Specialty Reversed Phase HPLC Columns for Polar Analytes

Ultra Aqueous C18 and Ultra IBD Columns Solve Retention Dilemmas

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- Both columns provide sharp peaks for basic analytes.
- Both columns compatible with 100% aqueous mobile phases.
- Complementary selectivity for acidic and basic analytes.

Over the past several years, HPLC column manufacturers have been creating new stationary phases in attempts to address some of the separation problems encountered by analytical chemists.

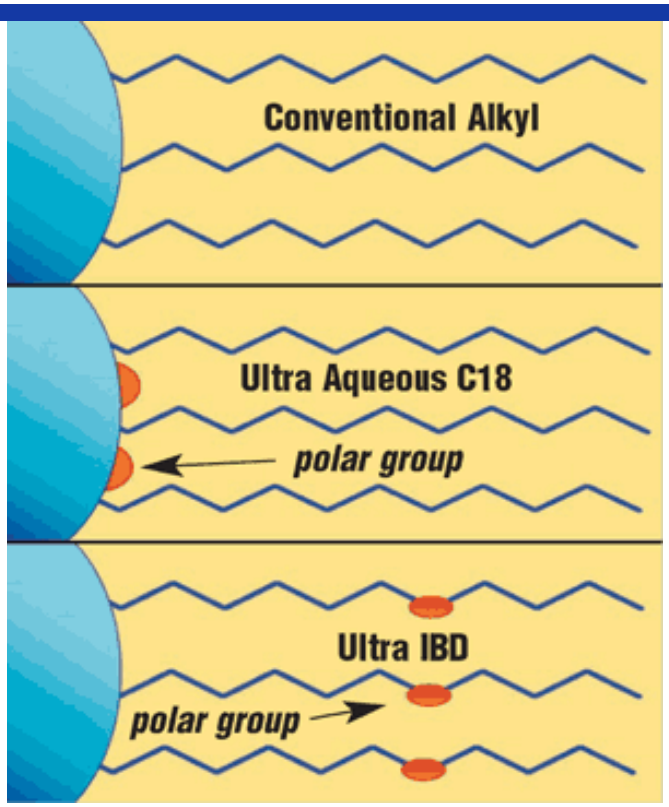
Stationary phases in traditional reversed phase columns are strictly non-polar alkyls, like C18. In contrast, many newer specialty reversed phase columns have stationary phases that are primarily alkyls, but with some secondary polar functionality. The polar functionality offers several advantages, including: unique selectivity, enhanced retention of polar compounds, and compatibility with completely aqueous mobile phases.

These specialty reversed phase columns can differ either in the type of polar group they incorporate or in how the polar group is incorporated into the stationary phase. Restek offers two specialty reversed phase columns that represent two different approaches to introducing secondary polar groups into a straight chain alkyl ligand (Figure 1). The stationary phase in the Ultra Aqueous C18 column has small polar groups attached to the silica surface, between the C18 chains. In contrast, the Ultra IBD stationary phase is a "polar embedded" type stationary phase, because its polar groups are embedded within a straight alkyl chain.

Figure 1
The stationary phase in an Ultra Aqueous C18 column has small polar groups attached to the silica surface; in the Ultra IBD stationary phase polar groups are embedded in the alkyl chain.

One drawback to these specialty columns is that their potential for mixed mode interactions makes it more difficult to predict which column will perform best for a particular application. With this in mind, we used a series of simple tests to directly compare the performance characteristics of Ultra Aqueous C18 and Ultra IBD columns. From the results of these tests, we can offer some useful guidelines for selecting a specialty reversed phase column.

The first test measured the hydrophobic retention of each column, using a sample mixture of completely nonpolar analytes and a mobile phase containing a high proportion of organic solvent. For pure alkyl stationary phases, hydrophobic retention usually is directly proportional to



the percent carbon (%C) in the bonded phase silica, if the phases are bonded on silica particles of comparable surface area. Figure 2A shows that the hydrophobic retention of Ultra Aqueous C18 columns is approximately twice that of Ultra IBD columns (Figure 2B), based on capacity factors for pyridine, despite the two bonded phases' similar surface area and %C (Ultra Aqueous C18: 100Å pores, 14%C; Ultra IBD: 100Å pores, 12%C). The hydrophobic retention of Ultra Aqueous C18 columns is equivalent to that of conventional C18 columns with the same surface area and %C. The considerably reduced hydrophobic retention of Ultra IBD columns can be attributed to the embedded polar group in the stationary phase shielding the lower portion of the alkyl chain from the nonpolar analytes.

We compared the columns' base deactivation by measuring the peak shape for a basic analyte, pyridine. Both Ultra Aqueous C18 (Figure 3A & Figure 3B) and Ultra IBD (Figure 3C) columns show excellent base deactivation, with pyridine peak symmetry values better than those for highly base-deactivated C18 phases made through conventional chemistry. Although they are similarly base-deactivated, Ultra Aqueous C18 columns exhibit much greater retention of pyridine than do Ultra IBD columns.

Next, we compared Ultra Aqueous C18 and Ultra IBD columns' ability to separate small carboxylic acids. It is difficult for conventional reversed phase columns to retain these molecules. A very weak, highly aqueous mobile phase is required. Many C18 phases are not compatible with highly aqueous mobile phases, and show a gradual or sudden loss of retention that is attributed to "chain folding" or "phase collapse." Both Ultra Aqueous C18 (Figure 4A) and Ultra IBD (Figure 4B) columns are completely compatible with 100% aqueous mobile phases. Neither column showed any loss of retention, even after mobile phase flow was temporarily stopped. (Absence of pressure maximizes the potential for phase collapse, thus exposure to 100% aqueous mobile phase under no flow is the most extreme test of phase integrity.) This comparison did reveal some differences between Ultra Aqueous C18 and Ultra IBD columns, however. Note that the elution orders in Figure 4 A & B are different, demonstrating significant differences in selectivity. Also, the malonic acid peak tails on the Ultra IBD column. We believe this is because malonic acid has two carboxylic acid groups -- several dicarboxylic acids tail on Ultra IBD columns and similar columns.

Finally, we used the same 100% aqueous mobile phase to evaluate selectivity and retention for mixtures containing both an acid (ascorbic acid/Vitamin C) and a base (thiamin/Vitamin B1). Note that Ultra Aqueous C18 (Figure 5A) and Ultra IBD (Figure 5B) columns produce opposite elution order, again demonstrating their complementary selectivity. Of several columns evaluated from various manufacturers, the Ultra Aqueous C18 column was the only column to exhibit significant retention of thiamin. This is consistent with the enhanced retention of pyridine shown in Figure 3 A & B. Thiamin is barely retained by an Ultra IBD column, using the uracil peak in Figure 2B as the measure of column void volume (t_0).

Highly base-deactivated Ultra Aqueous C18 and Ultra IBD columns are powerful tools for analyzing polar compounds by reversed phase HPLC. They offer alternate selectivity

to each other, as well as to conventional C18 stationary phases. Their secondary polar functionalities enhance retention of polar analytes, contribute to their unique selectivity, and make them compatible with a complete spectrum of mobile phase compositions, from 100% organic to 100% aqueous. An Ultra Aqueous C18 column is the better choice for small dicarboxylic acids, or for maximum retention of bases in a highly aqueous mobile phase.

Product Listing

Ultra Aqueous C18 5µm Columns

Ultra IBD 5µm Columns

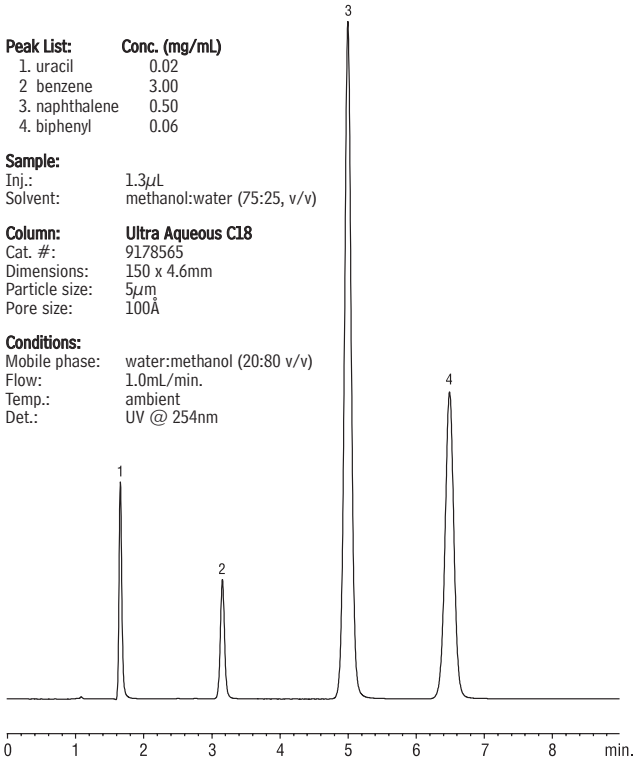
Reversed Phase Test Mix on Ultra Aqueous C18

Peak List:	**Conc. (mg/mL)**
1. uracil	0.02
2 benzene	3.00
3. naphthalene	0.50
4. biphenyl	0.06

Sample:
Inj.: 1.3µL
Solvent: methanol:water (75:25, v/v)

Column: **Ultra Aqueous C18**
Cat. #: 9178565
Dimensions: 150 x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: water:methanol (20:80 v/v)
Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 254nm



LC_0233

Reversed Phase Test Mix on Ultra IBD

Peak List: **Conc. (mg/mL)**

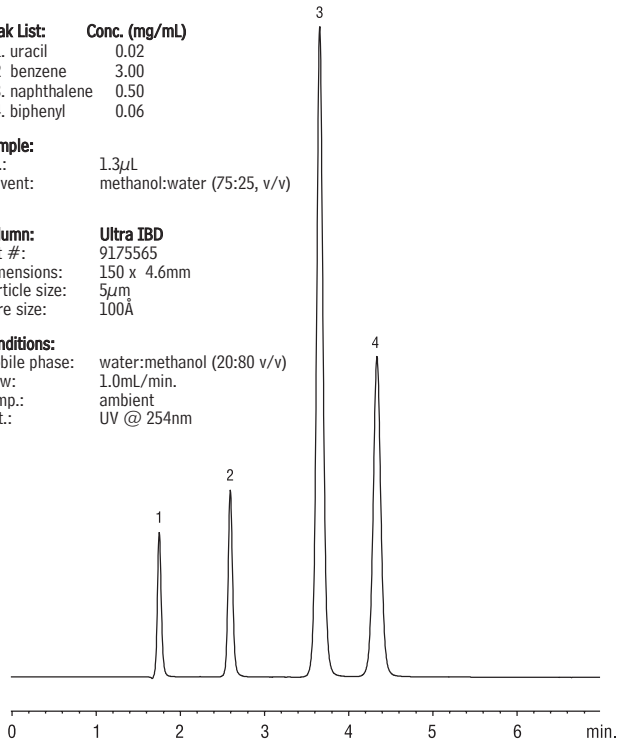
1.	uracil	0.02
2	benzene	3.00
3.	naphthalene	0.50
4.	biphenyl	0.06

Sample:

Inj.: 1.3µL
Solvent: methanol:water (75:25, v/v)

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

Conditions:
Mobile phase: water:methanol (20:80 v/v)
Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 254nm



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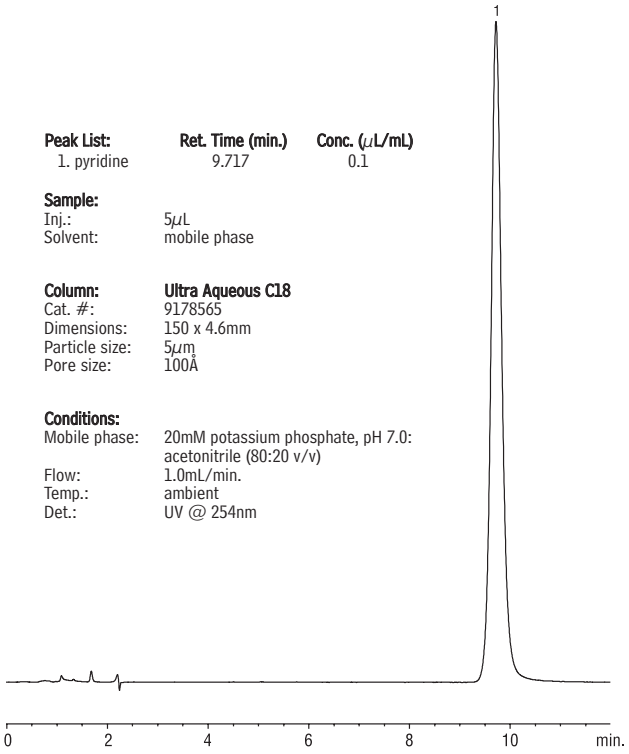
Pyridine on Ultra Aqueous C18



LC_0230

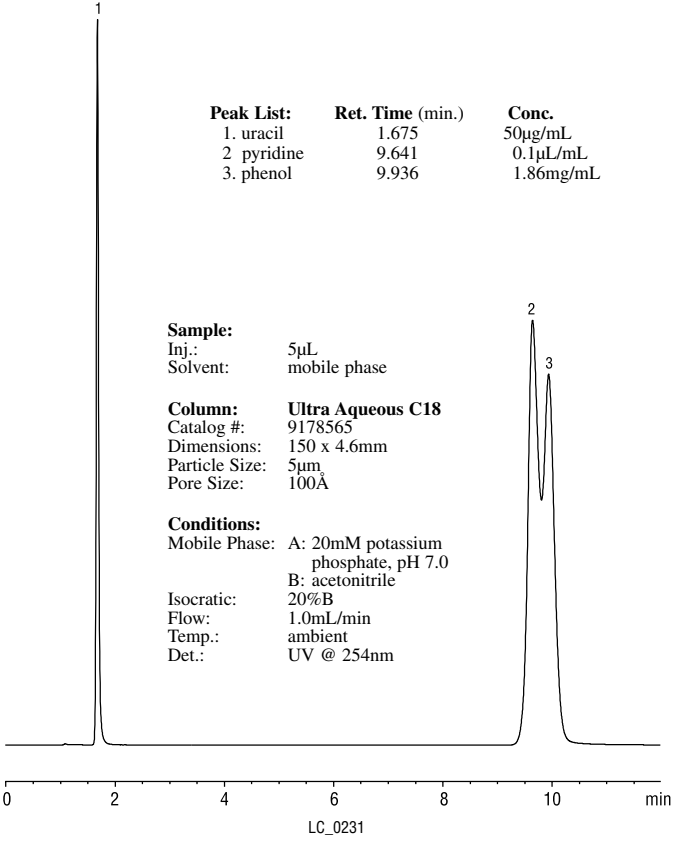
Peak List:	Ret. Time (min.)	Conc. (µL/mL)
1. pyridine	9.717	0.1

Sample:
Inj.: 5µL
Solvent: mobile phase

Column: **Ultra Aqueous C18**
Cat. #: 9178565
Dimensions: 150 x 4.6mm
Particle size: 5µm
Pore size: 100Å

Conditions:
Mobile phase: 20mM potassium phosphate, pH 7.0: acetonitrile (80:20 v/v)
Flow: 1.0mL/min.
Temp.: ambient
Det.: UV @ 254nm

Pyridine/Phenol Test Mix on Ultra Aqueous C18



Peak List:	**Ret. Time** (min.)	**Conc.**
1. uracil	1.675	50µg/mL
2 pyridine	9.641	0.1µL/mL
3. phenol	9.936	1.86mg/mL

Sample:
Inj.: 5µL
Solvent: mobile phase

Column: **Ultra Aqueous C18**
Catalog #: 9178565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 100Å

Conditions:
Mobile Phase: A: 20mM potassium phosphate, pH 7.0
B: acetonitrile
Isocratic: 20%B
Flow: 1.0mL/min
Temp.: ambient
Det.: UV @ 254nm

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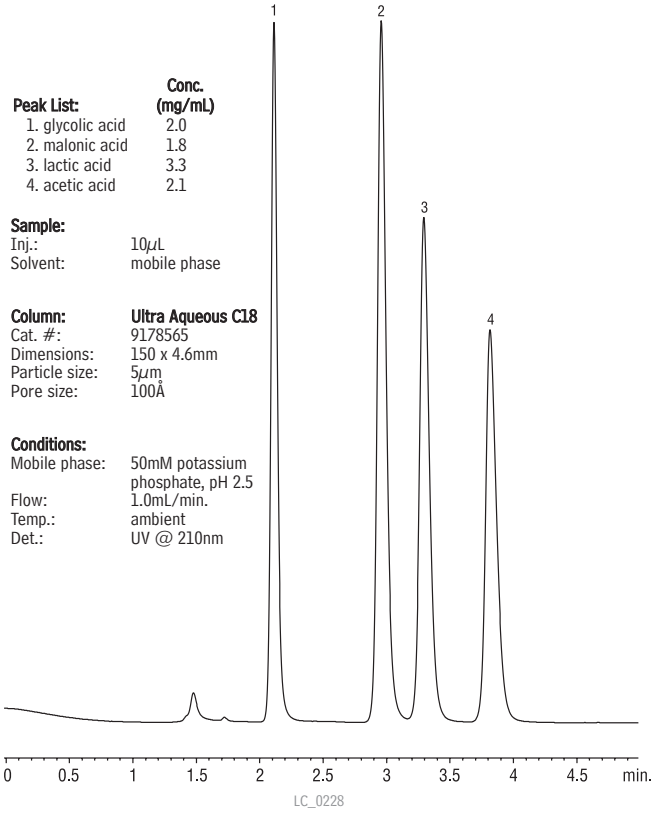
Carboxylic Acids on Ultra Aqueous C18



Peak List:	Conc. (mg/mL)
1. glycolic acid	2.0
2. malonic acid	1.8
3. lactic acid	3.3
4. acetic acid	2.1

Sample:

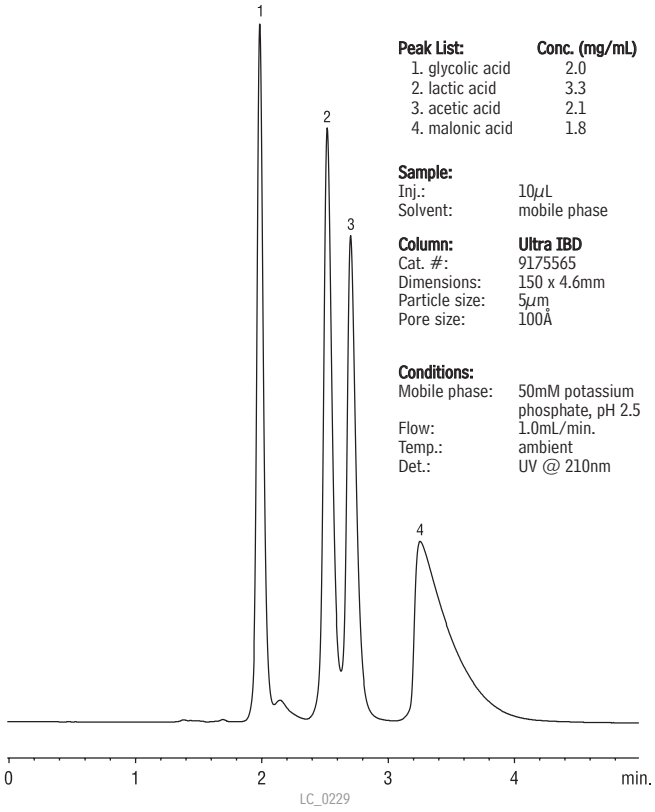
Inj.:	10µL
Solvent:	mobile phase

Column:	**Ultra Aqueous C18**
Cat. #:	9178565
Dimensions:	150 x 4.6mm
Particle size:	5µm
Pore size:	100Å

Conditions:

Mobile phase:	50mM potassium phosphate, pH 2.5
Flow:	1.0mL/min.
Temp.:	ambient
Det.:	UV @ 210nm

Carboxylic Acids on Ultra IBD



Peak List:	**Conc. (mg/mL)**
1. glycolic acid	2.0
2. lactic acid	3.3
3. acetic acid	2.1
4. malonic acid	1.8

Sample:

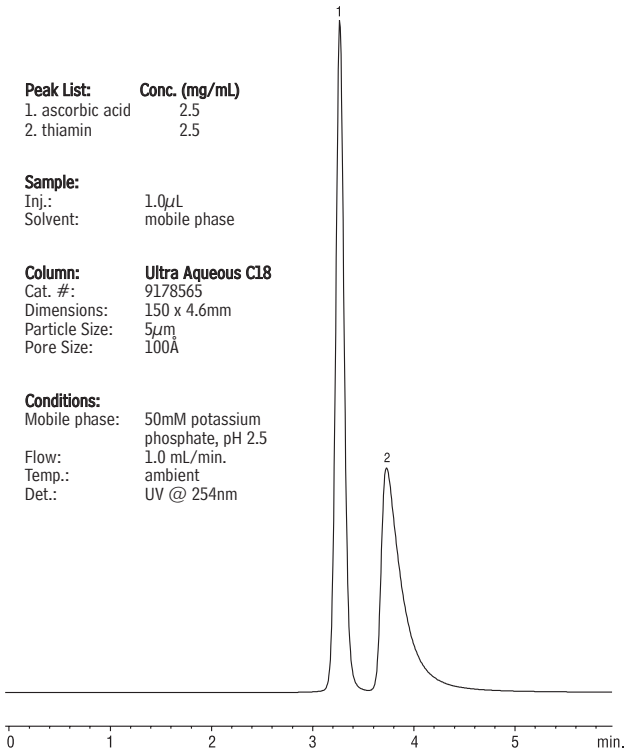
Inj.:	10µL
Solvent:	mobile phase

Column:	**Ultra IBD**
Cat. #:	9175565
Dimensions:	150 x 4.6mm
Particle size:	5µm
Pore size:	100Å

Conditions:

Mobile phase:	50mM potassium phosphate, pH 2.5
Flow:	1.0mL/min.
Temp.:	ambient
Det.:	UV @ 210nm

Vitamins Thiamin (Vitamin B1) and Ascorbic Acid (Vitamin C) on Ultra Aqueous C18



Peak List:	**Conc. (mg/mL)**
1. ascorbic acid	2.5
2. thiamin	2.5

Sample:

Inj.:	1.0µL
Solvent:	mobile phase

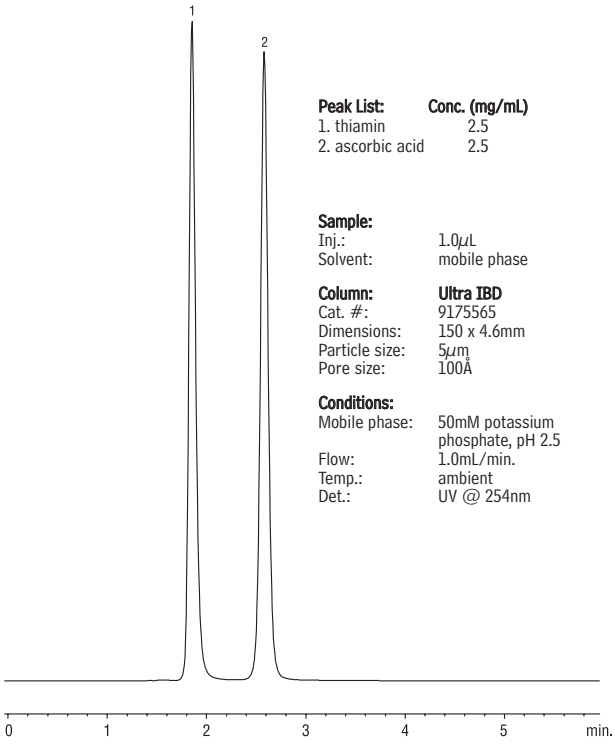
Column:	**Ultra Aqueous C18**
Cat. #:	9178565
Dimensions:	150 x 4.6mm
Particle Size:	5µm
Pore Size:	100Å

Conditions:

Mobile phase:	50mM potassium phosphate, pH 2.5
Flow:	1.0 mL/min.
Temp.:	ambient
Det.:	UV @ 254nm

LC_0226

Vitamins Thiamin (Vitamin B1) and Ascorbic Acid (Vitamin C) on Ultra IBD



Peak List:	**Conc. (mg/mL)**
1. thiamin	2.5
2. ascorbic acid	2.5

Sample:

Inj.:	1.0µL
Solvent:	mobile phase

Column:	**Ultra IBD**
Cat. #:	9175565
Dimensions:	150 x 4.6mm
Particle size:	5µm
Pore size:	100Å

Conditions:

Mobile phase:	50mM potassium phosphate, pH 2.5
Flow:	1.0mL/min.
Temp.:	ambient
Det.:	UV @ 254nm