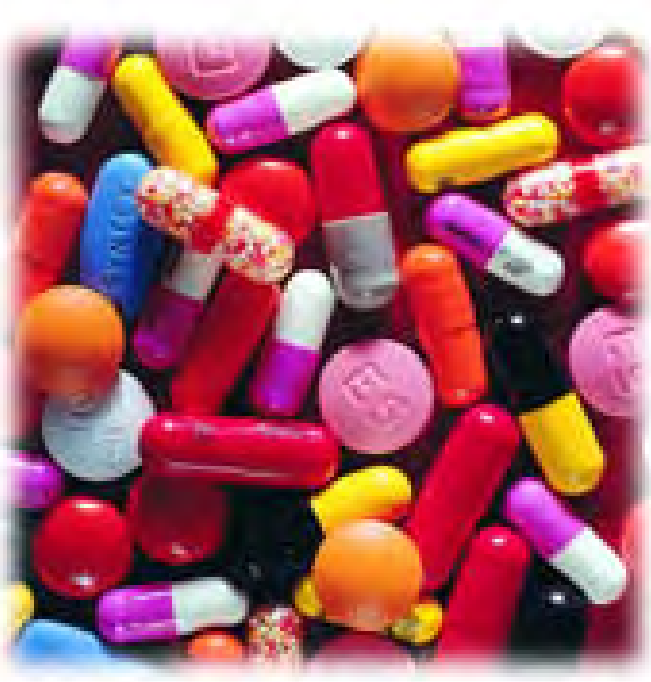
HAMILTON

HPLC COLUMNS

New, HxSil™ C18 and C8 HPLC Columns



A Word about HPLC Columns

For over 20 years Hamilton HPLC columns have been solving problems no other columns could. Now we have introduced HxSil silica-based reversed phase C18 and C8 HPLC columns to complement our selection of polymer-based columns. At Hamilton we believe that your separation requirements dictate the type of column needed. Whether it's a silica-based or polymer-based reversed phase column we are ready to help. Call us at ***800-648-5950*** *for expert technical assistance and FREE method development.*

Retention

Hamilton HxSil C18 columns exhibit greater retention than most columns. This allows you to separate compounds that are not sufficiently retained on other C18 columns. Figures 1 and 2 illustrate the separation of polycyclic aromatic hydrocarbons (PAH's) on an HxSil C18 column versus a Nova-Pak® C18 column of the same length. The flow rate has been reduced for the Nova-Pak C18 column to compensate for the smaller I.D. The Hamilton HxSil C18 column provides about twice the retention for these compounds (23 minute run time versus 11 minutes). The greater retention of the HxSil C18 column provides better separation of these PAH compounds. With the HxSil C18 column, peaks 2 through 6 (naphthalene, acenaphthylene, acenaphthene, phenanthrene, anthracene) are baseline resolved. The Nova-Pak C18 column does not fully separate peaks 2 and 3; peaks 5 and 6 are only partially resolved. Peaks 9 and 10 are better separated on the Hamilton HxSil C18 column.

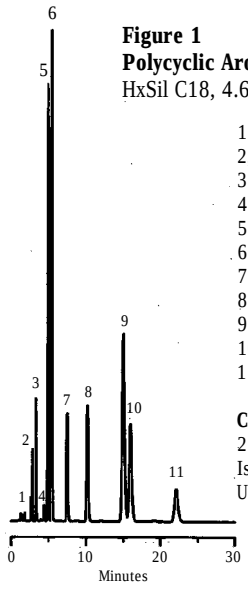


Figure 1
Polycyclic Aromatic Hydrocarbons (PAH's)
HxSil C18, 4.6 x 150 mm, 5 µm (P/N 79868)

1. Benzene 50 ppm
2. Naphthalene 50 ppm
3. Acenaphthylene 113 ppm
4. Acenaphthene 38 ppm
5. Phenanthrene 50 ppm
6. Anthracene 50 ppm
7. Pyrene 50 ppm
8. Chrysene 50 ppm
9. Benzo[b]fluoranthene 50 ppm
10. Benzo[k]fluoranthene 50 ppm
11. 1,2,5,6-Dibenzanthracene 50 ppm

Conditions: 7:3 Acetonitrile : Water.
2 mL/min, 50 µL,
Isocratic. Ambient.
UV at 254 nm.

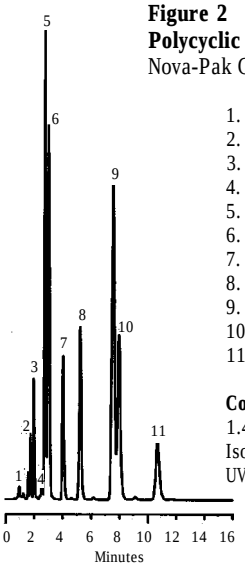


Figure 2
Polycyclic Aromatic Hydrocarbons (PAH's)
Nova-Pak C18, 3.9 x 150 mm, 5 µm

1. Benzene 50 ppm
2. Naphthalene 50 ppm
3. Acenaphthylene 113 ppm
4. Acenaphthene 38 ppm
5. Phenanthrene 50 ppm
6. Anthracene 50 ppm
7. Pyrene 50 ppm
8. Chrysene 50 ppm
9. Benzo[b]fluoranthene 50 ppm
10. Benzo[k]fluoranthene 50 ppm
11. 1,2,5,6-Dibenzanthracene 50 ppm

Conditions: 7:3 Acetonitrile : Water.
1.4 mL/min, 50 µL,
Isocratic. Ambient.
UV at 254 nm.

Selectivity

Each manufacturer's reversed phase column is different. Choosing the best column to separate your sample can be difficult. Hamilton manufactures both silica-based and polymeric reversed phase HPLC columns, providing you with a wide range of column retention selectivities and performance benefits. The applications which follow, demonstrate the performance characteristics of Hamilton silica-based reversed phase columns.

Small Molecules

Figures 3 and 4 compare Hamilton HxSil C18 and Inertsil® ODS-3 columns of the same length and particle size. The HxSil C18 column provides slightly longer retention (1 minute), and better separation of peaks 7 and 8 (amylbenzene and o-terphenyl). On the Inertsil ODS-3 column these two compounds coelute.

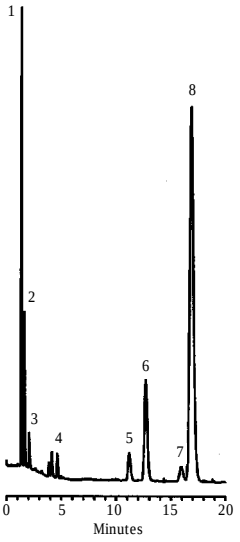


Figure 3
Eight Small Molecules
HxSil C18, 4.6 x 150 mm,
5 µm (P/N 79868)

1. Uracil 10 ppm
2. Caffeine 10 ppm
3. Phenol 10 ppm
4. Toluene 10 ppm
5. n-Butylbenzene 100 ppm
6. Triphenylene 10 ppm
7. Amylbenzene 100 ppm
8. o-Terphenyl 10 ppm

Conditions: 7:3 Acetonitrile :
Water. 1 mL/min, 50 µL,
Isocratic. Ambient.
UV at 254 nm.

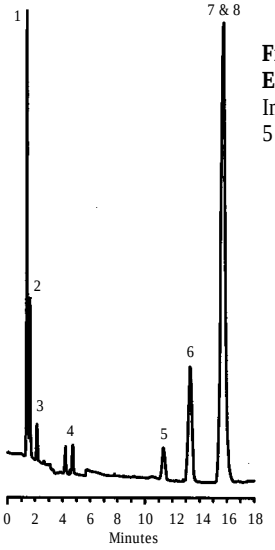


Figure 4
Eight Small Molecules
Inertsil ODS-3, 4.6 x 150 mm,
5 µm

1. Uracil 10 ppm
2. Caffeine 10 ppm
3. Phenol 10 ppm
4. Toluene 10 ppm
5. n-Butylbenzene 100 ppm
6. Triphenylene 10 ppm
7. Amylbenzene 100 ppm
8. o-Terphenyl 10 ppm

Conditions: 7:3 Acetonitrile :
Water. 1 mL/min, 50 µL,
Isocratic. Ambient.
UV at 254 nm.

USP Methods

The Hamilton HxSil C18 column meets the requirements of a USP L1 column and the HxSil C8 column meets the requirements for an L7 column. Figure 5 illustrates the analysis of tetracycline.

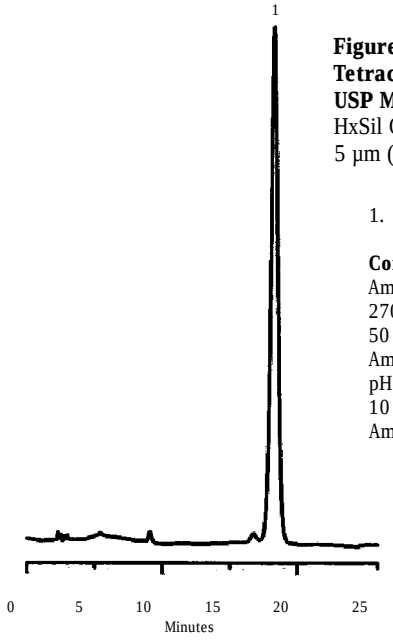


Figure 5
Tetracycline by USP Method
HxSil C18, 4.6 x 250 mm, 5 µm (P/N 79869)

1. Tetracycline 0.5 mg/mL

Conditions: 680 mL 0.1N Ammonium Oxalate, 270 mL Dimethylformamide, 50 mL 0.2 M Dibasic Ammonium Phosphate pH 7.6. 1 mL/min, 10 µL, Isocratic. Ambient. UV at 280 nm.

Figure 6 demonstrates the separation of folic acid from methotrexate on a HxSil C18 "L1" column following the USP 24 recommended conditions.

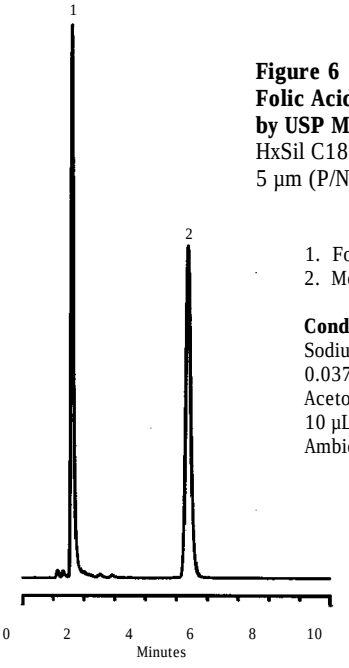


Figure 6
Folic Acid and Methotrexate by USP Method
HxSil C18, 4.6 x 150 mm, 5 µm (P/N 79868)

1. Folic Acid 100 µg/mL
2. Methotrexate 100 µg/mL

Conditions: 9:1 0.126 M Sodium Phosphate, dibasic, 0.037 M Citric Acid pH 6.0 : Acetonitrile. 1.2 mL/min, 10 µL, Isocratic. Ambient. UV at 302 nm.

Antibiotics

The separation of oxytetracycline and tetracycline using an HxSil C8 column is shown in Figure 7.

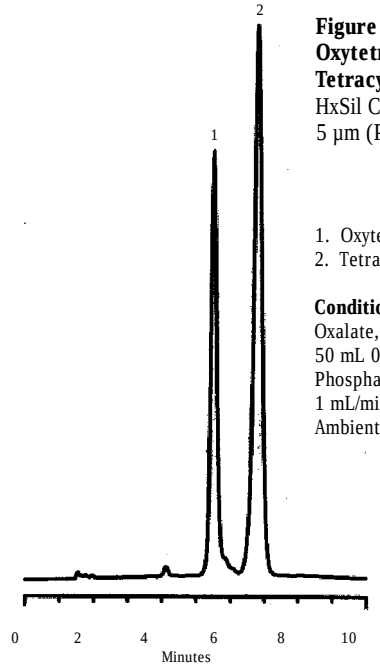


Figure 7
Oxytetracycline and Tetracycline
HxSil C8, 4.6 x 150 mm, 5 µm (P/N 79102)

1. Oxytetracycline 0.5 mg/mL
2. Tetracycline 0.5 mg/mL

Conditions: 680 mL 0.1N Ammonium Oxalate, 270 mL Dimethylformamide, 50 mL 0.2 M Dibasic Ammonium Phosphate pH 7.6. 1 mL/min, 20 µL, Isocratic. Ambient. UV at 280 nm.

Anticonvulsants

Three anticonvulsants and one antianxiety drug are separated in a 12 minute gradient run with only an acetonitrile, water mobile phase (see Figure 8). All four peaks have excellent symmetry, narrow band widths and good resolution.

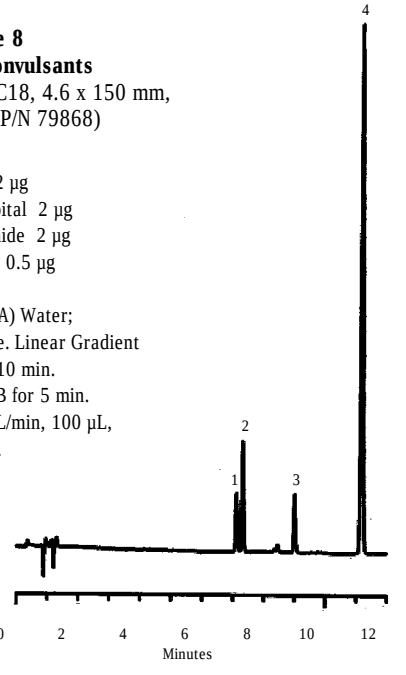


Figure 8
Anticonvulsants
HxSil C18, 4.6 x 150 mm, 5 µm (P/N 79868)

1. Ethotoin 2 µg
2. Phenobarbital 2 µg
3. Methsuximide 2 µg
4. Diazepam 0.5 µg

Conditions: A) Water; B) Acetonitrile. Linear Gradient 20-60% B in 10 min. Hold at 60% B for 5 min. Ambient. 1 mL/min, 100 µL, UV at 254 nm.

Meclizine in Motion Sickness Pills

In Figure 9 the antiemetic, meclizine is separated from excipients in motion sickness pills. This amine base drug exhibits excellent sym - metry (tailing factor of 1.061) on a Hamilton HxSil C18 column.

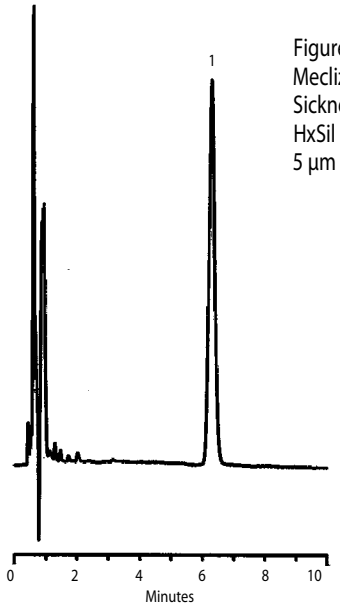


Figure 9
Meclizine in Motion Sickness Pills
HxSil C18, 4.6 x 150 mm, 5 µm (P/N 79868)

1. Meclizine 1 mg/mL

Conditions: 9:1 Methanol : 20 mM Potassium Hydrogen Phosphate pH 7.0. 2 mL/min, 5 µL, Isocratic. Ambient. UV at 254 nm.

Diphenhydramine in Itch Relief Gel

In Figure 10 the antihistamine diphenhydramine is separated in just over 2 minutes. Easy sample preparation is characterized by water dilution, and filtration. As in Figure 9, the compound of interest (diphenhydramine) is an amine base.

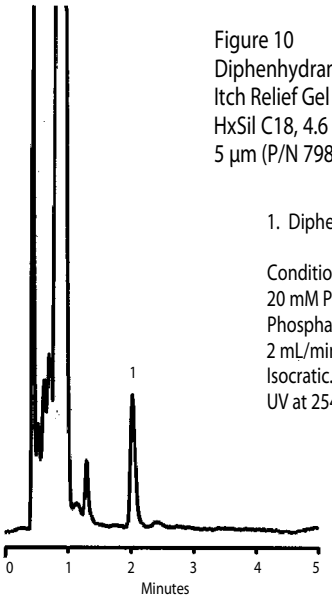


Figure 10
Diphenhydramine in Itch Relief Gel
HxSil C18, 4.6 x 150 mm, 5 µm (P/N 79868)

1. Diphenhydramine 10 ppm

Conditions: 9:1 Methanol : 20 mM Potassium Hydrogen Phosphate pH 7.0. 2 mL/min, 5 µL, Isocratic. Ambient. UV at 254 nm.

Steroids

Four steroids are separated on a Hamilton HxSil C18 (Figure 11) and C8 (Figure 12) column of the same length. Excellent selectivity is demonstrated by both columns (baseline separation of all four steroids). Two of the steroids, 4-androstene-3,17-dione (peak 2) and testosterone (peak 3) differ only by the oxidation state at the C-17 position; a carbonyl and hydroxyl group respectively. Note the reduced retention with the C8 column. 4.5 minute run time versus 7.5 with the C18.

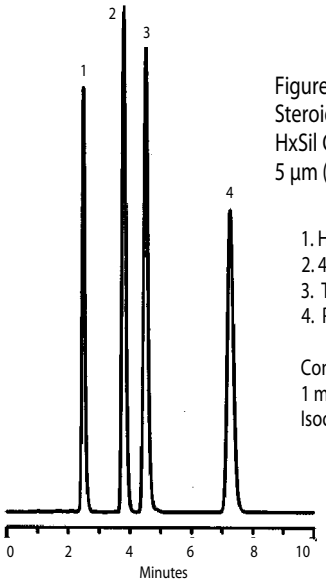


Figure 11
Steroids
HxSil C18, 4.6 x 150 mm, 5 µm (P/N 79868)

1. Hydrocortisone 250 ppm
2. 4-Androstene-3,17-dione 250 ppm
3. Testosterone 250 ppm
4. Progesterone 250 ppm

Conditions: 8:2 Methanol : Water . 1 mL/min, 20 uL, Isocratic. Ambient. UV at 240 nm.

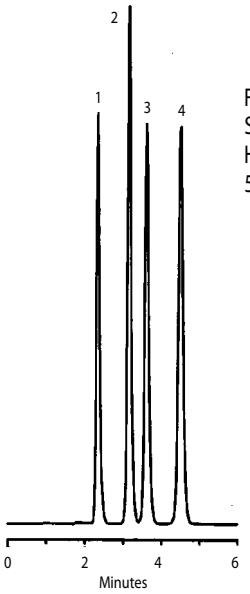


Figure 12
Steroids
HxSil C8, 4.6 x 150 mm, 5 µm (P/N 79102)

1. Hydrocortisone 250 ppm
2. 4-Androstene-3,17-dione 250 ppm
3. Testosterone 250 ppm
4. Progesterone 250 ppm

Conditions: 8:2 Methanol : Water . 1 mL/min, 20 µL, Isocratic. Ambient. UV at 240 nm.

Proteins

Hamilton Company offers four reversed phase column packings for the separation of proteins: two silica-based packings (HxSil C18 and HxSil C8) and two polymer-based packings (PRP®-1 and PRP-Infinity). In Figures 13 through 15 seven proteins are separated using the same conditions. In Figure 16 the protein mixture is a little different and the flow rate is optimized (increased) for the nonporous PRP-Infinity column.

The HxSil silica-based C18 and C8 columns show better resolution than the polymer-based PRP-1 and -Infinity columns for protein separations. The polymeric PRP-1 and -Infinity columns are used for protein purification when column cleaning/chemical sterilization is needed. Cleaning with acid or base does not harm these polymeric columns. The PRP-Infinity column (Figure 16) is well suited for the fast (less than 2 minute) separation of proteins.

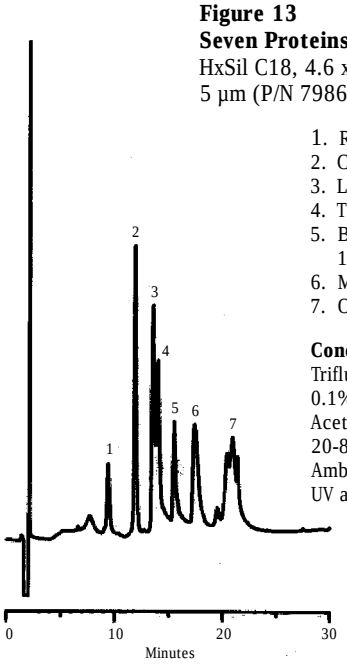


Figure 13
Seven Proteins
HxSil C18, 4.6 x 150 mm,
5 µm (P/N 79868)

1. Ribonuclease A 1mg/mL
2. Cytochrome c 1 mg/mL
3. Lysozyme 1 mg/mL
4. Transferrin 1 mg/mL
5. Bovine Serum Albumin 1 mg/mL
6. Myoglobin 1 mg/mL
7. Ovalbumin 1 mg/mL

Conditions: A) 0.1% Trifluoroacetic Acid in Water; B) 0.1% Trifluoroacetic Acid in Acetonitrile. Linear Gradient 20-80% B in 33.3 min. Ambient. 1 mL/min, 30 µL, UV at 215 nm.

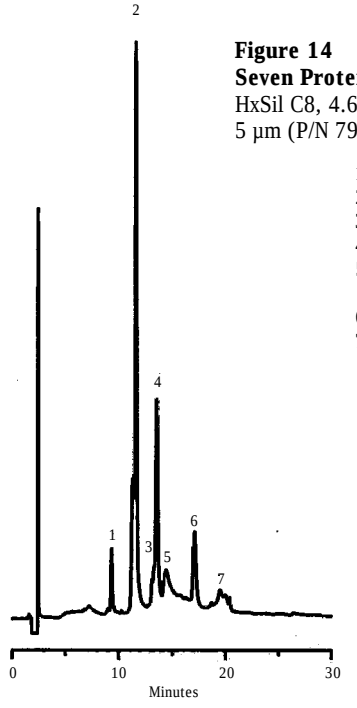


Figure 14
Seven Proteins
HxSil C8, 4.6 x 150 mm,
5 µm (P/N 79102)

1. Ribonuclease A 1mg/mL
2. Cytochrome c 1 mg/mL
3. Lysozyme 1 mg/mL
4. Transferrin 1 mg/mL
5. Bovine Serum Albumin 1 mg/mL
6. Myoglobin 1 mg/mL
7. Ovalbumin 1 mg/mL

Conditions: A) 0.1% Trifluoroacetic Acid in Water; B) 0.1% Trifluoroacetic Acid in Acetonitrile. Linear Gradient 20-80% B in 33.3 min. Ambient. 1 mL/min, 30 µL, UV at 215 nm.

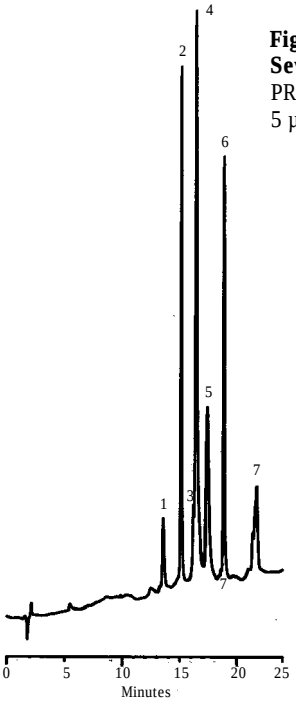


Figure 15
Seven Proteins
PRP-1, 4.1 x 150 mm,
5 µm (P/N 79444)

1. Ribonuclease A 1 mg/mL
2. Cytochrome c 1 mg/mL
3. Lysozyme 1 mg/mL
4. Transferrin 1 mg/mL
5. Bovine Serum Albumin 1 mg/mL
6. Myoglobin 1 mg/mL
7. Ovalbumin 1 mg/mL

Conditions: A) 0.1% Trifluoroacetic Acid in Water; B) 0.1% Trifluoroacetic Acid in Acetonitrile. Linear Gradient 20-80% B in 33.3 min. Ambient. 1 mL/min, 30 µL, UV at 215 nm.

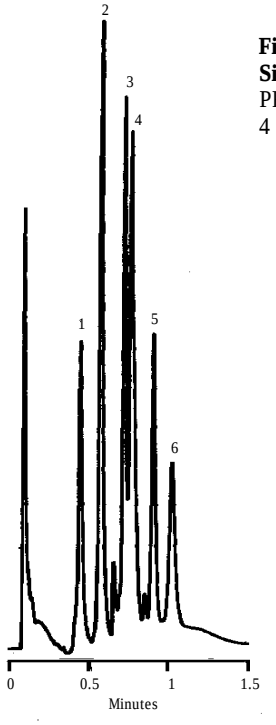


Figure 16
Six Proteins
PRP-Infinity, 4.1 x 30 mm,
4 µm (P/N 79470)

1. Ribonuclease A 1mg/mL
2. Cytochrome c 1 mg/mL
3. Transferrin 1 mg/mL
4. Bovine Serum Albumin 1 mg/mL
5. Concanavalin A 1 mg/mL
6. Ovalbumin 1 mg/mL

Conditions: A) 0.1% Trifluoroacetic Acid in Water; B) 0.1% Trifluoroacetic Acid in Acetonitrile. Linear Gradient 20-60% B in 1.2 min. Ambient. 2 mL/min, 20 µL, UV at 215 nm.

Peptides

Figures 17 through 19 illustrate the separation of a five component decapeptide standard on three Hamilton reversed phase columns.

The peptide standard contains the following five decapeptides;

1. NH_2-Arg-Gly-Ala-Gly-Gly-Leu-Gly-Leu-Gly-Lys-amide
2. Acetyl-Arg-Gly-Gly-Gly-Gly-Leu-Gly-Leu-Gly-Lys-amide
3. Acetyl-Arg-Gly-Ala-Gly-Gly-Leu-Gly-Leu-Gly-Lys-amide
4. Acetyl-Arg-Gly-Val-Gly-Gly-Leu-Gly-Leu-Gly-Lys-amide
5. Acetyl-Arg-Gly-Val-Val-Gly-Leu-Gly-Leu-Gly-Lys-amide

It is useful to note the unique selectivity of the PRP-1 polymeric, poly(styrene-divinylbenzene) HPLC column. It completely separates all five peptide standards. PRP-1 polymeric columns have the added advantage of pH stability from 0 to 14, and they are pressure stable to 5,000 psi. The HxSil C18 and C8 columns separate three of the standards and only partially resolve the first two peptide standards. None of the C18 silica columns tried (Discovery®, Vydac®, and Nova-Pak®) separated all five peptide standards.

Figure 17
Five Peptide Standards
PRP-1, 4.1 x 150 mm,
5 µm (P/N 79444)

Conditions: A) 0.1% Trifluoroacetic Acid; B) 50% Acetonitrile, 0.09% Trifluoracetic Acid, Linear Gradient 0-100% B in 50 min. Ambient. 1 mL /min, 100 µL, UV at 215 nm.

Figure 18
Five Peptide Standards
HxSil C18, 4.6 x 150 mm,
5 µm (P/N 79868)

Conditions: A) 0.1% Trifluoroacetic Acid; B) 50% Acetonitrile, 0.09% Trifluoracetic Acid, Linear Gradient 0-100% B in 50 min. Ambient. 1 mL /min, 100 µL, UV at 215 nm.

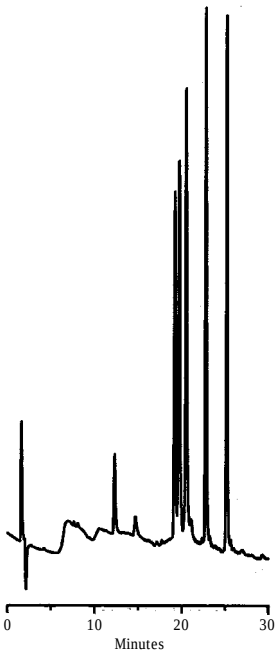


Figure 19
Five Peptide Standards
HxSil C8, 4.6 x 150 mm,
5 µm (P/N 79102)

Conditions: A) 0.1% Trifluoroacetic Acid; B) 50% Acetonitrile, 0.09% Trifluoracetic Acid, Linear Gradient 0-100% B in 50 min. Ambient. 1 mL /min, 100 µL, UV at 215 nm.

Technical Details

Hamilton HxSil C18 columns are functionalized monomerically with octadecyldimethylchlorosilane and end capped with trimethylchlorosilane. C8 columns are functionalized with octyldimethylchlorosilane and end capped with trimethylchlorosilane.

Efficiency

The tangent method is used to determine column efficiency. This method is common to a variety of column manufacturers and is easily performed by the user. Typical efficiencies are on the order of >10,000 plates per column for a 150 mm long column.

Reproducibility and Symmetry

HxSil C18 and C8 columns are manufactured to provide reproducible retention of your compound. Tight manufacturing controls and extensive characterization of the base silica enable us to manufacture C18 columns with reproducible retention. Just look at the results of four batches of HxSil C18.

% Carbon	16.6	17.6	17.9	17.9
µmol ligand/m^2	3.0	3.1	3.2	3.2
Particle Size	5 µm	5 µm	5 µm	3 µm

Test Probes

Each batch of HxSil C18 and C8 support is tested to determine retention and tailing of six model compounds. This mixture developed by Steffeck *et al*.[1] is used to determine the suitability of the C18 bonded phase and behavior of the underlying silica toward neutral, hydrophobic, acidic and basic compounds. The tailing factor for each compound is measured at 5% of peak height (more demanding than measurment at 10% of peak height).

The purpose of each probe is as follows:

Uracil is a void volume marker.

Pyridine is a basic compound used to test the activity of silanols toward bases.

Phenol is an acidic compound used along with pyridine to determine the underlying silica activity.

N,N-Dimethylaniline is a second basic compound used to determine the activity of residual silanols toward bases.

4-Butylbenzoic Acid is a second acidic compound used to test the activity of residual silanols toward acids.

Toluene tests the hydrophobicity of the column.

Both the Hamilton C18 (Figure 20) and C8 (Figure 21) columns separate all six test probes without a tailing peak (a tailing peak is defined as a peak with a tailing factor greater than 2.0). This demonstrates the suitability of Hamilton HxSil C18 and C8 columns for the analysis of acids, bases and neutral compounds. Of the 86 columns tested in the Steffeck *et al.* paper, 53 (62%) of the columns had 1 or more of the test probes listed as a tailing peak.

The HPLC Performance Report for each lot of HxSil C18 and C8 support includes information for the parameters listed below.

Silica Lot Number
Particle Size dv 50
Particle Distribution dv 90/10
Pore Volume
Silica Specific Surface Area
Metals
- Sodium
- Iron
- Aluminum

Bonded Phase Lot Number
Ligand
Carbon Loading %
µmol Ligand/m^2
Capping Agent

Hamilton Company's new HxSil silica-based reversed phase C18 and C8 HPLC columns provide greater retention of compounds than other C18 and C8 columns. This allows you to separate compounds that are not adequately retained on other reversed phase columns. When you combine HxSil columns with our selection of polymer-based reversed phase columns you have a wide range of column retention selectivity, and performance characteristics from which to choose.

At Hamilton we believe that your separation requirements dictate the type of column needed. Whether it's a silica-based or polymer-based reversed phase column we are ready to help. Call us at **800-648-5950** for expert technical assistance and FREE method development. We understand that sometimes the only way to know if an HPLC column will separate your compound is to see your sample run on that column.

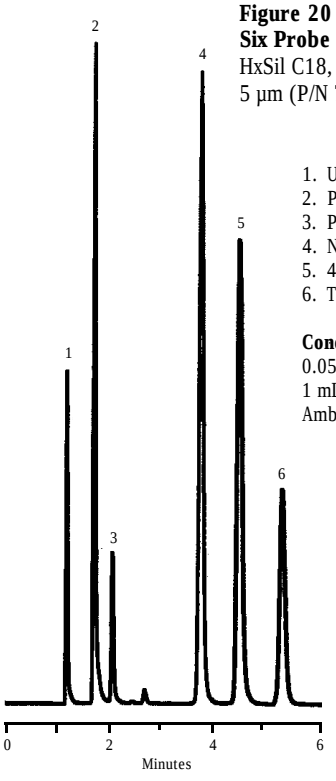


Figure 20
Six Probe Test Mix
HxSil C18, 4.6 x 150 mm,
5 µm (P/N 79868)

1. Uracil
2. Pyridine
3. Phenol
4. N,N-Dimethylaniline
5. 4-Butylbenzoic Acid
6. Toluene

Conditions: 65:35 Acetonitrile : 0.05M Sodium Citrate pH 3.15. 1 mL /min, 10 µL, Isocratic. Ambient. UV at 254 nm.

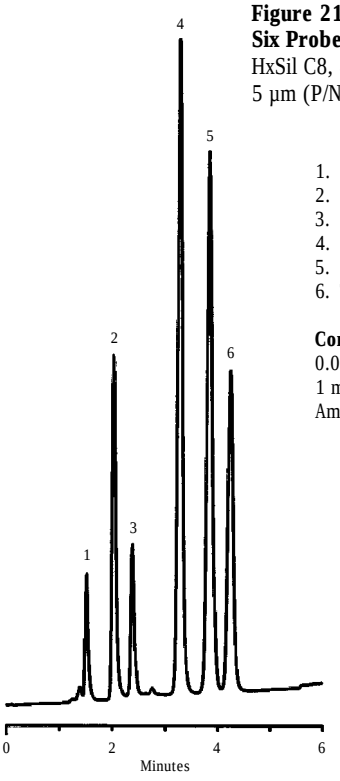


Figure 21
Six Probe Test Mix
HxSil C8, 4.6 x 150 mm,
5 µm (P/N 79102)

1. Uracil
2. Pyridine
3. Phenol
4. N,N-Dimethylaniline
5. 4-Butylbenzoic Acid
6. Toluene

Conditions: 65:35 Acetonitrile : 0.05M Sodium Citrate pH 3.15. 1 mL /min, 10 µL, Isocratic. Ambient. UV at 254 nm.

http://www.hamiltoncompany.com/hplc
Sales/Support USA 1-888-525-2123

References
1. Robert J. Steffeck, Susan L. Woo, Raymond J. Weigland and James M. Anderson, "A Comparison of Silica-Based C18 and C8 HPLC Columns to Aid Column Selection" LG* GC 13, 9 (1995): 720-726.

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