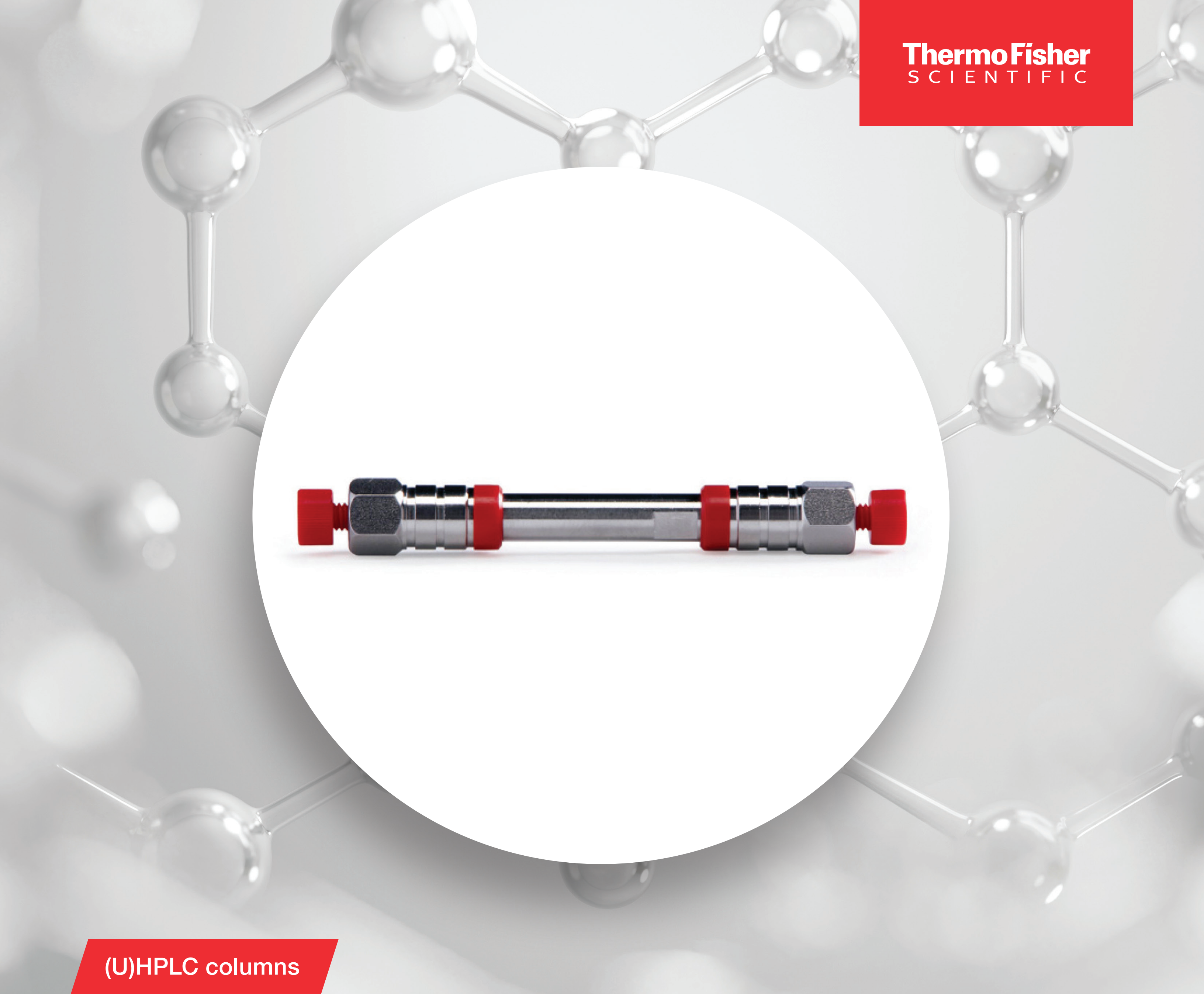


(U)HPLC columns

Hypersil BDS columns technical guide

Engineered for robust performance, reproducibility, and peak symmetry

thermo scientific

Contents

From silica to separation

When it comes to achieving peak performance in chromatography, every component matters. Since their introduction in 1989, Thermo Scientific™ Hypersil™ BDS Columns have earned a strong reputation for being among the most robust, reproducible, and reliable high-performance liquid chromatography (HPLC) columns available. Built on highly base-deactivated silica and enhanced with endcapping, Hypersil BDS columns consistently deliver:

- outstanding reproducibility
- minimal peak tailing
- exceptional durability and long column life
- superior peak symmetry for both basic and acidic compounds

This technical guide provides a comprehensive overview of the Hypersil BDS column portfolio and details the rigorous quality control processes used to ensure consistent phase and column performance.

Base deactivated silica and bonded phases

Advancing HPLC with silica-based stationary phases

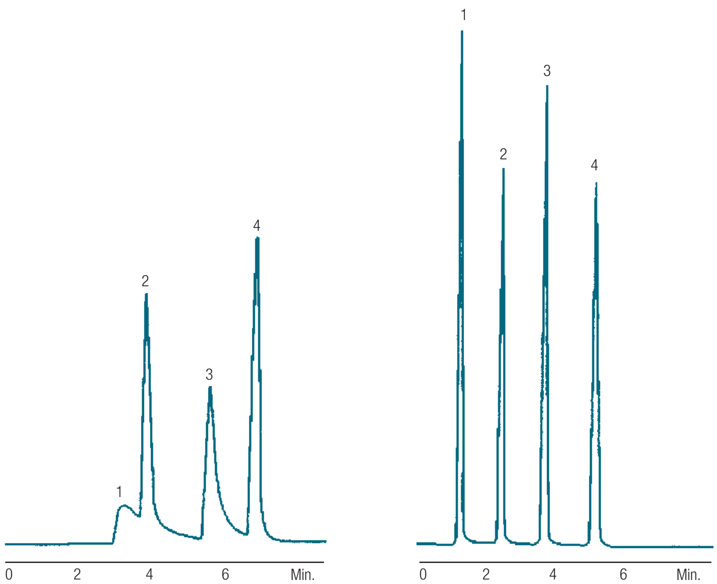
The use of covalently-bonded silica stationary phases in HPLC allows the analysis of a broad range of analytes. Along with rapid equilibration times, and significantly improved mass transfer characteristics over liquid-liquid partition chromatography, this has resulted in the hugely successful advancement 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 analyte 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 an improved range of chromatographic silicas (base deactivated silicas) that allow the analysis of both basic and acidic compounds without the requirement for 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 bonded silica surface no longer requires a competing acid or base in the mobile phase to achieve acceptable peak shapes for problematic analytes. This is illustrated in Figure 1.



Typical C18 column

Hypersil BDS C18 column

Figure 1: The Thermo Scientific™ Hypersil™ BDS C18 Column shows improved peak symmetry over traditional ODS for difficult analytes

The Thermo Scientific™ Hypersil™ BDS Packings were among the first base deactivated silica HPLC packings to offer the characteristics associated with these surface improvements, offering benefits such as:

- reduced silanol interactions
- reduced peak tailing
- reduced need for mobile phase additives
- excellent peak symmetry
- long column lifetimes
- improved performance with basic, neutral and acidic compounds

Hypersil BDS C18 column, 5 µm, 150 x 4.6 mm	
Cat. no.	28105-154630
Eluent	60% ACN/40% 0.05M KH_2PO_4, pH 4.5
Detector	UV at 254 nm
Sample	1. Pyridine
	2. N-methylaniline
	3. N, N-dimethylaniline
	4. Toluene

Base deactivation process

Understanding C18 column selectivity: The role of silica surface and bonding density

The popularity of columns packed with C18 derivatized silica is due to their wide breadth of application, encompassing 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 affect the density of the bonded phase on the surface.

This density of the bonded phase 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 nm^2 of surface on the silica, corresponding to 8-9 $mmol/m^2$. It is stereochemically impossible to react more than ~50% of the silanol groups even with ligands as small as trimethylsilane (C1).

The surface composition of silica prior to derivatization is very important. As illustrated in Figure 2A, most silica surfaces typically have a variety of silanol groups: (1) lone silanols, (2) siloxanes, (3) geminal silanols and vicinal silanols. The presence of these silanols in a derivatized silica can result in unwanted silanol interactions with the analytes which can give rise to peak tailing and changes in retention and selectivity on a typical alkyl C18 packing, such as:

- Si -OH •••••••NH_2 -R

(Hydrogen bonding with base)

- Si - OH•••••••O=RCOH

(Hydrogen bonding with acid)

- Si – O- $+NH_3$-R

(Ion exchange with base)

Figure 2A

Figure 2B

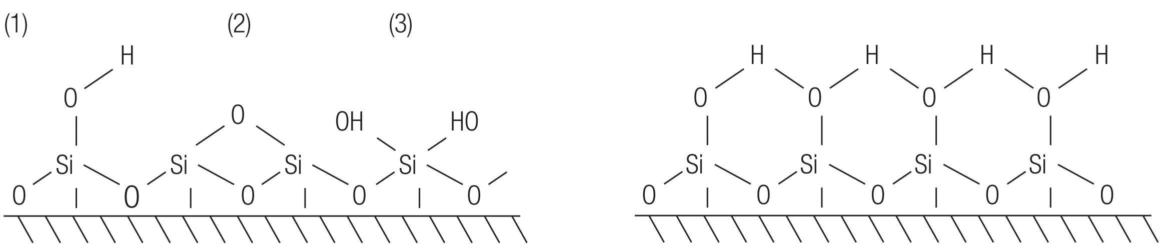


Figure 2: The base deactivation process produces a homogenous surface

Using a proprietary production process, the surface of the Hypersil BDS column silica is made much more homogeneous, so that all silanols are of the same type (vicinal silanols), as illustrated in Figure 2B. The resulting silica surface is more uniform and 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 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 analytes. With these 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

HPLC's success as an analytical technique can be attributed to several factors. One of the most important has been the ability to transfer newly developed methods to other laboratories 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 Fisher Scientific 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. Our stringent quality control measures ensure that 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 Thermo Scientific Hypersil BDS columns.

Batch testing procedure

Hypersil BDS columns are manufactured to the highest standards, and are rigorously quality controlled. The fully documented ISO9001:2000 control procedures for both media and column production ensure that only the highest quality columns are released to end users. Derivatization only takes place once the BDS silica has passed almost thirty (30) physical and chromatographic test specifications. Once bonded, every production lot of the BDS C18 media is tested for its chromatographic properties and for carbon load. This testing is done both prior to and after end-capping has taken place.

The chromatographic test compares the selectivity, efficiency, and asymmetry for the range of analytes against a standard column which is prepared from a blend of up to 50 previous batches of Hypersil BDS C18 column packing. The test mixture employed contains compounds such as pyridine and dimethylaniline, which are known to be sensitive to the stationary phase silanol content and can cause peak tailing and varying selectivity on many older HPLC phases.

For each batch of BDS material, all selectivity parameters (k and alpha values) must be within 5% of those measured for the standard column, while efficiency parameters and asymmetry values must also meet strict specifications before it is made available for packing into columns. Figure 3 shows batch-to-batch reproducibility for the selectivity between toluene, NN dimethyl aniline, and toluene retention time.

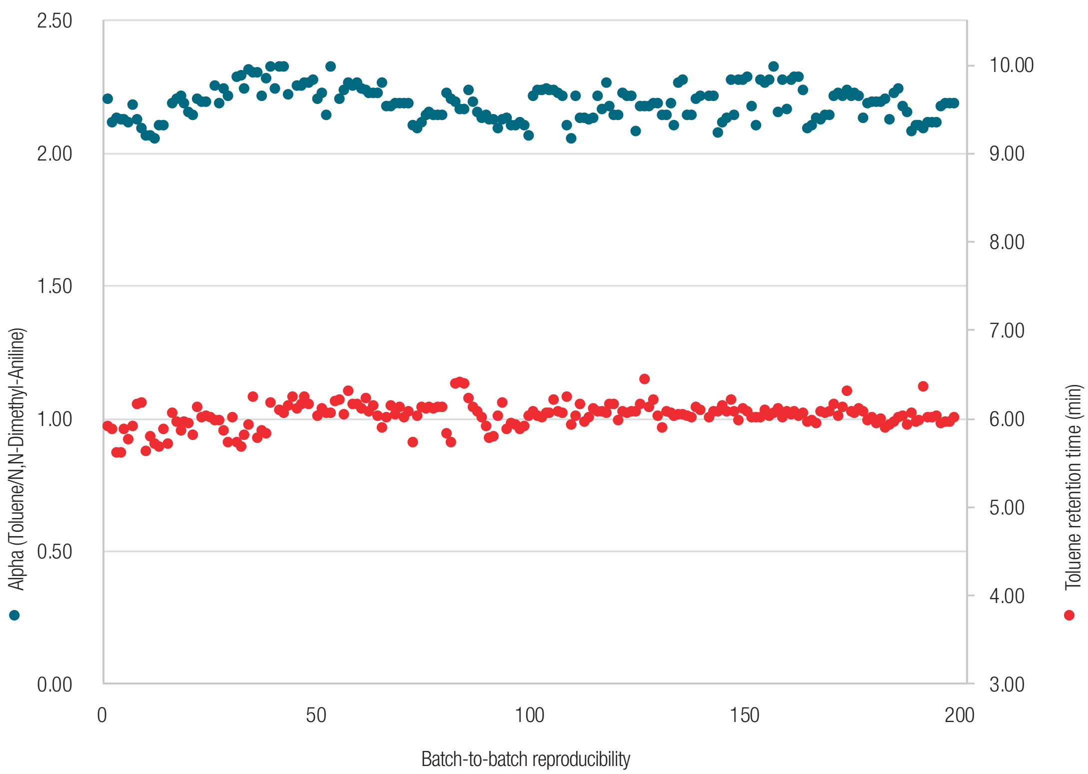


Figure 3: QC data illustrating selectivity (toluene/N,N-dimethyl-aniline) and toluene retention time for batch-to-batch Hypersil BDS column reproducibility

Column testing procedure

Column evaluation process

Peak asymmetry and decreased column efficiency are usually observed when a column deteriorates, but may also occur if the column is poorly packed. Every Hypersil BDS column is individually tested prior to shipment to ensure the quality of the column. Figure 4 demonstrates column performance in terms of efficiency for over 15,000 Hypersil BDS C18 columns (250 x 4.6 mm, 5 µm) . The peak efficiency (measured for o-xylene) is consistently above 80,000 plates/m. Also noticeable is the trend towards higher efficiency which reflects our commitment to continually improve our column packing process.

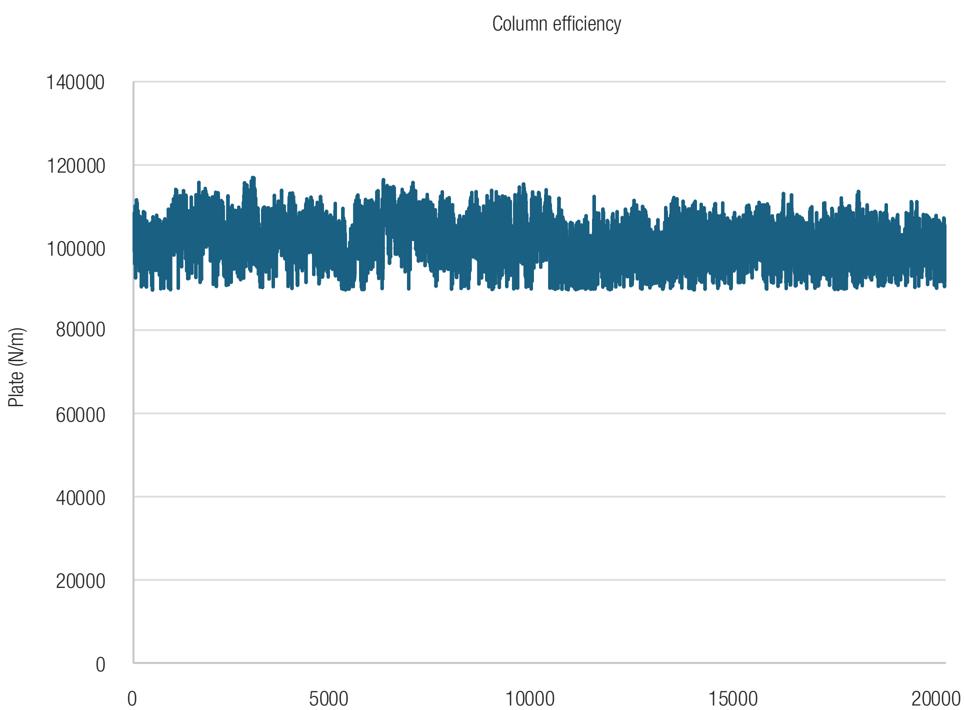


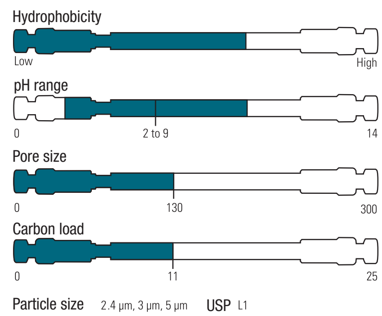
Figure 4. QC data illustrating efficiency for 20,000 Hypersil BDS columns

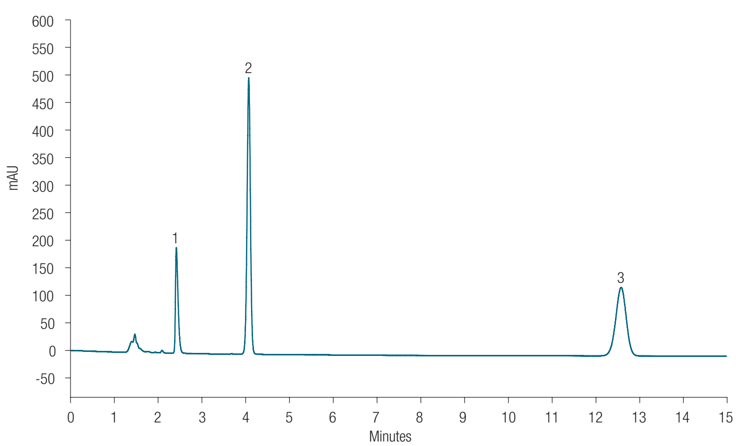
Bonded phases

Hypersil BDS columns are available in four bonded phases. All Hypersil BDS columns, the base deactivation procedure, and the endcapping process minimize peak tailing, even for basic drugs. Each Hypersil BDS column comes with a certificate of authenticity.

Hypersil BDS C18 columns

Thermo Scientific™ Hypersil™ BDS C18 Media is an excellent reversed-phase material for a variety of applications and is one of the most popular packing materials available. Hypersil BDS columns are a good choice for QA/QC labs as a robust general purpose column in applications where reproducibility and long column lifetimes are required. Hypersil BDS C18 columns are applicable to a wide range of analytes including acids, bases and neutrals as shown, and are popular in methods worldwide.





Hypersil BDS C18 column, 150 x 4.6 mm, 5 µm	
Mobile phase	30% MeCN 70% 50 mM KH_2PO_4, pH 3.5
Flow rate	1.0 mL/min
Detection	UV at 254 nm
Injection	20 µL
Temperature	30°C
Pressure	92 bar
Sample	1. Lidocaine
	2. Methyl paraben
	3. Propyl paraben

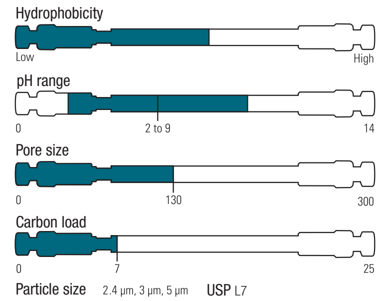
Figure 5: Separation of lidocaine and parabens

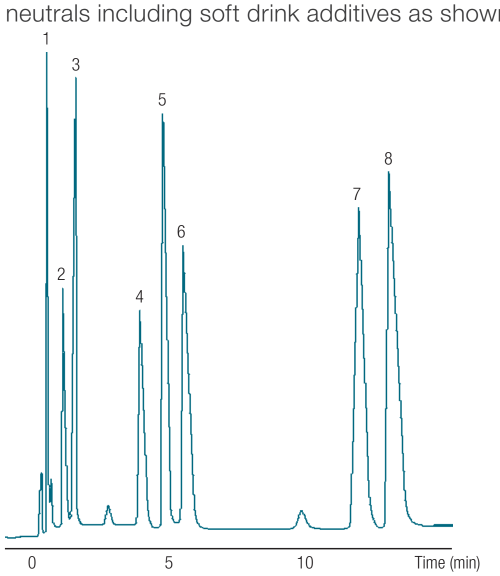
Hypersil BDS C18 columns

Particle size	Diameter	Length	Quantity	Cat. No
2.4 µm	2.1 mm	30 mm	Each	28102-032130
		50 mm	Each	28102-052130
		100 mm	Each	28102-102130
		150 mm	Each	28102-152130
	4.6 mm	30 mm	Each	28102-034630
		50 mm	Each	28102-054630
		100 mm	Each	28102-104630
		150 mm	Each	28102-154630
3.0 µm	2.1 mm	30 mm	Each	28103-032130
		50 mm	Each	28103-052130
		100 mm	Each	28103-102130
		150 mm	Each	28103-152130
	3.0 mm	30 mm	Each	28103-033030
		50 mm	Each	28103-053030
		100 mm	Each	28103-103030
		150 mm	Each	28103-153030
	4.0 mm	30 mm	Each	28103-034030
		50 mm	Each	28103-054030
		100 mm	Each	28103-104030
		150 mm	Each	28103-154030
	4.6 mm	30 mm	Each	28103-034630
		50 mm	Each	28103-054630
		100 mm	Each	28103-104630
		150 mm	Each	28103-154630
5.0 µm	2.1 mm	50 mm	Each	28105-052130
		100 mm	Each	28105-102130
		150 mm	Each	28105-152130
	3.0 mm	50 mm	Each	28105-053030
		100 mm	Each	28105-103030
		125 mm	Each	28105-123030
		150 mm	Each	28105-153030
		200 mm	Each	28105-203030
		250 mm	Each	28105-253030
	4.0 mm	100 mm	Each	28105-104030
		125 mm	Each	28105-124030
		150 mm	Each	28105-154030
		200 mm	Each	28105-204030
		250 mm	Each	28105-254030
	4.6 mm	50 mm	Each	28105-054630
		100 mm	Each	28105-104630
		125 mm	Each	28105-124630
		150 mm	Each	28105-154630
		200 mm	Each	28105-204630
		250 mm	Each	28105-254630

Hypersil BDS C8 columns

Thermo Scientific™ Hypersil™ BDS C8 Columns offer the same high quality base deactivated, fully endcapped phase as Hypersil BDS C18 columns, with similar selectivity but slightly less retention. They are applicable to the analysis of acids, bases, and neutrals including soft drink additives as shown.





Hypersil BDS C8 column, 5 µm, 250 x 4.6 mm (P/N 28205-254630)	
Mobile phase	A: 0.85% v/v H_2SO_4 in 17.5 mM KH_2PO_4, at pH 1.8
Flow rate	1.5 mL/min
Detection	UV at 254 nm
Sample	1. Asorbic acid
	2. Quinine
	3. Caffeine
	4. Saccharin
	5. Vanillin
	6. Aspartame
	7. Sorbic acid
	8. Benzoic acid

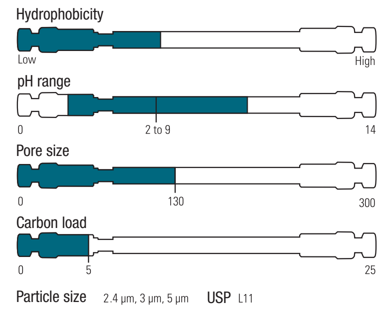
Figure 6: Additives in soft drinks can be readily separated using a Hypersil BDS C8 column

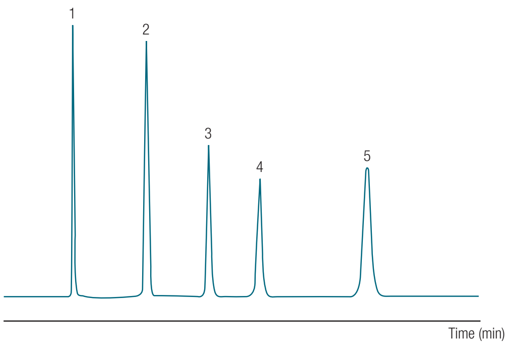
Hypersil BDS C8 columns

Particle size	Diameter	Length	Quantity	Cat. No
3.0 µm	2.1 mm	50 mm	Each	28203-052130
	3.0 mm	100 mm	Each	28203-103030
		150 mm	Each	28203-153030
	4.0 mm	50 mm	Each	28203-054030
		100 mm	Each	28203-104030
		150 mm	Each	28203-154030
	4.6 mm	50 mm	Each	28203-054630
		100 mm	Each	28203-104630
		150 mm	Each	28203-154630
5.0 µm	2.1 mm	50 mm	Each	28205-052130
		100 mm	Each	28205-102130
	3.0 mm	50 mm	Each	28205-053030
	4.0 mm	125 mm	Each	28205-124030
		150 mm	Each	28205-154030
		200 mm	Each	28205-204030
		250 mm	Each	28205-254030
	4.6 mm	50 mm	Each	28205-054630
		100 mm	Each	28205-104630
		125 mm	Each	28205-124630
		150 mm	Each	28205-154630
		200 mm	Each	28205-204630
		250 mm	Each	28205-254630

Hypersil BDS Phenyl columns

Thermo Scientific™ Hypersil™ BDS Phenyl Columns offer alternative selectivity to Hypersil BDS C18 and C8 columns. It is well established that phenyl phases offer clear advantages over alkyl chains when certain types of compounds must be resolved. The exceptional stability and unique selectivity of Hypersil BDS Phenyl columns can be used to accomplish a difficult separation.





Hypersil BDS Phenyl column, 5 µm, 150 x 4.6 mm (P/N 28905-154630)	
Eluent	90% 0.5M KH_2PO_4 /10% ACN
Flow rate	1.25 mL/min
Detection	UV at 254 nm
Sample	1. Uracil
	2. Procainamide
	3. N-acetyl procainamide
	4. Caffeine
	5. N-propionyl procainamide

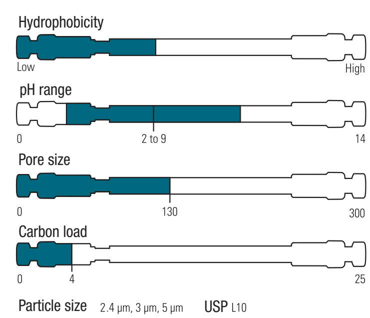
Figure 7: Analysis of procainamides using a Hypersil BDS Phenyl column

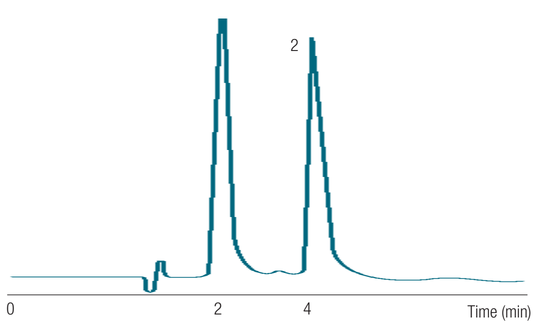
Hypersil BDS Phenyl columns

Particle size	Diameter	Length	Quantity	Cat. No
3.0 µm	4.6 mm	150 mm	Each	28903-154630
5.0 µm	4.0 mm	250 mm	Each	28905-254030
	4.6 mm	150 mm	Each	28905-154630
		250 mm	Each	28905-254630

Hypersil BDS Cyano columns

Thermo Scientific™ Hypersil™ BDS Cyano Columns can be used in both reversed phase and normal phase chromatography. In reversed-phase, they offer different selectivity compared to C18 or C8 phases. In normal phase, they are less retentive than silica columns.





Hypersil BDS Cyano column, 5 µm, 150 x 4.6 mm (P/N 28905-154630)	
Eluent	5% ACN / 95% 0.05M KH_2PO_4, pH 3.5
Flow rate	1.25 mL/min
Detection	UV at 254 nm
Sample	1. Morphine
	2. Nalorphine

Figure 8: Polar compounds (morphine and nalorphine) are retained and separated using a Hypersil BDS Cyano column

Hypersil BDS Cyano columns

Particle size	Diameter	Length	Quantity	Cat. No
5 µm	4.6 mm	150 mm	Each	28805-154630
		250 mm	Each	28805-254630

2.4 µm particle size for faster separations

2.4 µm particles give higher efficiency than 3 or 5 µm particles and this efficiency is delivered over a greater range of optimum linear velocity. This makes it possible to operate at higher flow rates without losing performance. Because shorter columns packed with 2.4 µm particles give equivalent efficiency to longer columns packed with 5 µm particles faster analysis and solvent savings for the chromatographer become a reality.

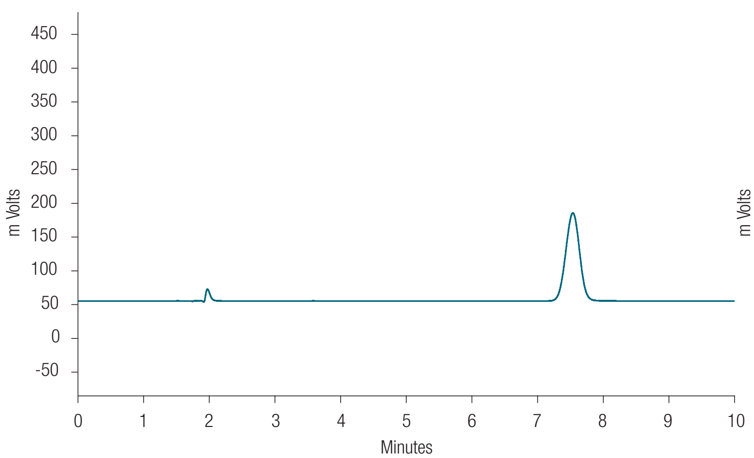
When transferring methods to columns packed with 2.4 µm particles, the following three tips should be considered:

1. to maintain an equivalent separation when transferring a method it is important to keep the reduced linear velocity constant between the original and new method.

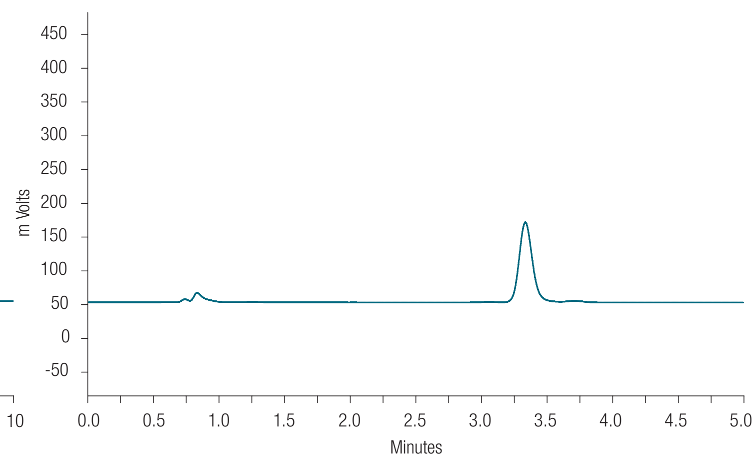
2. 2.4 µm based methods are most often transferred to smaller volume columns, so the same injection volume will take up a larger proportion of the new column, possibly leading to column omeprazole or band broadening. It is therefore important to scale down the injection volume to match the change in column volume.

3. Geometrical transfer of the gradient requires calculation of the number of column volumes of mobile phase in each segment (time interval) of the gradient in the original method to ensure that the new calculated gradient takes place over the same number of column volumes, for the new column.

Figure 9 illustrates method transfer to 2.4 µm particles using omeprazole as an example. The separation using the column packed with 2.4 µm particles, omeprazole elutes in 3.3 minutes, compared with 7.5 minutes for the column packed with 5 µm particles and delivers 20% more efficiency.



Hypersil BDS C8 column, 150 x 4.6 mm, 5 µm	
Mobile phase	75% ACN, 25% phosphate buffer
Flow rate	1.0 mL/min
Detection	UV at 254 nm
Injection	10 µL
Temperature	30°C
Pressure	90 bar



Hypersil BDS C8 column, 100 x 4.6 mm, 2.4 µm	
Mobile phase	75% ACN, 25% phosphate buffer
Flow rate	0.43 mL/min
Detection	UV at 254 nm
Injection	2 µL
Temperature	30°C
Pressure	278 bar

Figure 9: Columns packed with 2.4 µm particles give faster, more efficient chromatography than columns packed with 5 µm particles

One advantage of using 2.4 µm particle size columns is that high speed, high efficiency separations are achievable using conventional HPLC systems, even for narrow columns. For example, the backpressure for a 100 x 2.1 mm ID column packed with 2.4 µm particles is shown in Figure 10. The optimum flow rate for this column is 0.4 – 0.5 mL/min, which will give a backpressure within the limits for a conventional HPLC system.

There are some system considerations to remember when using short columns packed with 2.4 µm particles in order to maximise their performance. Firstly, to avoid dispersion which can lead to peak broadening, the system volume (connecting tubing ID and length, injection volume, UV detector flow cell volume) should be minimized. Secondly, because peak widths are narrower with fast chromatography, the detector time constant and sampling rate need to be carefully selected. If fast gradients are being used, then a pump with a low dwell volume is desirable to transfer the gradient to the column quickly.

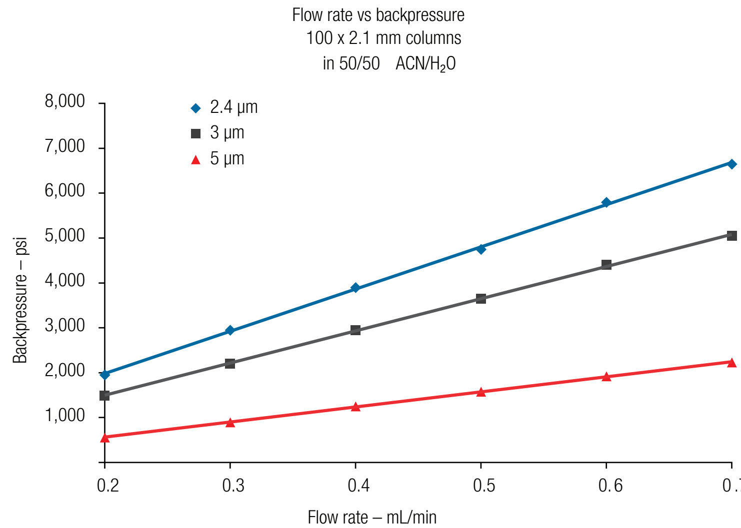


Figure 10: Column backpressure as a function of flow rate for 2.4 µm, 3 µm, and 5 µm particle packed columns

Guard cartridges for column protection

Drop-in guard cartridges and holders offer convenience, economy and effective protection for extending column lifetimes. The 10 mm design offers maximum protection with minimal increase in retention. Thermo Scientific™ Hypersil™ BDS Drop-in Guard Cartridges are provided in packs of 4 each.

Guard cartridges

Description	Particle size	Diameter	Length	Quantity	Cat. No
C18 guard cartridges	2.4 µm	4.6 mm	10 mm	Each	28102-014001
	3.0 µm	2.1 mm	10 mm	Each	28103-012101
		3.0 mm	10 mm	Each	28103-013001
		4.0 mm	10 mm	Each	28103-014001
		4.6 mm	10 mm	Each	28103-014001
	5.0 µm	2.1 mm	10 mm	Each	28105-012101
		3.0 mm	10 mm	Each	28105-013001
		4.0 mm	10 mm	Each	28105-014001
		4.6 mm	10 mm	Each	28105-014001
C8 guard cartridges	5.0 µm	2.1 mm	10 mm	Each	28205-012101
		3.0 mm	10 mm	Each	28205-013001
		4.0 mm	10 mm	Each	28205-014001
		4.6 mm	10 mm	Each	28205-014001

Chromatography columns and consumables

As the world's sole manufacturer of Thermo Scientific™ Hypersil™ Silica Columns, we have set a very high standard in HPLC and continue to uphold it with innovative new products spanning sample preparation extraction (SPE), HPLC, and gas chromatography (GC). We offer one of the broadest selections of premier chromatographic phases and innovative hardware designs available, combined with superb technical support and customer service. Whether you use Thermo Scientific™ HyperSep™ Cartridges for rapid sample preparation, Hypersil BDS columns for routine separations, or are seeking new solutions for your most challenging methods like Thermo Scientific™ Hypersil GOLD™ Columns, we have the options to meet your needs.

Learn more at **thermofisher.com/lccolumns**