Validation of Vancomycin Assay in Rat Plasma by Liquid Chromatography/Tandem Mass Spectrometry

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OVERVIEW

Purpose: Support a vancomycin preclinical GLP rat toxicity study at ITR Laboratories Inc.

Method: Protein precipitation with detection on a Shimadzu/AB Sciex API4000 platform

Result: Robust vancomycin rat plasma assay developed with a calibration range of 10.0 to 10 000 ng/mL.

INTRODUCTION

Antibiotic-resistant bacterial infection is one of the most serious clinical problems worldwide.
The glycopeptide vancomycin is a frequently used antibiotic for the treatment of multidrug-resistant bacterial infections[1].

We investigated several different bioanalytical methods for vancomycin and developed a new one most suitable for analysis of vancomycin in rat plasma at ITR Laboratories Inc.

The LC-MS/MS method developed and validated is sensitive, selective, and simple for the quantitation of vancomycin in rat plasma containing K_3EDTA as an anticoagulant with an LLOQ of 10.0 ng/mL.

[1] TSai, et al., Talanta 2013 Nov. 15, 116: 593-603
[2] Zhang, et al., J Anal Bioanal Tech 2014 5, 3:1-9
[3] Shen, www.tandemlabs.com, 2008

METHODS

Rat plasma with a volume of 50 µL was combined with 50 µL internal standard (IS), aminopterin[2], followed by the addition of 200 µL of 1 % formic acid in acetonitrile.

After centrifugation, 100 µL of supernatant was diluted with 300 µL water and then injected onto the Schimadzu HPLC/ AB Sciex API4000 mass spectrometer.

Vancomycin (Figure 1) and IS aminopterin (Figure 2) were separated on an Agilent, Zorbax SB-C8 column[3] with mobile phases consisting of 1 % formic acid in water and methanol at a flow rate of 1 mL/min.

The analyte and IS were detected using positive TurboIonSpray® ionization in multiple reaction monitoring (MRM) mode (see MRM transitions in Table 1). The total analysis time was 4.5 minutes.

Table 1. MRM Transitions of Vancomycin and Aminopterin

Compounds	MRM Transitions
vancomycin	725.60 to 144.10
aminopterin	441.10 to 294.10

RESULTS

Vancomycin is water soluble and has a molecular mass 1449.3 amu. Its polarity played a significant role in the final developed method. Numerous items were optimized and are listed in Table 2.

Table 2. Method Optimization (final are underlined)

HPLC columns	Phenomenex Kinetex C18, 2.6 µ
	Phenomenex Gemini C18, 5 µ
	Waters XBridge C18, 3.5 µ
	Agilent Zorbax SB-C8, 3.5 µ
	Agilent Zorbax SB-C18,1.8 µ
Mobile Phases	**1% formic acid in water** : acetonitrile or **methanol**
	0.1% formic acid in water : acetonitrile or methanol
	1 % formic acid in water : acetonitrile/methanol (50/50)
Extraction Solutions	Methanol or acetonitrile
	1% or 2% **formic acid in acetonitrile**
	1% TFA in acetonitrile
Internal Standards	dalbavancin
	monodeschloro vancomycin
	aminopterin

Figure 1. The structure of vancomycin

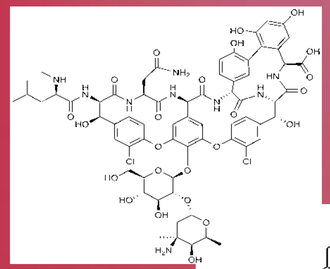
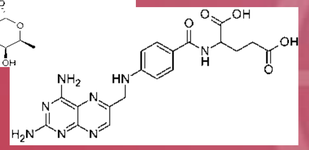
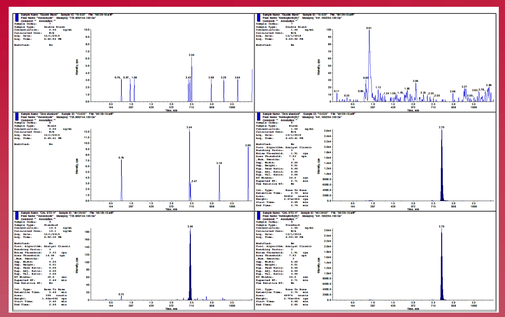


Figure 2. The structure of aminopterin



The chromatograms of a double blank (without IS), a single blank (with IS) and an LLOQ (10.0 ng/mL) are shown in Figure 3 below.

Figure 3. Representative chromatograms



CONCLUSION

The data successfully met the FDA and EMA guidelines for linearity (range 10.0 to 10000 ng/mL), selectivity, intra-day and inter-day precision and accuracy, matrix effect, hemolysis effect, stability in rat plasma for short term (at room temperature) and long term at ≤ -80ºC (149 days), stability of processed samples in autosampler, three cycles of freeze-thaw stability and vancomycin and aminopterin stock stability in diluent (96 days for vancomycin at ca. -20 °C).

The method has been used successfully in a preclinical GLP rat toxicity study at ITR Laboratories Inc. where 10.1% of the toxicity study samples were selected for ISR and 39 out of 40 samples met the acceptance criteria.