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Application Note

Increasing Chromatographic Performance of Acidic Peptides in RPLC-MS-based Assays with ACQUITY PREMIER featuring MaxPeak HPS Technology

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Abstract

Metal-ion mediated adsorption of analytes as a contributing factor in poor peak shape, tailing, and diminished recovery of compounds in LC-based techniques can negatively impact data quality and assay robustness. Analytes exhibiting phosphate groups, uncharged amines, and deprotonated carboxylic acids are particularly susceptible to these phenomena and are commonly encountered in the development and manufacturing of protein-based therapeutics. Current methods for addressing analyte/surface adsorption include ion-pairing additives, hardware passivation, and high-ionic strength mobile phase. These strategies, while proven effective, can be challenging to deploy in terms of instrument/technique compatibility in the case of MS-based methods as well ensuring they can be implemented in a safe and efficient manner with respect to lengthy passivation procedures that can involve corrosive reagents. The newly introduced ACQUITY PREMIER brand columns with MaxPeak HPS Technology is Waters solution to these challenges. Waters ACQUITY PREMIER Columns are designed to deliver exceptional chromatographic performance while minimizing analyte/surface interactions of sensitive compounds. In this study, the performance gain of ACQUITY PREMIER Columns with MaxPeak HPS Technology is demonstrated with increased recovery, reproducibility, and robustness of RPLC-MS-based peptide mapping assays using the Waters NIST mAb tryptic digest standard. Collectively, this study establishes MaxPeak HPS Technology can be broadly applied in the development and manufacturing of therapeutic drug products to deliver the chromatographic performance expected from Waters technologies while increasing reproducibility, peak shape, and recovery of analytes prone to surface interactions.

Benefits

- Increased sensitivity through improved recovery and lower peak tailing
- Compatibility with legacy methods without the use of additional additives
- Improved method robustness through increased assay reproducibility
- Increased productivity through reduced method development time

Introduction

Analyte/surface adsorption in liquid chromatography (LC) has been established as a contributing factor in

poor peak shape, tailing, and diminished recovery of compounds in LC-based techniques.[1-3] Recently, metal-ion mediated adsorption has been identified as a specific adsorption mechanism for analytes that exhibit Lewis acid/base characteristics.[4] The hypothesis being analytes bearing electron rich moieties such as phosphate groups, uncharged amines, and deprotonated carboxylic acids act as Lewis Bases which can adsorb in a non-covalent manner to electron deficient sites on the metal surface which act as a Lewis Acid. Conventional strategies to suppress metal-ion mediated adsorption include ion-pairing, hardware passivation, and high-ionic strength mobile phases. While largely successful, challenges still exist in certain instances where analytes exhibit inordinately strong interactions with metal surfaces (e.g. bearing multiple electron-rich moieties) and/or assays are performed with non-optimal conditions (e.g. weak vs. strong ion-pairing). This is particularly evident in RPLC-MS-based peptide analyses wherein peptide fragments containing aspartic acid (D) or glutamic acid (E) residues can interact with metal surfaces which can exacerbate adsorption characteristics resulting in increased tailing and reduced sensitivity of analytes prone to metal-ion mediated adsorption as shown in Figure 1. Recently, it was shown that metal chelators can be used as mobile phase additives to mitigate adsorption artifacts with notable success.[5] However, incorporation of such additives is not always ideal as they can introduce new chromatographic artifacts as well as suppress ionization in MS-based analyses. These challenges highlight the need for novel columns and instrumentation that can mitigate metal-ion mediated adsorption without the need for additional additives or lengthy passivation processes.

The newly introduced ACQUITY PREMIER brand columns with MaxPeak HPS Technology is Waters solution to these challenges. The ACQUITY PREMIER Columns are designed to deliver exceptional chromatographic performance while minimizing analyte/surface interactions of sensitive compounds. The objective of this application note is to demonstrate how ACQUITY PREMIER with MaxPeak HPS Technology can increase productivity in the lab and mitigate risk through increased reproducibility, recovery, and robustness of assays performed in the development and manufacturing of biopharmaceutical drug products.

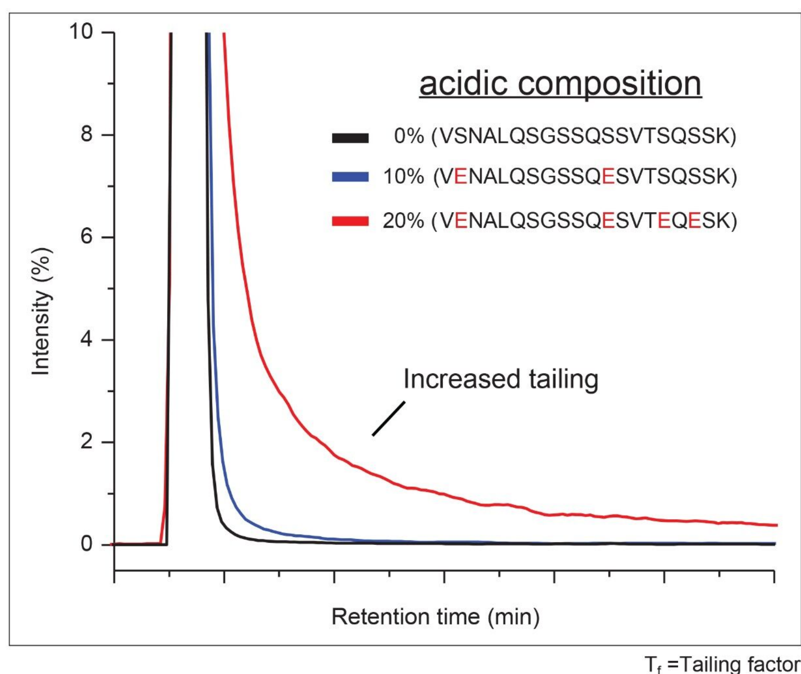


Figure 1. Synthetic acidic peptide ladder. Tailing was evaluated for 3 synthetic peptides manufactured with 0, 2, and 4 glutamic acid (E) residues representing 0%, 10%, and 20% acidic content by composition. Under isocratic conditions (MP A: 89%, MP B: 11%), tailing was observed to increase significantly for peptides containing multiple acidic residues.

Experimental

Columns were conditioned as outlined by the column care and use manual. Waters mAb Tryptic Digestion Standard (p/n: 186009126) was reconstituted in MS-grade water with 0.1% FA at a concentration of 0.2 mg/mL (SYNAPT XS) and 0.5 mg/mL (ACQUITY QDa), aliquoted and stored at -80 °C prior to use.

LC Conditions

LC system:	ACQUITY UPLC H-Class Binary Bio PLUS

Detection:	TUV, 10 mm Analytical FC, λ=214 nm
Vials:	QuanRecovery with MaxPeak HPS (300 µL/25 pk, p/n: 186009242)
Column(s):	ACQUITY CSH 130 Å C_{18} Column (2.1 x 100 mm, 1.7 µm p/n: 186005297) ACQUITY PREMIER Peptide CSH 130 Å C_{18} Column (2.1 x 100 mm, 1.7 µm p/n: 186009461)
Column temp.:	60 °C
Sample temp.:	6 °C
Injection volume:	ACQUITY QDa = 10 µL, SYNAPT XS = 1.0 µL
Flow rate:	0.200 mL/min
Mobile phase A:	H_2O, 0.1 % formic acid
Mobile phase B:	Acetonitrile, 0.1 % formic acid

Gradient Table (Figure 2–7)

Time	Flow (mL/min)	% A	% B	Curve
Initial	0.200	99	1	6
2.00	0.200	99	1	6
52.00	0.200	65	35	6
57.00	0.200	15	85	6
62.00	0.200	15	85	6
67.00	0.200	99	1	6
80.00	0.200	99	1	6

MS Conditions (Figure 1 and 6)

MS system:	ACQUITY QDa
Ionization mode:	ESI+
Acquisition range:	250–1250 *m/z*
Capillary voltage:	1.5 kV
Cone voltage:	10 V
Probe temp.:	600 °C

MS Conditions (Figure 2-5)

MS system:	SYNAPT XS
Ionization mode:	ESI+
Acquisition range:	50–2000 *m/z*
Capillary voltage:	2.2 kV
Cone voltage:	20 V
Source temp.:	120 °C
Desolvation temp.:	350 °C
Cone gas:	35 L/hr
Desolvation gas:	500 L/hr
Lockmass:	Glu fibrinopeptide B at 100 fmol/mL in 75/25 acetonitrile/water, 0.1% formic acid

Data Management

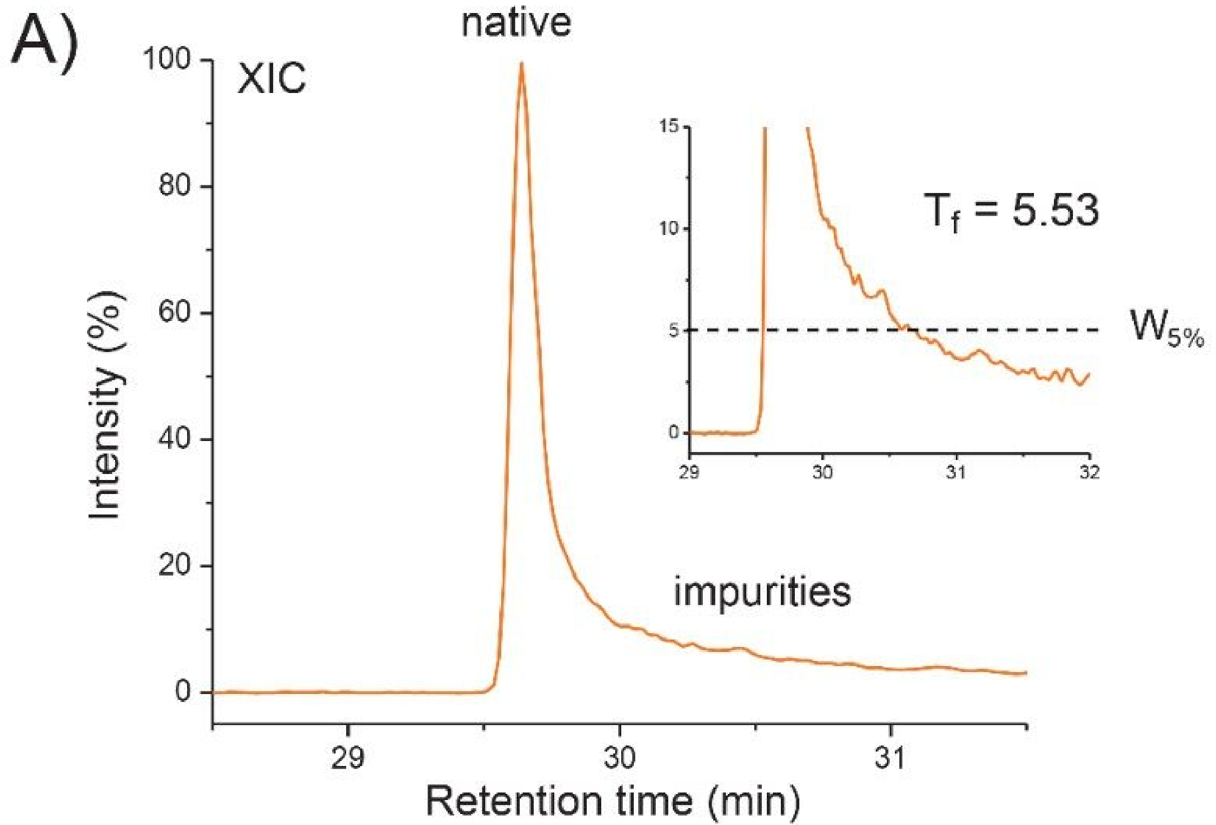
Chromatography software:	Empower 3 FR4
MS software:	MassLynx 4.2
Informatics:	UNIFI 1.9.4

Results and Discussion

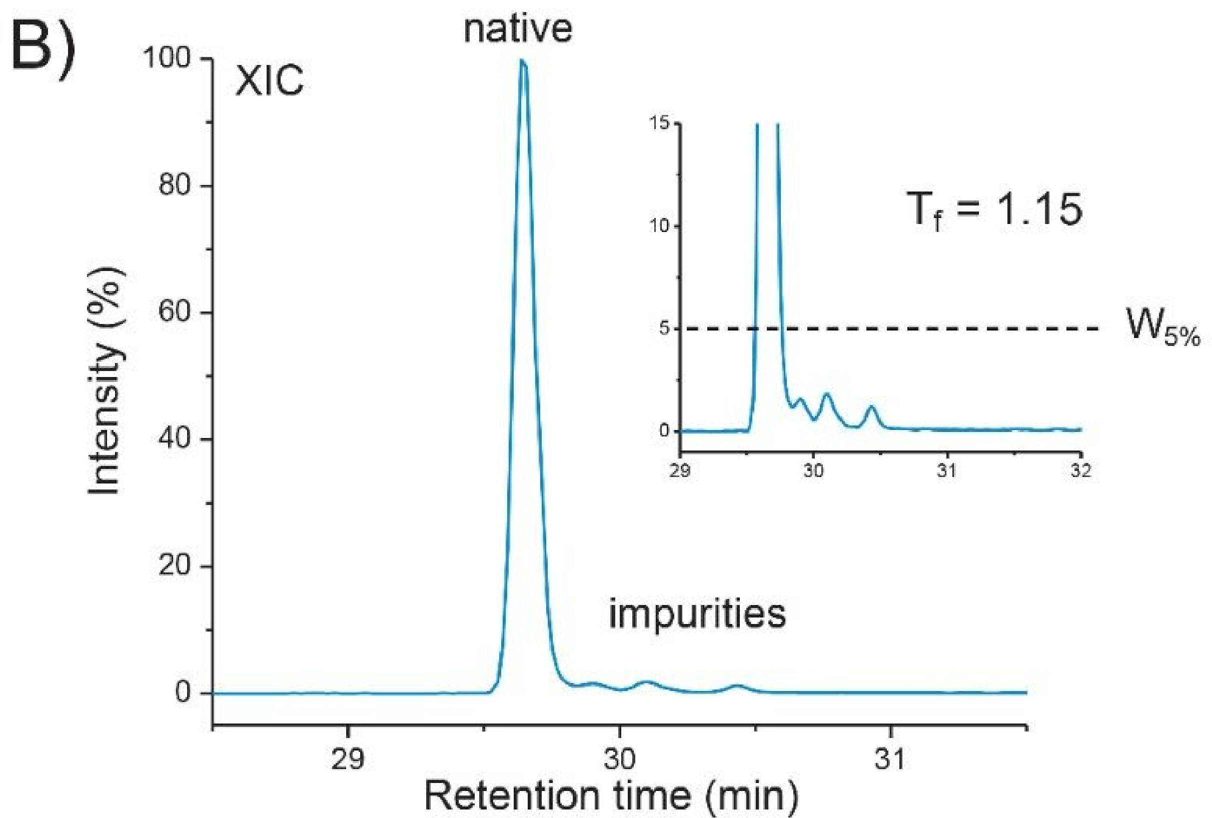
Industry Relevance:

Peptide based analyses have proven to be an invaluable tool in the characterization and quality control of protein-based therapeutics as part of a quality lifecycle management process. As part of this process, LC-MS-based data plays a critical role in providing information used to determine primary sequence protein modifications and their related impurities. The value of mass information in these analyses has contributed in part to renewed interest in expanding the role of MS-based methods to improve productivity and data quality in the development and manufacturing of drug products. However, peptide assays with MS detection are often deployed with weaker mobile phase additives such as formic acid in favor of sensitivity over chromatographic performance. This can be problematic for trace impurities that are prone to metal-ion mediated adsorption (e.g. "acidic" peptides) as assay reproducibility and accuracy of results can vary based on the severity of the analyte/surface interaction. An example of this is shown in Figure 2A in the case of deamidation of asparagine.

Conventional technology



ACQUITY PREMIER with MaxPeak HPS Technology



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ACQUITY UPLC H-Class PLUS Bio System <https://www.waters.com/waters/nav.htm?cid=10166246>

Empower Chromatography Data System <https://www.waters.com/513188>

ACQUITY UPLC Tunable UV Detector <https://www.waters.com/514228>

ACQUITY QDa Mass Detector <https://www.waters.com/134761404>

SYNAPT XS High Resolution Mass Spectrometer <https://www.waters.com/135020928>

UNIFI Scientific Information System <https://www.waters.com/134801648>

MassLynx MS Software <https://www.waters.com/513662>

720007003, September 2020

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