Monitoring Quality Attributes of Biotherapeutic Products Using a Mass Spectrometry Based Analytical Platform

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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 been greeted with both excitement regarding reducing the dependence on a cadre of low information content assays, and concerns over their appropriateness for deployment into regulated development and QC/lot release roles. The selectivity of High-Resolution-MS (HRMS) methodologies must be weighed against the challenges of deploying and operating these complex systems in regulated environments, and more established and routine nominal mass detection approaches require more rigorous evaluation for establishing the extent to which they can be applied to MAM based analysis.

In this study, we have generated a common set of Trastuzumab forced degradation samples, subjected to various levels of oxidative and high pH stress. Characterization results from the reference samples were parsed to select peptides for targeted monitoring of product attributes using both HRMS and nominal mass detection strategies. Results from these studies have been compiled to enable data-based discussions of fit-for-purpose MS for implementing peptide map based attribute monitoring in regulated environments.

UPLC/High Resolution MS:
Biopharmaceutical Platform Solution with UNIFI

UPLC/Mass Detection:
ACQUITY UPLC, TUV, QDa Mass Detection with Empower CDS

Figure 1: Two compliant-ready LC/UV/MS solutions for flexible deploying MS in Biopharmaceutical late development and QC laboratories.

METHODS

Sample Preparation:

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

LC/MS:

LC System: ACQUTIY UPLC H-Class Bio System
Column: ACQUITY UPLC CSH C_{18}, 1.7 µm, 2.1 mm x 100 mm
Column temperature: 65 °C
Mobile phase: A. 0.1% FA in water,
B. 0.1% FA in acetonitrile
Gradient: 3-33 %B over 120 min
TUV Detection: 215 nm

HRMS System: *Vion IMS QTof MS*
Data Acquisition: MS^E Mode: ESI positive mode
Capillary Voltage: 3.0 kV Cone Voltage: 30 V
Source Temperature:100 °C
Desolvation Temperature: 250 °C

Nominal Mass System: ACQUITY *QDa Mass Detector*
Sample Rate: 2points/sec Mode: ESI positive mode
Capillary Voltage: 1.5 kV Cone Voltage: 15 V
Probe Temperature:500 °C Desolvation Temperature: 250 °C
Mass Range (*m/z*): 350 – 1250

Informatics:

- UNIFI Scientific Information System v1.8 Service Release 2
- Empower 3 Chromatography Data Software

CONCLUSION

- **Using UNIFI/HRMS platform, product attribute characterization and monitoring data can be acquired using a standardized mapping data acquisition methodology (UPLC/UV/MSE), but different informatics processing workflows optimized for each analysis. This enables a common platform for both analyses, and efficient transfer of analytical platforms and methods between groups responsible for their execution.**
- **The AQUITY QDa mass detector can be easily added to existing Empower/ACQUITY UPLC/UV systems, with minimal maintenance and training requirements for analysts.**

	HRMS Xevo G2-XS/Vion IMS QTof	Mass Detection ACQUITY QDa
Mass Accuracy	< 5 PPM	< 0.5 Dalton
Dynamic Range	4+ Orders	3+ Orders
Typical Sample Loading	ng~µg	Low µg
Fragment Ion Confirmation	✓	N/A
New Peak Detection	✓	N/A
Costs (Capital & Operation)	$$$$	$
Required Expertise	MS Analyst	Chromatographer
Regulatory Compliance	✓	✓

RESULTS AND DISCUSSION

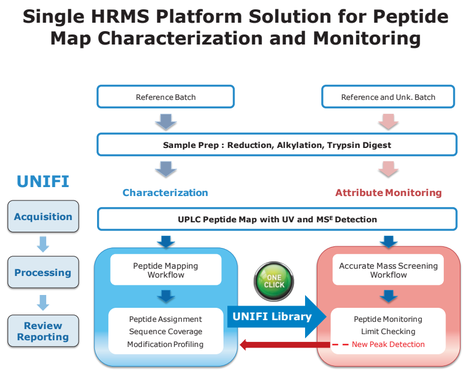


Figure 2: Transitioning from characterization to attribute monitoring workflows within the UNIFI Platform Solution.

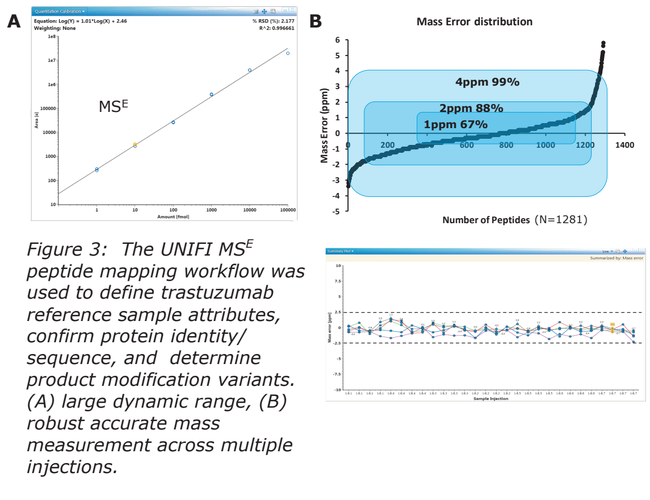


Figure 3: The UNIFI MS^E peptide mapping workflow was used to define trastuzumab reference sample attributes, confirm protein identity/ sequence, and determine product modification variants. (A) large dynamic range, (B) robust accurate mass measurement across multiple injections.

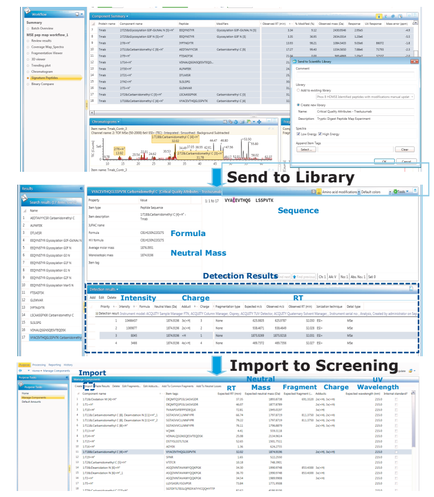
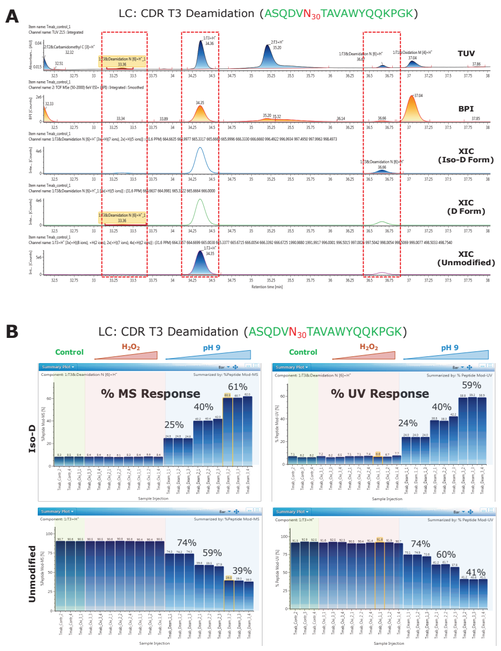


Figure 4: The UNIFI Scientific Library is a repository where information from multiple characterization runs can be aggregated and managed to produce target lists for subsequent screening based analyses.

Monitoring Product Attributes using the UNIFI Accurate Mass Screening Workflow



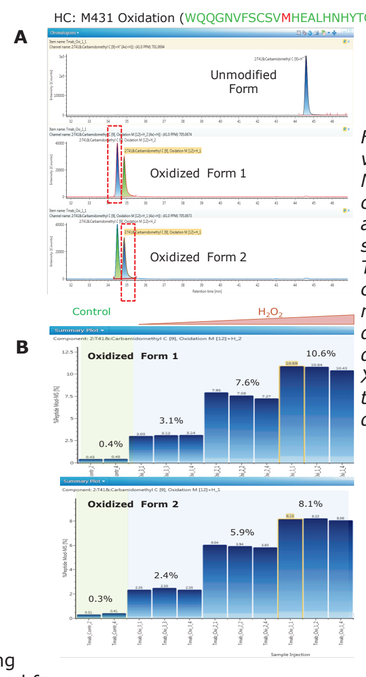


Figure 6: Monitoring variation in Methionine oxidation levels across control and stressed samples. The oxidized heavy chain peptide T41 is monitored and quantified as diastereomers.(A) XIC (B) mod% of two oxidation diastereomers.

Figure 8: Monitoring Asn deamidaton level. (A) Reviewing processed and integrated results in UNIFI can be obtained for optical and MS data channels, including XIC for each targeted component. (B) Summary plots demonstrate relative % abundance of UV and MS response.

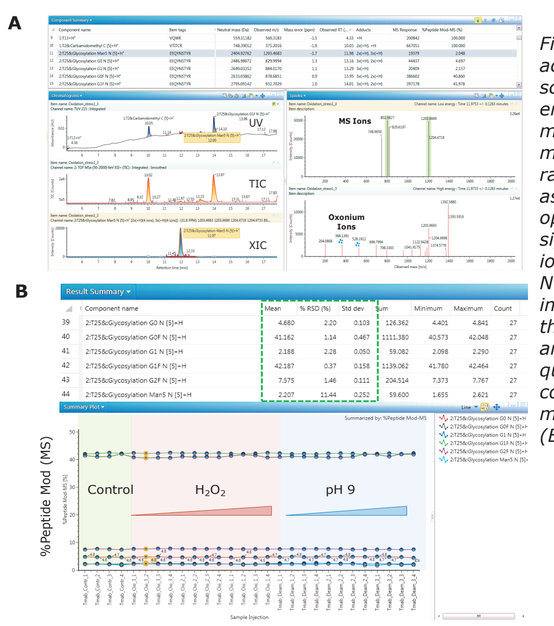


Figure 7: The UNIFI accurate mass screening workflow enabled targeted multiattribute monitoring, providing rapid qualitative assessment with an option to flag signature fragment ions (oxonium ions for N-Glycopeptides) to improve confidence in the assignments (A) and for robust quantification and comparison across multi-batch data sets (B).

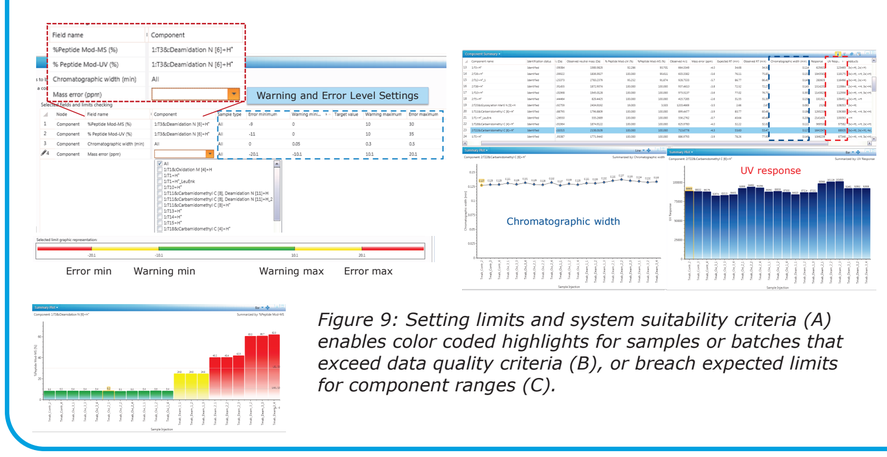


Figure 9: Setting limits and system suitability criteria (A) enables color coded highlights for samples or batches that exceed data quality criteria (B), or breach expected limits for component ranges (C).

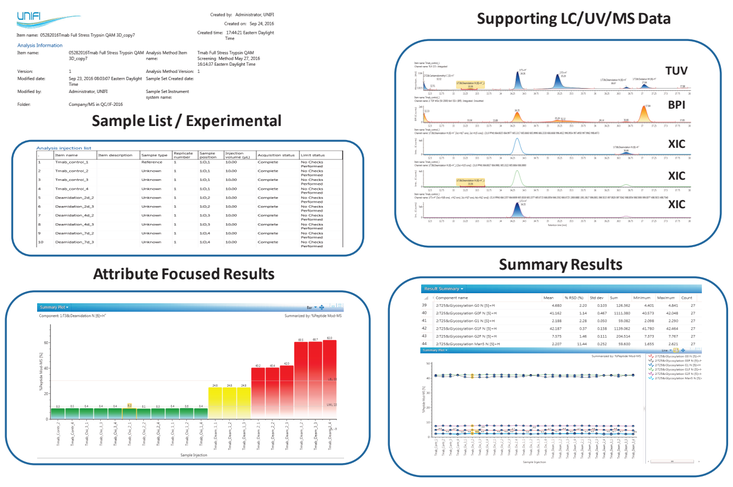


Figure 10: Analysts can report key product attributes in customized reports in UNIFI. Chapters can be created inside a report to focus on a particular attribute.

Monitoring Product Attributes using the Empower/QDa Platform

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	168.0	140.2	120.3	105.4	93.8	84.5
T30	838.0	**839.0**	**420.0**	280.3	210.5	168.6	140.7	120.7	105.8	94.1	84.8
T9	969.1	**970.1**	**485.5**	324.0	243.3	194.8	162.5	139.4	122.1	108.7	97.9
T6	1084.2	**1085.2**	**543.1**	**362.4**	272.1	217.8	181.7	155.9	136.5	121.5	109.4
T3	1089.2	**1090.2**	**545.6**	**364.1**	273.3	218.8	182.5	156.6	137.2	122.0	109.9
T36*	1161.4	**1162.4**	**581.7**	**388.1**	291.3	233.3	194.6	166.9	146.2	130.0	117.1
T2*	1167.4	**1168.4**	**584.7**	**390.1**	292.8	234.5	195.6	167.8	146.9	130.7	117.7
T8-9	1182.3	**1183.3**	**592.2**	**395.1**	296.6	237.5	198.1	169.9	148.8	132.4	119.2
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	267.9	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**	313.1	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	223.8
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**	349.3	310.4	279.5
T41*	2802.1	2803.1	1402.1	**935.0**	**701.5**	**561.4**	**468.0**	**401.3**	**351.3**	312.1	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 using TFA and FA based methods, affords significant flexibility in method development of monitoring assays using the ACQUITY QDa.

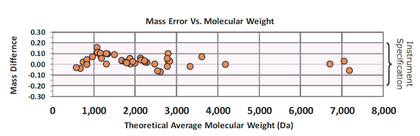
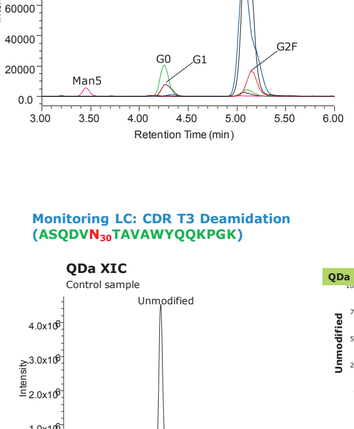


Figure 11: Peptide mass accuracy. The ACQUITY QDa is capable of providing mass information for peptides over a broad molecular weight range in assays routinely employed during the analysis of biotherapeutics.



Component	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 12: Glycopeptide profiles are obtained using the MS SIR scan for enhanced sensitivity to monitor co-eluted peptides with improved quantification robustness. Quantification using QDa with SIR scan provide compatible glycopeptide profiles vs UNIFI/HRMS results.

Figure 13: Quantification using the ACQUITY QDa with MS full scan provide compatible deamidation profiles (Green) of the CDR domain peptide T3, compared to the UNIFI/ HRMS results (Pink).

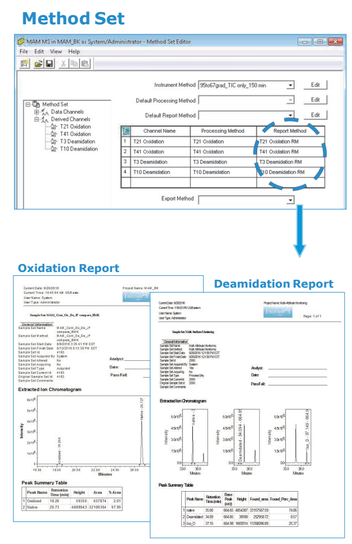


Figure 14: Reports can be automatically generated when linked to the acquisition through the method set, automating the monitoring process in a regulated environment.

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