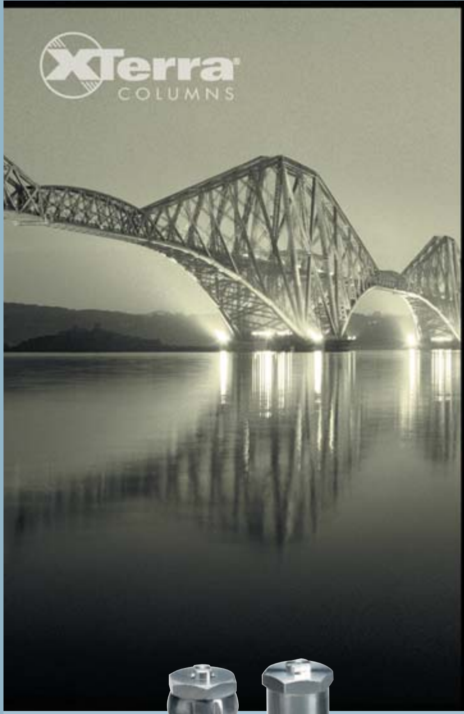
Waters

Optimum Bed Density (OBD™) Preparative Columns



Bridging the performance gap from analytical to prep


Scotland's Firth of Forth Bridge


Japan's Seto Ohashi Bridge


Boston's Leonard P. Zakim Bridge

Contents

Introduction

The technique of laboratory scale chromatographic isolation and purification has gone through many changes in recent years. The use of columns packed with large particle size material and manually controlled instrumentation is often insufficient to meet the demands of today's customer.

At Waters, we are committed to providing state of the art purification solutions from the HPLC instrument, data acquisition and control, technical support, as well as innovative chemistry solutions designed to meet the needs of all our purification customers.

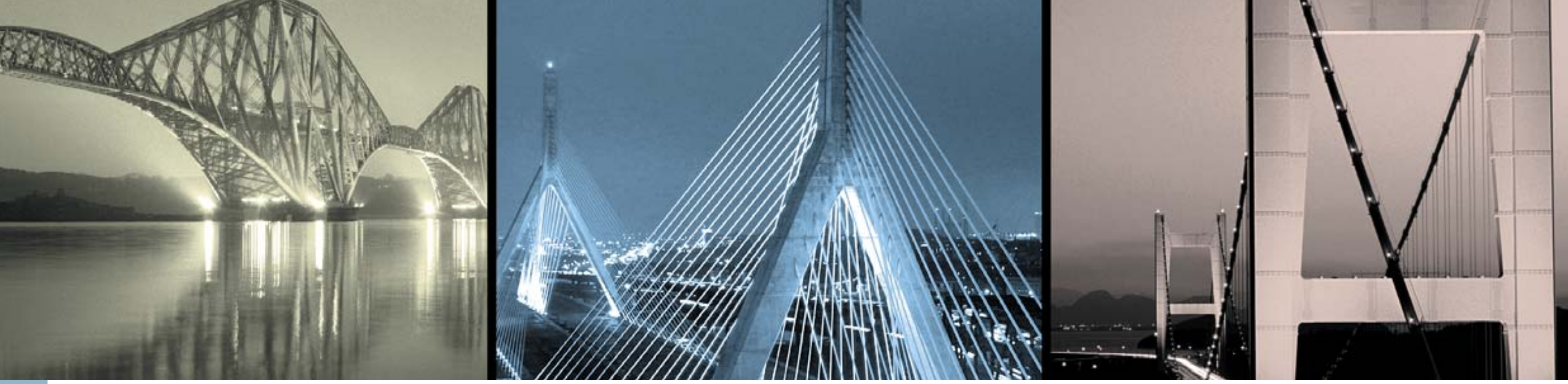
This brochure outlines one of these innovative solutions, the Optimum Bed Density (OBD™) design preparative columns, and introduces some key purification chemistries.

Full details of the Waters family of
preparative solutions may be found at **www.waters.com/prepcolumn**

XTerra® COLUMNS

SunFire™ COLUMNS

Atlantis® Columns



Optimum Bed Density* (OBD™) Preparative Column Design

—Bridging the Performance Gap from Analytical to Prep

Column Performance—Identifying the Problem

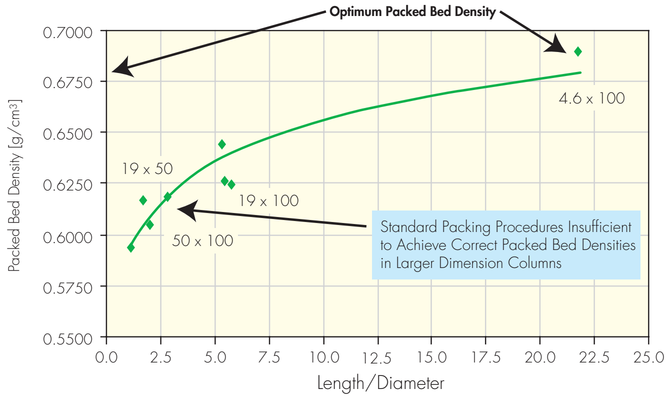
For a column to remain stable during operation, the bed must be packed sufficiently dense to withstand the compressive fluid forces encountered during use. In the case of analytical column dimensions, the necessary packed bed density can be generally achieved using traditional slurry packing methods. As the diameter and length of the column increases, it becomes increasingly difficult to reach the bed density required for stable, long term performance when using small particles. The optimum bed density depends on the specific properties of the chromatographic particles and column design being used.

Laboratory–scale HPLC purification presents many challenges to the chromatographer. One of the most frustrating challenges relates to the preparative column itself. Inconsistency in column-to-column performance and lifetimes often result in lost samples, repeat purification runs and poor scalability from small to larger volume columns. Scientists at Waters recognized this problem and, over a 3-year period, studied all aspects of the packing processes as well as the column design. The patent-pending Optimum Bed Density (OBD™) design is a direct result of these investigations.

Maximum Preparative Column Efficiency

Preparative columns packed to their optimum bed density exhibit the expected chromatographic performance characteristics of the equivalent analytical column. A major benefit of the OBD™ design is a significant improvement in peak efficiency and therefore resolution when compared to a column packed using standard methods as shown in Figure 2 below.

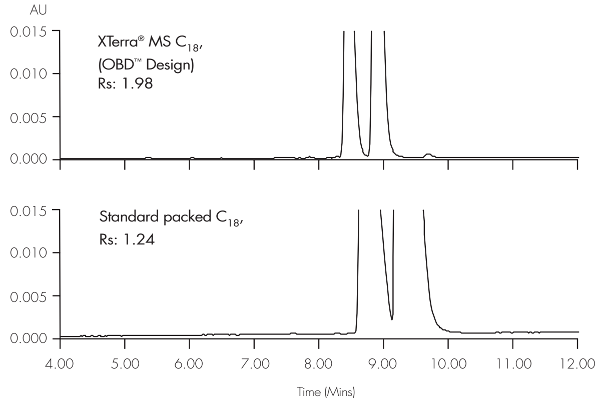
Figure 1: Conventional Packing Procedures
— Analytical vs. Preparative



New packing procedures/design enables correct packed bed densities in preparative dimensions leading to much greater column stability

Figure 2: Comparisons of Column Resolutions
under Same Unit Loading

Column A:	Manufactured with OBD™ process XTerra® Prep MS C_{18}, 19 x 100 mm, 5 µm
Part Number:	186001934
Column B:	Manufactured with the conventional process
Mobile phase A:	0.1 % TFA in water
Mobile phase B:	0.1 % TFA in acetonitrile
Gradient:	5% to 90% B in 10 min
Samples:	econazole and miconazole (3.2 mg/mL each in DMSO)
Inj. Vol.:	1 mL
System:	Waters AutoPurification™ System
Detector:	UV



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* Patent pending

Column Stability and Reliability

The demand for rapid high purity compound isolation places strong emphasis on the integrity and stability of the preparative column. Complex, sparingly soluble starting materials are often dissolved with strong solvents such as DMSO. This combination of poor solubility, and pressure shocks associated with large injection volumes of pure organic solvent, are the primary contributors to early column failure and chromatographic bed collapse.

The OBD™ design exhibits exceptional resistance to mechanical chromatographic bed failure and delivers consistent column-to-column performance.

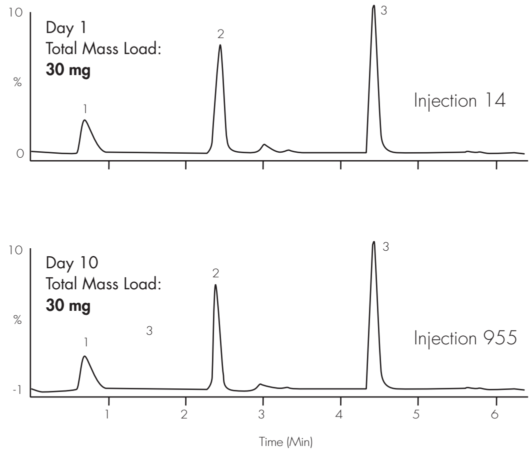
Scalability

Successful scaling of methods from analytical to preparative dimensions requires the use of chromatographically equivalent columns. All too often, even when the same chemistry phase and particle size are used, methods do not scale either due to loss of resolution and/or lower than expected loading. By matching the analytical and preparative column bed densities, scalability is assured eliminating the need for any time consuming method re-development.

Figure 3: Consistent Column-to-Column Performance

Column:	XTerra® Prep OBD™ MS C_{18} 19 x 50 mm, 5 µm
Part Number:	186001930
Mobile phase A:	0.1 % HCOOH in water
Mobile phase B:	0.1 % HCOOH in ACN at a flow rate of 18.0 mL/min
Gradient:	5% to 75% B in 4.5 min.
Inj. Vol.:	400 µL (samples in DMSO)
Instrument:	Waters AutoPurification™ Factory.

Compounds
1. Tylosin
2. Sulfathiazole
3. Ketoprofen

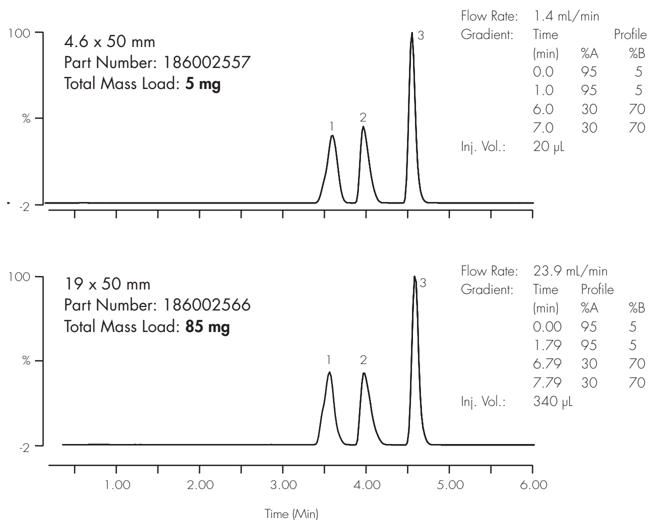


Column stability testing results over 10 days of continuous injections. Chromatograms are for the 14th and 955th injection.

Figure 4: Accurate Scale-up on SunFire™ Columns in 0.1% TFA

Column:	SunFire™ C_{18}
Mobile Phase A:	0.1% TFA in Water
Mobile Phase B:	0.1% TFA in ACN
Temperature:	Ambient
Detection:	UV @ 260 nm
Instrument:	Waters AutoPurification™ System

Compounds
1. Nadolol (100 mg/mL)
2. Metoprolol (100 mg/mL)
3. Propranolol (50 mg/mL)



Flow Rate: 1.4 mL/min

Gradient: Time (min)	%A	Profile %B
0.0	95	5
1.0	95	5
6.0	30	70
7.0	30	70

Inj. Vol.: 20 µL

Flow Rate: 23.9 mL/min

Gradient: Time (min)	%A	Profile %B
0.00	95	5
1.79	95	5
6.79	30	70
7.79	30	70

Inj. Vol.: 340 µL

Table 1. Reproducibility results for four XTerra® Prep OBD™ MS C_{18} columns.

Column	New Columns		Post 1000 Injections		% Change in Efficiency
	Plates	USP Tail	Plates	USP Tail	
1	4,278	1.14	3,927	1.17	**-8.2**
2	4,430	1.05	4,129	1.07	**-6.8**
3	3,943	1.02	4,054	1.08	**2.8**
4	4,093	1.03	3,945	1.13	**-3.6**
Average	**4,186**	**1.06**	**4,014**	**1.11**	**-4.0**

The Optimum Bed Density (OBD™) preparative columns are packed to bed densities which closely match the equivalent analytical column. This innovative procedure produces preparative columns with excellent stability, reproducibility and efficiency.

XTerra® Hybrid Particle Technology

—Purification Method Development Flexibility

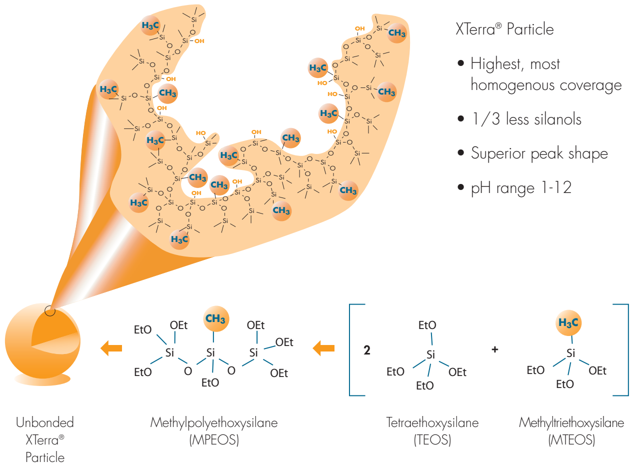
Breaking Traditional Boundaries

For over 30 years, scientists have been forced to work within certain boundaries when performing HPLC separations. Restrictions on speed, resolution, pH, temperature, and loading capacity were imposed upon the chromatographer by limitations of the stationary phase material. The patented* Hybrid Particle Technology of XTerra® columns allows chromatographers to break these boundaries and realize the full potential of their analytical and preparative separations.

In Hybrid Particle Technology, one out of every three silanols is replaced with a methyl group during synthesis. This hydrophobicity is distributed throughout the entire structure of the particle backbone. The result is a rugged hybrid (inorganic/organic) particle that can be operated at high speeds, high temperatures, and high pH. The presence of 33% fewer residual silanols (after endcapping and bonding) also means that XTerra® columns give exceptionally sharp, high-efficiency peaks for basic compounds.

*US Patent No. 6,686,035 B2

Figure 5: XTerra® Manufacturing Process: Much More Than a Surface Modification



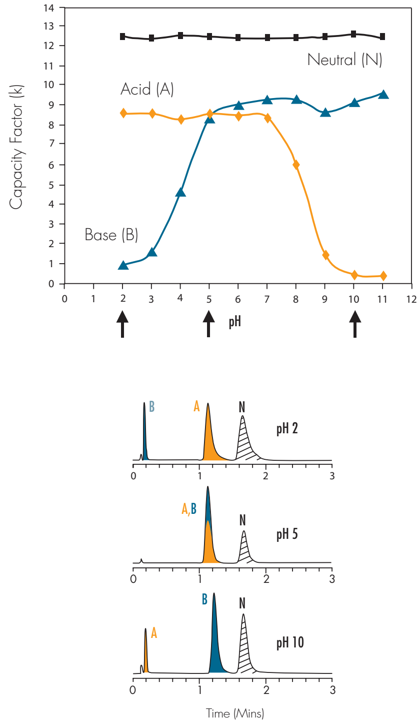
XTerra® Particle

- Highest, most homogenous coverage
- 1/3 less silanols
- Superior peak shape
- pH range 1-12

Optimize Selectivity for Ionizable Compounds

One of the greatest challenges facing the method development scientist is creating a robust separation in the most time efficient manner possible. Tools available to the separations scientist that influence elution order (selectivity) include solvent type, pH column chemistry and/or temperature. The unique Hybrid Particle Technology of XTerra® columns allows the greatest flexibility for selecting whatever chromatographic conditions are necessary for optimal selectivity and separation.

Figure 6: Maximize Selectivity with the Wider pH Range



The wider usable pH range of XTerra® columns is a powerful tool for changing the selectivities of ionizable analytes.

Increase Mass Capacity and Resolution

Interaction between basic analytes and ionizable silanols on silica surfaces results in poor peak shape. Separation of basic compounds is often performed at low pH to suppress silanol ionization, thereby reducing peak tailing. However, in this mode, basic analytes are protonated and are not retained well by reversed-phase sorbents.

XTerra® Prep columns with Hybrid Particle Technology—unlike traditional silica columns—can be used routinely at high pH so that basic compounds can be isolated in their neutral form. This results in a tremendous improvement, not only in peak shape, but also resolution and loadability. Moving from an isocratic to a gradient separation mode further increases capacity.

Minimize Final Fraction Volumes

XTerra® Prep packings are engineered specifically for preparative chromatography allowing you to operate routinely over a wider pH range (1-12). Choosing an optimum pH enables maximum column loading (low pH for acids and high pH for bases).

Once the correct pH has been chosen for the separation, the wide range of column dimensions and particle sizes available allows for further optimization of the purification method.

For a high throughput application, method cycle time, final fraction volumes and compound recoveries are key parameters. Accurate scaling of method conditions to smaller high efficiency columns enables faster purifications, smaller fraction volumes while maintaining peak resolution.

Figure 7: Low vs High pH Loading

Column: XTerra® Prep OBD™ MS C_{18} OBD™ 19 x 100 mm, 5 µm
Part Number: 186001934
Mobile Phase A: 0.1% TFA pH 2.3
Mobile Phase B: 0.1% TFA in ACN
Mobile Phase A: 10 mm NH_4HCO_3 pH 10
Mobile Phase B: 100 mm NH_4HCO_3/ACN (10/90)

Gradient: Time (min)	Profile %A	Analytes: %B
0	95	5
1.0	95	5
11.0	10	90
16.0	10	90

Inj. Vol. 1 mL pH 2.3
4 mL pH 10.0
Temperature: Ambient
Detection: UV @ 270 nm
Instrument: Waters AutoPurification™ System

Compounds
1. Miconazole
2. Econazole

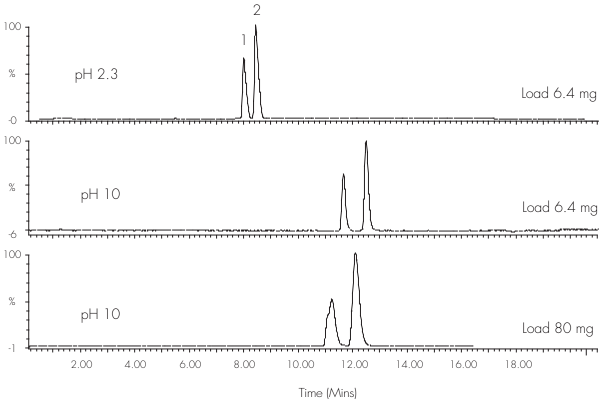
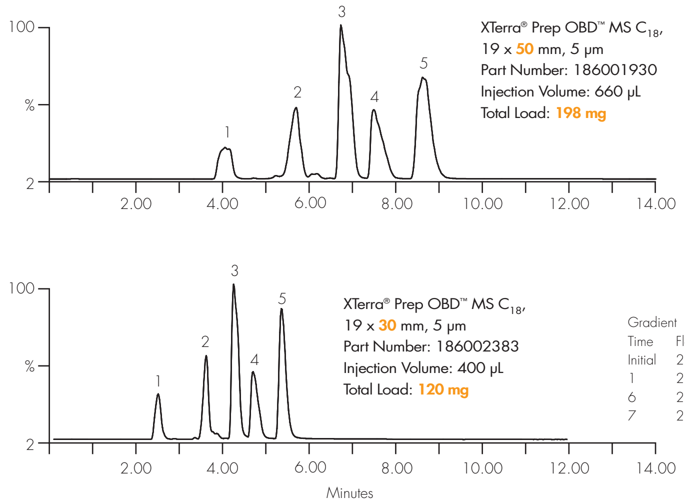


Figure 8: Optimization of High Throughput Applications



XTerra® Prep OBD™ MS C_{18},
19 x **50** mm, 5 µm
Part Number: 186001930
Injection Volume: 660 µL
Total Load: **198 mg**

Gradient

Time	Flow Rate	%A	%B
Initial	23.9	95	5
1.84	23.9	95	5
10.17	23.9	5	95
11.84	23.9	5	95

XTerra® Prep OBD™ MS C_{18},
19 x **30** mm, 5 µm
Part Number: 186002383
Injection Volume: 400 µL
Total Load: **120 mg**

Gradient

Time	Flow Rate	%A	%B
Initial	23.9	95	5
1	23.9	95	5
6	23.9	5	95
7	23.9	5	95

Mobile Phase A: Water with 0.1% DEA
Mobile Phase B: Acetonitrile with 0.1% DEA
Flow Rate: 23.9 mL/min
Make-up Pump Flow Rate: 1 mL/min

Compounds	
1. Labetolol	(50 mg/mL)
2. Quinine	(50 mg/mL)
3. Diltiazem	(50 mg/mL)
4. Verapamil	(100 mg/mL)
5. Amitriptyline	(50 mg/mL)

Column Dimension (mm)	**Peak Volume @ 5% Height (mL)**				
	Peak 1	**Peak 2**	**Peak 3**	**Peak 4**	**Peak 5**
19 x 30 mm, 5 µm	7.6	10.3	7.7	8.8	8.6
19 x 50 mm, 5 µm	12.9	12.9	9.32	13.8	16.0
Recovery (%) (19 x 50 mm)	**89**	**99**	**93**	**99**	**90**

XTerra® Prep OBD™ columns provide the flexibility required for fast purification method development. Wide pH operating range and high loadability have ensured that the XTerra® phase remains to be one of the most popular purification materials.

SunFire™ State-of-the-Art Silica Columns

—Optimized for Low pH Purifications

Setting a New Standard for Peak Shape

SunFire™ columns set a new standard as the state-of-the-art silica based HPLC column. By combining new bonding and end-capping technologies with a perfectly controlled particle synthesis route, Waters has developed a phase with superior peak shape performance. SunFire™ columns provide symmetrical peaks for improved resolution and purification of acidic, neutral and basic compounds at low and intermediate pH ranges.

Low pH Phase Stability

Many routine purifications are carried out using formic acid or TFA as the mobile phase modifier. At the low pH range of 2-3, the primary mode of column failure relates to the acid hydrolysis of the chemically bonded ligand. The proprietary bonding and end-capping techniques utilized in the manufacture of SunFire™ products results in a phase with much greater low pH chemical stability and extended column lifetime.

Silica based columns have traditionally been the first choice for routine purification methods. Many different phases are commercially available, but few have been specifically designed to meet the demands of the purification laboratory. Our R&D synthetic chemists, through many years of expertise in chromatographic particle synthesis, have designed the SunFire™ phase to meet all of the basic requirements of high mass loading, pH stability, efficiency and scalability.

Figure 9: SunFire™ Columns — Best Peak Shape

Isocratic separation

Mobile Phase A:	35% 20 mM K_2HPO_4/ 20 mM KH_2PO_4, pH 7.0
Mobile Phase B:	65 % Methanol
Wavelength:	254 nm
Flow rate:	1.0 mL/min
Injection volume:	14 µL
Temperature:	23 °C
Instrument:	Waters Alliance® 2695 2487 Dual Wavelength Absorbance Detector

Compounds
1. Uracil
2. Propranolol
3. Butylparaben
4. Naphthalene
5. Acenaphthene
6. Amitriptyline

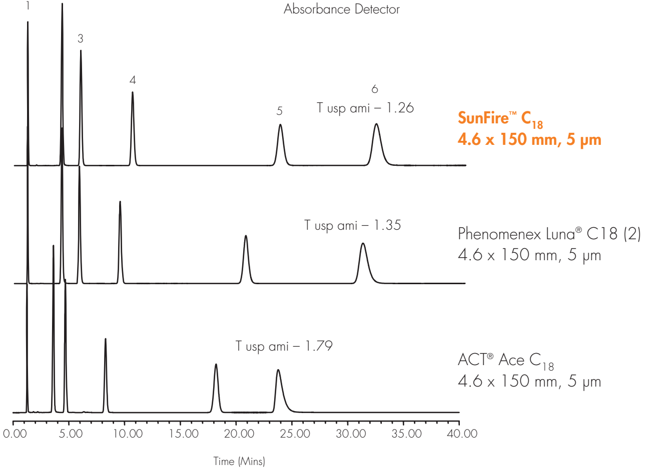
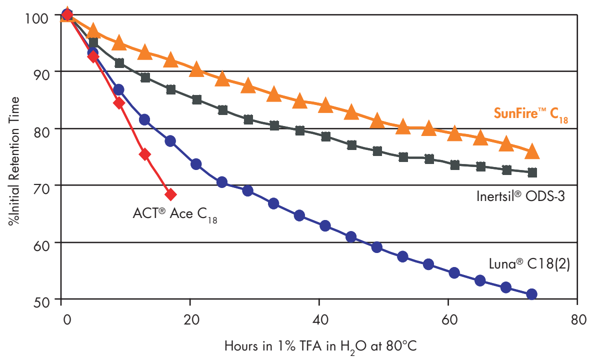


Figure 10: SunFire™ Columns — Low pH Stability



Loadability by Design

A successful isolation and purification process is not only a function of column efficiency, but is also related to the loadability of the chromatographic material for different analytes, and under different mobile phase conditions.

The physical characteristics of preparative phases, such as surface area and ligand density, do not always correlate well with the mass loadability of ionizable compounds. SunFire™ Prep silica particles have been synthetically designed under cGMP protocols to deliver maximum loadability in acidic to moderate pH mobile phase conditions.

Efficiency and Scalability

The isolation and purification of critical compound pairs is a major challenge to purification scientists. Often a successful analytical separation will not scale up directly due to the reduced performance of the preparative column.

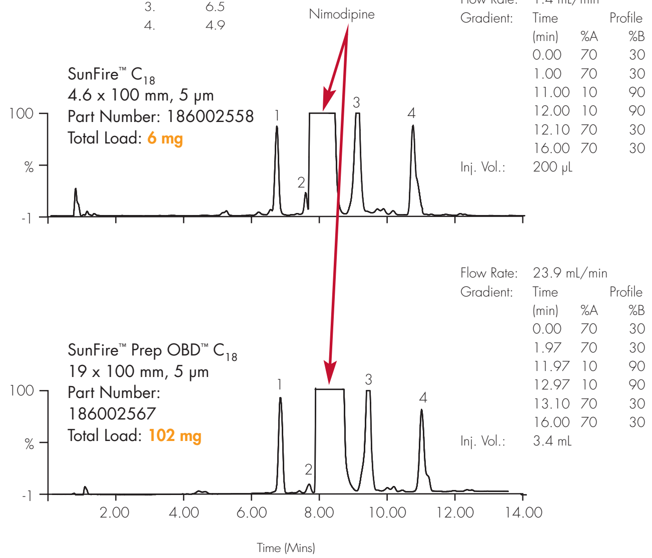
The OBD™ design in combination with the efficient SunFire™ particles ensures equivalent chromatographic performance from analytical to preparative dimensions, eliminating the need for any subsequent time consuming method redevelopment.

Figure 11: SunFire™ Columns — Isolation of Nimodipine and it's Impurities

Mobile Phase A:	Water with 0.1% FA
Mobile Phase B:	ACN with 0.1% FA
Analyte:	Nimodipine at 30 mg/mL in DMSO
Detection:	UV @ 290 nm
Instrument:	Waters AutoPurification™ Systems

Impurity levels:

Peak	% by Area
1.	3.1
2.	0.6
3.	6.5
4.	4.9



Flow Rate: 1.4 mL/min

Gradient:

Time (min)	Profile %A	%B
0.00	70	30
1.00	70	30
11.00	10	90
12.00	10	90
12.10	70	30
16.00	70	30

Inj. Vol.: 200 µL

Flow Rate: 23.9 mL/min

Gradient:

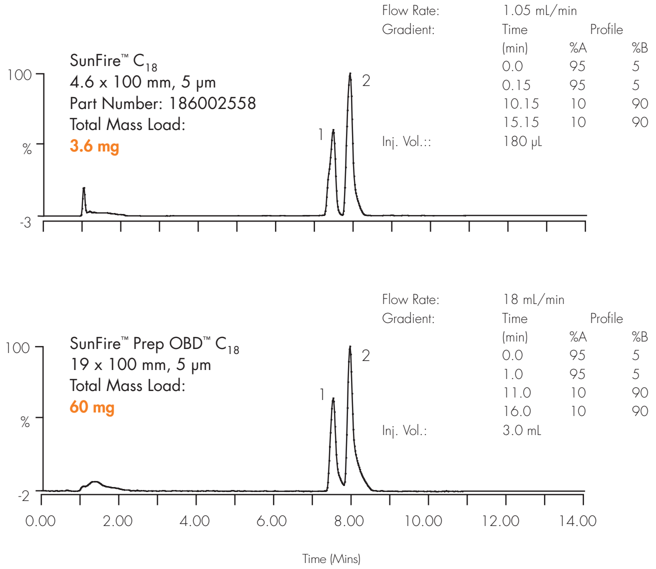
Time (min)	Profile %A	%B
0.00	70	30
1.97	70	30
11.97	10	90
12.97	10	90
13.10	70	30
16.00	70	30

Inj. Vol.: 3.4 mL

Figure 12: Scale-up of the Separation of Two Antifungal Critical Pairs on SunFire™ Columns

Mobile Phase A:	0.1% TFA in water
Mobile Phase B:	0.1% TFA in acetonitrile
Sample Concentration:	20 mg/mL in DMSO
Temperature:	Ambient
Instrument:	Waters AutoPurification™ System
Detection:	UV @ 285 nm

Compounds	
1. Econazole	(10 mg/mL)
2. Miconazole	(10 mg/mL)



Flow Rate: 1.05 mL/min

Gradient:

Time (min)	Profile %A	%B
0.0	95	5
0.15	95	5
10.15	10	90
15.15	10	90

Inj. Vol.:: 180 µL

Flow Rate: 18 mL/min

Gradient:

Time (min)	Profile %A	%B
0.0	95	5
1.0	95	5
11.0	10	90
16.0	10	90

Inj. Vol.: 3.0 mL

SunFire™ Prep OBD™ columns were specifically designed to meet the requirements of a low pH purification method with respect to ligand stability, peak efficiency and loadability. Columns are currently available in both C_8 and C_{18} bonded phases.

Atlantis® dC_{18}

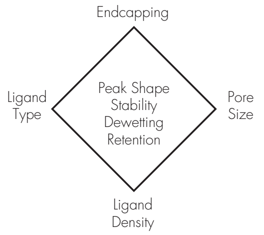
—Perfect Balance of Polar and Non-Polar Compound Retention

Designing the Ideal Column

In order to create a reversed-phase HPLC column for the retention and separation of polar, water-soluble compounds, a new and unique stationary phase packing material had to be created. The result of this two year stationary phase creation project was the silica-based, difunctionally bonded C_{18} material of Atlantis® dC_{18} columns. Stationary phase physical attributes such as endcapping, silica pore size, bonded phase ligand density and ligand type were all optimized in order to create a column that exhibits superior peak shape, low pH stability, resistance to dewetting (hydrophobic collapse) and enhanced polar compound retention.

Waters studied the effects of the stationary phase on polar compound retention and created a column that not only retains polar compounds, but provides excellent peak shape for all compounds and is fully LC/MS compatible. Atlantis® dC_{18} columns combine all the desirable characteristics of an ideal reversed-phase HPLC column, making it suitable for separating polar compounds as well as standard reversed-phase applications.

Figure 13: Atlantis® dC_{18} Columns—an Intelligent Design



Optimizing key stationary phase attributes results in the optimal combination of peak shape, low pH stability, resistance to dewetting and polar compound retention.

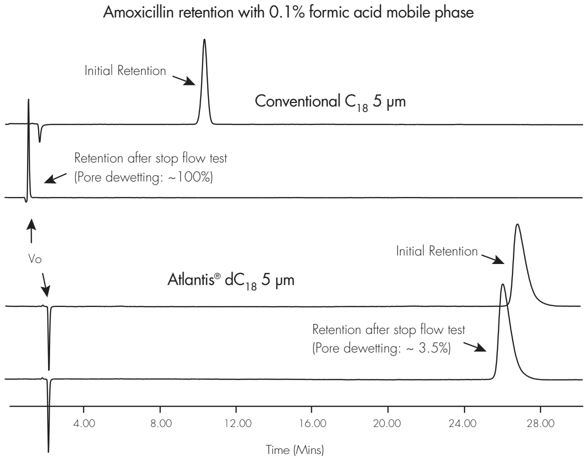
Optimized for Aqueous Mobile Phases

With conventional reversed-phase HPLC columns you may experience difficulties retaining and separating highly polar, water-soluble organic compounds. Retention of these types of analytes requires the use of mobile phases that contain little or no organic modifier. Under these aqueous conditions, conventional C_{18} stationary phases can exhibit a sudden loss of retention. In the past, this was attributed to a proposed phenomenon where the hydrophobic C_{18} chains "collapse."

Tests at Waters have revealed that the silica pores (where the majority of the surface area lies) actually expel aqueous mobile phase in the absence of pressure. Under these conditions, analytes do not migrate into the pores and, therefore, pass through the column unretained. This phenomenon is termed "dewetting."

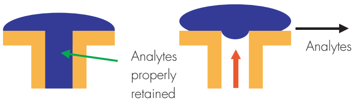
Figure 14: Atlantis® dC_{18} Columns Resist Dewetting

Waters "stop flow" test determines the susceptibility of a stationary phase to pore dewetting using 100% aqueous mobile phases. Under these difficult testing conditions, Atlantis® dC_{18} columns resist phase dewetting. Note also the increased retention of amoxicillin on the Atlantis® column compared to the conventional C_{18} column.



Pore Dewetting Mechanism

Flow stoppage relieves the pressure that forces aqueous mobile phase into the pores. When this pressure is decreased, the hydrophobic pore surface expels the polar mobile phase and the pore "dewets," resulting in retention loss.



Note: When the column is restricted, the mobile phase pressure is not sufficient to fully rewet all the pores throughout the column length. This results in the loss of retention.

Waters Atlantis® dC_{18} columns were developed specifically for operating in aqueous mobile phases without fear of dewetting.

Purification of Polar Analytes

Polar compounds present a unique and difficult challenge since these unretained and/or poorly separated analytes must be re-analyzed separately, thus becoming a bottleneck in the high-throughout laboratory. If some analyte separation is realized, the peak fraction is a highly aqueous, non-volatile solvent (i.e., very weak mobile phase) that requires long evaporation times. Since Atlantis® dC$_{18}$ preparative columns retain compounds longer, stronger mobile phases and/or steeper gradient profiles can now be used. This optimal retention results in more volatile peak fractions, faster fraction evaporation, less sample handling and higher recoveries.

Scalability and Mass Loading

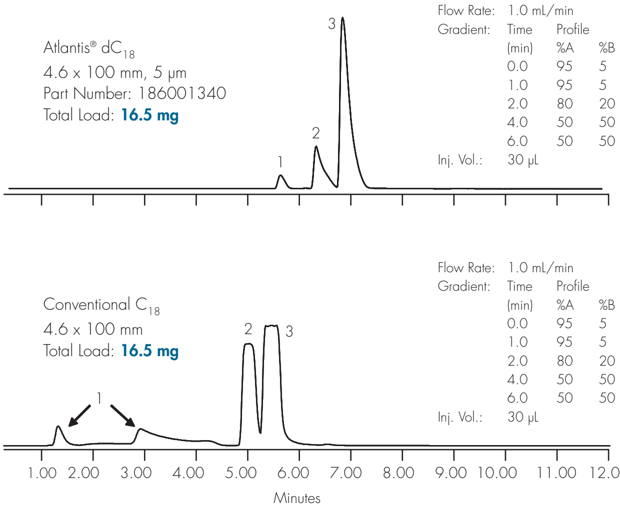
One of the most frustrating and time-consuming aspects of the purification process occurs when an analytical separation does not scale-up linearly to semi-preparative and preparative dimensions. All Waters® preparative column chemistries contain the same high-quality, fully tested stationary phase material as their equivalent analytical dimension.

Atlantis® dC$_{18}$ preparative columns also provide high mass loading for polar compounds. The isolation and purification scientist can choose to use either a larger preparative column which provides a capacity safety margin for unknown sample sets or a smaller preparative column to decrease solvent consumption, operating backpressures and peak volumes. This ability to inject high mass loads translates into less preparative runs and faster library screening and purification.

Figure 15: Polar β-Blockers

Mobile Phase A:	0.1% FA in water
Mobile Phase B:	0.1% FA in acetonitrile/water (90/10)
Gradient:	As indicated on the chromatograms
Flow Rate:	As indicated on the chromatograms
Sample Concentration:	Atenolol, metoprolol, propranolol at 5, 25, 55 mg/mL each in DMSO
Inj. Vol.:	As indicated in the chromatograms.
Detection:	UV @ 280 nm
Instrument:	Waters AutoPurification™ System

Compounds	
1. Atenolol	(5 mg/mL)
2. Metoprolol	(25 mg/mL)
3. Propranolol	(25 mg/mL)



Flow Rate: 1.0 mL/min

Gradient:

Time (min)	Profile %A	%B
0.0	95	5
1.0	95	5
2.0	80	20
4.0	50	50
6.0	50	50

Inj. Vol.: 30 µL

Flow Rate: 1.0 mL/min

Gradient:

Time (min)	Profile %A	%B
0.0	95	5
1.0	95	5
2.0	80	20
4.0	50	50
6.0	50	50

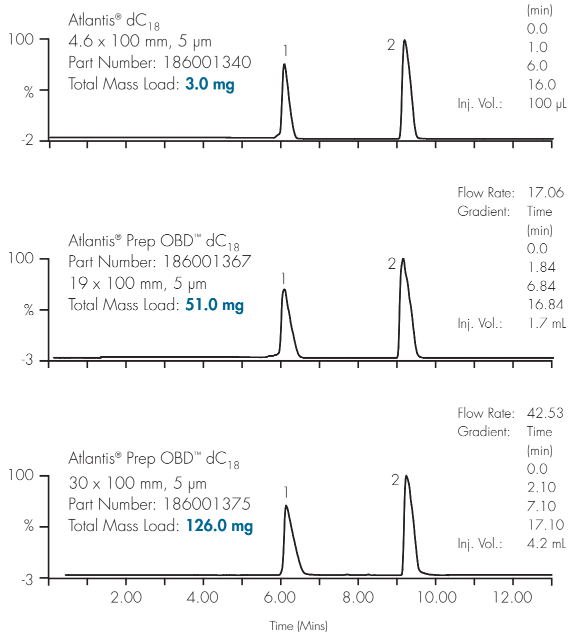
Inj. Vol.: 30 µL

Very polar analyte not sufficiently retained on conventional C$_{18}$ column.

Figure 16: High Mass Loading of Water Soluble Vitamins

Column:	Atlantis® dC$_{18}$
Mobile Phase A:	0.1% TFA in H$_2$O
Mobile Phase B:	0.1% TFA in ACN
Detection:	UV @ 290 nm
Temperature:	Ambient
Instrument:	Waters AutoPurification™ System

Compounds	
1. Pyridoxine	(5 mg/mL)
2. Caffeine	(25 mg/mL)



Flow Rate: 1.0 mL/min

Gradient:

Time (min)	Profile %A	%B
0.0	100	0
1.0	100	0
6.0	80	20
16.0	60	40

Inj. Vol.: 100 µL

Flow Rate: 17.06 mL/min

Gradient:

Time (min)	Profile %A	%B
0.0	100	0
1.84	100	0
6.84	80	20
16.84	60	40

Inj. Vol.: 1.7 mL

Flow Rate: 42.53 mL/min

Gradient:

Time (min)	Profile %A	%B
0.0	100	0
2.10	100	0
7.10	80	20
17.10	60	40

Inj. Vol.: 4.2 mL

Atlantis® dC$_{18}$ Prep OBD™ columns have been designed to retain polar analytes, without excessive retention of more hydrophobic analytes. This phase is the first in a family of application driven chemistry solutions.

At-Column Dilution*

Principle

At-Column Dilution (ACD) is a technique that you could use in all forms of liquid chromatography, preferably for reversed-phase preparative separations and it was developed specifically for injecting large volumes of relatively strong sample diluents. Such injections may distort the chromatography in a conventional system. If injection artifacts limit mass capacity or chromatographic resolution, the effects can be ameliorated by applying At-Column Dilution. In addition, At-Column Dilution often increases system ruggedness and column life by preventing bulk precipitation in the sample loop or in the column itself.

Conventional HPLC System

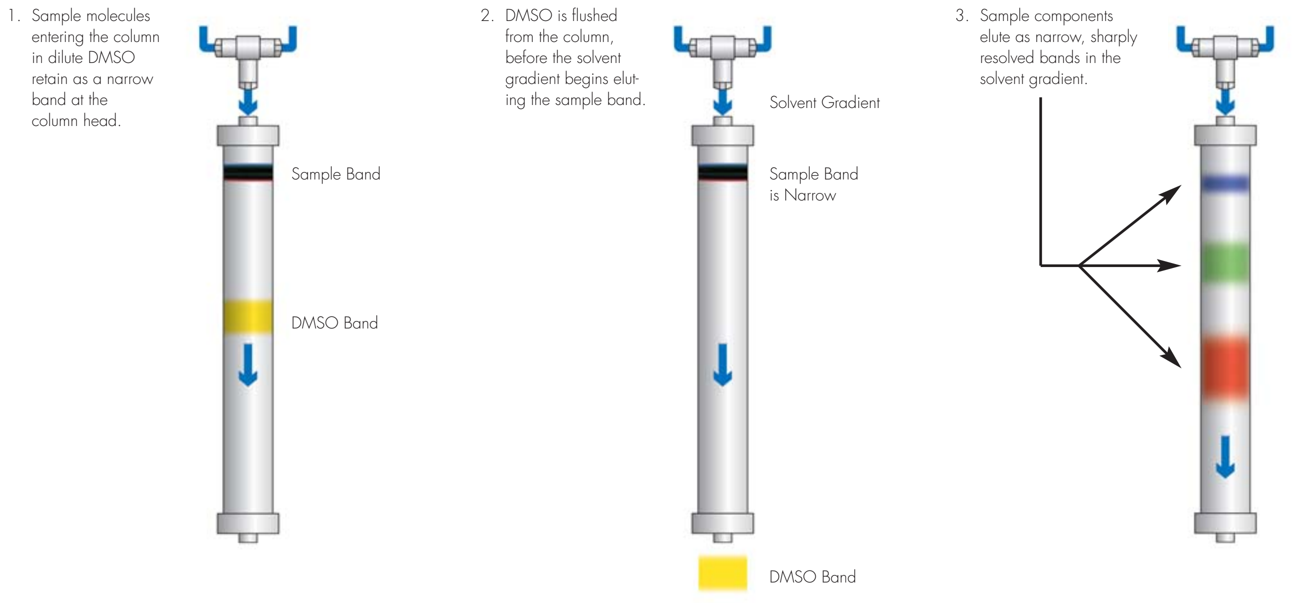
In a conventional system, the sample is dissolved in a strong diluent, such as DMSO, and carried from the injector to the head of the column as a plug sandwiched in a stream of weak solvent, often 95% aqueous. Precipitation might occur at the edges of this plug where the strong sample solvent is diluted with the weak chromatographic solvent. This precipitation might occlude the fluid path and lead to a high-pressure shutdown. In the absence of such precipitation, the sample enters the column, but there will be no retention until the sample plug is diluted with the initial-strength mobile phase in the pores of the column. With larger injections, the volume required to dilute the sample can only be derived by moving a substantial distance along the column. In such cases, the sample is deposited as a broad band that occupies a large fraction of the column volume. Elution of such samples gives incomplete resolution with the overlapping peaks spread over large volumes of eluent and fractions.

These problems may be reduced by strictly limiting both the volume and mass of sample injected. The alternative is substantial dilution of the sample with water, or most generally, a weak solvent, to ensure adequate retention. Neither approach is completely satisfactory because throughput and recovery are compromised. The required larger injection volume may be incompatible with the injector and fluidics present in the system.

At-Column Dilution System

In an At-Column Dilution system, the system is reconfigured to allow the sample plug to be carried to the head of the column in a stream of strong solvent. At the entrance of the column, this stream is continuously diluted with a stream of aqueous mobile phase. The rate of transfer into the column is so high that precipitation cannot occur. The sample molecules are then adsorbed to the packing material as very narrow bands that can be eluted as well-resolved, small-volume peaks as illustrated in Figure 17.

Figure 17: At-Column Dilution Separations



1. Sample molecules entering the column in dilute DMSO retain as a narrow band at the column head.
2. DMSO is flushed from the column, before the solvent gradient begins eluting the sample band.
3. Sample components elute as narrow, sharply resolved bands in the solvent gradient.

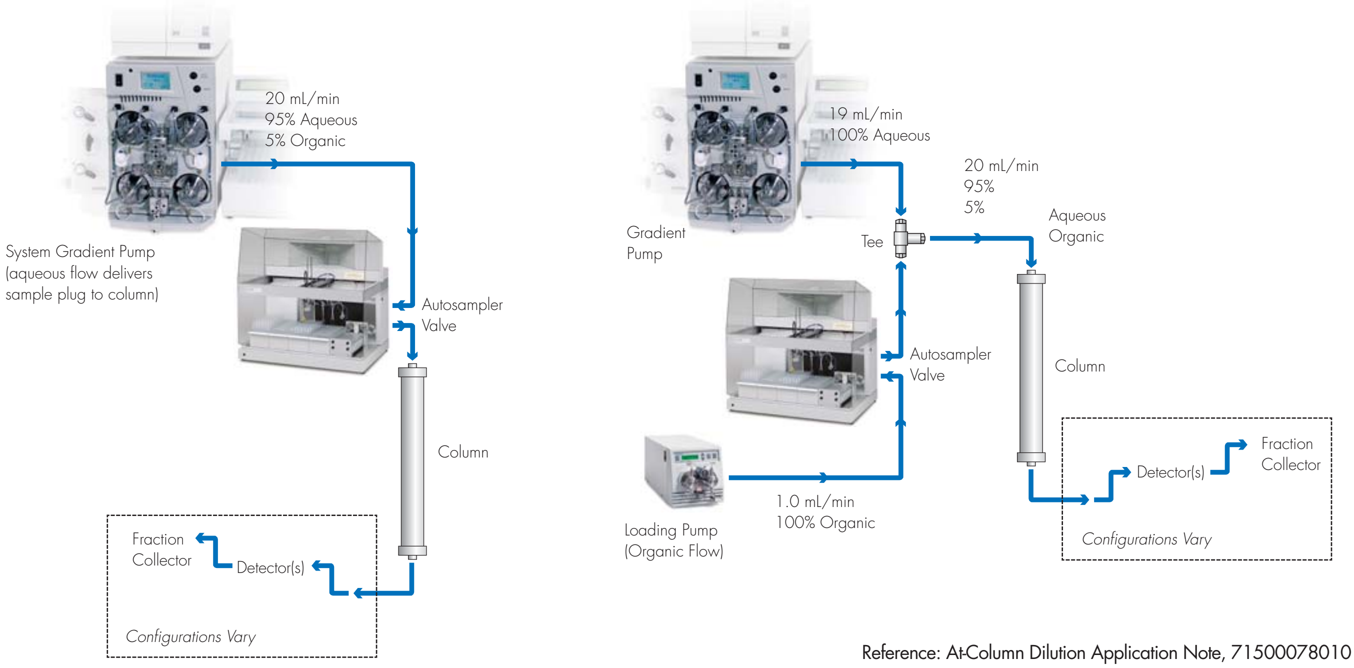
---

* U.S. Patent # 6,790,361 B2

Methods for At-Column Dilution General Principles

At-Column Dilution methods mimic the separation methods used for standard separations. In general, the flow rate and solvent composition generated with the gradient pump in a standard system are distributed between the gradient pump and the loading pump in an At-Column Dilution system. The solvent entering the column is the same in both systems as illustrated in Figure 18. With total flow and percentage composition thus modified, alter the gradient table to include an isocratic composition period (initial hold) sufficient to completely transfer the sample through the At-Column Dilution tee to the head of the column. Add the separation and other segments of the gradient to this initial hold. There is seldom any reason to further modify the gradient to account for the contribution of the loading pump to the percentage of strong solvent.

Figure 18: At-Column Dilution and Conventional Separation Methods Example



Reference: At-Column Dilution Application Note, 71500078010

Figure 19: Loading of Acids —Conventional Separation Method

Mobile Phase A: Water with 0.1% FA
Mobile Phase B: Acetonitrile with 0.1% FA
Flow Rate: 23.9 mL/min

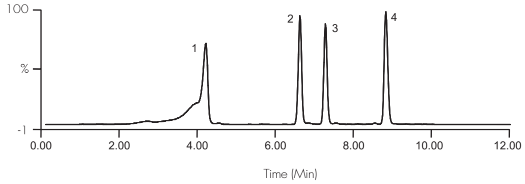
Compounds:

1. Sulfamethazine	(10 mg/mL)
2. Althiazide	(10 mg/mL)
3. Bendroflumethiazide	(10 mg/mL)
4. Amcinonide	(10 mg/mL)

Gradient

Time	Flow Rate	%A	%B
Initial	23.9	85	15
2.00	23.9	85	15
10.00	23.9	5	95
11.00	23.9	5	95

SunFire™ Prep OBD™ C_{18}, 19 x 100 mm, 5 µm
Part Number: 186002567
Injection Volume: 1 mL DMSO
Total Load: **40 mg**



Distortion of peak 1 is due to the large volume of DMSO injection. This effect limits the loading capacity for this component.

Figure 20: Loading of Acids —At-Column Dilution Method

Mobile Phase A: Water with 0.1% FA
Mobile Phase B: Acetonitrile with 0.1% FA
Flow Rate: 22.7 mL/min
Loading Pump Flow Rate: 1.2 mL/min

Compounds:

1. Sulfamethazine	(25 mg/mL)
2. Althiazide	(25 mg/mL)
3. Bendroflumethiazide	(25 mg/mL)
4. Amcinonide	(25 mg/mL)

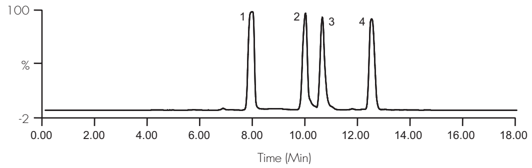
Extra holding time is used to ensure sample is fully loaded before gradient starts

Same gradient slope is used as to the separation without ACD.

Gradient

Time	Flow Rate	%A	%B
Initial	22.7	100	0
4.00	22.7	100	0
14.00	22.7	5	95
17.34	22.7	5	95

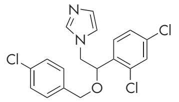
SunFire™ Prep OBD™ C_{18}, 19 x 100 mm, 5 µm
Part Number: 186002567
Injection Volume: 1 mL DMSO
Total Load: **100 mg**



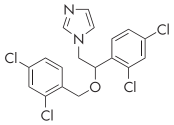
The At-Column Dilution procedure eliminates the sample solvent effect enabling significantly higher sample loading.

Chemical Structures

Figure 2, Page 4

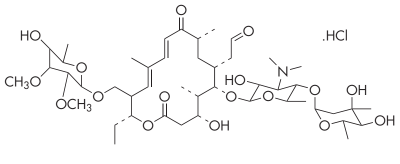


Econazole

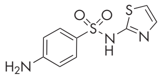


Miconazole

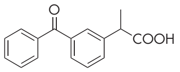
Figure 3, Page 5



Tylosin

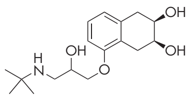


Sulfathiazole

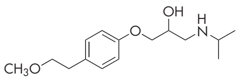


Ketoprofen

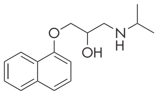
Figure 4, Page 5



Nadolol

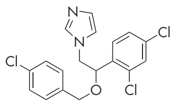


Metoprolol

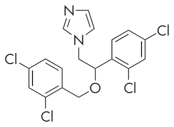


Propranolol

Figure 7, Page 7

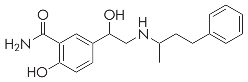


Econazole

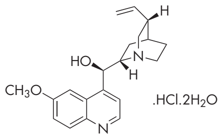


Miconazole

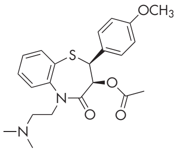
Figure 8, Page 7



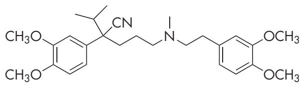
Labetolol



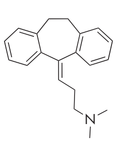
Quinine



Diltiazem

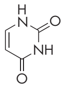


Verapamil

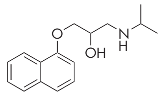


Amitriptyline

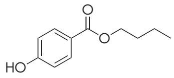
Figure 9, Page 8



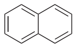
Uracil



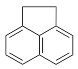
Propranolol



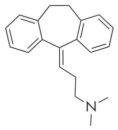
Butylparaben



Naphthalene

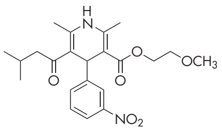


Acenaphthene



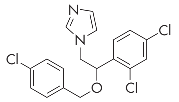
Amitriptyline

Figure 11, Page 9



Nimodipine

Figure 12, Page 9


Econazole

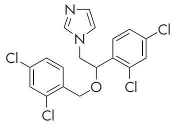
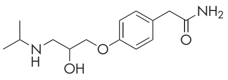
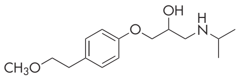

Miconazole

Figure 15, Page 11


Atenolol


Metoprolol

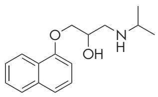
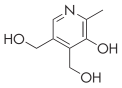

Propranolol

Figure 16, Page 11


Pyridoxine

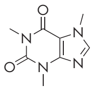
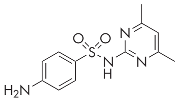
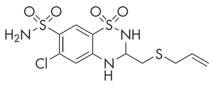
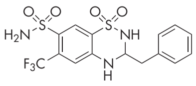

Caffeine

Figure 19, Page 13


Sulfamethazine


Althiazide


Bendroflumethiazide

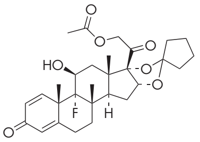
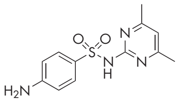
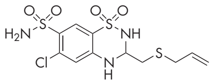
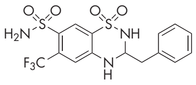
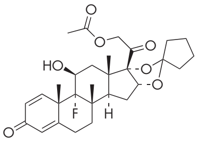

Amcinonide

Figure 20, Page 13


Sulfamethazine


Althiazide


Bendroflumethiazide


Amcinonide

How to Scale Up a Separation

Step 1

Once the analytical separation has been optimized a loading study on the analytical column is performed to determine the capacity of the particular packing material. Because the large scale separation should be identical to the small scale separation, the maximum sample load will be dependent upon the complexity of the analytical separation.

Step 2

The next step is to determine how much mass you need to purify or isolate.

Step 3

Once the desired purified mass is established some simple equations may be used to determine the required column size for purification.

Scale-up factor

$$\text{Scale-up factor} = \frac{(\text{Diameter prep})^2 \times \text{Length prep}}{(\text{Diameter analytical})^2 \times \text{Length analytical}}$$

Once determined, the scale-up factor is used to multiply the analytical column sample loading capacity to predict the loading capacity for the larger dimension column. Table 1 has general mass loading guidelines for common column dimensions. Remember, many factors affect the mass capacity of preparative columns. A brief list of modifying factors should be considered:

1. Capacity is higher for strongly retained material.
2. Capacity is higher for simple mixtures
3. Capacity is higher for gradient separations vs isocratic
4. Capacity is lower where higher resolution is required
5. Capacity is limited by loading volume
6. Capacity is limited by sample solubility
7. Capacity can be affected by choice of sample solvent
8. Capacity for ionisable solutes may be dramatically influenced by mobile phase pH

Consider scaling up from a 3.9 x 150 mm column to a 19 x 150 mm column:

$$\text{Scale-up factor} = \frac{(19)^2 \times 150}{(3.9)^2 \times 150} = 23.7$$

Applying the scale-up factor, we can predict that approximately 24–142 mg of sample could be applied to the larger column (packed with the same material as the analytical column). This range is based on an analytical (3.9 mm i.d.) mass load of 1–6 mg.

Flow Rate

$$\text{Flow rate (prep)} = \text{Flow rate (anal)} \times \frac{(\text{Diameter prep})^2}{(\text{Diameter anal})^2}$$

The calculated flow rate may be used for the larger column to ensure the same linear velocity of mobile phases as used in the analytical run. However, reasonable flow rates are based on column diameters. Systems will be limited by increasing backpressure with increasing length and decreasing particle size.

Gradient Duration (GD)

$$\text{GD (prep)} = \text{GD (anal)} \times \frac{\text{Length (prep)}}{\text{Length (anal)}} \times \frac{\text{Diameter (prep)}^2}{\text{Diameter (anal)}^2} \times \frac{\text{Flow rate (anal)}}{\text{Flow rate (prep)}}$$

The calculated prep gradient duration is entered into the pump's gradient separation over the same number of column volumes as was used in the analytical run.

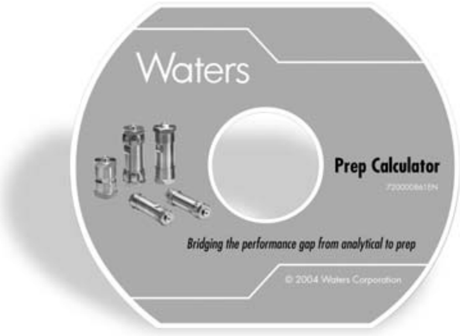
Mass Load Chart (mgs)*

	Diameter (mm)											
Length (mm)	3.9 mm	4.6 mm	7.8 mm	8 mm	10 mm	19 mm	20 mm	25 mm	30 mm	40 mm	47 mm	50 mm
30 mm	1	2			7	27						
50 mm	2	3	8		15	45	50		110			310
100 mm	4	5	15	15	25	90	100	155	225	400		620
150 mm	6	8	25		40	135	150		335			930
200 mm				30				310		795		
250 mm	10	13	40		60	225	250		560			1551
300 mm	12	16	45	50	75	270	300	470	670	1195	1650	1860
Reasonable Flow Rate (mL/min)	1.0	1.4	4.0	4.2	6.6	24	27	42	60	105	145	164
Reasonable Injection** Volume (µL)	15	20	60	65	100	350	390	610	880	1565	2160	2450

* *Mass loads were obtained experimentally using water-soluble compounds: oxacillin, cloxacillin and dicloxacillin. These are general mass loads, please refer to the scale factor section above for more information on mass loading.*

** *Reasonable injection volumes are based on column diameter at a length of 50 mm with relatively strong solvents. Increased length is compatible with larger injection, but not proportionately so. Weaker solvents allow for significantly increased injection volumes.*

Register to receive a FREE, updated Preparative OBD™ Column Calculator at **www.waters.com/prepcolumn**



720000861EN

Designed to simplify common preparative calculations:

- Column/tubing backpressure
- Mass load scale-up
- Gradient scale-up
- Gradient designs

Ordering Information

XTerra®
COLUMNS

XTerra® Prep Columns

ID (mm)	Length (mm)	Particle Size	Type	MS C_{18}	MS C_8	RP_{18}	RP_8
7.8 mm	10 mm	5 µm	Cartridge	186001168[1]	186001169[1]	186001170[1]	186001171[1]
7.8 mm	10 mm	10 µm	Cartridge	186001172[1]	186001173[1]	186001174[1]	186001175[1]
7.8 mm	50 mm	5 µm	Column	186001152	186001153	186001154	186001155
7.8 mm	100 mm	5 µm	Column	186001156	186001157	186001158	186001159
7.8 mm	150 mm	5 µm	Column	186001475	186001476	186001477	186001478
7.8 mm	150 mm	10 µm	Column	186001160	186001161	186001162	186001163
7.8 mm	300 mm	10 µm	Column	186001164	186001165	186001166	186001167
10 mm	10 mm	5 µm	Cartridge	186001001[2]	186001004[2]	186001006[2]	186001008[2]
10 mm	10 mm	10 µm	Cartridge	186001002[2]	186001005[2]	186001007[2]	186001009[2]
10 mm	30 mm	5 µm	Column	186001010	186001011	186001012	186001013
10 mm	50 mm	5 µm	Column	186001014	186001015	186001016	186001017
10 mm	100 mm	5 µm	Column	186001018	186001019	186001020	186001021
10 mm	150 mm	5 µm	Column	186001479	186001480	186001481	186001482
10 mm	150 mm	10 µm	Column	186001022	186001023	186001024	186001025
10 mm	250 mm	10 µm	Column	186001026	186001027	186001028	186001029
10 mm	300 mm	10 µm	Column	186001030	186001031	186001032	186001033
19 mm	10 mm	5 µm	Cartridge	186001104[3]	186001105[3]	186001106[3]	186001107[3]
19 mm	10 mm	10 µm	Cartridge	186001034[3]	186001035[3]	186001036[3]	186001037[3]
19 mm	30 mm	5 µm	OBD™ Column	186002383	186002384	186002385	186002386
19 mm	50 mm	5 µm	OBD™ Column	186001930	186001931	186001932	186001933
19 mm	50 mm	10 µm	OBD™ Column	186002254	—	—	—
19 mm	100 mm	5 µm	OBD™ Column	186001934	186001935	186001936	186001937
19 mm	150 mm	5 µm	OBD™ Column	186002379	186002380	186002381	186002382
19 mm	150 mm	10 µm	OBD™ Column	186002255	186002256	186002257	186002258
19 mm	250 mm	10 µm	OBD™ Column	186002259	186002260	186002261	186002262
19 mm	300 mm	10 µm	OBD™ Column	186002263	186002264	186002265	186002266
30 mm	50 mm	5 µm	OBD™ Column	186001938	186001939	186001940	186001941
30 mm	75 mm	5 µm	OBD™ Column	186002387	186002388	186002389	186002390
30 mm	100 mm	5 µm	OBD™ Column	186001942	186001943	186001944	186001945
30 mm	150 mm	10 µm	OBD™ Column	186002267	186002268	186002269	186002270
30 mm	250 mm	10 µm	OBD™ Column	186002271	186002272	186002273	186002274
30 mm	300 mm	10 µm	OBD™ Column	186002275	186002276	186002277	186002278
50 mm	50 mm	5 µm	OBD™ Column	186002218	186002219	186002220	186002221
50 mm	50 mm	10 µm	OBD™ Column	186002279	186002280	186002281	186002282
50 mm	100 mm	5 µm	OBD™ Column	186002222	186002223	186002224	186002225
50 mm	150 mm	10 µm	OBD™ Column	186002843	186002844	186002845	186002846
50 mm	250 mm	10 µm	OBD™ Column	186002847	186002848	186002849	186002850

[1] Requires 7.8 x 10 mm Cartridge Holder Part No. 186000708

[2] Requires 10 x 10 mm Cartridge Holder Part No. 289000779

[3] Requires 19 x 10 mm Cartridge Holder Part No. 186000709

SunFire™ COLUMNS

SunFire™ Prep 5 µm Columns

Particle Size	Dimensions	C_{18}	C_8
5 µm	10 x 50 mm	186002561	186002746
5 µm	10 x 100 mm	186002562	186002747
5 µm	10 x 150 mm	186002563	186002748
5 µm	10 x 250 mm	186002564	186002749
5 µm	10 x 10 mm Guard	186002565[1]	186002750[1]
5 µm	OBD™ 19 x 30 mm	186002879	186002881
5 µm	OBD™ 19 x 50 mm	186002566	186002751
5 µm	OBD™ 19 x 100 mm	186002567	186002752
5 µm	OBD™ 19 x 150 mm	186002568	186002753
5 µm	19 x 10 mm Guard	186002569[2]	186002754[2]
5 µm	OBD™ 30 x 50 mm	186002570	186002755
5 µm	OBD™ 30 x 75 mm	186002571	186002756
5 µm	OBD™ 30 x 100 mm	186002572	186002757
5 µm	OBD™ 30 x 150 mm	186002797	186002795
5 µm	OBD™ 50 x 50 mm	186002867	186002868
5 µm	OBD™ 50 x 100 mm	186002869	186002870

SunFire™ Prep 10 µm Columns

Particle Size	Dimensions	C_{18}	C_8
10 µm	10 x 150 mm	186002664	186002759
10 µm	10 x 250 mm	186002665	186002760
10 µm	10 x 10 mm Guard	186002663[1]	186002758[1]
10 µm	OBD™ 19 x 50 mm	186002667	186002762
10 µm	OBD™ 19 x 150 mm	186002668	186002763
10 µm	OBD™ 19 x 250 mm	186002669	186002764
10 µm	19 x 10 mm Guard	186002666[2]	186002761[2]
10 µm	OBD™ 30 x 150 mm	186002670	186002765
10 µm	OBD™ 30 x 250 mm	186002671	186002766
10 µm	OBD™ 50 x 50 mm	186002871	186002872
10 µm	OBD™ 50 x 150 mm	186002672	Custom
10 µm	OBD™ 50 x 250 mm	186002673	Custom

Atlantis® Columns

Atlantis® dC_{18} Prep 5 µm Columns

Type	Dimensions	dC_{18}
5 µm	10 x 10 mm Guard	186002300[1]
5 µm	10 x 50 mm	186002298
5 µm	10 x 100 mm	186002299
5 µm	19 x 10 mm Guard	186001361[2]
5 µm	OBD™ 19 x 50 mm	186001365
5 µm	OBD™ 19 x 100 mm	186001367
5 µm	OBD™ 19 x 150 mm	186002800
5 µm	OBD™ 30 x 50 mm	186001373
5 µm	OBD™ 30 x 100 mm	186001375
5 µm	OBD™ 30 x 150 mm	186002801

Atlantis® dC_{18} Prep 10 µm Columns

Type	Dimensions	dC_{18}
10 µm	10 x 10 mm Guard	186002452[1]
10 µm	10 x 150 mm	186002453
10 µm	10 x 250 mm	186002454
10 µm	19 x 10 mm Guard	186001363[2]
10 µm	OBD™ 19 x 150 mm	186001369
10 µm	OBD™ 19 x 250 mm	186001371
10 µm	OBD™ 30 x 150 mm	186002417
10 µm	OBD™ 30 x 250 mm	186002418

[1] Requires 10 x 10 mm Prep Guard Cartridge Holder 289000779

[2] Requires 19 x 10 mm Prep Guard Cartridge Holder 186000709

Purification and Isolation Cartridge Holders

Description	Part No.
7.8 x 10 mm Cartridge Holder	186000708
10 x 10 mm Cartridge Holder	289000779
19.0 x10 mm Cartridge Holder	186000709
Replacement o-ring 7.8 mm, 2 pkg	700001019
Replacement o-ring 10 mm, 2 pkg	700001436
Replacement o-ring 19 mm, 2 pkg	700001020
Replacement Rigid Connector	405000981

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