

For Research Use Only. NOT for Use in Diagnostic Procedures.

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

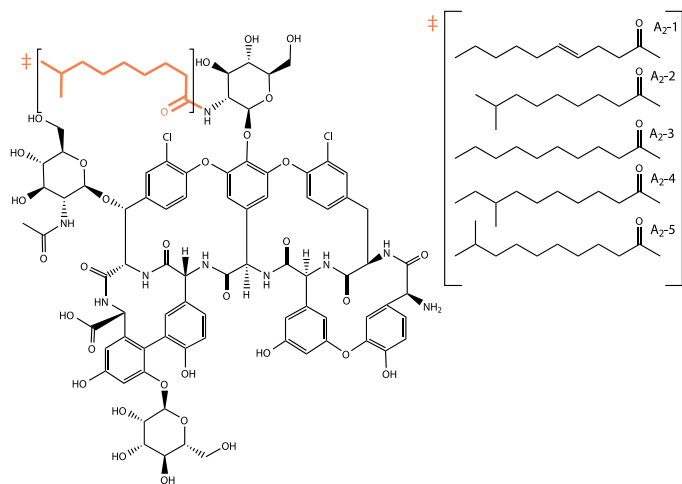


Figure 1. Teicoplanin A₂-2 (Major side-chain variants shown in parenthesis).

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⁻¹ and 60 mg L⁻¹. Teicoplanin has a UV chromophore at 279 nm (**Figure 2**), this was used for quantitation.

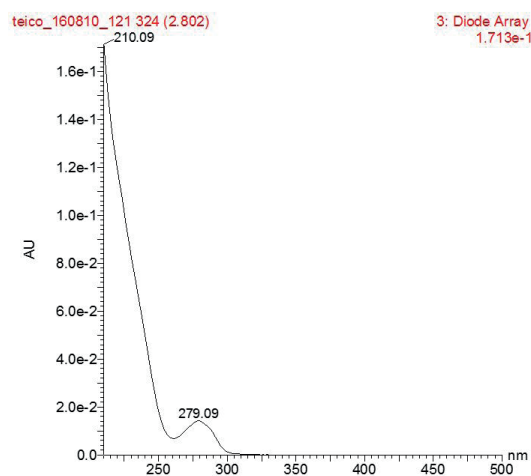


Figure 2. Teicoplanin A₂ UV Spectrum.

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 A₂ 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⁻¹

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 ²	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 r². An example calibration curve is shown in **Figure 3**.

Compound name: teicoplanin UV279
 Correlation coefficient: r = 0.998245, r² = 0.998492
 Calibration curve: 55.8053 * x + -18.2845
 Response type: External Std, Area
 Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None

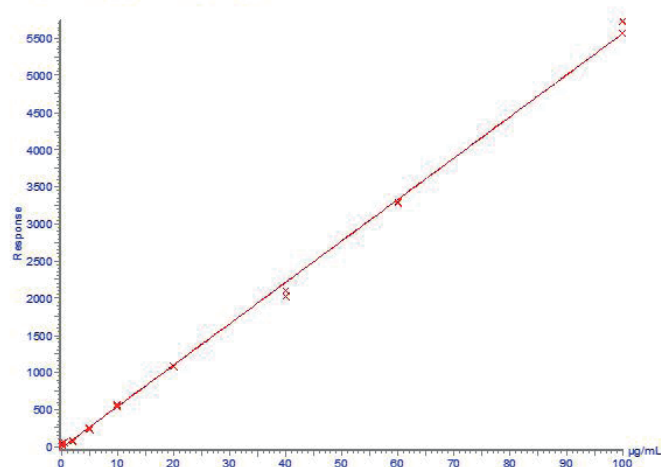


Figure 3. Teicoplanin Calibration Curve (0.1 to 100 µg/mL).

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⁻¹ teicoplanin A₂ 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)**.

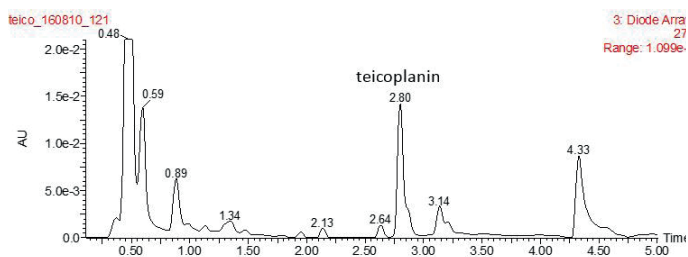


Figure 4(a). Teicoplanin Pre-Extraction Spike in Pooled Human Plasma, 20 µg mL⁻¹, λ279nm.

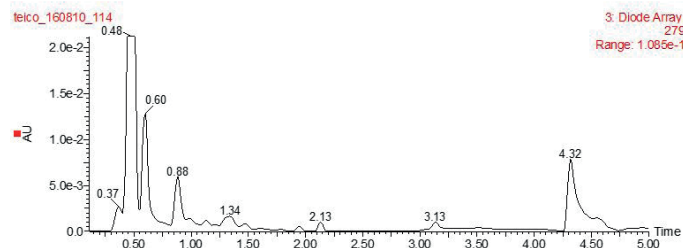


Figure 4(b). Blank Pooled Human Plasma, λ279nm.

Ordering Information

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

Additional information

Eluent Preparation

10 mM ammonium acetate pH 4: dilute 360 µL 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.

EUROPE

Main Office: +46 18 565900
 Toll Free: +800 18 565710
 Fax: +46 18 591922
 Order Tel: +46 18 565710
 Order Fax: +46 18 565705
 order@biotage.com
 Support Tel: +46 18 56 59 11
 Support Fax: +46 18 56 57 11
 eu-1-pointsupport@biotage.com

NORTH & LATIN AMERICA

Main Office: +1 704 654 4900
 Toll Free: +1 800 446 4752
 Fax: +1 704 654 4917
 Order Tel: +1 704 654 4900
 Order Fax: +1 434 296 8217
 ordermailbox@biotage.com
 Support Tel: +1 800 446 4752
 Outside US: +1 704 654 4900
 us-1-pointsupport@biotage.com

JAPAN

Tel: +81 3 5627 3123
 Fax: +81 3 5627 3121
 jp_order@biotage.com
 jp-1-pointsupport@biotage.com

CHINA

Tel: +86 21 2898 6655
 Fax: +86 21 2898 6153
 cn_order@biotage.com
 cn-1-pointsupport@biotage.com

To locate a distributor, please visit our website www.biotage.com

Part Number: AN869.V.1

© 2016 Biotage. All rights reserved. No material may be reproduced or published without the written permission of Biotage. Information in this document is subject to change without notice and does not represent any commitment from Biotage. E&OE. A list of all trademarks owned by Biotage AB is available at www.biotage.com/legal. Other product and company names mentioned herein may be trademarks or registered trademarks and/or service marks of their respective owners, and are used only for explanation and to the owners' benefit, without intent to infringe. **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**