

Separations of β -Lactam Antibiotics by Reversed Phase HPLC, Using Discovery Columns

Three β -lactam antibiotics were separated by reversed phase HPLC on Discovery C18 and Discovery C8 columns. These columns offer excellent resolution for this application.

Key Words

- antibiotics • β -lactam antibiotics • aztreonam
- penicillin • piperacillin

There are more than 100 antibiotics on the market today, and many more are in development. Resistance to antibiotics is a significant problem (1,2): an antibiotic that takes a decade to bring to market can induce resistance within months of its introduction into clinical practice (3). The frequency of resistance in bacteria and the numbers of drugs to which they are resistant are increasing. Therefore, it is critical to monitor the level of antibiotics given to humans and animals.

HPLC is a powerful tool for isolation and quantification of antibiotics. In this application, three β -lactam antibiotics (Figure A) were analyzed by HPLC, using Discovery™ C18 and Discovery C8 columns. Aztreonam, the first totally synthetic monocyclic β -lactam antibiotic, has a high degree of resistance to β -lactamases and shows specific activity against aerobic Gram-negative bacilli (4). Aztreonam is a promising antibiotic effective for a broad range of infections (5). Piperacillin is an extended-spectrum penicillin with activity against Gram-negative bacilli. The piperacillin/tazobactam combination is highly active *in vitro* against Gram-positive cocci (6,7).

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 and Discovery C8 reversed phase HPLC columns were used without guard columns or filters. The packing particles in both columns were 5 μ m in diameter.

Aztreonam and piperacillin were purchased from U.S. Pharmacopoeia. Penicillin G potassium was obtained from Sigma Chemical Co. Piperacillin was dissolved in methanol, then diluted with 50mM KH₂PO₄ buffer, pH 3. The other antibiotics were dissolved in the phosphate buffer.

The β -lactam antibiotics were separated on each column by isocratic elution. Column temperature was controlled at 35°C. Column pressure was 1050psi or less in all cases. Detailed conditions for each analysis are presented with the corresponding chromatogram.

The chromatograms in Figures B and C show the excellent resolution and peak shape obtained for aztreonam, penicillin G potassium, and piperacillin on the Discovery C18 and Discovery C8 columns, respectively.

This study showed that mixtures of β -lactam antibiotics can be separated by reversed phase HPLC, using Discovery C18 and Discovery C8 columns. Excellent resolution is achieved.

Figure A. Structures of β -Lactam Antibiotics

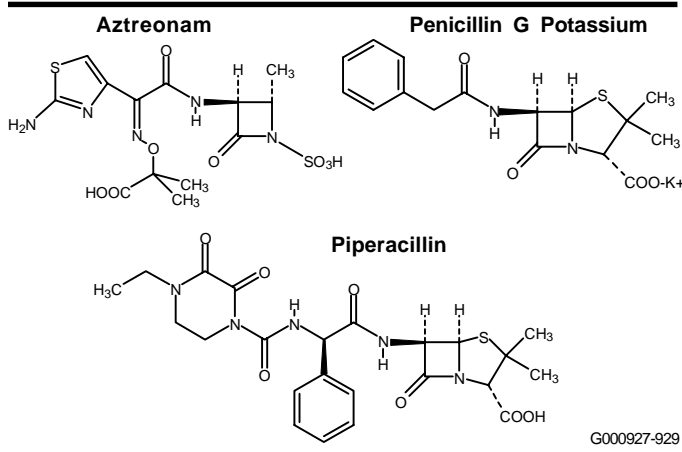


Figure B. β -Lactam Antibiotics on a Discovery C18 HPLC Column

Column: **Discovery C18, 15cm x 4.6mm ID, 5 μ m particles**
 Cat. No.: **504955**
 Mobile Phase: 50mM KH₂PO₄, pH 3 / acetonitrile, 70:30
 Flow Rate: 1mL/min
 Pressure: 1000psi
 Temperature: 35°C
 Detection: UV, 220nm
 Injection: 15 μ L 50mM KH₂PO₄, pH 3 containing 33 μ g/mL each analyte

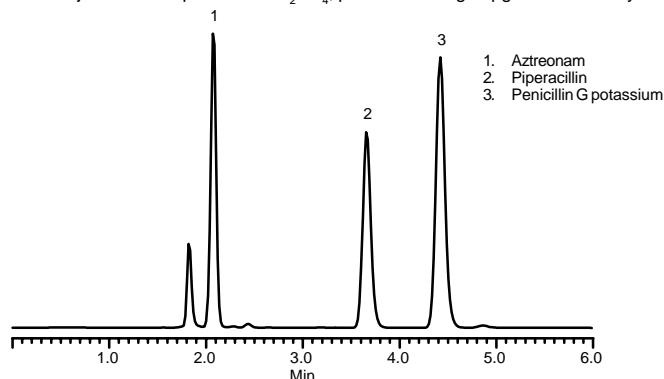
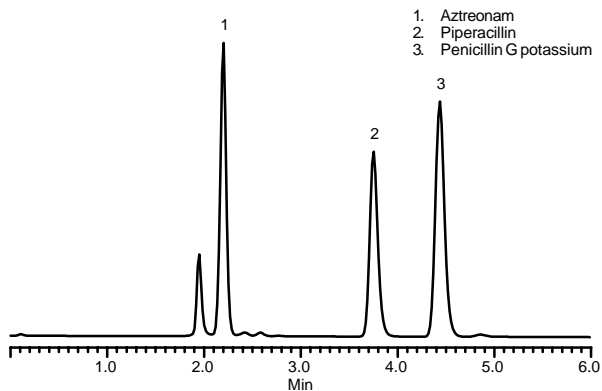


Figure C. β -Lactam Antibiotics on a Discovery C8 HPLC Column

Column: **Discovery C8, 15cm x 4.6mm ID, 5 μ m particles**
 Cat. No.: **59353-U**
 Mobile Phase: 50mM KH₂PO₄, pH 3 / acetonitrile, 70:30
 Flow Rate: 1mL/min
 Pressure: 1050psi
 Temperature: 35°C
 Detection: UV, 220nm
 Injection: 15 μ L 50mM KH₂PO₄, pH 3 containing 33 μ g/mL each analyte



G001135

Ordering Information:

Discovery Columns

15cm x 4.6mm ID, 5 μ m particles

Discovery C8

59353-U

Discovery C18

504955

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