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BETASIL™ HPLC Columns

Technical Guide

Highly Efficient, Retentive and Selective HPLC Columns

Analyze • Detect • Measure • Control™

Thermo
ELECTRON CORPORATION

BETASIL™ Columns

Introduction

BETASIL columns from Thermo Electron are based on a highly pure, 100Å silica designed for small molecule analysis. All reversed phases are end-capped with high phase loading, providing exceptional chromatography of acids, bases and neutral compounds. The dense bonding means that mobile phases require more organic solvent, making these columns a popular choice for LC/MS applications.

- **100Å high purity silica**
- **High surface area and phase loading**
- **Dense bonding results in excellent reproducibility and stability**
- **Excellent performance with acids, bases and neutral compounds**

Specifications

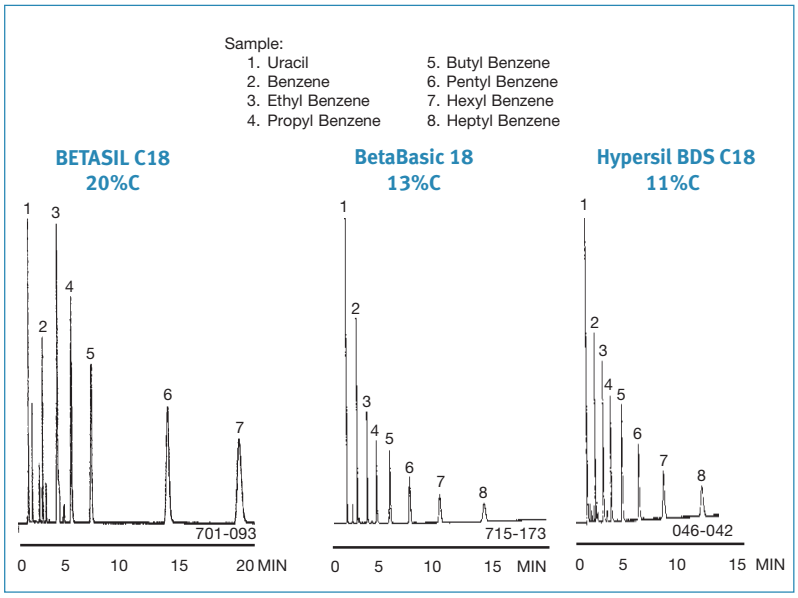
Phase	Particle size	Carbon Load	Pore Size	Endcapping	Silica type
BETASIL C18	3, 5 and 10µm	20%	100Å	Yes	High purity, base deactivated
BETASIL C8	3, 5 and 10µm	12%	100Å	Yes	High purity, base deactivated
BETASIL C6	3 and 5µm	11%	100Å	Yes	High purity, base deactivated
BETASIL C1	3 and 5µm	4%	100Å	Yes	High purity, base deactivated
BETASIL Phenyl	5µm	11%	100Å	Yes	High purity, base deactivated
BETASIL Phenyl/Hexyl	3 and 5µm	11%	100Å	Yes	High purity, base deactivated
BETASIL Cyano	5µm	7%	100Å	Yes	High purity, base deactivated
BETASIL Diol 100	5µm	6%	100Å	Yes	High purity, base deactivated
BETASIL Silica 100	5µm	N/A	100Å	N/A	High purity, base deactivated

BETASIL Chromatographic Characterization

The BETASIL C18 phase is a high carbon load packing that offers up to twice the retention of other C18 packings with lower percent carbon loading. Figure 1 shows the increased retention of the BETASIL C18 column when compared to other Thermo Electron columns. The increased retention associated with the BETASIL column is directly attributed to the increased surface area of theparticles, and higher carbon loading than both Hypersil™ BDS C18 and BetaBasic™ 18 columns.

Where longer retention is required or there is a requirement to use a higher percentage of organic solvent in themobile phase, e.g. to increase LC/MS sensitivity, the BETASIL C18 packing offers an excellent choice.

Figure 1. Increased Hydrophobic Retention of Neutral Molecules



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

Stability at Low pH

All surface bonded chemistries belonging to the BETASIL family are highly stable under chemical attack. Hydrolysis of the silane ligand can occur under acidic conditions, resulting in a loss of retention and/or selectivity. The effect becomes more severe as ligand chain length is reduced, as is observed for the BETASIL C1 phase. Wettable stationary phases such as the BETASIL Cyano packing can also show slightly less resistance to chemical attack, and consequently are recommended only for use between pH 2 and 8.

In general, BETASIL packings show superior stability to many other packings. Figure 2 shows BETASIL phases subjected to 25,000 column volumes of aggressive mobile phase at pH 1.8 and 50°C. The BETASIL C18 phase exhibits virtually no change in retention for the analysis of a sensitive drug mixture.

Base Deactivated for Better Peak Shape with Basic Compounds

All BETASIL packings are base deactivated and show excellent peak shape for basic compounds. Figure 3 shows the analysis of a procainamide test mixture, a mixture that would typically have given rise to broad tailing peaks for the more basic compounds on traditional C18 silicas prepared on a Type A silica. In terms of silanol activity, the BETASIL packing is similar to other Type B silica-based phases such as BetaBasic and BetaMax™ Neutral phases.

Phase Collapse Phenomenon

Mobile phases that contain a high proportion of water often are employed to retain highly polar compounds when using RP-HPLC. Many C18 columns will show a reversible loss of retention when exposed to highly aqueous mobile phase. The rate and degree of retention loss can vary greatly among different columns. A common explanation for this retention loss is that the hydrophobic alkyl chains of the stationary phase are not wettable and appear to "fold" down on the silica surface to avoid a highly aqueous, hydrophilic mobile phase (Figure 4).

Figure 2. BETASIL Phase Stability At Low pH

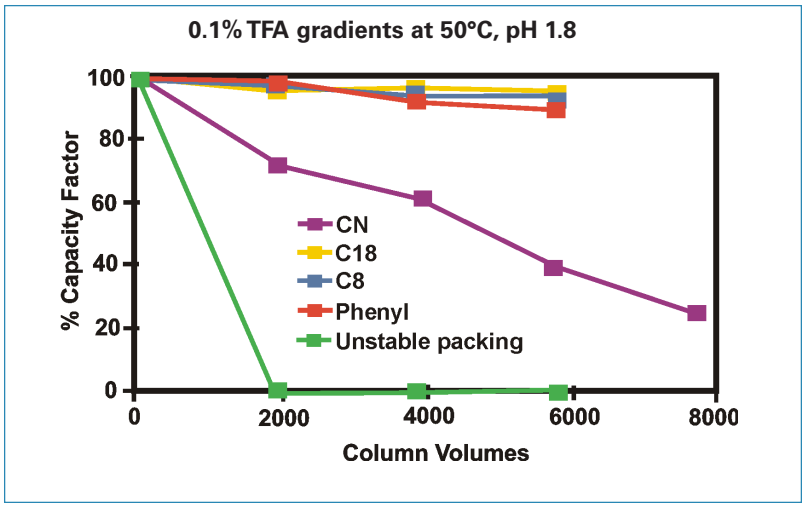
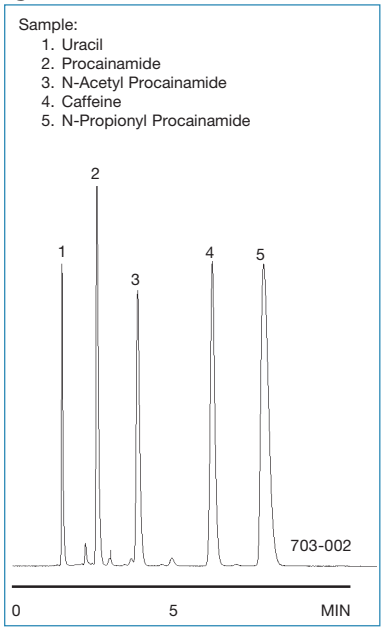
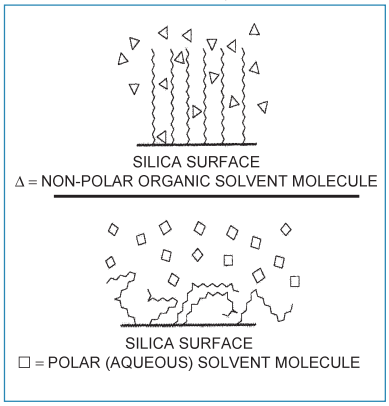


Figure 3. Base Deactivation of BETASIL C6



BETASIL C6, 5µm, 150x4.6mm
Eluent: 10% ACN / 90% 0.05M Phosphate buffer, pH 3.5 with H_3PO_4
Flow: 1.25 mL/min
Detector: UV @ 254

Figure 4. Phase Collapse



Regeneration After Phase Collapse

In this folded or collapsed state, the alkyl chains are much less able to interact with solutes, resulting in a loss of retention. We use the term "chain folding" to refer to this reversible loss of retention induced by highly aqueous mobile phases, although the exact mechanism of reversible retention loss is not proven.

The BETASIL phase is a high carbon load, high density C18 and consequently shows the characteristics associated with chain folding, i.e. loss in retention. However, the situation is completely reversible and the column can be regenerated simply by flushing with 40% ACN / 60% H_2O for 10 minutes (Figure 5).

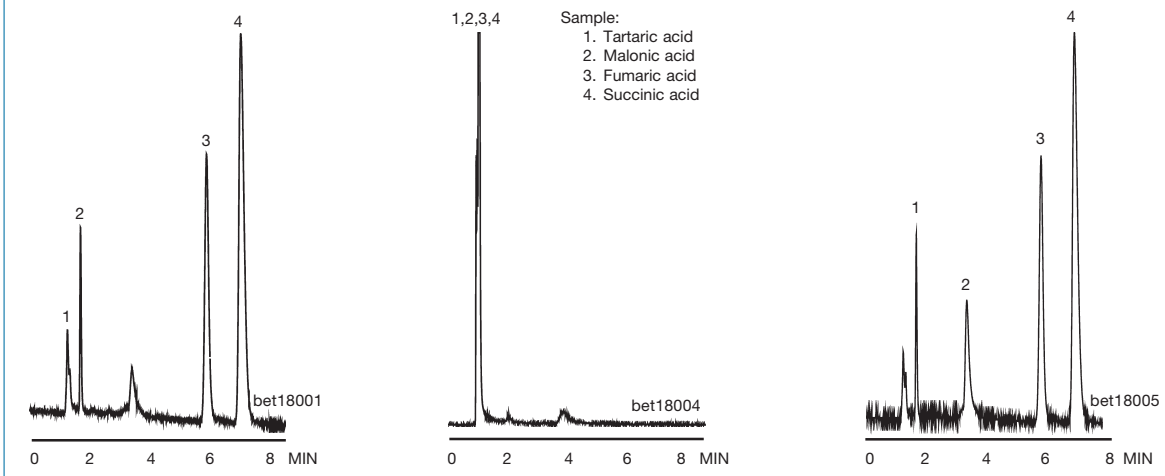
The phenomena of chain folding can be almost completely overcome by including trace amounts of organic solvent in the mobile phase. Figure 6 shows how just 0.3% n-propanol has been used to retard the loss in retention associated with chain collapse.

Choices in Stationary Phase Chemistries

BETASIL columns are available in a wide range of reversed phase packings, including C18, C8, C6, Phenyl, Cyano, and Phenyl/Hexyl. The BETASIL Phenyl/Hexyl phase provides a combination of straight-chain C6 groups and phenyl groups, resulting in a mixed-mode separation. The C6 chain exhibits classical reversed phase retention, while the phenyl ring provides special selectivity for polar groups (Fig. 7).

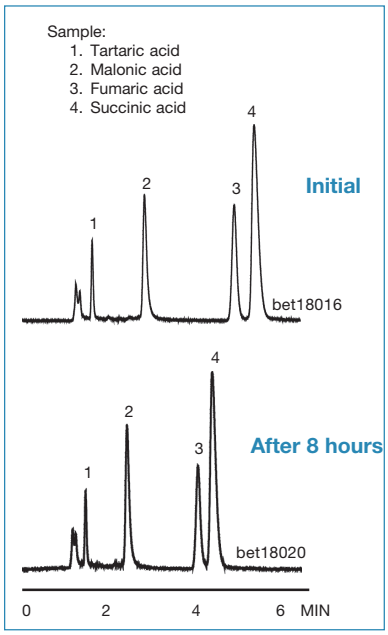
BETASIL columns also offer choices for normal phase chromatography, including silica and diol phases in 4 different pore sizes from 60Å to 300Å, and cyano. Diol phases provide higher polar selectivity than cyano with less water sensitivity than bare silica.

Figure 5. High Carbon Load C18 in 100% Aqueous Mobile Phase



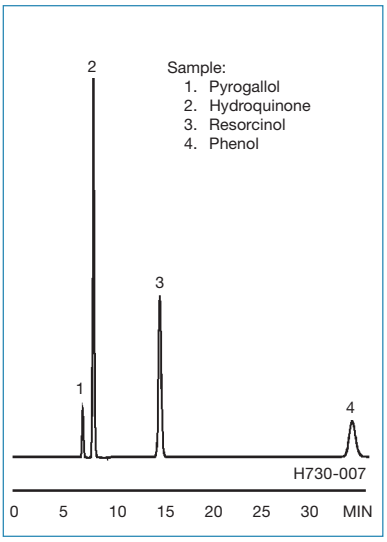
BETASIL C18, 150x4.6mm
Eluent: 0.05M KH_2PO_4 + 0.03M H_3PO_4
Flow: 1.0 mL/min
Detector: UV @ 210

Figure 6. Trace Organics in Mobile Phase Retard Chain Folding



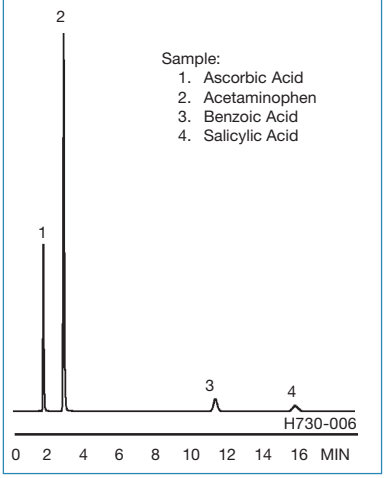
BETASIL C18, 150x4.6mm
Eluent: 0.05M KH_2PO_4 + 0.03M H_3PO_4 + 0.3% n-propanol
Flow: 1.0 mL/min
Detector: UV @ 210

Figure 7. Polyphenols



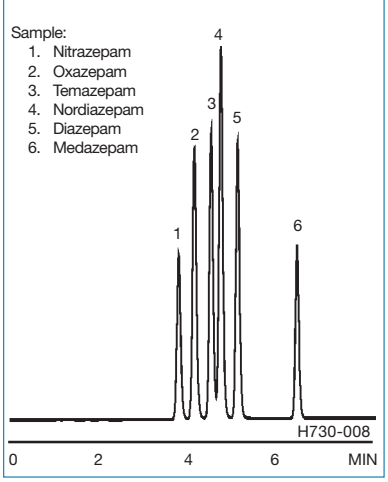
BETASIL Phenyl/Hexyl, 5µm, 150x4.6mm
Eluent: 5mM Acetic Acid
Flow: 1.0 mL/min
Detector: UV @ 280
Temp.: 25°C

Organic Acids



BETASIL® Phenyl/Hexyl, 5µm, 150x4.6mm
Eluent: 80% 20mM KH_2PO_4, pH 2 / 20% MeOH
Flow: 1.0 mL/min
Detector: UV @ 254
Temp.: 25°C

Benzodiazepines



BETASIL Phenyl/Hexyl, 5µm, 150x4.6mm
Gradient: A: H_2O
B: MeOH

Time	%B
0	15
7	85

Flow: 1.0 mL/min
Detector: UV @ 235
Temp.: 25°C

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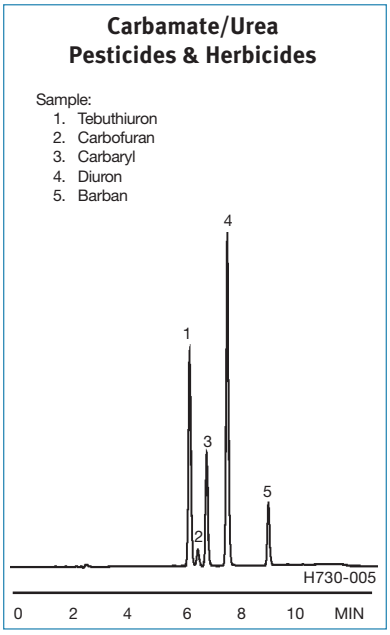
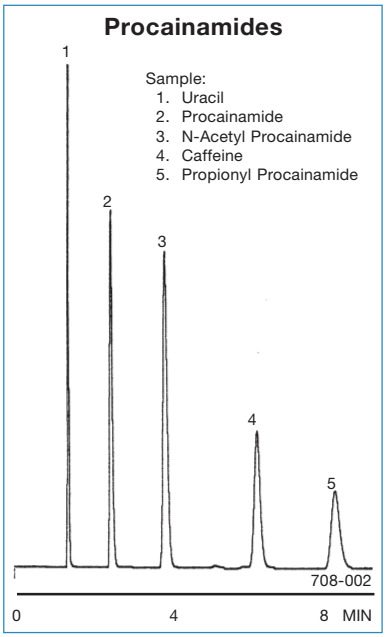


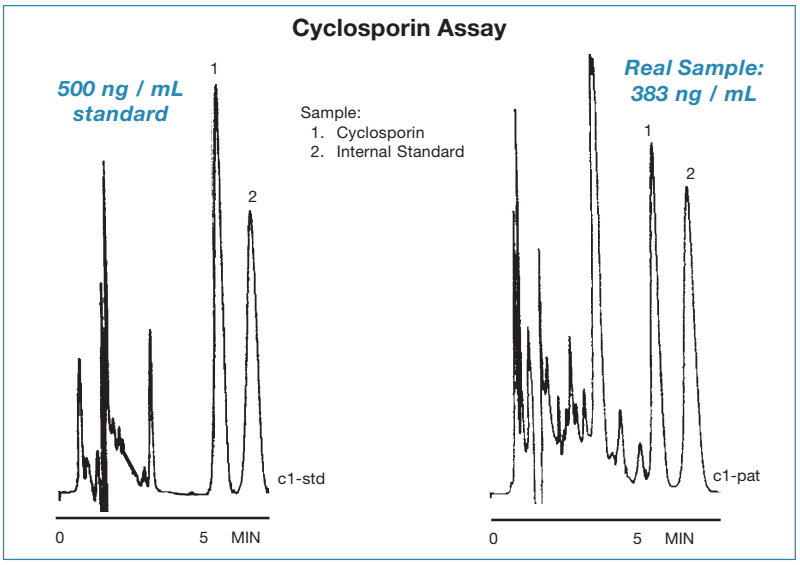
BETASIL Phenyl/Hexyl, 5µm, 150x4.6mm
Gradient: A: H_2O
B: ACN

Time	%B
0	15
7	85

Flow: 1.5 mL/min
Detector: UV @ 254
Temp.: 25°C



BETASIL C6, 5µm, 150x4.6mm
Eluent: 10% ACN / 90% 0.05M KH_2PO_4, pH 3.5 with H_3PO_4
Flow: 1.25 mL/min
Detector: UV@ 254



BETASIL C1, 5µm, 150x4.6mm
Eluent: 35% ACN / 65% 0.05M KH_2PO_4, pH 7
Flow: 1.25 mL/min
Detector: UV @ 254

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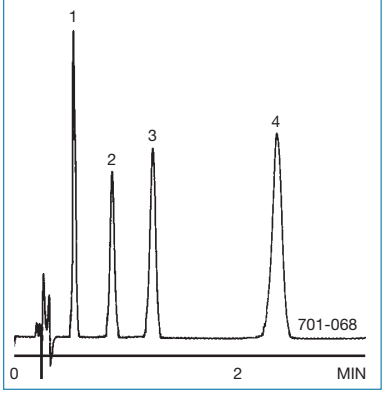
Common Analgesic

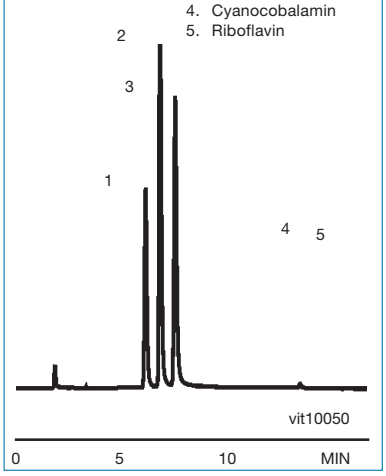
Sample:
1. Acetaminophen
2. Caffeine
3. Acetylsalicylic Acid
4. Benzoic Acid



BETASIL® C18 PIONEER™, 5µm, 50x3mm
Eluent: A: 0.05M Ammonium Acetate, pH 4
B: 70% ACN% / 30% MeOH
85:15 A:B
Flow: 2.5 mL/min
Detector: UV@ 254

Water-Soluble Vitamins

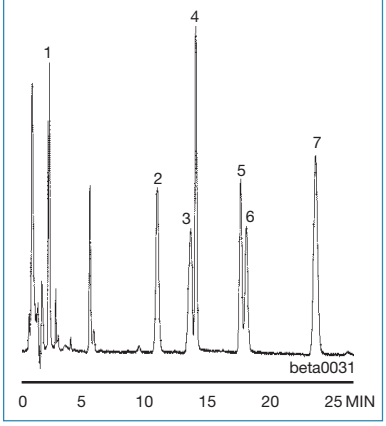
Sample:
1. Pyridoxine
2. Thiamine
3. Niacinamide
4. Cyanocobalamin
5. Riboflavin



BETASIL C18, 5µm, 150x4.6mm
Gradient: A: 0.05M K_2HPO_4, pH 6.5
B: ACN
2% B to 20% B in 15 min
Flow: 1.0 mL/min
Detector: UV @ 265

Pesticides

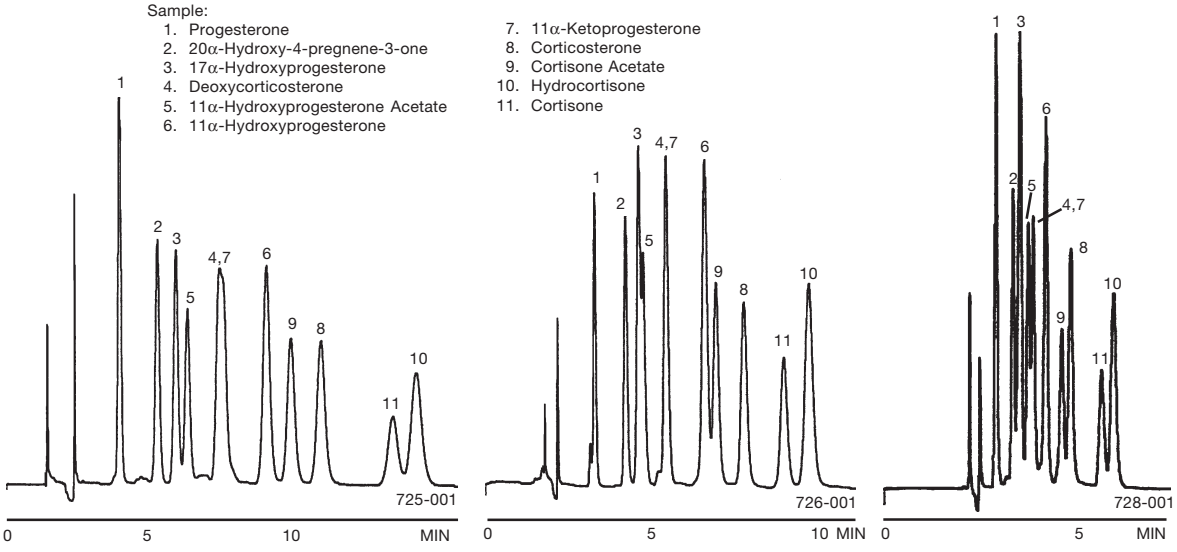
Sample:
1. Dimethoate
2. Methyl Parathion
3. Malathion
4. Fenitrothion
5. Ethyl Parathion (DNTP)
6. Diazinon
7. EPN



BETASIL C18, 5µm, 150x4.6mm
Gradient: 50% ACN / 50% H_2O, step to
60% ACN / 40% H_2O at 12 min.
Flow: 1.0 mL/min
Detector: UV @ 235

Steroids by Normal Phase

Sample:
1. Progesterone
2. 20α-Hydroxy-4-pregnene-3-one
3. 17α-Hydroxyprogesterone
4. Deoxycorticosterone
5. 11α-Hydroxyprogesterone Acetate
6. 11α-Hydroxyprogesterone
7. 11α-Ketoprogesterone
8. Corticosterone
9. Cortisone Acetate
10. Hydrocortisone
11. Cortisone



BETASIL Diol 60, 5µm, 150x4.6mm
Eluent: 85% Isooctane / 14.7% EtOH / 0.3% H_2O
Flow: 1.2 mL/min
Detector: UV @ 254

BETASIL Diol 100, 5µm, 150x4.6mm
Eluent: 85% Isooctane / 14.7% EtOH / 0.3% H_2O
Flow: 1.2 mL/min
Detector: UV @ 254

BETASIL Diol 300, 5µm, 150x4.6mm
Eluent: 85% Isooctane / 14.7% EtOH / 0.3% H_2O
Flow: 1.2 mL/min
Detector: UV @ 254

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