ProntoSIL C18-EPS Reversed-Phase HPLC Columns

- **Provides excellent separation of polar compounds**
- **Better peak shape for acids and bases**
- **Stabilized bonded phase for rugged, robust HPLC methods**
- **More retentive than ordinary polar embedded phases**

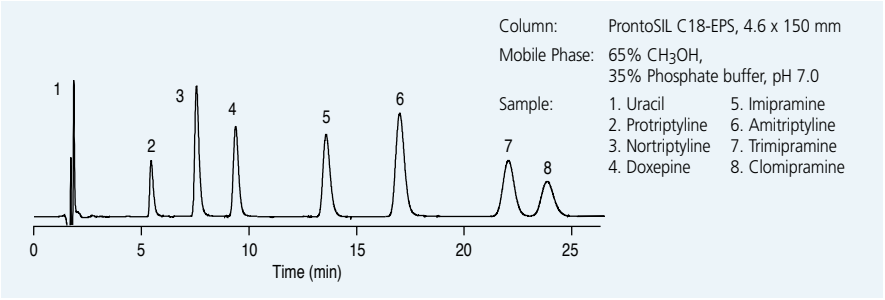
Enhanced Polar Selectivity for High Resolution Separations of Polar Compounds

A high performance base deactivated column with enhanced polar selectivity specifically designed for high resolution separation of polar compounds.

Provides Excellent Separation of Polar Compounds

Base deactivated stationary phases generally provide better peak shape, increased column efficiency, and improved lot-to-lot reproducibility when separating polar compounds. They have been particularly useful in improving the separation of samples containing basic compounds. However, there are occasions when typical base deactivated phases lack adequate selectivity.

FIGURE 1
Enhanced Polar Selectivity and Excellent Peak Shape for Basic Compounds



ProntoSIL C18-EPS is specifically designed for high resolution separations of polar compounds. A unique polar embedded group adds polar selectivity to this highly retentive phase and also shields the silica surface so that excellent peak shape for basic compounds can be achieved.

Specifications

Phase: C18 with amide embedded group

Particle Size: 3 and 5 µm

Pore Size: 120Å

Surface Area: 300 m^2/g

Carbon Load: 18%

pH Range: 1 - 10

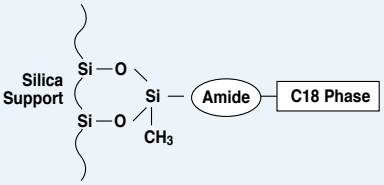
Manufacturers of base deactivated columns try to minimize polar-polar interactions between analytes and the stationary phase. Because of this, most base deactivated columns have similar selectivity for polar compounds. This means that if one brand of base deactivated column lacks selectivity to adequately separate a pair of polar solutes, other brands of base deactivated columns will also probably lack adequate selectivity for these solutes.

Stationary phases that permit polar-polar interaction through silanol activity offer alternate selectivity to base deactivated phases, but it often comes at a cost of poor peak shape and uncertain reproducibility. ProntoSIL C18-EPS solves this problem by providing both enhanced polar selectivity and excellent peak shape (Figure 1).

Enhanced Polar Selectivity

The ProntoSIL C18-EPS has an amide group strategically placed in the bonded phase (Figure 2). This polar amide group adds polar characteristics to this very hydrophobic stationary phase (Figure 3). With this enhanced polar selectivity, ProntoSIL C18-EPS provides a powerful alternate selectivity to typical base deactivated phases.

FIGURE 2
ProntoSIL C18-EPS Bonded Phase

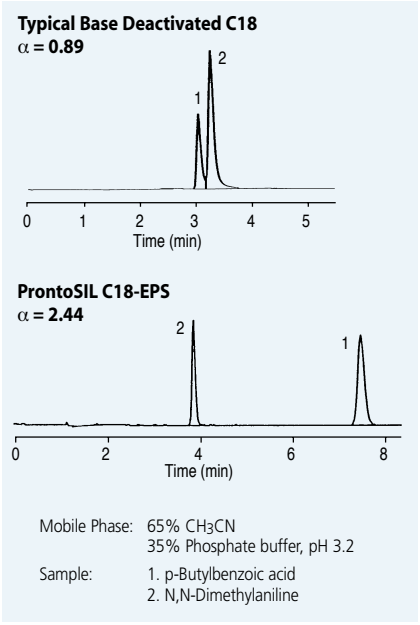


The ProntoSIL C18-EPS uses an amide group to add polar selectivity to this highly hydrophobic stationary phase.

Better Peak Shape for Acids and Bases

ProntoSIL C18-EPS has an amide group strategically placed close to the surface of the silica support. This not only permits the amide group to provide polar characteristics to the stationary phase, it also shields the silica surface and inhibits polar interactions between solutes and silanols. This "shielding" allows for exceptionally good peak shape for bases. Unlike some other polar embedded phases, ProntoSIL C18-EPS also provides excellent peak shape for acids.

FIGURE 3
Enhanced Polar Selectivity



The ProntoSIL C18-EPS column with enhanced polar selectivity provides significantly better selectivity for this pair of polar solutes than a typical base deactivated column.

Stabilized Bonded Phase for Rugged, Robust HPLC Methods

A major cause of column failure is the loss of bonded phase from the silica support due to hydrolysis of the siloxane bond. ProntoSIL C18-EPS uses a unique, proprietary bidentate bonding chemistry that provides a dual bond to the silica surface that stubbornly resists loss of bonded phase (Figure 4). This stabilized bonded phase greatly extends the lifetime of ProntoSIL C18-EPS columns.

The proprietary bonding chemistry also inhibits dissolution of the silica. The result is an unusually stable column that can confidently be used over a pH range much wider than other reversed phase columns, 1 to 10!

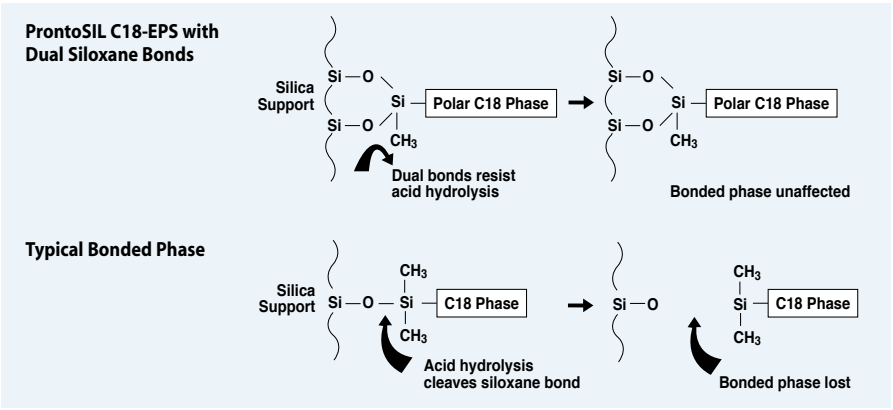
More Retention than Ordinary Polar Embedded Phases

Other polar embedded phases use either less hydrophobic C14 or C16 phases, or have part of their alkyl phase shielded by their polar embedded group. ProntoSIL C18-EPS is a true C18 phase with an octadecyl phase placed after the polar amide group. As a result, ProntoSIL C18-EPS provides more retention than other polar embedded phases (Figure 5).

Summary

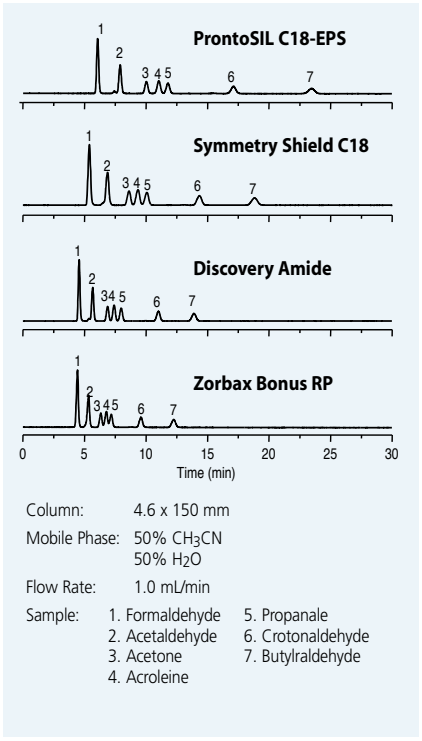
ProntoSIL C18-EPS columns with enhanced polar selectivity give you a powerful advantage when developing separations of polar compounds. Not only will this column provide you with excellent peak shape for both acids and bases, its stabilized bonded phase will allow you to develop rugged, robust HPLC methods over a broad pH range – 1 to 10! Best of all, ProntoSIL columns are sold and supported by MAC-MOD Analytical, the people you have come to trust when it comes to HPLC columns.

FIGURE 4
Dual Bonding Resists Loss of Bonded Phase



Acid hydrolysis of the siloxane bond and the resulting loss of bonded phase (i.e., C18 Phase) is one of the major reasons for column failure. ProntoSIL C18-EPS uses bidentate bonding chemistry to secure the polar C18 phase to the silica support through a dual siloxane bond. The dual bonding inhibits the loss of bonded phase and makes these columns unusually stable, even under conditions that quickly kill other columns.

FIGURE 5
Comparison of Retention



ProntoSIL C18-EPS is more hydrophobic than other polar embedded phases and, therefore, provides more retention.

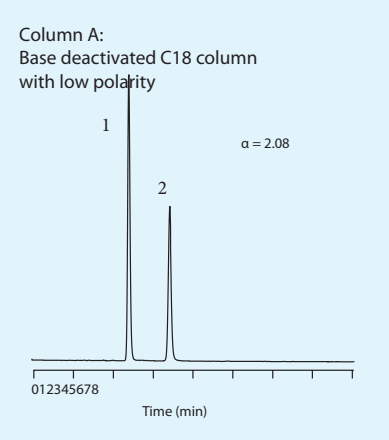
ProntoSIL C18-EPS Ordering Information

Dimensions (mm)	Particle Size (µm)	Part Number
2.0 x 50	3	0502F18APS030
2.0 x 50	5	0502F18APS050
2.0 x 75	3	0702F18APS030
2.0 x 75	5	0702F18APS050
2.0 x 100	3	1002F18APS030
2.0 x 100	5	1002F18APS050
2.0 x 150	3	1502F18APS030
2.0 x 150	5	1502F18APS050
2.0 x 250	5	2502F18APS050
4.6 x 50	3	0546F18APS030
4.6 x 50	5	0546F18APS050
4.6 x 75	3	0746F18APS030
4.6 x 75	5	0746F18APS050
4.6 x 100	3	1046F18APS030
4.6 x 100	5	1046F18APS050
4.6 x 150	3	1546F18APS030
4.6 x 150	5	1546F18APS050
4.6 x 250	5	2546F18APS050
8.0 x 250	5	2580F18APS050
20.0 x 250	5	2520F18APS050
Analytical Guard Cartridges (Guard Catridge Holder required)		
2.0 x 10 (5pk)	5	6321F18APS050
4.0 x 10 (5pk)	5	6301F18APS050
Guard Cartridge Holder		15010508
Semi-Preparative and Preparative Guard Columns		
8.0 x 30	5	0480F18APS050G
8.0 x 30	10	0480F18APS100G
20.0 x 33	5	0320F18APS050G
20.0 x 33	10	0320F18APS100G

ProntoSIL C18-EPS Reversed-Phase HPLC Columns

Using New Polar Embedded Phases to Optimize Reversed Phase Separations

Figure 1
High Polarity Stationary Phases Offer Alternate Selectivity to Base Deactivated Phases



Column B (significant silanol activity) provides greater selectivity than Column A for these polar solutes but at a cost of poorer peak shape for salicylic acid.

Introduction

Base deactivated stationary phases have been a real asset to chromatographers who develop HPLC separations of polar compounds. Columns packed with these base-deactivated phases provide better peak shape, increased column efficiency, and improved lot-to-lot reproducibility, especially when separating bases.

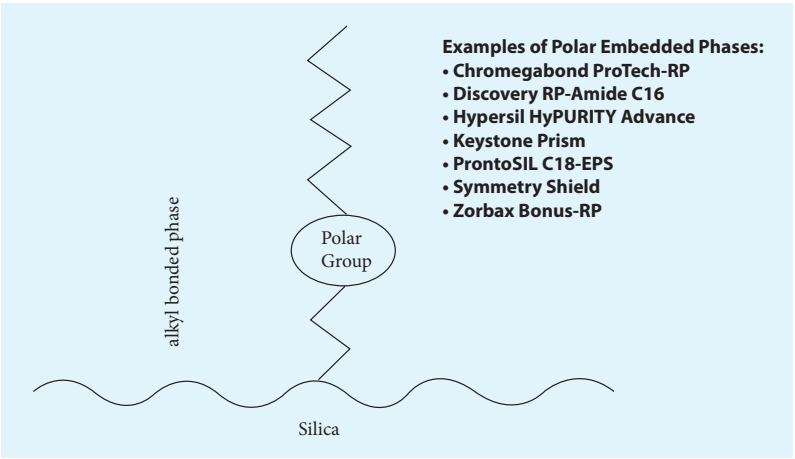
However, there are occasions when a typical base deactivated phase may not provide an optimum separation. Manufacturers of base deactivated columns try to minimize interactions between polar solutes and silanol groups on the surface of the stationary phase support. This produces stationary phases with low polarity and these low polarity phases will all have similar selectivity for polar compounds. This means that if one brand of base deactivated column lacks selectivity to adequately separate a pair of polar solutes, other brands of base deactivated columns will also probably lack adequate selectivity.

To optimize the separation, a more polar stationary phase may be necessary. Unfortunately, stationary phases that have polar characteristics due to silanol activity will often exhibit poor peak shape and uncertain reproducibility when separating polar compounds (Figure 1).

To solve this problem, column manufacturers have developed a new type of base deactivated stationary phase with polar groups, such as amides or carbamates, "embedded" in the bonded phase (Figure 2).

These polar embedded phases provide polar selectivity without the poor chromatographic performance associated with stationary phases that have high silanol activity.

Figure 2
Polar Embedded Phases



By embedding a polar group, such as an amide or carbamate, in the bonded phase a new type of base deactivated column with polar selectivity can be created.

Strategy for Developing Reversed Phase Separations of Ionic Compounds

Table 1 provides a brief outline of a typical strategy used to develop an isocratic separation of polar compounds.

As mentioned before, typical base deactivated columns sometimes fail to provide adequate selectivity for mixtures of polar compounds.

This is where polar embedded phases should be used.

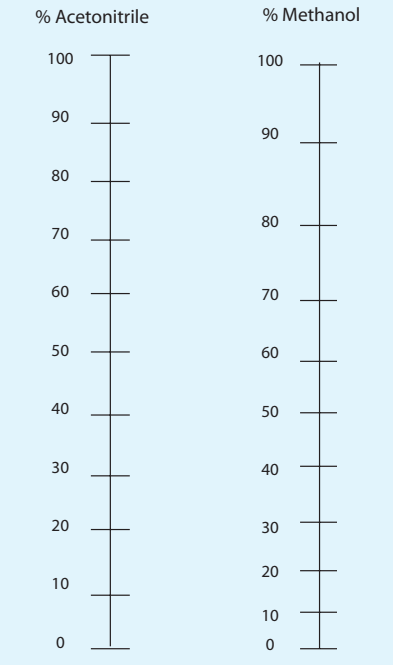
The polar characteristics of these special base deactivated columns will often provide significantly better selectivity for mixtures of acids and bases.

Therefore, if during the process of developing a separation you find that a typical C18 or C8 column does not provide adequate selectivity, your next step should be to select a polar embedded phase column to evaluate.

Table 1
Strategy for Developing Reversed Phase Separations of Polar Compounds

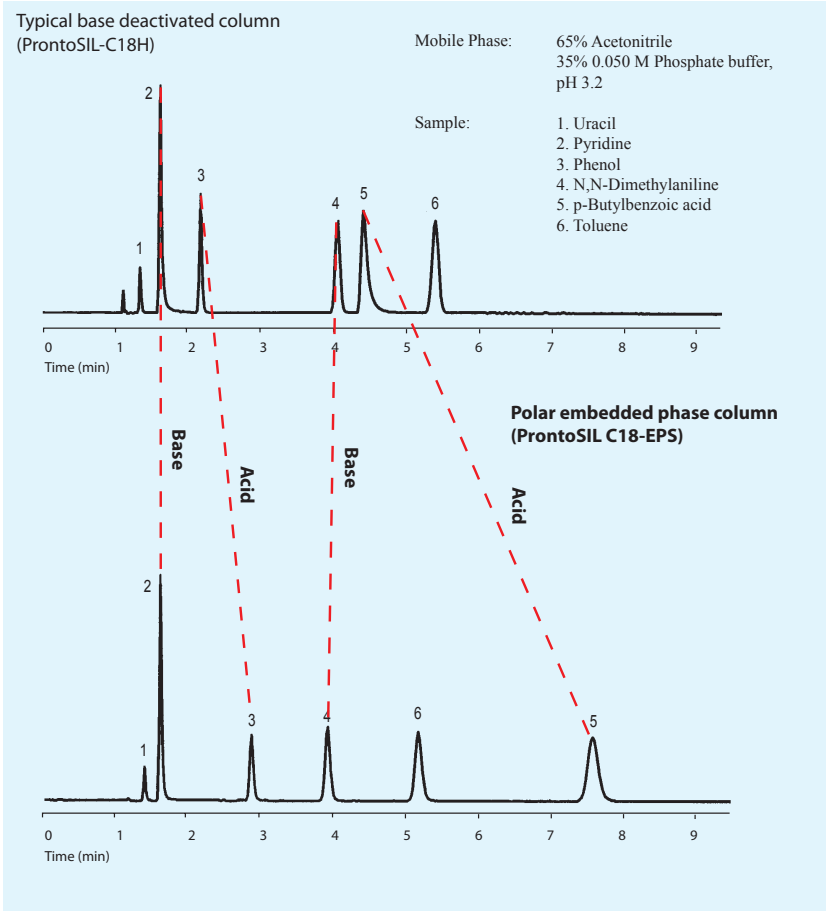
- Select a base deactivated C18 or C8 column.
- Use a mobile phase consisting of acetonitrile and 0.025 — 0.050 M potassium phosphate at a pH less than 3.0.
- Adjust the amount of acetonitrile in the mobile phase until the k values of all peaks are between 1 and 10.
- Evaluate the selectivity, and if it is unacceptable, (i.e., not all peaks of interest are adequately separated), substitute methanol for acetonitrile and again evaluate selectivity. Figure 4 will help you calculate the amount of methanol needed in the mobile phase to keep the elution strength constant.
- If selectivity is still unacceptable, choose a different stationary phase to evaluate. This can be a different brand of C18, but your best bet is to try a different bonded phase chemistry such as CN, Phenyl, or a polar embedded phase.
- Once the selectivity is acceptable, optimize the separation by adjusting the mobile phase strength (% organic solvent composition) and flow rate until all peaks are adequately separated (Rs > 1.5) and the system back pressure and overall separation time is suitable for the application. In a rigorous method development process, mobile phase pH, mobile phase additives (buffer concentration, ion pair reagents, amine modifiers, etc.), column temperature, and column configuration should also be evaluated and optimized.

Figure 3
Solvent Elution Strength Comparison



Acetonitrile is a stronger solvent than methanol for reversed phase HPLC. This nomograph will help you keep the mobile phase elution strength constant when you switch from acetonitrile to methanol. For example, you will need about 62% methanol in the mobile phase to equal the eluting strength of 50% acetonitrile.

Figure 4
Polar Embedded Phases Offer Alternate Selectivity to Typical Base Deactivated Phases



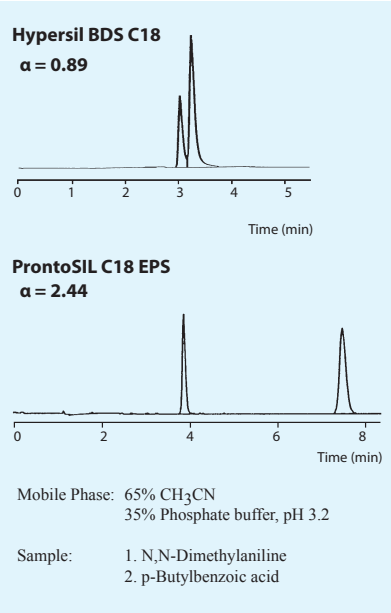
In general, polar embedded phases will retain acids longer and bases slightly less than typical base deactivated columns.

Using The Polar Selectivity of Polar Embedded Phases to Optimize a Separation

Columns packed with polar embedded phases are used in the same way as typical reversed phase columns and the separation strategy outlined in Table 1 is also appropriate for these columns.
However, you should expect significant differences in selectivity from polar embedded phases due to their polar selectivity.

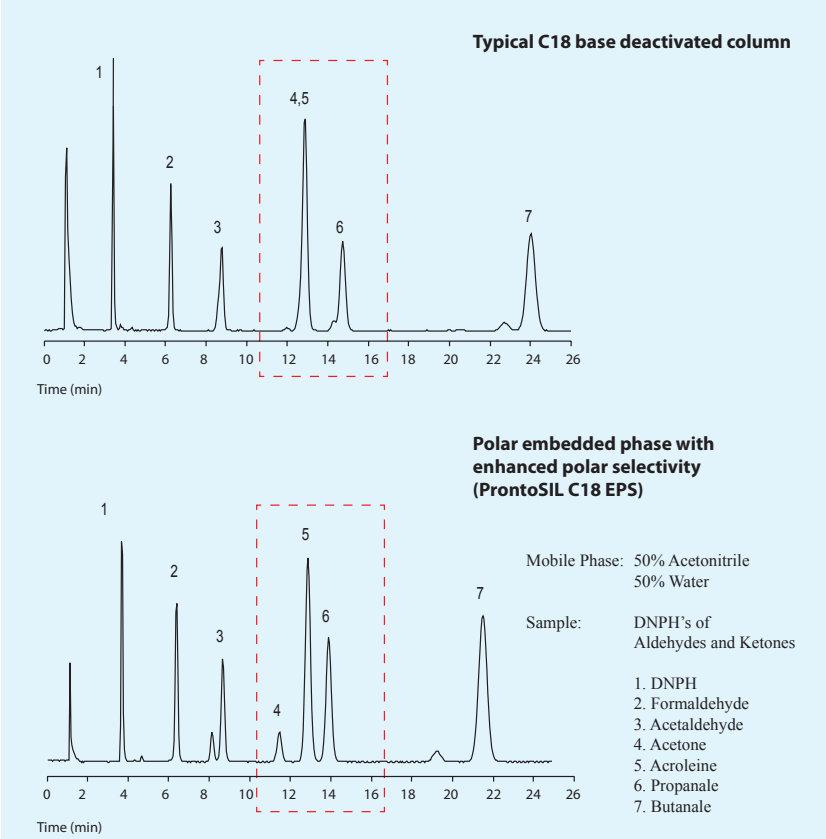
The polar selectivity of polar embedded phases comes from the interaction between the amide or carbamate group and polar solutes. In general you can expect that acidic compounds will be retained longer and basic compounds will be retained slightly less on polar embedded phases compared to typical reversed phase columns (Figure 4) . Sometimes the selectivity differences can be dramatic, as shown in Figure 5, and sometimes they may be more subtle but still significant in optimizing a separations, as shown in Figure 6.

FIGURE 5
Polar Embedded Phases Can Offer Dramatic Improvements in Selectivity



The ProntoSIL C18-EPS with enhanced polar selectivity provides significantly better selectivity for this pair of polar solutes than a typical base deactivated column (Hypersil BDS C18).

Figure 6
Subtle Differences in Stationary Phase Selectivity Can Be Useful in Optimizing HPLC Separations

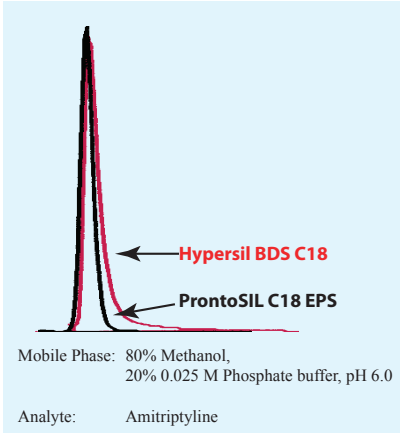


The polar selectivity offered by polar embedded phases will often provide a better separation. In this separation of DNPH's of aldehydes and ketones, compounds 4 and 5 co-elute on a typical base deactivated phase, but are well separated on a polar embedded phase.

Improved Peak Shape

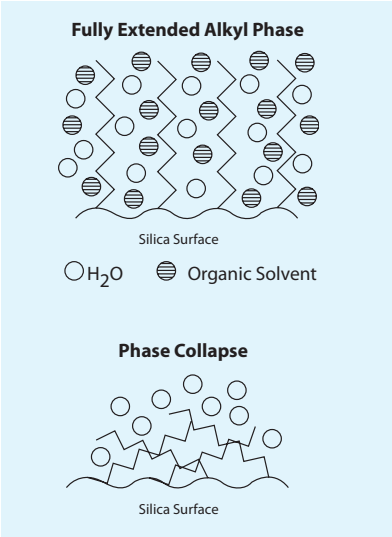
Most polar embedded phases use high purity silica as the stationary phase support and thoroughly cover the silica with bonded phase to reduce interaction between acidic silanols and polar solutes.
This in itself would produce a stationary phase with good peak shape for basic compounds, but polar embedded phases also have the advantage of further deactivating the stationary phase support
by means of the amide or carbamate groups.
Although the mechanism is not fully understood, the prevailing view is that the polar embedded groups interact with unbonded silanols on the silica stationary phase support and thereby block them from interacting with polar solutes.
The effect is similar to adding an amine modifier to the mobile phase.
This "polar shielding," as some manufacturers call it, gives these phases excellent peak shape for basic compounds over a broad pH range (Figure 7).

Figure 7
Polar Embedded Phases Provide Excellent Peak Shape for Basic Compounds



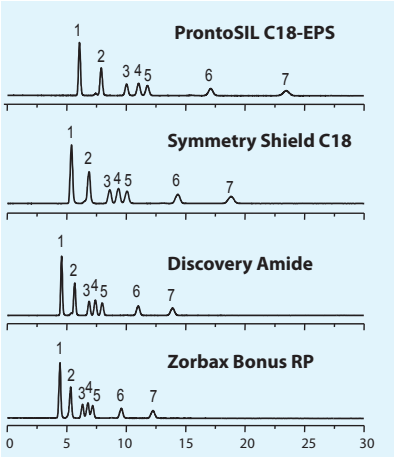
The amide group of the ProntoSIL C18-EPS shields the silica surface and prevents solutes from interacting with silanol groups. The result is exceptionally good peak shape for even difficult basic compounds.

Figure 8
Phase Collapse



When operating with less than 10% organic modifier in the mobile phase, typical C18 and C8 phases are susceptible to phase collapse.
Polar embedded phases do not have this problem and are preferred over typical base deactivated phases for high aqueous mobile phase conditions.

Figure 9
Comparison of Retention of Some Popular Polar Embedded Phases



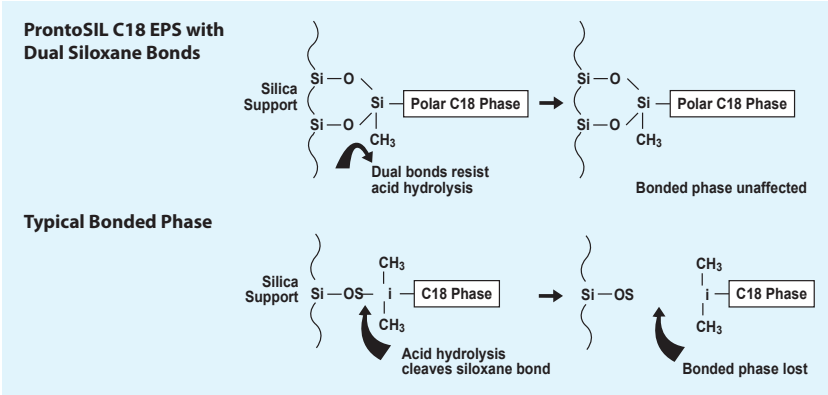
Column: 4.6 x 150 mm
Mobile Phase: 50% CH_3CN
50% H_2O

Flow Rate: 1.0 mL/min

Sample:
1. Formaldehyde
2. Acetaldehyde
3. Acetone
4. Acroleine
5. Propanale
6. Crotonaldehyde
7. Butylraldehyde

Most polar embedded phase columns are less hydrophobic, and thus less retentive than typical reversed phase columns. ProntoSIL C18-EPS, however, is an exception and provides comparable retention to other C18 phases. In this example, we see that ProntoSIL C18-EPS is the most retentive of these four polar embedded phases.

Figure 10
Bonded Phase on the ProntoSIL C18-EPS is Secured Through Dual Siloxane Bonds



Acid hydrolysis of the siloxane bond and the resulting loss of bonded phase (i.e., C18 Phase) is one of the major reasons for column failure. ProntoSIL C18-EPS uses unique bonding chemistry to secure the polar C18 phase to the silica support through dual siloxane bonds. The dual bonding inhibits the loss of bonded phase and makes these columns unusually stable.

Polar Embedded Phases Are Preferred for High Aqueous Mobile Phase Conditions

The alkyl bonded phase of typical C18 and C8 columns undergo what many researchers call "phase collapse," or "matting," when operating with less than 10% organic modifier in the mobile phase (Figure 8). As phase collapse progresses, the availability of the alkyl phase to interact with solutes decreases and retention time decreases.
Polar embedded phases can be used with high aqueous mobile phases without the problem of phase collapse.
The polar embedded groups permit the stationary phase surface to remain "wet" even under 100% aqueous mobile phase conditions.
This keeps the bonded phase fully extended into the mobile phase, eliminates phase collapse, and facilitates the retention of highly water soluble compounds that may be poorly retained on typical reversed phase columns.
Ion pair reagents can be avoided so that chromatographic conditions are simpler and methods are more rugged.

Retention Comparison

In general, polar embedded phase columns are less hydrophobic and therefore less retentive than typical reversed phase columns.
ProntoSIL C18-EPS, however, is an exception. It provides similar retention as other C18 phases for neutral and hydrophobic compounds and slightly
more retention for some highly water soluble compounds. Figure 9 provides a comparison of retention for four popular polar embedded phases.

Bonded Phase Stability

There is no reason to believe that polar embedded phases are any less stable than typical C18 and C8 phases.
Most manufacturers report stability data to support this. One column, Zorbax Bonus RP, uses unique silanes with bulky side groups to add stability to its bonded phase.
Another column, ProntoSIL C18-EPS, uses dual siloxane bonds to increase bonded phase stability and actually demonstrates much greater durability than other C18 phases as well as other polar embedded phases (Figure 10).
The next time you encounter poor selectivity while trying to separate polar solutes on a base deactivated phase, try one of these new polar embedded phases. You will be more likely to achieve a satisfactory separation with the polar embedded phase than with another brand of base deactivated column.