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Application Note

Innovative Silica-Based RPLC Preparative Columns: Enhanced Loadability and Peak Shapes

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Abstract

In the following experiments, we demonstrate superior peak shapes (under both low and high mass loading), enhanced efficiency, direct scale-up, and the benefit of these attributes in an impurity isolation and identification application utilizing Waters SunFire analytical- and prep-scale reversed-phase HPLC columns.

Introduction

Maximizing column load is one of the goals of preparative chromatography. However, tailing peaks limit the resolution and hence the mass loading of a column. Waters SunFire family of HPLC columns was designed to provide symmetric peak shapes and high mass loading. Innovative bonding and end-capping processes were used to synthesize these silica-based reversed-phase packing materials with higher surface coverage and reduced silanol activity.

SunFire column chemistry provides higher efficiencies, more symmetric peak shapes, and extremely high mass loading for acids, bases and neutral analytes. Two chemistries (C_{18} and C_8) were developed to meet the requirements of the chemist. Additionally, years of research led to the discovery that the packed bed density of a column is the key to manufacturing stable and efficient preparative columns. Manufactured with the unique patent-pending Optimum Bed Density (OBD) design, SunFire Prep OBD Columns have the same packed bed density as analytical columns.

Experimental Conditions and Results

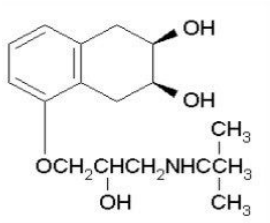
1. Peak Shape - Low Mass Load

Three beta-blocker drugs were separated on both a SunFire C_{18} column and a Luna $C_{18}(2)$ column (Phenomenex Inc., Torrance, CA). The tailing factors of all the analytes are listed on the chromatograms. Less peak tailing is observed on the SunFire column at 250 µg total mass load, as shown in Figure 1.

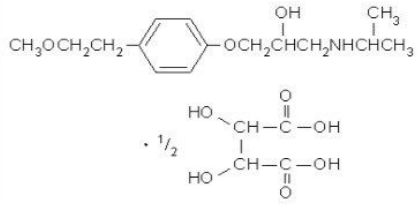
LC Conditions

Columns:	SunFire C_{18} and Luna $C_{18}(2)$, 4.6 × 50 mm, 5 µm
Mobile phase A:	0.1% TFA in H_2O
Mobile phase B:	0.1% TFA in ACN
Flow rate:	1.4 mL/min
Gradient:	5 min gradient, 5% to 70% B
Sample:	(1) nadolol (10 mg/mL) (2) metoprolol (10 mg/mL) (3) propranolol (5 mg/mL), all in DMSO

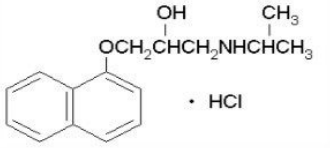
Injection volume:	10 µL
Mass load:	250 µg
Detection:	UV @ 260 nm
LC system:	Waters AutoPurification System



Nadolol



Metaprolol



Propranolol

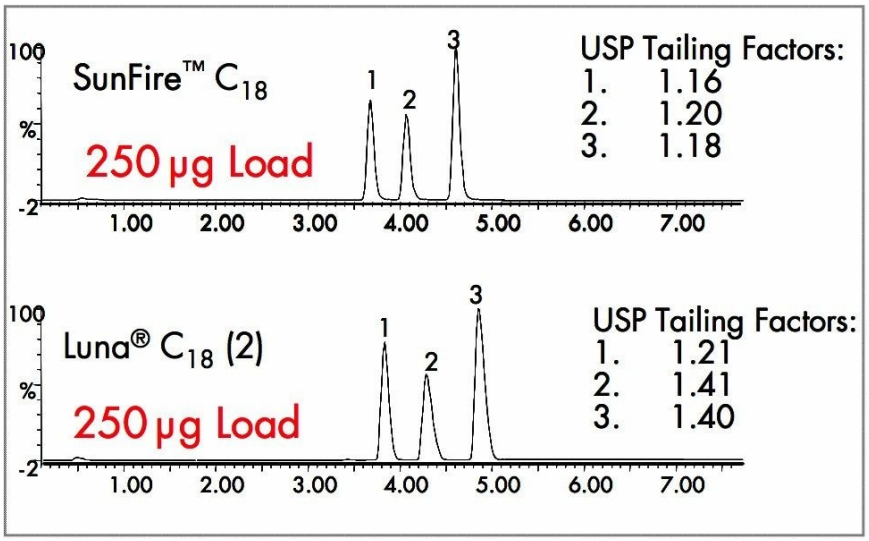


Figure 1. Peak shapes under low mass load conditions.

2. Peak Shape - High Mass Load

Three beta-blocker drugs were separated under high load on both a SunFire C_{18} and Luna $C_{18}(2)$. The tailing factors of all the analytes are listed on the chromatograms. Superior peak shapes are observed on the SunFire column under 4.5 mg total mass load, as seen in Figure 2.

LC Conditions

Columns:	SunFire C_{18} and Luna $C_{18}(2)$, 4.6 × 50 mm, 5 µm
Mobile phase A:	0.1% TFA in H_2O
Mobile phase B:	0.1% TFA in ACN
Flow rate:	1.4 mL/min
Gradient:	5 min gradient, 5% to 70% B
Sample:	(1) nadolol (100 mg/mL)

	(2) metoprolol (100 mg/mL)
	(3) propranolol (50 mg/mL), all in DMSO
Injection volume:	18 µL
Mass load:	4.5 µg
Detection:	UV @ 260 nm
LC system:	Waters AutoPurification System

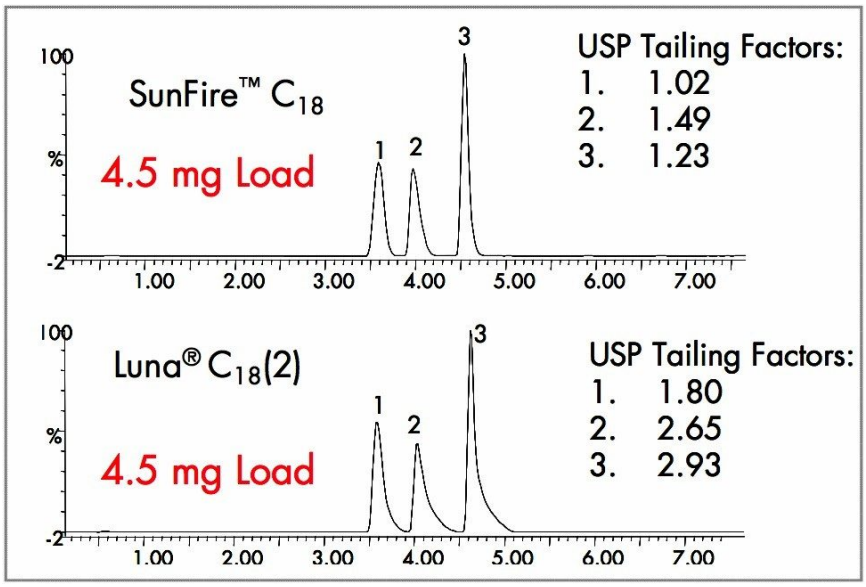


Figure 2. Peak shape under high mass load conditions.

3. Mass Loading Capacity

Three beta-blocker drugs were separated on a SunFire Prep OBD C_{18} column. The total mass load on this column is 100 mg. As shown in Figure 3, even under such high mass load, the peak shapes for all three analytes are excellent.

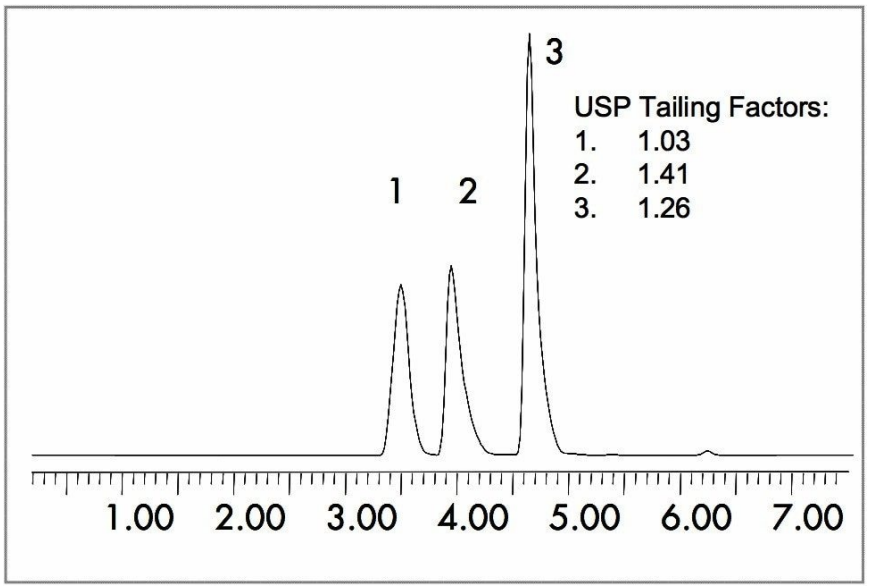


Figure 3. Mass loading capacity on a 19 x 50 mm SunFire Prep OBD C_{18} column.

4. Efficiency

Separations of three antifungal drugs at 1250 µg loading on six popular commercial RPLC C_{18} columns are shown in Figure 4. The same mass loading and gradient conditions were applied on all the columns. Econazole (peak 2) and miconazole (peak 3) have very similar structures with only a chlorine atom difference, which challenges column efficiency.

Resolution values of the last two peaks are listed on each chromatogram. Baseline resolutions are achieved on both SunFire C_{18} and Waters Symmetry C_{18} columns. None of the other C_{18} columns achieve resolution higher than 1.50.

LC Conditions

Columns:	SunFire Prep OBD C_{18}, 19 × 50 mm, 5 µm
Mobile phase A:	0.1% TFA in H_2O

Mobile phase B:	0.1% TFA in ACN
Flow rate:	23.9 mL/min
Gradient:	5 min gradient, 5% to 70% B
Sample:	(1) nadolol (100 mg/mL) (2) metoprolol (100 mg/mL) (3) propranolol (50 mg/mL), all in DMSO
Injection volume:	400 µL
Mass load:	100 µg
Detection:	UV @ 260 nm
LC system:	Waters AutoPurification System

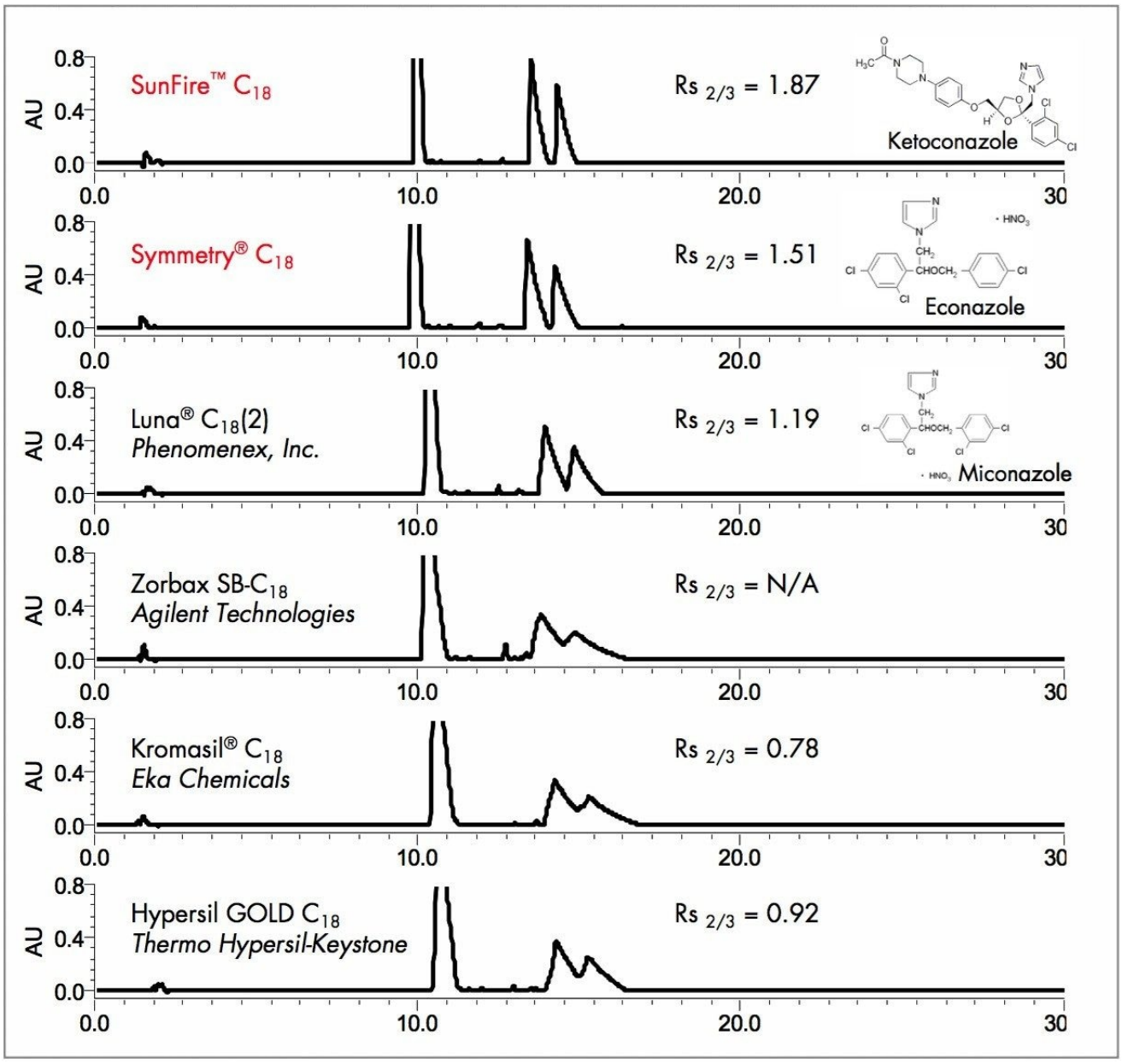


Figure 4. Comparison of column efficiencies among leading commercially available C_{18} columns.

5. Direct Scale-up

The separation of three acidic analytes was successfully scaled-up from an analytical-scale to a preparative-scale SunFire C_{18} column without the need for additional method development – a significant time saver.

LC Conditions

Columns:	Six brands listed in Figure 4, 4.6 × 150 mm, 5 µm
Mobile phase A:	0.1% TFA in H_2O
Mobile phase B:	0.1% TFA in ACN
Flow rate:	1.0 mL/min
Gradient:	15 min gradient, 20% to 85% B
Sample:	(1) ketoconazole (25 mg/mL) (2) econazole (50 mg/mL) (3) miconazole (50 mg/mL), all in DMSO
Injection volume:	10 µL
Temperature:	30 °C
Detection:	UV @ 254 nm
LC system:	Waters Alliance HT System with a Waters 2996 PDA Detector

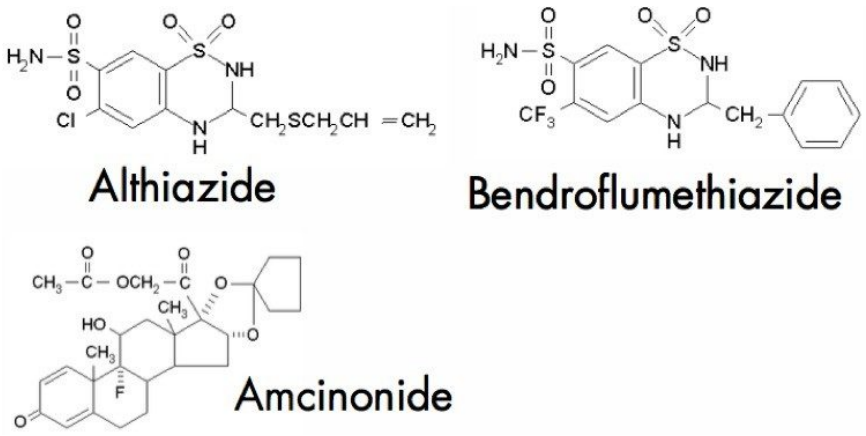
Analytical-Scale Gradient

Time (min)	%A	%B
0.00	80	20
1.00	80	20

Time (min)	%A	%B
6.00	5	95
7.00	5	95
7.01	80	20
11.00	80	20

Prep-Scale Gradient

Time (min)	%A	%B
0.00	80	20
1.91	80	20
6.91	5	95
7.91	5	95
8.01	80	20
12.00	80	20



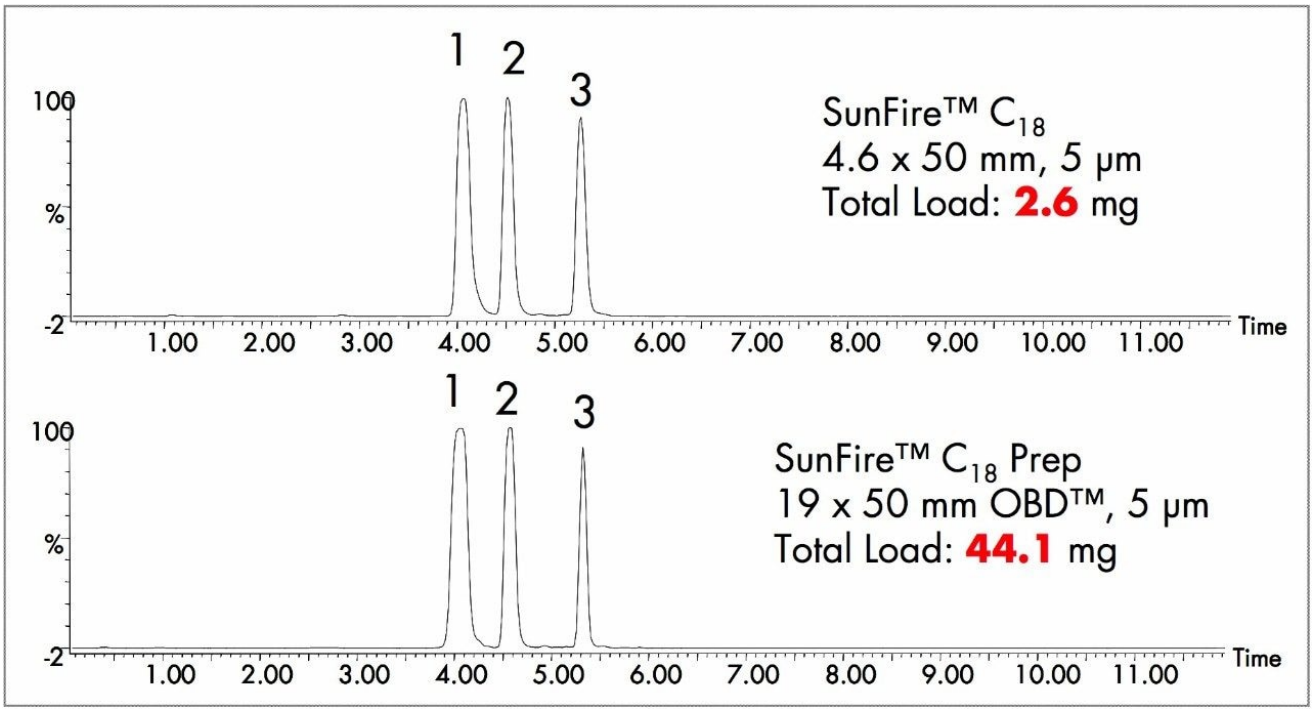


Figure 5. Direct scale-up from an analytical-scale to a prep-scale SunFire C_{18} column.

6. Impurity Isolation and Identification

Nimodipine is a calcium channel blocker, which acts on blood and heart vessels to increase blood flow to injured tissue. Several impurities may be formed during synthesis. The existence of impurities even in small amounts may influence the efficacy and safety of the active pharmaceutical ingredient. Therefore, it is important to identify

impurities.

Highly efficient isolation and direct scale-up was observed on SunFire analytical and preparative C_{18} columns, as shown in Figure 6. Each impurity was separated and collected on the preparative column and then analyzed by MS/MS. Daughter ion scans of nimodipine and its impurities resulted in structural identification of the impurities.

LC Conditions

Columns:	SunFire C_{18} (analytical-scale), 4.6 × 100 mm, 5 µm; SunFire Prep OBD C_{18} (prep-scale), 19 x 100 mm, 5 µm
Mobile phase A:	0.1% Formic acid in H_2O
Mobile phase B:	0.1% Formic acid in ACN
Flow rate:	1.4 mL/min (analytical-scale); 23.9 mL/min (prep-scale)
Gradient:	10min gradient, 30% to 90% B
Sample:	Crude nimodipine in DMSO (30 mg/mL)
Detection:	UV @ 290 nm
LC system:	Waters AutoPurification System

MS Conditions

Interface:	ESI+
Capillary:	3.0 kV

Source temp.:	150 °C
Cone:	30 V
Desolvation temp.:	350 °C
Extractor:	3.0 V
Cone gas:	50 L/hr
RF lens:	0.3 V
Desolvation gas:	550 L/hr
MS system:	Waters Micromass Quattro micro Mass Spectrometer

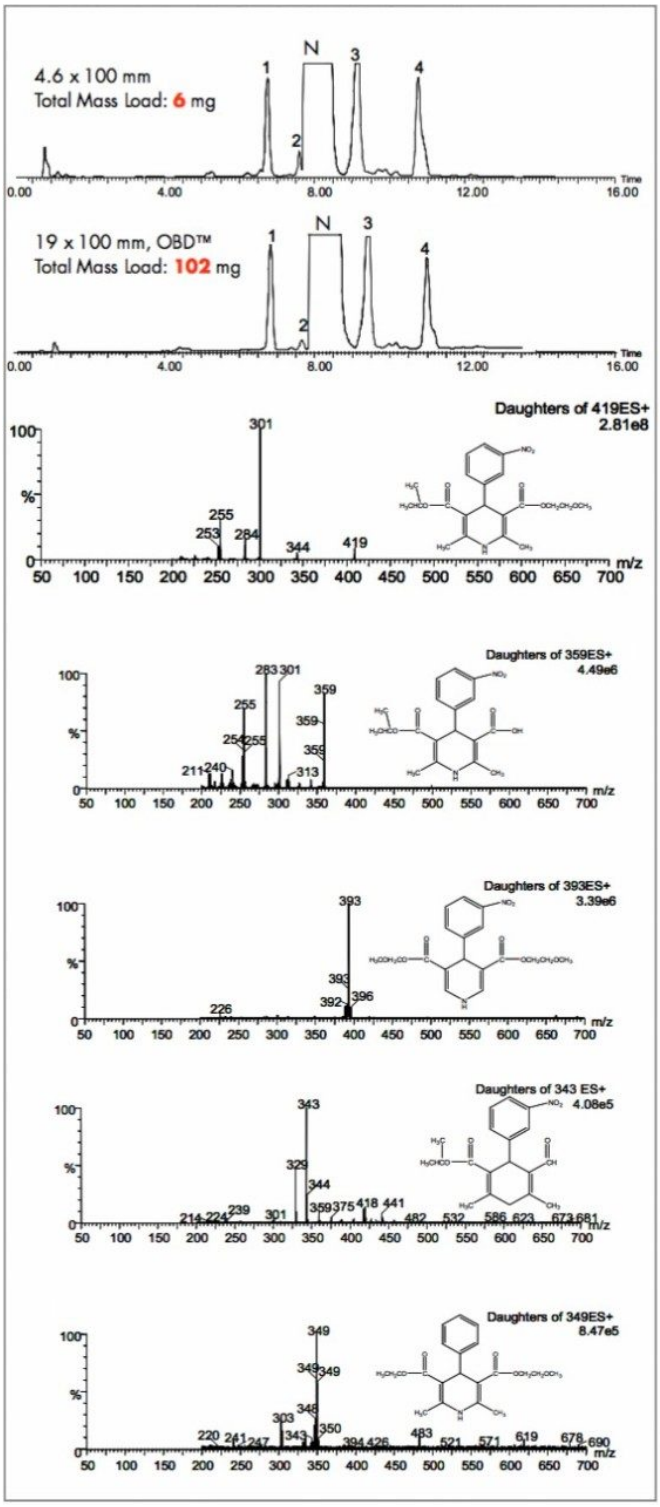


Figure 6. Isolation and structural identification of nimodipine and its

impurities on SunFire C_{18} columns.

Conclusion

Waters SunFire HPLC columns are synthesized with innovative bonding technology to achieve significantly higher surface coverage, as well as a more efficient end-capping technology to successfully reduce surface silanol activity. Therefore, for bases, acids, and neutrals, more symmetric and less tailing peak shapes can be achieved using SunFire columns, which leads to higher mass loading on preparative columns. Additionally, SunFire Prep OBD columns are created with the unique Optimum Bed Density design process, whichproduces more stable columns and ensures linear and streamlined scale-up from analytical to preparative applications. SunFire columns are also compatible with mass spectrometry detection, providing sharp peaks, good sensitivity, large peak capacities, and very low bleed – ideal for impurity isolation and identification.

Featured Products

AutoPurification System <https://www.waters.com/10007147>

Alliance HPLC <https://www.waters.com/514248>

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