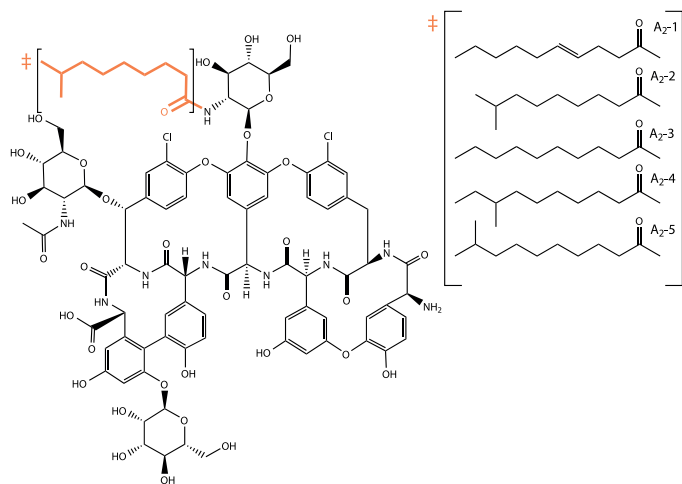


# Extraction of Teicoplanin from Plasma Using EVOLUTE® EXPRESS ABN Prior to HPLC-DAD Analysis

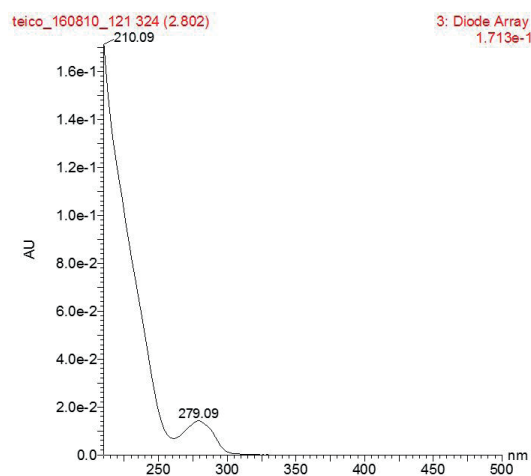


**Figure 1.** Teicoplanin A<sub>2</sub>-2 (Major side-chain variants shown in parenthesis).

## Introduction

This application note describes a solid phase extraction (SPE) protocol for the extraction of teicoplanin from plasma prior to HPLC-DAD analysis.

Teicoplanin (**Figure 1**) is a glycopeptide antibiotic used in the treatment of serious infections including methicillin-resistant *Staphylococcus aureus* (MRSA) as it has an activity spectrum similar to Vancomycin, inhibiting peptidoglycan (cell wall) synthesis. Trough concentrations can be between 15 mg L<sup>-1</sup> and 60 mg L<sup>-1</sup>. Teicoplanin has a UV chromophore at 279 nm (**Figure 2**), this was used for quantitation.



**Figure 2.** Teicoplanin A<sub>2</sub> UV Spectrum.

The method described in this application demonstrates high, reproducible recovery of teicoplanin from human plasma. Samples were extracted using an EVOLUTE® EXPRESS ABN 30 mg 96-well plate using 200 µL plasma volumes. EVOLUTE EXPRESS ABN products provide clean, rapid, robust, efficient, high throughput and automatable extraction solutions for this analyte.

## Analytes

Teicoplanin (as a mixture of A<sub>2</sub> variants)

## Sample Preparation Procedure

### Format:

EVOLUTE EXPRESS ABN 30 mg plate, part number 600-0030-PX01

### Sample Pre-treatment:

Dilute 200 µL plasma in a 1:3 ratio using 2% formic acid (aq).

### Condition:

Condition each well with methanol (1 mL).

### Equilibration:

Equilibrate each well with 0.1% HCOOH (aq) (1 mL).

### Sample Loading:

Load pre-treated sample (800 µL) at a flow rate of approximately 1 mL/min.

### Wash:

Elute interferences with water (1 mL).

### Elution:

Elute analyte with methanol/water (70/30, v/v, 500 µL).

### Post Elution:

Dry in a stream of air or nitrogen at 40° C using a Biotage® SPE Dry 96 Sample Concentrator System.

### Reconstitution:

Acetonitrile : 10 mM ammonium acetate pH 4.4 (10/90, v/v, 250 µL).

## HPLC Conditions

### Instrument

Waters Alliance 2795 Separations Module

### Column

Shimadzu ShimPack ODS-XR 50 x 3.0 mm, 2.2 µm

### Flow Rate

0.8 mL min<sup>-1</sup>

### Column Temperature

Room Temperature

### Sample Temperature

12 °C

### Injection Volume

25 µL partial (50 µL loop)

### Mobile Phase

A: 10 mM ammonium acetate pH 4.4

B: Acetonitrile

**Table 1.** Gradient Conditions.

Time	%A	%B	Curve
0.00	80	20	1
0.80	80	20	6
1.90	70	30	6
3.40	70	30	6
3.41	5	95	6
4.40	5	95	6
4.41	80	20	6
6.00	80	20	6

Curve 6: Linear Gradient

### Detector

Waters 996 PDA

### Detection λ

279 nm

### Resolution

1.2 nm

### Frequency

2 Hz

## Results

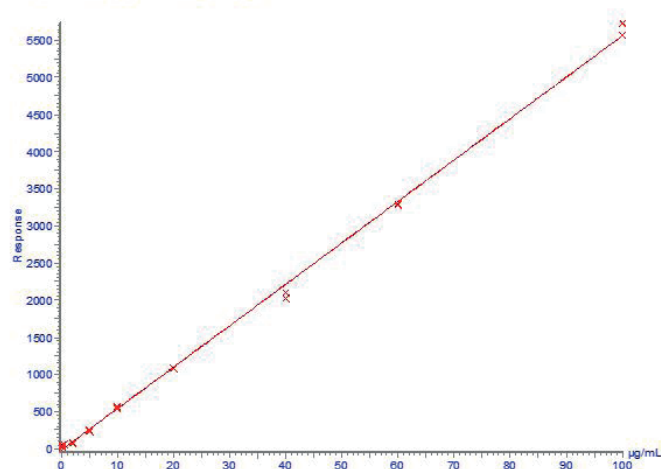
Assay performance is summarized below in Table 2.

**Table 2.** Teicoplanin Assay Performance.

Parameter	Value	Accuracy	Precision (RSD)
Linear range	2–100 µg/mL	-	-
Linearity coefficient, r <sup>2</sup>	0.9985	-	-
LOQ	2 µg/mL	88%	6.4% (n=4)
Recovery	20 µg/mL	102%	4.8% (n=6)

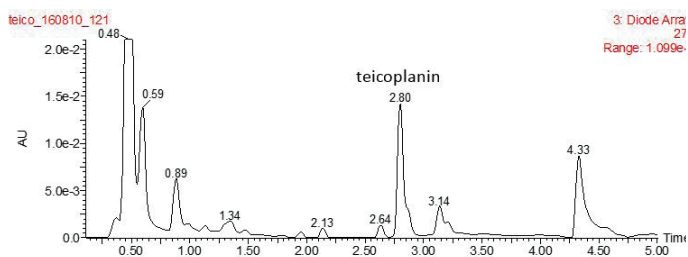
Linear range was determined from a ten-point calibration curve of spiked pooled plasma (n=4) where replicate means were within 80% to 120% of the stated value and RSD were ≤ 15%. Assay linearity was estimated using r<sup>2</sup>. An example calibration curve is shown in **Figure 3**.

Compound name: teicoplanin UV279  
 Correlation coefficient: r = 0.998245, r<sup>2</sup> = 0.998492  
 Calibration curve: 55.8053 \* x + -18.2845  
 Response type: External Std, Area  
 Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None

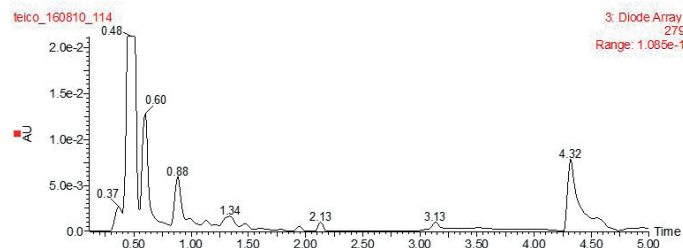


**Figure 3.** Teicoplanin Calibration Curve (0.1 to 100 µg/mL).

LOQ was estimated as the lowest concentration spiked standard demonstrating 80% to 120% accuracy and repeatability ≤ 15% RSD. Extraction recovery was determined at a spike level of 20 µg mL<sup>-1</sup> teicoplanin A<sub>2</sub> in pooled human plasma (Welsh Blood Service, Pontyclun). Extraction repeatability was estimated using the % RSD of the post extraction spike samples (n=6). Example spike and blank chromatograms are shown in **Figure 4(a)** and **Figure 4(b)**.



**Figure 4(a).** Teicoplanin Pre-Extraction Spike in Pooled Human Plasma, 20  $\mu\text{g mL}^{-1}$ ,  $\lambda 279\text{nm}$ .



**Figure 4(b).** Blank Pooled Human Plasma,  $\lambda 279\text{nm}$ .

## Ordering Information

Part Number	Description	Quantity
<b>600-0030-PX01</b>	EVOLUTE® EXPRESS ABN 30 mg Solid Phase Extraction Fixed Well Plate	1
<b>121-9600</b>	VacMaster-96 Sample Processing Manifold	1
<b>PPM-96</b>	Biotage® PRESSURE+ 96 Positive Pressure Manifold 96 Position	1
<b>SD-9600-DHS-EU</b>	Biotage® SPE Dry 96 Sample Concentrator System 220/240V	1
<b>SD-9600-DHS-NA</b>	Biotage® SPE Dry 96 Sample Concentrator System 100/120V	1

## Additional information

### Eluent Preparation

**10 mM ammonium acetate pH 4:** dilute 360  $\mu\text{L}$  of glacial acetic acid and 252 mg of ammonium acetate in 1 L of LC-MS grade water, titrate to pH 4.4 using monovalent strong base or acid as required.

### Reconstitution Solvents

Reconstitution solvents should be fully evaluated depending on collection vessel type in order to avoid issues with reproducibility associated with non-specific binding effects.

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