

## Separations of Peptide Antibiotics by Reversed Phase HPLC, Using Discovery Columns

*Peptide antibiotics bacitracin A and virginiamycin M1 were separated by reversed phase HPLC on Discovery C18, Discovery C8, and Discovery RP-AmideC16 columns. These columns offer excellent resolution for this application.*

### Key Words

- antibiotics • peptide antibiotics • bacitracin
- virginiamycin

Because antibiotic resistance has become a major clinical problem, there is an urgent need to develop agents that act via novel mechanisms. With the realization that they are prime effectors through which multicellular organisms eradicate bacteria, peptide antibiotics have received considerable attention. Bacitracin A and virginiamycin M1 (Figure A) are two antibiotics of this class (1-4). The main chemical characteristic of analytical interest is that these compounds are similar to biological matrix components; this also is one of the major reasons peptides are difficult to analyze in biological matrices. In this application, bacitracin A and virginiamycin M1 were analyzed by HPLC, using Discovery™ C18, Discovery C8, and Discovery RP-AmideC16 columns.

Chromatographic separations were performed on a Waters Alliance HPLC system. All injections were made through an autosampler. A Waters 2487 dual wavelength UV detector was used to monitor the UV absorbance of samples at 220nm. The 15cm x 4.6mm ID Discovery C18, Discovery C8, and Discovery™ RP-AmideC16 reversed phase HPLC columns were used without guard columns or filters. The packing particles in all columns were 5µm in diameter.

Bacitracin A and virginiamycin M1 were purchased from Sigma Chemical Co. They were dissolved in 25mM KH<sub>2</sub>PO<sub>4</sub> buffer, pH 3, at 50µg/mL each.

Bacitracin A and virginiamycin M1 were separated on each of the three columns by isocratic elution. Column temperature was controlled at 35°C. Column pressure was below 1000psi in all cases. Detailed conditions for each analysis are presented with the corresponding chromatogram.

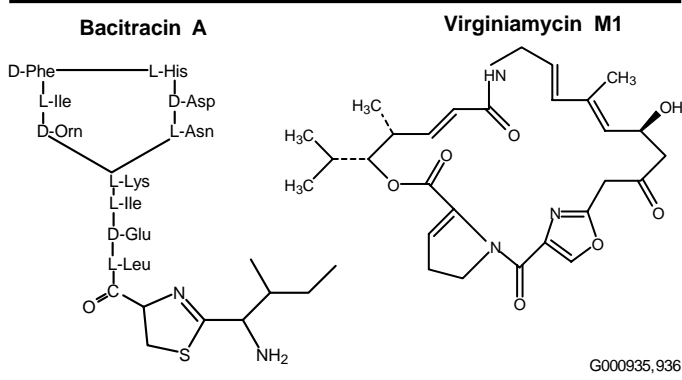
Results for the isocratic elution of these two antibiotics on Discovery C18 and Discovery RP-AmideC16 columns are illustrated in Figures B and C, respectively. Results for the analysis on a Discovery C8 column were identical to those for the Discovery C18 column. In each analysis, resolution and peak shape are excellent.

When we used the 60:40 phosphate:acetonitrile mobile phase with the Discovery RP-AmideC16 column, bacitracin A eluted among a group of impurity peaks. The 65:35 mobile phase used to obtain

Figure C improves the elution of bacitracin A, but causes virginiamycin M1 to elute 2 minutes later. The 60:40 mobile phase can be used when samples contain only virginiamycin M1, or if the particular samples of bacitracin A do not contain potentially interfering components.

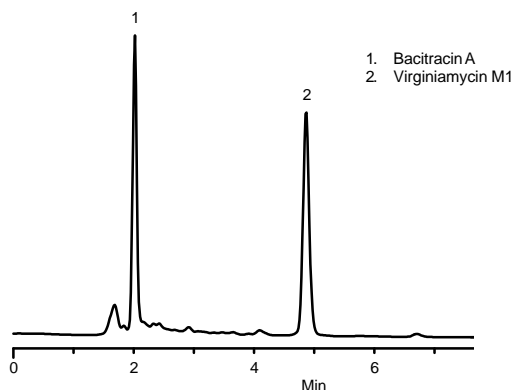
This study showed that bacitracin A and virginiamycin M1 could be separated by reversed phase HPLC, using Discovery C18, Discovery C8, and Discovery RP-AmideC16 columns. Excellent resolution was achieved in each separation.

**Figure A. Structures of the Peptide Antibiotics Bacitracin A and Virginiamycin M1**



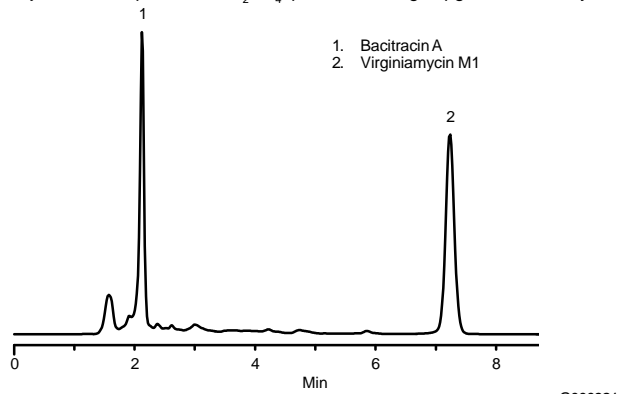
**Figure B. Bacitracin A and Virginiamycin M1 on a Discovery C18 HPLC Column**

Column: **Discovery C18, 15cm x 4.6mm ID, 5µm particles**  
Cat. No.: **504955**  
Mobile Phase: 25mM KH<sub>2</sub>PO<sub>4</sub>, pH 3 / acetonitrile, 60:40  
Flow Rate: 1mL/min  
Pressure: 890psi  
Temperature: 35°C  
Detection: UV, 220nm  
Injection: 10µL 25mM KH<sub>2</sub>PO<sub>4</sub>, pH 3 containing 50µg/mL each analyte



**Figure C. Bacitracin A and Virginiamycin M1 on a Discovery RP-AmideC16 HPLC Column**

Column: **Discovery RP-AmideC16, 15cm x 4.6mm ID, 5µm particles**  
 Cat. No.: **505013**  
 Mobile Phase: 25mM KH<sub>2</sub>PO<sub>4</sub>, pH 3 / acetonitrile, 65:35  
 Flow Rate: 1mL/min  
 Pressure: 900psi  
 Temperature: 35°C  
 Detection: UV, 220nm  
 Injection: 10µL 25mM KH<sub>2</sub>PO<sub>4</sub>, pH 3 containing 50µg/mL each analyte



**Ordering Information:**

Description	Cat. No.
<b>Discovery Columns</b>	
15cm x 4.6mm ID, 5µm particles	
Discovery C8	59353-U
Discovery C18	504955
Discovery RP-AmideC16	505013
<b>Discovery Selectivity Packs<sup>1</sup></b>	
5cm x 2.1mm ID Columns	55720-U21
15cm x 2.1mm ID Columns	55722-U21
5cm x 4.6mm ID Columns	55720-U
15cm x 4.6mm ID Columns	55722-U
25cm x 4.6mm ID Columns	55724-U

<sup>1</sup>Four columns of equal dimensions, one of each Discovery phase (C18, RP-AmideC16, C8, Cyano).

**References**

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References not available from Supelco.  
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