A WORKFLOW DRIVEN PLATFORM SOLUTION FOR MAM-BASED CRTICAL QUALITY ATTRIBUTE MONITORING OF BIOTHERAPEUTICS IN PROCESS DEVELOPMENT AND QC

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INTRODUCTION

- Biotherapeutics undergo rigorous characterization and monitoring during development and production to maintain product quality and safety.
- Peptide-based monitoring is often used to monitor multiple attributes affecting the product quality.
- A compliance-ready, easy-to-use, high performance LC-MS system with automated methods is highly desired.
- The BioAccord is an SmartMS-enabled LC-MS system purposefully designed with integrated workflow methods for biopharmaceutical analyses including peptide-based quality attribute profiling and monitoring.

- Small foot print benchtop system
- Easy-to-use & maintain
- Workflow-driven
- cGMP compliance-ready

METHODS

Sample Preparation:

- The Trastuzumab (Genentech, USA) sample was subjected pH, heat and oxidative stress conditions as given below.
- Protein digestion: the intact mAb samples were digested with trypsin (Promega, Madison, USA) for 4 h at 37˚C (20:1 protein to enzyme) following reduction, alkylation and buffer exchange steps. The samples were acidified and diluted prior to LC-MS analysis.

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

LC-MS System: BioAccord System

- Column: ACQUITY UPLC CSH C18, 1.7 µm, 2.1 x 100 mm
- Column temperature: 60 ºC
- Mobile phase: A. 0.1% FA in water, B. 0.1% FA in Acetonitrile
- Total run Time: 80 min
- Sample temperature: 6 ºC
- Injection volume: 5 µL
- Flow rate: 0.2 mL/min
- Gradient: 1%-35% Acetonitrile + 0.1% formic acid over 52 min

- Ionization: ESI+
- Acquisition mode: MS with fragmentation
- Acquisition range: *m/z* 50-2000
- Capillary voltage: 1.2 kV

METHODS

- Cone voltage: 30 V
- Collision energy: 60-120 V
- Desolvation energy: 350 ºC
- Intelligent data capture: on

- Informatics: UNIFI Scientific Information v1.9.4
  - Peptide mapping method
  - Accurate mass screening method
  - UNIFI scientific library

End-to-end Workflow

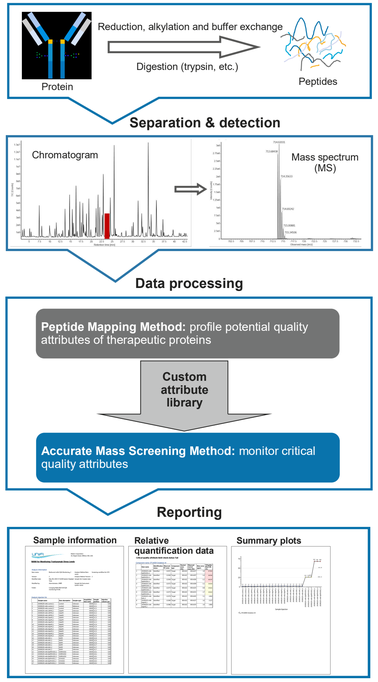
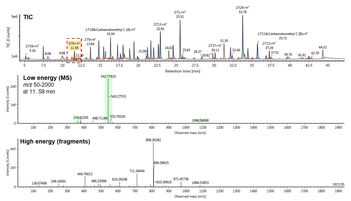


Figure 1: The end-to-end workflow for peptide attribute profiling and monitoring. The UNIFI processing methods are purposefully designed with automated data acquisition, processing and reporting capabilities.

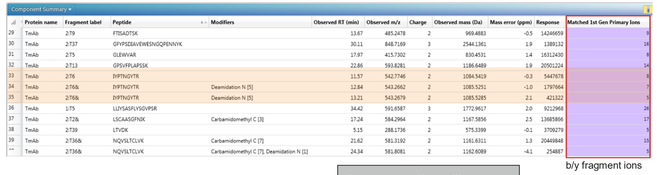
RESULTS

Peptide Mapping Method

(A) **Chromatographic separation of peptides and detection**



(B) **Identify peptides and their modifications**



	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	2:79	FTSSADTSK		13.67	485.2478	2	969.4883	-0.5	14246959	8
30	TmAb	2:737	GFYPSDIAVEWESNGQPENNYK		30.11	848.7169	3	2544.1361	1.9	1389132	16
31	TmAb	2:75	GLEWVAR		17.97	415.7302	2	830.4531	1.4	16312430	6
32	TmAb	2:713	GPSVFPLAPSSK		22.86	593.8281	2	1186.6489	1.9	20501224	14
33	TmAb	2:76	IYPTNGYTR		11.57	542.7746	2	1084.5419	-0.3	5447078	8
34	TmAb	2:76b	IYPTNGYTR	Deamidation N [5]	12.84	543.2962	2	1085.5251	-1.0	1787664	7
35	TmAb	2:76b	IYPTNGYTR	Deamidation N [5]	13.21	543.2679	2	1085.5285	2.1	421322	9
36	TmAb	1:75	LLDSADFLVSGVPSR		34.42	591.6587	3	1772.9617	2.0	9212968	29
37	TmAb	2:72b	LSCAASGFNIK	Carbamidomethyl C [3]	17.24	594.2964	2	1187.5856	2.5	13685866	17
38	TmAb	2:739	LTVDK		5.15	288.1758	2	575.3399	-0.1	3706279	5
39	TmAb	2:736b	NQVSLTCLVK	Carbamidomethyl C [7]	21.62	581.3192	2	1161.6311	1.3	20449848	15
40	TmAb	2:736b	NQVSLTCLVK	Carbamidomethyl C [7], Deamidation N [1]	24.34	581.8081	2	1162.6089	-4.1	254887	5

b/y fragment ions

Custom attribute library
- Sequence & modifications
- Mass & retention time
- Detected charge states, etc.

(C) **Sequence confirmation using fragment ions**

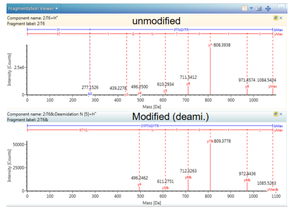
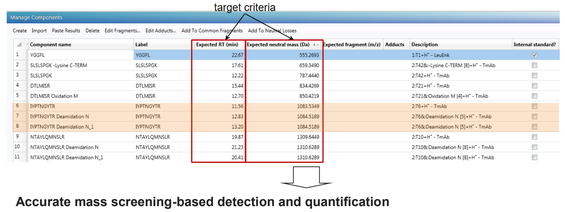


Figure 2: (A) The peptides and their modifications are chromatographically separated prior to MS analysis. This figure shows the total ion chromatogram of one of the pH stressed samples. (B) The component table provides all identified peptide sequences and modifications with their respective retention times, mass tolerance, *m/z*, number of primary fragment ions, etc. (C) The figure shows annotated fragment ion spectra for native and modified IYPTNGYTR. The fragmentation information is used in data filtering for high confidence peptide attribute identification.

Peptide Attribute Monitoring

(A) **Target peptide attribute list**

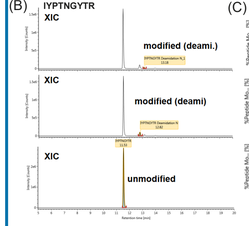
target criteria



	Component name	Label	Expected RT (min)	Expected neutral mass (Da)	Expected fragment (m/z)	Adducts	Description	Internal standard?
1	YGGFL	YGGFL	22.67	555.2693			1:T1+H+ - Leu/Ile	✓
2	SLSLSPGK -Lysine C-TERM	SLSLSPGK	17.61	659.3490			2:T42b--Lysine C-TERM [8]+H+ - Trn4b	
3	SLSLSPGK	SLSLSPGK	12.22	787.4440			2:T42+H+ - Trn4b	
4	DTLMISR	DTLMISR	15.44	834.4299			2:T22+H+ - Trn4b	
5	DTLMISR Oxidation M	DTLMISR	12.70	850.4219			2:T22b:Oxidation M [4]+H+ - Trn4b	
6	IYPTNGYTR	IYPTNGYTR	11.56	1083.5560			2:T6+H+ - Trn4b	
7	IYPTNGYTR Deamidation N	IYPTNGYTR	12.83	1084.5189			2:T6b:Deamidation N [5]+H+ - Trn4b	
8	IYPTNGYTR Deamidation N_1	IYPTNGYTR	13.20	1084.5189			2:T6b:Deamidation N [6]+H+ - Trn4b	
9	NTAVLQMNSLR	NTAVLQMNSLR	19.87	1309.6449			2:T10+H+ - Trn4b	
10	NTAVLQMNSLR Deamidation N	NTAVLQMNSLR	21.23	1310.6289			2:T10b:Deamidation N [8]+H+ - Trn4b	
11	NTAVLQMNSLR Deamidation N_1	NTAVLQMNSLR	20.41	1310.6289			2:T10b:Deamidation N [8]+H+ - Trn4b	

Accurate mass screening-based detection and quantification

(B)



(C)

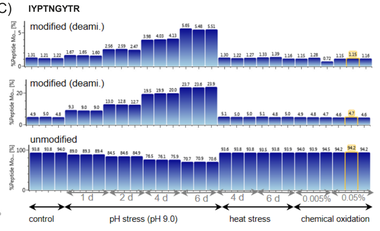
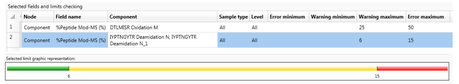


Figure 3: (A) The target peptide list is imported from custom attribute library created using peptide mapping results. Each library entry contains peptide sequence, neutral mass and retention time information (and fragment ions). Both neutral mass and retention time are used to identify peptide CQAs in the accurate mass screening method. (B) The XICs of target peptides (native and modified) are extracted for the monoisotopic masses or multiple isotopes. The peak area of each XIC is used to generate relative %modifications. (C) The summary plot shows native and deamidated IYPTNGYTR peptide %modification levels across samples. Each relative modification level is determined based on the total MS response for the respective peptide sequence.

Limit check capability

(A)



	Node	Field name	Component	Sample type	Level	Error minimum	Warning minimum	Warning maximum	Error maximum
1	Component	%Peptide Mod-MS (%)	DTLMISR Oxidation M	All	All		25	50	
2	Component	%Peptide Mod-MS (%)	IYPTNGYTR Deamidation N; IYPTNGYTR Deamidation N_1	All	All		6	15	

(B)

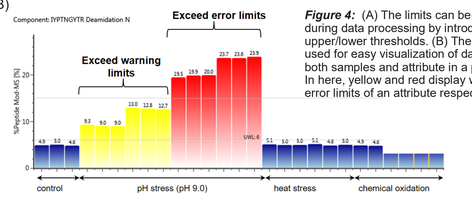


Figure 4: (A) The limits can be applied during data processing by introducing upper/lower thresholds. (B) The limits are used for easy visualization of data that flags both samples and attribute in a preset color. In here, yellow and red display warning and error limits of an attribute respectively.

New peak detection

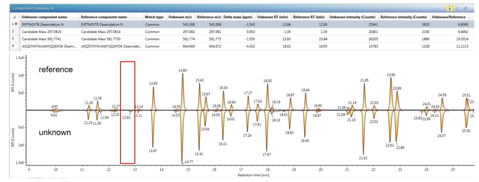


Figure 5: Comparison mode feature compares sample to its reference/control. This figure shows a chromatographic comparison of Trastuzumab control and a pH stressed sample. Specific data filters are applied to isolate unique peptide peaks or peptides present at a higher level (>5-fold) in the stress sample compared to the control.

Comparable quantification data obtained with and without fragmentation

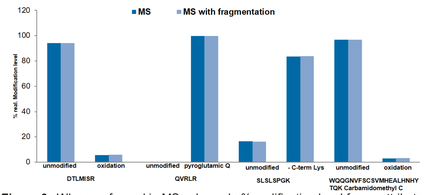


Figure 6: When performed in MS only mode %modification level for an attribute provides comparable results to that of the MS with fragmentation mode. This allows the user to chose either method in routine peptide attribute monitoring.

CONCLUSION

- The BioAccord is a compact LC-MS system that can be easily deployed across biopharmaceutical organizations from discovery thru QC.
- Integrated workflows (UNIFI Informatics) for intact protein, released-glycan and peptide analysis support automated attribute screening and monitoring workflows.
- Accessibility to non MS experts is facilitated by one-button startup, intelligent diagnostics, and automated tuning/calibration routines.
- Information transfer between peptide characterization and monitoring workflows is enabled by the UNIFI scientific library functionality, allowing lists of attributes to be incrementally updated for later use in targeted monitoring analyses.
- Automated data acquisition, processing, review, reporting, and signoff streamlines operations within regulated laboratories where data integrity is a concern.
- The ability to automatically highlight product attributes exceeding warning or error limits and denote new data features (potential attributes) vs. a reference sample greatly simplifies review and reporting of multiple attribute monitoring (MAM) studies.

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