Separation of Synthetic Peptides on Preparative RPLC Columns

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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 overloading profiles of synthetic peptides on analytical columns are shown first. 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%).

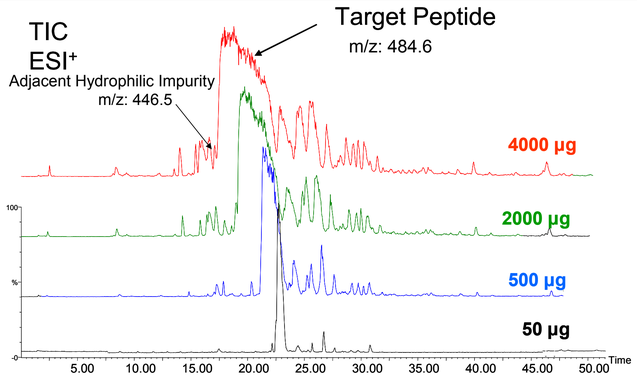
These results indicate that Atlantis™ dC_{18} RPLC columns provide good efficiency, high mass loading, and ease of scale-up for synthetic peptide crude samples.

INTRODUCTION

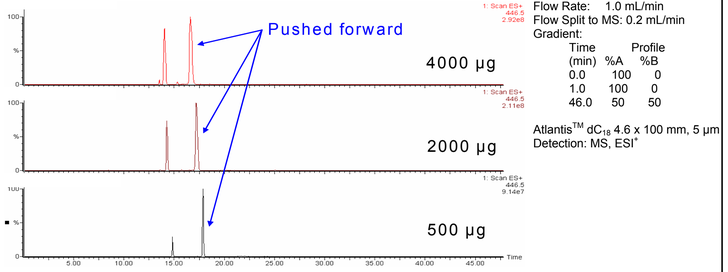
Synthetic peptides are too polar to retain on traditional RPLC columns, because traditional columns dewet under 100% aqueous conditions necessary for retaining polar analytes. The Atlantis™ columns are specially designed to meet the needs of chromatographers looking for polar compound retention.

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.

MASS OVERLOADING



MS Scan of the Adjacent Hydrophilic Impurity



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

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

Atlantis™ dC_{18} 4.6 x 100 mm, 5 µm
Detection: MS, ESI+

Figure 1: Mass overloading of synthetic peptide crude samples on an analytical Atlantis™ dC_{18} columns. Mobile phase A: 0.1% trifluoroacetic acid in water; Mobile phase B: 0.1% trifluoroacetic acid in acetonitrile.

- Atlantis™ dC_{18} columns are capable of retaining **synthetic peptide crude** under highly aqueous conditions.
- **Displacement profile** were observed when overloading the column with synthetic peptide, because the adjacent hydrophilic impurity were pushed forward by the enrichment of the target peptide.
- Displacement helps load even more samples on column without losing resolution.

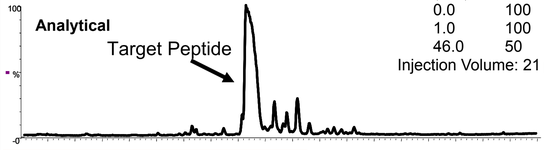
SEPARATION OF A SYNTHETIC PEPTIDE

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)	%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)	%A	%B
0.0	100	0
2.73	100	0
47.73	50	50

Injection Volume: 100 µL

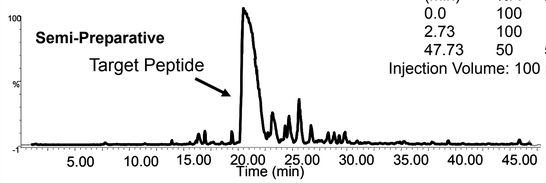


Figure 2: Separation of synthetic peptides on analytical and semi-preparative Atlantis™ dC_{18} columns. Mobile phase A: 0.2% formic acid; in water; Mobile phase B: 0.2% formic acid in acetonitrile. Target peptide sequence information: NH_2-DRNFLRF-COOH.

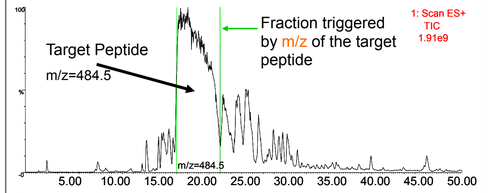
Waters ZQ™
ESI+

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



Re-injection of the collected fractions to check purity

MS Spectrum of the Peak

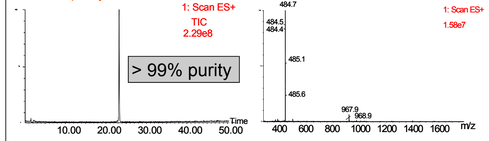


Figure 3: Mass triggered fraction collection and the re-injection result.

- 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 **synthetic peptide crude** under highly aqueous mobile phase conditions.
- Displacement profile is observed when overloading peptide, which helps load preparative amount of peptide on an analytical column.
- 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.