



# Determination of Vancomycin in Human Plasma using LC/MS/MS

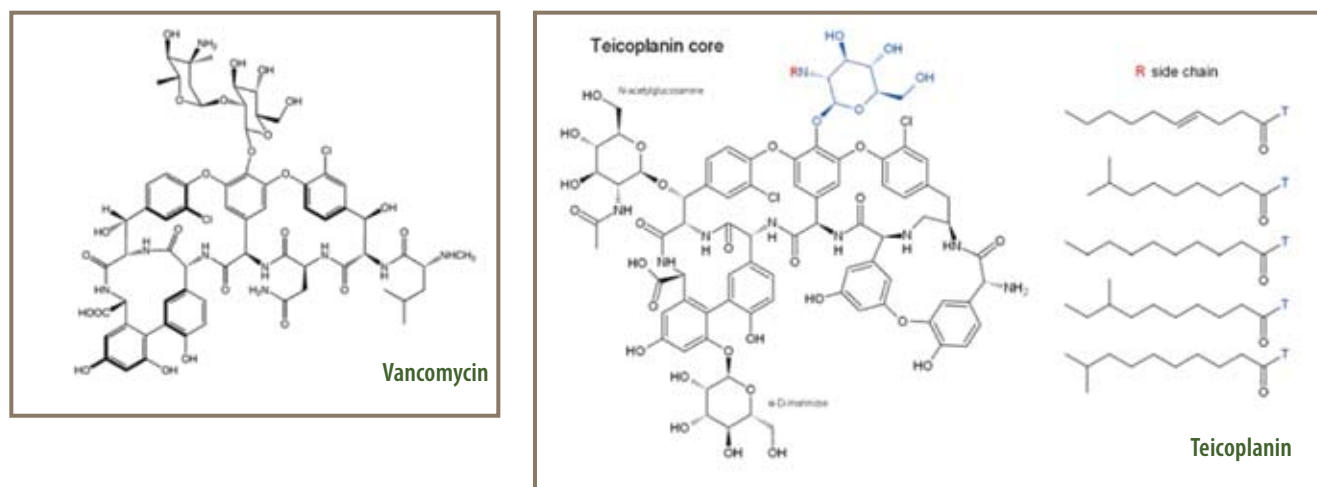
## Authors

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## Introduction

Vancomycin, a glycopeptide antibiotic, is used for the treatment of bacterial infection. With relatively large molecular weight and extremely high polarity, Vancomycin in human plasma presents a challenge of extraction and MS detection because of its unique physiochemical properties. This assay focuses on extraction from the matrix and consistent ionization of the compound.

Figure 1: Vancomycin and Teicoplanin molecular structure





## Method

A 50.0 µL aliquot of each human plasma sample was used for assay extraction followed by the addition of internal standard (Teicoplanin). The mixture was then diluted with 0.1% formic acid and injected into a cohesive HTLC system coupled to a Sciex API-3000. A LLOQ of 50.0 ng/mL was achieved with an ULOQ of 10.0 µg/mL. A Cohesive HTLC phenyl column and Fortis phenyl column were used as the extraction and analytical column, respectively. The total run time for each sample is 5.0 minutes.

Mobile phase: (including both loading pump and eluting pump):

A: 0.1% formic acid in water

B: 0.1% formic acid in acetonitrile

TABLE 1: HTLC program			
<b>STEP: 1</b>		<b>Start: 0 s</b>	
Loading sample		<b>Duration: 15 s</b>	
LOADING PUMP		ELUTING PUMP	
Flow Rate:	1.000	Flow Rate:	0.400
Flow Type:	Step	Flow Type:	Step
%A: 100.0	%B: 0.0	%A: 100.0	%B: 0.0
<b>STEP: 2</b>		<b>Start: 15 s</b>	
Slow pump		<b>Duration: 5 s</b>	
LOADING PUMP		ELUTING PUMP	
Flow Rate:	1.000	Flow Rate:	0.400
Flow Type:	Step	Flow Type:	Step
%A: 75.0	%B: 25.0	%A: 100.0	%B: 0.0
<b>STEP: 3 (Tee, loop in)</b>		<b>Start: 20 s</b>	
Analyte transfer		<b>Duration: 30 s</b>	
LOADING PUMP		ELUTING PUMP	
Flow Rate:	0.200	Flow Rate:	0.300
Flow Type:	Step	Flow Type:	Step
%A: 75.0	%B: 25.0	%A: 100.0	%B: 0.0
<b>STEP: 4 (loop in)</b>		<b>Start: 50 s</b>	
Slow pump		<b>Duration: 5 s</b>	
LOADING PUMP		ELUTING PUMP	
Flow Rate:	0.100	Flow Rate:	0.400
Flow Type:	Step	Flow Type:	Step
%A: 75.0	%B: 25.0	%A: 90.0	%B: 10.0

<b>STEP: 5 (loop in)</b>		<b>Start: 55 s</b>	
Fill loop and elute		<b>Duration: 60 s</b>	
LOADING PUMP		ELUTING PUMP	
Flow Rate:	1.000	Flow Rate:	0.400
Flow Type:	Step	Flow Type:	Ramp
%A: 75.0	%B: 25.0	%A: 80.0	%B: 20.0
<b>STEP: 6</b>		<b>Start: 115 s</b>	
Wash and elute		<b>Duration: 60 s</b>	
LOADING PUMP		ELUTING PUMP	
Flow Rate:	1.000	Flow Rate:	0.400
Flow Type:	Step	Flow Type:	Ramp
%A: 100.0	%B: 0.0	%A: 90.0	%B: 10.0
<b>STEP: 7</b>		<b>Start: 175 s</b>	
Wash and elute		<b>Duration: 60 s</b>	
LOADING PUMP		ELUTING PUMP	
Flow Rate:	1.000	Flow Rate:	0.400
Flow Type:	Step	Flow Type:	Ramp
%A: 0.0	%B: 100.0	%A: 60.0	%B: 40.0
<b>STEP: 8</b>		<b>Start: 235 s</b>	
Wash		<b>Duration: 45 s</b>	
LOADING PUMP		ELUTING PUMP	
Flow Rate:	2.000	Flow Rate:	0.600
Flow Type:	Step	Flow Type:	Step
%A: 100.0	%B: 0.0	%A: 90.0	%B: 10.0
<b>STEP: 9</b>		<b>Start: 280 s</b>	
re-equilibrate		<b>Duration: 20 s</b>	
LOADING PUMP		ELUTING PUMP	
Flow Rate:	1.000	Flow Rate:	0.400
Flow Type:	Step	Flow Type:	Step
%A: 100.0	%B: 0.0	%A: 100.0	%B: 0.0



**Method** continued

TABLE 2: MS condition	
Compound	MRM
Vancomycin	724.8 → 144.1
Teicoplanin	940.8 → 144.1

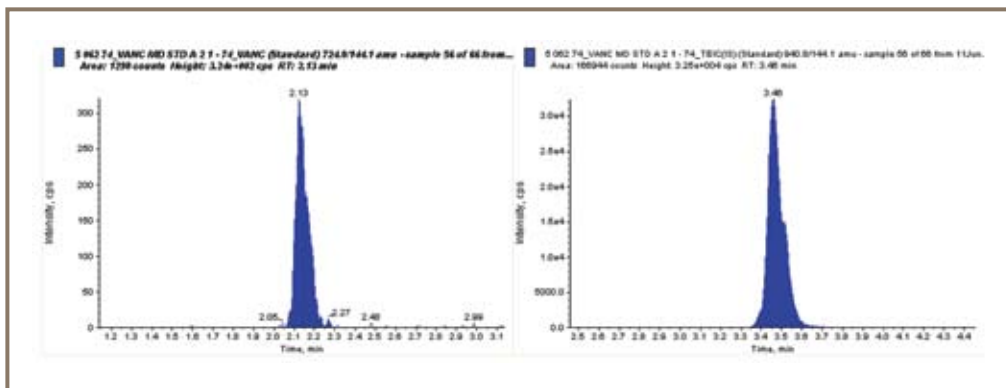
**Experimental**

The assay validates the linearity, accuracy and precision (both less than 10% at low, mid and high level) in human plasma. Stability of the compound in matrix was demonstrated for bench top, freeze-thaw and long-term storage stability as well as for solution stability. Auto-sampler stability was acceptable after 125 hours at room temperature. Selectivity, recovery and matrix effects were also investigated. A Vancomycin assay in human plasma using a Cohesive HTLC online extraction system was successfully developed and validated. With the advantages of using online extraction, the sample treatment and instrument setup are simple providing a robust and efficient method.

Recovery of Vancomycin and internal standard on Cohesive HTLC online extraction system was determined to compare the peak area with extracted pure solution vs. regular binary pump system with similar gradient. The recovery of Vancomycin at low (150 ng/mL), mid (4000 ng/mL) and high (8000 ng/mL) levels are 22.4%, 21.4% and 21.8%, respectively, while teicoplanin at 10.0 µg/mL is 52.2%.

Six (6) lots of human plasma were prepared at LLOQ levels to test the selectivity. The average of six (6) lots LLOQ is 102.2% of theoretical with 4.7% of %CV.

FIGURE 2: Extracted LLOQ (50.0 ng/mL) in human plasma





## Experimental continued

**TABLE 3: Validation summary**

Method	LC/MS/MS
Matrix	Human Plasma
Anti-coagulant	K <sub>2</sub> EDTA
LLQ	50.0 ng/mL
ULQ	10,000 ng/mL
Sample Volume	50.0 µL
Internal Standard	Analog (Teicoplanin)
<b>Accuracy: %Bias</b>	
LLQ	-2.0
LQC	6.0
GMQC	3.4
MQC	-0.5
HQC	-1.9
DilQC (DF=10)	2.6
<b>Precision: %CV</b>	
LLQ	0.0
LQC	0.0
GMQC	2.8
MQC	2.9
HQC	3.9
DilQC (DF=10)	3.3
Storage Stability in Matrix	20 hrs at room temperature 63 days at -20°C nominal 63 days at -70°C nominal
Reinjection Reproducibility	125.25 hrs at room temperature
Freeze/Thaw Stability	4 cycles from -20°C to room temperature 4 cycles from -70°C to room temperature
Vancomycin Solution Stability	24 hrs at room temperature in acetonitrile:water (20:80, v:v) 84 days at -20°C in acetonitrile:water (20:80, v:v)



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## Conclusion

Online extraction system provides clean extraction to obtain a robust selectivity between matrix lots to meet the acceptance criteria of GLP studies.