

Hypersil® BDS Columns

TG01-05



Highly base deactivated

Analyze • Detect • Measure • Control™

Thermo
ELECTRON CORPORATION

Introduction

Hypersil BDS columns have gained a reputation over the years as one of the most robust, reproducible and reliable HPLC column brands available. This Technical Guide provides an overview of the Hypersil BDS product range, its strengths, and how quality and reproducibility of the phases and columns are monitored.

- Highly base deactivated
- Excellent reproducibility
- Reduced tailing
- Very robust and rugged with long column lifetimes
- Excellent peak symmetry for basic and acid compounds
- All phases are endcapped

Base Deactivated Silica and Bonded Phases

The use of covalently-bonded silica stationary phases in HPLC allows the analysis of a broad range of analytes. Along with rapid equilibration times, and significantly improved mass transfer characteristics over liquid-liquid partition chromatography, this has resulted in the hugely successful advancement of HPLC as a modern day analytical technique. However, covalently-bonded silica stationary phases often have specific limitations.

Many chemical properties associated with derivatized silicas used in HPLC have a strong effect on analyte interactions. These properties are specific to either the derivatized ligand itself, or the remaining underivatized silanol groups on the silica surface. In particular, the number and acidity of these remaining silanol groups is

of significance. It is the silanol groups that are responsible for the acid-base properties of the base silica, contributing to the overall polarity of the surface even when the surface method is derivatized. Their type and acidity play an important role in determining resolution and peak shape for various classes of compounds being analyzed.

Peak tailing and low efficiencies of both basic and acidic compounds can occur due to unwanted silanol interactions. The effect is most apparent with some of the earlier silicas developed for HPLC, in which silanol groups are quite acidic.

For these types of silicas, the observed effect on peak shape requires the mobile phase to include either a competing base such as triethylamine or a competing acid such as acetic acid. Both peak shape and column performance are improved dramatically when the appropriate competing agent is used. The effect of the additive is to compete with any silanol interactions that interfere with an analyte's retention and peak shape. Consequently, the additive must be present in fairly high concentrations, often as much as 1% volume fraction of the mobile phase. The use of additives in such a concentration can often have a deleterious effect on the column lifetime and also on reproducibility of the method.

These difficulties have provided the impetus for the development of a newer range of chromatographic silicas (base deactivated silicas) that allow the analysis of both basic and acidic compounds without competing additives in the mobile phase. A

proprietary treatment to the silica surface results in significant improvements to the homogeneity of the surface silanol population prior to derivatization. The result has been that the derivatized silica surface (typically C18, C8, Phenyl, etc.) no longer requires a competing acid or base in the mobile phase to achieve acceptable peak shapes for problematic analytes.

We have called this improvement in silica surface properties 'Base Deactivation' and the resulting silica, 'Base Deactivated Silica' or BDS.

Hypersil BDS packings are among the first of the newer HPLC packings to offer the characteristics associated with these surface improvements.

Base deactivated stationary phases provide the following benefits:

- Reduced silanol interaction
- Reduced tailing
- Reduced need for mobile phase additives
- Excellent peak symmetry
- Long column lifetimes
- Improved performance with basic, neutral and acidic compounds

Specifications:

Phase	Pore Size	Particle Size	Carbon Loading
Hypersil BDS C18	130Å	3 and 5µm	11%
Hypersil BDS C8	130Å	3 and 5µm	7%
Hypersil BDS Phenyl	130Å	3 and 5µm	7%
Hypersil BDS Cyano	130Å	3 and 5 µm	5%

The Base Deactivation Process

The popularity of columns packed with C18 derivatized silica is due to their wide breadth of application, including non-polar and neutral, acidic, and basic analytes. The selectivity of a given C18 phase can depend on the type of silane used and the synthetic conditions, as both of these factors will effect the density of the bonded phase on the surface.

This density is important since the greater the access of an analyte to the underlying silica support, the greater the opportunity for secondary interactions such as hydrogen bonding. There are approximately five silanol (Si-OH) groups per nm² of surface on the silica, corresponding to 8-9 mmol/m². It is stereochemically impossible to react more than ~50% of the silanol groups even with ligands as small as trimethylsilane (C₁).

The following silanol interactions can give rise to peak tailing and changes in retention and selectivity on a typical alkyl C18 packing:

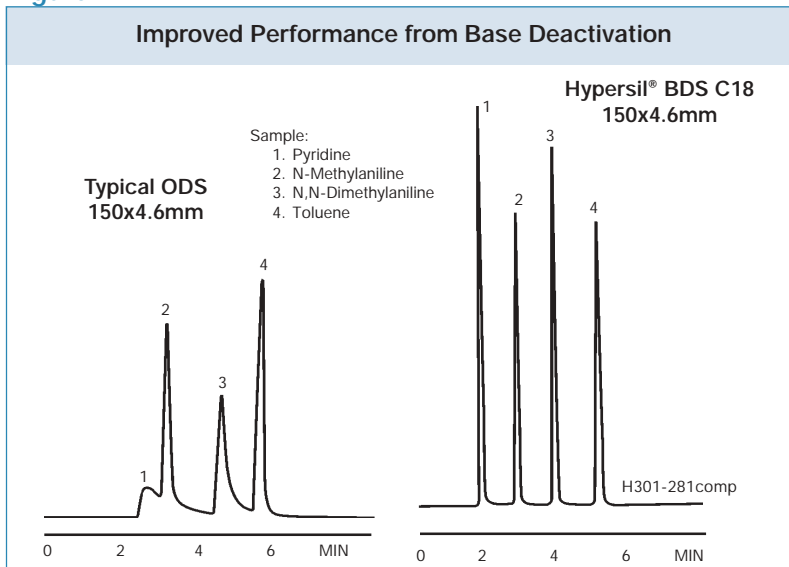
- Si-OH •••••NH₂-R
(Hydrogen bonding with base)
- Si-OH•••••O=RCOH
(Hydrogen bonding with acid)
- Si-O⁻NH₃⁺-R
(Ion exchange with base)

The surface composition of a silica prior to derivatization is very important. At most silica surfaces it is usual to have a variety of silanol groups: (1) lone silanols, (2) siloxanes, (3) geminal silanols and (4) vicinal silanols (Figure 2a). The presence of these silanols in a derivatized silica can result in unwanted silanol interactions with the analyte.

Using a proprietary production process, the surface of the Hypersil BDS silica is made much more homogeneous (Figure 2b), ideally so that all silanols are of the same type (vicinal silanols).

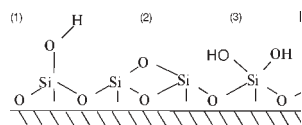
The more uniform silica surface is then ready for surface derivatization. Special care is taken to ensure a high density of coverage followed by thorough end-capping in order to further reduce the possibility of any silanol interactions. As a consequence of the base deactivation process, the silanols that are still present after surface derivatization

Figure 1



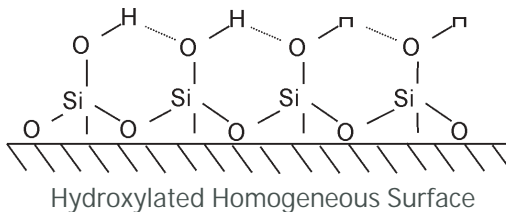
Columns: 5µm, 150x4.6mm
 Eluent: 60% ACN / 40% 0.05M KH₂PO₄, pH 4.5
 Flow: 1.0 mL/min
 Detector: UV @ 254

Figure 2a



Hydroxylated Heterogeneous Surface

Figure 2b



Silica Surface

Silica Substrate



become much more 'friendly' toward basic and acidic compounds, and the packing material becomes an excellent choice to develop highly reproducible methods. Silanols are less acidic and are less likely to be available for ion exchange interaction with ionized basic analytes, and are also less likely to hydrogen bond with polar analyte. With reduced silanol interactions, Hypersil BDS columns are ideally suited for analysis of a wide range of analytes including both acids and bases, with peak shape and column performance significantly improved.

Reproducibility of Hypersil® BDS C18 Columns

HPLC owes its success as an analytical technique to several factors. One of the most important has been the ability to transfer newly developed methods to other labs around the world. In this respect, column reproducibility has played a crucial role. Column performance parameters are key factors in determining reproducibility from column to column. A column with poor efficiency may lead to loss in resolution, while stationary phase differences may lead to a change in selectivity that can result in loss of resolution. Thermo Hypersil-Keystone strives continuously to provide HPLC columns of the highest standard with a strong focus on reproducibility.

Different brands of C18 media may differ from one another significantly. This is largely due to differences in the properties of the underlying silica. Differences in surface area and silanol population give rise to stationary phases that differ in carbon content, ligand type or silanol content.

Differences within a particular column brand may also occur simply due to the amorphous properties of the base silica itself. Strict control over the processes employed to manufacture both the stationary phase and columns are therefore of paramount importance. At Thermo Hypersil-Keystone, stringent quality control measures are employed to ensure the required column-to-column reproducibility is achieved.

In the following discussion, we describe some of the quality control measures that ensure the continued quality and reproducibility associated with Hypersil BDS packings and columns.

Batch Testing Procedure

As with all Thermo Hypersil-Keystone products, Hypersil BDS packings are manufactured to the highest standards, and are rigorously quality controlled. The fully documented ISO9001 control procedures for both media and column production ensure that only the highest quality columns are released to end users.

Derivatization of the BDS silica only takes place once it has passed almost thirty physical and chromatographic test specifications. Once bonded, the BDS C18 packing is tested chromatographically (Figure 3) and for carbon content. This testing is done both prior to and after end-capping has taken place. The test mixture employed contains compounds such as pyridine and dimethylaniline, known to be sensitive to the stationary phase silanol content. Such compounds can cause peak tailing and varying selectivity on many older HPLC phases. Each batch of BDS material must meet strict specifications for selectivity and peak shape.

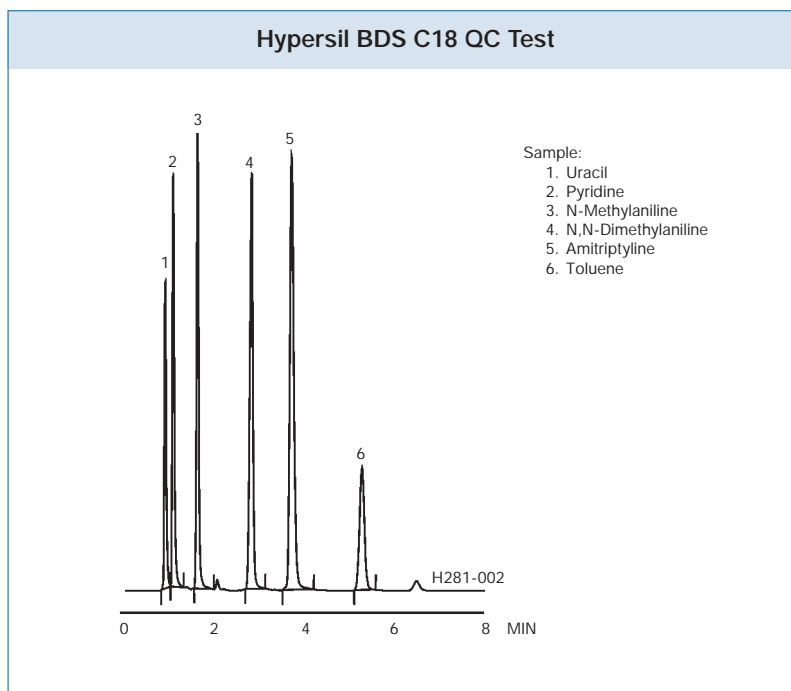
The chromatographic test consists of comparing the selectivity,



efficiency and asymmetry for the range of analytes as shown in Figure 3 against a standard column. A standard column is one which is prepared from a blend of up to 50 previous batches of Hypersil BDS C18 packing. All selectivity parameters (k and α values) must be within 5% of those measured for the standard column, while efficiency parameters and asymmetry values must also meet strict specifications.

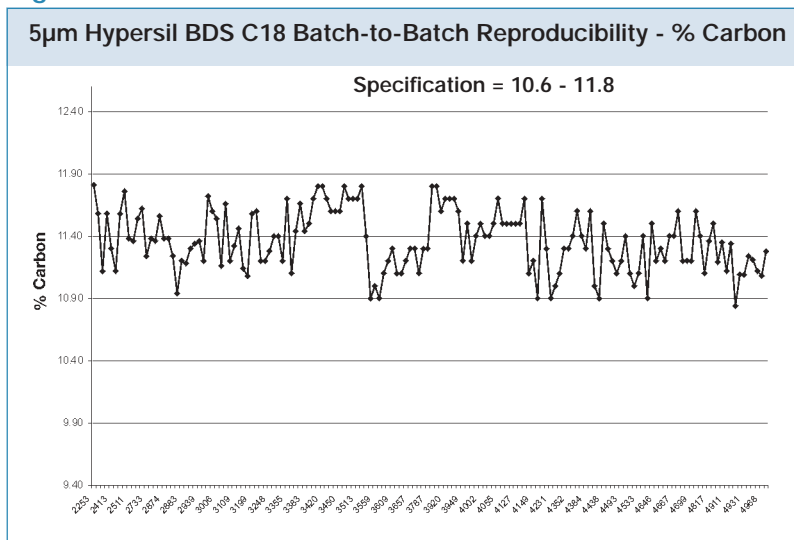
Figure 4 shows the % carbon reproducibility observed for batches of 5 μ m Hypersil BDS C18 manufactured over the last six years. The % carbon is measured by a Leco Carbon analyzer and is accurate to within $\pm 0.1\%$. Note the continuous tightening of the band over the last few years, illustrating the commitment to continually improving the quality of Thermo Hypersil-Keystone products.

Figure 3



Hypersil BDS C18, 3 μ m, 100x4.6mm
 Eluent: 60% MeOH / 40% 0.05M KH_2PO_4 , pH 3.5
 Flow: 1.2 mL/min
 Detector: UV @ 254

Figure 4



Selectivity, Peak Asymmetry and Column Efficiency

Selectivity, peak asymmetry and column efficiency measures indicate column reproducibility. Changes in the bonded phase will be reflected by changes in these sensitive performance parameters.

Figure 5 shows batch-to-batch reproducibility for two selectivity measures. Alpha values are calculated as a ratio of capacity factors, k_3/k_2 . A change in either capacity factor will cause a significant change in the alpha value. Alpha values for Hypersil BDS C18 columns must fall $\pm 5\%$ of the standard column.

Peak asymmetry and decreased

column efficiency are usually observed when a column deteriorates, but also occur if the column is poorly packed. The asymmetry ratio for a given peak is calculated at 10% of the peak height. To pass specification, asymmetry must fall between 1.2 and 0.9, and efficiency must be greater than 75,000 theoretical plates for the final eluting peak (anthracene).

Figure 5

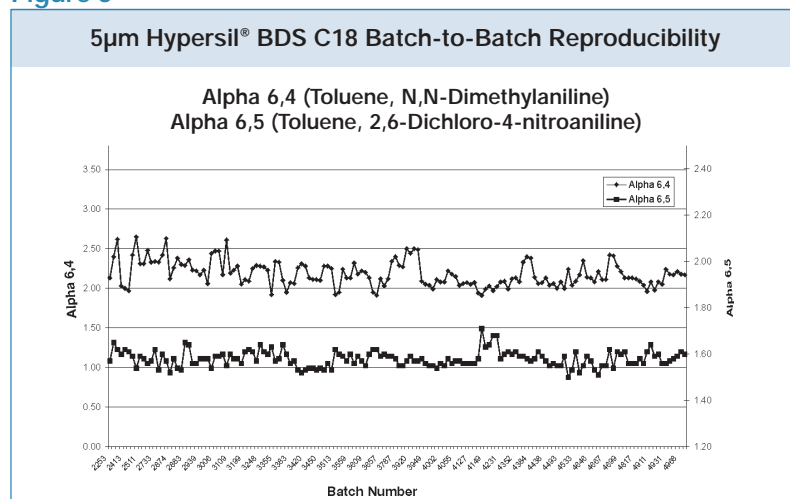


Figure 6

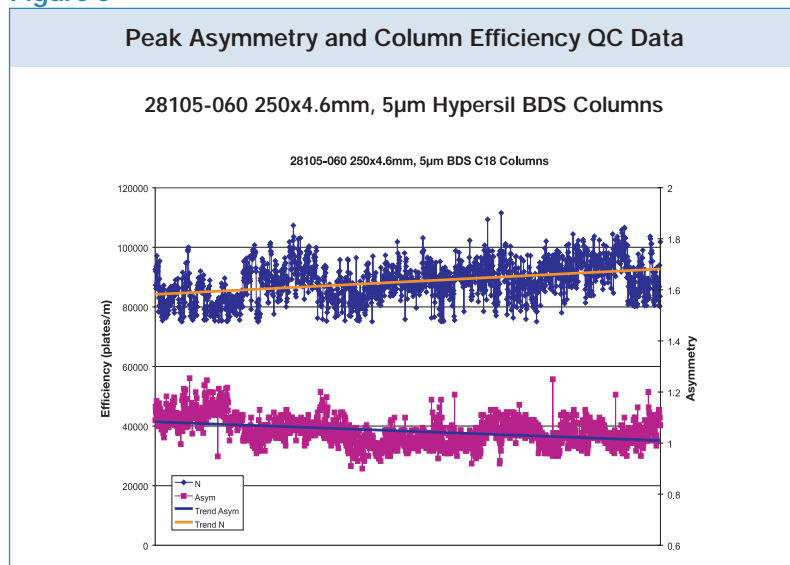


Figure 6 demonstrates column performance for over 10,000 Hypersil BDS C18 columns. Note the continual improvement in quality as the average asymmetry value approaches 1.0, and column efficiency approaches 90,000 plates per meter. A typical test chromatogram with conditions and results is presented in Figure 7 and Table 1.

Figure 7

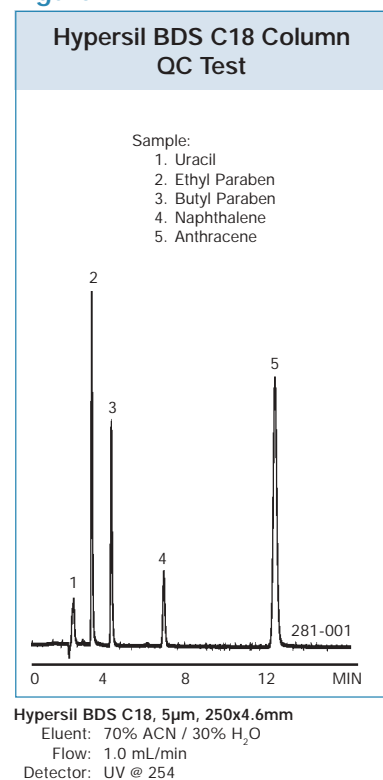
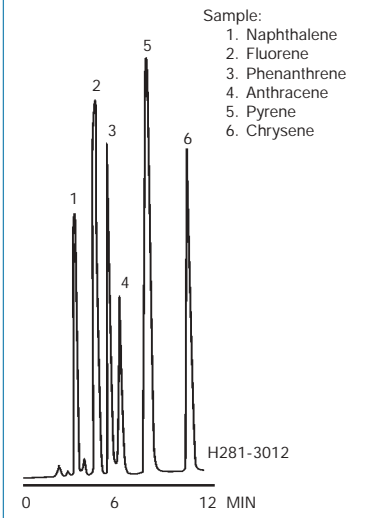


Table 1

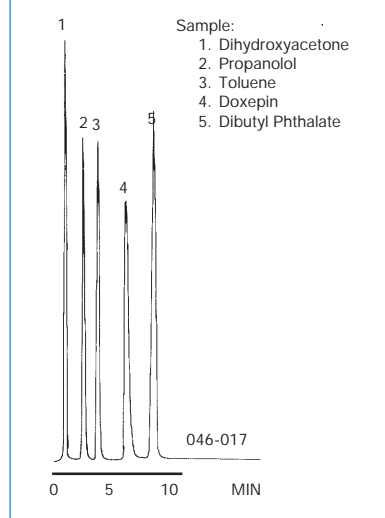
Component	R_t (min)	N (plates / meter)	Asym (10%)
T_o (Uracil)	2.56	7213	0.74
Ethyl Paraben	3.43	58237	1.33
Butyl Paraben	4.38	69791	1.25
Naphthalene	6.93	90993	1.14
Anthracene	12.32	92419	1.07

Polynuclear Aromatic Hydrocarbons



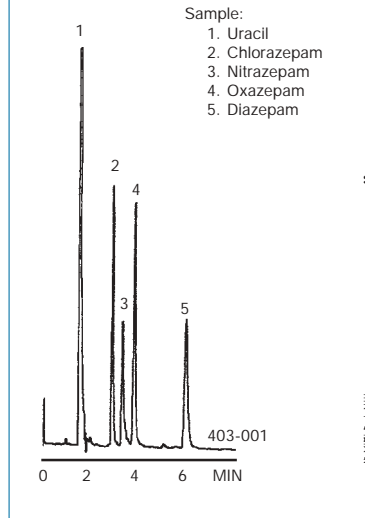
Hypersil® BDS C18, 5µm, 150x4.6mm
 Eluent: 75% ACN / 25% H₂O
 Flow: 1.0 mL/min
 Detector: UV @ 254

Base - Neutral Mix



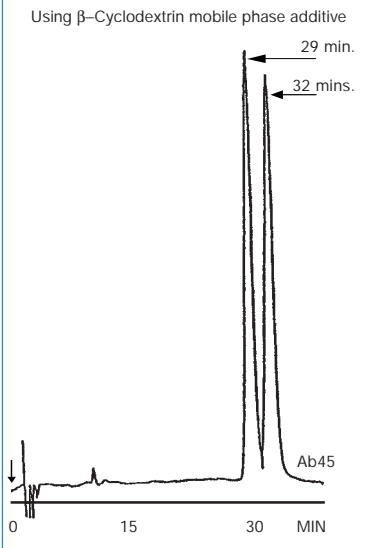
Hypersil BDS C18, 5µm, 150x4.6mm
 Eluent: 75% MeOH / 25% 0.05M Na₂HPO₄, pH 7.0
 Flow: 1.0 mL/min
 Detector: UV @ 254

Benzodiazepines



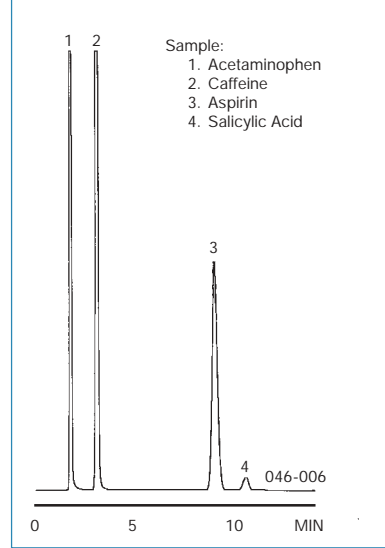
Hypersil BDS Phenyl, 5µm, 150x4.6mm
 Eluent: 40% ACN / 60% H₂O
 Flow: 1.0 mL/min
 Detector: UV @ 254

Enantiomers of Methyphenobarbitone



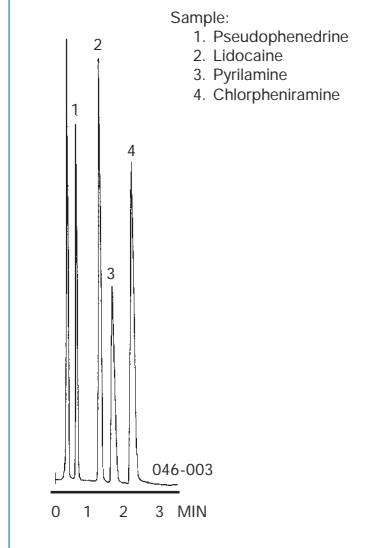
Hypersil BDS C18, 5µm, 100x4.6mm
 Eluent: 10mM Na₂HPO₄ / Methanol (80/20) with 5mM β-Cyclodextrin
 Flow: 1.0 mL/min
 Detector: UV @ 240

Commercial Pain Reliever



Hypersil BDS C18, 5µm, 150x4.6mm
 Eluent: 720 mL H₂O / 250 mL MeOH / 3 mL Acetic Acid
 Flow: 1.25 mL/min
 Detector: UV @ 275

Pyrilamine & Chlorpheniramine



Hypersil BDS C18, 3µm, 100x4.6mm
 Eluent: 20% ACN / 80% 0.05M KH₂PO₄, pH 2.5
 Flow: 2.0 mL/min
 Detector: UV @ 220

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