Technical Guide Version 2

Thermo Scientific AQUASIL C18 HPLC Columns

Exceptional Chromatography of Polar Compounds

Part of Thermo Fisher Scientific

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Thermo Scientific AQUASIL C18 HPLC Columns

Introduction

The Thermo Scientific AQUASIL C18 columns provide a versatile C18 phase for a wide range of applications.

Combining a C18 phase with a hydrophilic end-capping, Aquasil C18 columns offer a unique material for reversed phase chromatography, offering alternative selectivity, up to twice the retention for polar compounds, and no phase collapse under 100% aqueous conditions. Ideal for use with LC/MS, these columns maintain selectivity with reduced concentrations of buffers and additives.

In this Technical Guide, we review the AQUASIL C18 packing, designed to go beyond the limitations of traditional C18 packing materials, including:

- **Different retention and selectivity to conventional C18**
- **Excellent peak shapes for basic, acidic and neutral compounds**
- **Polar molecule retention twice as strong as conventional C18**
- **Compatible with 100% aqueous mobile phase**
- **Excellent results with low buffer concentrations**
- **Stable for LC/MS applications**

Chromatographic Characterization

Packings that offer additional interaction modes give rise to quite different retention behavior and selectivity. Additionally, analytes with the greatest polar functionality will typically show significant changes in selectivity and retention.

Figure 1 illustrates the behavior differences between the AQUASIL C18 column and the Thermo Scientific BetaBasic 18 column (a C18 column that is highly base deactivated and densely bonded and also has a very similar percent carbon value).

Figure 1a demonstrates that the AQUASIL C18 column is slightly less retentive than the BetaBasic™ 18 column where analyte interactions are based purely on hydrophobic (or dispersive) interactions. 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).

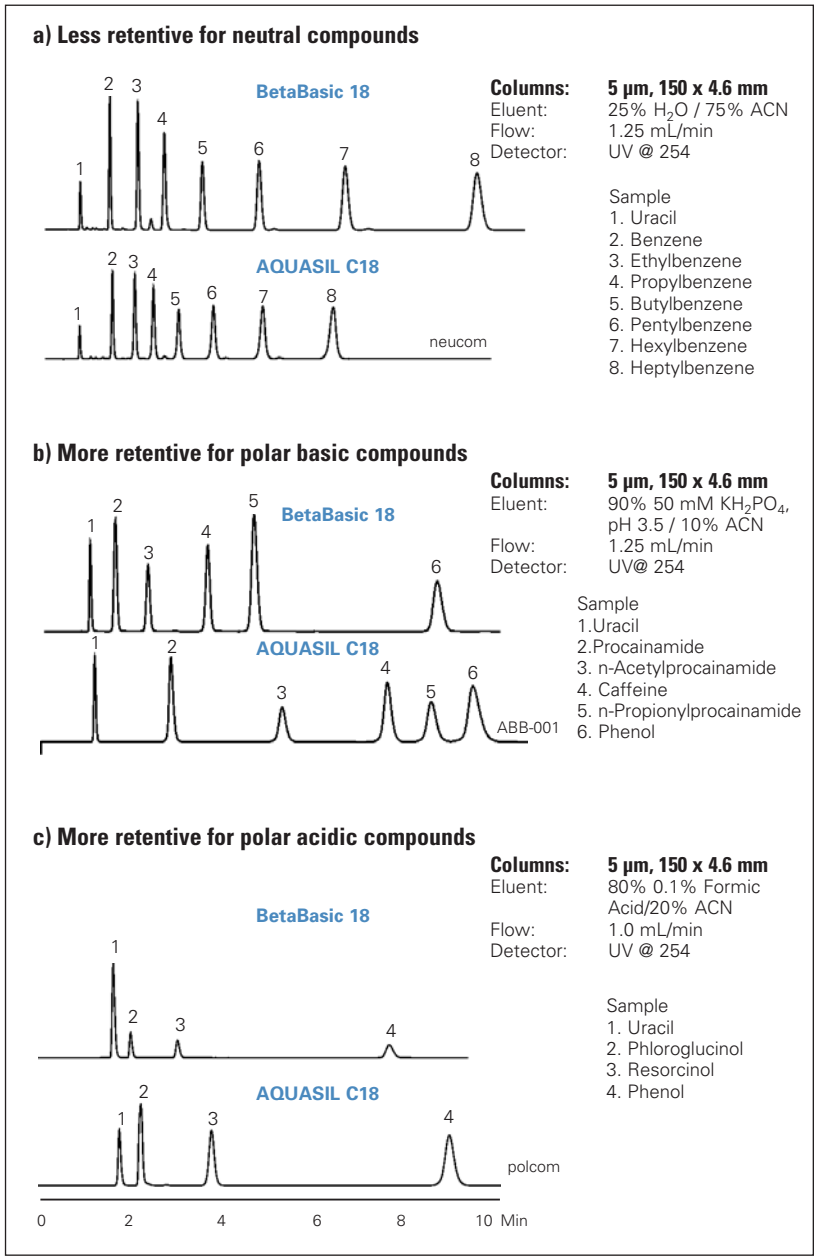
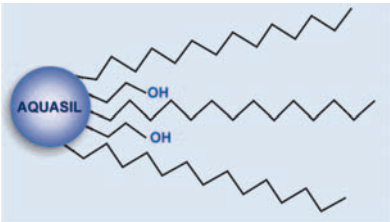


Figure 1: Chromatographic Retention Behavior AQUASIL C18 vs BetaBasic 18 Columns

Figure 1b shows how the AQUASIL C18 column offers the nearly twice the retention of several polar, basic compounds when compared to a BetaBasic 18 column. The retention of basic compounds and polar acidic compounds on the AQUASIL C18 column are both significantly increased compared to the BetaBasic 18 column. This illustrates how useful the AQUASIL C18 column can be when increased retention of polar compounds or alternative selectivity is required.



AQUASIL media combines C18 with hydrophilic endcapping

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.

Applications

- Highly Polar Compounds
- Nucleosides and Nucleotides
- Organic acids
- Vitamins
- Peptides
- Catecholamines

Chromatographic Interactions

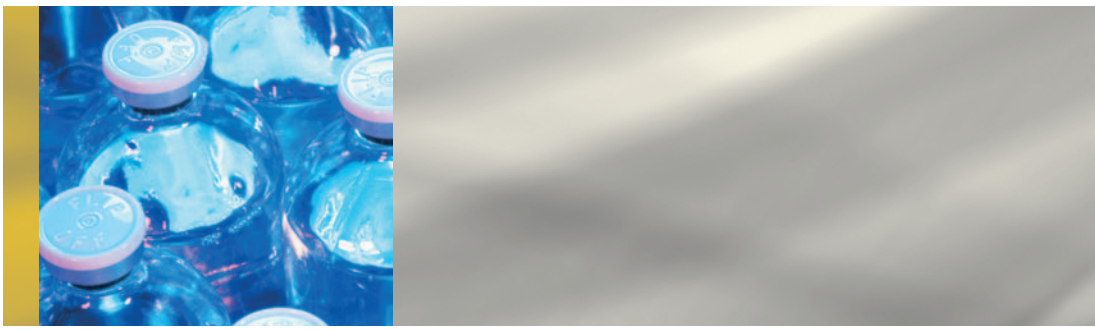
Dispersive interactions are the primary interactions generally associated with retention using traditional alkyl C18 type packings.

Secondary interactions associated with residual silanols have been significantly reduced by end-capping, improvements in silica quality and increased density of the derivatized ligand. Therefore, silanol interactions that previously gave rise to broad tailing peaks for basic analytes have been somewhat 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.

Aquasil Phase Specifications

PHASE	PARTICLE SIZE	PORE SIZE	CARBON LOAD	SILICA TYPE
AQUASIL C18	3 and 5 µm	100Å	12%	High purity, base deactivated



This is illustrated in Figure 2 which demonstrates the capability of the AQUASIL C18 column's ability 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 (Figures 3 and 4) and shows no tendency towards phase collapse. 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 are 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. Figure 4 demonstrates that reproducible chromatography is maintained even after flushing with 100% aqueous mobile phase for 113 hours.

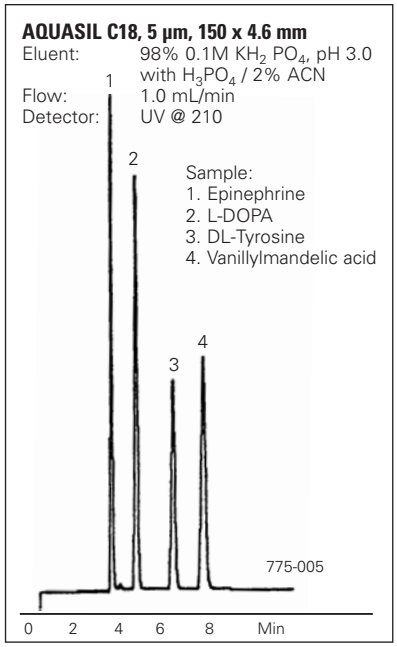


Figure 2: Catecholamines

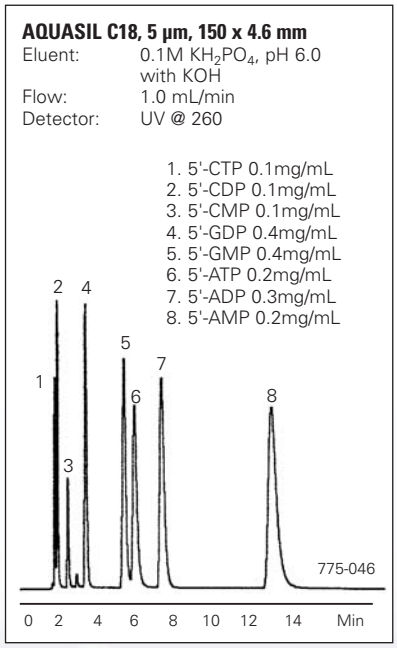


Figure 3: Separation of nucleotides with 100% aqueous mobile pahse

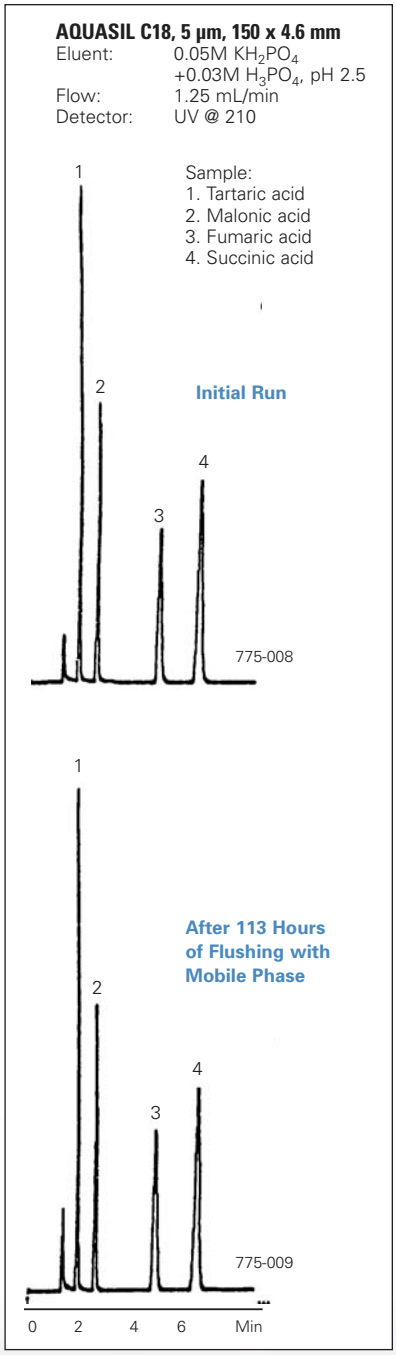


Figure 4: AQUASIL C18 compatibility with 100% aqueous mobile phase

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.

To enhance ionization, it is good practice to use volatile mobile phase additives in LC/MS methods. 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.

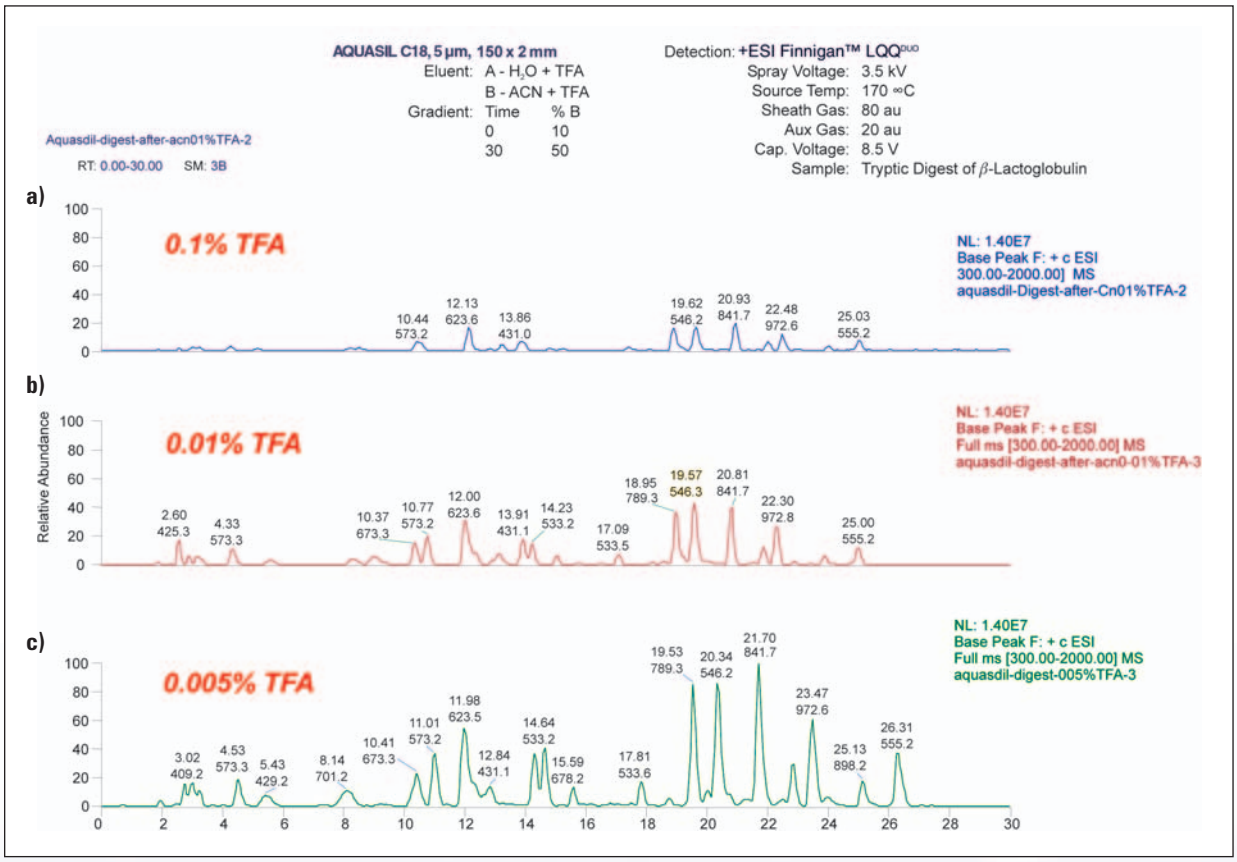


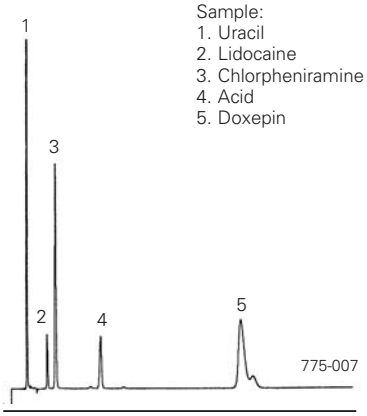
Figure 5: Tryptic Digest of β-Lactoglobulin

Example Applications

Acid Base Mix

AQUASIL C18, 5 µm, 150 x 4.6 mm
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

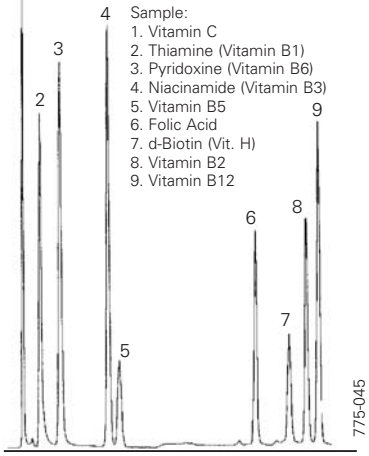
Sample:
1. Uracil
2. Lidocaine
3. Chlorpheniramine
4. Acid
5. Doxepin



Water-Soluble Vitamins

AQUASIL C18, 5 µm, 50 x 4.6 mm
Eluent: 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
Detector: UV @ 205

Sample:
1. Vitamin C
2. Thiamine (Vitamin B1)
3. Pyridoxine (Vitamin B6)
4. Niacinamide (Vitamin B3)
5. Vitamin B5
6. Folic Acid
7. d-Biotin (Vit. H)
8. Vitamin B2
9. Vitamin B12

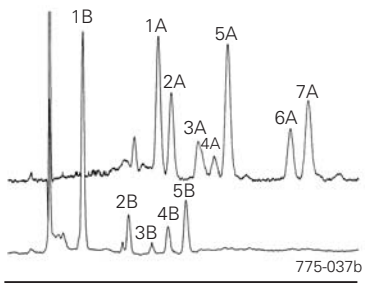


Fat-Soluble Vitamins

AQUASIL C18, 5 µm, 250 x 4.6 mm
Eluent: 83%ACN / 16%Dioxane/ 1%MeOH
Flow: 1.5 mL/min
Detector: chromatogram A: UV@450 nm
chromatogram B: UV@325/300 nm

Sample: A)
1. Lutein
2. Zeaxanthin
3. & 4. Lycopene
5. β-cryptoxanthin
6. α-Carotene
7. β-Carotene

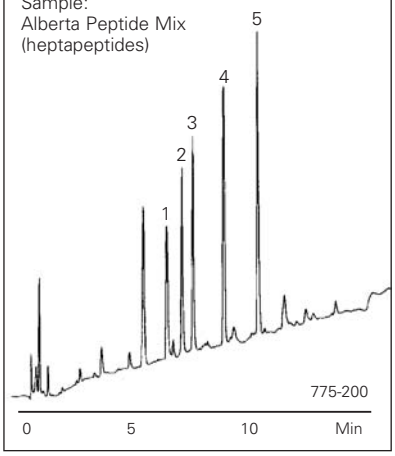
Sample:B)
1. Retinol
2. Tocol
3. σ-Tocopherol
4. γ-Tocopherol
5. α-Tocopherol



Peptides

AQUASIL C18, 3 µm, 100 x 4.6 mm
Eluent: A: 0.1% TFA in H_2O
B: 0.1% TFA in ACN
0%B to 50%B in 20 min.
Flow: 1.0 mL/min
Detector: UV @ 210

Sample:
Alberta Peptide Mix
(heptapeptides)

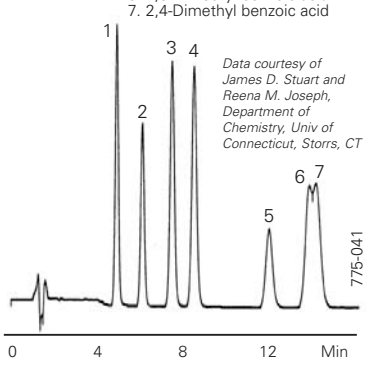


Bio-Remediation Acids

AQUASIL C18, 5 µm, 150 x 4.6 mm
Eluent: 50% MeOH / 50% 0.025M KH_2PO_4, pH 2.5
Flow: 1.5 mL/min
Detector: UV @ 230
Temperature: 22.0 +/- 0.2o C

Sample:
1. Benzoic acid
2. 4-Fluoro benzoic acid
3. 2-Toluic acid
4. 4-Toluic acid
5. 2,4,6-Trimethyl benzoic acid
6. 2,5-Dimethyl benzoic acid
7. 2,4-Dimethyl benzoic acid

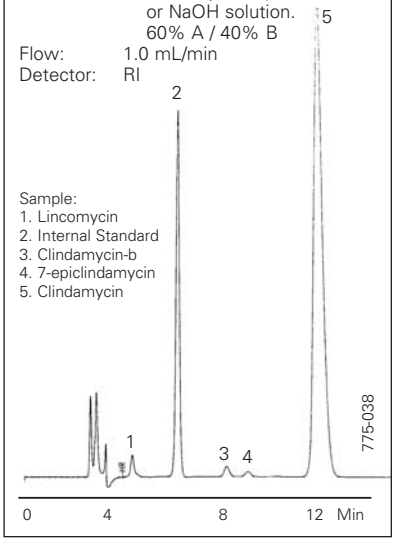
Data courtesy of James D. Stuart and Reena M. Joseph, Department of Chemistry, Univ of Connecticut, Storrs, CT



Clindamycin

AQUASIL C18, 5 µm, 250 x 4.6 mm
Eluent: A: MeOH
B: 4g/L dl-10- camphorsulfonic acid, Adjust to pH 6 with HCL or NaOH solution.
60% A / 40% B
Flow: 1.0 mL/min
Detector: RI

Sample:
1. Lincomycin
2. Internal Standard
3. Clindamycin-b
4. 7-epiclindamycin
5. Clindamycin

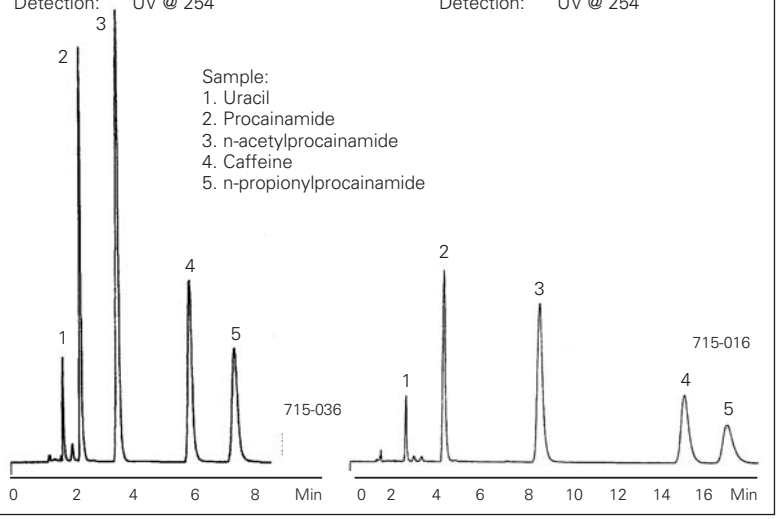


Procainamides Comparison

BetaBasic 18, 5 µm, 150 x 4.6 mm
Eluent: 10% ACN/90% 0.05M KH_2PO_4, pH 3.5
Flow: 1.25 mL/min
Detection: UV @ 254

AQUASIL C18, 5 µm, 150 x 4.6 mm
Eluent: 10% ACN / 90% 0.05M KH_2PO_4, pH 3.5
Flow: 1.25 mL/min
Detection: UV @ 254

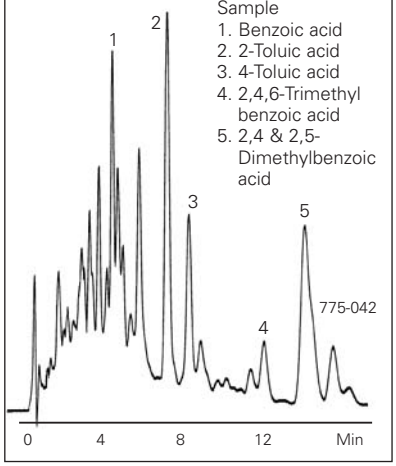
Sample:
1. Uracil
2. Procainamide
3. n-acetylprocainamide
4. Caffeine
5. n-propionylprocainamide



Ground Water Extract

AQUASIL C18, 5 µm, 150 x 4.6 mm
Eluent: 50% MeOH/50% 0.025M KH_2PO_4, pH 2.5
Flow: 1.5 mL/min
Detection: UV @ 230
Temperature: 22°C

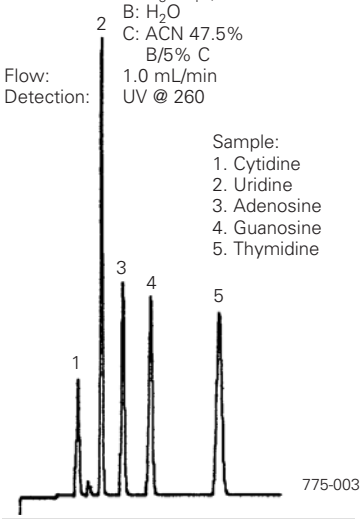
Sample
1. Benzoic acid
2. 2-Toluic acid
3. 4-Toluic acid
4. 2,4,6-Trimethyl benzoic acid
5. 2,4 & 2,5-Dimethylbenzoic acid



Nucleosides

AQUASIL C18, 5 µm, 150 x 4.6 mm
Eluent: A: 0.05M KH_2PO_4, & 0.03M H_3PO_4, pH 2.4
B: H_2O
C: ACN 47.5%
B/5% C
Flow: 1.0 mL/min
Detection: UV @ 260

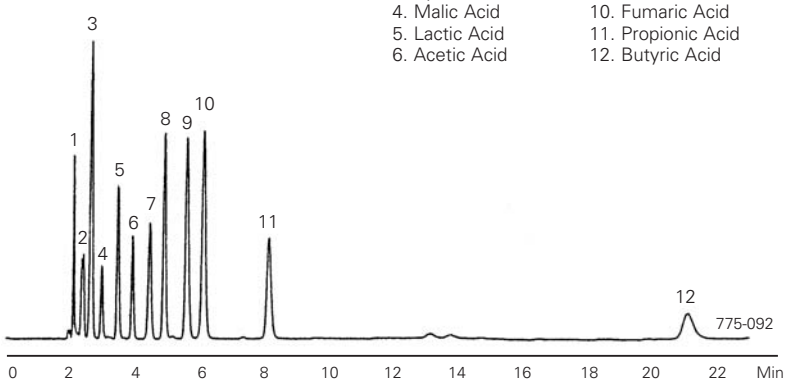
Sample:
1. Cytidine
2. Uridine
3. Adenosine
4. Guanosine
5. Thymidine



Organic Acids in Highly Aqueous Mobile Phase

AQUASIL C18, 5 µm, 250 x 4.6 mm
Eluent: 1% ACN / 99% 0.05M KH_2PO_4, pH 2.8
Flow: 1.25 mL/min
Detection: UV @ 210

Sample:
1. Oxalic Acid
2. Tartaric Acid
3. Pyruvic Acid
4. Malic Acid
5. Lactic Acid
6. Acetic Acid
7. Acid
8. Maleic Acid
9. Succinic Acid
10. Fumaric Acid
11. Propionic Acid
12. Butyric Acid



Ordering Information

AQUASIL C18 Columns

Particle Size	Length (mm)	4.6 µm ID	4.0 µm ID	3.0 µm ID	2.1 µm ID	1.0 µm ID
3 µm	30	77503-034630	77503-034030	77503-033030	77503-032130	77503-031030
	50	77503-054630	77503-054030	77503-053030	77503-052130	77503-051030
	100	77503-104630	77503-104030	77503-103030	77503-102130	77503-101030
	150	77503-154630	77503-154030	77503-153030	77503-152130	77503-151030
5 µm	30	77505-034630	77505-034030	77505-033030	77505-032130	77505-031030
	50	77505-054630	77505-054030	77505-053030	77505-052130	77505-051030
	100	77505-104630	77505-104030	77505-103030	77505-102130	77505-101030
	125	77505-124630	77505-124030	77505-123030	77505-122130	77505-121030
	150	77505-154630	77505-154030	77505-153030	77505-152130	77505-151030
	250	77505-254630	77505-254030	77505-253030	77505-252130	77505-251030

Other column dimensions are also available. Please call Customer Service for more information.

AQUASIL C18 Drop-In Guard Cartridges (pk/4)

Particle Size	Length (mm)	4.6 µm ID	4.0 µm ID	3.0 µm ID	2.1 µm ID	1.0 µm ID
3 µm	10	77503-014001	77503-014001	77503-013001	77503-012101	77503-011001
5 µm	10	77505-014001	77505-014001	77505-013001	77505-012101	77505-011001
UNIGUARD™ Direct-Connect Drop-in Guard Cartridge Holder 		850-00	850-00	852-00	852-00	851-00

AQUASIL C18 columns are available in other column formats. Please contact your local customer support for more details.

www.thermo.com/columns

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