

Development of an HPLC/MS/MS Method for the Quantitative Bioanalysis of Vancomycin from the Plasma of Several Species: A Lesson Learned When Conventional Methods Are Unsuccessful

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Overview

➤ **Purpose** - Develop an HPLC/MS/MS method to determine concentrations of vancomycin in rat plasma when typical liquid-liquid and precipitation methods are unsuccessful

➤ **Methods** – 96 well-plate extraction and HPLC/ESI/MS/MS (API3000)

➤ **Results** – Range from 0.01 to 50 µg/mL with accuracies and precision better than ±20%

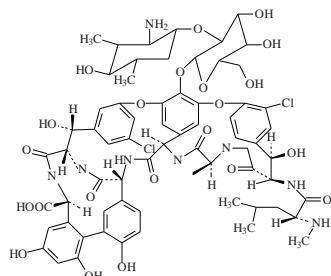
Introduction

Vancomycin is a glycopeptide antibiotic that is often used as a last-line of defense against bacterial infections. Since bacteria strains are becoming resistant to current antibiotics new glycopeptides are being developed

Typically, glycopeptides are water-soluble and have molecular weights > 1400 amu. The water solubility of glycopeptides makes these compounds difficult to extract from plasma with typical liquid-liquid extraction.

The goal of this study is to find an HPLC/MS/MS method that accurately quantifies vancomycin at limits of quantitation (LLOQ) near 10 ng/mL.

Structure of Vancomycin



Molecular Weight 1449.3 amu

Methods

Extraction

- Extraction of vancomycin or other glycopeptides from 100 µL of rat plasma in 96 well plates
- Investigate several methods of extraction at pH 2, pH 7 and pH 10
 - Hexane
 - Acetonitrile
 - Ethyl Acetate
 - MTBE
 - N-Butyl Chloride
 - 10% Isobutanol in Hexane
 - 96 well plate SPE extraction: C2, C8, C18, Phenyl, CN and Ion-exchange
- Extract, transfer aliquot, evaporate and reconstitute in aqueous mobile phase

HPLC

- Gradient from 5% to 90% organic in 3 minutes.
- Flow rate = 0.2 mL/minute
- 2 mM ammonium formate, 0.1% formic acid mobile phase with MeOH
- Advantage Armor C8 (Analytical Sales and Service) 2.1 x 30 mm column
- Thirty µL injections

Note: Decreasing the flow rate from 0.6 mL/min. to 0.2 mL/min. increased MS signal by 10X!

Mass Spectrometry

- Sciex API3000 operating in MRM mode
- Turbionspray (450 °C)
- Positive ion mode
- MRM transitions for Vancomycin– 725.5 (M+2H)⁺⁺ → 144.2

Table 1. Development of an Extraction Method for the HPLC/MS/MS Analysis of Vancomycin from Rat Plasma

Extraction Procedure (pH 2, pH 7, pH 11)	% Recovery
Acetonitrile Precipitation	<10
Ethyl Acetate	<10
Hexane	<10
Methyl-Tert-Butyl Ether	<10
N-Butyl Chloride	<10
10% Isobutanol in Hexane	<10
SPE 96 Well C2*	91

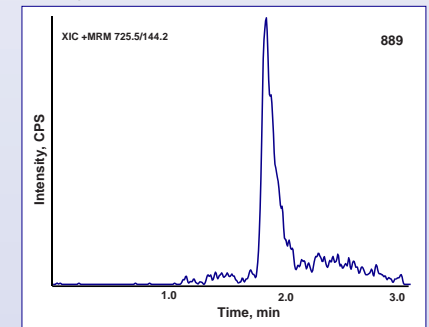
*C8 and C18 gave slightly lower recoveries. The Ph, CN and ion-exchange phases gave recoveries <50%.

Final SPE C2 Extraction Method: 100 µL of plasma with 100 µL of water. Add to C2 well. Wash with 50 µL of water. Rinse with 50 µL of MeOH. Elute with 500 µL 10% formic acid in MeOH.

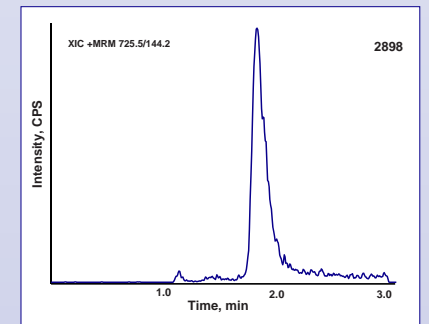
Table 2. HPLC/MS/MS Analysis of Vancomycin from Rat Plasma.

Standard Curve Level (µg/mL)	Calculated Concentration (µg/mL)	% Accuracy
0.01	0.0114	114
0.01	0.00819	81.9
0.05	0.0563	113
0.05	0.488	97.7
0.1	0.107	107
0.1	0.112	112
0.5	0.566	113
0.5	0.553	111
1.0	1.10	110
1.0	1.10	110
10	8.90	89.0
10	9.51	95.1

HPLC/MS/MS Chromatogram from the Analysis of a Standard Fortified with Vancomycin at 0.01 µg/mL and Extracted from Rat Plasma



HPLC/MS/MS Chromatogram from the Analysis for Vancomycin of a Rat Plasma Sample 8 Hours Post-Dose



Conclusions

- Developed HPLC/MS/MS method to quantify vancomycin from rat and mouse plasma
- Found that a SPE C2 96 well plate extraction gave best recoveries compared to other extractions
- Continue to support PK studies for vancomycin and other glycopeptides