Automated Rapid Antibody screening with a UHR ESI QTOF Mass Spectrometer and BioPharma Compass

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Introduction

- A comprehensive characterization of therapeutic mAbs is critical. PTMs including oxidation, deamidation, glycosylation may significantly alter the efficacy and safety of the protein drug.
- Regulatory agencies require a full characterization of the biological and chemical heterogeneity of any biological drug product.
- This work focuses on the utility of the maXis II UHR–QTOF for rapid screening of mAbs at the intact protein and peptide level for sequence characterization and MAM analysis.
- The automated workflow is implemented using BioPharma Compass software which encompasses data acquisition, processing and reporting using a seamless workflow solution.

Materials and Methods

- A 96-well plate of reduced mAb samples from eight different monoclonal antibodies and tryptic digests of two proteins were provided by The Institute for Protein Innovation (Boston, MA).
- The workflow included the maXis II UHR-QTOF connected to the Elute HT (Bruker Daltonics, Billerica, MA) equipped with a PAL3 autosampler (CTC Analytics, Switzerland). Data acquisition and processing was done with BioPharma Compass software (Bruker Daltonics, Billerica, MA).

Reduced mAb characterization

During IgG production, cells were co-transfected with two vectors; one expressing a heavy chain and the other a light chain. Errors in the process could result in discrepancies between the observed and expected mass. Assaying the reduced mAbs allowed quick determination of the mass of the light and heavy chains that co-elute during the fast 1.2 min LC runs.

The high resolving power of the maXis II enabled isotopic resolution of the reduced mAb heavy and light chain while maintaining the true isotopic pattern™ (TIP) under fast HPLC conditions. This allowed protein modifications to be easily determined and accurately quantified. The SNAP II peak picking algorithm was used to determine the monoisotopic mass of the mAbs with high mass accuracy.

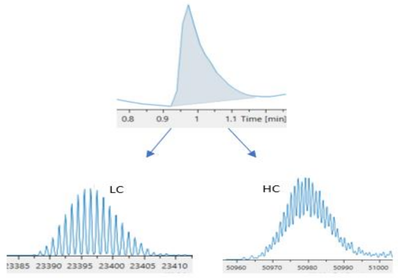
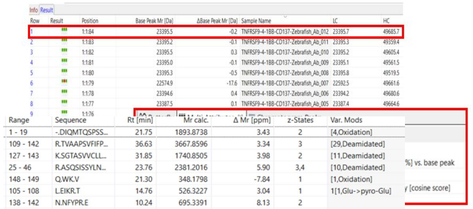


Fig. 1 Isotopic resolution of co-eluting light and heavy chain on a reduced mAb in a 1.2 min LC method.

The Multi-Target Screening ESI workflow in BioPharma Compass enabled rapid processing of a 96-well plate batch of reduced mAbs in 2.5 hours. Batch screening is facilitated through similarity scores and mass accuracy attributes which are the basis for an automatic pass/fail assessment.



Range	Sequence	Rt [min]	Mr calc.	Δ Mr [ppm]	z-States	Var. Mods
1 - 19	-.DIQMTQSPSS...	21.75	1893.8738	3.43	2	[4,Oxidation]
109 - 142	R.TVAAPSVFIFP...	36.63	3667.8596	3.34	3	[29,Deamidated]
127 - 143	K.SGTASVVCLL...	31.85	1740.8505	3.98	2	[11,Deamidated]
25 - 46	R.ASQSISSYLN...	23.76	2381.2016	5.90	3,4	[10,Deamidated]
148 - 149	Q.WK.V	21.30	348.1798	-7.84	1	[1,Oxidation]
105 - 108	L.EIKR.T	14.76	526.3227	3.04	1	1[1,Glu->pyro-Glu]
138 - 142	N.NFYPR.E	10.24	695.3391	8.13	2	

Fig. 2 The list result view indicated the success of the analysis (confirming expected result) with several reporting attributes using the MAM reporting icon .

Peptide Mapping

The variable region contained the library specific variable CDR sequences. A fast 5 min trypsin digest was done to obtain high throughput. The digested peptides were separated on an Acquity BEH C18, 1.7um 1mm x 100mm column with a gradient of 60 min. An Instant Expertise™ auto MS/MS acquisition method with a dynamic MSMS acquisition rate of 2 - 12 Hz fragmented the tryptic peptides and yielded high sequence coverage in a single experiment. The MS/MS fragment spectra obtained were of high quality and demonstrated excellent signal intensity for fragments across the m/z range (100-2000 m/z)

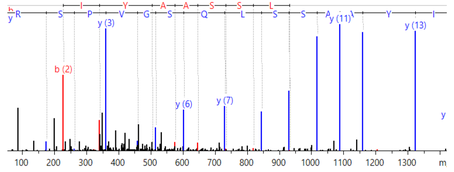


Fig. 4 High quality MS/MS spectrum yielding good fragment coverage.

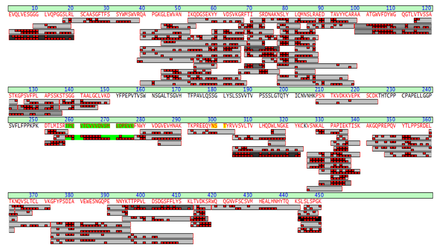


Fig. 3 The protein sequence view shows the identified peptides mapped to the protein sequence. The individual fragments detected in the MS/MS data are represented with red bricks (top row and bottom row of bricks represent N- and C-terminal matches, respectively).

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Fig. 4 The peptides list shows PTMs including oxidation, deamidation and glycosylations

Conclusions

- The high resolving power of the maXis II enabled isotopic resolution of the co-eluting heavy and light chains while the SNAP II peak picking algorithm calculated the monoisotopic molecular weight from isotopically resolved peaks with high isotopic fidelity (True Isotopic Pattern™)
- The Instant Expertise™ MS/MS capabilities of the maXis II enabled excellent sequence coverage for the peptide maps and detection of several PTMs despite a limited trypsin digest of 5 min.
- The Elute HT system coupled to a PAL3 autosampler allowed analysis of the 96-well plate of reduced mAbs to be completed in 2.5 hours without any carryover.