Measurement of teicoplanin by liquid chromatography-tandem mass spectrometry: Development of a novel method

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INTRODUCTION

•Teicoplanin is an antibiotic that protects against infection by inhibiting peptidoglycan synthesis in the bacterial cell wall.

•Vancomycin has historically been the drug of choice for treating staphylococci and enterococci infections; however, its potential nephrotoxicity is one of the major limitations for its routine use.

•Due to its similar structure and spectrum of activity, teicoplanin is increasingly considered as an effective alternative to vancomycin.

•Therapeutic drug monitoring (TDM) plays an important role in the optimisation of drug therapy, especially for drugs with narrow therapeutic ranges (25-75μg/mL for teicoplanin). It is widely accepted that an optimal loading dose followed by appropriate maintenance doses should achieve trough serum teicoplanin concentrations of >25μg/mL rapidly and steadily, increasing the chances of full recovery for the patient.

•More than 90% of Teicoplanin in circulation is protein bound, and has a long half-life (>150 hours) in individuals with normal renal function. Due to the importance of a correct loading dose, timing of administration, and health status of the patient, accurate teicoplanin administration is of paramount importance.

•To date, there lacks a routine method for teicoplanin measurement in the clinical setting by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), possibly due to the complex nature of this antibiotic and its heterogeneity. Five variations (A_2-1 through A_2-5) comprise the majority of detectable teicoplanin, 93% of which are A_2-2 and A_2-3. In addition, the lack of an obvious internal standard adds to the difficulty of method design.

•The work presented here details the development of a novel LC-MS/MS method for measuring teicoplanin concentrations (and ristocetin as the internal standard) in patient serum within a clinical setting.

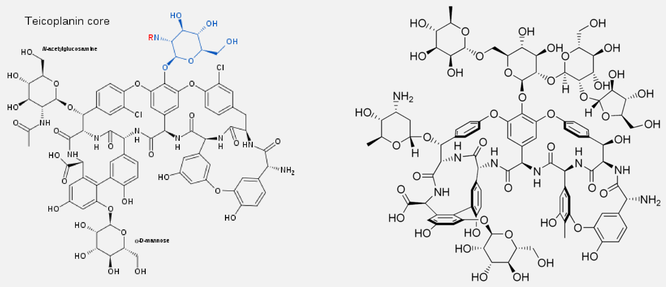


Figure 1 Structure of teicoplanin (left) and ristocetin (right)

Molecular structure of teicoplanin (left), with its glycopeptide core and point of attachment for its side chains at R. There are five major variations of teicoplanin, with A_2-2 and A_2-3 comprising up to 93% in detectable teicoplanin

Molecular structure of ristocetin (right), with a similar core and side chain as teicoplanin. This allows ristocetin to be a suitable internal standard for the teicoplanin methodology

Images courtesy of Wikipedia

METHODS

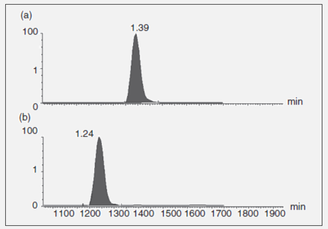
•A Waters Acuity Quattro Premier XE loaded with an ultra-pressure liquid chromatography (UPLC) column was used to determine the precursor and product ions of teicoplanin (Insight Biotechnology, Middlesex, UK; M_r = 1881) and ristocetin (Sigma, UK; M_r = 2061)

•Patient samples, external quality assurance (EQA) samples, and Quality Control (QC) material (25μL) were spiked with ristocetin (in water, 50μL), and protein precipitated by methanol. After mixing and centrifugation, the clear supernatant (25μL) was injected directly into the Acuity operating in positive electrospray ionisation mode.

•Liquid chromatography was performed on a Waters Acquity™ UPLC system. 25μL of extracted sample was injected onto an Acquity UPLC BEH (Bridged Ethane-Silicon Hybrid) C18 1.7μm (2.1 x 50mm) column (Waters, Hertfordshire, UK) in reversed-phase mode. 99% mobile phase A (1% ammonium acetate, 0.1% formic acid) was introduced from initial sample injection, switched to 1% mobile phase A at time 1.00, then 99% mobile phase A at time 1.45. Strong wash consisting of 70%-30% (ACN and propan-2-ol, respectively) were used in between sample injections.

•Transitions were m/z 940.5 > 316.5 (teicoplanin) and m/z 1030.4 > 725 (ristocetin)

RESULTS



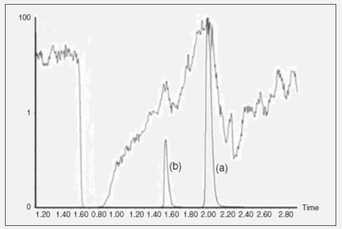


Figure 2a (left) Typical chromatograms for teicoplanin and ristocetin Chromatogram A is a typical teicoplanin peak with an elution time of 1.39 min and a detector response of 1.09 x 105 cpm (200μg/mL). Chromatogram B is a typical ristocetin peak with an elution time of 1.24 min and a detector response of 1.43 x 104 cpm (2μg/mL)

Figure 2b (right) Typical ion suppression study from direct infusion Teicoplanin was continuously infused directly into the mass spectrometer to provide a constant signal in the specific MRM channel. A drug free serum extract was subsequently injected into the system by UPLC. Chromatogram A represents a teicoplanin transition (1.09 x 105 cpm) and chromatogram B represents a ristocetin transition (1.43 x 104 cpm)

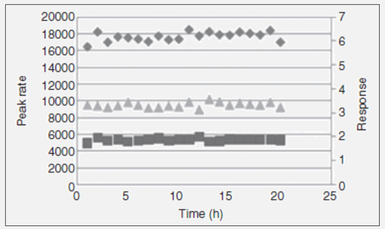


Figure 3 Detector stability of the assay Injections of 25μL were performed over a 20 hour period, and the individual response of teicoplanin and ristocetin are plotted in addition to their response ratios. The primary y-axis refers to the peak areas of teicoplanin (♦) and internal standard, ristocetin (■). The secondary y-axis refers to the response ratio of each injection (▲, teicoplanin: ristocetin peak-area ratio) and is plotted against each corresponding data point

	1	2
X (*n*=10)	6.1	5.5
Y (*n*=10)	0.4	0.5
Z (*n*=10)	2.8	3.1
Matrix effects	6.6%	9.1%
Recovery efficiency	93.0%	93.8%

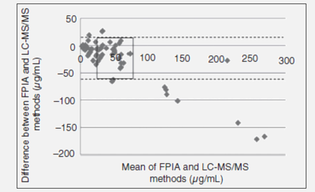
Figure 4a (left) Matrix effects and recovery efficiency Two independent experiments - Aqueous teicoplanin standards (100μg/mL) were spiked with ristocetin (250μg/mL), and their response denoted as 'X'. Drug-free serum teicoplanin samples were protein precipitated and then spiked with ristocetin as response 'Y'. Drug-free serum teicoplanin samples were spiked with ristocetin, protein precipitated, and their response represents 'Z'. The matrix effect is determined by the ratio of Y: X, and the recovery efficiency is calculated by the ratio of Z: Y

Target teicoplanin value (μg/mL)	SD	CV (%)
Within batch (*n* = 10)		
20	0.8	4.0
50	3.0	6.3
100	3.7	4.4
200	22.4	11.3
Between batch (*n* = 20)		
20	2.1	9.9
50	4.7	9.8
100	8.8	9.4
200	25.9	13.4

Figure 4b (middle) Intra-assay and inter-assay standard deviation (SD) and coefficient of variation (CV) Teicoplanin at four concentrations (20, 50, 100, 200μg/mL) were evaluated for intra-assay (n=10) and inter-assay (n=20) imprecision

	TP	A	G	T	V	AG	AT	AV
Variation (%)	100	101	116	117	77	112	123	77
CV (%)	5.9	7.8	5.8	3.2	8.4	6.6	2.8	1.6
	GT	GV	TV	AGT	AGV	GTV	AGTV	
Variation (%)	106	117	123	121	141	127	104	
CV (%)	10.6	10.2	24.0	3.2	6.8	8.6	16.2	

Figure 4c (right) Antibiotic interference Teicoplanin (TP) samples spiked with amikacin (A), gentamycin (G), tobramycin (T), and vancomycin (V) were compared against teicoplanin alone, expressed as a percentage difference from 100. CVs of each group of samples were also expressed as a percentage



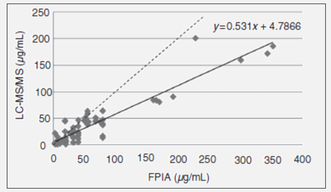


Figure 5a (left) Altman-Bland difference plot comparing the FPIA method (Abbott TDx) and LC-MS/MS method Dashed lines represent the 1 SD limits of agreement for the mean difference between the two methods. Rectangle represents the therapeutic range of teicoplanin (25-75μg/mL). FPIA: fluorescence polarization immunoassay; LC-MS/MS: liquid chromatography-tandem mass spectrometry

Figure 5b (right) Linear regression graph comparing FPIA versus LC-MS/MS The relationship between results generated by FPIA and LC-MS/MS are illustrated in this linear regression plot. The line of best fit generated a slope of 0.53 and an r^2 value of 0.86. Dashed line represents y = x

SUMMARY

•Teicoplanin, despite its molecular complexity, can be accurately measured by LC-MS/MS

•There are a number of advantages in this method: small sample volume, minimal sample preparation steps, rapid analysis, and accurate read-outs

•Method comparison exhibits a negative bias for LC-MS/MS compared to immunoassay, highlighting the potential improvement in accuracy and sensitivity by LC-MS/MS

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