RESTEK pharmaceutical

# Applications note

## 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 ß-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.

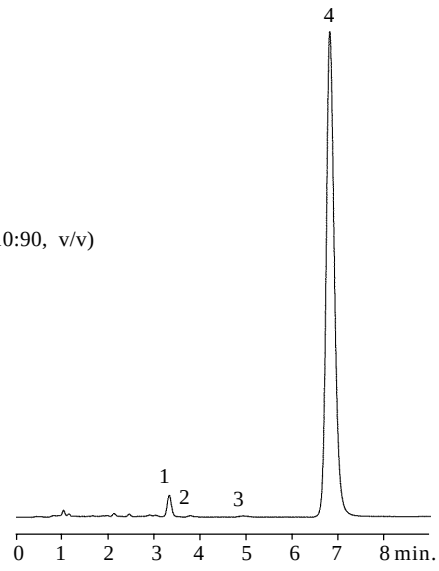
Unfortunately, biosynthetic fermentation does not produce a "pure" antibiotic. Even after cleanup of the fermentation mash, some side reaction products will remain. Many of these side products are closely related to the primary analyte (Figure 1). Desired products, however, are created in the semi-synthetic 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.: 2.5µL
Conc.: 1.2mg/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 (60:40, v/v) |
| Flow: | 1.2mL/min. |
| Temp.: | 30°C |
| Det.: | UV @ 270nm |

LC_0096

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a hydride ion to create a phenyl ring is the only structural difference between cephradine and its side product cephalexin (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

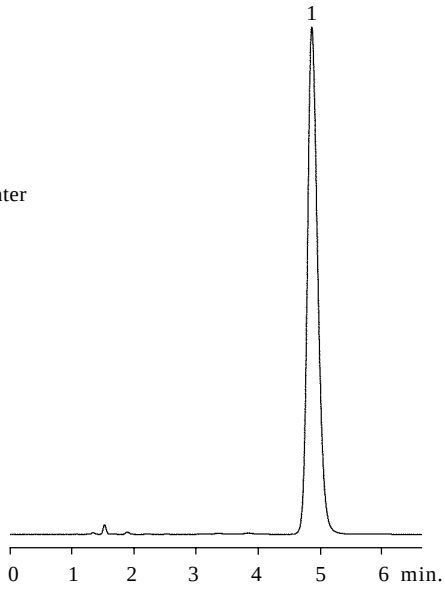
The Restek Ultra IBD phase provides greater versatility for the LC/MS analysis of penicillins and cephalosporins compared to a C18 column. The Ultra IBD column is capable of providing retention for cephaloridine in reverse phase mode with up to 45% organic solvent in the mobile phase. A conventional 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

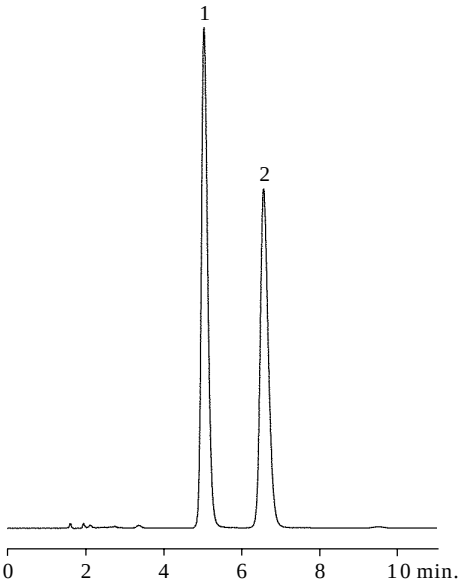
LC_0095

### Figure 3

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

**Peak List:**
1. cephalexin
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

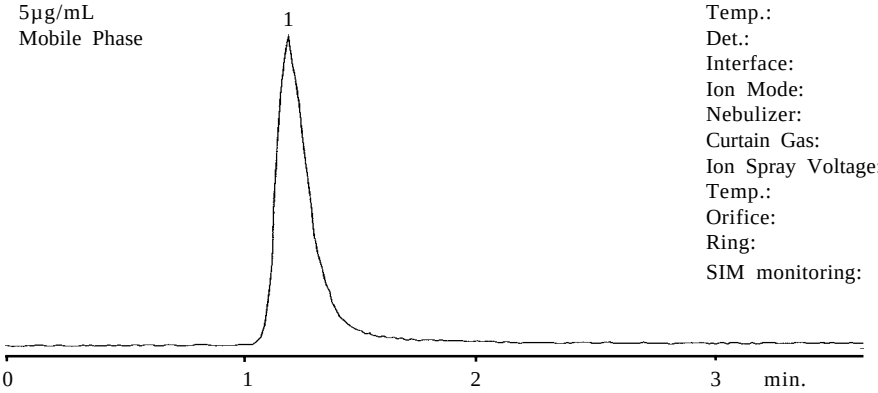
*Ultra IBD allows increased LC/MS sensitivity of cephaloridine in normal phase mode.*

**Peak List:**
1. cephaloridine

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

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

**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.



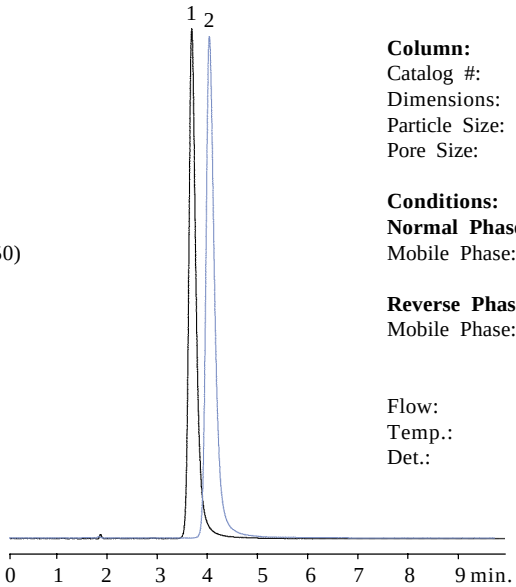

LC_0124

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




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

**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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