Maximizing Throughput of Synthetic Peptides in Reversed-Phase Liquid Chromatography

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INTRODUCTION

Synthetic peptides sometimes are difficult to retain on traditional RPLC columns, because the traditional columns de-wet under 100% aqueous conditions necessary for retaining polar analytes. The Atlantis™ columns are specially 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.

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

In this poster, the overloading profiles of synthetic peptides on analytical columns are shown first. Then the scalability of separating crude synthetic peptides from analytical to semi-preparative columns are studied. Further, the LC/MS/MS analysis was employed to identify the sequences of each impurity in the synthetic peptide crude. Finally, 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. Displacement helps loading preparative amount of peptides on small analytical dimension columns.

EXPERIMENT

HPLC Conditions:
As indicated in the chromatograms.

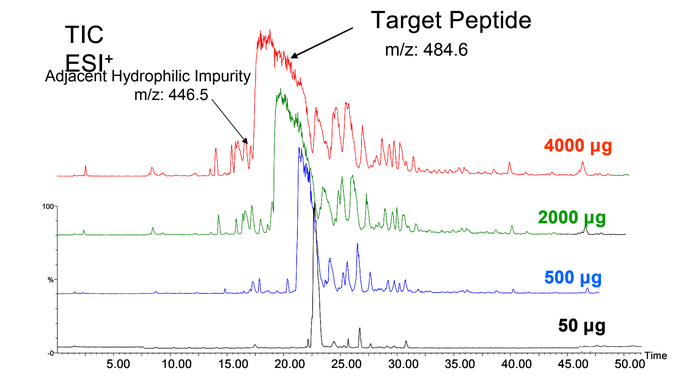
Waters ZQ™ ESI+

Capillary (kV)	3.5	LM Resolution	15
Cone (V)	25	HM Resolution	15
Extractor (V)	3.0	Ion Energy (V)	0.3
RF Lens	0.3	Multiplier (V)	650
Source Temp (°C)	100	Scan Range (m/z)	320-1920
Desolvation Temp (°C)	250	Scan Mode	Continuum

Waters Q-Tof micro™

Capillary (kV)	3.5	LM Resolution	15
Cone (V)	35	HM Resolution	15
Extractor (V)	0	Ion Energy (V)	0.3
Source Temp (°C)	135	Scan Range (m/z)	320-1920
Desolvation Temp (°C)	350	Scan Mode	Continuum
Cone Gas Flow (L/Hr)	50	Scan Time (sec)	2.2
Desolvation Gas Flow (L/Hr)	500	Delay Time (sec)	0.1

MASS OVERLOADING



MS Scan of the Adjacent Hydrophilic Impurity

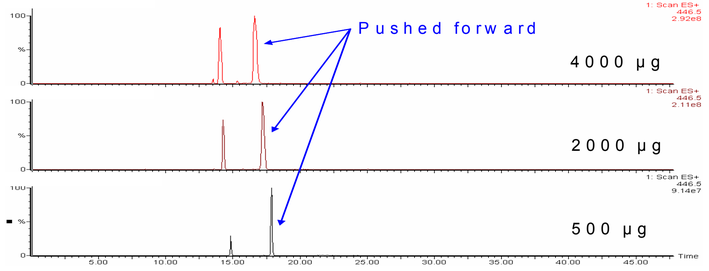


Figure 1: Mass overloading of synthetic peptide crude samples on an analytical Atlantis™ dC_{18} columns. Mobile phase A: 0.2% formic acid in water; Mobile phase B: 0.2% formic acid in acetonitrile. 45 min gradient from 0% to 50% B. Atlantis™ dC_{18}, 4.6×100mm, 5µm. Peptide sequence: NH_2-DRNFLRF-COOH.

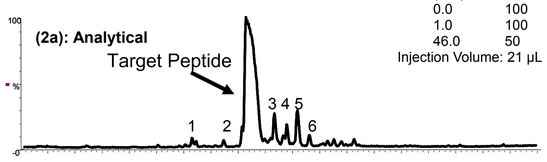
- Atlantis™ dC_{18} columns are capable of retaining **synthetic peptide crude** under highly aqueous conditions.
- **Displacement profile** were observed when overloading the column with synthetic peptide, which is indicated by the fact that the adjacent hydrophilic impurity were pushed forward by the enrichment of the target peptide.
- Displacement phenomenon helps load more samples (e.g. 4000 µg in this case) on a small column without losing resolution.

SEPARATION OF A SYNTHETIC PEPTIDE

Atlantis™ dC_{18} 4.6 x 100 mm, 5 µm
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
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

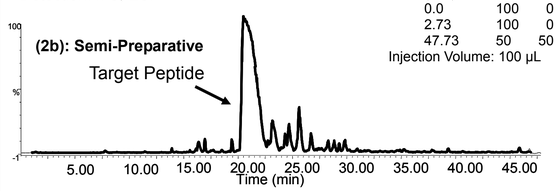


Figure 2: Separation of synthetic peptides on analytical and semi-preparative Atlantis™ dC_{18} columns. (2a): analytical; (2b): semi-preparative. Mobile phase A: 0.2% formic acid; in water; Mobile phase B: 0.2% formic acid in acetonitrile. Target peptide sequence information: NH_2-DRNFLRF-COOH.

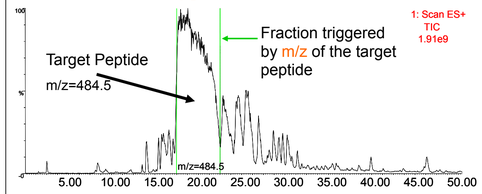
LC/MS/MS Study on Peptide Impurities as Shown in Figure 2a

Peaks	Sequence
Target Peptide	NH2-DRNFLRF-COOH
1	NH2-DRNALRF-COOH
2	NH2-DRRNFLRF-COOH
3	NH2-PRNFL-COOH
4	Aggregate or dimer
5	NH2-DRDLFLRF-COOH
6	NH2-DRNFFLRF-COOH

- Achieve **linear scale-up** from analytical to preparative chromatography.
- LC/MS/MS is capable of identify every impurity in the peptide crude.

MASS DIRECTED FRACTION COLLECTION

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



Re-injection of the collected fractions to check purity

MS Spectrum of the Peak

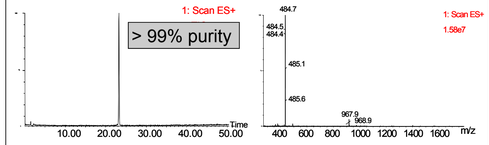


Figure 3: Mass triggered fraction collection and the re-injection result.

- Achieve **preparative** peptide loading on an **analytical** column.
- MS directed fraction collection ensures 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