

## BETASIL® Columns

TG01-03



100Å high purity silica

Analyze • Detect • Measure • Control™

**Thermo**  
ELECTRON CORPORATION

## Introduction

- 100Å high purity silica
- High surface area and phase loading
- Designed for small molecules
- Type B silica gives good results with acids or bases
- High phase loading means mobile phases require more organic solvent (popular for mass spec)
- All reversed phases are end-capped
- Dense bonding results in excellent reproducibility and stability
- Excellent performance with acids, bases and neutral compounds

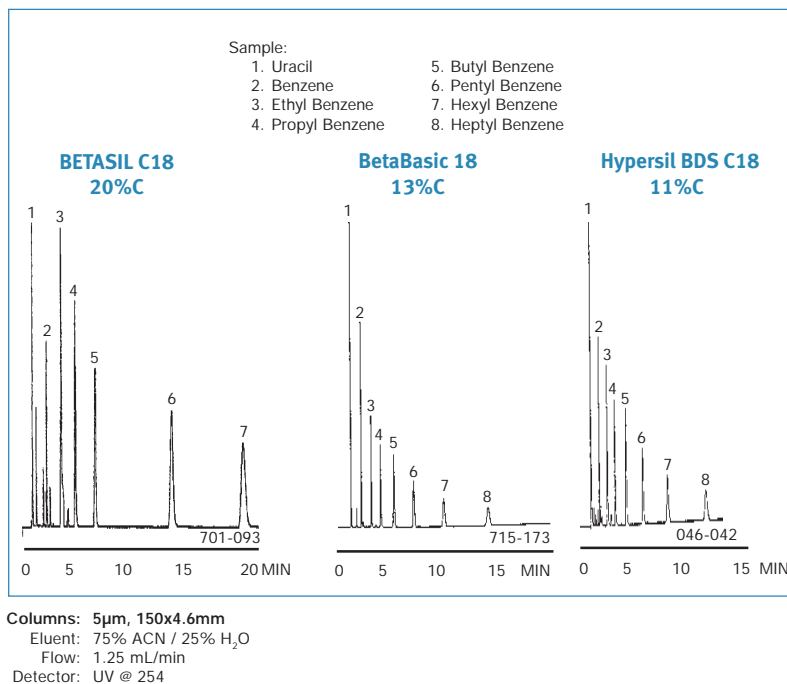
Specifications					
Phase	Particle size	Carbon Load	Pore Size	Endcapping	Silica type
BETASIL C18	3, 5 and 10µm	20%	100Å	Yes	High purity, base deactivated
BETASIL C8	3, 5 and 10µm	12%	100Å	Yes	High purity, base deactivated
BETASIL C6	3 and 5µm	11%	100Å	Yes	High purity, base deactivated
BETASIL C1	3 and 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 Hypersil-Keystone columns. The increased retention associated with the BETASIL column is directly attributed to the increased surface area of the particles, 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 the mobile phase, e.g. to increase LC-MS sensitivity, the BETASIL C18 packing offers an excellent choice.

Figure 1. Increased Hydrophobic Retention of Neutral Molecules



## 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.

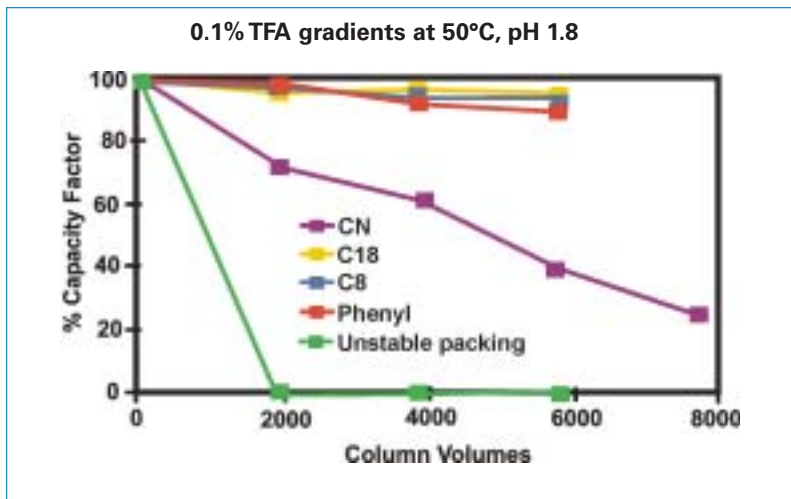
## Base Deactivated for Better Peak Shape with Basic Compounds

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

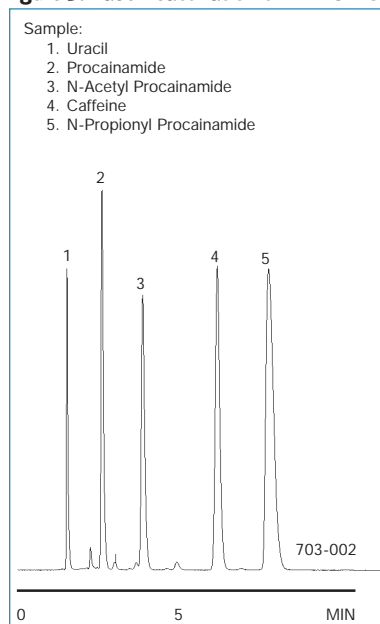
Figure 2. BETASIL Phase Stability At Low pH



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

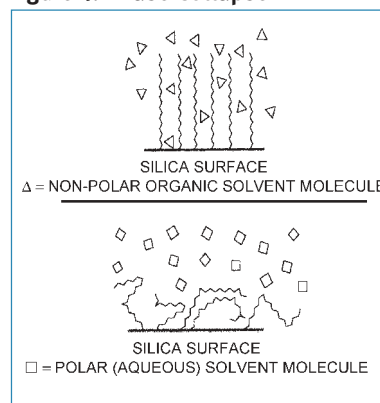
retention loss is that the hydrophobic alkyl chains of the stationary phase are not wettable and appear to "fold" down on the silica surface to avoid a highly aqueous, hydrophilic mobile phase (Figure 4).

Figure 3. Base Deactivation of BETASIL C6



BETASIL C6, 5µm, 150x4.6mm  
 Eluent: 10% ACN / 90% 0.05M Phosphate buffer, pH 3.5 with H<sub>3</sub>PO<sub>4</sub>  
 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<sub>2</sub>O 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. Further information on chain collapse can be found in Technical Bulletin TB99-01.

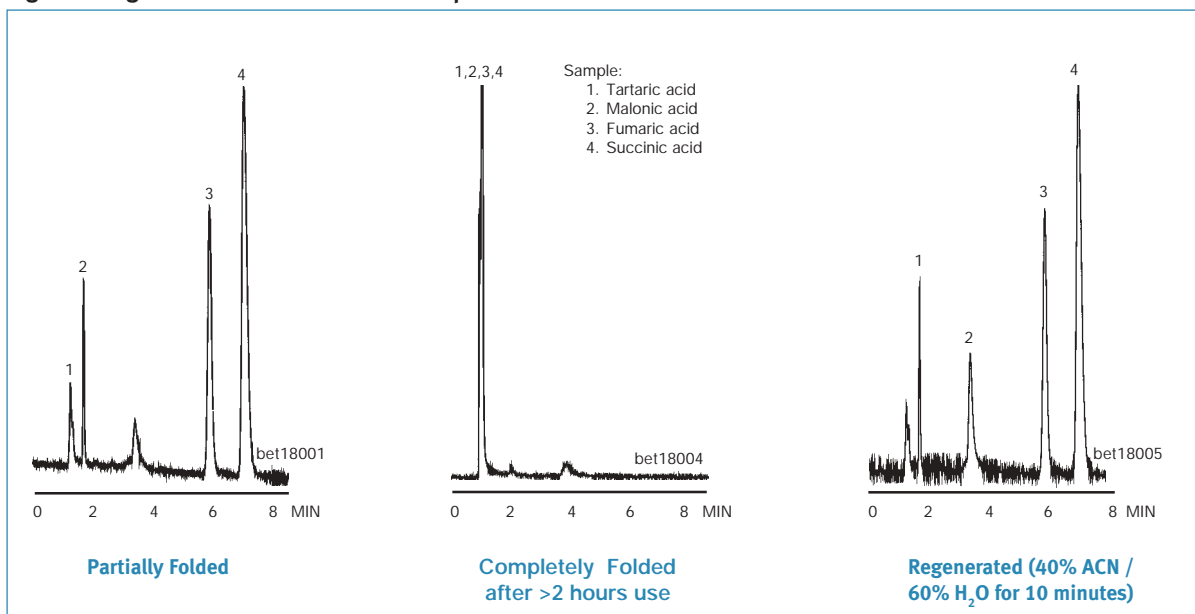
### Choices in Stationary Phase Chemistries

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).

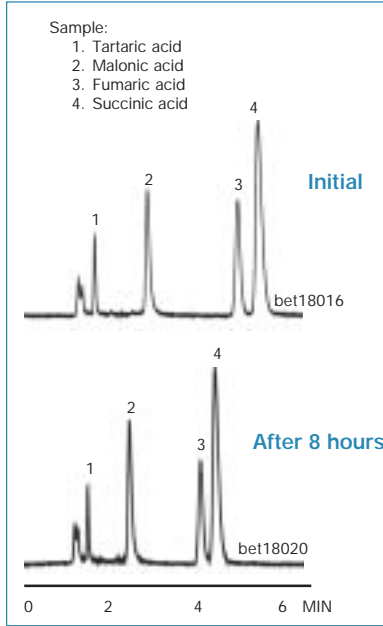
BETASIL columns also offer choices for normal phase chromatography, including silica and diol phases in 4 different pore sizes from 60Å to 300Å, and cyano. Diol phases provide higher polar selectivity than cyano with less water sensitivity than bare silica.

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



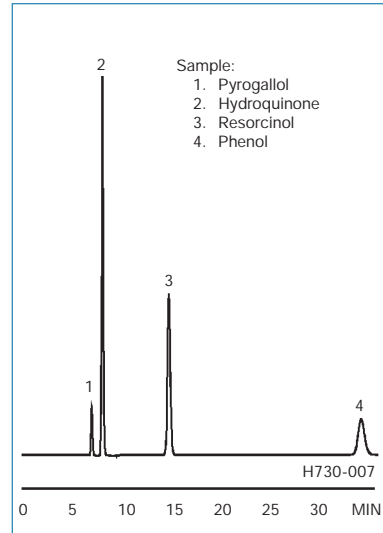
BETASIL C18, 150x4.6mm  
 Eluent: 0.05M KH<sub>2</sub>PO<sub>4</sub> + 0.03M H<sub>3</sub>PO<sub>4</sub>  
 Flow: 1.0 mL/min  
 Detector: UV @ 210

**Figure 6. Trace Organics in Mobile Phase Retard Chain Folding**

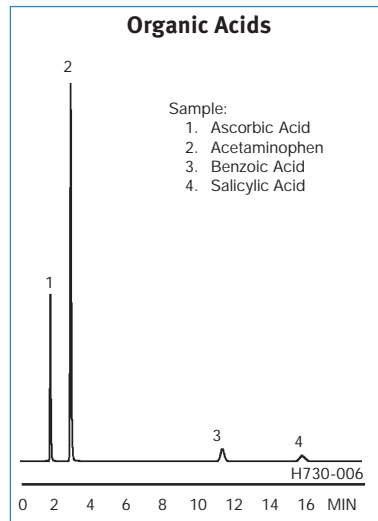


**BETASIL C18, 150x4.6mm**  
 Eluent: 0.05M  $\text{KH}_2\text{PO}_4$  + 0.03M  $\text{H}_3\text{PO}_4$  + 0.3% n-propanol  
 Flow: 1.0 mL/min  
 Detector: UV @ 210

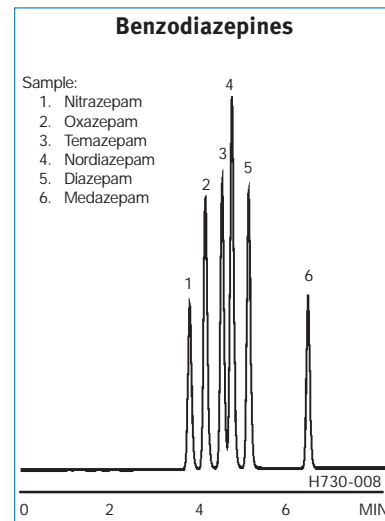
**Figure 7. Polyphenols**



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



**BETASIL® Phenyl/Hexyl, 5µm, 150x4.6mm**  
 Eluent: 80% 20mM  $\text{KH}_2\text{PO}_4$ , pH 2 / 20% MeOH  
 Flow: 1.0 mL/min  
 Detector: UV @ 254  
 Temp.: 25°C



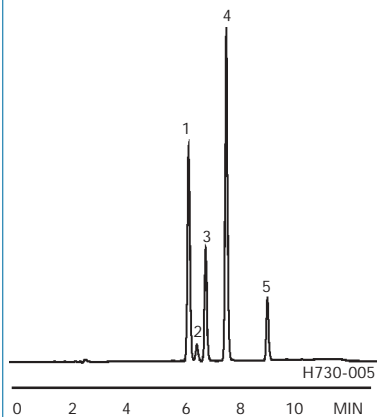
**BETASIL Phenyl/Hexyl, 5µm, 150x4.6mm**  
 Gradient: A:  $\text{H}_2\text{O}$   
 B: MeOH

Time	%B
0	15
7	85

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

### Carbamate/Urea Pesticides & Herbicides

- Sample:
1. Tebuthiuron
  2. Carbofuran
  3. Carbaryl
  4. Diuron
  5. Barban



BETASIL Phenyl/Hexyl, 5µm, 150x4.6mm

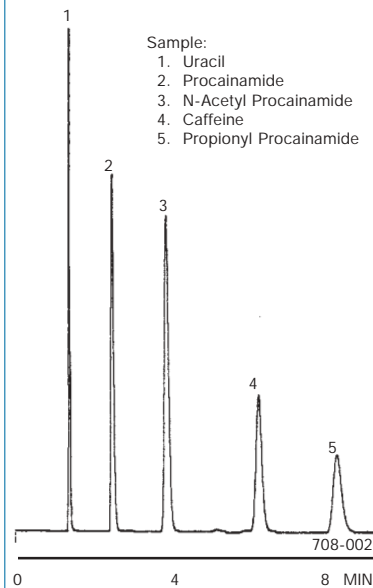
Gradient: A: H<sub>2</sub>O  
B: ACN

Time	%B
0	15
7	85

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

### Procainamides

- Sample:
1. Uracil
  2. Procainamide
  3. N-Acetyl Procainamide
  4. Caffeine
  5. Propionyl Procainamide



BETASIL C6, 5µm, 150x4.6mm

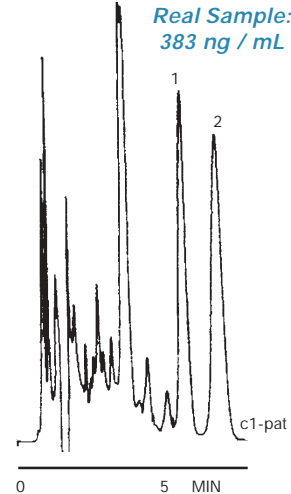
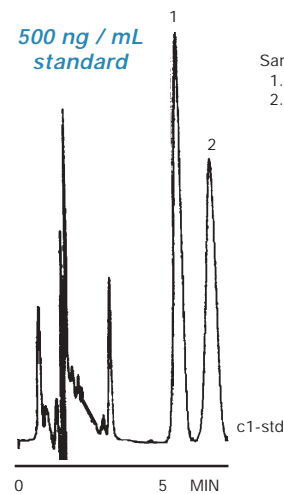
Eluent: 10% ACN / 90% 0.05M KH<sub>2</sub>PO<sub>4</sub>,  
pH 3.5 with H<sub>3</sub>PO<sub>4</sub>  
Flow: 1.25 mL/min  
Detector: UV@ 254

### Cyclosporin Assay

500 ng / mL  
standard

Real Sample:  
383 ng / mL

- Sample:
1. Cyclosporin
  2. Internal Standard



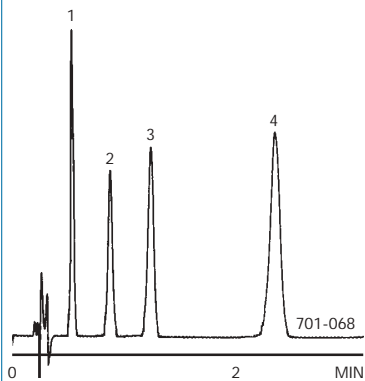
BETASIL C1, 5µm, 150x4.6mm

Eluent: 35% ACN / 65% 0.05M KH<sub>2</sub>PO<sub>4</sub>, pH 7  
Flow: 1.25 mL/min  
Detector: UV @ 254

### Common Analgesic

Sample:

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

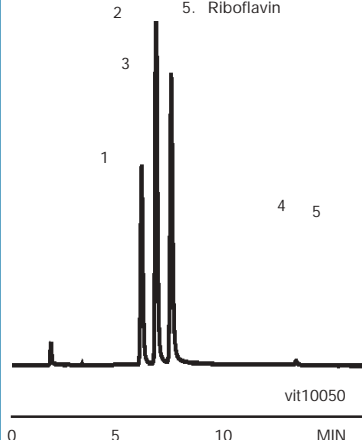


**BETASIL® C18 PIONEER™, 5µm, 50x3mm**  
 Eluent: A: 0.05M Ammonium Acetate, pH 4  
 B: 70% ACN / 30% MeOH  
 85:15 A:B  
 Flow: 2.5 mL/min  
 Detector: UV @ 254

### Water-Soluble Vitamins

Sample:

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

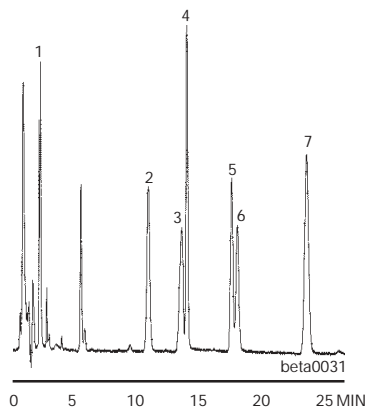


**BETASIL C18, 5µm, 150x4.6mm**  
 Gradient: A: 0.05M K<sub>2</sub>HPO<sub>4</sub>, pH 6.5  
 B: ACN  
 2% B to 20% B in 15 min  
 Flow: 1.0 mL/min  
 Detector: UV @ 265

### Pesticides

Sample:

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

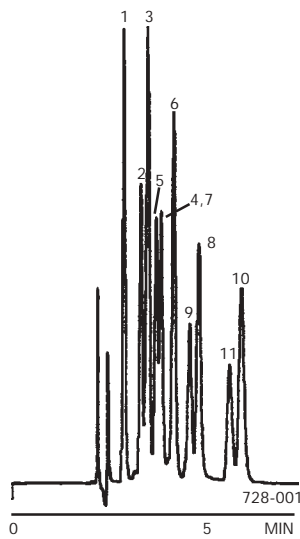
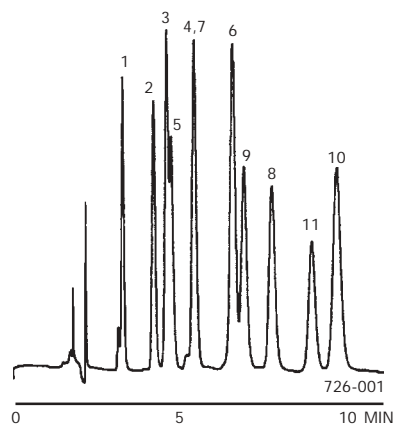
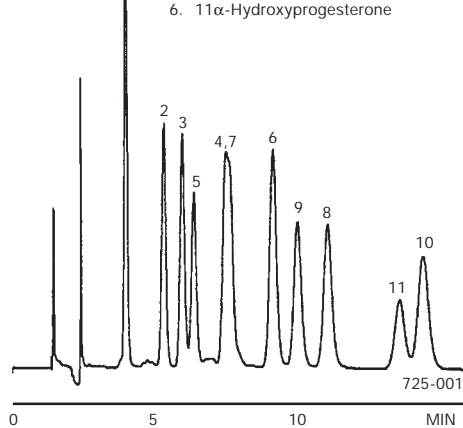


**BETASIL C18, 5µm, 150x4.6mm**  
 Gradient: 50% ACN / 50% H<sub>2</sub>O, step to  
 60% ACN / 40% H<sub>2</sub>O at 12 min.  
 Flow: 1.0 mL/min  
 Detector: UV @ 235

### Steroids by Normal Phase

Sample:

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



**BETASIL Diol 60, 5µm, 150x4.6mm**  
 Eluent: 85% Isooctane / 14.7% EtOH / 0.3% H<sub>2</sub>O  
 Flow: 1.2 mL/min  
 Detector: UV @ 254

**BETASIL Diol 100, 5µm, 150x4.6mm**  
 Eluent: 85% Isooctane / 14.7% EtOH  
 / 0.3% H<sub>2</sub>O  
 Flow: 1.2 mL/min  
 Detector: UV @ 254

**BETASIL Diol 300, 5µm, 150x4.6mm**  
 Eluent: 85% Isooctane / 14.7%  
 EtOH / 0.3% H<sub>2</sub>O  
 Flow: 1.2 mL/min  
 Detector: UV @ 254

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