

Development and validation of a bioanalytical assay for the measurement of total and unbound teicoplanin in human serum

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Background: The glycopeptide teicoplanin is considered first-line treatment for severe infections caused by Gram-positive bacteria. Individualized treatment of teicoplanin is gaining interest. As only protein-unbound drug is pharmacologically active, a sensitive assay measuring unbound and total teicoplanin is indispensable for pharmacological research and dose optimization.

Objectives: To develop and validate a UPLC-MS/MS method to quantify unbound and total teicoplanin in human serum.

Methods: The developed assay was validated according to the ICH guideline M10 on Bioanalytical Method Validation and study sample analysis. Unbound teicoplanin was obtained by ultrafiltration. The assay was cross-validated with a quantitative microsphere (QMS) immunoassay in a side-by-side comparison using 40 patient samples.

Results: With the developed and validated method, all main teicoplanin components (A2-1, A2-2/A2-3, A2-4/A2-5 and A3-1) can be quantified. Total run time was 5.5 min. Concentration range was 2.5–150 mg/L for total and 0.1–25 mg/L for unbound teicoplanin. Precision (coefficient of variation) and accuracy (bias) of total teicoplanin were 5.97% and 107%, respectively, and 7.17% and 108%, respectively, for unbound teicoplanin. Bland–Altman analysis showed total concentrations measured with the UPLC-MS/MS method were equivalent to the results of the QMS immunoassay. A total of 188 samples from 30 patients admitted to the ICU and haematology department were measured; total concentrations ranged between 2.92 and 98.5 mg/L, and unbound concentrations ranged between 0.37 and 30.7 mg/L.

Conclusions: The developed method provided rapid, precise and accurate measurement of unbound and total teicoplanin. The developed method is now routinely applied in pharmacological research and clinical practice.

Introduction

Teicoplanin is a glycopeptide antibiotic active against infections caused by Gram-positive bacteria, including MSSA, CoNS and MRSA.¹ It is a semi-synthetic antibiotic produced by *Actinoplanes teichomyeticus* and is a complex mixture of related molecules. The active subcomponents include five closely related glycopeptides (A₂₋₁–A₂₋₅) and the hydrolysis product A₃₋₁, comprising ≥95% of all subcomponents.² The subcomponents all share the same glycopeptide core, differing only in the alkyl side chain.³

Interest in optimizing teicoplanin therapy and utilization of pharmacokinetic (PK) data and therapeutic drug monitoring (TDM) in daily practice is increasing.⁴ This is standard practice for the glycopeptide vancomycin and aminoglycosides. Relationship between exposure and efficacy and/or toxicity has been demonstrated for these antibiotics, and target concentrations have been determined.⁵ TDM of teicoplanin is recommended, yet not common practice in the majority of clinics.⁴ A total trough concentration of 10–20 mg/L is usually targeted. In the case of deep-seated and/or severe infections, a target of

20–30 mg/L is suggested in the literature for the total trough concentration at steady-state.^{4,6}

Teicoplanin is reported to be ~90% protein-bound in healthy volunteers.⁷ Generally, critically ill patients have altered and variable PK and protein binding.⁵ As only the unbound concentration is responsible for the pharmacological effect, dose optimization of highly protein-bound drugs like teicoplanin should be performed on unbound concentrations.⁸ This shows the necessity to determine unbound concentrations of teicoplanin.

Several assays to measure teicoplanin concentrations have been reported in the literature. The often-used fluorescence polarization immunoassay (FPIA) is simple and fast, but expensive. Quantification of unbound teicoplanin concentrations with this method is not feasible, due to the relatively high lower limit of quantification (LLOQ).^{9–13} Description of an assay with a short turn-around time and proficient LLOQ that is able to measure total and unbound concentrations and for which the reliability of the results is investigated by a cross-validation is lacking.

In this paper, we present a rapid, validated UPLC–MS/MS method able to quantify total and unbound concentrations of all main subcomponents of teicoplanin in human serum in a clinically relevant concentration range.

Materials and methods

Reagents, chemicals and chromatographic and mass spectrometric conditions

An overview of the used reagents and chemicals and the chromatographic and spectrometric conditions of the developed method can be found in the [Supplementary Methods 1.1 and 1.2](#), [Figure S1](#) and [Tables S1 and S2](#), available as [Supplementary data](#) at JAC Online.

Preparation of calibration standards (CSs) and quality controls (QCs) for the measurement of total teicoplanin

Independently weighted stock solutions for CSs and QCs were created by reconstituting 400 mg of teicoplanin in 8 mL of Milli-Q water (final concentration of 50 000 mg/L). The stock solutions were diluted 1:10 with blank human serum to create 5000 mg/L working solutions for both the CSs and QCs. Seven CSs (2.5, 7.5, 15, 30, 50, 100 and 150 mg/L) were prepared from the working solution in duplicate freshly before each run. Five QCs were prepared in blank human serum: LLOQ 2.5 mg/L, low concentration (QCLow, 5 mg/L), medium concentration (QCMed, 37.5 mg/L), high concentration (QCHigh, 125 mg/L) and higher limit of quantification (HLOQ, 150 mg/L).

Preparation of CSs and QCs for the measurement of unbound teicoplanin

Stock solutions prepared for the measurement of total teicoplanin were diluted 1:10 with pH 9 Milli-Q water to create two 5000 mg/L working solutions, one for unbound CSs and one for QCs. The CS working solution was diluted with pH 9 Milli-Q water to achieve working stocks of 250, 80, 25, 8, 5, 2.4 and 1 mg/L. The QC working solution was diluted with pH 9 Milli-Q water to achieve QC working stocks of 250, 185.7, 35.71, 3.184 and 1 mg/L. Amicon Centrifree 30K filters (Merck Life Science BV, Hoeilaart, Belgium) were used for ultrafiltration.¹⁴ For the CSs, 500 µL of blank human serum was pipetted onto the filters, after which these were equilibrated for 60 min at 1 × g/41°C to reach a sample temperature of 37°C. These were then ultrafiltered by centrifuging for 20 min at 1650 × g/41°C. The CSs were created in duplicate by spiking the blank

human ultrafiltrate at seven concentration levels by adding 10 µL of the prepared CS working stocks (1–250 mg/L) to 90 µL of blank human ultrafiltrate. This resulted in unbound calibration standards of 0.1, 0.24, 0.5, 0.8, 2.5, 8 and 25 mg/L. LLOQ (0.1 mg/L), QCLow (0.318 mg/L), QCMed (3.57 mg/L), QCHigh (18.57 mg/L) and HLOQ (25 mg/L) were prepared by spiking blank human ultrafiltrate with QC working solutions. An additional QC of 25 mg/L was prepared in blank human serum by adding 50 µL of QC stock solution of 50 000 mg/L teicoplanin to 450 µL of serum and diluting 100 µL of the created solution with 19 900 µL of serum. This QC was ultrafiltered in triplicate in each run to create an unbound QC sample of approximately 4 mg/L. For both QCs and CSs, 100 µL of sample was transferred to a vial using the reverse pipetting method, along with 100 µL of internal standard (IS) solution and 100 µL of eluent A. The samples were subsequently sealed with a pre-slit septum cap and vortexed for 3 min at 2500 rpm.

Preparation of IS solution

Daptomycin (500 mg) was reconstituted in 10 mL of Milli-Q water to obtain a concentration of 50 000 mg/L. Two microlitres of this solution was diluted in 50 mL of acetonitrile hypergrade resulting in a final IS concentration of 2 mg/L.

Patient sample preparation for measurement of the total teicoplanin concentration

A volume of 50 µL of patient serum was mixed with 50 µL of Milli-Q water and 150 µL of IS solution. The mixture was vortexed for 3 min and centrifuged at 18 260 × g for 5 min. Fifty microlitres of supernatant was 1:1 diluted with eluent A, vortexed for 3 min and centrifuged at 1910 × g for 5 min.

Patient sample preparation for measurement of the unbound teicoplanin concentration

For each sample, an aliquot of 300 µL of patient serum was pipetted onto an Amicon Centrifree 30K filter and equilibrated for 60 min at 1 × g/41°C to reach a sample temperature of 37°C. The samples were then ultrafiltered by centrifuging for 20 min at 1650 × g/41°C. An aliquot of 100 µL of patient sample ultrafiltrate was transferred to a vial using the reverse pipetting method, along with 100 µL of IS solution and 100 µL of eluent A. The samples were subsequently sealed with a pre-slit septum cap and vortexed for 3 min at 2500 rpm.

Concentration calculation for total and unbound teicoplanin

First, the areas of the separate subcomponents were compared to the limit of quantification of the individual calibration curves of each component to ensure that the measured concentrations of all separate components fell within the validated range. Second, the areas of the peaks of all subcomponents were summed and divided by the peak area of the IS. A quadratic fit with a $1/x^2$ weight factor was used on the CSs to compensate for non-linearity in the instrument response to obtain slope, intercept and coefficient of determination (r^2) for each run using a worksheet for analytical calibration curves.¹⁵ The teicoplanin concentrations were subsequently calculated using the quadratic equation.

Validation procedures

The method was validated according to the most recent version of the EMA guideline on bioanalytical method validation and study sample analysis.¹⁶ All validation procedures are described below. Validation parameters specifically related to ultrafiltration, such as solubility, recovery of the ultrafiltration process (i.e. non-specific binding to the ultrafiltration

device) and batch-to-batch difference of the ultrafiltration, can be found in the [Supplementary Methods 1.3](#), [Supplementary Results 2.1](#) and [Table S9](#)). The impact of haemolysed and hyperlipidaemic sera on UPLC–MS/MS results was also validated and can be found in the [Supplementary Methods 1.3.4](#), [Supplementary Results 2.1.4](#) and [Tables S10 and S11](#).

Accuracy and precision

Accuracy and precision of both total and unbound teicoplanin were assessed by applying the developed method to measure QC (LLOQ, QCLow, QCMed, QCHigh and HLOQ) concentrations 5-fold in three runs spread out over two separate days. Accuracy was evaluated by calculating the bias (%) for each of the concentrations. Precision was expressed in terms of coefficient of variation (CV, %) and was calculated between and within runs. The maximum accepted value for both accuracy and precision was <20% for LLOQ and <15% for other QCs.

Selectivity

Selectivity was investigated for total and unbound teicoplanin by comparing the response of blank serum or ultrafiltrate of six individuals not receiving teicoplanin with the corresponding blank serum or ultrafiltrate spiked at LLOQ to determine whether the detection of teicoplanin or the IS was interfered by endogenous substances.

Carry-over

Carry-over was evaluated for both total and unbound teicoplanin concentrations by measuring blank serum or ultrafiltrate samples 5-fold immediately after an HLOQ sample. The difference in response between the blank samples and the LLOQ and IS was calculated. The acceptance criteria of the peak areas of the blank samples were that they could not exceed 20% of the peak area of the LLOQ samples for teicoplanin and 5% for IS.

LLOQ

The signal measured in the LLOQ samples should be at least five times the signal measured in a blank sample. Six blank serum samples from healthy volunteers were prepared as described for the QCs above, analysed and compared with the LLOQ of 2.5 mg/L. The same procedure was performed with the ultrafiltrate, with an LLOQ of 0.1 mg/L.

Dilution integrity

Dilution integrity of total teicoplanin was demonstrated by spiking blank human serum with a concentration of 1.5 times the HLOQ. Dilution integrity for unbound teicoplanin was determined by spiking blank ultrafiltrate to a concentration equal to QCHigh. These were diluted 5-fold two and five times, respectively, in blank serum. Samples were prepared and measured with the developed method. Concentration of the undiluted sample was back calculated using the dilution factor. Accuracy and precision were calculated as previously described and must be <15%.

Matrix effect

Matrix effect of total and unbound teicoplanin was assessed using six individual blank serum samples from healthy volunteers, spiked in duplicate with IS and teicoplanin to a concentration equal to the QCLow and QCHigh. These samples were prepared and measured. The measured signals were compared with the signal of spiked eluent A to a concentration equal to QCLow and QCHigh. The ratio between the measured signals in the serum samples and the eluent samples was determined for teicoplanin and the IS. These ratios were divided upon one another. The CV of these ratios of both concentrations were determined. Matrix effects were considered to be acceptable if CV was <15%.

Stability

The stability of QCs and stock solutions was evaluated under different conditions. Stability of the stock solution was assessed at 2°C–6°C and at –40°C. Stability of the QCs was tested after storage at room temperature, 2°C–6°C and –40°C, after 3 freeze–thaw cycles and while in the autosampler.

Cross-validation of UPLC–MS/MS–quantitative microsphere (QMS)

The developed method was cross-validated with a QMS immunoassay using a cobas C8000-2 with a C502 module (Roche Diagnostics, Basel, Switzerland). As described in the CLSI guidelines,¹⁷ 40 patient samples were used for the cross-validation. Serum samples for QMS were measured according to the instructions of the manufacturer. If concentrations were measured outside the range recommended by Roche (3–50 µg/mL) or outside the validated concentration range of the described UPLC–MS/MS method, samples were diluted in blank serum. Measurement error for UPLC–MS/MS was estimated by using the data generated by measuring the samples in duplicate. Measurement error for the QMS was estimated by measuring three QCs: low (9.4 mg/L), medium (36.29 mg/L) and high (83.17 mg/L) 14, 14 and 16 times, respectively. Agreement between the two methods was assessed using weighted Deming regression and Bland–Altman plots.^{16,18} Bland–Altman plots were generated using the NCSS statistical software V12.0.0. The difference between two values obtained from the different methods should be within 20% of the mean for at least 67% of the samples, as described under cross-validation in the EMA guideline for bioanalytical method validation.¹⁹ Deming regression was performed using R-4.1.3 (64-bit) using the MCR package V1.2.2.^{20,21} The 95% CI of the slope should include 1 and the 95% CI of the intercept should include 0 to be considered equivalent.¹⁸

Clinical application

To evaluate whether the UPLC–MS/MS method could be used for TDM and clinical research, the method was used to measure total and unbound teicoplanin in 188 patient samples from 30 patients admitted to the ICU and haematology department collected for research purposes. Samples were obtained on Days 2 and 5 of teicoplanin therapy. Ratios of the individual subcomponents were determined and compared with the ratios of the subcomponents in spiked samples to examine the differences in the ratio between the individual subcomponents.

Results

Method validation

Accuracy and precision

Accuracy and precision were accepted for all conditions for both total and unbound teicoplanin ([Table 1](#)).

Selectivity

There was no significant endogenous interference, proving that the selectivity was acceptable ([Table S3](#)).

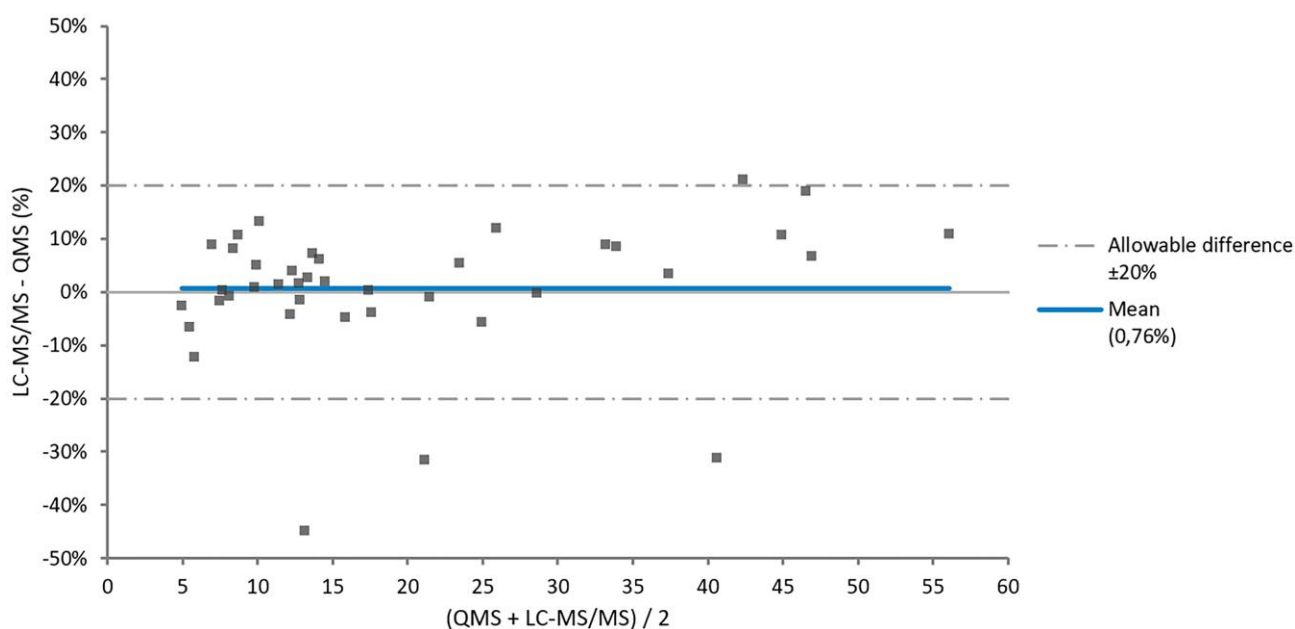
Carry-over

Carry-over effects were within limits ([Table S4](#)).

Table 1. Accuracy and precision of the UPLC–MS/MS method for the QC samples ($n=5$) of total teicoplanin in serum and unbound teicoplanin in ultrafiltrate

	LLOQ (%)	QCLow (%)	QCMed (%)	QCHigh (%)	HLOQ (%)
Total teicoplanin					
Within-run accuracy (bias, %)	105	98.7	101	103	107
Between-run accuracy (bias, %)	102	100	101	102	103
Within-run precision (CV, %)	5.97	5.68	4.66	3.25	5.03
Between-run precision (CV, %)	2.36	n.d.	n.d.	n.d.	2.75
Unbound teicoplanin					
Within-run accuracy (bias, %)	108	107	92.8	94.2	96.7
Between-run accuracy (bias, %)	101	100	100	100	98.9
Within-run precision (CV, %)	2.84	6.62	5.66	5.33	3.12
Between-run precision (CV, %)	7.17	6.15	6.00	4.80	2.45

n.d., not determined as within-run precision exceeded between-run precision.

**Figure 1.** Bland–Altman plot for the comparison of the developed UPLC–MS/MS assay and the QMS assay for measurement of total teicoplanin concentrations. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

Matrix effects

In both total and unbound concentrations, measured signals in the blank serum samples were well below the predetermined limits when compared with the LLOQ (Table S5).

Dilution integrity

Sample deviation parameters of variance were below the accepted limit after diluting and measuring a known concentration that was outside of the HLOQ (Table S6).

Stability

Results of stability tests of QC and stock solution analyses under different conditions are depicted in Tables S7 and S8. QCs were

stable at room temperature for up to 2 days, and at 2°C–6°C for up to 7 days. Long-term stability at –40°C was within predefined limits (Table S7). Samples were stable during three freeze–thaw cycles. Stock solutions were stable at 2°C–6°C for up to 24 months (Table S8).

Cross-validation

The Bland–Altman difference plot (Figure 1) showed that bias for the measured teicoplanin concentrations was within predefined limits: 0.76% (95% CI –3.29% to 4.81%) higher concentrations were measured using UPLC–MS/MS. Concentrations measured with the UPLC–MS/MS assay were within $\pm 20\%$ of the concentrations measured with the QMS immunoassay for 90% of the

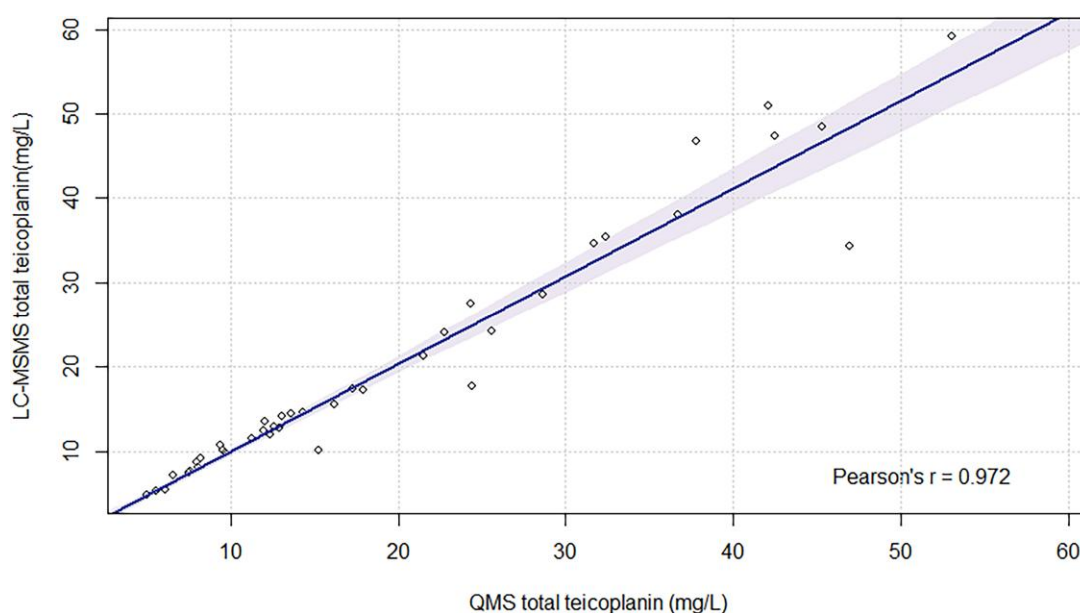


Figure 2. Comparison between the newly developed UPLC-MS/MS assay and the QMS assay for measurement of total teicoplanin concentrations. The line shows the weighted Deming regression with the grey area depicting the 95% CI. The slope was 1.06 (95% CI 0.98–1.15). The intercept was -0.81 (95% CI -1.19 to 0.14). This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

patient samples, and thereby within predefined acceptance criteria for cross-validation. Results of the weighted Deming regression are depicted in Figure 2. The slope was 1.06 (95% CI 0.98–1.15) and the intercept was -0.81 (95% CI -1.19 to 0.14), showing agreement between the methods.

Clinical application

The UPLC-MS/MS assay was used to analyse patient samples to investigate the PK of unbound and total teicoplanin in critically ill and haematology patients. The highest total concentration measured was 98.5 mg/L. Measured unbound concentrations were between 0.37 and 30.7 mg/L. On average, protein binding was 79.0% (range 55.3%–92.8%). Relationship between measured total and unbound teicoplanin concentrations is depicted in Figure 3. The slope was 3.83 (95% CI 3.66–4.01) and the intercept was 3.36 (95% CI 2.3–4.4). Out of 188 samples, 2 measured concentrations were outside the validated concentration range; one unbound concentration was above the validated range and one measured total concentration was below the validated range. The sample with a measured teicoplanin concentration above the range was diluted and re-measured according to the validated procedure. QC samples for unbound teicoplanin showed different ratios of the individual components of teicoplanin than patient serum samples, but a comparable protein binding (Table 2).

Discussion

A UPLC-MS/MS assay for the analysis of both total and unbound teicoplanin was successfully developed and validated. The method was able to distinguish teicoplanin components A_{2-1} , A_{2-2} / A_{2-3} , A_{2-4} / A_{2-5} and A_{3-1} without extensive preparation steps.

The method was shown to be accurate and precise and can be used for research purposes and TDM in daily practice.

Previously, QMS,²² HPLC-UV,²³ HPLC with electrochemical detection²⁴ and LC-MS^{14,25–29} methods for the quantification of teicoplanin have been described, some of which were able to quantify teicoplanin subcomponents separately.^{14,24,30} Few of the reported assays were able to determine unbound concentrations.^{11,12,14,23,30} None of these assays fulfilled all desired criteria for use in clinical practice and for research purposes. In comparison with previously published assays able to measure unbound teicoplanin,^{6,27,28,30} the assay described in this paper has a significantly reduced runtime of 5.5 mins. Labour intensity and sample preparation time facilitate application of this assay in clinical practice. The assay was able to measure teicoplanin concentrations in a clinically relevant range. In order to perform TDM in clinical practice these are essential improvements.

Several challenges were faced during method development, specifically concerning the ratios of the subcomponents and calculation of the subcomponents of teicoplanin. The ratios of the subcomponents observed in ultrafiltrated patient samples diverged from the ratios observed after ultrafiltrating spiked blank human serum. Differences in subcomponent ratios between samples of different origin have been reported.²⁸ We hypothesized that in order to appropriately assess the performance of UPLC-MS/MS results, ratios in QC samples should reflect patient samples. Ultrafiltrate is slightly basic (pH 9) and making QCs in pH 9 Milli-Q water and spiking ultrafiltrate, instead of spiking and ultrafiltrating blank serum, created an environment where subcomponent ratios were the most similar (see Table 2). To appropriately assess the ultrafiltration process, in each run a QCmed sample was prepared in human serum, ultrafiltrated in triplicate and processed according to the procedure described for the patient samples. Using this method we found components A_{2-2}

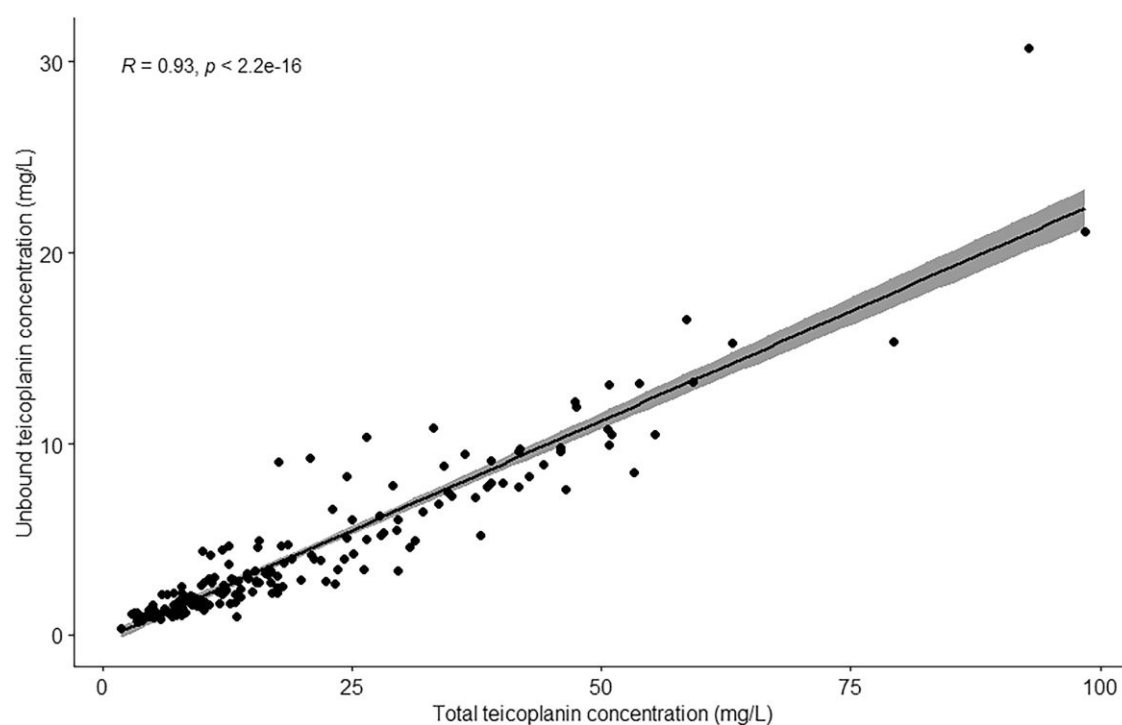


Figure 3. Spearman correlation of total and unbound teicoplanin concentrations ($n=188$) measured with the developed UPLC–MS/MS method. The slope was 3.83 (95% CI 3.66–4.01) and the intercept was 3.36 (95% CI 2.3–4.4). The grey area depicts the 95% CI.

Table 2. Mean ratios of the subcomponents of unbound teicoplanin and percentage protein bound in various samples

	A ₃₋₁ (%)	A ₂₋₁ (%)	A ₂₋₃₊₄ (%)	A ₂₋₄₊₅ (%)	Percentage (%) protein bound
Patient serum	8.39	27.5	48.9	15.2	79.0
Spiked serum	16.9	27.7	49.1	6.31	83.2
pH 9 Milli-Q water	4.74	28.5	50.8	16.0	—

and A₂₋₃ to account for approximately 49% of the total teicoplanin concentration in both patient and QC samples, which is consistent with previous reports^{14,26–28} and in compliance with the limits on the ratios as specified by the EMA.²

To ensure the individual subcomponents fell within the respective limits of the individual calibration curves, this was confirmed prior to summing the peaks of the subcomponents in every run. Determination of the teicoplanin concentrations was performed by summing the area of all components, which is comparable to methods previously described in the literature.^{14,28} A quadratic curve with a $1/x^2$ weight factor was the most appropriate fit on the summed peak areas for both total and unbound teicoplanin to compensate for non-linearity in the instrument response.

Accurate and reproducible determination of unbound drug concentrations is challenging.³¹ The described assay has been shown to reproducibly measure unbound teicoplanin concentrations. In order to provide accurate results, we aimed to reflect physiological conditions. Previously performed experiments revealed that the time for equilibration within our specific

centrifuge was 60 min at $1 \times g$ at a set temperature of 41°C to enable a sample temperature of 37°C.^{32,33} A limitation of the described method is that the pH of the samples before ultrafiltration was not adjusted to physiological conditions, as is suggested in some recent reports.^{31,34}

Our data on protein binding show a clear relationship between total and unbound teicoplanin concentrations, but suggest substantial variability in protein binding between patients. Protein binding in both patient samples and pooled serum of healthy subjects was lower than that published in the literature (79% and 83.2% versus 90%, respectively),^{7,14} indicating further research into exposure and PK of unbound teicoplanin is warranted.

During method validation it was noticed that haemolysed and hyperlipidaemic serum influence the UPLC–MS/MS assay. Additional validation in these samples was performed.

Results showed that the results obtained with the described UPLC–MS/MS assay are equivalent to the results obtained with a widely used QMS method, in line with previous findings that showed correlation between immunological and HPLC methods

is high when all teicoplanin subcomponents are taken into account.^{22,25,35,36}

Ideally, deuterated teicoplanin is used as IS. To the best of our knowledge, there is no commercially labelled teicoplanin available. As previously described in other MS-based teicoplanin assays, the use of an IS other than deuterated teicoplanin is proficient.^{14,25–28} Similar to Begou *et al.*,²⁸ daptomycin was used as IS.

Recently, a panel of experts recommended TDM for teicoplanin.⁴ To realize teicoplanin TDM in clinical practice, several prerequisites need to be met.³⁷ This method enables measurement of all major teicoplanin subcomponents without extensive sample preparation. The assay described in this report enables further research in determining total and unbound target exposures, PK of teicoplanin and can be used in daily clinical TDM practice.

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Transparency declarations

None to declare.

Supplementary data

Supplementary Methods, Supplementary Results, Figure S1 and Tables S1 to S11 are available as Supplementary data at JAC Online.

References

- Cavalcanti AB, Goncalves AR, Almeida CS *et al.* Teicoplanin versus vancomycin for proven or suspected infection. *Cochrane Database Syst Rev* 2010; **issue 6**: CD007022. <https://doi.org/10.1002/14651858.CD007022.pub2>
- EMA. Assessment report: Teicoplanin. https://www.ema.europa.eu/en/documents/referral/assessment-report-article-53-procedure-teicoplanin-containing-medicinal-products_en.pdf
- Borghesi A, Coronelli C, Faniuolo L *et al.* Teichomycins, new antibiotics from *Actinoplanes teichomyceticus* nov. sp. IV. Separation and characterization of the components of teichomycin (teicoplanin). *J Antibiot (Tokyo)* 1984; **37**: 615–20. <https://doi.org/10.7164/antibiotics.37.615>
- Abdul-Aziz MH, Alffenaar JC, Bassetti M *et al.* Antimicrobial therapeutic drug monitoring in critically ill adult patients: a position paper. *Intensive Care Med* 2020; **46**: 1127–53. <https://doi.org/10.1007/s00134-020-06050-1>
- Jager NGL, van Hest RM, Lipman J *et al.* Therapeutic drug monitoring of anti-infective agents in critically ill patients. *Expert Rev Clin Pharmacol* 2016; **9**: 961–79. <https://doi.org/10.1586/17512433.2016.1172209>
- Pea F. Teicoplanin and therapeutic drug monitoring: an update for optimal use in different patient populations. *J Infect Chemother* 2020; **26**: 900–7. <https://doi.org/10.1016/j.jiac.2020.06.006>
- Bernareggi A, Borgonovi M, Del Favero A *et al.* Teicoplanin binding in plasma following administration of increasing intravenous doses to healthy volunteers. *Eur J Drug Metab Pharmacokinet* 1991; **Spec No 3**: 256–60. <https://pubmed.ncbi.nlm.nih.gov/1840325/>
- Wong G, Briscoe S, McWhinney B *et al.* Therapeutic drug monitoring of β -lactam antibiotics in the critically ill: direct measurement of unbound drug concentrations to achieve appropriate drug exposures. *J Antimicrob Chemother* 2018; **73**: 3087–94. <https://doi.org/10.1093/jac/dky314>
- Rybak MJ, Bailey EM, Reddy VN. Clinical evaluation of teicoplanin fluorescence polarization immunoassay. *Antimicrob Agents Chemother* 1991; **35**: 1586–90. <https://doi.org/10.1128/AAC.35.8.1586>
- Yu L, Zhong M, Wei Y. Direct fluorescence polarization assay for the detection of glycopeptide antibiotics. *Anal Chem* 2010; **82**: 7044–8. <https://doi.org/10.1021/ac100543e>
- Urakami T, Maiguma T, Kaji H *et al.* Analysis using fluorescence polarization immunoassay for unbound teicoplanin concentration in serum. *J Clin Pharm Ther* 2008; **33**: 357–63. <https://doi.org/10.1111/j.1365-2710.2008.00923.x>
- Dykhuisen RS, Harvey G, Stephenson N *et al.* Protein binding and serum bactericidal activities of vancomycin and teicoplanin. *Antimicrob Agents Chemother* 1995; **39**: 1842–7. <https://doi.org/10.1128/AAC.39.8.1842>
- Cox H, Whitby M, Nimmo G *et al.* Evaluation of a novel fluorescence polarization immunoassay for teicoplanin. *Antimicrob Agents Chemother* 1993; **37**: 1924–6. <https://doi.org/10.1128/AAC.37.9.1924>
- Deltombe O, Mertens T, Eloit S *et al.* Development and validation of an ultra-high performance liquid chromatography—high resolution mass spectrometry method for the quantification of total and free teicoplanin in human plasma. *Clin Biochem* 2019; **65**: 29–37. <https://doi.org/10.1016/j.clinbiochem.2018.12.010>
- O'Haver T. Worksheets for analytical calibration curves. CalibrationQuadraticB.xlsx, 2008. <https://terpconnect.umd.edu/~toh/models/CalibrationCurve.html>
- EMA. ICH Guideline M10 on Bioanalytical Method Validation and Study Sample Analysis. 2022. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m10-bioanalytical-method-validation-step-5_en.pdf
- CLSI. *Method Comparison and Bias Estimation Using Patient Samples—Second Edition: EP09*. 2002.
- Gleason CR, Ji QC, Wickremsinhe ER. Evaluation of correlation between bioanalytical methods. *Bioanalysis* 2020; **12**: 419–26. <https://doi.org/10.4155/bio-2020-0019>
- EMA. Guideline on Bioanalytical Method Validation. 2011. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf
- R Core Team. R: A Language and Environment for Statistical Computing. 2021. <https://www.R-project.org/>
- Potapov S, Model F, Schuetzenmeister A *et al.* Method comparison regression. R package version 1.2.2. <https://CRAN.R-project.org/package=mcr>
- Dailly E, Fraissinet F, Deslandes G *et al.* Evaluation of the QMS[®] teicoplanin immunoassay (ThermoFisher Scientific) on Cobas[®] 8000 system (Roche diagnostics) and comparison to fluorescence polarization immunoassay for the determination of teicoplanin concentrations in human plasma. *J Clin Lab Anal* 2013; **27**: 96–8. <https://doi.org/10.1002/jcla.21567>
- Roberts JA, Stove V, De Waele JJ *et al.* Variability in protein binding of teicoplanin and achievement of therapeutic drug monitoring targets in critically ill patients: lessons from the DALI study. *Int J Antimicrob Agents* 2014; **43**: 423–30. <https://doi.org/10.1016/j.ijantimicag.2014.01.023>

- 24** Mochizuki N, Ohno K, Shimamura T et al. Quantitative determination of individual teicoplanin components in human plasma and cerebrospinal fluid by high-performance liquid chromatography with electrochemical detection. *J Chromatogr B* 2007; **847**: 78–81. <https://doi.org/10.1016/j.jchromb.2006.09.037>
- 25** Fung FH, Tang JC, Hopkins JP et al. Measurement of teicoplanin by liquid chromatography-tandem mass spectrometry: development of a novel method. *Ann Clin Biochem* 2012; **49**: 475–81. <https://doi.org/10.1258/acb.2012.011257>
- 26** Tsai IL, Sun HY, Chen GY et al. Simultaneous quantification of antimicrobial agents for multidrug-resistant bacterial infections in human plasma by ultra-high-pressure liquid chromatography-tandem mass spectrometry. *Talanta* 2013; **116**: 593–603. <https://doi.org/10.1016/j.talanta.2013.07.043>
- 27** Mueller DM, Eckardstein AV, Saleh L. Quantification of teicoplanin in plasma by LC-MS with online sample clean-up and comparison with QMS[®] assay. *Clin Chem Lab Med* 2014; **52**: 879–87. <https://doi.org/10.1515/cclm-2013-0974>
- 28** Begou O, Kontou A, Raikos N et al. An ultra-high pressure liquid chromatography-tandem mass spectrometry method for the quantification of teicoplanin in plasma of neonates. *J Chromatogr B* 2017; **1047**: 215–22. <https://doi.org/10.1016/j.jchromb.2016.01.042>
- 29** Chae H, Lee JJ, Cha K et al. Measurement of teicoplanin concentration with liquid chromatography-tandem mass spectrometry method demonstrates the usefulness of therapeutic drug monitoring in hematologic patient populations. *Ther Drug Monit* 2018; **40**: 330–6. <https://doi.org/10.1097/FTD.0000000000000498>
- 30** Hanada K, Kobayashi A, Okamori Y et al. Improved quantitative determination of total and unbound concentrations of six teicoplanin components in human plasma by high performance liquid chromatography. *Biol Pharm Bull* 2005; **28**: 2023–5. <https://doi.org/10.1248/bpb.28.2023>
- 31** Nilsson LB. The bioanalytical challenge of determining unbound concentration and protein binding for drugs. *Bioanalysis* 2013; **5**: 3033–50. <https://doi.org/10.4155/bio.13.274>
- 32** van Ewijk-Beneken Kolmer EWJ, Teulen MJA, van den Hombergh ECA et al. Determination of protein-unbound, active rifampicin in serum by ultrafiltration and ultra performance liquid chromatography with UV detection. A method suitable for standard and high doses of rifampicin. *J Chromatogr B* 2017; **1063**: 42–9. <https://doi.org/10.1016/j.jchromb.2017.08.004>
- 33** Berezhkovskiy LM. On the temperature dependence of the unbound drug fraction in plasma: ultrafiltration method may considerably underestimate the true value for highly bound drugs. *Drug Discov Ther* 2008; **2**: 74–6. <https://www.ddtjournal.com/article/106>
- 34** Dorn C, Schießer S, Wulkersdorfer B et al. Determination of free clindamycin, flucloxacillin or tedizolid in plasma: pay attention to physiological conditions when using ultrafiltration. *Biomed Chromatogr* 2020; **34**: e4820. <https://doi.org/10.1002/bmc.4820>
- 35** Davani S, Bérard M, Royer B et al. Comparison of fluorescence polarization immunoassay and high-performance liquid chromatography methods for assay of teicoplanin: can correlation be improved? *Pathol Biol* 2004; **52**: 584–8. <https://doi.org/10.1016/j.patbio.2004.07.033>
- 36** Mastin SH, Buck RL, Mueggler PA. Performance of a fluorescence polarization immunoassay for teicoplanin in serum. *Diagn Microbiol Infect Dis* 1993; **16**: 17–24. [https://doi.org/10.1016/0732-8893\(93\)90125-Q](https://doi.org/10.1016/0732-8893(93)90125-Q)
- 37** Ensom MH, Davis GA, Cropp CD et al. Clinical pharmacokinetics in the 21st century. Does the evidence support definitive outcomes? *Clin Pharmacokinet* 1998; **34**: 265–79. <https://doi.org/10.2165/00003088-199834040-00001>