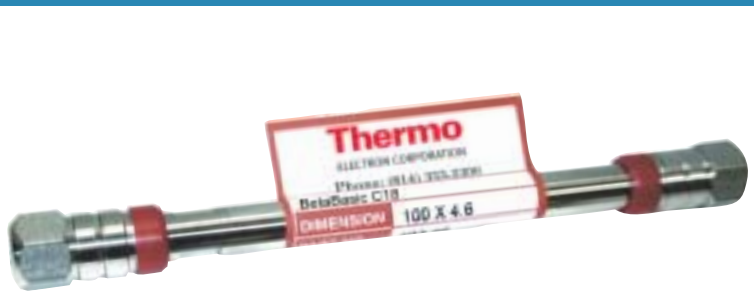
BetaBasic™ Columns

TG01-02



Excellent Peak Shapes for Basic Compounds

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Introduction

BetaBasic columns offer a broad range of applicability including acidic, basic and neutral compounds. BetaBasic packings are highly base deactivated with chemistries bonded to a high purity supporting spherical silica. The BetaBasic family of packings is very stable and offers reproducibility at high and low pH extremes. The pH stability of the BetaBasic 18 phase has become one of the most popular features associated with this packing. This makes it particularly useful for LC/MS applications that employ Atmospheric Chemical Ionization Interface (APCI) at high pH for the analysis of basic compounds in their neutral form.

- 150Å high purity silica
- Most deactivated general purpose packing, excellent choice for basic compounds
- Designed for both small and large molecules
- Moderate surface area and phase loading
- Type B silica with wider pores that result in a very dense, uniform bonding and excellent stability
- Suitable for peptides and small proteins

Specifications:

Phase	Particle size	Carbon Load	Pore Size	End-capping	Silica type
BetaBasic 18	3 and 5µm	13%	150Å	Yes	High purity, base deactivated
BetaBasic 8	3 and 5µm	7%	150Å	Yes	High purity, base deactivated
BetaBasic 4	3 and 5µm	6%	150Å	Yes	High purity, base deactivated
BetaBasic CN	3 and 5µm	5%	150Å	Yes	High purity, base deactivated
BetaBasic Phenyl	3 and 5µm	7%	150Å	Yes	High purity, base deactivated

A Wide Variety of Applications

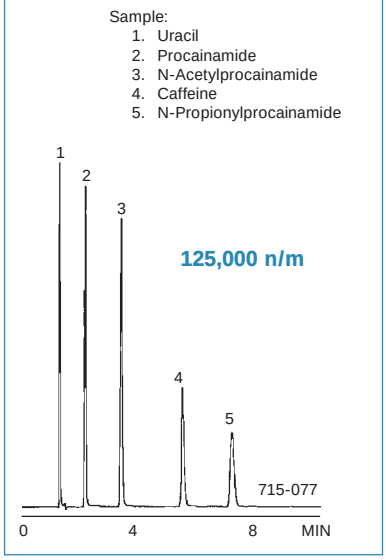
BetaBasic columns are proven for their performance for a wide variety of sample types. These range from small polar organics such as water-soluble vitamins to peptides and protein digests.

Excellent Peak Shapes for Basic Compounds

BetaBasic columns are designed for excellent chromatography of many molecules that can be considered difficult to analyze. Acids, bases, and polar compounds that present challenges to reversed phase columns show excellent results on BetaBasic packings. Available in a variety of stationary phases, including C18, C8, C4, CN, and phenyl, the outstanding stability, reproducibility and deactivation of BetaBasic columns make them the ideal first choice for general use.

Figure 1

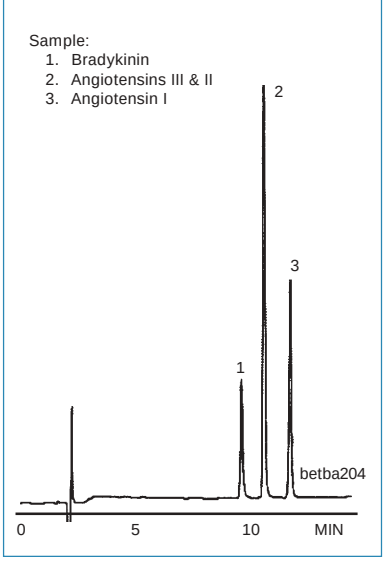
High Efficiency and Excellent Peak Shapes



BetaBasic 18, 3µm, 150x4.6mm
Eluent: 10% ACN / 90% 0.05M KH_2PO_4, pH 3.5
Flow: 1.0 mL/min
Detector: UV @ 254

Figure 2

Peptides



BetaBasic 8, 5µm, 150x4.6mm
Gradient: A: 0.05% TFA in H_2O
B: 0.05% TFA in ACN
10% B to 50% B in 20 min
Flow: 1.0 mL/min
Detector: UV@ 220

Chromatographic Characterization

BetaBasic™ 18 has retention characteristics that place it firmly among the most popular of the most rugged high performance columns used for developing new methods. It is highly base deactivated, which means excellent peak shapes are obtained for basic compounds.

Hydrophobic Retention Behavior

There are several factors that control the overall hydrophobic retention of a packing material:

1) Percent carbon loading
2) Silane density at the silica surface
3) The surface area of the silica support

Provided the density of the bonding is similar for different packings, retention will be determined by the %carbon per gram of packing. This is to a large extent determined by the underlying surface area and pore size of the silica. In Figure 3 we show the retention of a homologous series of alkylbenzenes on BetaBasic 18. The retention on three Thermo Hypersil-Keystone packings, BetaBasic 18, Hypersil™ BDS C18 and HyPURITY™ C18 are compared and found to be very similar, often allowing them to be interchanged.

Chromatography of Basic Compounds

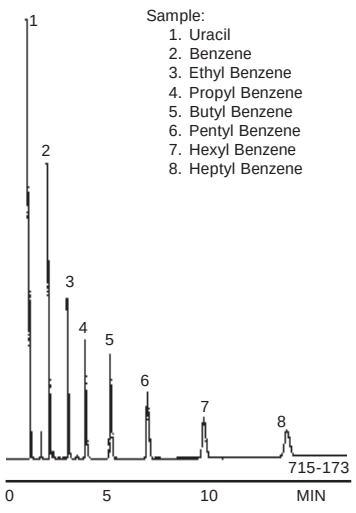
The factors that control peak shape and retention of basic compounds are related to the number and type of free silanols (silanol acidity) that remain after the bonding and endcapping processes. In Figure 4, the BetaBasic 18 column provides excellent peak shape under conditions that often lead to peak tailing.

Reproducibility and Stability

BetaBasic columns are designed to give excellent reproducibility, run to run and column to column. The dense bonding chemistry and high purity silica of BetaBasic columns provides excellent column lifetimes, even under demanding gradient conditions (Figure 5).

Figure 3

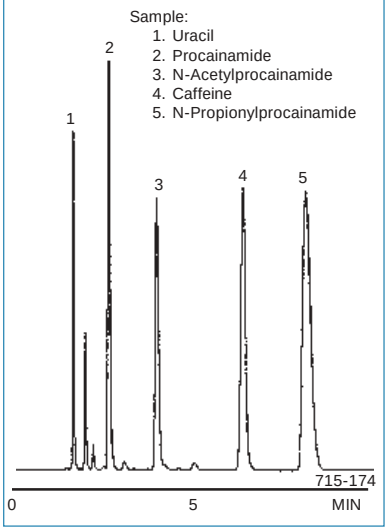
Alkylbenzenes - Hydrophobicity Test



BetaBasic 18, 5µm, 150x4.6mm
Eluent: 75% ACN / 25% H_2O
Flow: 1.25 mL/min
Detector: UV @ 254

Figure 4

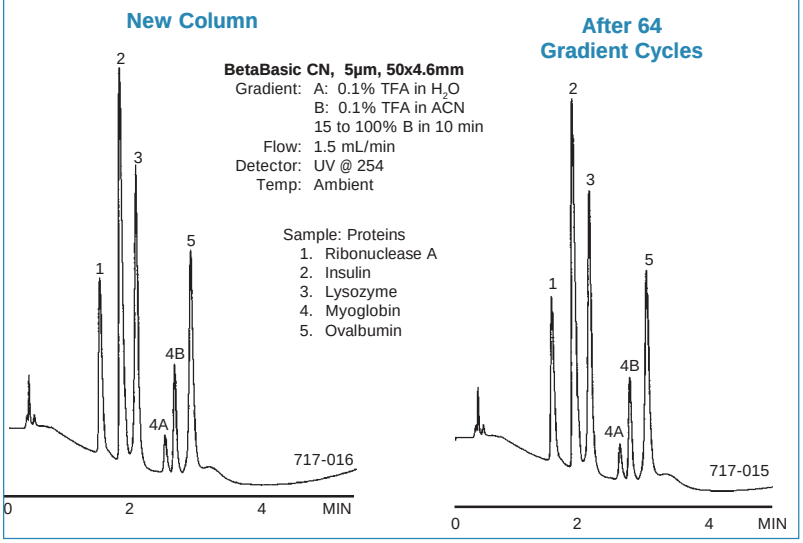
Procainamides



BetaBasic 18, 5µm, 150x4.6mm
Eluent: 10% ACN / 90% 0.5M KH_2PO_4, pH 3.5
Flow: 1.25 mL/min
Detector: UV @ 254

Figure 5

Reproducibility of Protein Separation on BetaBasic CN Column



Using BetaBasic™ Columns at Low pH

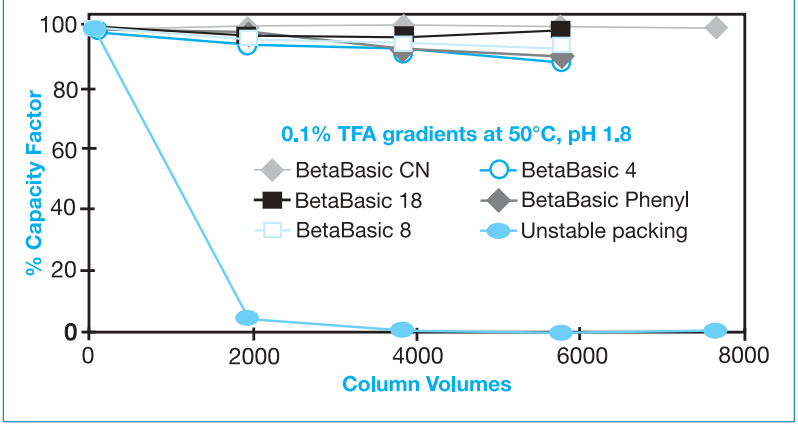
Figure 6 demonstrates that after over 25,000 column volumes of aggressive mobile phase (low pH and high temperature):

- BetaBasic 18 exhibits virtually no change in retention for the analysis of a sensitive drug mix ture
- Other packings belonging to the BetaBasic family are also shown to be highly stable to chemical attack (hydrolysis of the silane ligand under acidic conditions)

The high purity of the silica support and excellent bonding chemistry make BetaBasic bonded phases stable,selective and efficient.

Figure 6

BetaBasic Bonded Phase Stability at Low pH



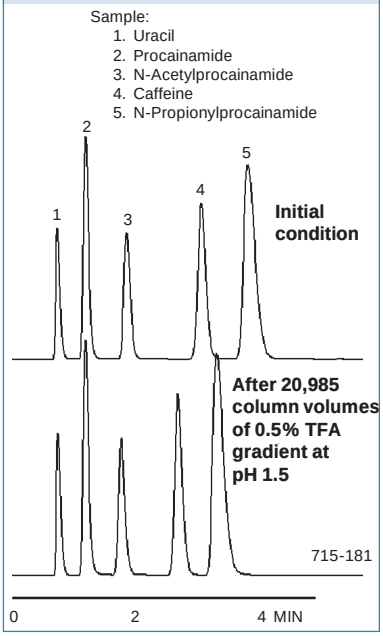
BetaBasic 18 Columns at Extreme Low pH

BetaBasic 18 columns are particularly stable at extreme low pH. In Figure 7a and 7b the column has been run extensively (20,000 column volumes) at pH 1.5. Some loss in retention is observed as might be expected for this pH where C18 ligand hydrolysis can occur, leading to reduced retention.

The loss of C18 phase is slow, however, as shown in Figure 7b. This suggests that while lifetime of the column may to some extent be compromised, the column can be used at this low pH for limited periods.

Figure 7a

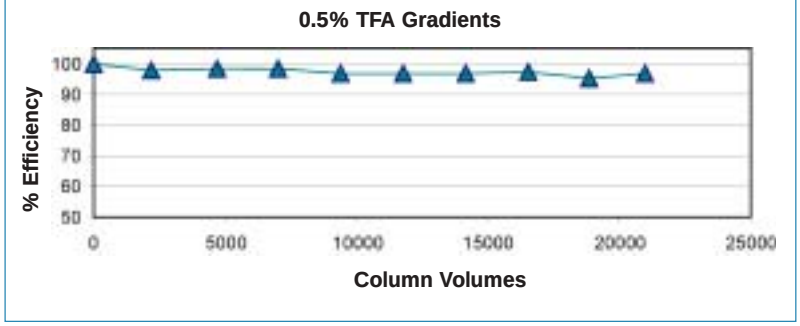
BetaBasic 18 at Extreme Low pH



BetaBasic 18, 5µm, 50x4.6mm
Eluent: 90% 0.5M KH_2PO_4 / 10% ACN, pH 3.5
Flow: 1.25 mL/min
Detector: UV @ 254
Temp.: 25° C

Figure 7b

TFA Hydrolysis of BetaBasic 18 Phases at pH 1.5



Using BetaBasic™ 18 Columns at High pH

Recently, there has been a growing interest in the analysis of highly polar compounds by chromatographers, particularly in the pharmaceutical industry. The analysis of polar basic compounds can give rise to difficulties through:

1. Unwanted interactions with silanol groups on the silica surface. These unwanted interactions can result in peak tailing and poor column efficiencies.
2. Ionization of compounds at lower pH where silica is stable (pH<7). Performing the analysis at low pH, where the basic species is essentially completely protonated, can increase the affinity of the analyte for the mobile phase, thereby resulting in elution at or near the void volume.

One way to overcome the difficulties mentioned above is to increase the pH of the mobile phase, so that the basic analyte is no longer protonated. At high pH (>1.5 units above the pK_a of the basic analyte) there are no ion exchange interactions that can lead to poor peak shape and tailing. The analyte will also have reduced affinity for the mobile phase leading to increased retention. The main disadvantage of working at high pH is that silica (the foundation for many traditional HPLC packings) begins to become soluble. The problem usually manifests itself by a sudden and severe loss in column efficiency as the particles begin to collapse.

There are two factors that can strongly affect the rate of particle collapse:

1. The type of mobile phase buffer used in the analysis.[1]
2. The density of C18 (or alkyl functionality) bonded to the surface.[2]

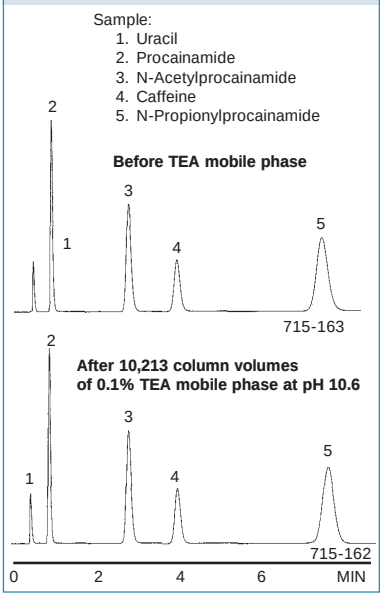
Phosphate buffers in particular have been shown to be very aggressive towards the silica surface. It has been proposed that at high pH, phosphate may form a complex with siloxane groups on the silica surface, facilitating hydrolysis by hydroxyl ions and increasing the solubility of the silica support.[2] Column lifetime at high pH can be extended by the choice of organic buffers (tris, pyrrolidine HCl, etc.) over their inorganic phosphate counterparts.

Under certain conditions, BetaBasic 18 columns can be used for the analysis of polar basic compounds at high pH (>10), where conventional silica-based stationary phases typically last only a few injections.

To establish a practical lifetime for BetaBasic 18 columns at high pH, a BetaBasic 18 column was subjected to a pH of 10.6. After 10,213 column volumes of 50:50 ACN/H_2O with 0.1% triethylamine (TEA), there was no change in the retention of a sensitive drug mixture of procainamides, as shown (Fig. 8) and virtually no evident loss of performance with over 15,000 column volumes (Fig 9).

Figure 8

Procainamides at pH 10.6

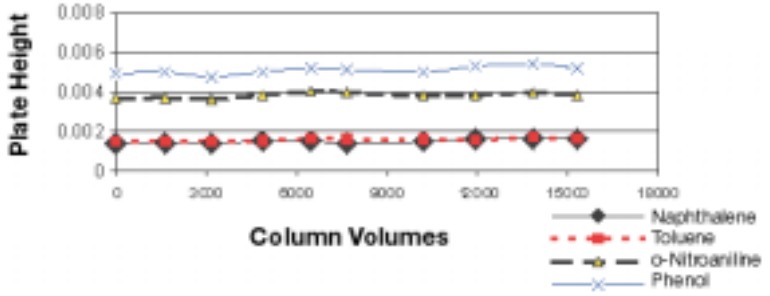


BetaBasic 18, 5µm, 50x4.6mm
Eluent: 85% 0.1% TEA (aq) / 1
5% TEA in ACN, pH 10.6
Flow: 1.25 mL/min
Detector: UV @ 254

Figure 9

Base Stability of BetaBasic 18 at pH 10.6

BetaBasic 18, 50x4.6mm, 5µm
Conditions: 50/50 0.1% TEA in ACN / 0.1% TEA in H_2O (pH 10.6)



To extend the lifetime of a silica-based column when using mobile phases with pH>8, consider the following:

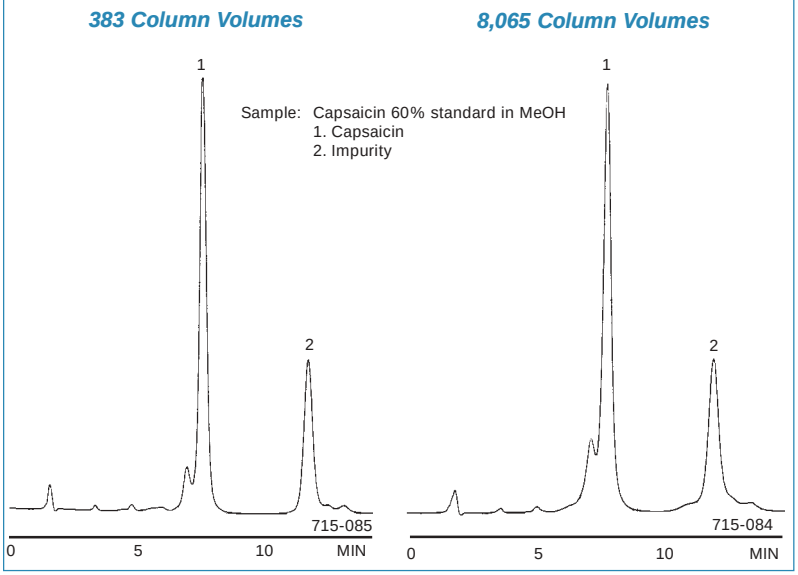
- Use organic or borate salts as buffers in the mobile phase
- Don't use phosphate buffers above pH 8
- Always use a guard column before the analytical column

In addition to organic buffers, borate buffers have shown potential for increased column stability at high pH. As shown (Fig. 10), essentially no change is seen in the chromatography of capsaicin on a BetaBasic™ 18 column at pH 12 after over 8,000 column volumes of borate-buffered mobile phase.

1. Kirkland, J. J.; Henderson, J. W.; DeStefano, J. J.; van Straten, M. A., Claessens, H.A., *J. Chrom. A*, 762 (1997) 97-112
2. Claessens, H. A., van Straten, M. A.; Kirkland, J. J., *J Chrom. A.,* 728, (1996), 259.

Figure 10

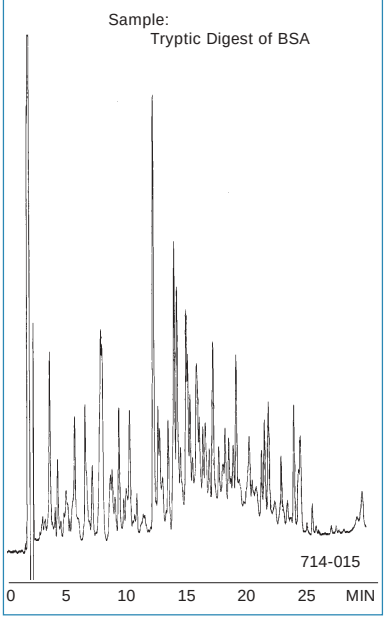
Stability Study at pH 12



BetaBasic 18, 5µm, 150x4.6mm
Eluent: 63% MeOH/37%10 mM Sodium Borate, pH 12
Flow: 1.0 mL/min
Detector: UV @ 280

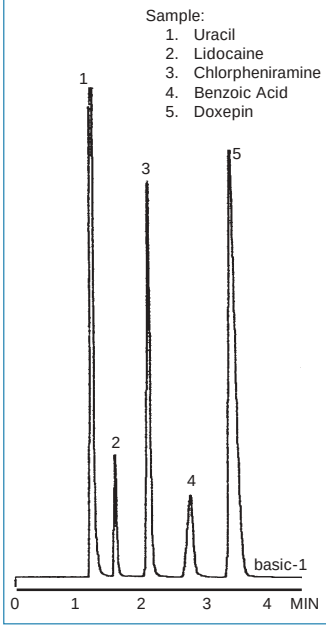
Data courtesy of Kristine Phillips, Dept of Chemistry Univ. of New Hampshire

Tryptic Digest of BSA



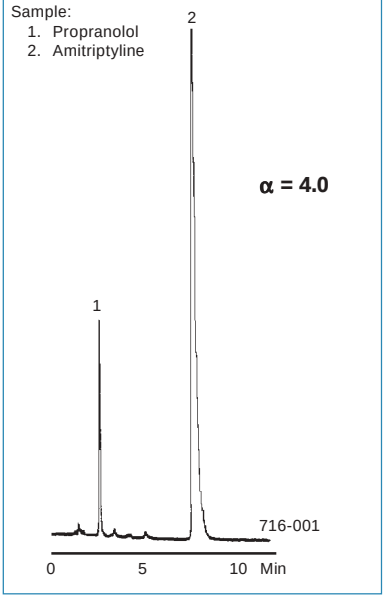
BetaBasic 8 , 3µm, 150x4.6mm
Gradient: A: 0.1% TFA in H_2O
B: 0.1% TFA in ACN
5% B to 50% B in 40 min
Flow: 1.0 mL/min
Detector: UV @ 220

Acids & Bases



BetaBasic 8, 5µm, 150x4.6mm
Eluent: 35% ACN / 65% 0.05M KH_2PO_4, pH 3
Flow: 1.25 mL/min
Detector: UV @ 254

Basic Drugs

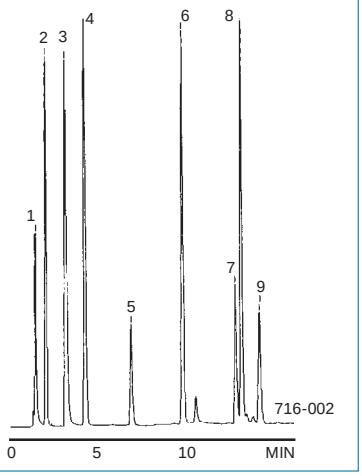


BetaBasic 4, 5µm, 150x4.6mm
Eluent: 65% MeOH / 35% 0.05M KH_2PO_4, pH 7
Flow: 1.0 mL/min
Detector: UV @ 254

Vitamins

Sample:

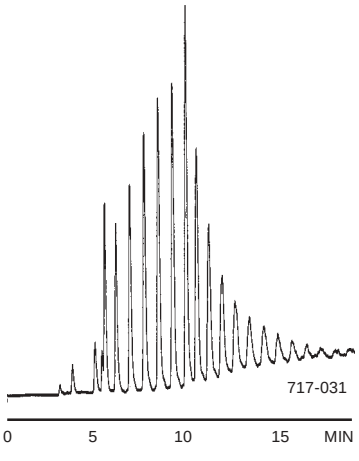
1. Vitamin C
2. Pyridoxine (Vitamin B6)
3. Thiamine (Vitamin B1)
4. Niacinamide (Vitamin B3)
5. Vitamin B5
6. Folic Acid
7. Vitamin B2
8. Vitamin B12
9. d-Biotin (Vitamin H)



BetaBasic® 4, 5µm, 150x4.6mm
Gradient: A: 0.05M KH_2PO_4, pH 3.5
B: 50/50 0.05M KH_2PO_4, pH 3.5 / ACN
4% B to 40% B in 15 min
Flow: 1.0 mL/min
Detector: UV @ 254

Volpo-10 Surfactant

Sample: Volpo-10

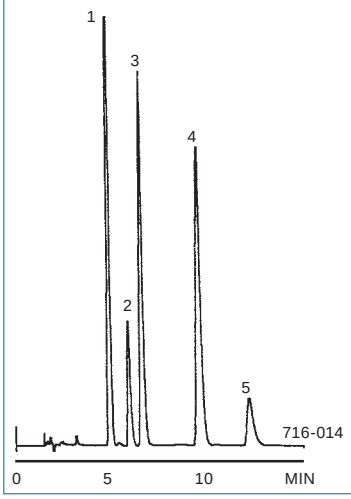


BetaBasic CN, 5µm, 150x4.6mm
Gradient: A: Hexane
B: EtOH
0% B to 25% B in 20 min
Flow: 1.0 mL/min
Temp.: Ambient
Detector: ELSD

Acids

Sample:

1. trans-2-Pentenoic Acid
2. Pentanoic acid
3. trans,trans-2,4-Hexadienoic Acid
4. trans-2-Hexenoic Acid
5. Hexanoic Acid



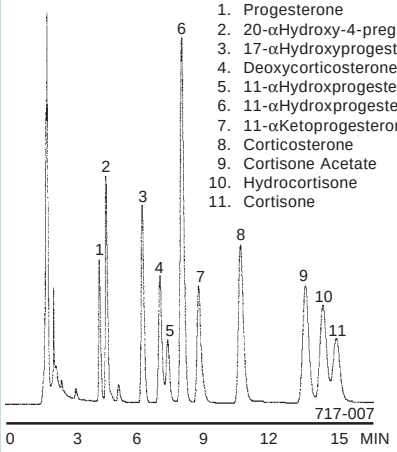
BetaBasic 4, 5µm, 150x4.6mm
Eluent: 21/79 (v/v) Acetonitrile/0.05% Trifluoroacetic Acid in HOH (v/v)
Flow: 1.0 mL/min
Detection: UV @ 210

Normal and Reversed Phase Separations on the Same BetaBasic Cyano Column

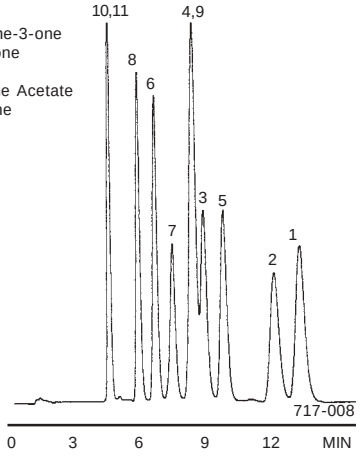
Steroids By NPLC Normal Phase

Sample: Steroids:

1. Progesterone
2. 20-αHydroxy-4-pregnene-3-one
3. 17-αHydroxyprogesterone
4. Deoxycorticosterone
5. 11-αHydroxprogesterone Acetate
6. 11-αHydroxprogesterone
7. 11-αKetoprogesterone
8. Corticosterone
9. Cortisone Acetate
10. Hydrocortisone
11. Cortisone



Steroids by RPLC Reversed Phase



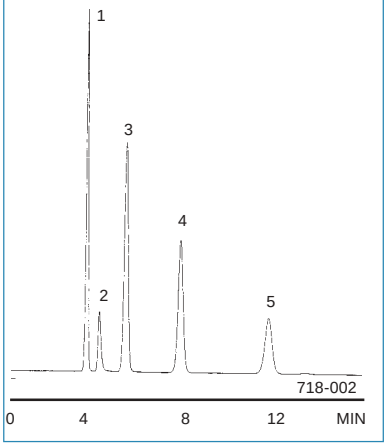
BetaBasic CN, 5µm, 150x4.6mm
Normal Phase Eluent: 97/3 Hexane/Ethanol
Reversed Phase Eluent: 35/65 Methanol/Water
Flow: 1.2 mL/min
Detector: UV @ 254nm

An ethanol flush is recommended between RP and NP use.

Carbamates

Sample:

1. 3-Aminophenol
2. 3-Dimethylaminomethyleneiminophenol
3. 3-Hydroxyformanilide
4. 3-Aminophenyl methylcarbamate
5. 3-Formaminophenyl methylcarbamate



BetaBasic Phenyl, 5µm, 150x4.6mm
Eluent: 15% MeOH / 85% 0.05M KH_2PO_4, pH 6.5
Flow: 1.0 mL/min
Detector: UV @ 254

Innovators in life and laboratory sciences, Thermo Electron Corporation provides advanced analytical technologies, scientific instrumentation, laboratory informatics solutions, and laboratory consumables to help scientists and clinicians to discover new drugs, improve manufacturing processes, and diagnose illness and disease. Unparalleled in our capabilities, we can help you every step of the way – from sample preparation and sample analysis through interpretation of results.

For more information on our products and services, please visit our website at: www.thermo.com/hypersil-keystone

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