Improving Multi-Attribute Method using LC-MS System with Novel Inert Fluidic Pathway

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Introduction

Non-specific adsorption of acidic peptides to metal surfaces is a well-known phenomenon affecting LC-MS analyses, causing asymmetric peaks, loss of peptides, and increased variability in detector response for quantitative measurements. This could affect the sensitivity and reproducibility of MAM assays. In this study, we optimize a MAM analytical workflow for low level CQA peptides using an LC fluidic pathway engineered with MaxPeak High Performance Surface (HPS) technology, that is inert towards metal-sensitive peptides. MAM data generated from HPS system and conventional stainless steel LC system are compared here.

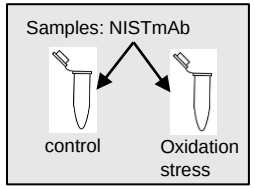
Experimental

BioAccord™ System with ACQUITY™ Premier System

System 1	UPLC/TOFMS with conventional stainless steel fluidic path and column
System 2	UPLC/TOFMS with HPS technology

Mobile phase A: H_2O, 0.1% FA, B: Acetonitrile, 0.1% FA

Time (min)	Flow (mL/min)	%A	%B	Curve
0.00	0.200	99.0	1.0	initial
3.00	0.200	99.0	1.0	6
78.00	0.200	65.0	35.0	6
85.70	0.200	15.0	85.0	6
93.00	0.200	15.0	85.0	6
100.70	0.200	99.0	1.0	6
120.00	0.200	99.0	1.0	6



MS System:	ACQUITY RDa Detector
Ionization Mode:	ESI+
Acquisition Range:	m/z 50- 2000
Capillary Voltage:	1.2 kV
Collision Energy:	60-120 V
Cone Voltage:	20 V
Column(s):	ACQUITY UPLC™ Peptide CSH C18 column (p/n 186006938) ACQUITY Premier Peptide CSH C18 column (p/n 186009489)

Results

Improved recovery of acidic peptide attributes

Recovery Comparison of HC:T37 peptide : GFYPSDIAVEWESNGQPENNYK and deamidated forms

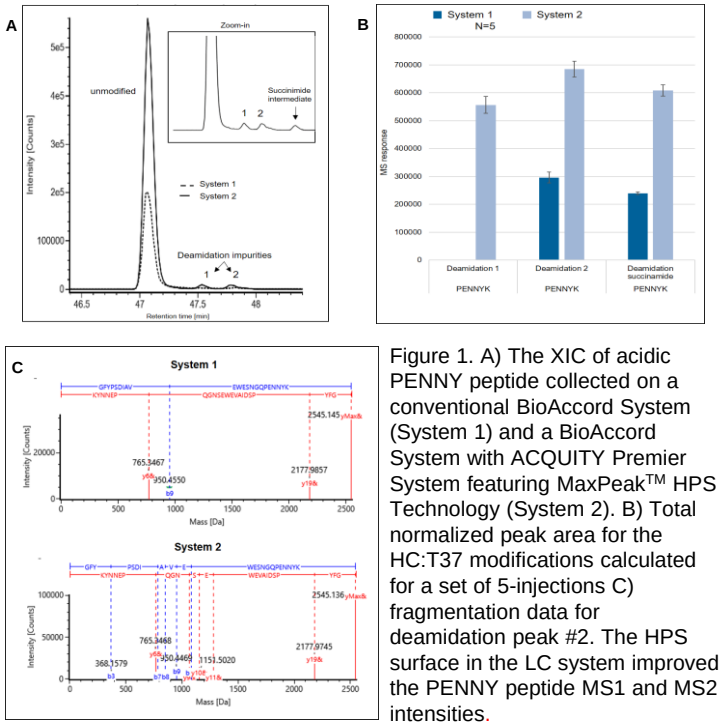


Figure 1. A) The XIC of acidic PENNY peptide collected on a conventional BioAccord System (System 1) and a BioAccord System with ACQUITY Premier System featuring MaxPeak™ HPS Technology (System 2). B) Total normalized peak area for the HC:T37 modifications calculated for a set of 5-injections C) fragmentation data for deamidation peak #2. The HPS surface in the LC system improved the PENNY peptide MS1 and MS2 intensities.

Improved RSD% measurement for CQA peptides

Table 1: %modification and %RSD levels for selected acidic CQA's of NISTmAb. Data shows comparable %modification and lower %RSD on System 2 (in blue).

Peptide sequence	Modification	%modification System 1	%Modification System 2	%RSD System 1	%RSD System 2
EEQYNSTYR	G0F	43.81	43.81	0.27	0.46
EEQYNSTYR	G1F	41.51	41.65	0.44	0.31
EEQYNSTYR	G2F	8.17	7.65	0.57	0.93
EEQYNSTYR	G0F-GlcNAc	2.36	2.47	2.68	1.41
EEQYNSTYR	G1F-GlcNAc	2.56	2.85	1.25	1.64
EEQYNSTYR	Man5	0.91	0.998	3.06	1.88
EEQYNSTYR		0.68	0.564	4.81	2.41
PENNYK	Deamidation 1	2.10	1.71	7.40	2.81
PENNYK	Deamidation 2	-	2.10	-	1.33
PENNYK	Deamidation succinimide	1.99	1.87	2.68	0.89
VTNMDPADTATYYCAR	Oxidation	0.46	0.67	7.45	2.40
VVSVLTVLHQDWLNGK		95.88	96.55	0.04	0.06
VVSVLTVLHQDWLNGK	Deamidation 1	0.92	0.92	3.44	1.48
VVSVLTVLHQDWLNGK	Deamidation succinimide	2.74	2.53	2.15	1.94

Improved %base peak response in New Peak Detection

Table 2:
%base peak values of DMIFNFYFDVWGQGTTV TVSSASTK oxidation [M] with System 1 &2

System	%base peak
System 1	1.24%
System 2	2.70%

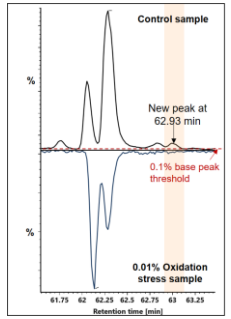


Figure 2. %base peak value is one of the criteria (thresholds) used in new peak detection. It is calculated relative to the intensity of the most abundant peptide of the chromatogram. The System 2 shows high %base peak values due to improved recovery of peptides. An example peak eluting at 62.89 min (DMIFNFYFDVWGQGTTVTVSSAS TK) in stressed sample of NISTmab is shown here

Conclusions

The study demonstrated that the MaxPeak HPS technology minimizes adsorption of metal sensitive analytes, enabling robust method execution with improved recovery, assay sensitivity, and method reproducibility. In summary, the BioAccord System with ACQUITY Premier System represents a robust and flexible LC-MS platform to develop Multi-Attribute-Method, that has the potential to be deployed across development, manufacturing, and quality organizations.