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

Francis HY Fung, Jonathan CY Tang, John PP Hopkins¹, Lisa M Bailey, Andrew S Davison

Department of Clinical Biochemistry and Metabolic Medicine, Royal Liverpool and Broadgreen University Hospital, Liverpool, L7 8XP, UK
¹Waters MS Technology Centre, Micromass Atlas Park, Simons Way, Manchester, M22 5PP, UK

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 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 (25–75g/mL for teicoplanin). It is widely accepted that an optimal loading dose followed by appropriate maintenance doses should achieve trough serum teicoplanin concentrations of >25g/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 complexity of this antibiotic and its heterogeneous. Five variations (A₁–A₅) comprise the majority of detectable teicoplanin, 93% of which are A₂ and A₃. 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.

METHODS

• A Waters Acuity Quadra 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 = 1861) and ristocetin (Sigma, UK; M = 2061).
• Patient samples, external quality assurance (EQA) samples, and Quality Control (QC) material (25g/L) were spiked with ristocetin (in water, 5g/L), and protein precipitated by methanol. After mixing and centrifugation, the clear supernatant (25g/L) was injected directly into the Acuity operating in positive electrospray ionisation mode.
• Liquid chromatography was performed on a Waters AcuityTM UPLC system. 25g/L extracted sample was injected onto an Acuity 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 B at time 1.00, then 99% mobile phase B at time 1.45. Strong wash consisting of 70%/30% (ADN 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 1a (left) Allman-Based difference plot comparing the FPIA method (abscissa T0) and LC-MS/MS method. Dotted lines represent the 1 SD limits of agreement for the mean difference between the two methods. Rectangles represent the 25CVs across the response range. No significant difference was observed between the methods - FPIA and LC-MS/MS. Figure 1b (right) Linear regression graph comparing FPIA versus LC-MS/MS. The relationship between results generated by FPIA and LC-MS/MS is illustrated in this linear regression plot. The line of best fit generated a slope of 0.52 and an r² value of 0.98. Dotted line represents y = x.

Figure 2a (left) Typical chromatogram for teicoplanin and ristocetin. Chromatogram A is a typical teicoplanin peak with an elution time of 1.35 min and a detected response of 193.9 x 10⁵ cps (230g/L). Chromatogram B is a typical ristocetin peak with an elution time of 1.28 min and a detected response of 143 x 10⁵ cps (230g/L).

Figure 2b (right) Ions suppression study from direct infusion. Teicoplanin was continuously infused directly into the mass spectrometer to provide a constant signal in the specific UPLC channel. A drug free serum extract was subsequently injected into the system by UPLC. Chromatogram A represents a teicoplanin transition (100, 105 cps) and chromatogram B represents a ristocetin transition (45, 104 cps).

Figure 3 Detector stability of the assays. Injections of 25g/L were performed over a 20 day period with a native autosampler-derived standard and a drug free serum-derived standard. Teicoplanin and ristocetin were plotted in addition to their response ratios. The primary y-axis refers to the peak area of teicoplanin, while the secondary y-axis refers to the peak area of ristocetin. The secondary y-axis refers to the response ratio of each standard (y-axis), teicoplanin (peak area ratio) and is plotted against each corresponding data point.

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 immunosassay, highlighting the potential improvement in accuracy and sensitivity by LC-MS/MS.

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