot [22,23]. Furthermore, before clinical implementation, a clinical study must be performed to investigate the correlation of dried blood spot concentrations to the plasma concentrations.

The DBS method may especially be an advantage for patients treated with vancomycin within outpatient parenteral antimicrobial therapy (OPAT) services. OPAT service provides antimicrobial therapy via parenteral infusion without hospitalization and is associated with a greater comfort for the patient and is cost saving for the health care system. Nonetheless, currently these vancomycin patients still need to visit the hospital to perform their blood sampling for TDM purposes. A great benefit will be achieved if sampling can be performed by DBS at home. Despite the clinical importance of TDM of vancomycin and the benefits of DBS, only one study [24] has described an analytical method for the simultaneous quantification of vancomycin and creatinine, currently.

Considering the importance of TDM of vancomycin, the many benefits of DBS for these patients, and our experience with several previously described DBS quantification methods [25–27], we developed and validated a patient friendly, accurate, sensitive, and selective DBS quantification method of vancomycin and creatinine using liquid chromatography coupled by mass spectrometry (LC-MS/MS).

2. Materials and methods

2.1. Chemicals and reagents

Vancomycin hydrochloride was obtained from Sigma-Aldrich Chemie B.V. (Zwijndrecht, the Netherlands) and from Cayman Chemical Company (Ann Arbor. Michigan, USA). Creatinine and creatinine-D_3 were purchased from Sigma-Aldrich Chemie B.V. (Zwijndrecht, the Netherlands). Vancomycin-D_{12} trifluoroacetate salt was purchased from Alsachim (Illkirch Graffenstaden, France). LC-MS grade methanol (99 %) was purchased from Biosolve BV (Valkenswaard, the Netherlands) and formic acid was purchased from Merck (Darmstadt, Germany). Ultra-pure water was prepared in-house using a MilliPore Advantage A10 System (Millipore, Bedford, MA, USA). DBS cards (Whatman™ 903 protein saver cards) were purchased from VWR International B.V. (Amsterdam, the Netherlands).

2.2. Stock solutions, calibration standards, quality control samples, and internal standard

Two freshly prepared stock solutions of vancomycin were used for preparation of the calibration standards and quality control samples (QCs). Stock solutions were prepared by dissolving 50 mg of vancomycin hydrochloride in 25 mL of MilliQ water to obtain stock concentrations of 2000 mg/L. Both concentrations were corrected for their salt factor. The stock solutions of the internal standards were prepared by dissolving 1 mg of vancomycin-D_{12} in 10 mL of MilliQ water and 10.2 mg of creatinine-D_3 in 250 mL of methanol. Stock concentrations were 100 mg/L for vancomycin-D_{12} and 350 μmol/L for creatinine-D_3. The internal standard (IS) working solution was prepared in methanol: MilliQ water 50: 50 % v/v and 1 % formic acid, by diluting the stock solution of vancomycin-D_{12} 40 times and the stock solution of creatinine-D_3 70 times, resulting in concentrations of 2.5 mg/L of vancomycin-D_{12} and 5 μmol/L of creatinine-D_3. Eight calibration standards and three quality control levels (low (L), medium (M), and high (H)) were prepared in DBS. Calibration standard 2 to 8 were prepared by diluting the stock solution of vancomycin with MilliQ water. Calibration standard 1 was prepared by diluting calibration standard 5 with MilliQ water. Quality control levels (low (L), medium (M), and high (H)) were prepared by diluting the stock solution with MilliQ water. Thereafter, 50 μL of each calibration standard and QC sample were pipetted into a cryo tube and diluted with 950 μL of whole blood based on their creatinine concentration. Since it is impossible to obtain creatinine-free blood, independent, vancomycin-free, whole blood samples of patients were used for each calibration standard and QC level. Residues of, vancomycin-free, patient samples were obtained from the laboratory of the clinical chemistry department. Before use, potential objection against the use of material for research purposes was checked for each patient in the laboratory information system of the Erasmus Medical Center. Finally, after mixing and homogenization, 50 μL was spotted on the DBS cards. DBS cards were stored in the desiccator and dried for at least 24 h at room temperature before use. A punched blank spot from the DBS card without whole blood was used as a blank, because of the presence of creatinine in whole blood,

2.3. Sample preparation

A 6 mm spot was punched out of the DBS cards into a cryo tube. 400 μL of the internal standard solution was added to all samples and vortexed for 10 s. After vortexing, samples were sonicated in a water bath for 30 min at 40 °C. Subsequently, 200 μL of each extract was pipetted into an autosampler insert vial.

2.4. Instrumentation

Both compounds including their internal standards were analyzed using a Waters Acquity UPLC® system connected to a Xevo TQ-S micro triple quadrupole mass spectrometer (Waters Corp., Milford, MA, USA). The UPLC® consisted of an Acquity binary solvent manager (chromatographic pump), a sample manager (autosampler), a sample organizer, and column manager. Data acquisition and processing were performed by using MassLynx™ V4.1 and Targetlynx V4.1 (Waters Corp.). Creatinine and hematocrit concentrations of the venous blood samples were performed at the laboratory of the clinical chemistry department. Creatinine plasma concentrations were measured by an enzymatic assay (Cobas8000 system, Roche Diagnostics, Basel, Switzerland). This enzymatic method is based on the conversion of creatinine with the aid of creatininase, creatinase, and sarcosine oxidase to glycine, formaldehyde and hydrogen peroxide. This method was used according to manufacturer's instructions and calibrated to the global IDMS method. Validation of this method was performed according to the Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures (CLSI Guideline EP15-A2) [28]. The bias and precision of this method are 3.7 % and 2.0 %, respectively. Hematocrit whole blood concentrations were measured on a Sysmex XN-1000 hemocytomery analyzer (Sysmex Corporation, Kobe, Japan). The bias and precision of this method are −1.9 % and 1.9 %, respectively.

2.4.1. Chromatographic conditions

Chromatographic separation was performed by using a Waters Acquity UPLC® HSS T3 C18 column (1.8 μm, 2.1 x 100 mm). A gradient elution program was applied using mobile phase A and mobile phase B. Mobile phase (MP) A consisted of 2 mM ammonium acetate and 0.1 % formic acid in 1L of MilliQ water. Mobile phase B (MP) consisted of 2 mM ammonium acetate and 0.1 % formic acid in 1L of methanol. Column temperature was set at 45 °C and the flow rate was set at 0.35 mL/

min. The initial condition of the elution program was 95 % of MP A and 5 % MP B. After 0.80 min MP B increased to 90 % for 2 min. From 2.8 min to 3.8 min MP B decreased to 5 % and was hold on starting conditions for 1.4 min to equilibrate for the next injection. Total run time was 5.2 min. 2 μL of each extract was injected into the UPLC® system. The retention time of each compound is presented in Table 1.

2.4.2. MS/MS conditions

The electrospray ionization was performed in positive mode. The final optimized MS settings were a capillary voltage of 3.0 kV, source temperature of 130 °C, desolvation temperature of 400 °C, cone gas flow of 10 L/Hr, and the desolvation gas flow was set at 500 L/Hr. The optimized parent- and daughter *m/z*, collision energy, and cone voltages are summarized in Table 1.

2.5. Validation of the method

Validation of this analytical method was performed based on the European Medicines Agency (EMA) guidelines and Food and Drug Administration (FDA) guidelines [29,30]. In our setting, we used stricter requirements for the linearity. The EMA and FDA describe that at least 75 % of the linearity should meet the acceptance criteria. In our setting, all linearity results should meet the acceptance criteria. The following parameters for analytical validation were included: linearity, limits of quantification, accuracy, inter-day and intra-day precision, carry-over, autosampler stability, short-term and long-term stability. Since this method was developed in DBS, additional parameters were validated to evaluate several aspects which may affect the measured concentrations of each compound when using DBS. These parameters were based on the previously published paper of Capiau *et al.* which describes guidelines specifically for the validation of DBS methods for TDM purposes [31]. Additional validation parameters for DBS were as follows: the influence of different spot volumes on the DBS cards, the drying time and storage condition of the spots, recovery and the hematocrit (Ht) effect.

2.5.1. Linearity

A blank sample (without internal standard) and zero-calibrator sample (with internal standard) and eight calibration standards were prepared and measured in duplicate for validation of the linearity. Measured concentrations should be within ± 15 % of the nominal concentrations, except at LLOQ, where the calibration standard should be within ± 20 % of the nominal concentration. Determination coefficients (r^2) and correlation coefficients (r) should be at least 0.995 and 0.990, respectively. The highest calibration standard which was measured within the 15 % accuracy, was set as the ULOQ. Concentrations of each calibration standard are presented in Table 2.

2.5.2. Accuracy

Quality control samples, including QC LLOQ, QC L, QC M, and QC H, were prepared and measured in quintuplicate on three different days. Measured concentrations were compared to the nominal concentrations. The bias should be within ± 20 % for the LLOQ and 15 % for QC L, M, and H.

2.5.3. Intra- and inter-day precision

Intra-day precision was established by measuring QC LLOQ, QC L, QC M, and QC H in quintuplicate on the same day. Inter-day precision was established by measuring all QCs in quintuple on three different days. All coefficient of variations (CVs) should be within 15 %, except for the LLOQ, where CVs should be within 20 %.

2.5.4. Carry-over

A blank sample after the highest calibration standard was measured in duplicate to assess the impact of any carry-over during sample analysis. Measured concentrations in the blank samples should not exceed 20 % of the LLOQ of each compound.

2.5.5. Stability

Autosampler stability was investigated by storing the extracts of three quality control samples in duplicate in the autosampler at 15 °C after sample analysis. The extracts of these samples were measured after 24 h against freshly prepared calibration standards.

Short-term stability was investigated by storing QC L, QC M, and QC H in triplicate at room temperature and in the freezer −20 °C during 24 h and 168 h. Long-term stability was investigated by storing QC L, QC M, and QC H samples at room temperature and in the freezer −20 °C during one month. QCs were measured after one month in triplicate. Measured concentrations were compared to the measured concentrations at T = 0 and recoveries should be between 85–115 %.

The stability of vancomycin in the stock solution was investigated by storing the stock solution in the refrigerator. After two months of storage, QCs (low, medium, and high) were prepared using the stored stock solution. QCs were measured in triplicate against a calibration curve which was prepared from a freshly prepared stock solution of vancomycin. Measured concentrations of the QCs should be between 85–115 % compared to the nominal concentrations.

2.5.6. Spot volumes

Based on the typically generated DBS volumes when blood drops are collected on the filter paper [31], three different volumes (30, 40, and 50 μL) were spotted on DBS cards at low and high concentrations to evaluate the volume effect on the measured concentrations. After drying the blood spots, all QCs were prepared and measured in duplicate against calibration standards which were prepared using a 50 μL spot. Back calculated concentrations and CVs should be within 15 %.

2.5.7. Drying time

The required drying time of the blood spots was assessed. QC L and QC H were prepared and measured after 3 h and 24 h. Back calculated concentrations and CVs should be within 15 %.

2.5.8. Hematocrit effect

Hematocrit effect was evaluated at three different hematocrit (Ht) concentrations based on the expected Ht concentrations of the target population. Hematocrit references values were used from the laboratory of the clinical chemistry department. Due to the natural presence of creatinine in blood, it was difficult to obtain QC samples with the desired creatinine and hematocrit values. Therefore, clinical care samples were used to investigate the Ht effect for both, the Ht concentration was measured in whole blood and creatinine and vancomycin were measured in plasma with the enzymatic assay. Thereafter, samples were homogenized and 50 μL of whole blood was spotted on the DBS cards.

Table 1
MS/MS settings and retention times.

Compound	Parent ion (*m/z*)	Product ion (*m/z*)	ESI mode	Cone Voltage (V)	Collision Energy (eV)	Retention time (min)
Creatinine	114.00	85.99	+	16.0	8.0	0.57
Creatinine-D_3	117.00	88.98	+	26.0	8.0	0.57
Vancomycin	725.60	144.00	+	14.0	14.0	1.10
Vancomycin-D_{12}	731.70	144.10	+	32.0	12.0	1.10

Table 2
Concentrations of the calibration standards (S) and lower limit of quantification (LLOQ) sample.

Compound	LLOQ	S1	S2	S3	S4	S5	S6	S7	S8
Creatinine (μmol/L)	41.8	41.8	56.1	77.9	96.0	187.2	317.3	694.5	722.0
Vancomycin (mg/L)	3.8	3.8	7.7	15.3	31.9	38.3	47.9	63.9	76.6

After drying the DBS, samples were prepared and measured in duplicate against calibration standards which were prepared in blood with median Ht values. Recoveries at each Ht concentration were calculated by comparing the measured DBS concentration to the plasma concentration. The mean CVs of the recoveries should be within 15 %.

2.5.9. Recovery

Recoveries were determined using three different batches of blood containing vancomycin and creatinine at three different concentration levels. A fixed volume of 15 μL was spiked on pre-punched DBS disks and dried for at least 24 h. DBS samples and 15 μL of the whole blood samples were simultaneously prepared and measured. Measured concentrations of DBS samples were compared to the whole blood concentrations. Recoveries and CVs should be within 85–115 %.

2.6. Clinical application

We compared the plasma concentrations to the DBS concentrations of 6 patient samples. First, 50 μL of each patient sample was pipetted on a DBS card. Hereafter, the same samples were used to measure the vancomycin and creatinine concentrations in plasma using an enzymatic assay. After drying the DBS, samples were prepared and measured using our validated LC-MS/MS method. The measured concentrations of vancomycin and creatinine in DBS were compared to the measured plasma concentrations.

3. Results

3.1. Method development

Reversed phase chromatography was applied for the chromatographic separation because of the highly polar groups in the chemical structures of creatinine and vancomycin. Several gradient elution programs and column temperatures were tested in order to achieve chromatographic separation and Gaussian peak shapes for both compounds including their internal standards. This resulted in the following elution program: an isocratic step for 0.8 min on 95 % MP A and 5 % MP B for the elution of creatinine, which was less retained compared to vancomycin. After 0.8 min elution strength was increased up to 90 % MP B for the elution of vancomycin. After the elution of vancomycin, the gradient was hold on 100 % MP B for 2 min as a washing step to elute strongly retained impurities, which is important to increase the column shelf-life. After the washing step, gradient changed in 1 min to the starting conditions and was held for 1.4 min to equilibrate for the next injection.

To optimize all MS settings, infusion solutions were prepared of each compound by diluting the stock solutions to concentrations of 1 mg/L in methanol. Each infusion solution was directly injected into the MS without passing the chromatographic system. MS settings as the parent mass, daughter mass, collision energy, spray voltage, cone voltage, and desolvation temperature were optimized during this infusion experiment.

Different extraction solutions were tested in order to achieve the highest recovery of vancomycin and creatinine from the DBS. The internal standard solution was used for the extraction of vancomycin and creatinine. Deuterated internal standards (vancomycin-D_{12} and creatinine-D_3) were used to correct for variations during sample preparation, sample extraction and analysis. First, an extraction solution was tested using the internal standard solution in methanol. However, extraction yields and recovery signals were too low for vancomycin to meet the desired limits of quantification. Therefore, the internal standard solution was adjusted and prepared in methanol:MilliQ water 50:50 % v/v to improve the solubility and extraction of vancomycin. Higher responses were obtained for vancomycin. Despite the increased extraction yield of vancomycin, the responses still did not meet the desired quantification limits. Hereafter, the internal standard solution was adjusted by adding 1 % of formic acid which resulted in the desired recoveries for both compounds, vancomycin and creatinine, which may be explained by a higher solubility at lower pH values.

3.2. Method validation

3.2.1. Linearity

Concentration ranges were found linear for creatinine from 41.8 μmol/L to 722 μmol/L and for vancomycin from 3.8 mg/L to 76.6 mg/L. Correlation coefficients (r) were 0.999 for creatinine and 0.997 for vancomycin. Determination coefficients (r^2) were 0.998 and 0.994 for creatinine and vancomycin, respectively. Back calculated concentrations of all calibration standards were within 15 % of the nominal concentrations. A least square regression, including origin and weighting factor 1/x was applied for both compounds. Following calibration curves were obtained: $y = 0.0068x + 0.00574$ (creatinine) and $y = 0.0114x + 0.00498$ (vancomycin). Chromatograms of the LLOQ samples are presented in Fig. 1 and Fig. 2 for creatinine and vancomycin, respectively.

3.2.2. Accuracy and precision

Accuracy, inter-day, and intra-day precision results were all within the requirements (Table 3).

3.2.3. Carry-over

No carry-over effect was observed for creatinine and vancomycin. Measured concentrations of blank samples after the highest calibration standard were < 20 % of the LLOQ concentrations.

3.2.4. Stability

Extracts of the quality control samples were found stable after 24 h of storage in the autosampler at 15 °C and therefore extracts could be measured until 24 h of storage in the autosampler. QC samples were stable for one month at room temperature and freezer −20 °C, for both compounds. Stock solution of vancomycin was found stable after two months of storage in the refrigerator, mean bias of the measured QCs against freshly prepared calibration standards were within 85–115 %. Stability results are presented in Supplemental Table 1 (S1).

3.2.5. Spot volumes

Three different spot volumes for QC L and QC H were investigated and no volume effect was observed on the measured concentrations. Mean back calculated concentrations and CVs were within 15 %. Spot volumes of 30, 40, and 50 μL could be used for the analysis of vancomycin in creatinine in DBS (Table 4).

3.2.6. Drying time

Mean back calculated concentrations after 3 h and 24 h were within 15 % of the nominal concentrations. Therefore, calibration standards, QCs, and patient samples could be prepared and measured after 3 h of drying (Table 5).

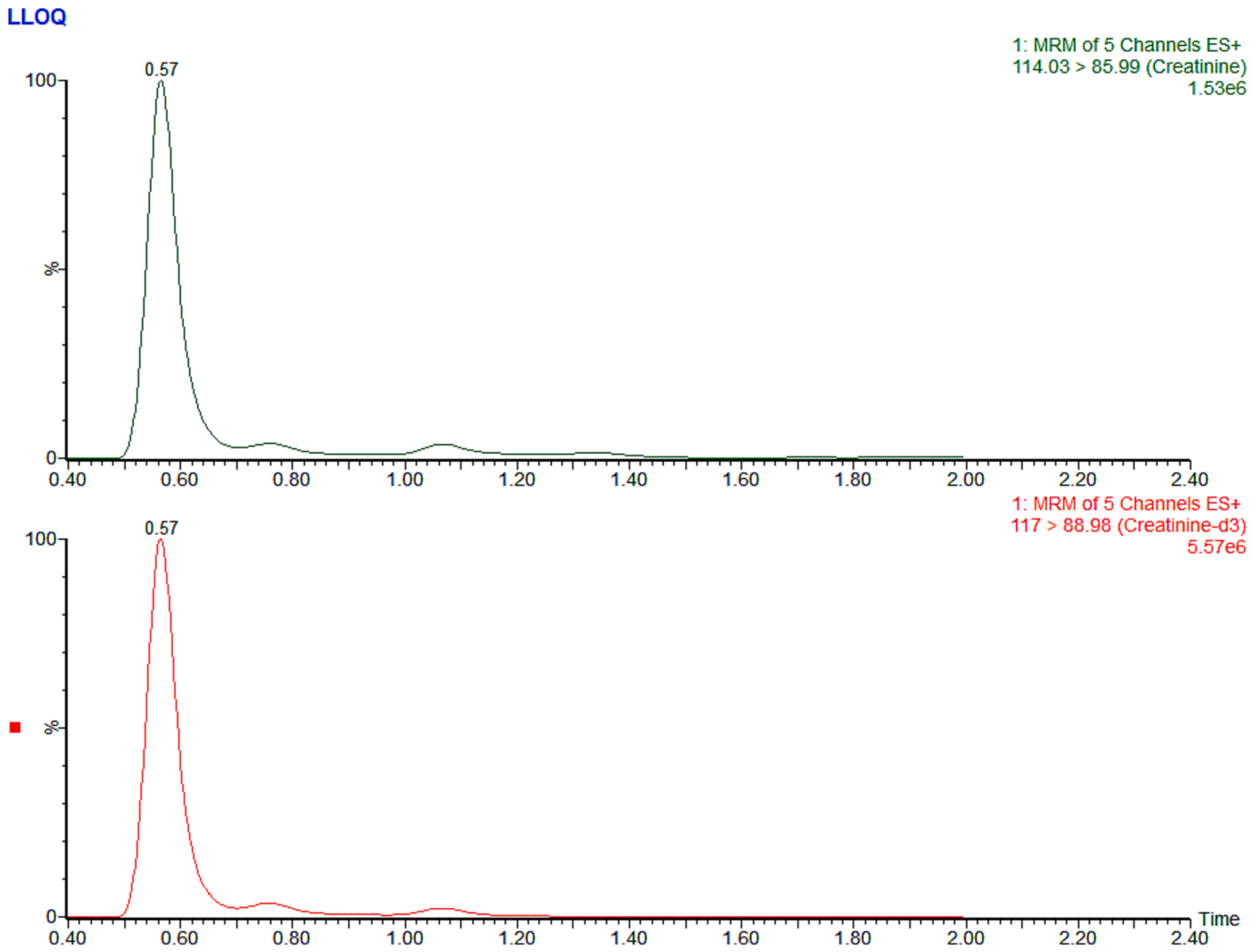

Fig. 1. MRM chromatograms of the lower limit of quantification (LLOQ) standard of creatinine (top) and the internal standard creatinine-D_3 (bottom). The retention time in minutes is given on top of the peak.

3.2.7. Hematocrit effect

Three different batches of blood with different hematocrit levels were used to prepare QC L and QC H. The hematocrit levels were 0,3 L/L, 0,38 L/L, and 0,42 L/L. No significant hematocrit effect was observed in these range. CVs were 3,9% and 4,4% for creatinine and vancomycin, respectively. Therefore, we can conclude that no significant hematocrit effect is observed for creatinine and vancomycin. Results for creatinine and vancomycin are presented in Figs. 3 and 4, respectively. The hematocrit level (L/L) is given on the horizontal axis, and the ratio (%) of DBS to plasma concentrations is given on the vertical axis.

3.2.8. Recovery

The averaged recoveries from the DBS were 99,5% with a CV of 4,6% and 106.0 % with a CV of 6.5 % for vancomycin and creatinine, respectively. Extraction procedure was found optimal.

3.3. Clinical application

A difference was observed between the correlation of creatinine and vancomycin in plasma concentrations to DBS concentrations. Measured concentrations of creatinine in DBS were 2.3 % lower compared to concentrations. Measured concentrations of vancomycin in DBS were consistently 40 % lower compared to the plasma concentrations (Table 6).

4. Discussion

We developed a highly accurate and rapid assay for the simultaneous quantification of vancomycin and creatinine in DBS using liquid chromatography combined with tandem mass spectrometry according to US Food and Drug Administration guidelines. DBS sampling can be performed at home, which makes this sampling method a sustainable alternative to venipuncture.

Due to the high speed and simplicity, enzymatic assays have commonly been used for the quantification of vancomycin in venous blood. However, cross-reactivity with metabolites and degradation products should be taken into account when using this technique. For example, an overestimation of vancomycin concentrations was observed up to 60 % in patients with impaired renal function [32]. This cross-reactivity was caused by cytidine diphosphate (CDP), which is a crystalline degradation product of vancomycin [33]. To overcome this issue, we used chromatography coupled by tandem mass spectrometry to develop a more accurate and selective quantification method compared to enzymatic assays.

Compared to previously published methods [24,34,35], our method is unique by using vancomycin-D_{12} as an internal standard which is identical in physical and chemical properties to vancomycin. The use of an isotope labeled internal standard is highly recommended to minimalize the influence of possible matrix effects [36]. Furthermore, with a short total run time of 5.2 min, we can conclude that this method is

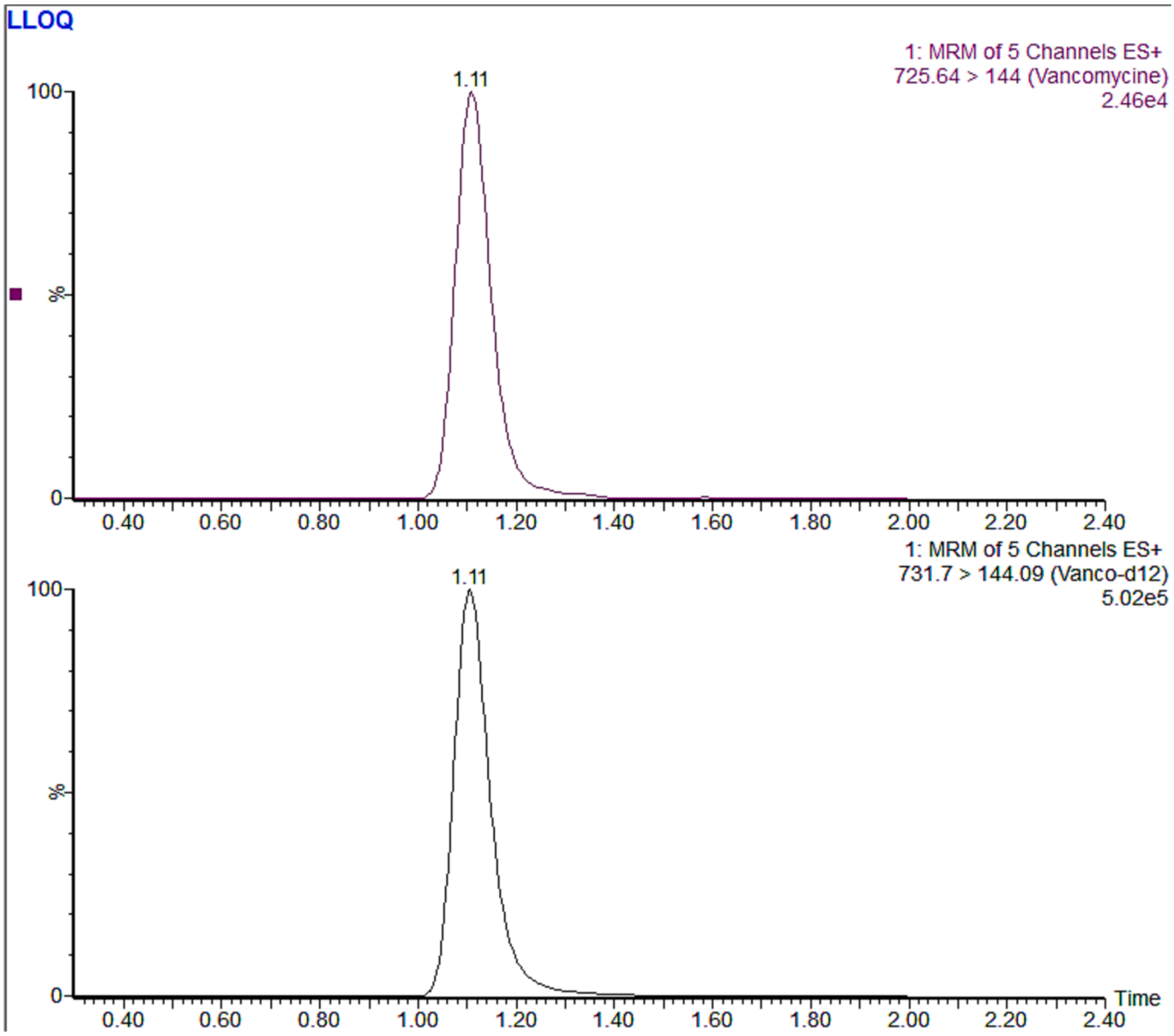

Fig. 2. MRM chromatograms of the lower limit of quantification (LLOQ) standard of vancomycin (top) and the internal standard vancomycin-D_{12} (bottom). The retention time in minutes is given on top of the peak.

Table 3
Validation results of the accuracy and precision for creatinine and vancomycin QC: Quality control samples, QC lower limit of quantification (LLOQ), QC low (L), QC medium (M), and QC high (H).

Compound	QC	Conc. (μmol/L)	Conc. (mg/L)	Accuracy Bias (%)	Intra-day precision RSD (%)	Inter-day precision RSD (%)
Creatinine	LLOQ	41.8	x	1.3	3.9	3.4
	L	77.9	x	−8.1	3.6	3.5
	M	114.0	x	−1.6	2.9	2.6
	H	413.3	x	−0.1	4.1	4.5
Vancomycin	LLOQ	x	3.8	−0.7	7.8	8.1
	L	x	14.6	5.8	4.9	12.5
	M	x	29.2	0.3	3.9	9.1
	H	x	58.5	0.1	6.4	9.7

Table 4
Results of different spot volumes (30 μL, 40 μL, and 50 μL).

	Mean bias (%)		Mean bias (%)		Mean bias (%)	
Compound	30 μL	CV (%)	40 μL	CV (%)	50 μL	CV (%)
Creatinine	4.5	1.8	−4.5	5.0	−4.9	0.7
Vancomycin	−2.1	4.2	−5.4	3.4	−10.7	7.3

highly suitable for daily routine analysis in hospital settings. Besides that, our run time is faster compared to previously published methods for the simultaneous quantification of vancomycin and creatinine in plasma and DBS using LC-MS/MS [24,34,35].

Moreover, since we make use of DBS, our method is more patient friendly than previously described methods [34,35], because of the less painful and less invasive sampling method compared to venous blood sampling. Only one validated method has previously been published by Scribel *et al* for the simultaneous quantification of creatinine and vancomycin in DBS [24]. Although the validated concentration ranges of our method were smaller compared to the method of Scribel *et al.,* our validated concentration ranges covered the clinically relevant concentrations in clinical care. Moreover, Scribel *et al.* [24] described a more time-consuming sample preparation by including an evaporation step and a 10-fold higher injection volume of 20 μL, which could reduce the lifespan of the chromatographic column. Recoveries at three different Ht levels were presented in Fig. 3 and Fig. 4. The CVs of the obtained recoveries were lower than 5 %. Therefore, we can conclude that hematocrit levels between 0.3 and 0.42 L/L did not affect the accuracy of vancomycin and creatinine measurements in DBS, which is comparable to the findings of Scribel *et al* [24]. Measured concentrations of creatinine in DBS were 2.3 % lower compared to plasma concentrations. This good correlation of creatinine in DBS to plasma concentrations are comparable to the study of Koster *et al.* [38]. The difference between the correlation of creatinine and vancomycin may be due to the lack of protein binding of creatinine which results in a more homogenous distribution into the red bloods cells compared to vancomycin [38]. Therefore, a clinical study should be performed to establish the correction factor of vancomycin for DBS concentrations before clinical implementation. In our center, a clinical validation study has been initiated and the protocol of this clinical study has been approved by The Medical Ethics Committee of the Erasmus MC.

5. Conclusion

We successfully developed and validated a fast and accurate quantification method for the simultaneous determination of vancomycin and creatinine in DBS. The use of DBS is a patient friendly and sustainable sampling method which is especially of added value for patients using vancomycin at home. The fast and efficient sample preparation and short analysis run time make this method highly suitable for hospitals and other clinical settings.

CRediT authorship contribution statement

Soma Bahmany: Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **Moska**

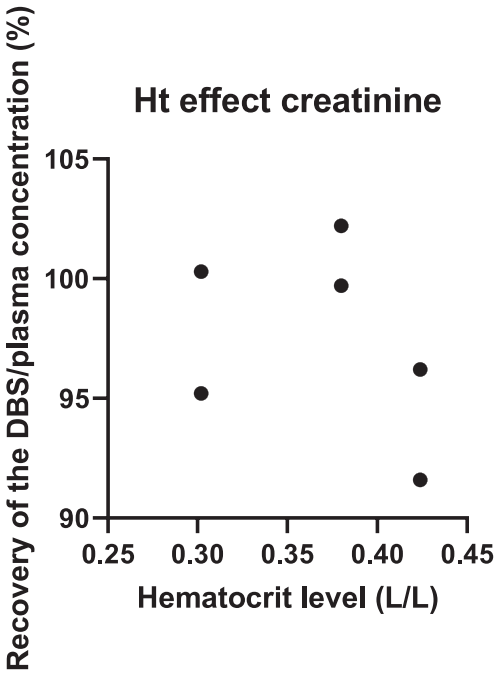

Fig. 3. Results of the hematocrit (Ht) effect on the recovery of creatinine for DBS versus plasma concentrations.

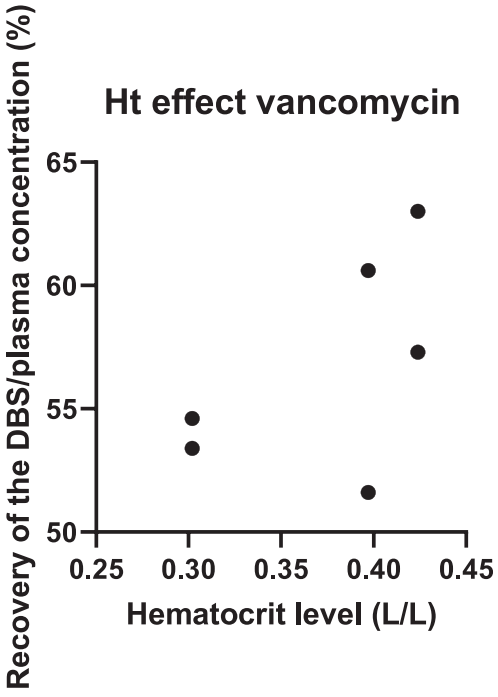

Fig. 4. Results of the hematocrit (Ht) effect on the recovery of vancomycin for DBS versus plasma concentrations.

Table 5
Results of the drying time after 3 h and 24 h.

	Bias 3 h (%)				Bias 24 h (%)			
Compound	QC L	CV (%)	QC H	CV (%)	QC L	CV (%)	QC H	CV (%)
Creatinine	−3.2	3.1	−1.7	1.7	−10.6	5.4	−2.8	3.3
Vancomycin	−4.1	6.3	−0.3	6.3	−0.8	13.4	−0.9	5.1

Table 6
Results of the measured clinical samples.

Compound	Sample	Plasma conc. (μmol/L)	DBS conc. (μmol/L)	Plasma conc. (mg/L)	DBS conc. (mg/L)	Ratio DBS/venous blood conc. (%)
Creatinine	1	81	74.2	x	x	8.4
	2	178	177.5	x	x	0.3
	3	69	77.9	x	x	−0.3
	4	70	77.7	x	x	−11
	5	77	73.2	x	x	4.9
	6	55	64.0	x	x	−16.4
Vancomycin	1	x	x	18.2	10.4	42.7
	2	x	x	23.1	14.0	39.4
	3	x	x	28.7	15.3	46.6
	4	x	x	22.8	12.9	43.6
	5	x	x	28.4	16.6	41.6
	6	x	x	22.7	13.3	41.5

Hassanzai: Conceptualization, Writing – original draft. **Robert B. Flint:** Conceptualization, Writing – original draft. **Hein A.W. van Onzenoort:** Conceptualization, Writing – original draft. **Brenda C.M. de Winter:** Conceptualization, Writing – original draft. **Birgit C.P. Koch:** Conceptualization, Supervision, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2023.117689.

References

[1] M.M. Bergman, R.H. Glew, T.H. Ebert, Acute interstitial nephritis associated with vancomycin therapy, Arch. Intern. Med. 148 (10) (1988) 2139–2140.
[2] M.A. Cimino, C. Rotstein, R.L. Slaughter, L.J. Emrich, Relationship of serum antibiotic concentrations to nephrotoxicity in cancer patients receiving concurrent aminoglycoside and vancomycin therapy, Am. J. Med. 83 (6) (1987) 1091–1097.
[3] M.J. Rybak, L.M. Albrecht, S.C. Boike, P.H. Chandrasekar, Nephrotoxicity of vancomycin, alone and with an aminoglycoside, J. Antimicrob. Chemother. 25 (4) (1990) 679–687.
[4] P.G. Traber, D.P. Levine, Vancomycin Ototoxicity in patient with normal renal function, Ann. Intern. Med. 95 (4) (1981) 458–460.
[5] L.K. Hidayat, D.I. Hsu, R. Quist, K.A. Shriner, A. Wong-Beringer, High-dose vancomycin therapy for methicillin-resistant Staphylococcus aureus infections: efficacy and toxicity, Arch. Intern. Med. 166 (19) (2006) 2138–2144.
[6] B.P. Howden, P.B. Ward, P.G. Charles, T.M. Korman, A. Fuller, P. du Cros, et al., Treatment outcomes for serious infections caused by methicillin-resistant Staphylococcus aureus with reduced vancomycin susceptibility, Clin. Infect. Dis. 38 (4) (2004) 521–528.
[7] T.P. Lodise, J. Graves, A. Evans, E. Graffunder, M. Helmecke, B.M. Lomaestro, et al., Relationship between vancomycin MIC and failure among patients with methicillin-resistant Staphylococcus aureus bacteremia treated with vancomycin, Antimicrob. Agents Chemother. 52 (9) (2008) 3315–3320.
[8] G. Sakoulas, P.A. Moise-Broder, J. Schentag, A. Forrest, R.C. Moellering Jr., G. M. Eliopoulos, Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant Staphylococcus aureus bacteremia, J. Clin. Microbiol. 42 (6) (2004) 2398–2402.
[9] J. Li, A.A. Udy, C.M. Kirkpatrick, J. Lipman, J.A. Roberts, Improving vancomycin prescription in critical illness through a drug use evaluation process: a weight-based dosing intervention study, Int. J. Antimicrob. Agents 39 (1) (2012) 69–72.
[10] G.T. Khotaei, S. Jam, S. SeyedAlinaghi, F. Motamed, F. Nejat, M. Taghi, et al., Monitoring of serum vancomycin concentrations in pediatric patients with normal renal function, Acta Med. Iran. 48 (2) (2010) 91–94.
[11] P. Mariani-Kurkdjian, H. Nebbad, Y. Aujard, E. Bingen, [Monitoring serum vancomycin concentrations in the treatment of Staphylococcus infections in children] Surveillance des taux seriques de vancomycine dans le traitement des infections a staphylocoque en pediatrie, Arch Pediatr 15 (11) (2008) 1625–1629.
[12] L.L. Marengo, S. Del Fiol Fde, J. Oliveira Sde, C. Nakagawa, E.L. Croco, S. Barberato-Filho, et al., Vancomycin: the need to suit serum concentrations in hemodialysis patients, Braz. J. Infect. Dis. 14 (2) (2010) 203–208.
[13] M.V. Miles, L. Li, H. Lakkis, J. Youngblood, P. McGinnis, Special considerations for monitoring vancomycin concentrations in pediatric patients, Ther. Drug Monit. 19 (3) (1997) 265–270.
[14] D. Commandeur, C. Giacardi, M. Danguy Des Deserts, S. Huynh, M.L. Buguet-Brown, M. Ould-Ahmed, et al., [Monitoring vancomycin in an intensive care unit: A retrospective survey on 66 patients] Monitorage de la vancomycine en reanimation: etude retrospective de 66 patients, Med Mal Infect 41 (8) (2011) 410–414.
[15] A.G. Reis, S.J. Grisi, [Monitorization of blood levels of vancomycin in children with multi-resistant bacterial infections] Monitorizacao das concentracoes sericas da vancomicina em criancas com infeccoes por bacterias multiresistentes, J Pediatr (Rio J) 72 (4) (1996) 225–229.
[16] F. Pea, L. Porreca, M. Baraldo, M. Furlanut, High vancomycin dosage regimens required by intensive care unit patients cotreated with drugs to improve haemodynamics following cardiac surgical procedures, J. Antimicrob. Chemother. 45 (3) (2000) 329–335.
[17] C. Hochart, C. Berthon, S. Corm, J. Gay, M. Cliquennois, S. Tricot, et al., Vancomycin serum concentration during febrile neutropenia in patients with acute myeloid leukemia, Med. Mal. Infect. 41 (12) (2011) 652–656.
[18] L.S. Eiland, T.M. English, E.H. Eiland 3rd, Assessment of vancomycin dosing and subsequent serum concentrations in pediatric patients, Ann. Pharmacother. 45 (5) (2011) 582–589.
[19] M.J. Rybak, J. Le, T.P. Lodise, D.P. Levine, J.S. Bradley, C. Liu, et al., Therapeutic Monitoring of Vancomycin for Serious Methicillin-resistant Staphylococcus aureus Infections: A Revised Consensus Guideline and Review by the American Society of Health-system Pharmacists, the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the Society of Infectious Diseases Pharmacists, Clin. Infect. Dis. 71 (6) (2020) 1361–1364.
[20] K. Matsumoto, Y. Takesue, N. Ohmagari, T. Mochizuki, H. Mikamo, M. Seki, et al., Practice guidelines for therapeutic drug monitoring of vancomycin: a consensus review of the Japanese Society of Chemotherapy and the Japanese Society of Therapeutic Drug Monitoring, J. Infect. Chemother. 19 (3) (2013) 365–380.
[21] A. Marsot, A. Boulamery, B. Bruguerolle, N. Simon, Vancomycin: a review of population pharmacokinetic analyses, Clin. Pharmacokinet. 51 (1) (2012) 1–13.
[22] M.I. Francke, L.E.J. Peeters, D.A. Hesselink, S.M. Kloosterboer, B.C.P. Koch, H. Veenhof, et al., Best practices to implement dried blood spot sampling for therapeutic drug monitoring in clinical practice, Ther. Drug Monit. 44 (5) (2022) 696–700.
[23] M.V. Antunes, M.F. Charao, R. Linden, Dried blood spots analysis with mass spectrometry: Potentials and pitfalls in therapeutic drug monitoring, Clin. Biochem. 49 (13–14) (2016) 1035–1046.
[24] L. Scribel, A.P. Zavascki, D. Matos, F. Silveira, T. Peralta, N. Goncalves Landgraf, et al., Vancomycin and creatinine determination in dried blood spots: Analytical validation and clinical assessment, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1137 (2020), 121897.
[25] C. Tron, S.M. Kloosterboer, B.C.H. van der Nagel, R.A. Wijma, B. Dierckx, G. C. Dieleman, et al., Dried blood spots combined with ultra–high-performance liquid chromatography–mass spectrometry for the quantification of the antipsychotics risperidone, aripiprazole, pipamperone, and their major metabolites, Ther Drug Monit 39 (4) (2017) 429–440.
[26] L.E.J. Peeters, S. Bahmany, T. Dekker, A. Aliawi, B. van Domburg, J. Versmissen, et al., Development and validation of a dried blood spot assay using UHPLC-MS/MS to Identify and quantify 12 antihypertensive drugs and 4 active metabolites: clinical needs and analytical limitations, Ther. Drug Monit. 44 (4) (2022) 568–577.
[27] M.I. Francke, B. van Domburg, S. Bouarfa, D. van de Velde, M.E. Hellemons, O. C. Manintveld, et al., The clinical validation of a dried blood spot method for simultaneous measurement of cyclosporine A, tacrolimus, creatinine, and hematocrit, Clin. Chim. Acta 535 (2022) 131–139.
[28] C.a.L.S. Institute, Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures 25(Second Edition) (2006).
[29] E.M. Agency, Guideline on bioanalytical method validation (2011).
[30] U.S.D.o.H.a.H. Services, Bioanalytical Method Validation Guidance for Industry (2018).
[31] S. Capiau, H. Veenhof, R.A. Koster, Y. Bergqvist, M. Boettcher, O. Halmingh, et al., Official International association for therapeutic drug monitoring and clinical toxicology guideline: development and validation of dried blood spot-based methods for therapeutic drug monitoring, Ther. Drug Monit. 41 (4) (2019) 409–430.
[32] H. Brozmanova, I. Kacirova, R. Urinovska, P. Sistik, M. Grundmann, New liquid chromatography-tandem mass spectrometry method for routine TDM of vancomycin in patients with both normal and impaired renal functions and comparison with results of polarization fluoroimmunoassay in light of varying creatinine concentrations, Clin. Chim. Acta 469 (2017) 136–143.
[33] L. Anne, M. Hu, K. Chan, L. Colin, K. Gottwald, Potential problem with fluorescence polarization immunoassay cross-reactivity to vancomycin

degradation product CDP-1: its detection in sera of renally impaired patients, Ther. Drug Monit. 11 (5) (1989) 585–591.
[34] A.C.C. da Silva, L. de Lima Feltraco, M.F. Lizot, M.V. Bastiani, N. Antunes, R. L. Brucker, Ready for TDM: Simultaneous quantification of amikacin, vancomycin and creatinine in human plasma employing ultra-performance liquid chromatography-tandem mass spectrometry, Clin. Biochem. 70 (2019) 39–45.
[35] N.B. Andriguetti, L.L. Lisboa, S.R. Hahn, L.R. Pagnussat, M.V. Antunes, R. Linden, Simultaneous determination of vancomycin and creatinine in plasma applied to volumetric absorptive microsampling devices using liquid chromatography-tandem mass spectrometry, J. Pharm. Biomed. Anal. 165 (2019) 315–324.
[36] R.N. Xu, L. Fan, M.J. Rieser, T.A. El-Shourbagy, Recent advances in high-throughput quantitative bioanalysis by LC-MS/MS, J. Pharm. Biomed. Anal. 44 (2) (2007) 342–355.
[37] I.R. Muller, G. Linden, M.F. Charao, M.V. Antunes, R. Linden, Dried blood spot sampling for therapeutic drug monitoring: challenges and opportunities, Expert. Rev. Clin. Pharmacol. (2023) 1–11.
[38] R.A. Koster, B. Greijdanus, J.W. Alffenaar, D.J. Touw, Dried blood spot analysis of creatinine with LC-MS/MS in addition to immunosuppressants analysis, Anal. Bioanal. Chem. 407 (6) (2015) 1585–1594.