A Highly Consistent and Chemically Stable Silica-based Octadecylsilane Stationary Phase for High Performance Liquid Chromatography: TSKgel Super-ODS Column for LC/MS

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Abstract

Octadecylsilane (ODS) columns have been most widely used for reversed phase HPLC. High throughput analysis with high sensitivity is now expected in various applications such as biological, pharmaceutical, and environmental testing. The TSK-GEL Super Series columns, composed of the TSKgel Super-ODS, TSKgel Super-Octyl, and the TSKgel Super-Phenyl are high performance columns that combine superior resolution and speed for reversed phased chromatography separations. These columns are provided in three functionalities for the selectivity required for a given separation.

The silica particles used in the Super Series columns are monodispersed spherical 2µm beads with a 110Å pore size. Due to the fact that the silica is high purity and metal free, there is no band broadening. In addition, the high level of endcapping minimizes secondary interactions between the sample and the column packing. The narrow particle size distribution results in low back pressure. The 2µm porous silica packing also provides a large surface area and higher loading capacity than similar non-porous materials. For fast separation of various compounds, the TSKGEL Super Series columns are packed in dimensions of 1mm, 2mm, and 4.6mm I.D. with 5cm and 10cm lengths. The data presented will include LC/MS separations on three different types of compounds: A) Pesticides. B) Tryptic Digest. and C) Small Peptides.

In this poster

- Comparisons between traditional 5µm particles (ODS-80Ts column) and ultra-fast 2µm particles used in the Super Series Reversed phase columns (Super-ODS, Super Octyl, and Super-Phenyl).
- Super Series columns availability in micro and semi-micro analytical format (such as 1mm and 2mm ID).
- The advantages of 2µm Silica.
- How to capitalize on the advantages by optimizing inter/extra column components.
- Interrelationship between sample injection volume, sample mass, flow rate, and column diameter.
- Examples of LC/MS Applications

TSK-GEL Super Series RPC Columns

Column	Functionality	Dimension	Pore size(Å)
Super-ODS	C18, polymeric 8% carbon	1, 2 or 4.6mm ID 5 or 10cm L	110
Super-Octyl	C8, polymeric 5% carbon	2 or 4.6mm ID 5 or 10cm L	110
Super-Phenyl	phenyl, polymeric 3% carbon	2 or 4.6mm ID 5 or 10cm L	110

Basic Properties of 2µm and 5µm Silica

	TSK-GEL Super Series	TSK-GEL ODS-80T$_S$
Silica	High purity	High purity
End-capped	Yes	Yes
Particle Size	2.2µm	5.06µm
Standard Deviation	0.27µm	0.87µm
Mean Pore Diam.	112Å	82Å
Pore Volume	0.25mL/g	0.63mL/g
Specific Surf. Area	96.8m^2/g	312.8m^2/g
Exclusion Limit	20,000Da	6,000Da
Carbon Content (C%)	~8	~15

Advantages of 2µm Silica

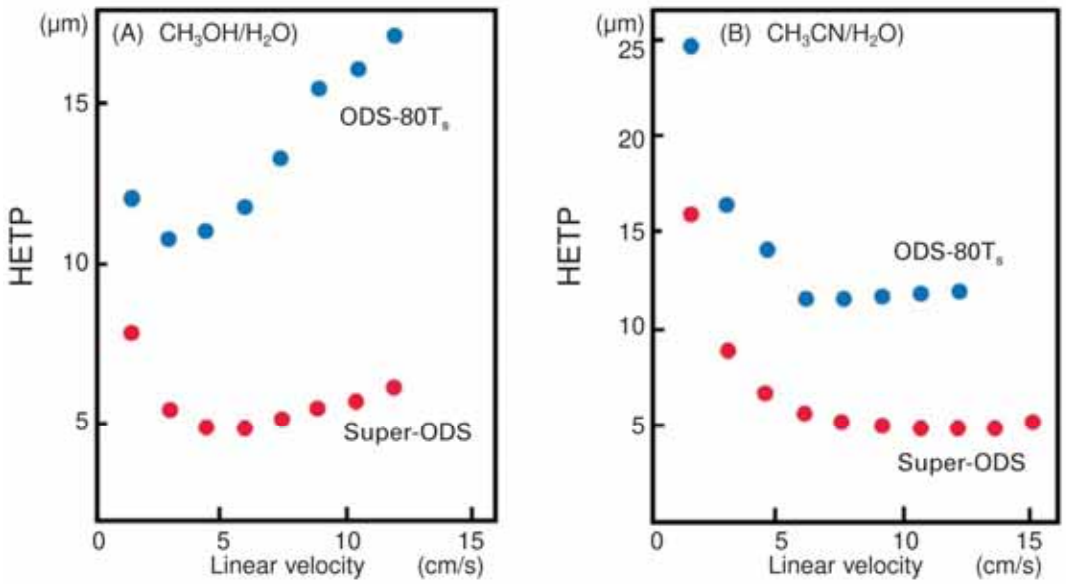
- **Reduces the diffusion path** (particle size)

- **Narrows peak bandwidth**
  - increases resolution (particle size)
  - increases sensitivity (small bore)

- **Reduces solvent consumption**
  - shorter column lengths
  - decreased analysis time
  - lower solvent strengths

Column

Optimization of Particle Size and Mobile Phase

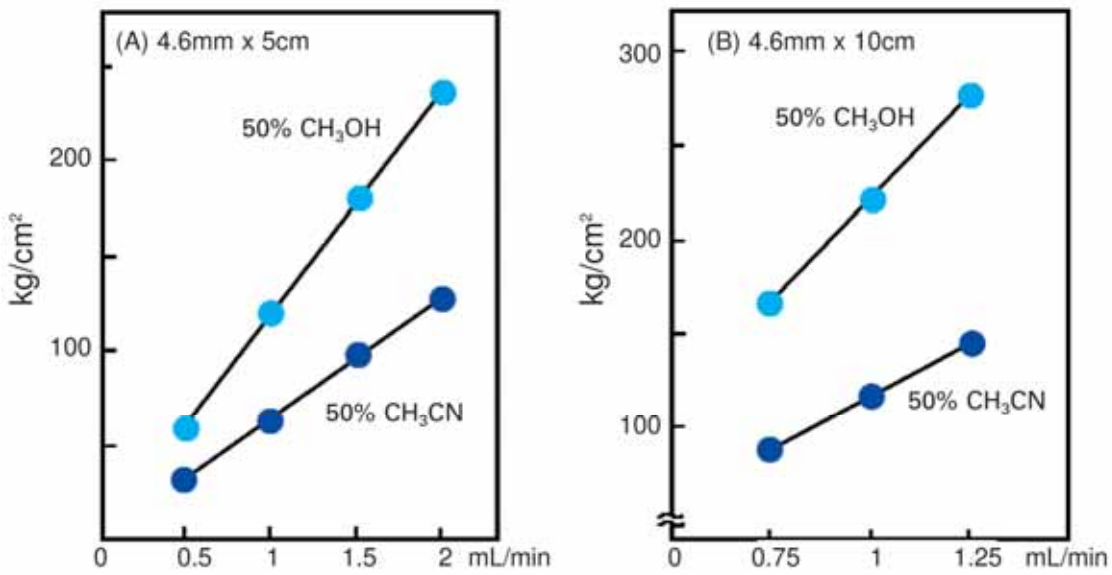


H/u Curve in Various Eluent Compositions

Column: ● TSKgel Super-ODS (4.6mm ID x 5cm)
● TSKgel ODS-80T$_s$ (4.6mm ID x 15cm)
Eluent: (A) 70% methanol (B) 50% acetonitrile
Flow rate: 0.25 to 2.5mL/min
Detection: UV (254nm) Temperature: 25°C
Sample: Fluorene

Column

Optimization of Column Size: Why Smaller is Better

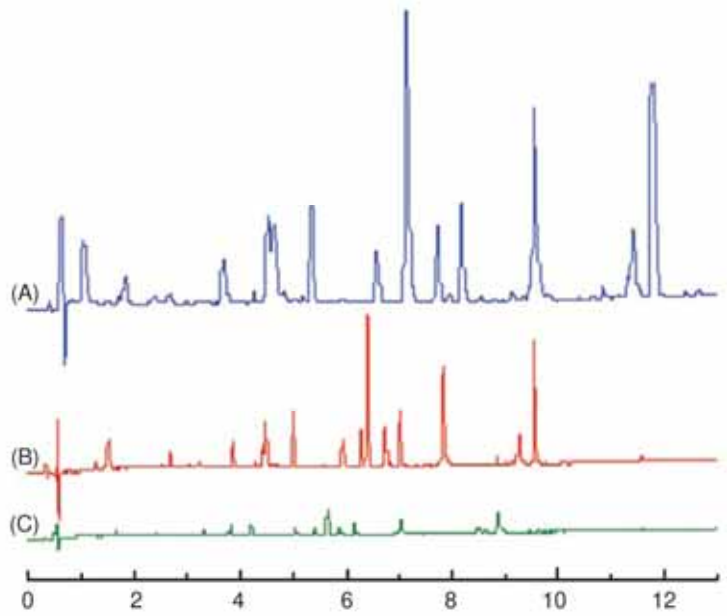


Relationship between Eluent Composition and Pressure

Column: A. TSKgel Super-ODS (4.6mm ID x 5cm)
B. TSKgel Super-ODS (4.6mm ID x 10cm)
Eluent: 50% CH_3OH, 50% CH_3CN
Flow rate: 0.5 to 2.0mL/min
Temperature: Ambient

Column

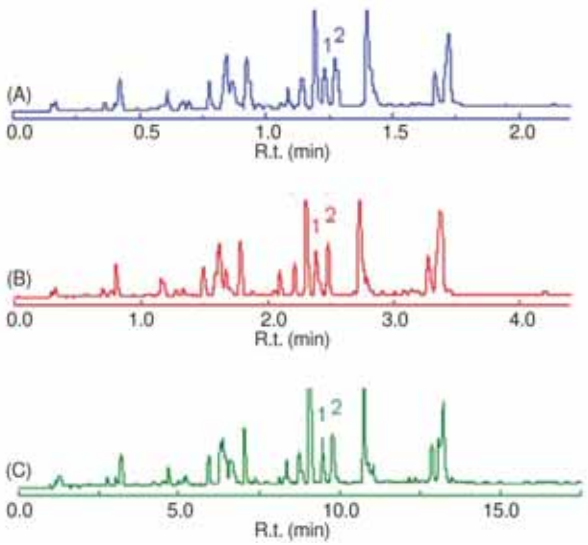
Effect on Column Diameter on Peak Height

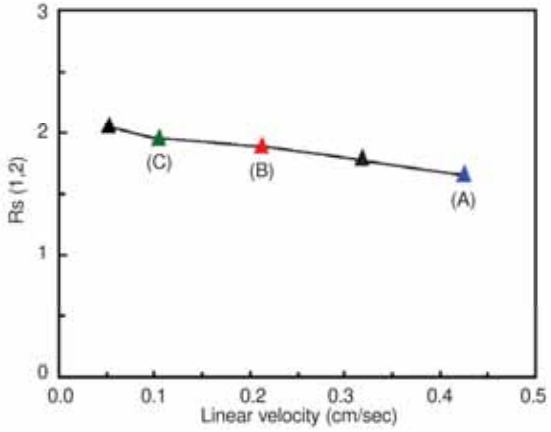


Column:	TSKgel Super-ODS (A) 1.0mm I.D. _ 5cm (B) 2.0mm I.D. _ 5cm (C) 4.6mm I.D. _ 5cm
Eluent;	A: H_2O/CH_3CN(95/5)+0.1% TFA B: H_2O/CH_3CN(50/50)+0.1% TFA
Gradient:	1-100% B(15min, Linear gradient)
Flow rate:	(A) 0.1mL/min (B) 0.4mL/min (C) 2.12mL/min
Inj. Vol.:	0.5µL
Temp.:	40°C
Sample:	β-Lactoglobulin tryptic digest

Conditions

Effect of Flow Rate on Resolution (gradient volume is constant)

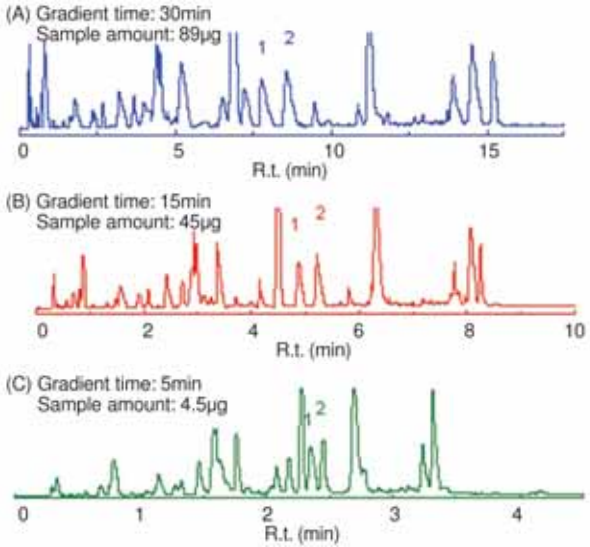


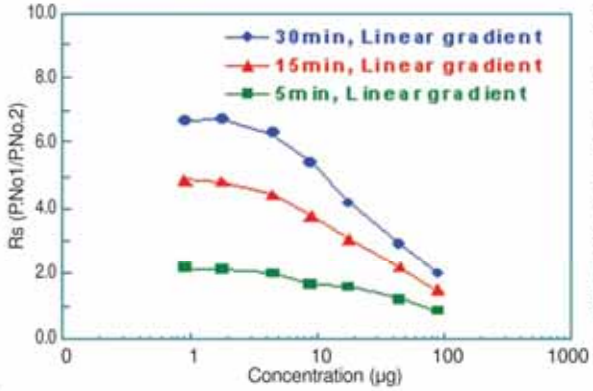


Column:	TSKgel Super-ODS (2.0mm I.D. x 5cm)
Eluent:	A: H_2O/CH_3CN(95/5)+0.1% TFA B: H_2O/CH_3CN(50/50)+0.1% TFA
Gradient:	0-100% B((A) 2.5min, (B) 5min, (C) 20min, Linear gradient)
Flow rate:	(A) 0.44cm/min, (B) 0.22cm/min, (C) 0.055cm/min
Inj. Vol.:	2µL
Temp.:	40°C
UV:	215nm
Sample:	β-Lactoglobulin tryptic digest

Conditions

Effect of Sample Mass on Resolution

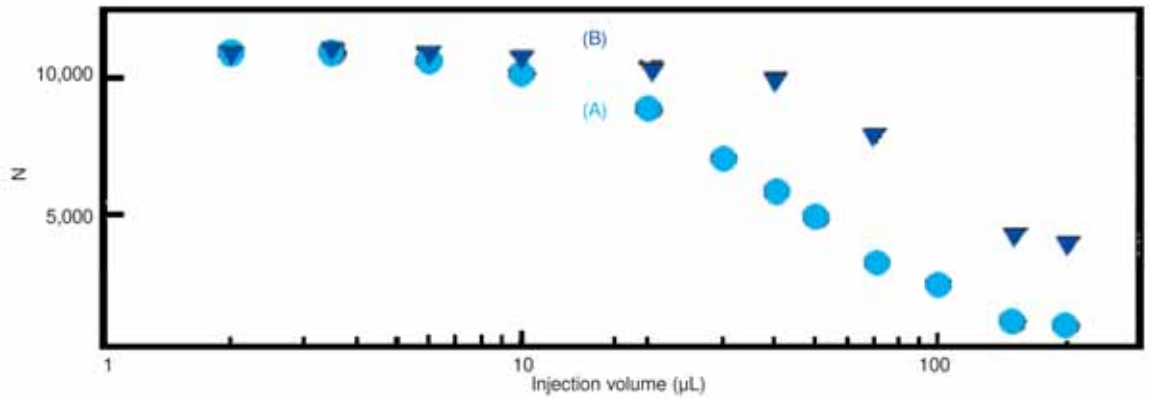




Column:	TSKgel Super-ODS (2.0mm I.D. x 5cm)
Eluent:	A: H_2O/CH_3CN(95/5)+0.1% TFA B: H_2O/CH_3CN(50/50)+0.1% TFA
Gradient:	0-100% B((A) 30min, (B) 15min, (C) 5min, Linear gradient)
Flow rate:	0.4mL/min
Inj. Vol.:	2μL
Temp.:	40°C
UV:	215nm
Sample:	β-Lactoglobulin tryptic digest ((A) 44.5mg/mL, (B) 22.5mg/mL, (C) 2.25mg/mL

Conditions

Sample Injection Volume and Column Efficiency (Theoretical Plates)



Column:	(A), (B) TSKgel Super-ODS (4.6mm I.D.x 5cm)
Eluent:	70% methanol
Flow rate:	1.0mL/min
Temperature:	25°C
Detection:	UV (254nm)
Samples:	Naphthalene (0.1g/L) dissolved in (A) 70% methanol , (B) dissolved in 40% methanol, (0.1 g/L)

Detector / Tubing

Optimization of Tubing and Its Effect on Column Efficiency

Injector/Column*			Column/Detector**		
Length of the tubing	Volume of tubing	HETP	Length of the tubing	Volume of tubing	HETP
(cm)	(µL)	(µm)	(cm)	(µL)	(µm)
10.00	0.79	4.66	10.00	0.79	4.66
15.00	1.19	4.70	15.00	1.19	4.70
30.00	2.36	5.23	30.00	2.36	4.74
50.00	3.93	5.51	50.00	3.93	5.35
70.00	5.50	5.89	70.00	5.50	5.54

* Distance between Injector/column
** Distance between Column/Detector

Column: TSKgel Super-ODS (4.6mm x 5cm)
Eluent: 70% methanol Flow rate: 1mL/min
Detection: UV (254nm), micro flow cell
Sample: Fluorene

Detector / Tubing

Relationship between Detector Response and Column Efficiency

Time Constant	Naphthalene Theoretical Plates TP/column (relative reduction)	Resolution Tol/Nap
50 msec	10529 (0%)	13.37
1 sec	6996 (34%)	10.37
3 sec	3420 (68%)	6.87
Eluent:	70% Methanol	
Samples:	Toluene (Tol), Naphthalene (Nap)	

Detector / Tubing

Optimization of Detector Cell Volume and Its Effect on Column Efficiency

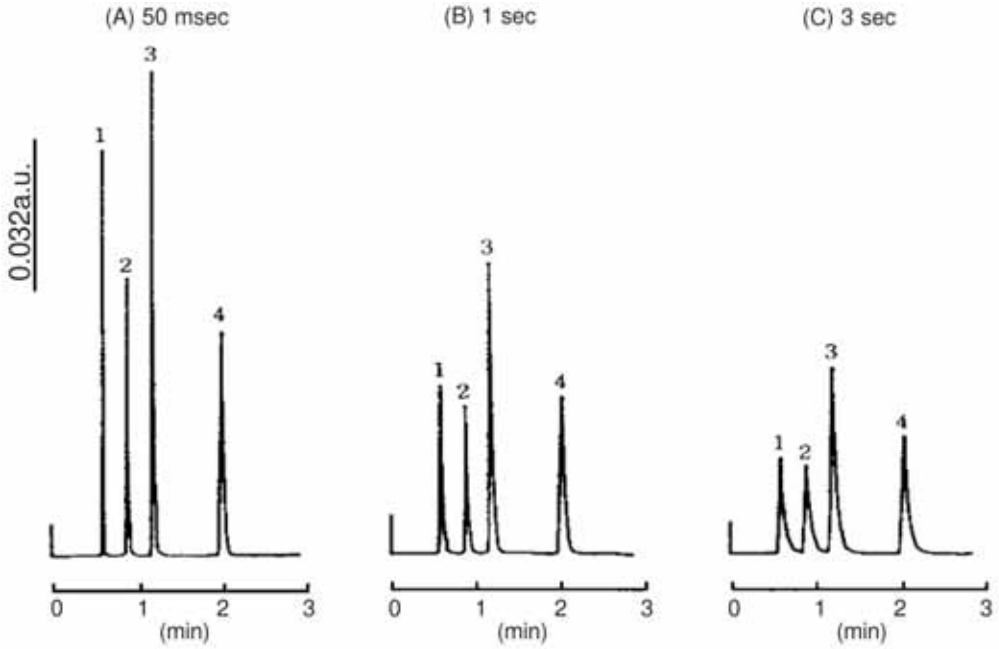
Cell Volume (µL)	Type	Column theoretical plates (relative reduction in theoretical plates) TP/5cm column
2	micro flow cell	10769 (0%)
10	low dead volume type	10150 (6%)
10	standard flow cell	3104 (71%)

Low dead volume means heat sink was removed.
Standard flow cell means heat sink was included.

Eluent: 70% Methanol
Sample: Fluorene

Detector / Tubing

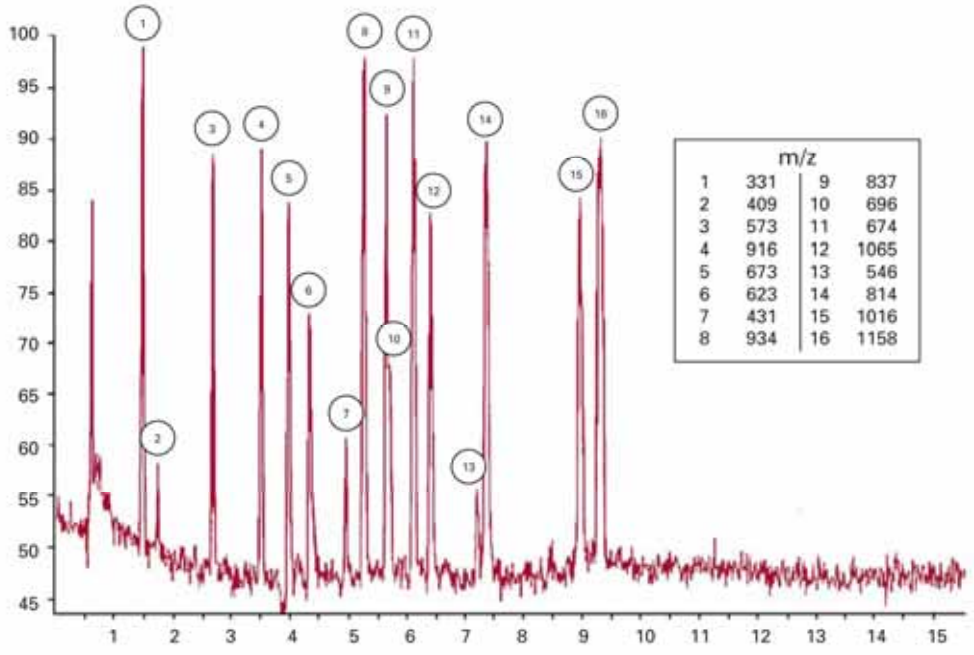
Effect of Detector Time Constant on Theoretical Plates



Column: TSKgel Super-ODS (4.6mm ID x 5cm)
Eluent: 70% Methanol
Flow rate: 1.0mL/min
Sample: Fluorene Temperature: 25°C
Detector: UV (254nm), micro flow cell
Time constant: (A) 50 msec, (B) 1 sec, (C) 3 sec

Applications

LC/MS Chromatogram (TIC) of β-lactoglobulin Tryptic Digest

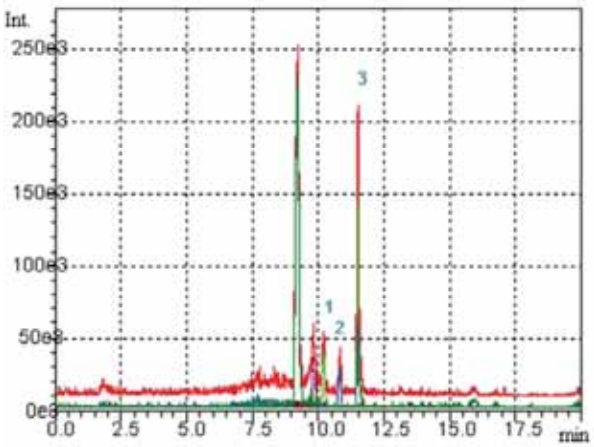


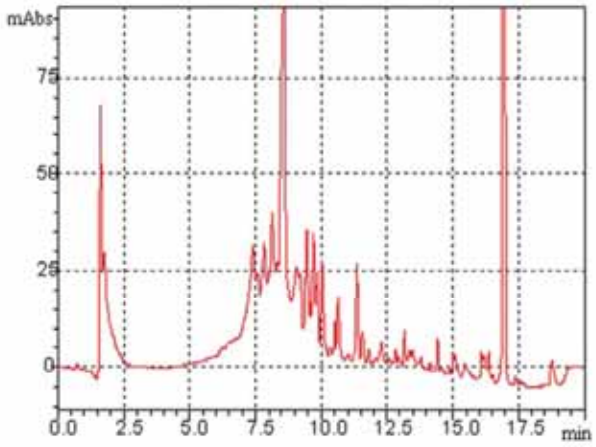
	m/z		
1	331	9	837
2	409	10	696
3	573	11	674
4	916	12	1065
5	673	13	546
6	623	14	814
7	431	15	1016
8	934	16	1158

Column:	TSKgel Super-ODS (2.0mm I.D. x 5cm)
Eluent:	A: H_2O/CH_3CN(95/5)+0.1% TFA
	B: H_2O/CH_3CN(50/50)+0.1% TFA
Gradient:	0-100% B(15 min. Linear gradient)
Flow rate:	0.4mL/min
Inj. Vol.:	2µL
Temp.:	40°C
UV:	215nm
Sample:	β-Lactoglobulin tryptic digest
Ion mode:	ESI+
Ion species:	Normal Ion [MF-Liner]
TIC Range:	m/z 50 to 2600

Applications

LC/MS Chromatogram Pesticides Extracted from Orange





Column:	TSKgel Super-ODS (2.0mmI.D.x10cm)
Flow rate:	0.4mL/min
Inj. Vol.:	2µL
Eluent:	(A) H_2O+0.1% TFA (B) CH_3CN+0.1% TFA
Temp.:	40°C
UV:	254nm
Gradient:	5% B-100% B(12min, Linear gradient)
Ion mode:	ESI(-)
Sample:	Orange extracts spiked std. pesticides 1. Azimsulfron 2. Flazasulfuron 3. Halosulfuron-methyl
Monitored ions:	406, 423, 433, 435, 835, 869, 889
TIC Range:	m/z 100 to 900

Conclusions

- The advantages of the Super Series Columns (2µm particles) over the more traditional 5µm particles are:
  - Increased efficiency
  - Reduction in analysis time
  - Higher resolution
- The Super Series columns are available in:
  - 1, 2, or 4.6mm ID
  - 5 or 10cm Length
- The advantages of 2µm silica:
  - Reduction of diffusion path caused by a narrowing of particle interstices.
  - Increased resolution due to small particle size and narrow pore size distribution.
- Optimization of the inter/extra column components (such as void volume, detector response, and sample injection volume) significantly increases column performance.
- These ultra efficient columns are coupled with the specificity of Mass Spec, which results in superior analytical power.