

Excellent LC/MS Separation of Penicillins and Cephalosporins Using Ultra IBD Columns

Antibiotics are the most widely used medications in the world. Whether by prescription, addition to animal feed stocks, or use of cleaning agents, everyone in the civilized world is either directly or indirectly exposed to antibiotics in daily life. The overuse of antibiotics, however, has allowed resistant bacteria to thrive. The death of 12,500 people in Guatemala from an episode of Shingella fever can be traced to a simple mutation of the bacterial strain. Research indicated that the bacterium incorporated a single plasmid into its RNA sequence and resultantly became resistant to four different antibiotics. This illustrates the danger of resistance caused by adaptation. To combat resistant bacteria, new antibiotic derivatives must be created to overcome the bacteria's new defense mechanisms. Typically, HPLC columns can be used to analyze penicillins and their structurally related cephalosporins. However, the similarity of many derivatives may require additional interactions to effectively separate related compounds. Restek's Ultra IBD column is better able to resolve these compounds using polar and hydrophobic interactions.

Background

Penicillins and cephalosporins represent nearly sixty percent of antibiotics worldwide. These antibiotics possess a sulfur

atom within a five- or six-membered ring, attached to a four-member β -lactam ring. They are produced by fermentation processes using either selected fungi or species of *Streptomyces* bacteria. Derivatives are produced in two fashions:

1. Biosynthetic process—The fungus or bacteria are genetically engineered to produce a new derivative, or the starting materials are altered to produce biosynthetic variants during fermentation.
2. Semi-synthetic processes—The materials from a biosynthetic process are converted to chemical derivatives. Penicillin derivatives are created from penicillin G or V, while cephalosporin derivatives are created from cephalosporin C or cephamycin C.

Unfortunately, biosynthetic fermentation does not produce a "pure" antibiotic. Even after cleanup of the fermentation mash, some side reaction products will remain. Many of these side products are closely related to the primary analyte (Figure 1). Desired products, however, are created in the semi-synthetic process. Penicillin V is converted to amoxicillin through chemical intermediates and varies only slightly in structure (Figure 2). Similar reactions also occur during production of cephalosporin derivatives. The loss of

Figure 1

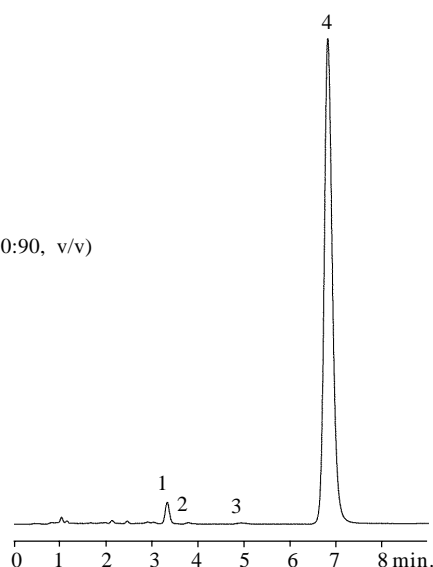
Ultra IBD separates penicillin V from other fermentation impurities.

Peak List:

1. unknown
2. unknown
3. unknown
4. penicillin V

Sample:

Inj.: 2.5 μ L
 Conc.: 1.2mg/mL
 Solvent: acetonitrile:water (10:90, v/v)



Column:

Ultra IBD
 Catalog #: 9175565
 Dimensions: 150 x 4.6mm
 Particle Size: 5 μ m
 Pore Size: 100 \AA

Conditions:

Mobile Phase: 10mM ammonium formate, pH 2.5: acetonitrile (60:40, v/v)
 Flow: 1.2mL/min.
 Temp.: 30°C
 Det.: UV @ 270nm

LC_0096

a hydride ion to create a phenyl ring is the only structural difference between cephradine and its side product cephalixin (Figure 3). Semi-synthetic processes are used to create derivatives like cephaloridine.

Unfortunately, many penicillins and cephalosporins are acid labile so that liquid chromatographic (LC) analysis of these molecules only should be performed if the sample is dissolved in a neutral media. Furthermore, if analysis time on the column is prolonged, breakdown of the analytes may occur *in situ* with a mobile phase that is not at a neutral pH. When measuring trace quantities of the analytes, especially by LC/mass spectrometry (MS), maintaining physiological

pH near 7.4 may become important for stability and accurate quantitation.

Discussion of Analysis

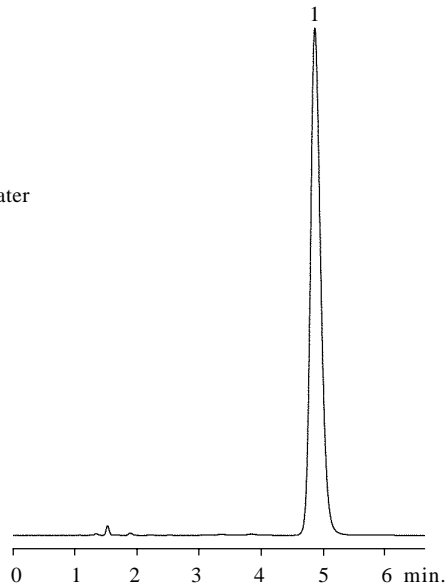
The Restek Ultra IBD phase provides greater versatility for the LC/MS analysis of penicillins and cephalosporins compared to a C18 column. The Ultra IBD column is capable of providing retention for cephaloridine in reverse phase mode with up to 45% organic solvent in the mobile phase. A conventional C18 column loses all retention near 35% organic solvent. Unlike a C18 column, the IBD is capable of polar interactions in a normal phase mode with analytes that possess charged functional groups. The ability

Figure 2

Ultra IBD shows excellent peak shape for amoxicillin.

Peak List:
1. amoxicillin

Sample:
Inj.: 5µL
Conc.: 1.5mg/mL
Solvent: acetonitrile:water
(10:90, v/v)



Column: Ultra IBD
Catalog #: 9175565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: 10mM ammonium formate, pH 2.5:acetonitrile (95:5, v/v)
Flow: 1.2mL/min.
Temp.: 30°C
Det.: UV @ 270nm

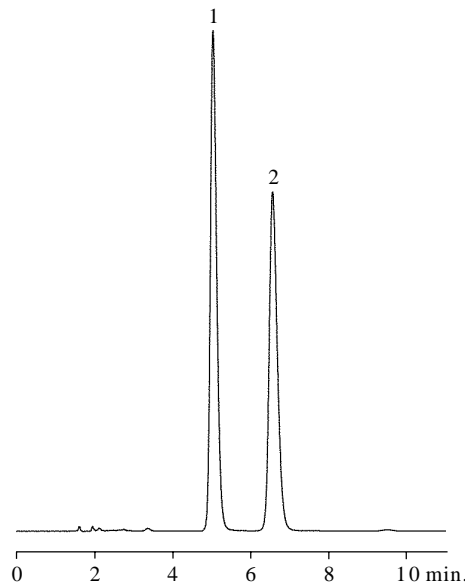
LC_0095

Figure 3

Ultra IBD shows great separation between cephalixin and cephradine, which differ only by a hydride ion.

Peak List:
1. cephalixin
2. cephradine

Sample:
Inj.: 10µL
Conc.: 500µg/mL
Solvent: acetonitrile:water
(10:90, v/v)



Column: Ultra IBD
Catalog #: 9175565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: 10mM ammonium formate, pH 2.5: acetonitrile (90:10, v/v)
Flow: 1.2mL/min.
Temp.: 30°C
Det.: UV @ 270nm

LC_0094

to retain a compound such as cephaloridine in normal phase mode using levels of organic solvents above 50% in the mobile phase, will allow increased sensitivity by LC/MS (Figure 4).

The IBD column also provides other chromatographic benefits. The excellent peak shape for cephaloridine in both the reverse and normal phase modes (Figure 5) increases sensitivity and improves quantitation. Furthermore, the retention of cephalosporin and cephaloridine is essentially unaffected by the pH. This allows full control in the pH range of 2 to 8 for optimum stabilization of the cephalosporins and penicillins during analysis, provided hydrolysis is

not an issue. The IBD column has a unique blend of hydrophobic and polar character for better resolution of closely related compounds.

Conclusion

Closely related compounds such as penicillins and cephalosporins may require more than one type of interaction for optimum resolution of closely related components. The Restek IBD phase provides those interactions using only simple mobile phases. The excellent peak shape, resolution enhancement, and wide pH make it the ideal choice for the analysis of penicillin- and cephalosporin-based antibiotics by HPLC or LC/MS.

Figure 4

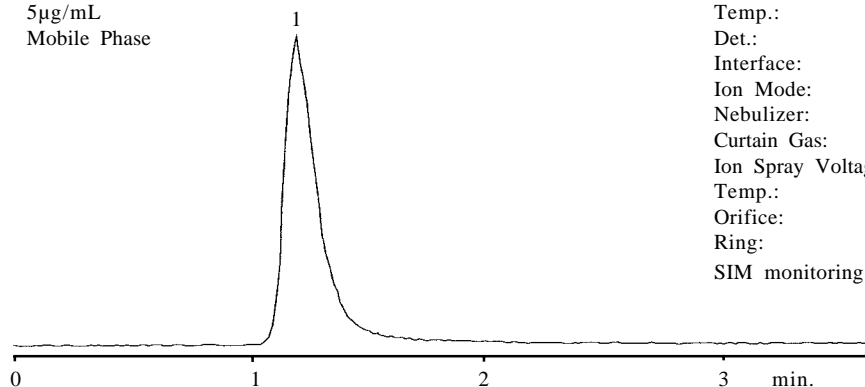
Ultra IBD allows increased LC/MS sensitivity of cephaloridine in normal phase mode.

Peak List:
1. cephaloridine

Sample:
Inj.: 5 µL
Conc.: 5 µg/mL
Solvent: Mobile Phase

Column: Ultra IBD
Catalog #: 9175552
Dimensions: 50 x 2.1mm
Particle Size: 5 µm
Pore Size: 100 Å

Conditions:
Mobile Phase: 5mM ammonium acetate pH 7.4: acetonitrile (20:80)
Flow: 0.2mL/min.
Temp.: ambient
Det.: PE/Sciex API 150 EX
Interface: Turbo Ion Spray
Ion Mode: Positive
Nebulizer: 8L/hour
Curtain Gas: 12L/Hour
Ion Spray Voltage: 4700.0v
Temp.: 350°C
Orifice: + 10.0v
Ring: + 25.0v
SIM monitoring: 416 ± 3dal.



LC_0124

Figure 5

Ultra IBD shows excellent peak shape for cephaloridine in both normal and reverse phase modes.

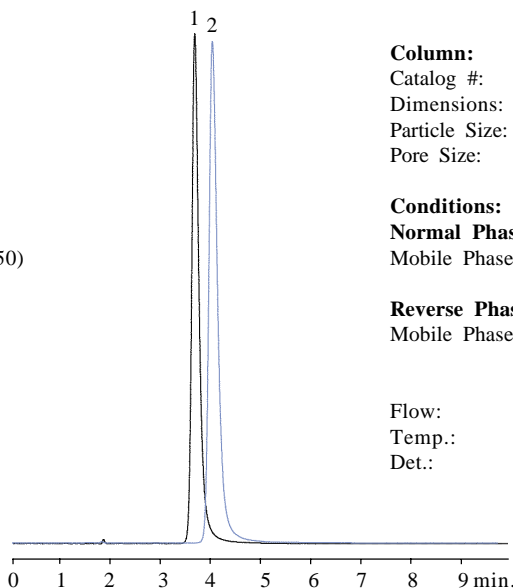
Peak List:
1. cephaloridine reverse phase
2. cephaloridine normal phase

Sample:
Inj.: 5 µL
Conc.: 1mg/mL
Solvent: acetonitrile:water (50:50)

Column: Ultra IBD
Catalog #: 9175565
Dimensions: 150 x 4.6mm
Particle Size: 5 µm
Pore Size: 100 Å

Conditions:
Normal Phase:
Mobile Phase: acetonitrile: pH 4.0 20mM ammonium phosphate (80:20, v/v)
Reverse Phase:
Mobile Phase: acetonitrile: pH 4.0 20mM ammonium phosphate (20:80, v/v)

Flow: 1.2mL/min.
Temp.: 27°C
Det.: UV @ 254nm



LC_0101&LC_0102

■ *Ultra IBD, 3µm Columns*

Particle Size: 3µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175331	9175332	9175333	9175335
50mm length	9175351	9175352	9175353	9175355
100mm length	9175311	9175312	9175313	9175315
150mm length	9175361	9175362	9175363	9175365

■ *Ultra IBD, 3µm Columns with Trident™ Inlet*

Particle Size: 3µm	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175332-700	9175333-700	9175335-700
50mm length	9175352-700	9175353-700	9175355-700
100mm length	9175312-700	9175313-700	9175315-700
150mm length	9175362-700	9175363-700	9175365-700

■ *Ultra IBD, 5µm Columns*

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175531	9175532	9175533	9175535
50mm length	9175551	9175552	9175553	9175555
100mm length	9175511	9175512	9175513	9175515
150mm length	9175561	9175562	9175563	9175565
200mm length	9175521	9175522	9175523	9175525
250mm length	9175571	9175572	9175573	9175575

■ *Ultra IBD, 5µm Columns with Trident™ Inlet*

Particle Size: 5µm	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9175532-700	9175533-700	9175535-700
50mm length	9175552-700	9175553-700	9175555-700
100mm length	9175512-700	9175513-700	9175515-700
150mm length	9175562-700	9175563-700	9175565-700
200mm length	9175522-700	9175523-700	9175525-700
250mm length	9175572-700	9175573-700	9175575-700

■ *Ultra IBD Guard Cartridges*

Dimensions	cat.#	qty.
10 x 2.1mm	917550212	3
10 x 4.0mm	917550210	3
20 x 4.0mm	917550220	2

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