

BioBasic® Columns

Introduction

BioBasic columns are designed specifically for biochromatography of proteins, peptides, and nucleic acids. The 300Å pore size, high purity silica, and stable bonding chemistry of BioBasic packings makes them ideal for life science applications.

Improved HPLC performance for biomolecules

Whether you're doing reversed phase or ion exchange, the BioBasic family of HPLC columns provides superior performance:

- improved resolution
- better reproducibility
- longer column lifetimes
- more efficient separations

Better Reproducibility

BioBasic columns provide superior chromatography, run after run, column after column. The extra dense bonding chemistry used for BioBasic reversed phase packings gives a highly stable, reproducible surface for reliable results. Figure 1 demonstrates the reproducible performance of 3 batches of BioBasic 18 packing materials.

Figure 1

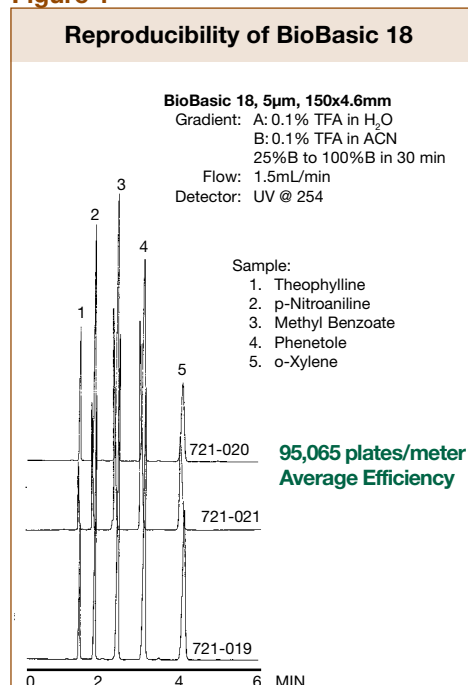


Table 1: BioBasic Packing Characteristics

Specifications					
Phase	Particle Size	Carbon Load	Pore Size	Endcapping	Silica Type
BioBasic 18	5µm	9%	300Å	yes	high purity base deactivated
BioBasic 8	5µm	5%	300Å	yes	high purity base deactivated
BioBasic 4	5µm	4%	300Å	yes	high purity base deactivated
BioBasic Phenyl	5µm	-	300Å	yes	high purity base deactivated
BioBasic CN	5µm	-	300Å	yes	high purity base deactivated
BioBasic AX	5µm	-	300Å	yes	high purity base deactivated
BioBasic SCX	5µm	-	300Å	yes	high purity base deactivated

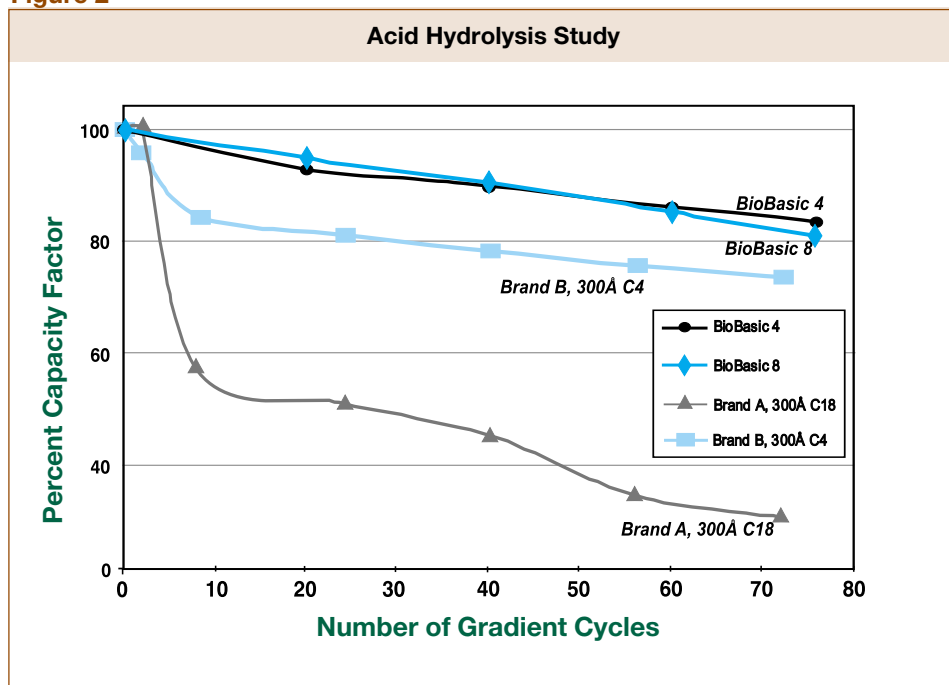
Longer column lifetimes

BioBasic packings are designed to hold up to harsh mobile phase conditions for longer column lifetimes. Even when subjected to acid hydrolysis conditions at pH 1.8 and 50°C using 0.1% TFA, BioBasic columns show superior results. Figure 2 demonstrates a comparison of column lifetime under these difficult gradient conditions.

A Range of Stationary Phases with Different Selectivities

BioBasic reversed phase packings are available in several chemistries, including C18, C8, C4, phenyl and cyano. BioBasic ion exchangers include BioBasic AX for anion exchange and SCX for cation exchange. Table 1 provides a summary of characteristics for each BioBasic packing.

Figure 2



300Å Pore Size for Better Protein and Peptide Separations

Pore size can exert a significant influence on the chromatography of biomolecules. Figure 3 shows the higher resolution of the 300Å pore size BioBasic® 18 column for a tryptic digest separation, compared to a 150Å pore size C18 column. In particular, the peaks eluting near 8 and 13 minutes show much higher resolution on the BioBasic 18 column. Figure 4 shows a comparison of three proteins separated on a 300Å BioBasic 4 column versus a 150Å C4 column. The BioBasic 4 column provides taller, sharper peaks with less tailing.

Figure 5 demonstrates the changes in selectivity that occur due to differences in bonded phase chain length. The BioBasic 18 and BioBasic 4 columns show a reversal of peak order for the two myoglobin peaks, which are not separated on the BioBasic 8 column.

Figure 3

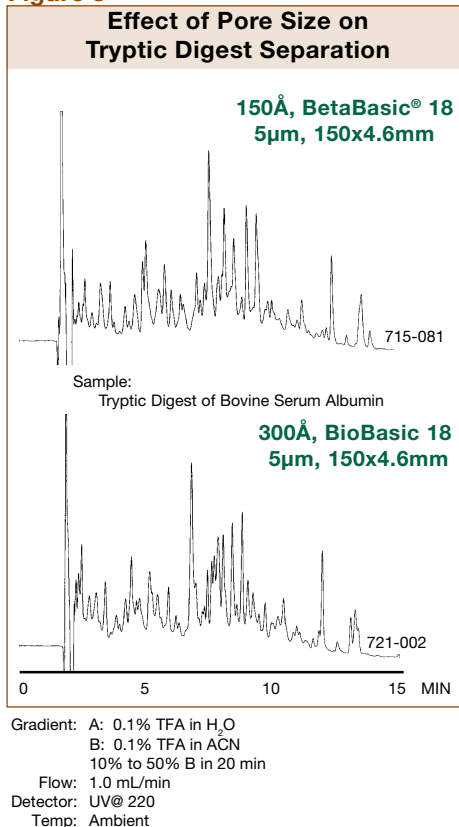


Figure 4

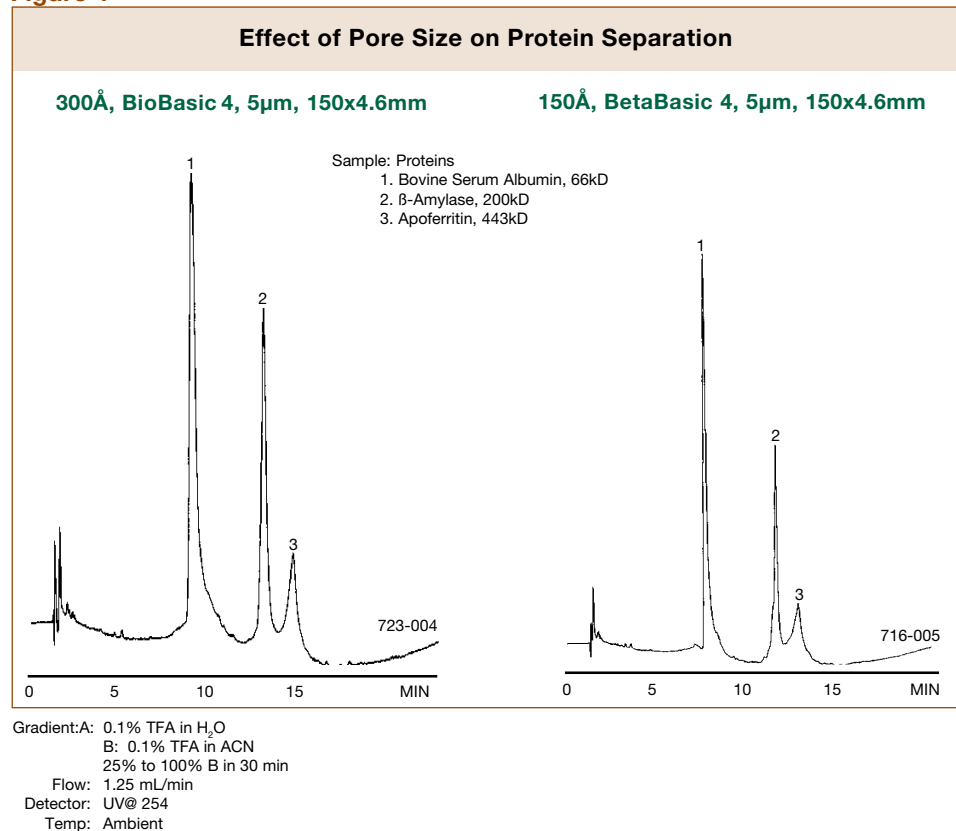
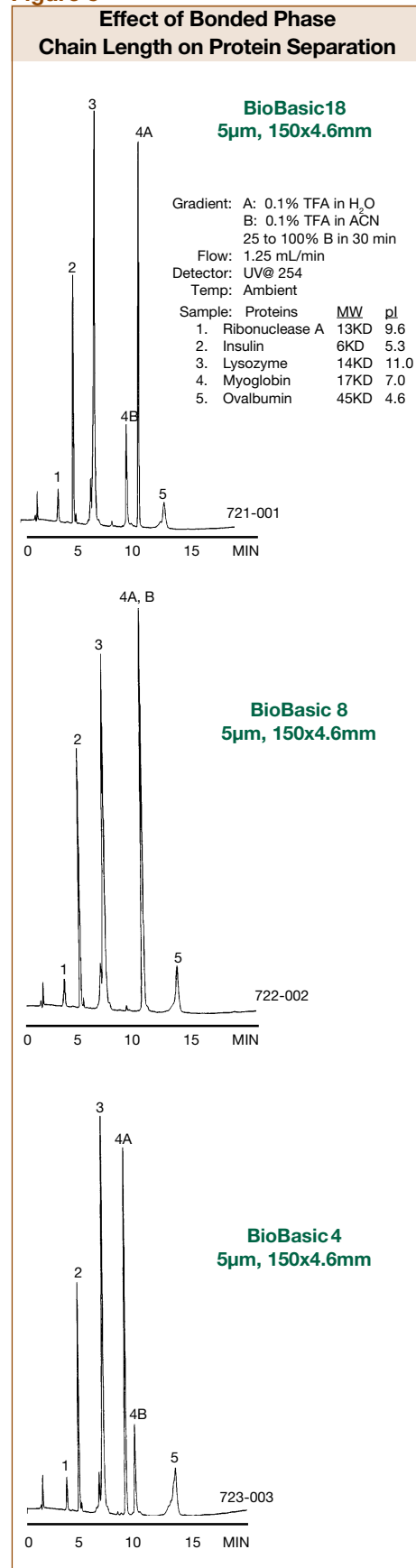


Figure 5



NEW! BioBasic® AX Anion Exchanger

The BioBasic family now includes BioBasic AX columns for anion exchange. BioBasic AX columns give you superior performance for proteins, peptides and nucleic acids (Figure 6), using protein-friendly ion exchange conditions.

BioBasic AX columns give you:

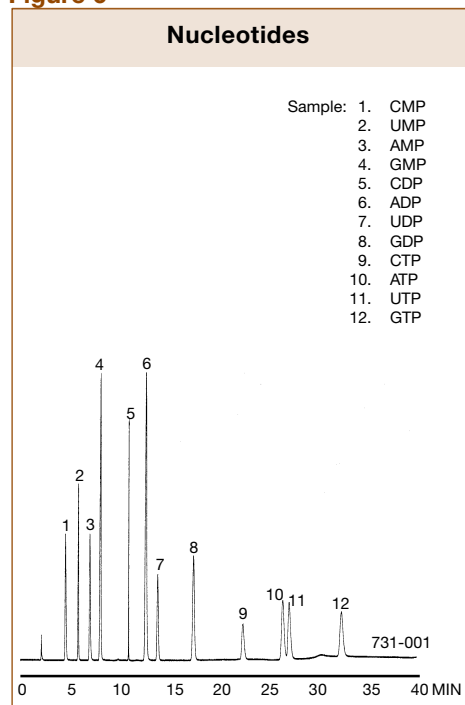
- better reproducibility
- longer column lifetimes
- more efficient separations
- multiple modes of interaction

What is BioBasic AX packing material?

BioBasic AX columns are made from polyethyleneimine (PEI) covalently bonded to a highly base deactivated 300Å 5µm silica (see Table 2). PEI forms a polymeric structure on the silica surface which is protein-friendly. The covalent bond to the silica surface provides better stability and longer column lifetime than typical PEI-coated particles.

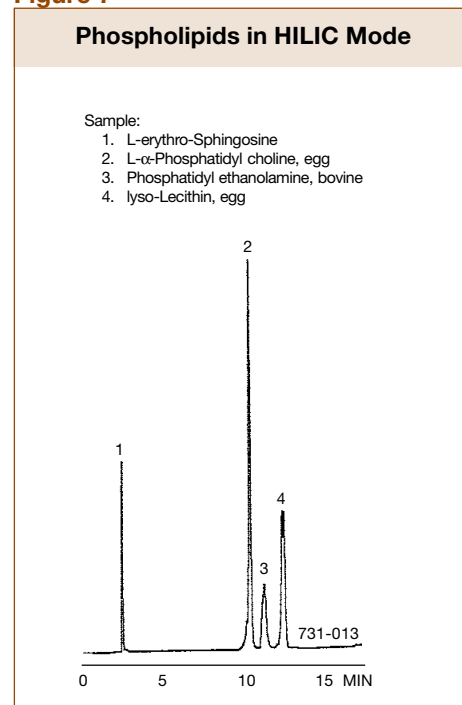
BioBasic AX columns can also be used in non-buffered conditions for Hydrophilic Interaction Liquid Chromatography (HILIC). HILIC is closely related to normal phase chromatography, although HILIC uses a high aqueous component in the mobile phase, as shown in Figure 7.

Figure 6



BioBasic AX, 5µm, 150x4.6mm
Gradient: A: 5mM KH_2PO_4 , pH 3.2
B: 0.75M KH_2PO_4 , pH 3.2
0-100%B in 30 min.
Flow: 1.0 mL/min
Detector: UV @ 254

Figure 7



BioBasic AX, 5µm, 150x4.6mm
Gradient: A: 95% ACN / 5% 5mM $\text{NH}_4\text{Formate}$, pH 6.13
B: 80% ACN / 20% 5mM $\text{NH}_4\text{Formate}$, pH 6.13
0% B 0-5 min., 0-100%B 5-15 min.
Flow: 1.0 mL/min
Detector: ELSD

Table 2

Specifications:

Phase	Particle size	Pore size	Ion exchange ligand	Ion exchange capacity
BioBasic AX	5µm	300Å	PEI	0.22 mequivalents/gram
BioBasic SCX	5µm	300Å	Sulfonic Acid	0.07 mequivalents/gram

NEW! Versatile BioBasic SCX Cation Exchange Columns

- NEW! Versatile cation exchanger
- 300Å pore size for better protein and peptide separations
- Superb stability under demanding pH conditions
- Exceptional efficiency from 5µm silica particles

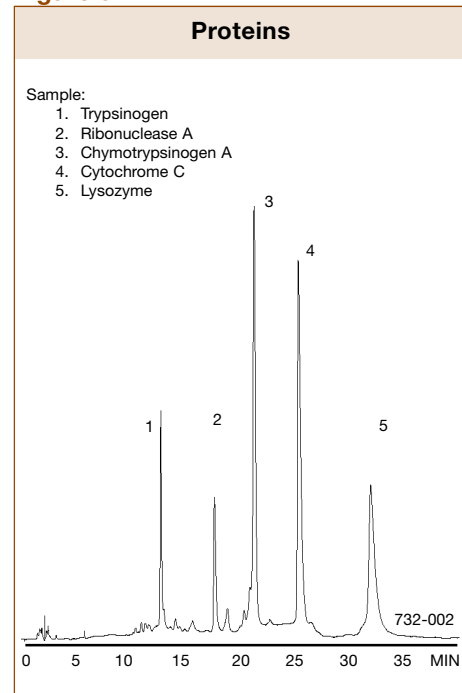
NEW! BioBasic SCX Cation Exchanger

BioBasic SCX columns are a versatile new cation exchange column providing you with superior performance for proteins, peptides and small molecules. BioBasic SCX columns are particularly useful for protein fractionations for proteomics analyses by LC-MS, including 2-dimensional fractionation when coupled to a BioBasic 18 capillary column.

Better Ion Exchange Performance

BioBasic SCX columns are designed to give superior reproducibility, both column-to-column and batch-to-batch. The 5µm 300Å silica provides significantly higher efficiency than typical polymer-based ion exchangers. Every BioBasic SCX column packed and tested shows over 80,000 plates per meter as tested under normal phase conditions.

Figure 8



BioBasic SCX, 5µm, 150x4.6mm
Gradient: A: 0.02M Tris, pH 6
B: A + 1.0M Na Acetate, pH 6
0-100%B in 60 min.
Flow: 1.0 mL/min
Detector: UV @ 280

"Tunable" Ion Exchange

Both the BioBasic® AX and BioBasic SCX packing materials can be used across a broad range of both pH and ionic strength. By manipulating buffer concentration, separations can be optimized for maximum retention, high efficiency, or rapid throughput.

Figures 9 and 10 demonstrate the effect of buffer concentration on retention for a series of organic acids using the BioBasic AX column. As buffer concentration decreases, retention increases. Figure 11 shows the same effect of buffer strength on the BioBasic SCX column for a series of basic analytes. In Figure 12, the change in retention with change in pH is demonstrated on the BioBasic SCX column, showing longer retention and improved separations with decreased pH for a series of peptides.

Figure 9

Effect of Buffer Concentration on Ion Exchange Retention

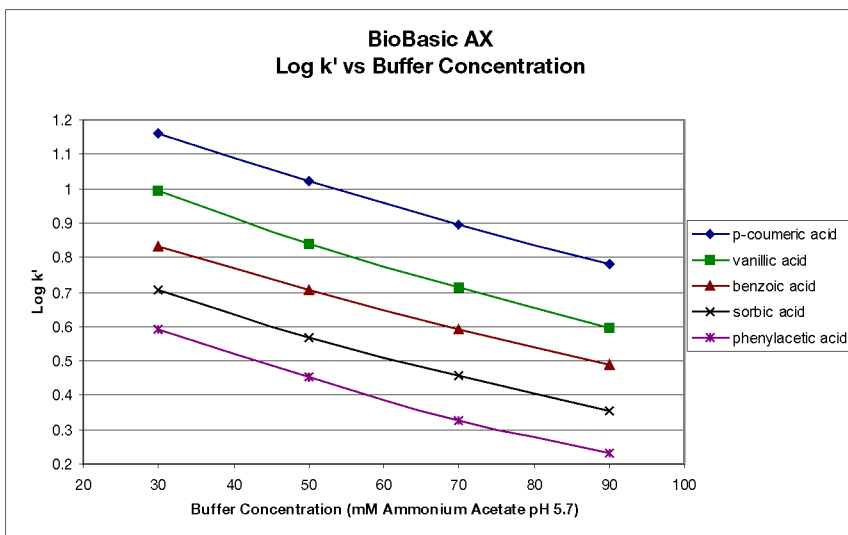
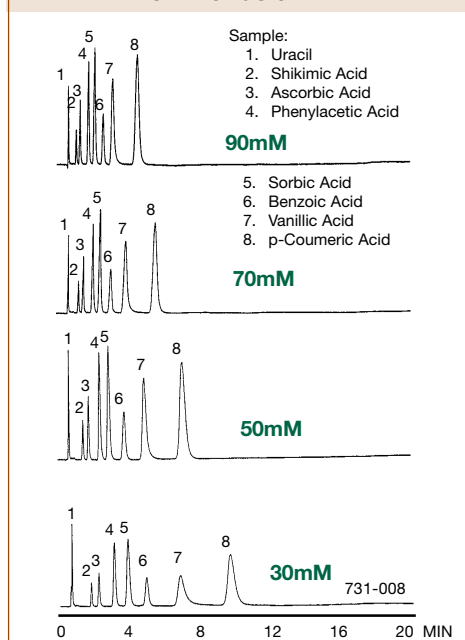


Figure 10

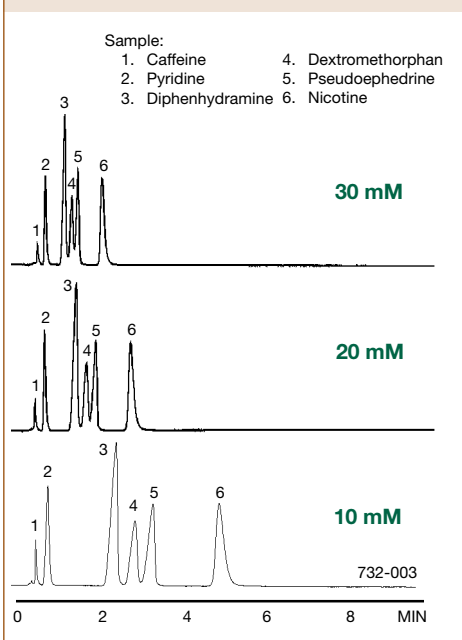
Effect of Buffer Concentration on BioBasic AX



BioBasic AX, 5µm, 50x4.6mm
Eluent: A: NH₄Acetate, pH 5.7
B: Acetonitrile
95%A / 5%B
Flow: 1.0 mL/min
Detector: UV @ 225

Figure 11

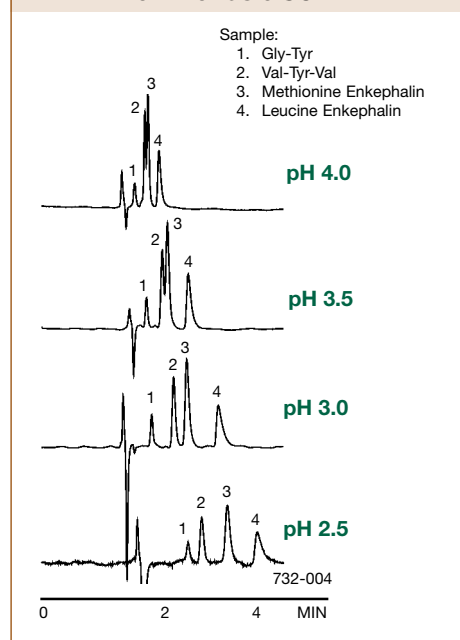
Effect of Buffer Concentration on BioBasic SCX



BioBasic SCX, 5µm, 150x4.6mm
Eluent: 60% NH₄Acetate, pH 4.5 / 40% ACN
Flow: 1.0 mL/min
Detector: UV @ 254

Figure 12

Effect of pH on BioBasic SCX



BioBasic SCX, 5µm, 150x4.6mm
Eluent: A: 5mM NH₄Formate
B: A + 1M NaCl
95% A / 5% B
Flow: 1.0 mL/min
Detector: UV @ 210

Better Performance from a Better Ion Exchange Column

BioBasic® AX columns are designed to give superior reproducibility, both column-to-column and batch-to-batch. Stringent quality control tests using ionic analytes and buffered mobile phase conditions ensure a reproducible ion exchange surface and consistent results. The exceptional ion exchange reproducibility of the BioBasic AX packing is demonstrated in Figure 13.

BioBasic AX columns provide superb stability at both low and high pH ranges, unlike many other silica-based ion exchangers. Figures 14 and 15 show column lifetime studies done with 14,000 – 16,000 column volumes at pH 2.5 and pH 8. At these pH extremes, many silica-based columns will show permanent degradation of the bonded phase (pH 2.5) or the underlying silica (pH 8). At either pH, the BioBasic AX columns are easily regenerated with a salt gradient to remove impurity buildup caused by the buffers, and return to their original performance.

Ion exchange columns often show poor efficiency, especially those that are based on polymeric materials. The BioBasic AX packing is manufactured on a base-deactivated silica, which provides exceptional efficiency in an ion exchange column. In addition, the surface characteristics of the PEI-bonded BioBasic AX stationary phase provide rapid mass transfer for more efficient separations. Figure 16 shows a standard quality control test for the BioBasic AX packing run under normal phase conditions, demonstrating a minimum efficiency of 80,000 plates per meter for each column packed and tested.

Figure 14

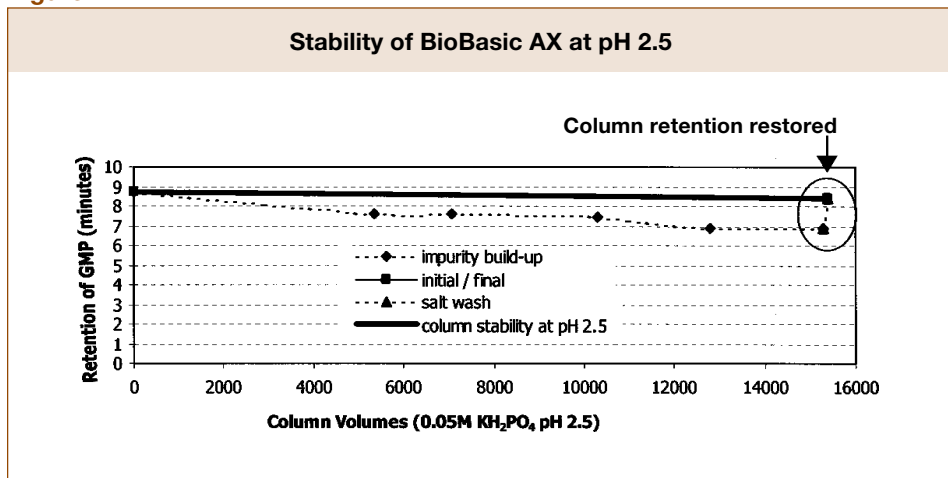


Figure 15

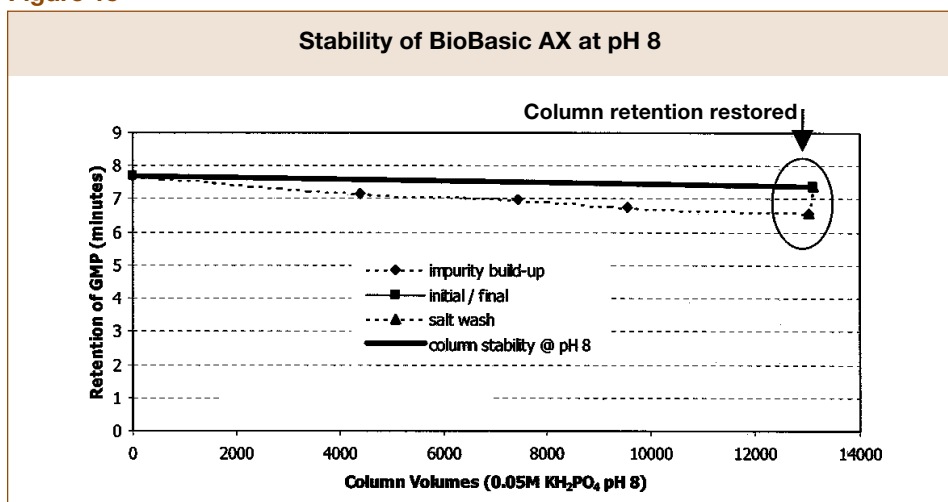
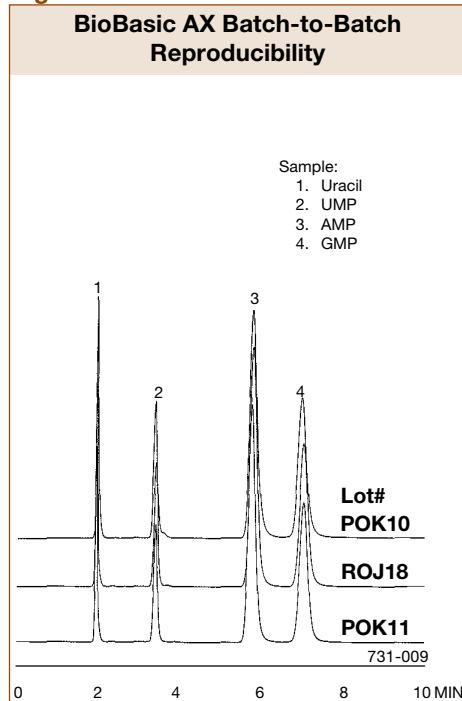
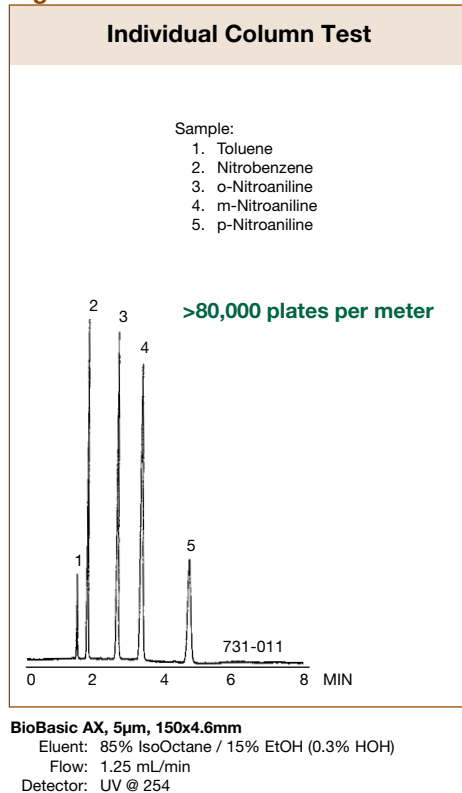


Figure 13



BioBasic AX, 5µm, 150x4.6mm
Eluent: 0.05M KH₂PO₄, pH 4.68
Flow: 1.0 mL/min
Detector: UV @ 254

Figure 16



Better Performance from Better Biochromatography Columns

BioBasic® AX columns provide better separations than many other choices for ion exchange of biological molecules. Figures 17 and 18 show comparisons of superior performance for both nucleic acids and proteins versus other choices for ion exchangers. Figure 17 demonstrates the excellent selectivity and resolution of the BioBasic AX column for a complex mixture of nucleotides compared to a popular 80Å pore size anion exchange column. Figure 18 shows three proteins chromatographed with good separation and efficiency on the BioBasic AX column versus a popular 300Å anion exchanger.

BioBasic 18 columns also demonstrate superior performance when compared to other wide-pore reversed phase HPLC columns. Compare the difference in resolution for a BioBasic 4 column and another 300Å C4 column. Figure 19 (shown on page 7) demonstrates the higher resolving power of the BioBasic 4 column for a complex protein mixture, showing an additional peak separated with baseline resolution.

Not Just for Large Molecules

BioBasic columns are designed to give superior results for the chromatography of proteins, peptides and biomolecules. However, BioBasic columns also give outstanding results for small molecule separations, whether by reversed phase or ion exchange. Please refer to pages 7-9 for chromatograms that demonstrate the usefulness of BioBasic columns for proteins, peptides, and oligonucleotides, as well as a number of small molecule applications.

Figure 17

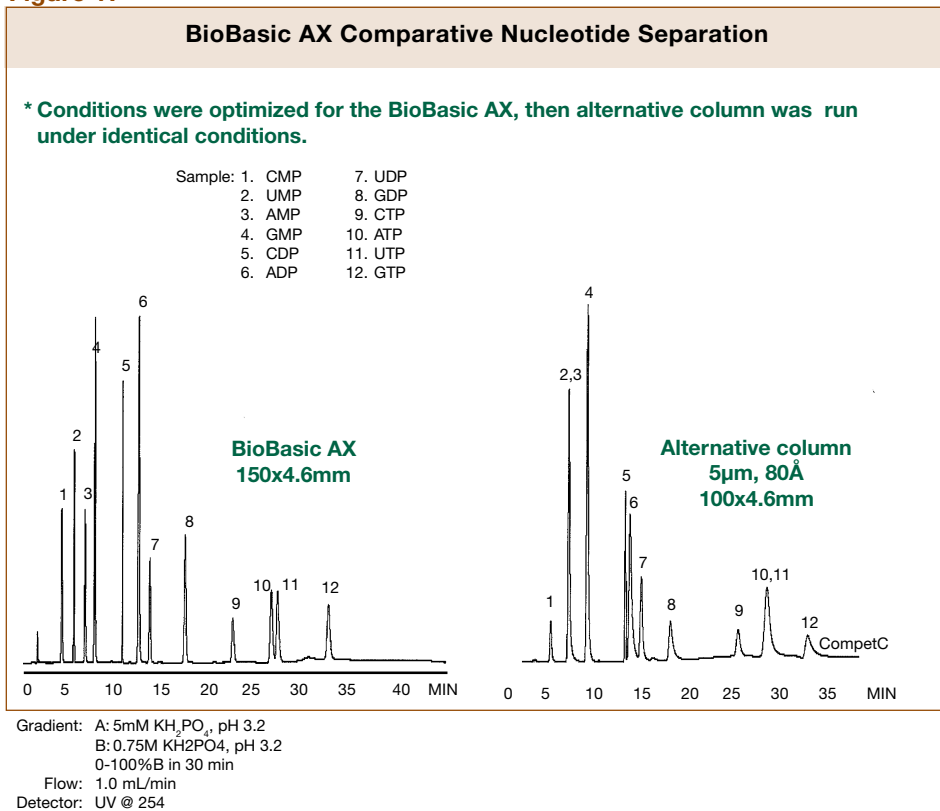


Figure 18

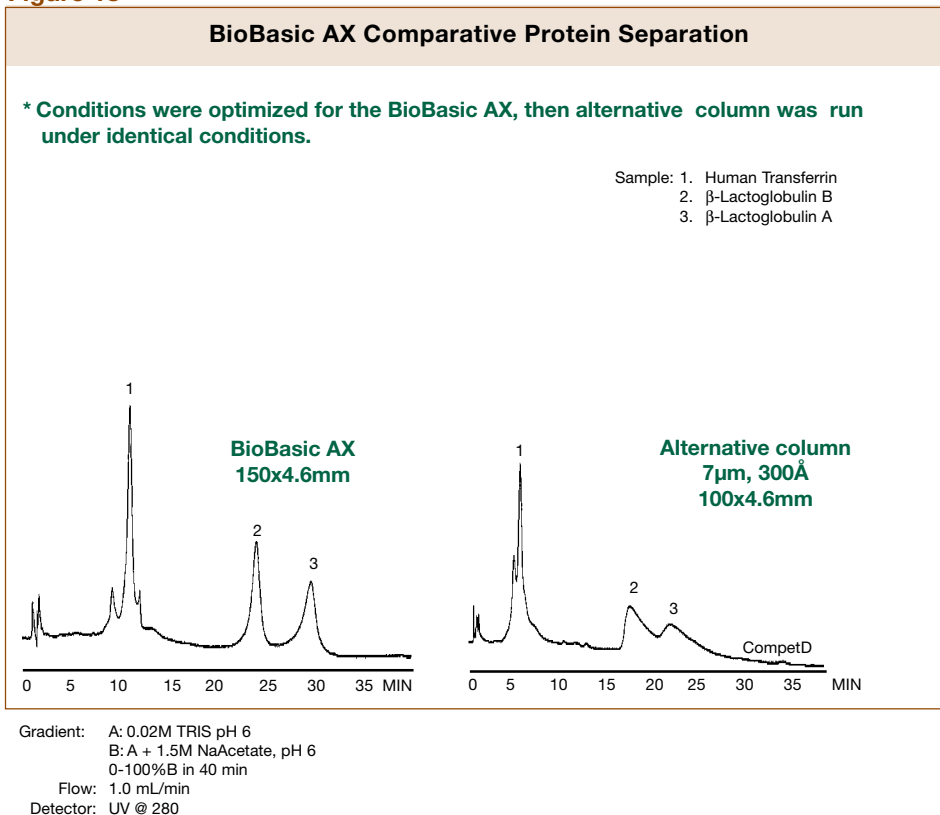
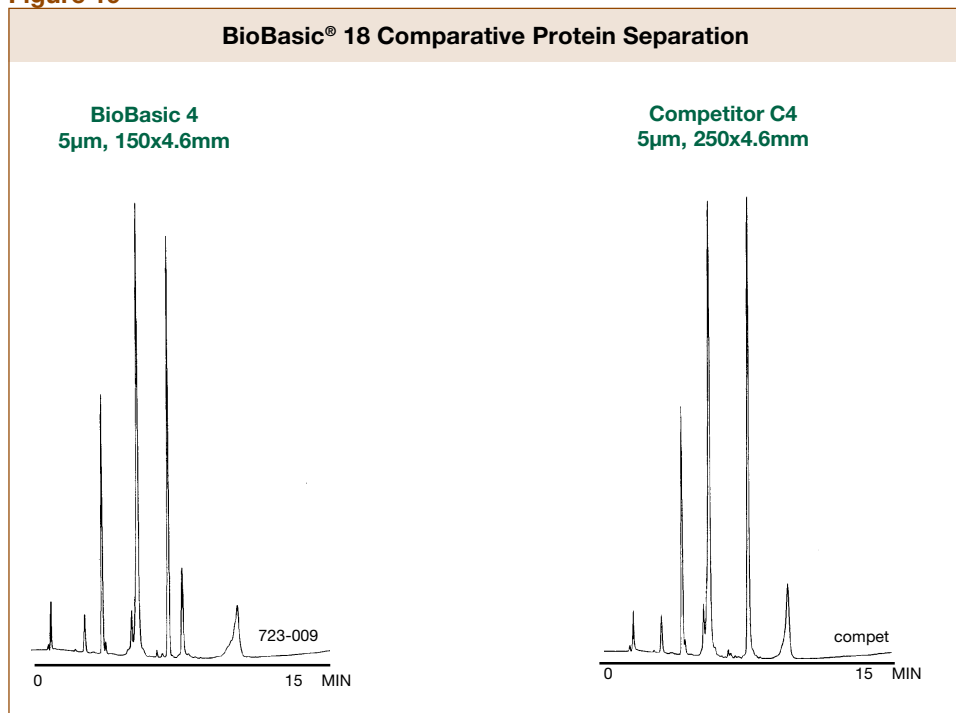
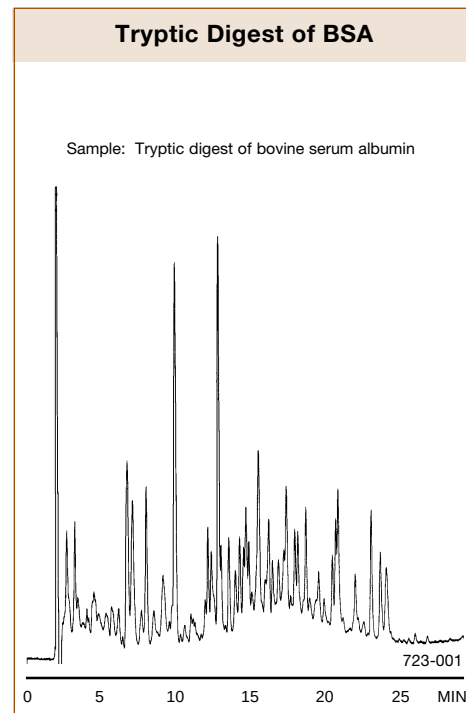


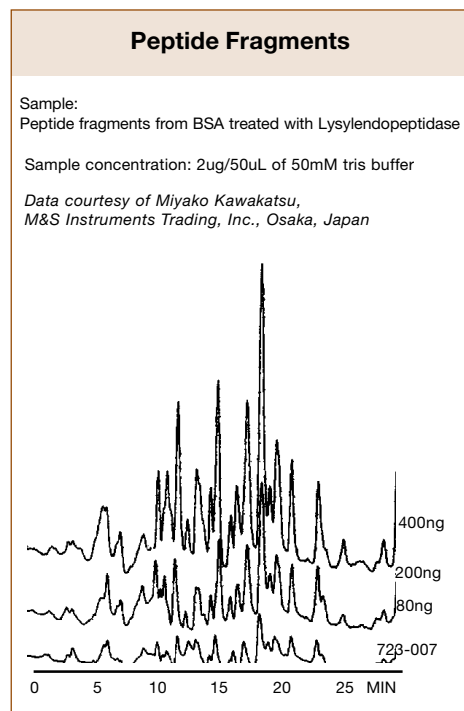
Figure 19



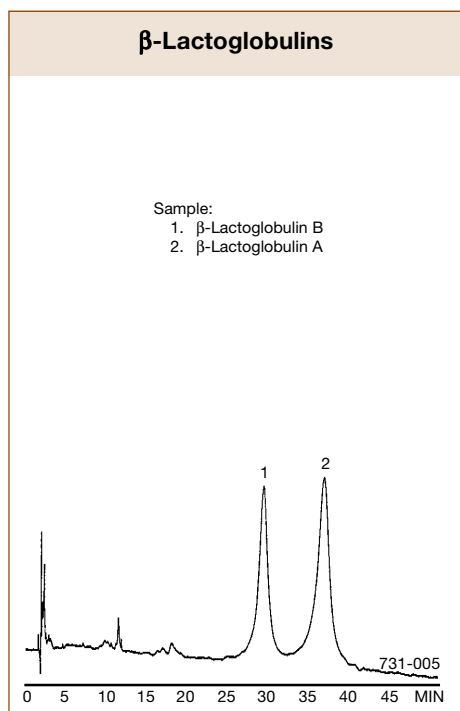
Gradient: A: 0.1% TFA in H₂O
B: 0.1% TFA in ACN
25%B to 100%B in 30 min
Flow: 1.5mL/min
Detector: UV @ 254
Sample: Protein Mix



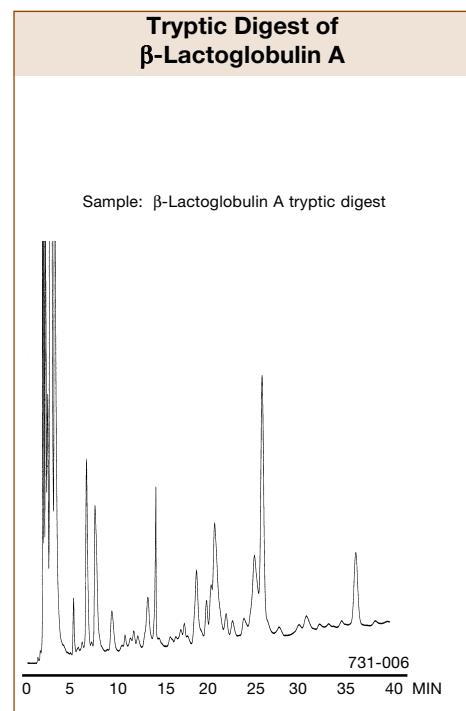
BioBasic 4, 5µm, 150x4.6mm
Gradient: A: 0.1% TFA in H₂O
B: 0.1% TFA in ACN
5% B - 50% B in 40 min.
Flow: 1.0 mL/min
Detector: UV @ 220



BioBasic 4, 5µm, 150x1mm
Gradient: A: 0.1% TFA in H₂O
B: 0.1% TFA in 59%ACN
5-100% to B in 30 min. Hold for 5 min.
Flow: 60 µL/min
Detector: UV@ 210
System: Gilson small bore HPLC



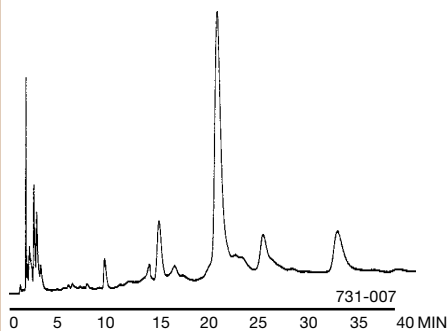
BioBasic AX, 5µm, 150x4.6mm
Gradient: A: 0.02M TRIS, pH 6
B: A + 1M NaAcetate, pH 6
0-100%B in 40 min.
Flow: 1.0 mL/min
Detector: UV @ 280



BioBasic AX, 5µm, 150x4.6mm
Gradient: A: 0.02M TRIS, pH 6
B: A + 1M NaAcetate, pH 6
0-100%B in 50 min.
Flow: 1.0 mL/min
Detector: UV @ 280

Tryptic Digest of Ovalbumin

Sample: Tryptic Digest of Ovalbumin



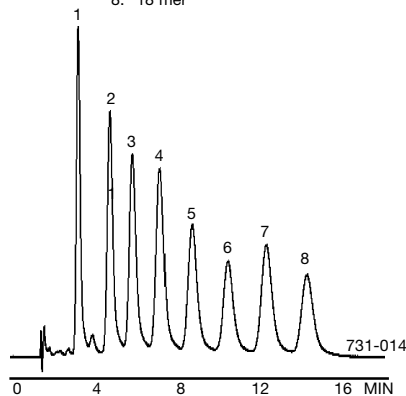
BioBasic® AX, 5µm, 150x4.6mm

Gradient: A: 0.02M TRIS, pH 6
B: A + 1M NaAcetate, pH 6
0-100%B in 50 min.
Flow: 1.0 mL/min
Detector: UV @ 280

Oligonucleotides

Sample: Oligothymidylic Acid

1. 10 mer
2. 12 mer
3. 13 mer
4. 14 mer
5. 15 mer
6. 16 mer
7. 17 mer
8. 18 mer



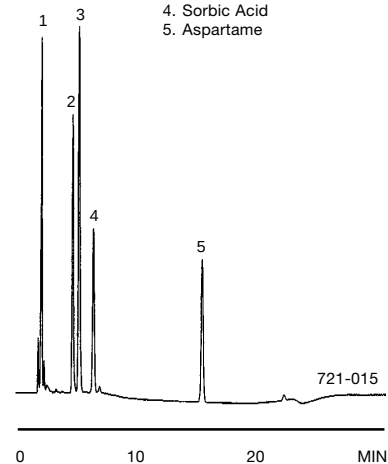
BioBasic AX, 5µm, 50x4.6mm

Gradient: A: 5mM KH_2PO_4 , pH 7.2
B: 0.15M KH_2PO_4 , pH 7.2
75-100%B in 15 min.
Flow: 0.4 mL/min
Detector: UV @ 265

Food Additives

Sample:

1. Uracil
2. Acesulfame
3. Benzoic Acid
4. Sorbic Acid
5. Aspartame



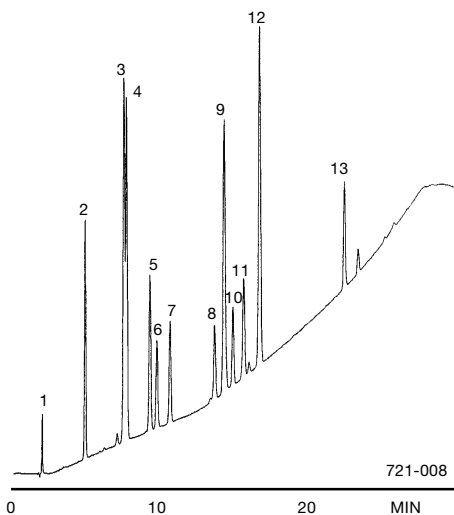
BioBasic 18, 5µm, 150x4.6mm

Gradient: A: 0.05M KH_2PO_4 , pH 6
B: MeOH
5% B to 50% B in 20 min.
Flow: 1 mL/min
Detector: UV @ 200

Aromatic Acids on BioBasic 18

Sample:

1. Uracil
2. Protocatechuic Acid
3. Caffeic Acid
4. p-Hydroxybenzoic Acid
5. Phthalic Acid
6. Vanillic Acid
7. Syringic Acid
8. Phenylacetic Acid
9. Benzoic Acid
10. Salicylic Acid
11. p-Coumaric Acid
12. Ferulic Acid
13. trans-Cinnamic Acid



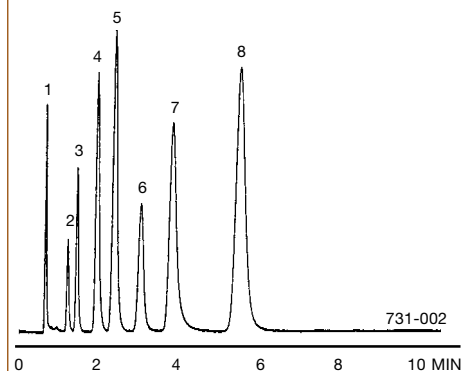
BioBasic 18, 5µm, 150x4.6mm

Gradient: A: 0.5M KH_2PO_4 , pH 3
B: MeOH
10%B to 60%B in 30 min.
Flow: 1.0 mL/min
Detector: UV @ 200

Aromatic Acids on BioBasic AX

Sample:

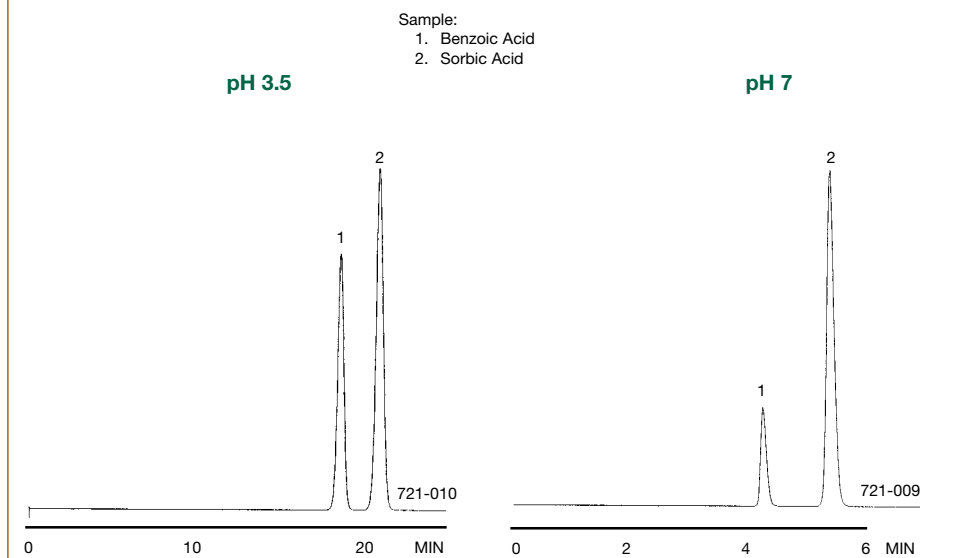
1. Uracil
2. Shikimic Acid
3. Ascorbic Acid
4. Phenylacetic Acid
5. Sorbic Acid
6. Benzoic Acid
7. Vanillic Acid
8. p-Coumaric Acid



BioBasic AX, 5µm, 50x4.6mm

Eluent: A: 70mM $\text{NH}_4\text{Acetate}$, pH 5.7
B: Acetonitrile
95%A / 5%B
Flow: 1.0 mL/min
Detector: UV @ 225

Small Organic Acids - Variation with pH



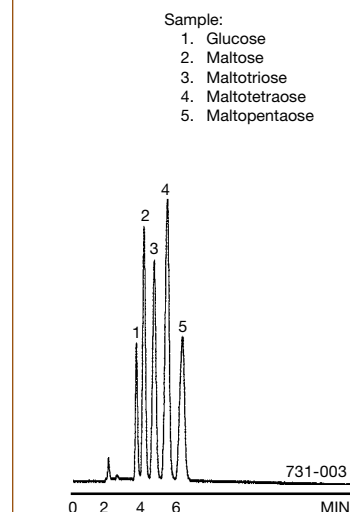
BioBasic® 18, 5µm, 150x4.6mm

Eluent: A: 80% 0.05M KH_2PO_4 , pH 3.5
B: 20% MeOH
Flow: 1.0 mL/min
Detector: UV @ 235

BioBasic 18, 5µm, 150x4.6mm

Eluent: A: 90% KH_2PO_4 in H_2O , pH 7
B: 10% MeOH
Flow: 1.0 mL/min
Detector: UV @ 235

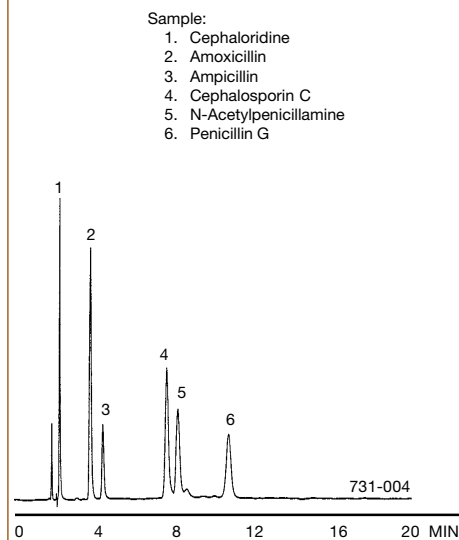
Sugars in HILIC Mode



BioBasic AX, 5µm, 150x4.6mm

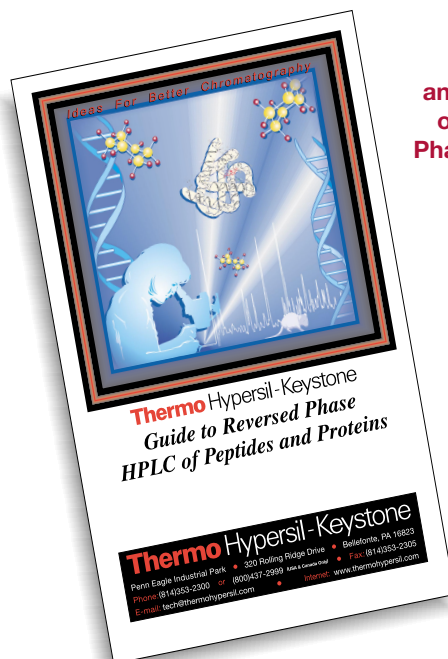
Eluent: 75% ACN / 25% H_2O
Flow: 1.0 mL/min
Detector: ELSD

Antibiotics



BioBasic AX, 5µm, 150x4.6mm

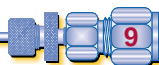
Eluent: A: 10mM $\text{NH}_4\text{Acetate}$
B: Acetonitrile
90%A / 10%B
Flow: 1.0 mL/min
Detector: UV @ 220
Temp. 35°C



For more information on protein and peptide chromatography, request our publication "Guide to Reversed Phase HPLC of Peptides and Proteins."

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