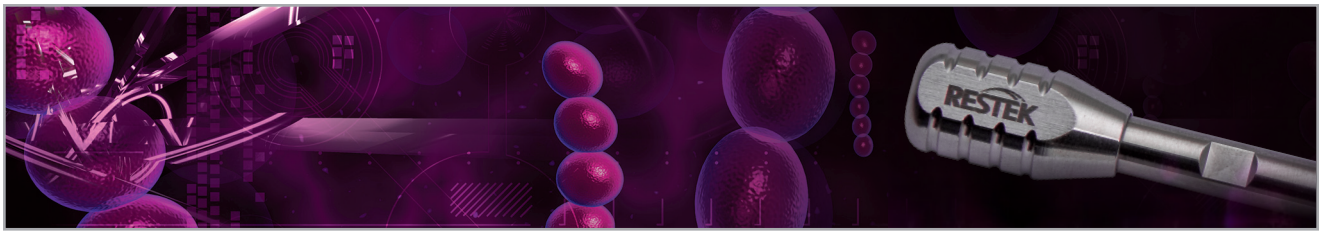


Pharmaceutical Applications

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

Introduction

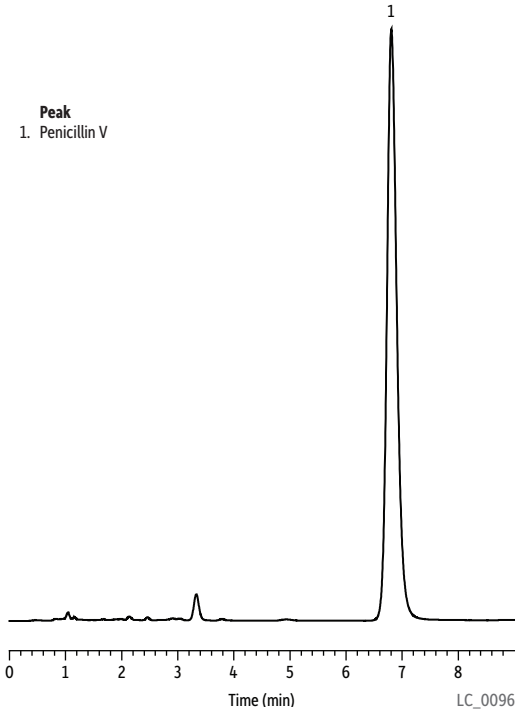
Antibiotics are the most widely used medications in the world. Whether by prescription, addition to animal feed stocks, or use of cleaning agents, everyone in the civilized world is either directly or indirectly exposed to antibiotics in daily life. The overuse of antibiotics, however, has allowed resistant bacteria to thrive. The death of 12,500 people in Guatemala from an episode of Shigella 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 fourmember 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.

Figure 1: Ultra IBD column separates penicillin V from fermentation impurities.

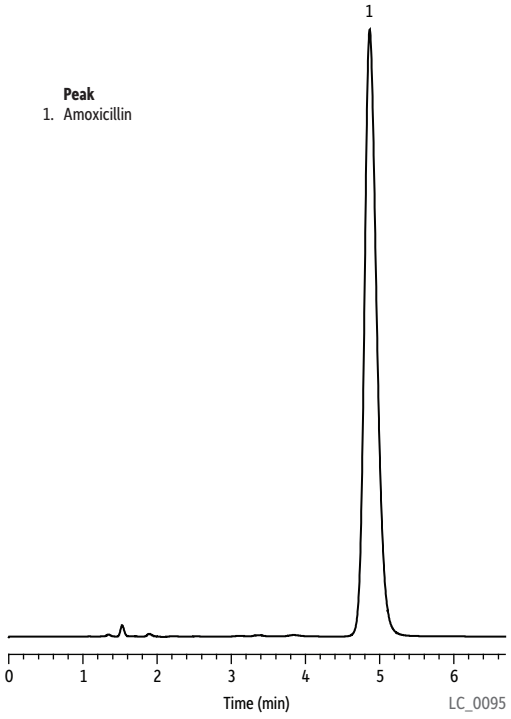


Column	Ultra IBD (cat.# 9175565)
Dimensions:	150 mm x 4.6 mm ID
Particle Size:	5 µm
Pore Size:	100 Å
Temp.:	30 °C
Sample	
Diluent:	acetonitrile:water (10:90, v/v)
Conc.:	1.2 mg/mL
Inj. Vol.:	2.5 µL
Mobile Phase	10 mM ammonium formate, pH 2.5:acetonitrile (95:5, v/v)
Flow:	1.2 mL/min
Detector	UV/Vis @ 270 nm

But 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 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.

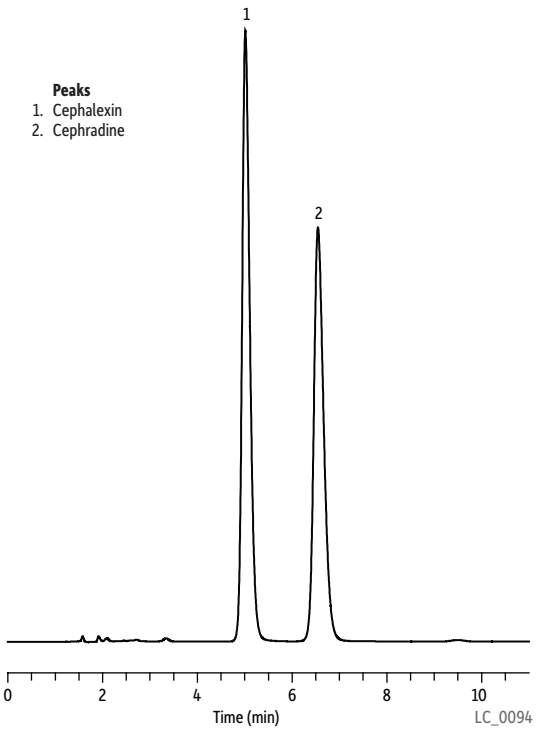
Unfortunately, many penicillins and cephalosporins are acid labile so that liquid chromatographic (LC) analysis of these molecules only should be performed if the sample is dissolved in a neutral media. Furthermore, if analysis time on the column is prolonged, breakdown of the analytes may occur *in 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 pH near 7.4 may become important for stability and accurate quantitation.

Figure 2: Ultra IBD column shows excellent peak shape for amoxicillin.



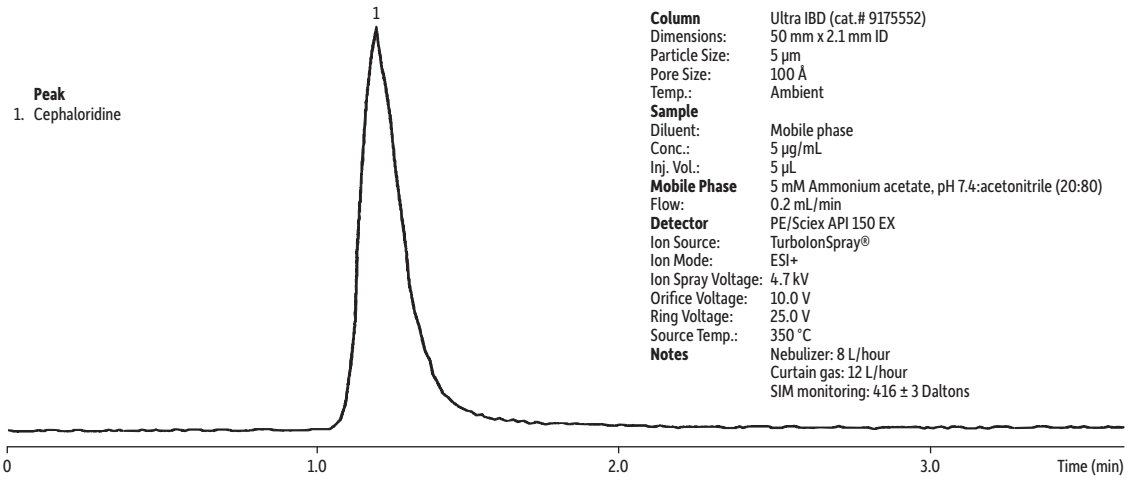
Column	Ultra IBD (cat.# 9175565)
Dimensions:	150 mm x 4.6 mm ID
Particle Size:	5 µm
Pore Size:	100 Å
Temp.:	30 °C
Sample	
Diluent:	acetonitrile:water (10:90, v/v)
Conc.:	1.5 mg/mL
Inj. Vol.:	5 µL
Mobile Phase	10 mM ammonium formate, pH 2.5:acetonitrile (95:5)
Flow:	1.2 mL/min
Detector	UV/Vis @ 270 nm

Figure 3: Ultra IBD column shows great separation between cephalexin and cephradine, which differ only by ring structure.



Column	Ultra IBD (cat.# 9175565)
Dimensions:	150 mm x 4.6 mm ID
Particle Size:	5 µm
Pore Size:	100 Å
Temp.:	30 °C
Sample	
Diluent:	acetonitrile:water (10:90, v/v)
Conc.:	500 µg/mL
Inj. Vol.:	10 µL
Mobile Phase	10 mM ammonium formate, pH 2.5:acetonitrile (90:10)
Flow:	1.2 mL/min
Detector	UV/Vis @ 270 nm

Figure 4: Ultra IBD column allows increased LC-MS sensitivity of cephaloridine in HILIC mode.



Column	Ultra IBD (cat.# 9175552)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	5 µm
Pore Size:	100 Å
Temp.:	Ambient
Sample	
Diluent:	Mobile phase
Conc.:	5 µg/mL
Inj. Vol.:	5 µL
Mobile Phase	5 mM Ammonium acetate, pH 7.4:acetonitrile (20:80)
Flow:	0.2 mL/min
Detector	PE/Sciex API 150 EX
Ion Source:	TurboIonSpray®
Ion Mode:	ESI+
Ion Spray Voltage:	4.7 kV
Orifice Voltage:	10.0 V
Ring Voltage:	25.0 V
Source Temp.:	350 °C
Notes	Nebulizer: 8 L/hour Curtain gas: 12 L/hour SIM monitoring: 416 ± 3 Daltons

Discussion of Analysis

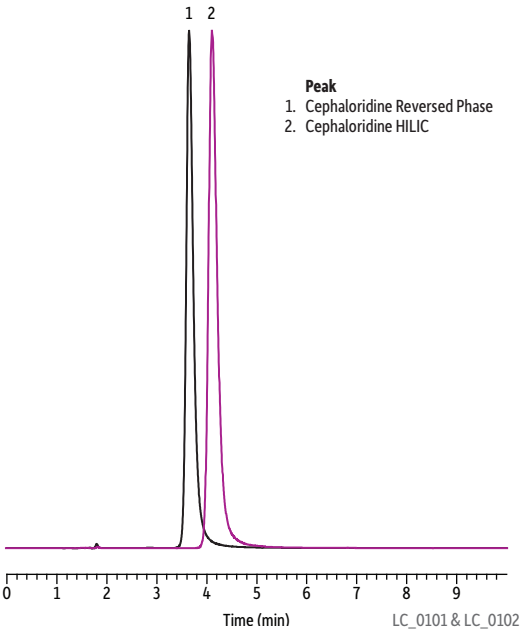
The Restek® Ultra IBD phase provides greater versatility for the LC-MS analysis of penicillins and cephalosporins compared to a C18 column. The Ultra IBD column is capable of providing retention for cephaloridine in reversed-phase mode with up to 45% organic solvent in the mobile phase. A conventional C18 column loses all retention near 35% organic solvent. Unlike a C18 column, the IBD is capable of polar interactions in a HILIC mode with analytes that possess charged functional groups. The ability to retain a compound such as cephaloridine in HILIC 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 reversed- and HILIC 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 2.5 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

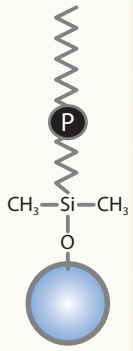
Closely related compounds such as penicillins and cephalosporins may require more than one type of interaction for optimum resolution of closely related components. The Restek® IBD phase provides those interactions using only simple mobile phases. The excellent peak shape, resolution enhancement, and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

Figure 5: Ultra IBD column shows excellent peak shape for cephaloridine in both HILIC and reversed-phase modes.



Column	Ultra IBD (cat.# 9175565)
Dimensions:	150 mm x 4.6 mm ID
Particle Size:	5 µm
Pore Size:	100 Å
Temp.:	27 °C
Sample	
Diluent:	acetonitrile:water (50:50, v/v)
Conc.:	1 mg/mL
Inj. Vol.:	5 µL
Mobile Phase	acetonitrile:20mM ammonium phosphate, pH 4.0 (20:80, v/v reversed phase; 80:20, v/v HILIC)
Flow:	1.2 mL/min
Detector	UV/Vis @ 254 nm

Product Listings

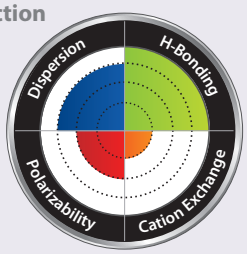


IBD

Column Characteristics:

particle size:	3 µm or 5 µm, spherical
pore size:	100 Å
carbon load:	12%
end-cap:	no
pH range:	2.5 to 8
temperature limit:	80 °C
USP phase code:	L68
phase category:	polar-embedded alkyl
ligand type:	proprietary polar functional embedded alkyl

USLC® Column Interaction Profile



Ultra IBD Columns (USP L68)

Chromatographic Properties

The Restek® IBD is a polar-embedded column that acts as a strong hydrogen bonder and may be the most versatile column available today. With a unique polar group, this column is very retentive and selective for acids. It also provides symmetrical peak shape for strong bases. Restek's IBD is compatible with 100% aqueous mobile phases and can be used under reversed-phase or HILIC conditions to retain very polar, ionic compounds in highly organic mobile phases.

Length	1.0 mm ID cat.#	2.1 mm ID cat.#	3.0 mm ID cat.#	4.6 mm ID cat.#
3 µm Columns				
30 mm	9175331	9175332	917533E	9175335
50 mm	9175351	9175352	917535E	9175355
100 mm	9175311	9175312	917531E	9175315
150 mm	9175361	9175362	917536E	9175365
5 µm Columns				
30 mm	9175531	9175532	917553E	9175535
50 mm	9175551	9175552	917555E	9175555
100 mm	9175511	9175512	917551E	9175515
150 mm	9175561	9175562	917556E	9175565
200 mm	9175521	9175522	917552E	9175525
250 mm	9175571	9175572	917557E	9175575

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Ultra IBD Guard Cartridges

Guard Cartridges	3-pk. (10 x 2.1 mm)	3-pk. (10 x 4.0 mm)
Ultra IBD Guard Cartridge	917550212	917550210

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