

AQUASIL 18 Columns

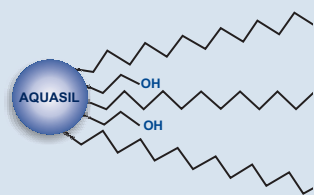
TG01-01



Compatible with 100% aqueous mobile phase

Analyze • Detect • Measure • Control™

Thermo
ELECTRON CORPORATION



AQUASIL C18 columns provide a versatile C18 phase for a wide range of application areas. AQUASIL C18 columns have a hydrophilic endcapping that gives up to twice the retention for polar compounds and alternative selectivity compared to traditional C18 phases. AQUASIL C18 columns are also compatible with 100% aqueous mobile phases without loss of performance.

- Exhibits different retention & selectivity than conventional C18
- Excellent peak shapes for basic, acidic and neutral compounds
- Retains polar molecules twice as strongly as conventional C18
- Compatible with 100% aqueous mobile phase
- Excellent results with low buffer concentrations
- Stable for LC/MS applications

Applications

- Highly polar compounds
- Nucleosides and Nucleotides
- Organic acids
- Vitamins
- Peptides
- Catecholamines



Specifications:

Phase	Particle Size	Carbon Load	Pore Size	Silica Type
AQUASIL C18	3 and 5µm	12%	100Å	High purity, base deactivated

AQUASIL C18 columns combine a C18 phase with polar endcapping to provide a unique material for reversed phase chromatography, offering alternative selectivity, increased retention of polar compounds, and no phase collapse under 100% aqueous conditions. These columns maintain selectivity with reduced concentrations of buffers and additives, making them ideal for use with LC/MS. In this Technical Guide we review the AQUASIL C18 packing, which has been tailored to go beyond the limitations of traditional C18 packing materials.

Chromatographic Characterization

Packings that offer additional modes of interaction give rise to quite different retention behavior and selectivity. In general, analytes with the greatest polar functionality will typically show the greatest changes in selectivity and retention. Figure 1 illustrates the difference in behavior between the AQUASIL C18 column and the BetaBasic® 18 column (a highly base deactivated and densely bonded C18 column that has a very similar percent carbon value).

Figure 1a demonstrates that where analyte interactions are based purely on hydrophobic (or dispersive) interactions, the AQUASIL C18 column is slightly less retentive than the BetaBasic 18 column. The AQUASIL C18 packing was designed for the reversed phase separation of polar molecules. Despite its relatively high concentration of C18 groups, it also has hydrophilic sites that help to provide increased retention of highly polar water soluble compounds (Figure 1b,1c). Figure 1b shows how the AQUASIL C18 column offers the nearly twice the retention for several polar, basic compounds when compared to a BetaBasic 18 column.

The retention of basic compounds and also polar acidic compounds on the AQUASIL C18 column are both significantly increased compared to the BetaBasic 18 column. This illustrates clearly how useful the AQUASIL C18 column can be when increased retention of polar compounds or alternative selectivity is required.

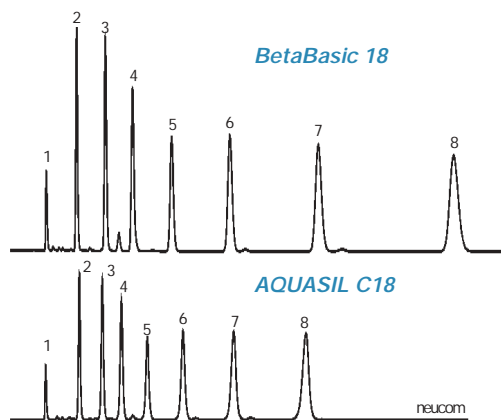
Increased Retention of Polar Compounds

Polar compounds often elute near or at the unretained marker when run on typical C18 HPLC columns. AQUASIL C18 columns provide additional analyte–ligand interactions to reversed phase hydrophobic interactions, leading to increased retention of analytes with polar functionality. AQUASIL C18 columns maintain retention of neutral compounds while offering increased retention for both acidic and basic compounds.

Figure 1

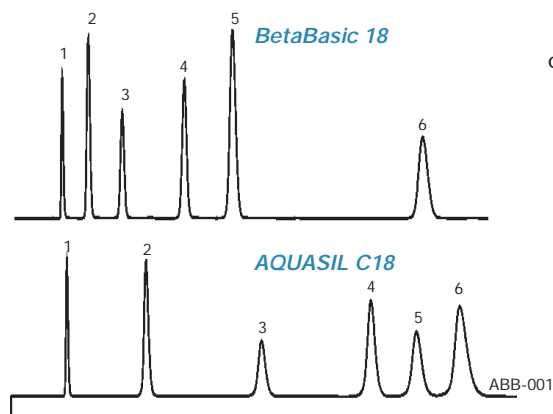
**Chromatographic Retention Behavior
AQUASIL C18 vs BetaBasic 18 Columns**

a) Less retentive for neutral compounds



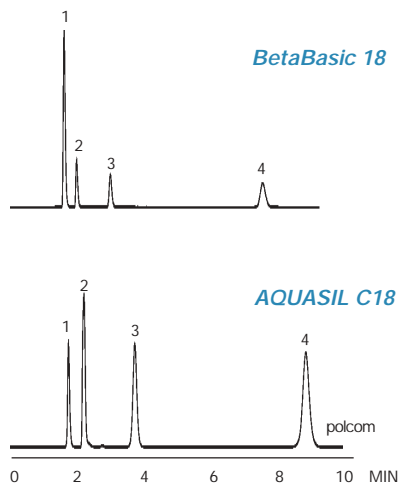
Columns: 5 μ m, 150x4.6mm
 Eluent: 25% H₂O / 75% ACN
 Flow: 1.25 mL/min
 Detector: UV @ 254
 Sample: 1. Uracil
 2. Benzene
 3. Ethylbenzene
 4. Propylbenzene
 5. Butylbenzene
 6. Pentylbenzene
 7. Hexylbenzene
 8. Heptylbenzene

b) More retentive for basic compounds



Columns: 5 μ m, 150x4.6mm
 Eluent: 90% 50mM KH₂PO₄,
 pH 3.5 / 10% ACN
 Flow: 1.25 mL/min
 Detector: UV@ 254
 Sample: 1. Uracil
 2. Procainamide
 3. N-Acetylprocainamide
 4. Caffeine
 5. N-Propionylprocainamide
 6. Phenol

c) More retentive for polar acidic compounds



Columns: 5 μ m, 150x4.6mm
 Eluent: 80% 0.1% Formic Acid / 20% ACN
 Flow: 1.0 mL/min
 Detector: UV @ 254
 Sample: 1. Uracil
 2. Phloroglucinol
 3. Resorcinol
 4. Phenol

Dispersive interactions are the primary interactions generally associated with retention on traditional alkyl C18 type packings. Secondary interactions associated with residual silanols have been significantly reduced by endcapping, improvements in silica quality and increased density of the derivatized ligand. Silanol interactions that previously gave rise to broad tailing peaks for basic analytes have therefore, to some extent, been eliminated. These secondary interactions are also responsible in part for the retention of compounds with polar functionality, either by hydrogen bonding interactions or via ion exchange interactions. The progressive elimination of the secondary silanol interactions has resulted in columns that give



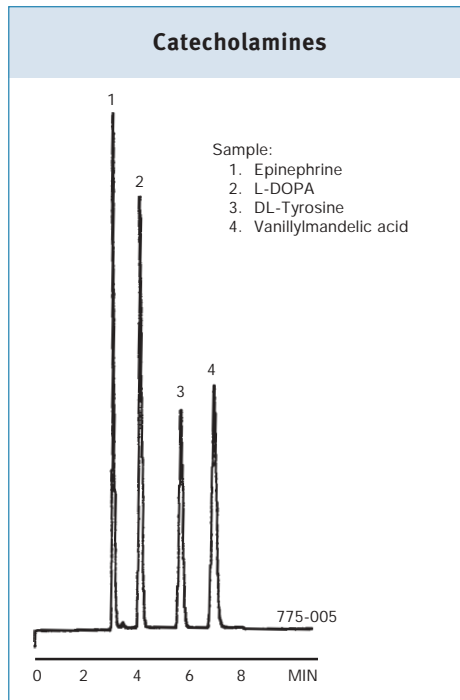
good peak shape for basic compounds but reduced retention of polar compounds in general. AQUASIL C18 columns provide an excellent combination of traditional reversed phase interactions and polar interactions to retain more polar analytes. Figure 2 demonstrates the capability of the AQUASIL C18 column to separate catecholamines without ion pair reagents in a highly aqueous mobile phase.

Highly Aqueous Mobile Phases

The inclusion of polar functionality to the stationary phase also increases the wetting characteristics of the packing in highly aqueous mobile phases. The AQUASIL C18 column can be run in 100% aqueous mobile phase conditions and shows no tendency towards phase collapse (Figures 3 and 4). Phase collapse is often seen with C18 packings unless a small amount of

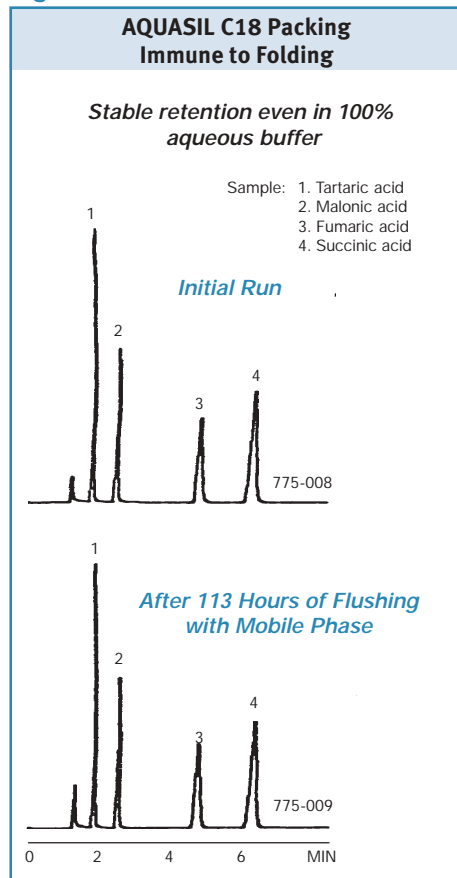
organic solvent (1-5%) is added to the mobile phase. As a result of phase collapse, the retention and selectivity of the phase is lost and the column must be regenerated using a pure organic solvent wash. The AQUASIL C18 packing is immune to this folding due its unique polar functionality. Refer to Technical Bulletin TB99-01 for further explanation of phase collapse.

Figure 2



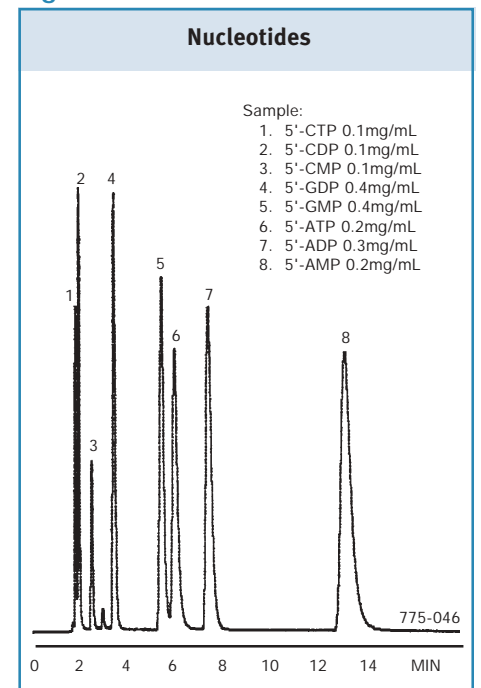
AQUASIL C18, 5 μ m, 150x4.6mm
 Eluent: 98% 0.1M KH_2PO_4 , pH 3.0
 with H_3PO_4 / 2% ACN
 Flow: 1.0 mL/min
 Detector: UV @ 210

Figure 3



AQUASIL C18, 5 μ m, 150x4.6mm
 Eluent: 0.05M KH_2PO_4
 +0.03M H_3PO_4 , pH 2.5
 Flow: 1.25 mL/min
 Detector: UV @ 210

Figure 4



AQUASIL C18, 5 μ m, 150x4.6mm
 Eluent: 0.1M KH_2PO_4 , pH 6.0 with KOH
 Flow: 1.0 mL/min
 Detector: UV @ 260

Reduced Buffer Concentrations and Increased MS Sensitivity

At any concentration, additives such as trifluoroacetic acid can cause ion suppression and consequently reduce sensitivity in LC/MS methods. The choice of HPLC column is of key importance for LC-MS applications, since the properties of the bonded stationary phase and underlying silica can strongly influence the concentration of additive required.

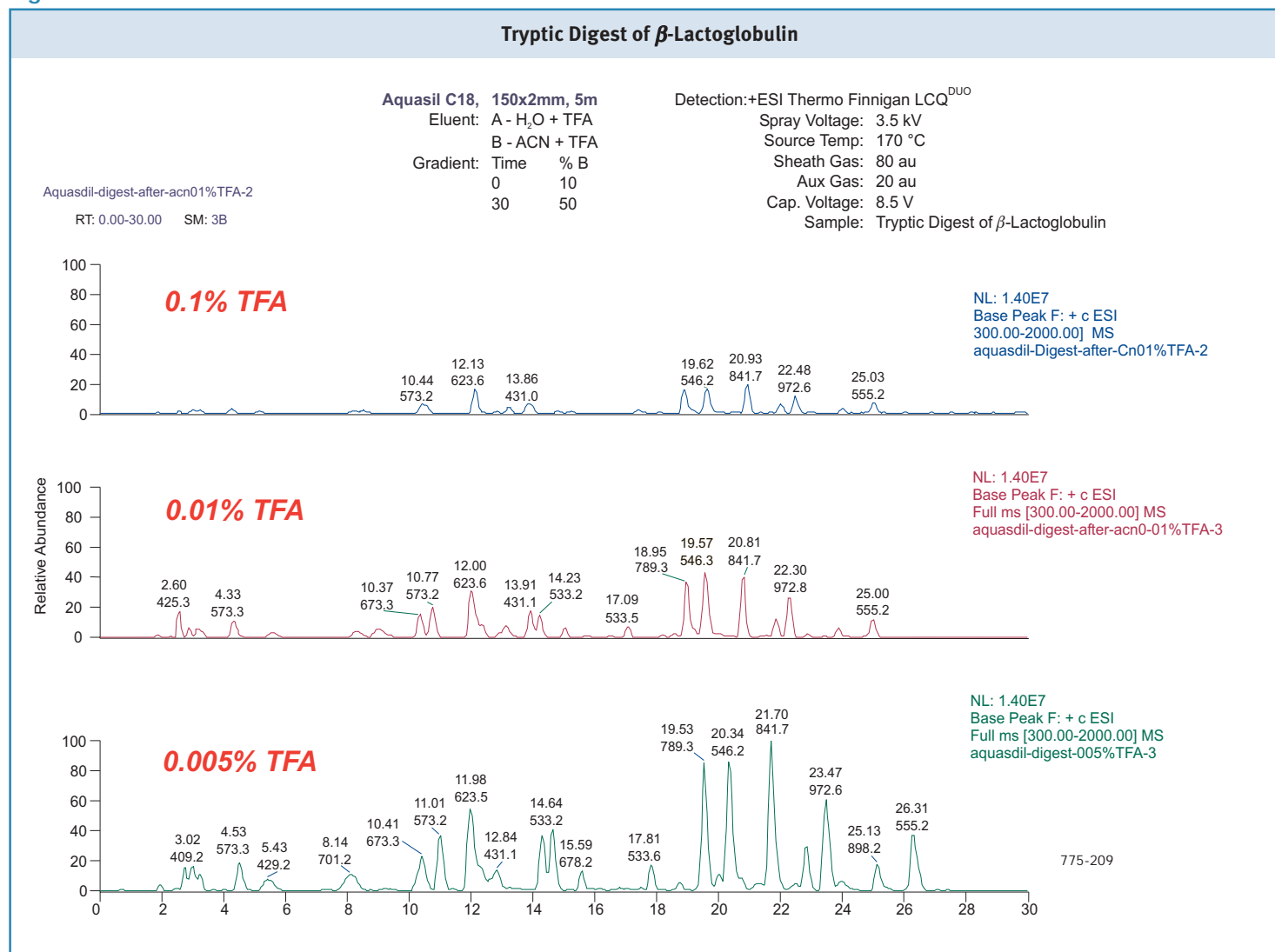
It is good practice to use volatile mobile phase additives in LC/MS methods to

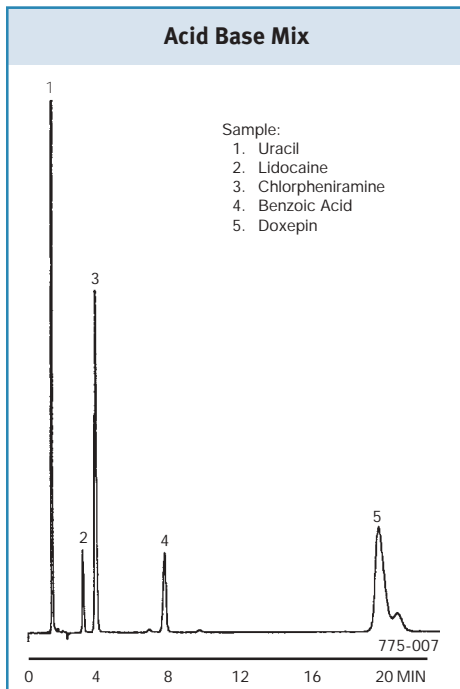
enhance ionization. The addition of acidic modifiers such as formic acid or TFA is commonplace when analyzing proteins and peptides by reversed phase chromatography. The additive solvates the analyte, displacing water molecules and creating a more hydrophobic analyte with stronger retention on traditional C18 packings.

The AQUASIL C18 packing can retain peptides in their water soluble state, reducing the need for TFA as an additive. The examples shown in Figure 5 illustrate how the AQUASIL C18 column can be used

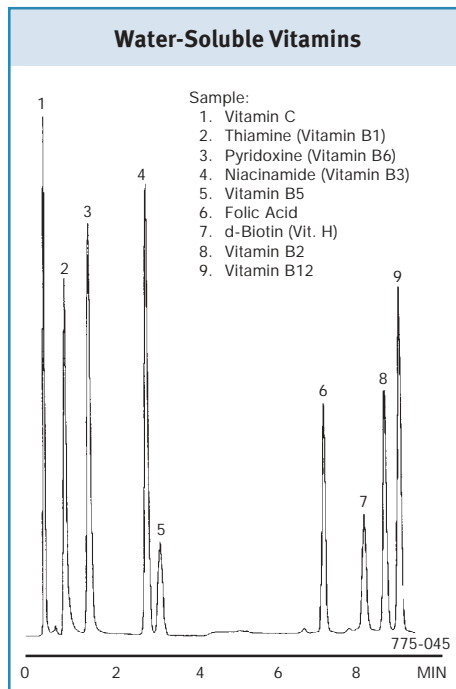
at very low TFA concentrations while maintaining retention and performance of many of the peptides of interest. Low level additive concentrations (Figure 5C) also offer the reward of increased sensitivity for MS. This is an important consideration when trying to identify trace quantities of a drug compound or impurity that may normally disappear into the noise of the baseline of the MS response.

Figure 5

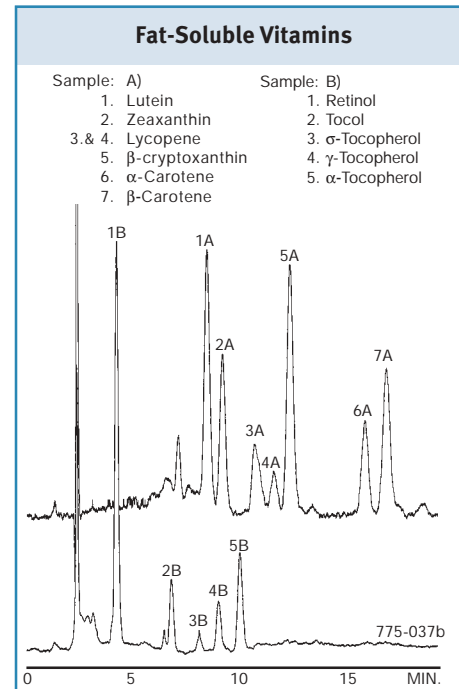




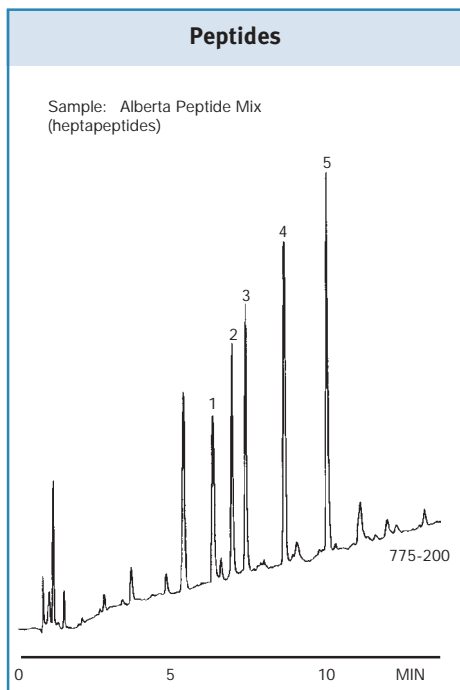
AQUASIL C18, 5 μ m, 150x4.6mm
 Eluent: A: 0.05M KH_2PO_4 + 0.03M H_3PO_4 , pH 2.47
 B: ACN
 75% A / 25% B
 Flow: 1.25 mL/min
 Detector: UV @ 254



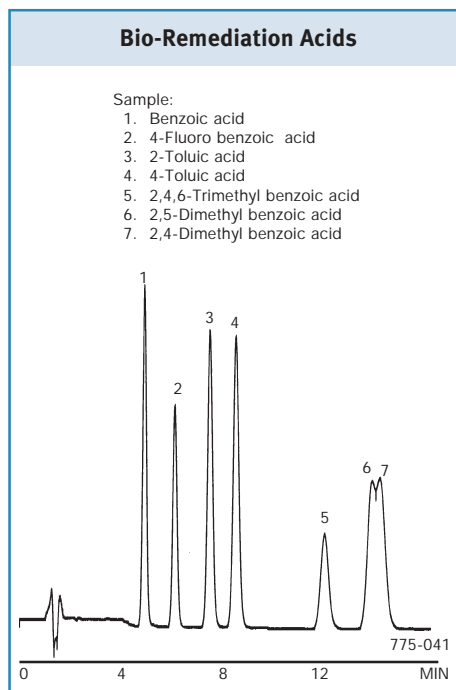
AQUASIL C18, 5 μ m, 50x4.6mm
 Gradient: A: 0.5M K_2HPO_4 , pH 3.5
 B: 50% ACN / 50% 0.05M K_2HPO_4 , pH 3.5
 4% B to 40% B in 9 min
 Flow: 1.0 mL/min
 Detector: UV @ 205



AQUASIL C18, 5 μ m, 250x4.6mm
 Eluent: 83%ACN / 16%Dioxane/1%MeOH
 Flow: 1.5 mL/min
 Detector: chromatogram A: UV@450 nm
 chromatogram B: UV@325/300 nm
 Data courtesy of Neal Craft, Craft Technologies, Inc.

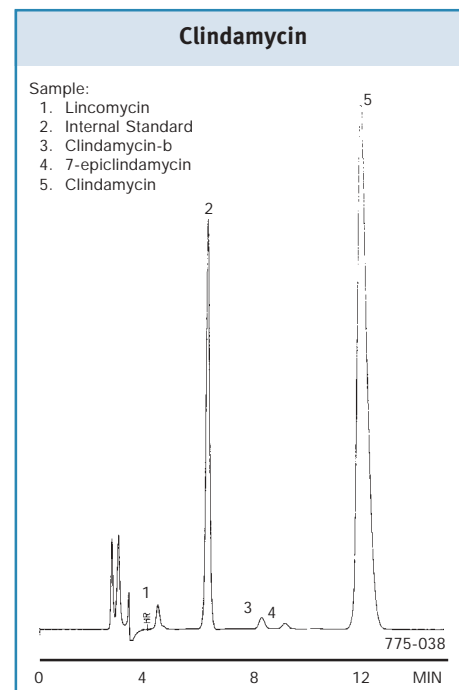


AQUASIL C18, 3 μ m, 100x4.6mm
 Gradient: A: 0.1% TFA in H_2O
 B: 0.1% TFA in ACN
 10%B to 50%B in 20 min.
 Flow: 1.0 mL/min
 Detector: UV @ 210



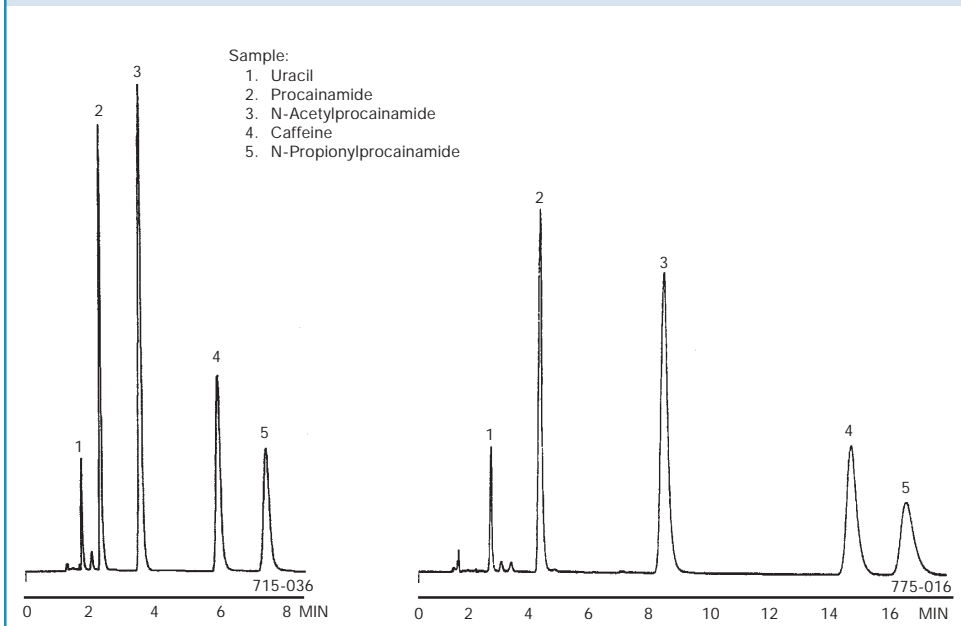
AQUASIL C18, 5 μ m, 150x4.6mm
 Eluent: 50% MeOH / 50% 0.025M KH_2PO_4 , pH 5.2
 Flow: 1.5 mL/min
 Detector: UV @ 230
 Temp: 22.0 +/- 0.2°C

Data courtesy of James D. Stuart and Reena M. Joseph,
 Department of Chemistry, Univ of Connecticut, Storrs, CT



AQUASIL C18, 5 μ m, 250x4.6mm
 Eluent: A: MeOH
 B: 4g/L dl-10-camphorsulfonic acid,
 2g/L NH_4 acetate, 0.2% glacial acetic acid.
 Adjust to pH 6 with HCL or NaOH solution.
 60% A / 40% B
 Flow: 1.0 mL/min
 Detector: RI

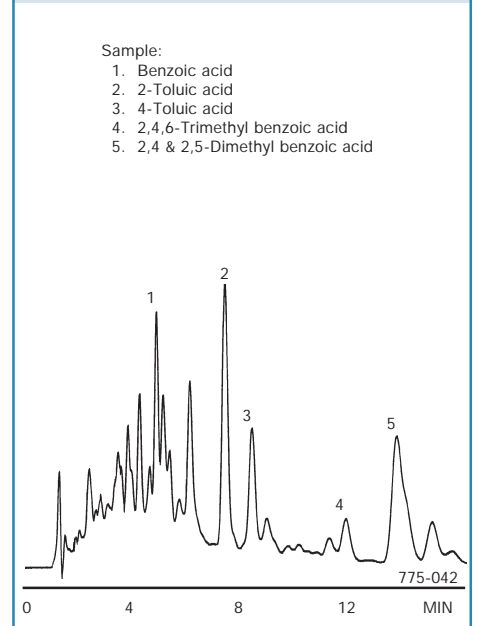
Procainamides Comparison



BetaBasic 18, 5µm, 150x4.6mm
 Eluent: 10% ACN / 90% 0.05M KH₂PO₄, pH 3.5
 Flow: 1.25 mL/min
 Detector: UV @ 254

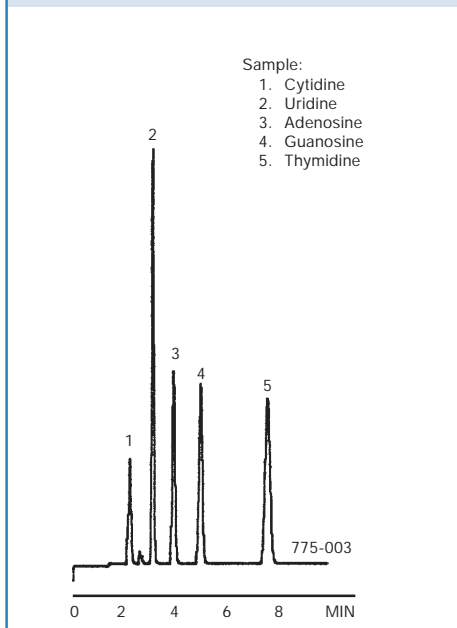
AQUASIL C18, 5µm, 150x4.6mm
 Eluent: 10% ACN / 90% 0.05M KH₂PO₄, pH 3.5
 Flow: 1.25 mL/min
 Detector: UV @ 254

Ground Water Extract



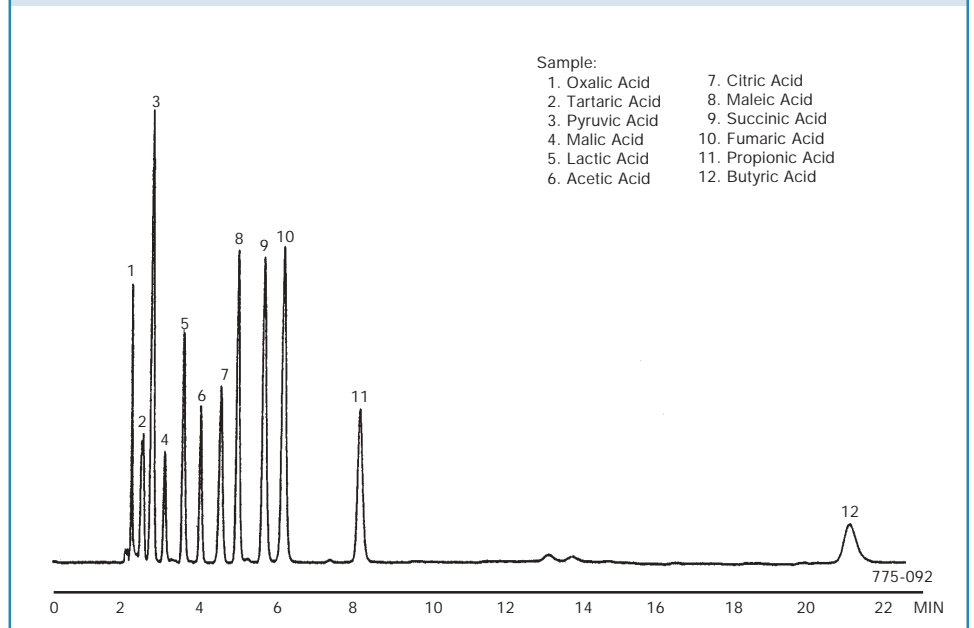
AQUASIL C18, 5µm, 150x4.6mm
 Eluent: 50% MeOH / 50% 0.025M KH₂PO₄, pH 2.5
 Flow: 1.5 mL/min
 Detector: UV @ 230
 Temp: 22°C

Nucleosides



AQUASIL C18, 5µm, 150x4.6mm
 Eluent: A: 0.05M KH₂PO₄, & 0.03M H₃PO₄, pH 2.4
 B: H₂O
 C: ACN
 47.5% A / 47.5% B / 5% C
 Flow: 1.0 mL/min
 Detector: UV @ 260

Organic Acids in Highly Aqueous Mobile Phase



AQUASIL C18, 5µm, 250x4.6mm
 Eluent: 1% ACN / 99% 0.05M KH₂PO₄, pH 2.8
 Flow: 1.25 mL/min
 Detector: UV @ 210

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AQUASIL™ Siliconizing Fluid for treating glass surfaces is sold by PierceChemical Co., Rockford, IL

AQUASIL C18 Standard Columns



Description	Particle Size(µm)	Length (mm)	Standard bore (4.6 mm)	Standard bore (4.0 mm)	Small bore (3.0 mm)	Small bore (2.1 mm)	Microbore (1.0 mm)
AQUASIL C18	3	20	77503-024630	77503-024030	77503-023030	77503-022130	77503-021030
	3	30	77503-034630	77503-034030	77503-033030	77503-032130	77503-031030
	3	50	77503-054630	77503-054030	77503-053030	77503-052130	77503-051030
	3	100	77503-104630	77503-104030	77503-103030	77503-102130	77503-101030
	3	125	77503-124630	77503-124030	77503-123030	77503-122130	77503-121030
	3	150	77503-154630	77503-154030	77503-153030	77503-152130	77503-151030
AQUASIL C18	5	30	77505-034630	77505-034030	77505-033030	77505-032130	77505-031030
	5	50	77505-054630	77505-054030	77505-053030	77505-052130	77505-051030
	5	100	77505-104630	77505-104030	77505-103030	77505-102130	77505-101030
	5	125	77505-124630	77505-124030	77505-123030	77505-122130	77505-121030
	5	150	77505-154630	77505-154030	77505-153030	77505-152130	77505-151030
	5	200	77505-204630	77505-204030	77505-203030	77505-202130	77505-201030
	5	250	77505-254630	77505-254030	77505-253030	77505-252130	77505-251030

Standard Columns with Integral Guard (COLUMNPLUS Guard, or CPG)



Description	Particle Size(µm)	Length (mm)	Standard bore (4.6 mm)	Standard bore (4.0 mm)	Small bore (3.0 mm)	Small bore (2.1 mm)	Microbore (1.0 mm)
AQUASIL C18	3	50	77503-054631	77503-054031	77503-053031	77503-052131	77503-051031
	3	100	77503-104631	77503-104031	77503-103031	77503-102131	77503-101031
	3	125	77503-124631	77503-124031	77503-123031	77503-122131	77503-121031
	3	150	77503-154631	77503-154031	77503-153031	77503-152131	77503-151031
AQUASIL C18	5	50	77505-054631	77505-054031	77505-053031	77505-052131	77505-051031
	5	100	77505-104631	77505-104031	77505-103031	77505-102131	77505-101031
	5	125	77505-124631	77505-124031	77505-123031	77505-122131	77505-121031
	5	150	77505-154631	77505-154031	77505-153031	77505-152131	77505-151031
	5	200	77505-204631	77505-204031	77505-203031	77505-202131	77505-201031
	5	250	77505-254631	77505-254031	77505-253031	77505-252131	77505-251031

Drop-In Guard Cartridges for UNIGUARD Holder and CPG Columns (pk/4)



NOTE: 4.0mm drop-ins are used for both 4.0 and 4.6mm analytical columns.

Description	Particle Size(µm)	Length (mm)	Standard bore (for 4.6mm)	Standard bore (4.0 mm)	Small bore (3.0 mm)	Small bore (2.1 mm)	Microbore (1.0mm)
AQUASIL C18	3	10	77503-014001	77503-014001	77503-013001	77503-012101	77503-011001
	5	10	77505-014001	77505-014001	77505-013001	77505-012101	77505-011001
UNIGUARD Direct-Connect Drop-in Guard Cartridge Holder for use with standard columns		10	850-00	850-00	852-00	852-00	851-00



Javelin Guard Columns



Available in 10mm and 20mm lengths and NEW 1.0mm ID!

Description	Particle Size(µm)	Length (mm)	Standard bore (4.0 mm)	Small bore (3.0 mm)	Small bore (2.1 mm)	Microbore (1.0 mm)
AQUASIL C18	5	10	77505-014006	77505-013006	77505-012106	77505-011006
	5	20	77505-024006	77505-023006	77505-022106	77505-021006