POSTER
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Simplifying workflows for MAM-based critical quality attribute monitoring of biotherapeutics in process development and QC using a novel LCMS platform

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PURPOSE

- Biotherapeutics undergo rigorous characterization and attribute monitoring during development/manufacturing to establish/maintain product quality and safety attributes (PQAs).
- Peptide-based Multi-Attribute Methods (MAM) that utilize LC-HRMS technology to multiplex monitoring of PQAs are now being implemented to reduce analytical testing and increase productivity.
- Developing GXP-friendly workflows that can identify PQAs, monitor known CQAs, identify newly emerging peaks and those that change in intensity, and yet be easily deployed across an organization continues to be a challenge.

OBJECTIVE

Here, we demonstrate a workflow-driven compact LC-TOF MS platform for PQA assignment, monitoring, and new peak detection using a Trastuzumab sample.

METHODS

Sample Preparation:
Trastuzumab (Genentech, USA) was subjected to pH, heat and oxidative stress conditions (Table).

Protein digestion: Reduced and alkylated mAb was trypsin digested (Promega, Madison, USA) for 4 h at 37˚C at a 20:1 ratio protein to enzyme. The samples were acidified and diluted prior to LC-MS analysis.

pH 9.0, at 37ºC			
1 day	2 days	4 days	6 days
Heat, 37ºC			
4 days		6 days	
H_2O_2, room temperature, 1 day			
0.005%		0.05%	

BioAccord System LCMS Conditions:

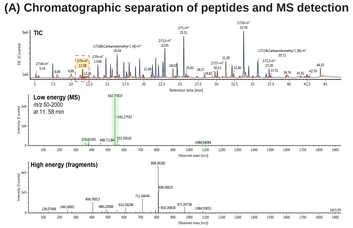
❖ Waters ACQUITY I-Class Plus UPLC System
- Column: ACQUITY UPLC CSH C18, 1.7 µm, 2.1 x 100 mm, 60 ˚C
- Mobile phase: (A) 0.1% FA, (B) 0.1% in Acetonitrile, 0.2 mL/min
- Cycle Time: 80 min (gradient 1 to 35 %B in 51 min)
- Autosampler 6 ˚C , 5 µL Injection

❖ Waters RDa TOF MS Detection
- ESI+, *m/z* 50-2000, MS with fragmentation mode , IDC on,
- Cone: 30 V, Desolvation Capillary: 350 ˚C , Collision energy: 60-120 V

❖ Informatics: Waters UNIFI Scientific Information System v1.9.4
- Peptide mapping workflow for characterization
- UNIFI scientific library for attribute library storage
- Accurate mass screening workflow for attribute monitoring

Peptide Mapping Method (Characterization)

(A) Chromatographic separation of peptides and MS detection



(B) Bioinformatic search identifies peptides and their modifications

Component Summary

	Protein name	Fragment label	Peptide	Modifiers	Observed RT (min)	Observed m/z	Charge	Observed mass (Da)	Mass error (ppm)	Response	Matched 1st Gen Primary Ions
29	TmAb	2?9	FTDLDQTSK		13.67	485.2478	2	969.4883	-0.5	14246659	9
30	TmAb	2?37	GPVPSDIAVEWESNGQPENVYK		30.11	848.7169	3	2544.1361	1.9	13891132	16
31	TmAb	2?5	GLEWVAR		17.97	415.7302	2	830.4531	1.4	16312430	9
32	TmAb	2?13	GPSVFPLAPSSK		22.86	593.8281	2	1186.6489	1.9	20501224	14
33	TmAb	2?6	IYPTNGYTR		11.57	542.7746	2	1084.5419	-0.3	5447878	9
34	TmAb	2?6b	IYPTNGYTR	Deamidation N [5]	12.84	543.2662	2	1085.5251	-1.0	1707066	9
35	TmAb	2?6b	IYPTNGYTR	Deamidation N [5]	13.21	543.2679	2	1085.5285	2.1	421322	5
36	TmAb	2?5	LDYSASFLYSDVPSR		34.42	591.6587	3	1772.9617	2.0	9212968	28
37	TmAb	2?2b	LSCAASGFNDK	Carbamidomethyl C [3]	17.24	584.2964	2	1167.5856	2.5	13685866	17
38	TmAb	2?3?	LTVDK		5.15	288.1736	2	575.3399	-0.1	3709279	3
39	TmAb	2?3?6b	NQVSLTCLVK	Carbamidomethyl C [7]	21.62	581.3192	2	1160.6311	1.3	20449848	10
**	TmAb	2?3?6b	NQVSLTCLVK	Carbamidomethyl C [7], Deamidation N [1]	24.34	581.8081	2	1162.6098	-4.1	254887	

ent ions

Custom attribute library
- Sequence & modifications
- Mass & RT & Charge States

(C) Sequence confirmation using fragment ions

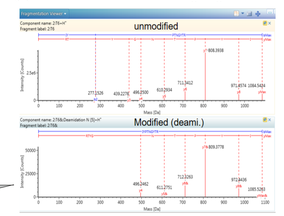


Figure 1: (A) Peptides and their modified variants were analyzed by RP-LCMS peptide mapping with fragmentation. A pH stressed sample is shown with a representative MS1 and fragmentation spectrum.

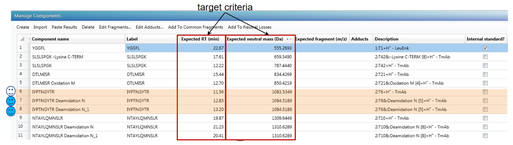
(B) Bioinformatics automatically assigns peptide spectra and modifications based on accurate mass with fragment Ion confirmation. This information can be used to populate an attribute library to search using a targeted screening (monitoring) workflow.

(C) Fragment ion spectra for native and a deamidated form of tryptic peptide IYPTNGYTR are displayed.

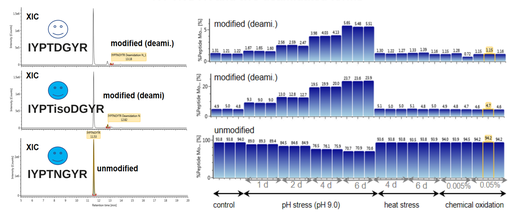
Peptide Attribute Monitoring

(A) Targeted peptide attribute list

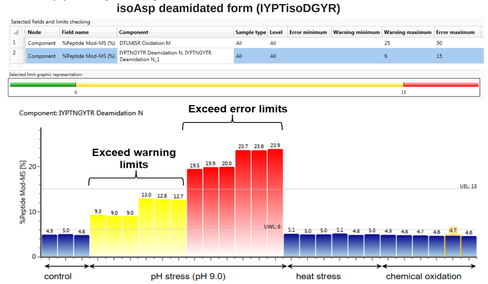
target criteria



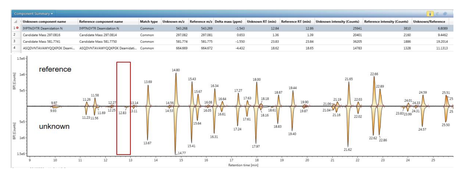
(B) Accurate mass screening-based detection and quantification of IYPTNGYR and two deamidated forms



(C) Setting warning and error limit checks for IYPTNGYR and its isoAsp deamidated form (IYPTisoDGYR)



(D) Detecting new/changed peaks that are not in the reference sample



(E) Using customized templates to generate attribute-centric reports

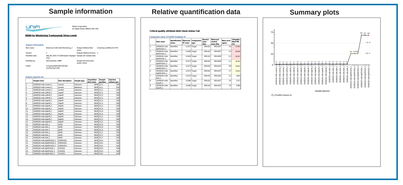


Figure 2: **(A)** The target peptide list is imported from the custom attribute library created using peptide mapping results. Each library entry contains peptide sequence, neutral mass and retention time information. **(B)** Quantitative data for each attribute is collected based on this targeting criteria from attribute monitoring runs. **(C)** Warning and Error Limits can be set for the quantified results and quality metrics for the collected attribute data. **(D)** "New Peaks" not seen in a reference sample, and peaks with 10x intensity differences, can be automatically flagged by the software for user review. **(E)** Sample Information, Results, and Results Summaries can be automatically generated using reporting templates designed to be attribute centric.

CONCLUSION(S)

❖ The BioAccord System, a novel LC-UV-TOFMS platform with integrated workflow-driven UNIFI informatics has been developed that simplifies deployment of peptide MAM-based analyses for the assessment and monitoring of CQAs within regulated and nonregulated environments.

❖ Testing using Trastuzumab forced degredation demonstrated the ability to define PQAs by peptide mapping, develop the targeted peptide PQA monitoring assay, and detect "new peaks" and significant intensity changes versus a reference sample.

❖ Practical deployment within manufacturing and quality organizations requires the ability to effectively communicate out of specification results and data quality issues. This has been demonstrated using color-based warning scheme and error flags in graphical and tabular review displays and reporting objects.

Data was acquired, processed, and reported on the **Waters BioAccord System**

A small footprint, benchtop LC-TOF MS system designed for high user accessibility, with compliance-ready workflow-driven UNIFI informatics for biopharmaceutical analysis.

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