Waters

Separation of Polar Analytes on Preparative RPLC Columns

Fang Xia*, Jie Y. Cavanaugh, Martin Gilar, Uwe D. Neue, Jeffrey R. Mazzeo, Diane M. Diehl
Waters Corporation, 34 Maple St., Milford, MA 01757-3696 (* Send Correspondence to Fang_Xia@waters.com)

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OVERVIEW

Atlantis™ dC_{18} columns are a fully LC/MS compatible line of reversed-phase (RP) columns designed for retaining and separating both polar and non-polar compounds. This new silica-based packing material was designed with the best combination of pore size, ligand density, ligand type, and is fully compatible with 100% aqueous mobile phases.

The separation of active pharmaceutical ingredients is shown on both analytical and preparative columns. We also show the separation of a crude synthetic peptide mixture on both analytical and semi-preparative columns. Finally, a preparative load of peptide is achieved successfully on an analytical dimension column. The fraction collector was triggered by the mass-to-charge ratio of the target peptide to obtain a high purity (>99%).

These results indicate that Atlantis™ dC_{18} RPLC columns provide good efficiency, high mass loading, and ease of scale-up for both small and large molecules.

INTRODUCTION

Polar analytes (small molecule drugs and peptides) are very difficult to retain on traditional RPLC columns, because traditional columns dewet under 100% aqueous conditions necessary for retaining polar analytes. The Atlantis™ columns are specially designed to meet the needs of chromatographers looking for polar compound retention.

The Waters AutoPurification™ system is capable of running preparative chromatography in an automated fashion. FractionLynx™ software installed with MassLynx™ triggers, tracks, controls and documents runs so the user can carry out purifications in an automated fashion and an unattended mode. This scalable MS- and UV-based system provides a simple but powerful automated purification process for successful compound discovery and development.

SEPARATION OF PHARMACEUTICALS

Column: Atlantis™ dC_{18} 4.6 x 100 mm, 5 µm
Part Number: 186001340
Total Mass Load: 280 µg
Detection: UV @ 300 nm

Flow Rate: 1.0 mL/min
Gradient:

Time (min)	Profile %A	%B
0.0	85	15
1.0	85	15
2.0	70	30
12.0	30	70
14.0	30	70

Injection Volume: 70 µL

Column: Atlantis™ dC_{18} 19 x 100 mm, 5 µm
Part Number: 186002116
Total Mass Load: 4800 µg
Detection: UV @ 280 nm

Flow Rate: 17.06 mL/min
Gradient:

Time (min)	Profile %A	%B
0.0	85	15
3.02	85	15
4.02	70	30
14.02	30	70
16.02	30	70

Injection Volume: 1200 µL

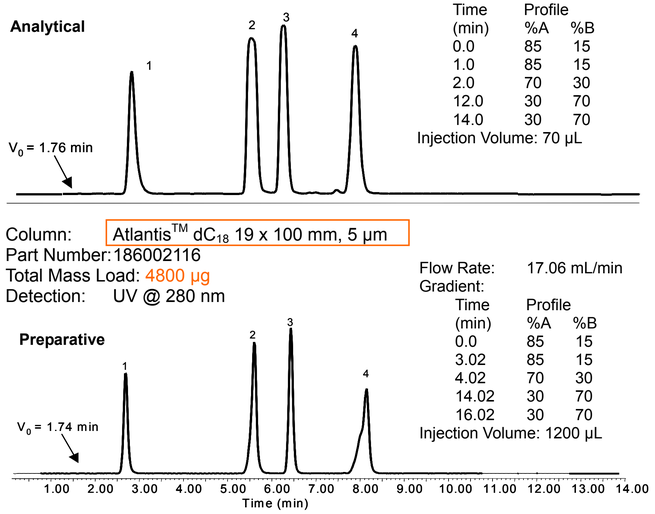
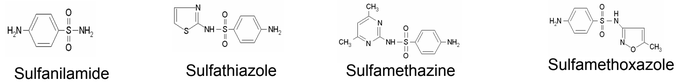


Figure 1: Separation of sulfonamides on analytical and preparative Atlantis™ dC_{18} columns. Mobile phase A: 0.1% formic acid in water; Mobile phase B: acetonitrile/1% formic acid (90/10). Analytes in order of elution: sulfanilamide (1), sulfathiazole (2), sulfamethazine (3), sulfamethoxazole (4).



The flattened peak profiles on the analytical chromatogram reflect the saturation of the PDA detector, not column overload. Higher UV wavelength is chosen due to the saturation of the PDA at lower wavelength.

- Atlantis™ dC_{18} columns are capable of retaining **polar small molecules** under highly aqueous conditions.
- Achieve linear scale-up from analytical to preparative chromatography.

SEPARATION OF A SYNTHETIC PEPTIDE

Atlantis™ dC_{18} 4.6 x 100 mm, 5 µm
Part Number: 186001340
Total Mass Load: 210 µg
Detection: MS, ESI^+

Flow Rate: 1.0 mL/min
Flow Split to MS: 0.2 mL/min
Gradient:

Time (min)	Profile %A	%B
0.0	100	0
1.0	100	0
46.0	50	50

Injection Volume: 21 µL

Atlantis™ dC_{18} 10 x 100 mm, 5 µm
Part Number: 186002299
Total Mass Load: 1000 µg
Detection: MS, ESI^+

Flow Rate: 4.73 mL/min
Flow Split to MS: 0.25 mL/min
Gradient:

Time (min)	Profile %A	%B
0.0	100	0
2.73	100	0
47.73	50	50

Injection Volume: 100 µL

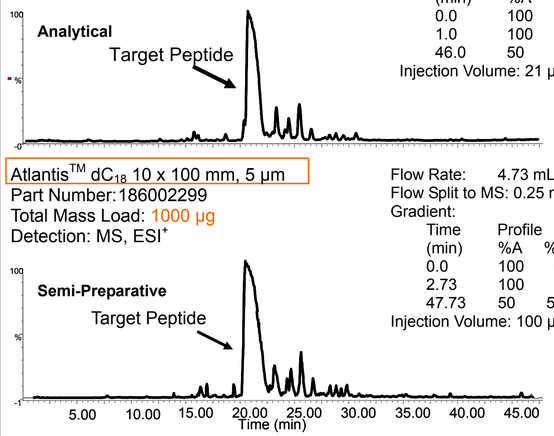


Figure 2: Separation of synthetic peptides on analytical and semi-preparative Atlantis™ dC_{18} columns. Mobile phase A: 0.2% formic acid; in water; Mobile phase B: 0.2% formic acid in acetonitrile. Target peptide sequence information: NH_2-DRNFLRF-COOH.

Waters ZQ™
ESI^+

Capillary (kV)	3.5	LM Resolution	15
Cone (V)	25	HM Resolution	15
Extractor	3.0	Ion Energy	0.3
RF Lens	0.3	Multiplier (V)	650
Source Temp (ºC)	100	Scan Range (m/z)	320-1920
Desolvation Temp (ºC)	250	Continuum	
Cone Gas Flow (L/Hr)	50	Scan Time (sec)	2.2
Desolvation Gas Flow (L/Hr)	260	Delay Time (sec)	0.1

- Atlantis™ dC_{18} columns are capable of retaining **synthetic peptides** under highly aqueous conditions.
- Achieve linear scale-up from analytical to preparative chromatography.

MASS DIRECTED FRACTION COLLECTION

Atlantis™ dC_{18} 4.6 x 100 mm, 5 µm
Part Number: 186001340
Total Mass Load: 4000 µg

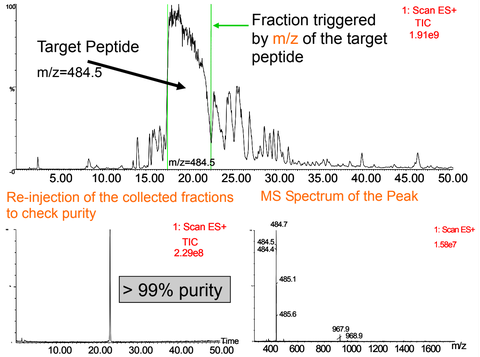


Figure 3: Mass triggered fraction collection and the re-injection result.

- Achieve **preparative** peptide loading on an **analytical** column.
- MS directed fraction collection results in high purity of the target samples.

CONCLUSIONS

- Atlantis™ dC_{18} RPLC columns are useful tools for the purification of **both polar and non-polar** small molecule drugs under highly aqueous mobile phase conditions.
- Atlantis™ dC_{18} RPLC columns are also suitable for the purification of **synthetic peptides** on both analytical and preparative scales.
- The mass-directed fraction collection purification systems ensure high purity of target peptide samples.
- Atlantis™ dC_{18} preparative columns are available in various dimensions for ease of scale-up, and high mass loading.