Back to Table of Contents

Hypersil ™ Classical Phases including Hypersil ODS Columns

Technical Guide

Exceptionally reliable and reproducible columns for neutral and polar compounds

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Hypersil™ Classical Phases and Hypersil ODS Columns

Introduction to Hypersil Classic Column Range

Thermo Electron columns offer exceptional performance and documentation of quality, batch and colum QA information and validation going back to 1978. Accredited under ISO9001:2000, the Thermo Electron HPLC column manufacturing plants insure strict adherence to quality, through the initial silica production, bonded phase production and finally to the manufacture of the HPLC columns themselves. In this Technical Guide we review the different Hypersil Classical columns in terms of physical properties and usage, and then focus in greater detail on the quality assurance protocols associated with Hypersil ODS columns.

Hypersil Classical Columns

- Exceptionally reliable and reproducible columns for neutral and polar compounds
- All columns supplied with test certificates
- Proven, reproducible column efficiency
- Long column lifetimes, even under basic conditions
- Wide range of bonded phases with very low pressure drop
- One of the world's most widely referenced column packing materials with a proven track record

Specifications:

Bonded Phase	Particle Size	Pore Size (Å)	Pore Volume (cc/gm)	Surface Area (m^2/gm)	%Carbon	End-Capped
Silica	3, 5, 10	120	0.65	170	--	No
ODS (C18)	3, 5, 10	120	0.65	170	10.0	Yes
MOS (C8)	3, 5, 10	120	0.65	170	6.5	No
MOS-2 (C8)	3, 5, 10	120	0.65	170	6.5	Yes
Phenyl	3, 5, 10	120	0.65	170	5.0	No
Phenyl-2	3, 5, 10	120	0.65	170	5.0	Yes
SAS (C1)	3, 5, 10	120	0.65	170	2.5	Yes
APS-2 (NH_2)	3, 5, 10	120	0.65	170	1.9	No
CPS (Cyano)	3, 5, 10	120	0.65	170	4.0	No
CPS-2 (Cyano)	3, 5, 10	120	0.65	170	4.0	Yes
SAX (-NMe_3)	5	120	0.65	170	2.5	No
SCX	5	100	0.65	300	--	No

Phase Summary

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 HPLCmarket in the mid 1970's, the Classical Hypersil silica is a Type A silica having 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, even at high pH

A number of bonded phase chemistries are available in the Classic Hypersil family.

Hypersil ODS is an excellent reversed phase packing for a large 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 is a highly efficient chromatographic medium, showing the quality and reproducibility typical of the Hypersil Classical family. 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 including narrow bore columns (1.0 and 2.1mm I.D). Narrow bore columns provide the proven characteristics of Hypersil ODS in a format that allows higher sensitivity for critical applications, together with a dramatic reduction in solvent consumption.

Thermo Electron 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 columns is provided.

Hypersil MOS -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.

Hypersil SAS has a 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.

Hypersil APS-2 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 phasematerial excels for carbohydrate analysis.

Hypersil CPS is a 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 complementaryselectivity, 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 complements the alkyl chain bonded phases. Short equilibration times make it an ideal choice for analytes needing gradient elution. The CPS-2 phase is endcapped to produce a high quality stationary phase.

Hypersil SAX is a 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.

Hypersil SCX is a silica based strong cation 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 nucleosides, and organic bases.

Hypersil® ODS Columns in Detail

Hypersil ODS is an excellent packing for a wide range of reversed phase applications, and is one of the world's most popular stationary phase packings. Made with the renowned Hypersil as a silica backbone, a monolayer of octadecyl silane is covalently bonded to the silica surface. It is then fully endcapped to minimize secondary interaction 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

Hypersil columns direct from Thermo Electron – the original source

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

Batch Testing Procedure

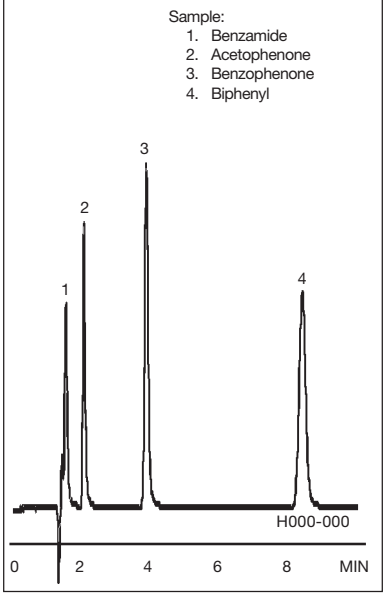
As with all Thermo Electron columns, Hypersil ODS columns are manufactured to highest standards, and are rigorously quality controlled. The fully documented ISO9001:2000 control procedures for both media and column production insure that only the highest quality columns are released.

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, Hypersil ODS is tested chromatographically (Figure 1), and is tested for carbon content. This testing takes place both before and after the material is end-capped.

The final Hypersil ODS chromatographic test is 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 alpha 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.

Figure 1

Chromatographic QC Test for Hypersil ODS



Hypersil ODS, 5µm, 100x4.6mm
Eluent: 70% MeOH / 30% H_2O
Flow: 0.8 mL/min
Detector: UV @ 254

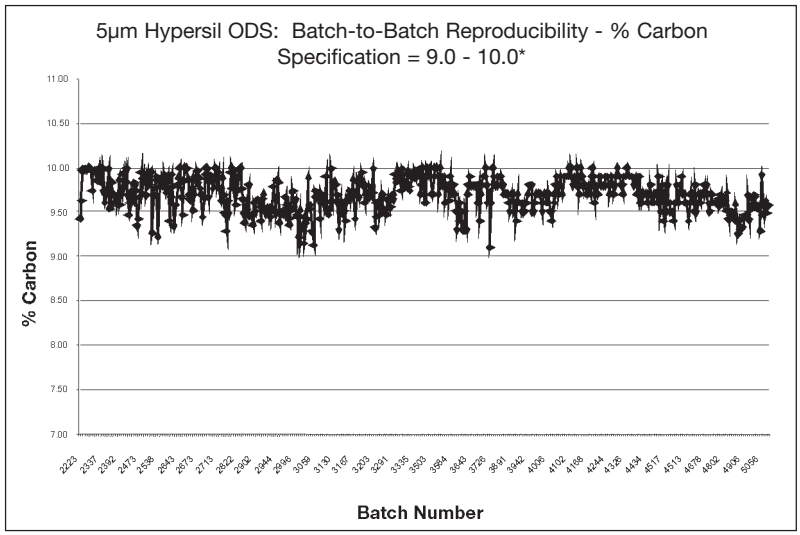
Figure 2 shows the reproducibility of the % carbon observed for batches of Hypersil ODS media manufactured over the last six years. The percent carbon is an important parameter to measure as it will directly influence the chromatographic retention of analytes run under reversed phase conditions. The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

Figure 3 illustrates the batch to batch reproducibility of chromatographic selectivity for Hypersil ODS media. Alpha values represent a ratio of capacity factors (k values measured for twodifferent analytes, k_3/k_2 within a given test mixture). It is a useful parameter 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 (a standard column is column packed with a blend of at least 50 previous Hypersil ODS batches).

Column Reproducibility

Final column perormance 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 10,000 Hypersil ODS columns.

Figure 2



**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

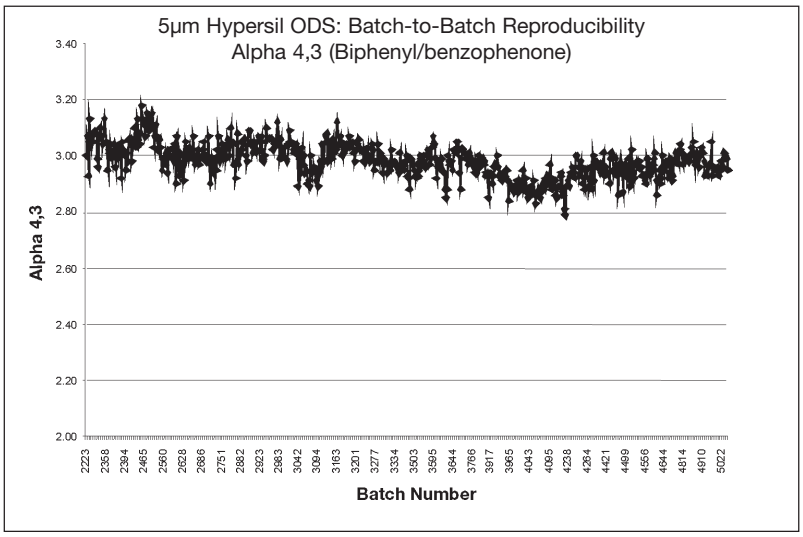
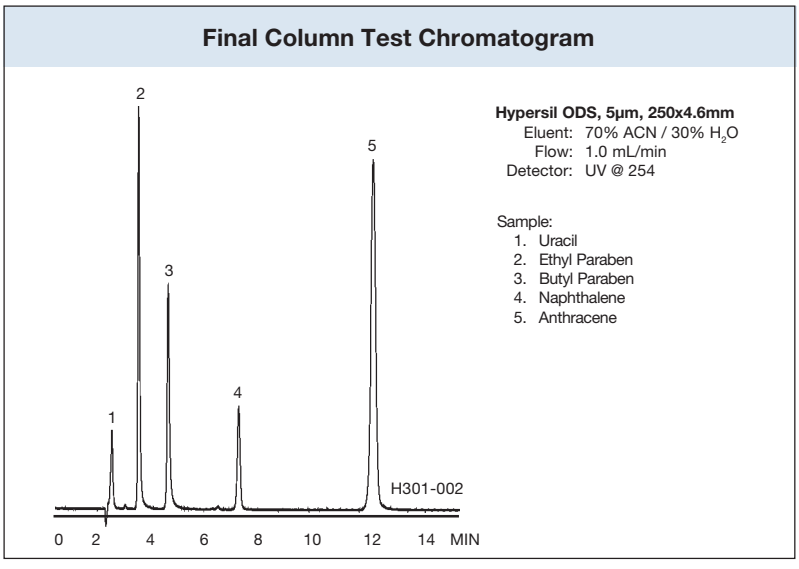


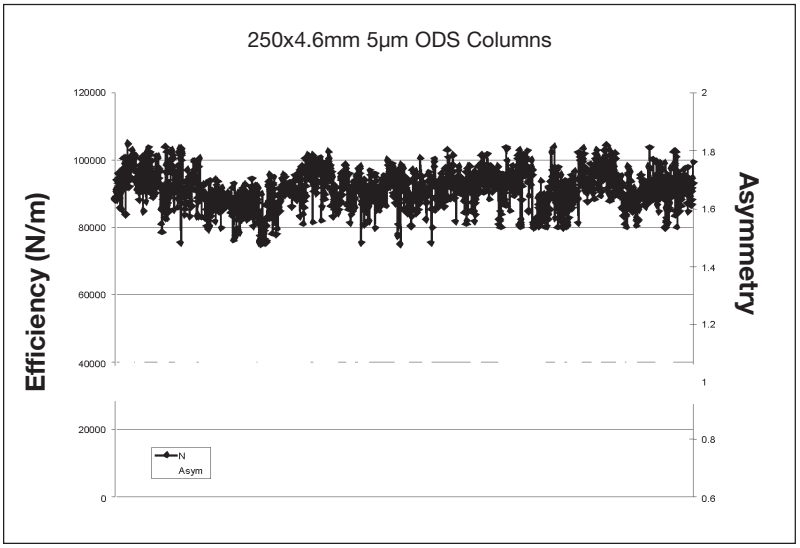
Figure 4



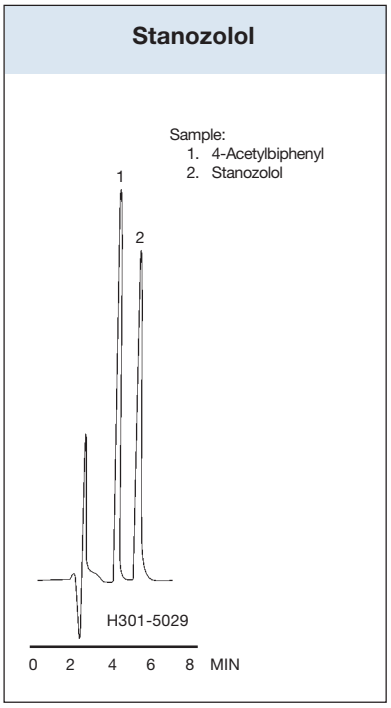
Peak Asymmetry

Peak asymmetry (peak tailing) gives a useful measure of the quality of a column. Peak tailing is usually observed when a column deteriorates, but may also be observed 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 pass specification the asymmetry measurement must fall within a range of 0.4-1.2. Figure 5 demonstrates asymmetry measurements that are used to monitor the effectiveness of the packing procedure.

Figure 5

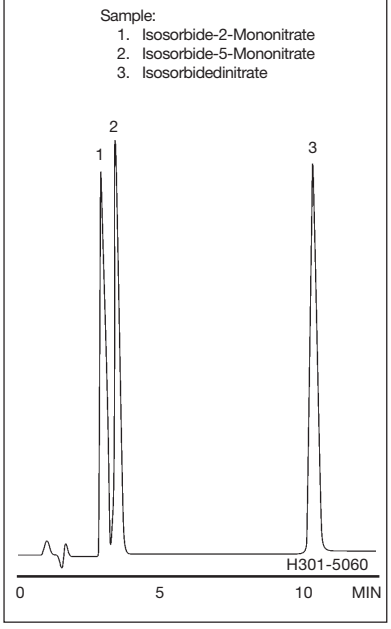


Stanozolol



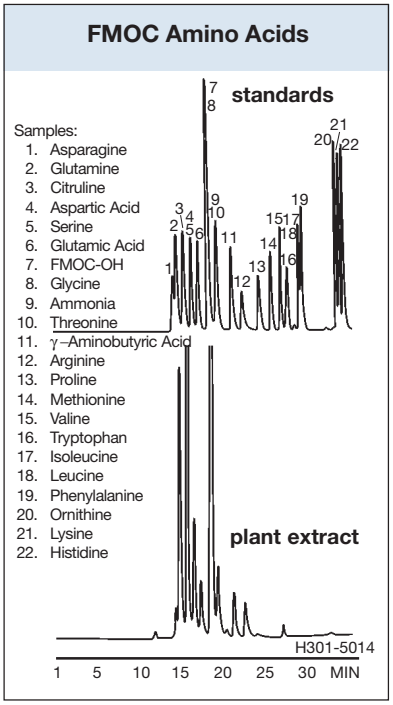
Hypersil® ODS, 5µm, 250x4.6mm
Eluent: 85% MeOH / 15% 0.05M Ammonium Phosphate
Flow: 1.0 mL/min
Detector: UV @ 230
Courtesy of Cavrini V. et al Analyst 112 (1987) 1671

Isosorbide



Hypersil ODS, 5µm, 200x2.1mm
Eluent: 80% H_2O / 20% MeOH
Flow: 1.0 mL/min
Detector: UV @ 210
Courtesy of Azcona T. et al J. Pharm Biom An 09 (1991) 728

FMOC Amino Acids



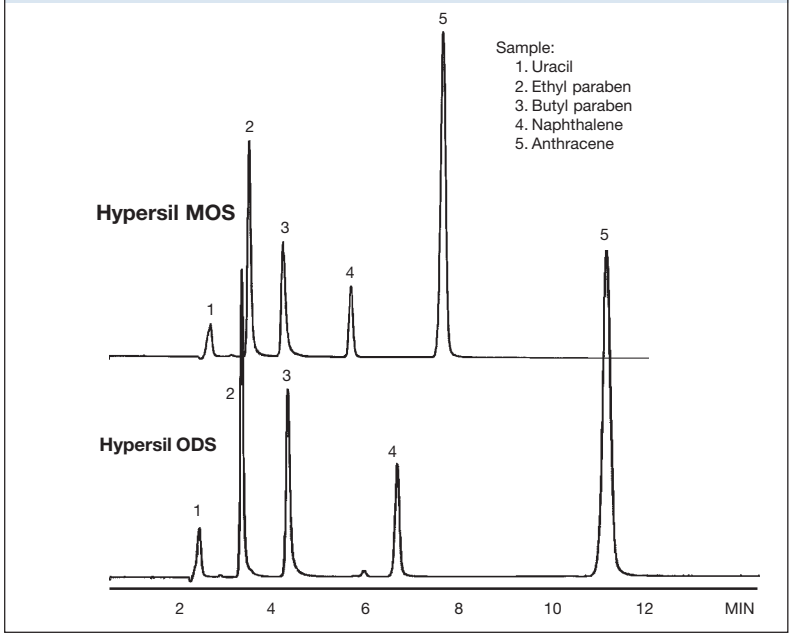
Hypersil ODS, 5µm, 250x4.6mm
Eluent: A: 0.5M Acetic Acid pH 2.8
B: Methanol
Flow: 1 mL/min
Gradient:

Time	%B
0	50
20	70
30	90

Detector: Fluorescence

Back to Table of Contents

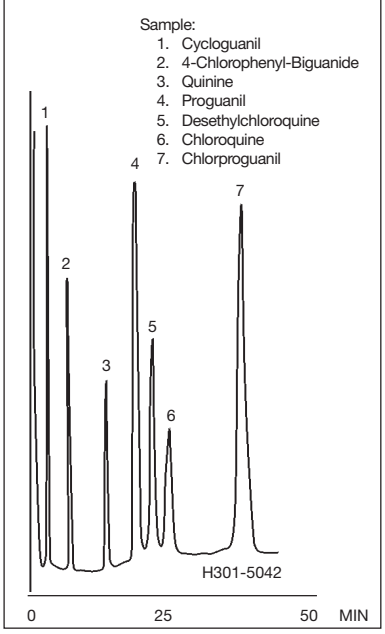
Comparison of Retention on Hypersil ODS and MOS Columns



Hypersil ODS , 5µm, 150x4.6mm
Eluent: 60% ACN / 40% H_2O
Flow: 1.0 mL/min
Detector: UV @ 254

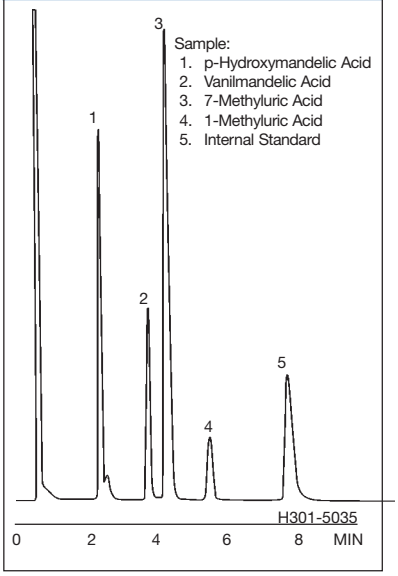
Hypersil MOS, 5µm, 150x4.6mm
Eluent: 60% ACN / 40% H_2O
Flow: 1.0 mL/min
Detector: UV @ 254

Peptides



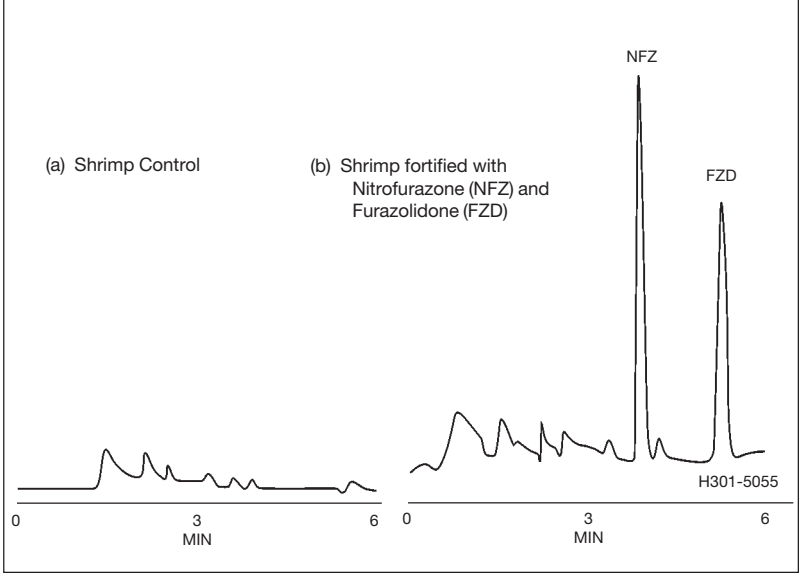
Hypersil ODS, 100x2.1mm
Eluent: 50% ACN / 50% 0.02M KH_2PO_4, pH 2.5 containing 60mM SLS and 10 mM TBA
Flow: 1.0 mL/min
Detector: UV @ 254
Ref: Taylor R.B. et al J.Pharm Biom. An 10(1992) 867

Urine Metabolites



Hypersil ODS, 150x4.6mm
Eluent: 0.8% EtOH in 10mM KH_2PO_4, pH 2.3
Flow: 2.0 mL/min
Detector: UV @ 280

Nitrofurazones in Shrimp



Hypersil ODS, 5µm, 200x4.6mm
Eluent: 25% ACN / 75% 1% aq. Acetic Acid
Flow: 1.0 mL/min
Detector: UV @ 375

Back to Table of Contents

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