



(U)HPLC columns

Hypersil classical columns technical guide

Exceptionally reliable and reproducible
columns for neutral and polar compounds

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Introduction to Hypersil classical column range

Thermo Scientific™ Hypersil™ Classical (U)HPLC Columns offer exceptional performance and documentation of quality, batch, and column QA information, with validation dating back to 1976. Accredited under ISO9001:2000, the Hypersil classical columns manufacturing plants ensure strict adherence to quality, from the initial silica production and bonded phase phase to the final manufacture of the (U)HPLC columns.

Hypersil classical columns:

- are exceptionally reliable and reproducible columns for neutral and polar compounds
- are individually tested and supplied with a test certificate
- offer proven, reproducible column efficiency and performance
- feature a wide range of bonded phases with very low pressure drop
- are among the world's most trusted and extensively referenced HPLC columns with demonstrated success

This technical guide provides a comprehensive overview of the different Hypersil classical columns in terms of physical properties and usage, and then focuses in greater detail on the quality assurance protocols associated with Thermo Scientific™ Hypersil™ ODS Columns.

Hypersil classical columns phase summary

Proven silica technology for efficient separations

Hypersil silica is the base media for the Hypersil classical range of bonded phase columns. The silica itself is also an HPLC media that is a powerful and efficient tool in the chromatography of non-polar and moderately polar organic compounds.

Designed and first introduced to the HPLC market in the mid-1970s, the classical Hypersil silica is a Type A silica with the following characteristics:

- Made from aqueous sol with controlled amounts of metal ion
- Active toward bases; requires basic additives in the mobile phase
- One of the most stable silicas

A number of bonded phase chemistries are available in the Hypersil classical columns portfolio.

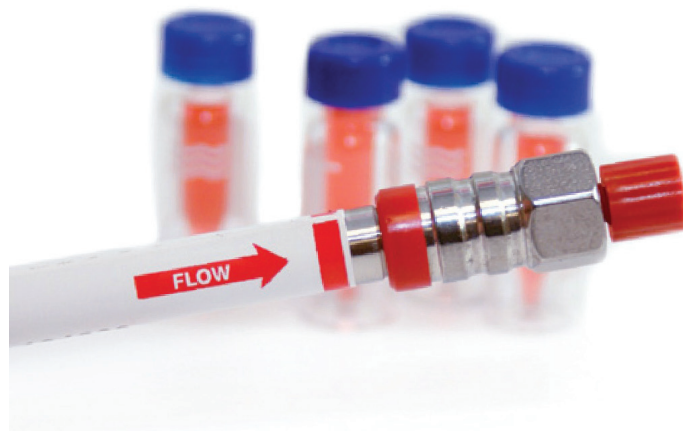


Table 1. Hypersil ODS columns specifications

Bonded phase	Particle size (µm)	Pore size (Å)	Pore volume (cc/gm)	Surface area (m ² /gm)	Carbon (%)	End-capped
Silica	3, 5	120	0.65	170	—	No
ODS (C18)	3, 5, 10	120	0.65	170	10.0	Yes
MOS (C8)	5, 10	120	0.65	170	6.5	No
MOS-2 (C8)	5	120	0.65	170	6.5	Yes
Phenyl	3, 5	120	0.65	170	5.0	No
Phenyl-2	5	120	0.65	170	5.0	Yes
SAS (C1)	5	120	0.65	170	2.5	Yes
APS-2 (NH ₂)	3, 5, 10	120	0.65	170	1.9	No
CPS (Cyano)	5	120	0.65	170	4.0	No
CPS-2 (Cyano)	5	120	0.65	170	4.0	Yes
SAX (-NMe ₃)	5	120	0.65	170	2.5	No

Phase properties

Hypersil ODS columns

The excellent reversed-phase packing is suitable for a wide range of applications and is one of the world's most popular packings. Made with Hypersil silica as a base, a monolayer of octadecyl silane (C18) is bonded on to the silica surface. It is then fully endcapped to minimize secondary interactions of analytes with residual silanol groups.

Hypersil ODS columns is a highly efficient chromatographic medium, showing the quality and reproducibility typical of the Hypersil classical portfolio. ODS is suitable for the analysis of non-polar to moderately polar acids, neutrals and lipophilic compounds. It is available in a range of sizes to improve your productivity and reduce solvent consumption.

Thermo Fisher Scientific manufacturing prides itself on quality and stability. The ODS bonded phase has a documented history of achievement in reproducibility and column efficiency. A full review of the quality assurance protocols for Hypersil ODS column is provided.

Thermo Scientific™ Hypersil™ MOS Columns

This phase has a monolayer coverage of octylsilane (C8 alkyl chain) chemically bonded onto the Hypersil silica surface. The MOS-2 phase is end-capped to produce a high quality stationary phase. MOS phases are highly efficient reversed phase materials that exhibit similar selectivity to ODS, but are less retentive.

Thermo Scientific™ Hypersil™ SAS Columns

This phase has the short alkyl chain (C1 or trimethyl) chemically bonded onto the silica surface. This material is the least retentive of all the alkyl group bonded phases for non-polar solutes. SAS has unique selectivity for polar and multi-functional compounds and has been successfully used for ion-pair separations.

Thermo Scientific™ Hypersil™ APS-2 Columns

This phase has propyl amino groups bonded to the silica surface. This versatile phase exhibits excellent chromatographic properties in three modes: weak anion exchange, reversed-phase and normal phase. The APS-2 phase can be used with common buffers and an organic modifier as a weak ion-exchange material for the analysis of anions and organic acids. When used as a normal phase material, APS-2 offers a different selectivity than silica with less sensitivity to water in the mobile phase. APS-2 used as a reversed phase material excels for carbohydrate analysis.

Thermo Scientific™ Hypersil™ CPS Columns

The cyanopropyl phase that can be used for both normal and reversed-phase HPLC. Under normal phase conditions this material offers different selectivity than Hypersil Silica and APS-2. In addition to complementary selectivity, CPS equilibrates very rapidly with the mobile phase, and is not sensitive to small quantities of water, making it the ideal choice for separations where gradient elution is necessary.

As a reversed-phase material, the selectivity of Hypersil CPS column complements the alkyl chain bonded phases, offering alternative selectivity with lower hydrophobicity. Ideal for retaining and separating polar compounds based on polarity.

Thermo Scientific™ Hypersil™ SAX Columns

The silica based strong anion exchanger, which is highly stable and robust, working well in aqueous and low pH mobile phases. It is suitable for the analysis of smaller organic molecules including nucleotides and organic acids.

Versatile reversed-phase HPLC packing

Overview of stationary phase performance

The Hypersil ODS columns are an excellent choice for a wide range of reversed-phase applications and are among the world's most popular stationary phase packings.

Made with the renowned Hypersil silica backbone, a monolayer of octadecyl silane is covalently bonded to the silica surface. It is then fully endcapped to minimize secondary interactions of analytes with residual silanol groups.

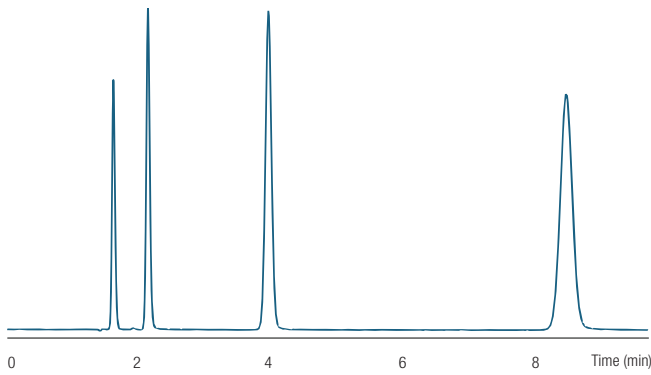
- Industry standard, used for many existing methods around the world
- High efficiency
- Proven reproducibility
- Long column lifetimes
- Wide range of bonded phases
- Not recommended for strongly basic compounds

The Hypersil ODS column was made commercially available in 1978. With a wide range of applications, the Hypersil ODS column provides excellent separation of moderately polar analytes, including acids, neutrals, and lipophilic compounds. The media has significant silanol interaction with the analytes and it is often this interaction that provides the Hypersil ODS column with its unique selectivity. Due to these silanol interactions, Hypersil ODS columns are only recommended for use with basic compounds when a competing base, such as triethylamine or dimethylamine, is used in the mobile phase.

Batch testing procedure

As Hypersil ODS columns are manufactured to 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. Prior to bonding with the C18 organosilane ligand used to prepare Hypersil ODS, the Hypersil base silica must pass almost thirty physical and chromatographic test specifications. Once bonded, the Hypersil ODS is tested chromatographically (Figure 1), for carbon content. This testing takes place both before and after the material is end-capped. The final Hypersil ODS chromatographic test involves a comparison against a standard column, evaluating a range of analytes for selectivity, efficiency, and asymmetry. A standard column is one which is prepared from a blend of up to 50 previous batches of Hypersil ODS. The standard column is run on the same day, on the same HPLC system, and with the same mobile phase solvent as the batch under test. All selectivity parameters (k and α values) must fall within 5% of those measured for the standard column, while efficiency parameters and asymmetry values must also meet strict specifications. Figure 1 illustrates the chromatographic test procedure employed. Reproducibility for all parameters is then recorded and monitored on an ongoing basis.





Hypersil ODS column, 5 μm, 150 x 4.6 mm	
Eluent	70% MeOH / 30% H ₂ O
Flow	0.8 mL/min
Detector	UV at 254nm
Sample	1. Benzamide
	2. Acetophenone
	3. Benzophenone
	4. Biphenyl

Figure 1. Chromatographic QC test for Hypersil ODS columns

Figure 2 shows the reproducibility of the % carbon observed for batches of Hypersil ODS media manufactured over the last six years. The percent carbon is an important parameter to measure as it will directly influence the chromatographic retention of analytes run under reversed-phase conditions. The percent carbon is measured by a carbon analyzer and is accurate to within plus or minus 0.1%. This tight specification insures reproducible column-to-column performance.

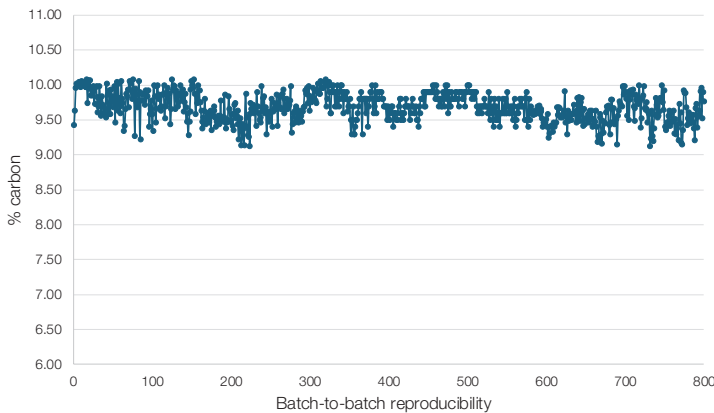


Figure 2. Hypersil ODS column (5 μm). Batch-to-batch reproducibility % carbon. Specification = 9.0 - 10.0. (Note the continuous tightening of the band over the last few years illustrating our commitment to continually improving the quality of our products).

Figure 3 illustrates the batch-to-batch reproducibility of chromatographic selectivity for Hypersil ODS media. Alpha values represent a ratio of capacity factors (k_3/k_2 within a given test mixture). This parameter is useful when comparing the performance of one column to another, as any change in one capacity factor will result in a significant change in the alpha value. Alpha values measured for Hypersil ODS must fall within plus or minus 10% of the standard column values.

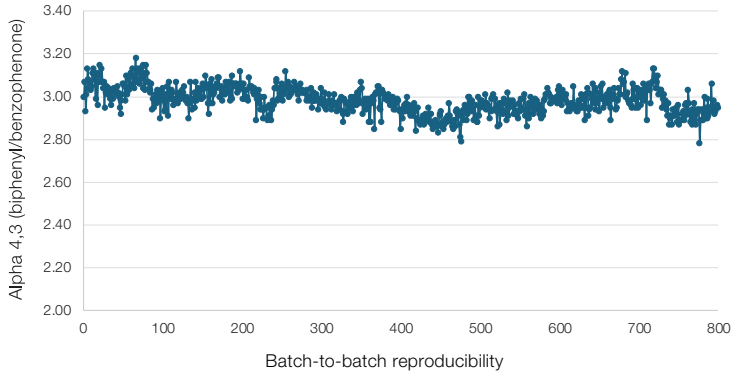
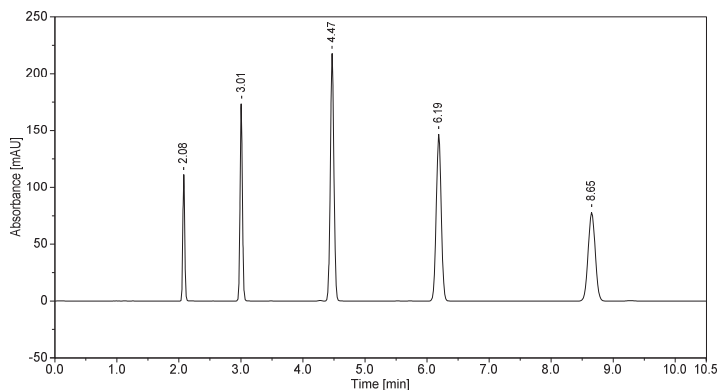


Figure 3. Hypersil ODS column (5 μm). Batch-to-batch reproducibility Alpha 4,3 (biphenyl/benzophenone).

Column reproducibility

Final column performance is checked using a different test procedure (Figure 4). Column-to-column performance is monitored for peak tailing (asymmetry) and column efficiency (theoretical plates) on every column. Figure 5 demonstrates how both of these parameters monitor column performance for over 1000 Hypersil ODS columns.



Hypersil ODS column, 5 μm , 250 x 4.6 mm

Eluent	60% ACN / 40% H ₂ O
Flow	1.25 mL/min
Detection	UV at 254nm
Sample	1. Theophylline
	2. p-Nitroaniline
	3. Methyl benzoate
	4. Phenetole
	5. o-Xylene

Figure 4. Final column test chromatogram

Peak asymmetry

Peak asymmetry (peak tailing) provides a useful measure of the quality of a column. Peak tailing is usually observed when a column deteriorates, but may occur if the columns are not well packed. The asymmetry ratio for a given peak is the width of the tail to the width of the front at 10% of the peak height. To meet specifications, the asymmetry measurement must fall within a range of 0.9-1.2. Figure 5 demonstrates the asymmetry measurements used to monitor the effectiveness of the packing procedure.

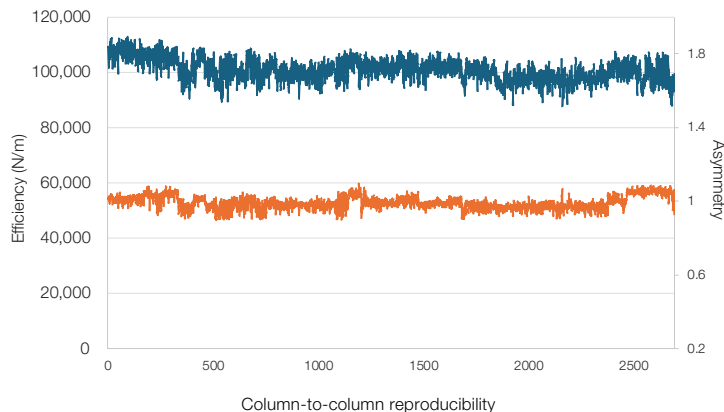
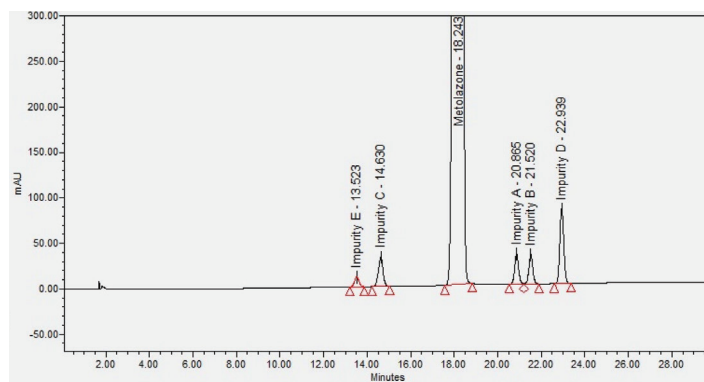


Figure 5. Hypersil ODS column (5 μm). Column-to-column reproducibility—peak asymmetry and efficiency.

Metolazone

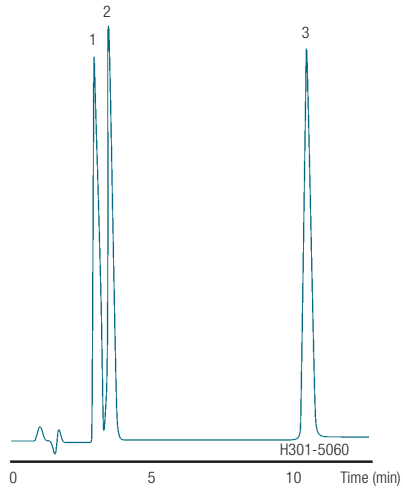


Hypersil ODS column, 5 μm , 250 x 4.6 mm

Eluent	A: 5.44 g/L KH ₂ PO ₄ in water B: Methanol
Flow	1.5 mL/min
Detection	UV at 230 nm
Sample	Ph Eur Reference standard: API metolazone and the impurities A, B, C, D, and E

Figure 6. Analysis of API metolazone and its five impurities¹

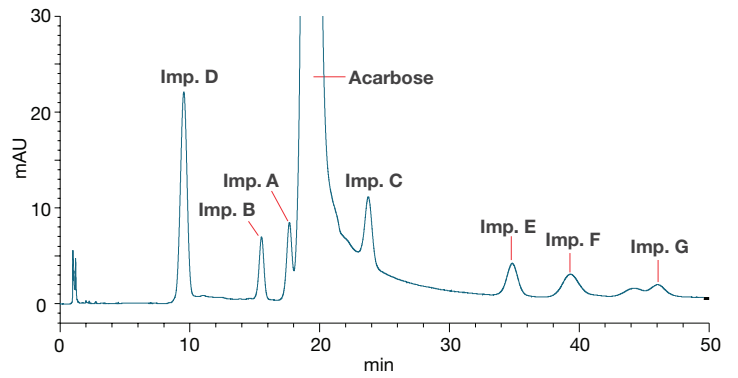
Isosorbide



Hypersil ODS column, 5 m, 200 x 2.1 mm	
Eluent	80% H ₂ O / 20% MeOH
Flow	1.0 mL/min
Detection	UV at 210 nm
Sample	1. Isosorbide-2-Mononitrate 2. Isosorbide-5-Mononitrate 3. Isosorbidedinitrate

Figure 7. Isosorbide²

Acarbose

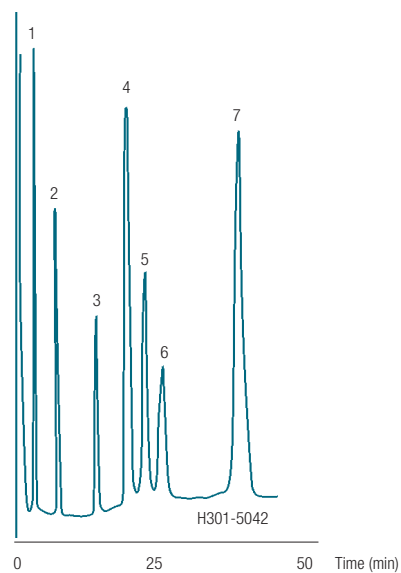
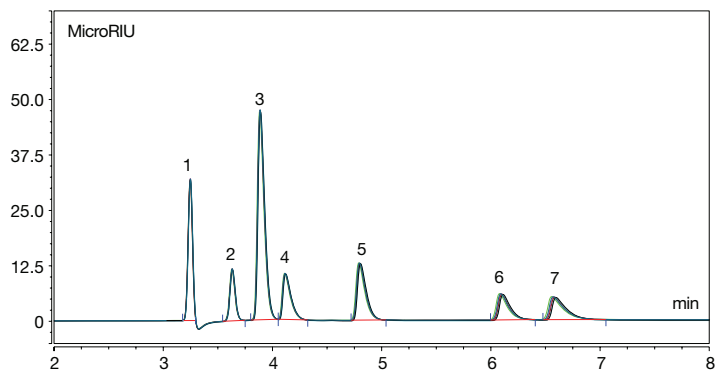


Hypersil APS-2 columns, 5 µm, 250 x 4.0 mm	
Eluent	A: aqueous solution of 0.60 g/L potassium dihydrogen phosphate and 0.35 g/L sodium hydrogen phosphate dihydrate B: acetonitrile
Flow	1.0 mL/min
Detection	UV at 210 nm
Sample	Acarbose certified reference standard (CRS) Acarbose for peak identification CRS

Figure 8. Impurity analysis of acarbose reference solution (1) by means of the current Ph. Eur. related substances test for acarbose. Hypersil APS-2 column (250 × 4 mm, 5 µm), UV-detection at 210 nm.

Polyaromatic hydrocarbons

Antimalarial drugs



Hypersil APS-2 column, 3 μ m, 250 x4 .6 mm

Eluent n-Heptane

Flow 1.0 mL/min

Detection RI detector

Sample

1. Cyclohexane
2. Dodecylbenzene
3. o-Xylene
4. Hexamethylbenzene
5. Naphthalene
6. Dibenzothiophene
7. 9-Methylantracene

Hypersil ODS column, 100 x 2.1 mm

Eluent 50% ACN / 50% 0.02M KH_2PO_4 , pH 2.5 containing 60mM SLS and 10 mM TBA

Flow 1.0 mL/min

Detection UV at 254 nm

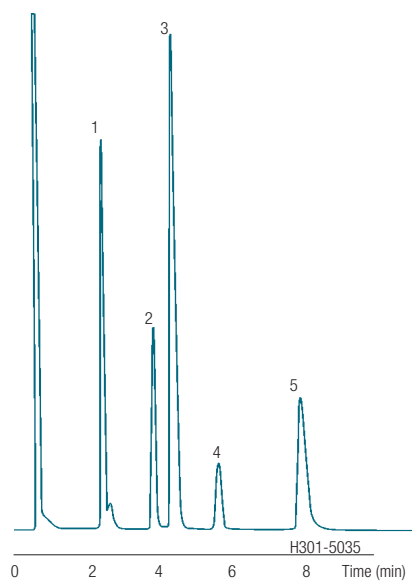
Sample

1. Cycloguanil
2. 4-Chlorophenyl-Biguanide
3. Quinine
4. Proguanil
5. Desethylchloroquine
6. Chloroquine
7. Chlorproguanil

Figure 9. Overlay of six replicate SPS solution injections

Figure 10. Antimalarial drugs³

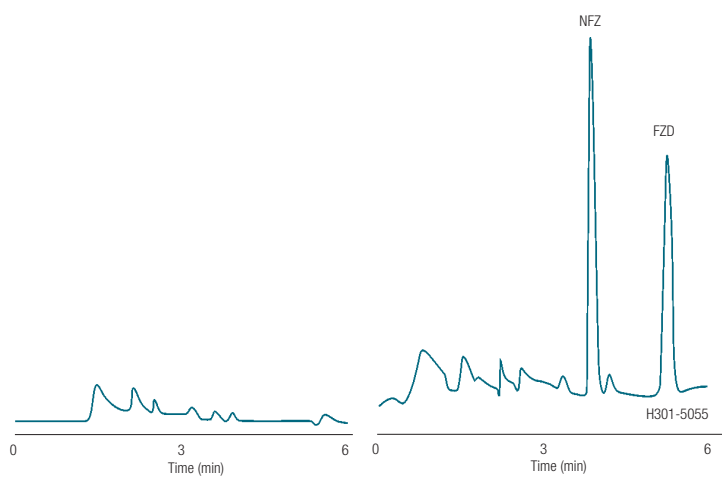
Urine metabolites



Hypersil ODS column, 150 x 4.6 mm	
Eluent	0.8% EtOH in 10mM KH ₂ PO ₄ , pH 2.3
Flow	2.0 mL/min
Detector	UV at 280 nm
Sample	1. p-Hydroxymandelic acid
	2. Vanilmandelic acid
	3. 7-Methyluric acid
	4. 1-Methyluric acid
	5. Internal standard

Figure 11. Urine metabolites

Nitrofurazones in shrimp



Hypersil ODS column, 5 µm, 200 x 4.6 mm	
Eluent	25% ACN / 75% 1% aq. acetic acid
Flow	1.0 mL/min
Detector	UV at 375 nm
Sample	(a) Shrimp control
	(b) Shrimp fortified with nitrofurazone (NFZ) and furazolidone (FZD)

Figure 12. Nitrofurazones in shrimp

Ordering information

Hypersil ODS columns

Description	Particle size	Diameter	Length	Quantity	Cat. No	
Hypersil ODS columns	3 µm	2.1 mm	10 mm	Each	30103-012101	
			100 mm	Each	30103-102130	
			150 mm	Each	30103-152130	
			150 mm	Each	30103-252130	
		3.0 mm	10 mm	Each	30103-013001	
			50 mm	Each	30103-053030	
			100 mm	Each	30103-103030	
			150 mm	Each	30103-153030	
		4.0 mm	10 mm	Each	30103-014001	
			100 mm	Each	30103-104030	
			125 mm	Each	30103-124030	
			150 mm	Each	30103-154030	
	250 mm		Each	30103-254030		
	4.6 mm	50 mm	Each	30103-054630		
		100 mm	Each	30103-104630		
		125 mm	Each	30103-124630		
		150 mm	Each	30103-154630		
	Hypersil ODS columns	5 µm	2.1 mm	250 mm	Each	30105-252130
				200 mm	Each	30105-202130
				150 mm	Each	30105-152130
125 mm				Each	30105-122130	
100 mm				Each	30105-102130	
10 mm				Each	30105-012101	
3.0 mm			250 mm	Each	30105-253030	
			150 mm	Each	30105-153030	
			125 mm	Each	30105-123030	
			100 mm	Each	30105-103030	
			50 mm	Each	30105-053030	
4.0 mm			10 mm	Each	30105-014001	
		50 mm	Each	30105-054630		
		100 mm	Each	30105-104030		
		125 mm	Each	30105-124030		
		150 mm	Each	30105-154030		
		200 mm	Each	30105-204030		
		250 mm	Each	30105-254030		
		50 mm	Each	30105-054630		
4.6 mm		100 mm	Each	30105-104630		
	125 mm	Each	30105-124630			
	150 mm	Each	30105-154630			
	200 mm	Each	30105-204630			
	250 mm	Each	30105-254630			
	300 mm	Each	30105-304630			
10 µm	4.0 mm	10 mm	Each	30110-014001		
		250 mm	Each	30110-254030		
		300 mm	Each	30110-304030		
	4.6 mm	100 mm	Each	30110-104630		
		200 mm	Each	30110-204630		
		250 mm	Each	30110-254630		
		300 mm	Each	30110-304630		

Ordering information (continued)

Hypersil ODS-2 columns

Description	Particle size	Diameter	Length	Quantity	Cat. No
Hypersil ODS-2 columns	3 µm	4.0 mm	10 mm	Each	31603-014001
		4.6 mm	50 mm	Each	31603-054630
			100 mm	Each	31603-104630
			150 mm	Each	31603-154630
	5 µm	4.0 mm	10 mm	Each	31605-014001
		4.0 mm	125 mm	Each	31605-124030
			250 mm	Each	31605-254030
		4.6 mm	50 mm	Each	31605-054630
			100 mm	Each	31605-104630
			125 mm	Each	31605-124630
			150 mm	Each	31605-154630
			200 mm	Each	31605-204630
250 mm	Each		31605-254630		

Hypersil MOS (C8) and Hypersil MOS-2 (C8) columns

Description	Particle size	Diameter	Length	Quantity	Cat. No		
Hypersil MOS (C8) columns	5 µm	4.0 mm	10 mm	Each	30205-014001		
			150 mm	Each	30205-154030		
			250 mm	Each	30205-254030		
		4.6 mm	50 mm	Each	30205-054630		
	100 mm		Each	30205-104630			
	150 mm		Each	30205-154630			
	200 mm		Each	30205-204630			
	Hypersil MOS-2 (C8) columns	5 µm	4.0 mm	10 mm	Each	30305-014001	
125 mm				Each	30305-124630		
4.6 mm			150 mm	Each	30305-154630		
			250 mm	Each	30305-254630		
			5 µm	4.0 mm	10 mm	Each	30903-014001
				150 mm	Each	30903-154630	

Hypersil Phenyl and Phenyl-2 columns

Description	Particle size	Diameter	Length	Quantity	Cat. No
Hypersil Phenyl columns	3 µm	3.0	150 mm	Each	30903-153030
		4.0	10 mm	Each	30903-014001
		4.6	150 mm	Each	30903-154630
	5 µm	4.0	10 mm	Each	30905-014001
		4.6	250 mm	Each	30905-254030
			150 mm	Each	30905-154630
Hypersil Phenyl-2 columns	5 µm	4.6	250 mm	Each	30905-254630

Ordering information

Hypersil SAS (C1) columns

Description	Particle size	Diameter	Length	Quantity	Cat. No
Hypersil SAS (C1) columns	5 µm	4.0 mm	10 mm	Each	30505-014001
		4.6 mm	125 mm	Each	30505-124630
	150 mm		Each	30505-154630	
	200 mm		Each	30505-204630	
	250 mm	Each	30505-254630		

Hypersil CPS (Cyano) and Hypersil CPS-2 columns

Description	Particle size	Diameter	Length	Quantity	Cat. No
Hypersil CPS (Cyano) columns	3 µm	4.6 mm	100 mm	Each	30803-104630
			150 mm	Each	30803-154630
	5 µm	4.0 mm	125 mm	Each	30805-124030
			250 mm	Each	30805-254030
		4.6 mm	100 mm	Each	30803-104630
	150 mm		Each	30805-154630	
	200 mm		Each	30805-204630	
	Hypersil CPS-2 columns	5 µm	4.6 mm	250 mm	Each
150 mm				Each	31805-154630
			250 mm	Each	31805-254630

Hypersil APS-2 columns

Description	Particle size	Diameter	Length	Quantity	Cat. No
Hypersil APS-2 columns	3 µm	2.1 mm	10 mm	Each	30703-012101
			100 mm	Each	30703-102130
			150 mm	Each	30703-152130
		4.0 mm	10 mm	Each	30703-014001
			125 mm	Each	30703-124030
			50 mm	Each	30703-054630
	4.6 mm	100 mm	Each	30703-254630	
		150 mm	Each	30703-154630	
		3.0 mm	100 mm	Each	30705-103030
	5 µm	4.0 mm	10 mm	Each	30705-014001
			250 mm	Each	30705-254030
		4.6 mm	300 mm	Each	30705-304030
			150 mm	Each	30705-154630
	10 µm	4.0 mm	250 mm	Each	30705-254630
			300 mm	Each	30710-304030
4.6 mm		300 mm	Each	30710-254630	

Ordering information (continued)

Hypersil Silica columns

Description	Particle size	Diameter	Length	Quantity	Cat. No
Hypersil Silica columns	3 µm	4.0 mm	10 mm	Each	30003-014001
			150 mm	Each	30003-154030
		4.6 mm	150 mm	Each	30003-154630
			250 mm	Each	30003-254630
		2.1 mm	50 mm	Each	30005-052130
			100 mm	Each	30005-102130
	5 µm	4.0 mm	10 mm	Each	30005-014001
			50 mm	Each	30005-054030
			150 mm	Each	30005-154030
		4.6 mm	250 mm	Each	30005-254030
			300 mm	Each	30005-304030
			50 mm	Each	30005-054630
4.6 mm	150 mm	Each	30005-154630		
	200 mm	Each	30005-204630		
	300 mm	Each	30005-254630		

Hypersil SAX columns

Description	Particle size	Diameter	Length	Quantity	Cat. No
Hypersil SAX columns	5 µm	3.0 mm	250 mm	Each	34105-253030
			10 mm	Each	34105-014001
		4.6 mm	150 mm	Each	31405-154630
			250 mm	Each	34105-254630

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2. Azcona, T., et al. "Journal of Pharmaceutical and Biomedical Analysis." J. Pharm. Biom. Anal., vol. 9, 1991, pp.
3. Taylor, R.B., et al. "Journal of Pharmaceutical and Biomedical Analysis." J. Pharm. Biom. Anal., vol. 10, 1992, pp. 867.

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