MONITORING PRODUCT AND PROCESS ATTRIBUTES IN BIOPHARMACEUTICAL DEVELOPMENT AND QC

Robert Birdsall, Jing Fang, Brooke Koshel, Min Du, Joe Fredette, and Ying Qing Yu
Waters Corporation, Milford, MA, United States

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INTRODUCTION

The proposal of "Multi-Attribute Method (MAM)" based LCMS peptide mapping methods for semi-targeted monitoring of biotherapeutic protein attributes has the potential to reduce manufacturing costs and bring products to market faster.

Currently, MAM-based approaches are being evaluated for their appropriateness to streamline characterization processes as industry moves towards continuous manufacturing strategies as well as their ability to transfer to QC/lot release roles.

Deploying and operating High-Resolution-MS (HRMS) methodologies in regulated environments must be weighed against more routine nominal mass detection approaches that may require more rigorous evaluation in establishing their suitability for MAM based analyses.

In this study, we have generated a common set of Trastuzumab forced degradation samples, subjected to various levels of oxidative and high pH stress to enable data-based discussions of fit-for-purpose MS for peptide map based attribute monitoring in regulated environments.

METHODS

Sample Preparation:
Trastuzumab samples were treated with alkaline and oxidation stress, followed by denaturation, alkylation and tryptic digestion.

Control	No treatment		
Alkaline Stress	pH 9.0, 37 °C, Time		
	2d	4d	7d
Oxidation stress	H_2O_2% (v/v), 24 hr @ RT		
	0.003%	0.01%	0.015%

LC System: ACQUTIY UPLC H-Class Bio System
Column: ACQUITY UPLC CSH300 C18, 1.7 µm, 2.1 x 100 mm
Column temperature: 65 °C

Mobile phase:
A. 0.1% FA in H_2O
B. 0.1% FA in MeCN

Gradient: 3-33 %B over 120 min
TUV Detection: 215 nm

Characterizing Product Attributes using High Resolution Accurate Mass

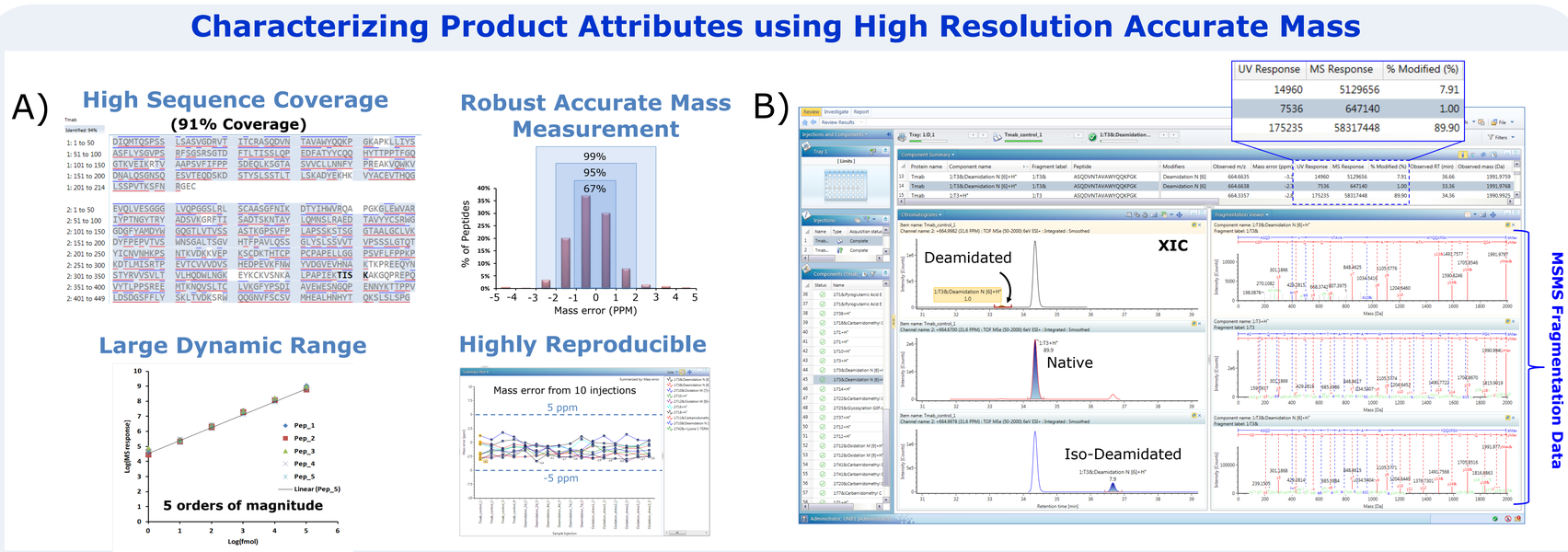


Figure 1: A) The Vion QTof enables robust accurate mass measurement of peptides with high sensitivity over a large dynamic range with high sequence coverage. B) A MS^E peptide mapping workflow in conjuction with the high MSMS fragmentation efficiency of the Vion was used to define product attributes of the trastuzumab reference sample, confirm protein identity/sequence, and determine product modification variants for confident assignment of peptides and their post-translational and chemical modifications.

Building an Accurate Mass Based Scientific Library for Multi-Attribute Monitoring

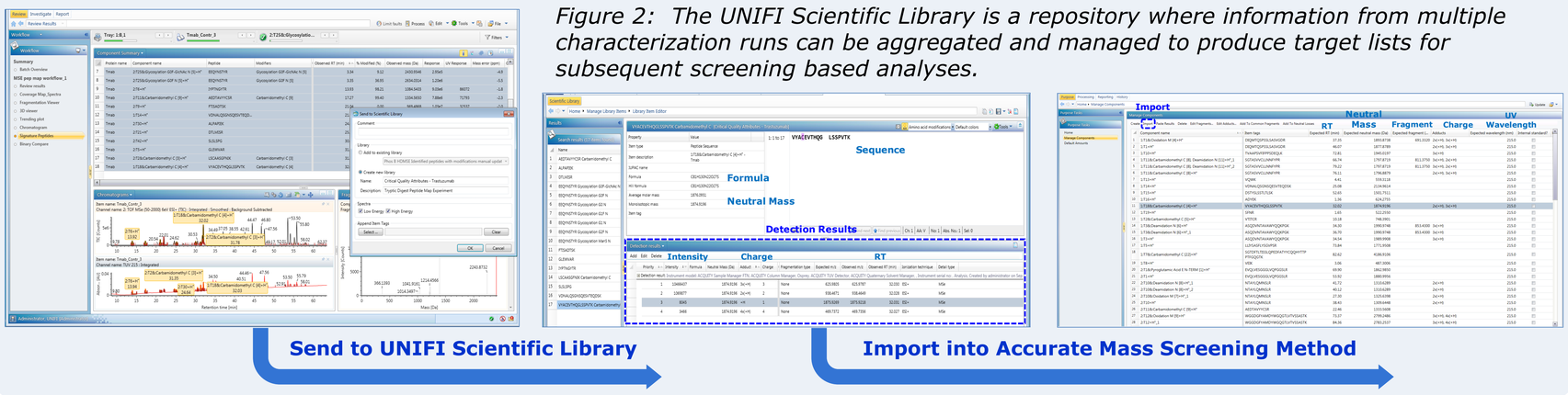


Figure 2: The UNIFI Scientific Library is a repository where information from multiple characterization runs can be aggregated and managed to produce target lists for subsequent screening based analyses.

RESULTS AND DISCUSSION

Monitoring Product Attributes with a HRMS Accurate Mass Workflow

Accurate Mass System:
Vion & Xevo QTof MS
Data Acquisition: MS^E
Capillary Voltage: 3.0 kV
Cone Voltage: 30 V
Source Temperature: 100 °C
Desolvation Temperature: 250 °C
Mass Range (*m/z*): 100-2000

Lock mass used:
LeuEnk ($[M+2H]^{2+}$, 556.2763)
MS^E settings:
Low/high Energy Scan rate: 0.5 sec
Low energy: 6 V
High energy ramp: 20-45 V

Deamidated Peptide Monitoring

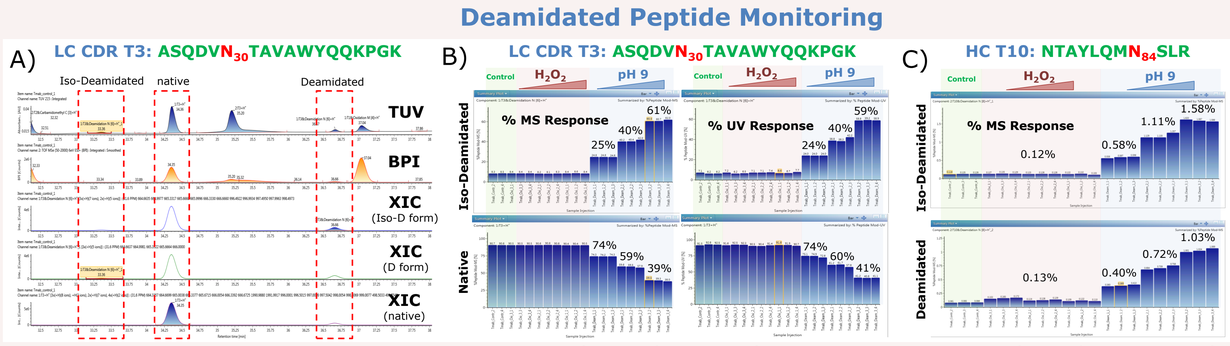


Figure 3: Monitoring variation in asparagine deamidation. A) Reviewing processed and integrated results in UNIFI can be obtained for optical and MS data channels, including XICs for individual components. B) Summary plots demonstrate relative % abundance of UV and MS response. Robust quantification was also obtained for peptides less susceptible to alkaline stress.

Glycopeptide Profiles Monitoring (HC T25: EEQYN300STYR)

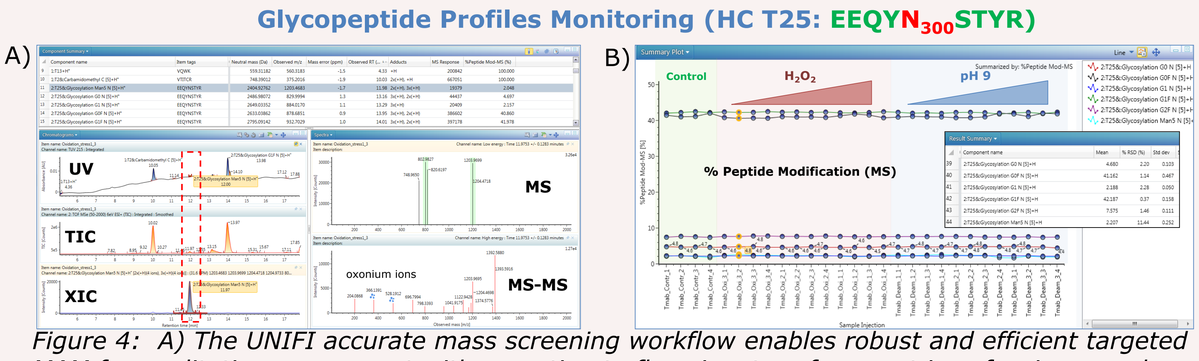


Figure 4: A) The UNIFI accurate mass screening workflow enables robust and efficient targeted MAM for qualitative assessment with an option to flag signature fragment ions for increased confidence in assignments B) across large multi-batch data sets.

Setting Limits and System Suitability

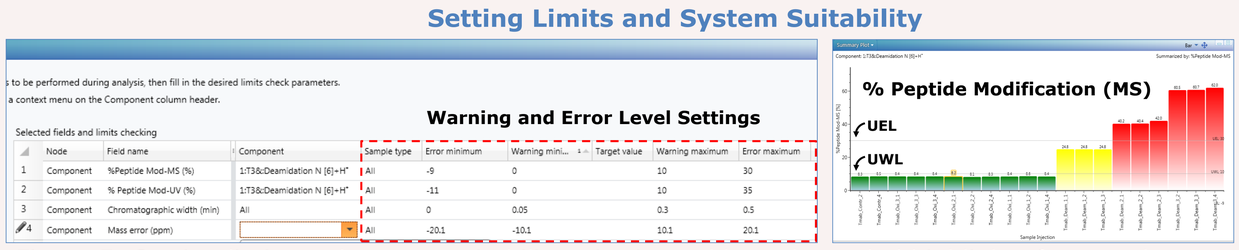


Figure 5: Setting limits and system suitability criteria enables color coded highlights for samples or batches that exceed data quality criteria or breach expected limits for component ranges.

Using the UNIFI/HRMS platform, characterization and monitoring data can be acquired using a single acquisition methodology (UPLC/UV/MSE), but with different informatics processing workflows optimized for each analysis.

- ✓ A compliant-ready UNIFI platform
- ✓ Robust Quantification (UV and MS)
- ✓ New/differential peak detection
- ✓ Ability to update the scientific library

Monitoring Product Attributes with a Nominal Mass Workflow

Nominal Mass System:
ACQUITY QDa Mass Detector
Sample Rate: 2points/sec
Capillary Voltage: 1.5 kV
Cone Voltage: 15 V
Probe Temperature: 500 °C
Mass Range (*m/z*): 350 – 1250

Fragment	Average Mass	$[CH+1H]^{+1}$	$[CH+2H]^{+2}$	$[CH+3H]^{+3}$	$[CH+4H]^{+4}$	$[CH+5H]^{+5}$	$[CH+6H]^{+6}$	$[CH+7H]^{+7}$	$[CH+8H]^{+8}$	$[CH+9H]^{+9}$	$[CH+10H]^{+10}$
T39	574.3	575.3	288.2	192.4	144.6	115.9	96.7	83.0	72.8	64.8	58.4
T7	681.3	682.3	341.7	228.1	171.3	137.3	114.6	98.3	86.2	76.7	69.1
T5	830.0	831.0	416.0	277.7	208.5	167.0	139.3	119.6	104.7	93.2	84.0
T21	835.0	836.0	418.5	279.3	209.7						
T30	838.0	839.0	420.0	280.3	210.5						
T9	969.1	970.1	485.5	324.0	243.3						
T6	1084.2	1085.2	543.1	362.4	272.1						
T3	1089.2	1090.2	545.6	364.1	273.3						
T36*	1161.4	1162.4	581.7	388.1	291.3						
T2*	1167.4	1168.4	584.7	390.1	292.8						
T8-9	1182.3	1183.3	592.2	395.1	296.6						
T13	1186.4	1187.4	594.2	396.5	297.6	238.3	198.7	170.5	149.3	132.8	119.6
T10	1310.5	1311.5	656.3	437.8	328.6	263.1	219.4	188.2	164.8	146.6	132.1
T4-5	1311.5	1312.5	656.8	438.2	328.9	263.3	219.6	188.4	164.9	146.7	132.2
T14*	1321.5	1322.5	661.8	441.5	331.4	265.3	221.3	189.8	166.2	147.8	133.2
T11*	1334.4	1335.4	668.2	445.8	334.6	267.0	223.4	191.6	167.8	149.3	134.4
T23	1677.8	1678.8	839.9	560.3	420.5	336.6	280.6	240.7	210.7	187.4	168.8
T33-34	1724.9	1725.9	863.5	576.0	432.2	346.0	288.5	247.4	216.6	192.7	173.5
T26	1808.1	1809.1	905.1	603.7	453.0	362.6	302.4	259.3	227.0	201.9	181.8
T38	1874.1	1875.1	938.0	625.7	469.5	375.8	313.3	268.7	235.3	209.2	188.4
T1	1882.1	1883.1	942.1	628.4	471.5	377.4	314.7	269.9	236.3	210.1	189.2
T22*	2139.4	2140.4	1070.7	714.1	535.8	428.9	357.6	306.6	268.4	238.7	214.9
T26-27	2228.6	2229.6	1115.3	743.9	558.1	446.7	372.4	319.4	279.6	248.6	223.9
T2-3*	2238.6	2239.6	1120.3	747.2	560.6	448.7	374.1	320.8	280.8	249.7	224.9
T37	2544.7	2545.7	1273.3	849.2	637.2	509.9	425.1	364.5	319.1	283.7	255.5
T12	2785.0	2786.0	1393.5	929.3	697.3	558.0	465.2	398.9	349.1	310.4	279.5
T41*	2802.1	2803.1	1402.1	935.0	701.5	561.4	468.0	401.3	351.3	312.3	281.2
T19-20*	3335.9	3336.9	1669.0	1113.0	835.0	668.2	557.0	477.6	418.0	371.7	334.6
T15*	6716.5	6717.5	3359.2	2239.8	1680.1	1344.3	1120.4	960.5	840.6	747.3	672.6
T15-16*	7058.9	7059.9	3530.4	2354.0	1765.7	1412.8	1177.5	1009.4	883.4	785.3	706.9
T15-17*	7187.0	7188.0	3594.5	2396.7	1797.8	1438.4	1198.8	1027.7	899.4	799.6	719.7

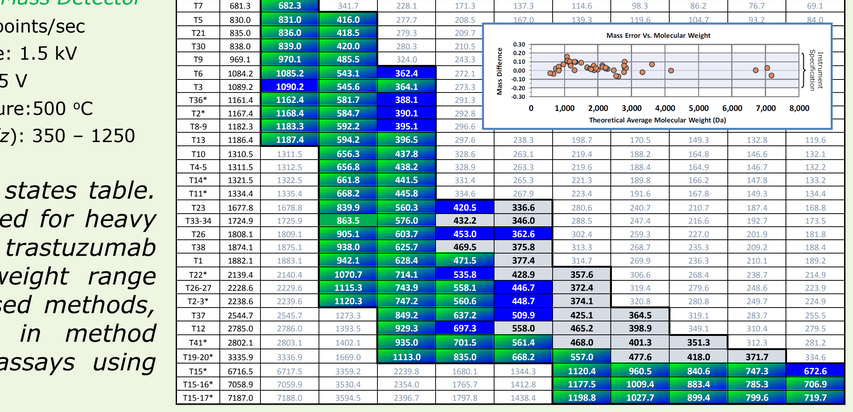


Table 1: Peptide map charge states table. Multiple charge states observed for heavy chain tryptic peptides of trastuzumab across a broad molecular weight range (inset) using TFA and FA based methods, affords significant flexibility in method development of monitoring assays using the ACQUITY QDa.

Monitoring LC: CDR T3 Deamidation

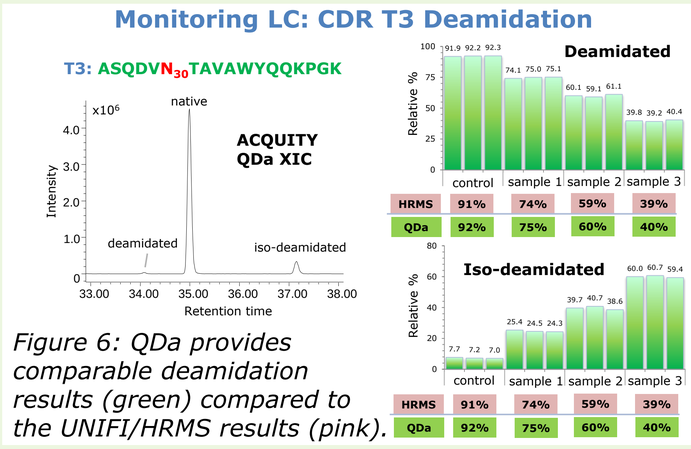
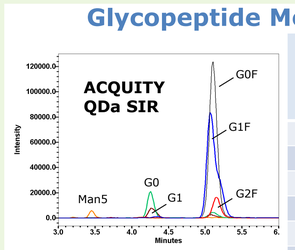


Figure 6: QDa provides comparable deamidation results (green) compared to the UNIFI/HRMS results (pink).

Glycopeptide Monitoring



	Modification (%)	
	HRMS	QDa + SIRs
G0	4.7	6.0
G0F	41.2	44.9
G2F	7.6	6.2
G1	2.2	2.7
G1F	42.2	38.7
Man5	2.2	1.4

Figure 7: Glycopeptide profiles are obtained using the MS SIR scan for enhanced sensitivity to monitor co-eluted peptides. Relative modification results using QDa were comparable to UNIFI/HRMS results.

CDR Peptide Monitoring using ACQUITY QDa

Light Chain
1: **ASQDVNTAVA**WYQQKPGK
2: LLIY**SASFLYS**GVPSR
3: SGTDFTLTISSLQPEDFA
TYYC**QQHYTTPPT**FGQGTK

Heavy Chain
4: **DTYIH**WVR
5: **IYPTNGYTR**/(6)**YADSVKG**
7: **WGGDGFYAMDY**WGQGTLV
TVSSASTK

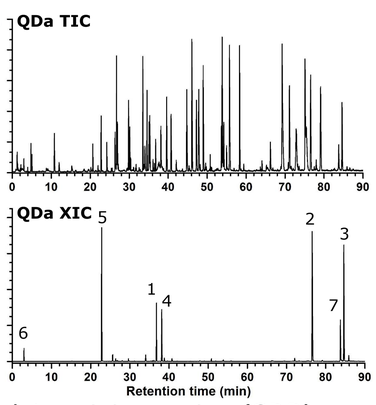


Figure 8: Complementary determining region (CDR) peptide profiles are effectively extracted from the MS full scan TIC for the rapid determination of product identity.

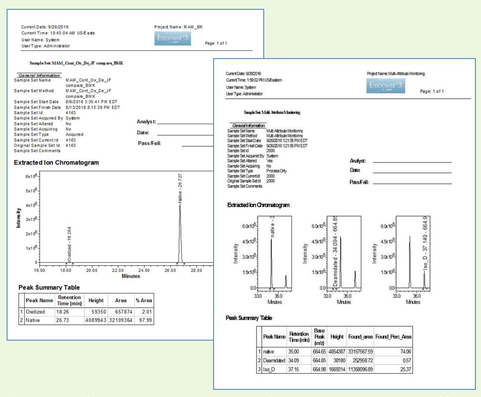


Figure 9: Reports can be automatically generated when linked to the method, automating the monitoring process in a regulated environment.

The AQUITY QDa mass detector can be readily added to existing Empower/ACQUITY UPLC/UV systems for expanded dynamic range in attribute monitoring over optical only assays

- ✓ Compliant-ready Empower platform
- ✓ Accessible technology
- ✓ Small footprint, easier deployment

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