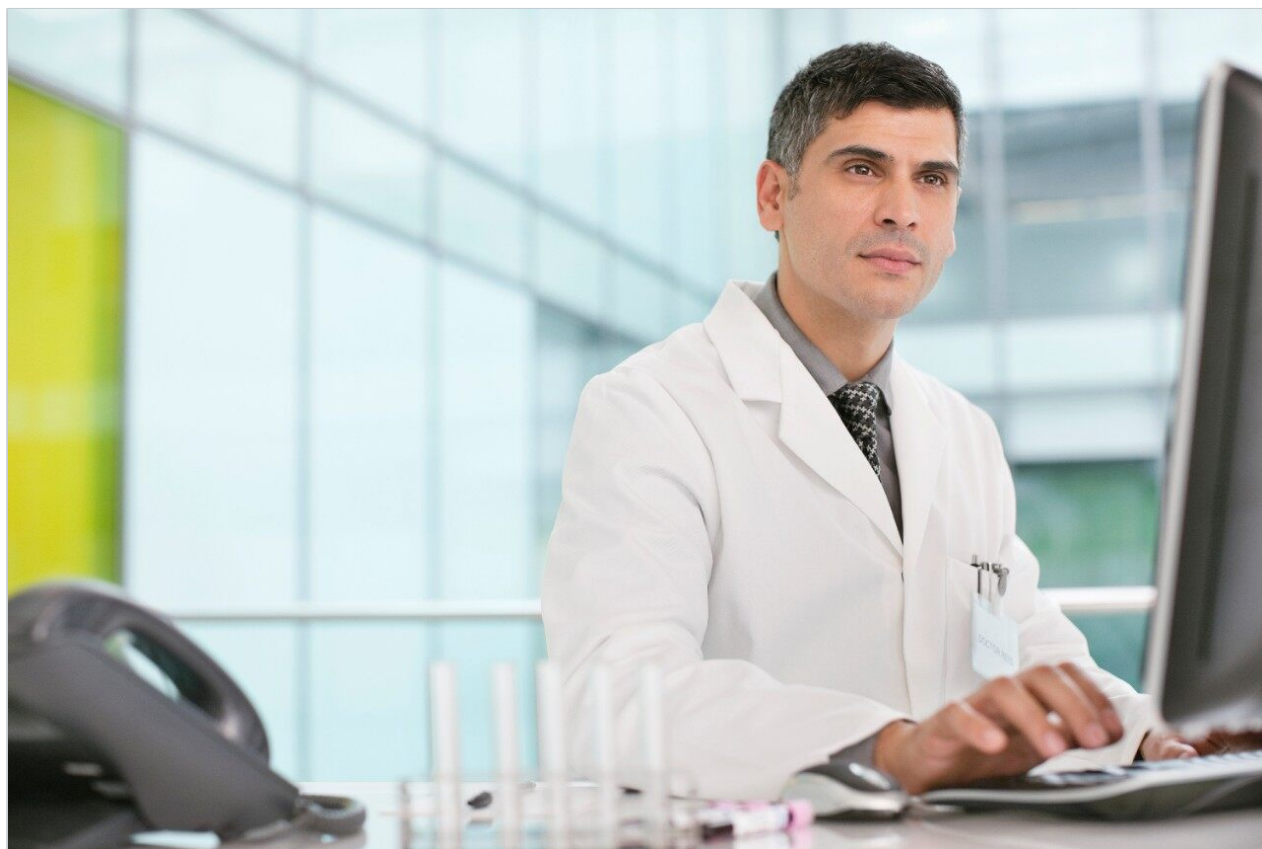


Streamlining Compliant and Non-Compliant Intact Mass Analysis of Biotherapeutic mAbs with the Biopharmaceutical Platform Solution with UNIFI

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Abstract

The intact mass analysis workflows within the Biopharmaceutical Platform Solution with UNIFI enable automated data acquisition, processing, and reporting of a typical method validation sample set. This demonstrates UNIFI Software's ability to facilitate robust glycoform profiling of a recombinant mAb, removes the necessity of manual data processing, and improves the process of data review and reporting.

Benefits

The Biopharmaceutical Platform Solution with UNIFI enables a fully integrated workflow for intact mass analysis, including acquisition, processing, and reporting, for organizations in early development and those operating under regulatory compliant environments. The ability to automate and standardize intact mass analysis enables laboratories to deploy their scientific resources with greater efficiency and effect.

Introduction

Intact mass analysis is a rapid and convenient method for confirming protein identity and profiling product-related variants. In conjunction with other analytical techniques, such as peptide mapping and released glycan analysis, intact mass analysis can help determine if the biomolecule had been correctly cloned, expressed, purified, and formulated during the biopharmaceutical drug development process.

Intact mass analysis can provide a semi-quantitative view of product heterogeneity and is often employed to determine relative composition of product glycoforms. As a lot release test, intact protein mass analysis often provides a quick identity test using the mass of a major variant, sometimes in conjunction with a purity test with defined product variation for peaks corresponding to variants displaying critical product attributes. Demonstration of process consistency through such comparability exercises is critical to obtain initial regulatory approval and for later process improvement studies.

Data processing and report generation often become productivity-limiting tasks for organizations responsible for biotherapeutic protein characterization and analysis. It is still common for LC-MS intact protein data to be manually processed, an inefficient process that lacks standardization and is prone to human error. Further inefficiency and sources of error result from scientists having to reformat results into graphical and tabular formats suitable for communicating information to their organizations.

The ability to automate and standardize data acquisition, processing, and reporting for intact mass analysis allows laboratories to deploy their scientific resources with greater efficiency and effect. The

Waters UNIFI Scientific Information System enables these benefits, as well as regulatory compliance, to be realized throughout discovery, development, and quality management organizations.

In this application note, an integrated and compliant-ready solution for intact mass analysis is described. The combination of UPLC separations, optimized application-tested protein column chemistries, the Xevo G2-S QToF for mass detection, all used under control of the UNIFI Scientific Information System, achieves the goal of total workflow automation and standardization.

Experimental

LC conditions

System:	ACQUITY UPLC H-Class Bio System
Detector:	ACQUITY UPLC Tunable UV Detector
Column:	ACQUITY UPLC Protein BEH C ₄ Column, 300Å, 1.7 µm, 2.1 mm X 50 mm (p/n 186004495)
Column temp.:	80 °C
Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Mobile phase C:	1% formic acid
Optical detection:	UV 280 nm
Total run time:	6.5 min

LC gradient table:

Time(min)	Flow(mL/min)	%A	%B	%C	%D	Curve
Initial	0.40	85.0	5.0	10.0	0	Initial

Time(min)	Flow(mL/min)	%A	%B	%C	%D	Curve
1.00	0.40	85.0	5.0	10.0	0	6
1.01	0.20	85.0	5.0	10.0	0	6
3.50	0.20	5.0	95.0	0.0	0	6
3.70	0.40	5.0	95.0	0.0	0	6
4.00	0.40	10.0	80.0	10.0	0	6
4.50	0.40	10.0	80.0	10.0	0	6
5.00	0.40	85.0	5.0	10.0	0	6
5.50	0.40	85.5	5.0	10.0	0	6

MS conditions

Mass spectrometer:	Xevo G2-S QTof
Capillary:	2.5 kV
Sampling cone:	80 V
Extraction cone:	4 V
Source temp.:	150 °C
Desolvation temp.:	350 °C
Cone gas flow:	0 L/h
Desolvation gas flow:	800 L/h

Informatics

Results derived from an intact IgG1 mAb mass analysis are used to illustrate how this integrated system solution can help the biopharmaceutical laboratories to streamline a common analytical workflow, shown in Figure 1, and more quickly and efficiently communicate key information needed to bring better molecules to market faster.

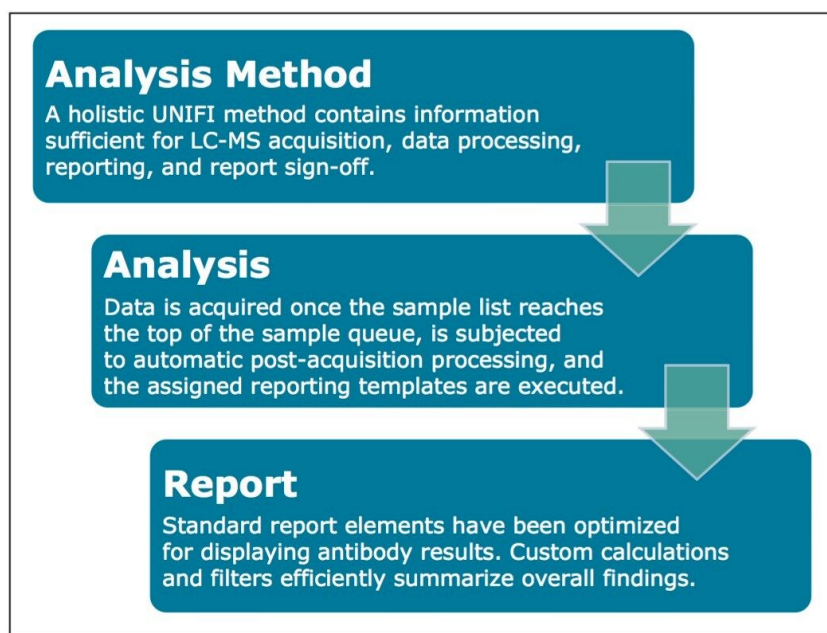


Figure 1. Intact mass analysis workflow with the UNIFI Scientific Information System.

Biopharmaceutical System Solution with UNIFI

- ACQUITY UPLC H-Class Bio System
- Protein Separation Technology (PrST) Columns
- Xevo G2-S QToF with an ACQUITY UPLC Tunable UV Detector
- UNIFI Scientific Information System

Sample preparation

Waters Intact mAb Mass Check Standard (p/n 186006552) was analyzed by solubilizing the standard (10 mg/mL or 67 μ M, 100 μ L DI water to standard vial, 5 min sonication), and diluting 20X (Final 3.3 μ M, 0.50 μ g/ μ L) with eluent A for Xevo G2 QToF analysis or 200X (0.33 μ M, 0.05 μ g/ μ L) for Xevo G2-S analysis.

Results and Discussion

An automated mAb LC-MS analysis set of 11 injections was automatically acquired, processed, and reported as specified in a single UNIFI method. Data are representative of a simple method development set, where the goal of the researcher is to assess the extent of product glycovariation and determine analytical reproducibility.

For the processed results, a single injection is represented in the review panel of the UNIFI analysis center, shown in Figure 2. This panel is configured to convey chromatographic information (integrated total ion chromatogram), the MaxEnt deconvoluted MS spectrum corresponding to the summed spectra under the detected peak, and a component summary window filtered to display the top five most intensely assigned glycoforms (G0F/G0F, G0F/G1F, G1F/G1F, G1F/G2F, or G2F/G2F).



Figure 2. The review panel in UNIFI displays automatically processed experimental results.

This combined panel enables a researcher to assess chromatographic quality, the quality of MS data processing, and the quality of glycoform assignments in a single display. Closer examination also reveals

the relative abundance of each glycoform was automatically calculated as part of the processing.

Having designated one sample as the reference enables a researcher to select the comparative mode display of the review tab. The binary comparison display, shown in Figure 3, provides a means to visually examine the differences between the two samples, thus revealing the extent of variation between samples. In this display, comparative chromatograms and spectra (A280 and summed m/z spectra) are depicted, along with the component summary, now reformatted to address comparative questions. Since both injections were from the same sample, minimal experimental result differences are predictably observed.



Figure 3. UNIFI's review panel (compare mode) displays automatically processed experiment results, with a focus on identifying similarities and differences between a reference sample and unknown samples.

The summary plot tool within the review tab enables researchers to quickly compare trends and differences within the larger data set. The variation of mAb glycoform MS response, as shown in Figure 4, would be a common application of this capability, as would comparisons of observed retention time or mass error across the sample set. The consistent MS response of glycoforms across all injections illustrates the expected reproducibility of the intact mass analysis of replicated injections.

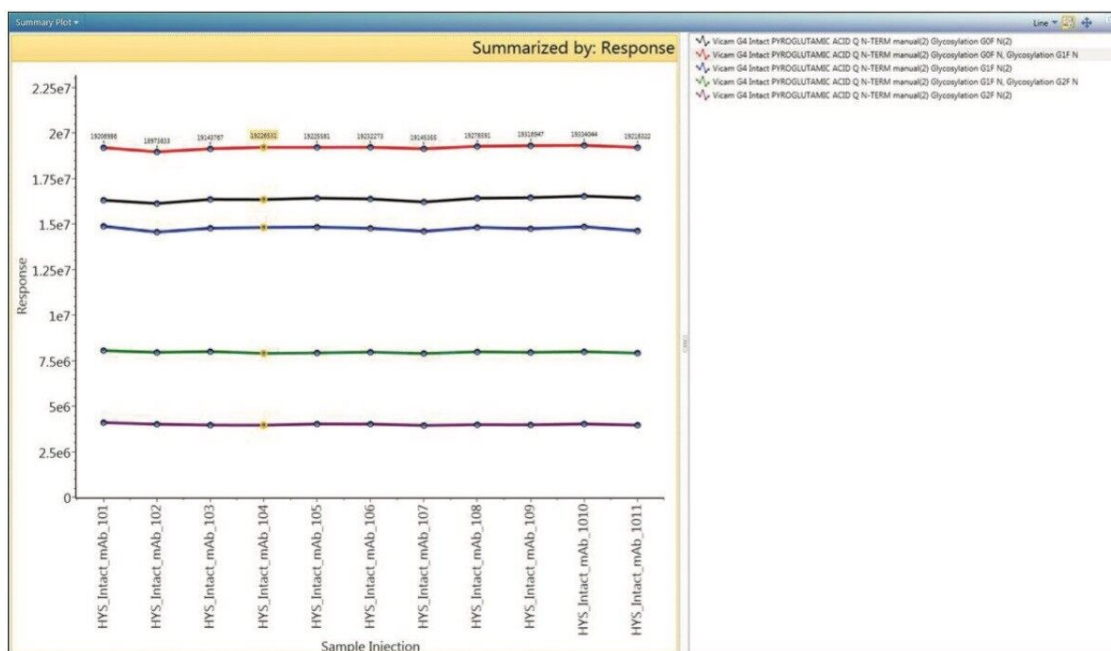


Figure 4. summary plot view of MS response for 5 mAb glycoforms ($n = 11$ injections).

UNIFI reporting

The reporting functionality within UNIFI Software is powerful, addressing one of the common bottlenecks encountered by organizations when generating and managing large volumes of complex scientific data. The ability to customize common report objects by means of filters, formatting, and the use of custom fields and calculations enables report content to be automatically generated by an entire organization with high quality on a consistent basis. Based on the analytical objectives, one or more report templates can be attached to the analysis method.

The first page of a typical intact mass analysis experimental report contains a summary of sample information and acquisition status, as shown in Figure 5. More detailed experimental results (such as TUV and TIC chromatograms, raw and deconvoluted MS spectra, and identified component response summary table) are often grouped for each injection, as shown in Figure 6.



mAb Intact Protein Report

Analysis Information

Item name: HYS_Intact_mAb July 2012 for noes Analysis Method Item name: HYS_Intact_mAb July 2012
Version: 1 Analysis Method Version: 1
Modified date: Dec 20, 2012 16:25:23 Eastern Standard Time Sample Set Created date:
Modified by: Shion, Henry Sample Set Created by:

Sample List

.	Item name	Sample type	Replicate number	Sample position	Acquisition start time	Acquisition time (min)	Acquisition status	Injection volume (µL)
1	HYS_Intact_mAb_101	Unknown	1	1:B,1	7/18/2012 5:14:08 PM	6.5	Complete	4.00
2	HYS_Intact_mAb_102	Unknown	1	1:B,1	7/18/2012 5:22:02 PM	6.5	Complete	4.00
3	HYS_Intact_mAb_103	Unknown	1	1:B,1	7/18/2012 5:29:26 PM	6.5	Complete	4.00
4	HYS_Intact_mAb_104	Unknown	1	1:B,1	7/18/2012 5:37:16 PM	6.5	Complete	4.00
5	HYS_Intact_mAb_105	Unknown	1	1:B,1	7/18/2012 5:44:43 PM	6.5	Complete	4.00
6	HYS_Intact_mAb_106	Unknown	1	1:B,1	7/18/2012 5:52:36 PM	6.5	Complete	4.00
7	HYS_Intact_mAb_107	Unknown	1	1:B,1	7/18/2012 6:00:00 PM	6.5	Complete	4.00
8	HYS_Intact_mAb_108	Unknown	1	1:B,1	7/18/2012 6:07:51 PM	6.5	Complete	4.00
9	HYS_Intact_mAb_109	Unknown	1	1:B,1	7/18/2012 6:15:22 PM	6.5	Complete	4.00
10	HYS_Intact_mAb_1010	Unknown	1	1:B,1	7/18/2012 6:23:12 PM	6.5	Complete	4.00
11	HYS_Intact_mAb_1011	Reference	1	1:B,1	7/18/2012 6:30:44 PM	6.5	Complete	4.00

Figure 5. Typical first page of an intact protein LC-MS report in UNIFI Software that summarizes sample and acquisition details.

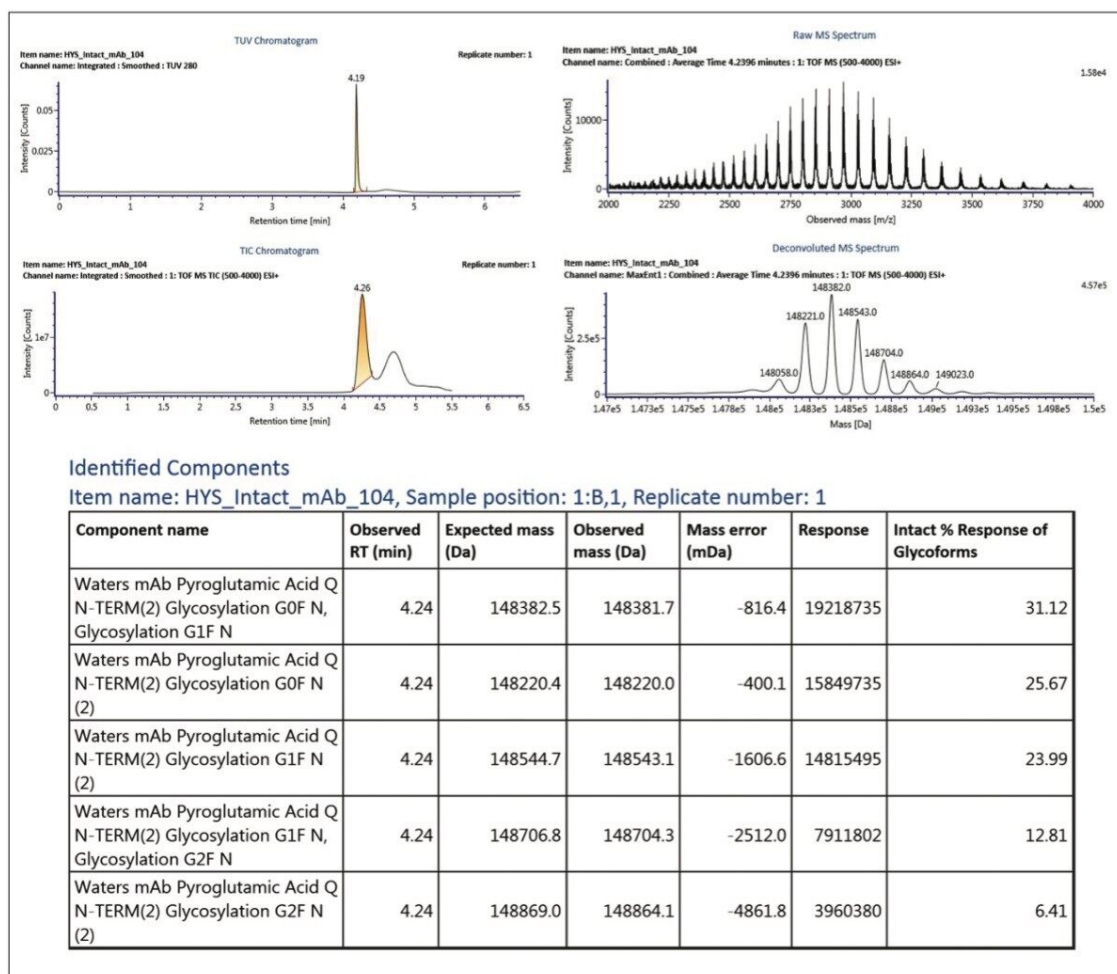


Figure 6. Example report object grouping (TUV and TIC chromatograms, raw and deconvoluted MS spectra, and identified component response summary table) from a single injection within the analysis

In the case of mAbs, generic report objects were tuned to account for the rapid desalting LC-MS method that was used, the acquisition of UV and MS data, and the typical input m/z and output mass ranges encountered during antibody ESI mass analysis.

In addition, the ability to automate reporting summary results across the sample sets eliminates the use of external software for data aggregation, as shown in Figure 7. This not only greatly increases the timeliness of communicating results, but avoids the human errors and validation efforts that cost analytical organizations time and money. In the case of this typical method validation injection set, the precision of MS response and mass accuracy is reported for one of the observed glycoforms.

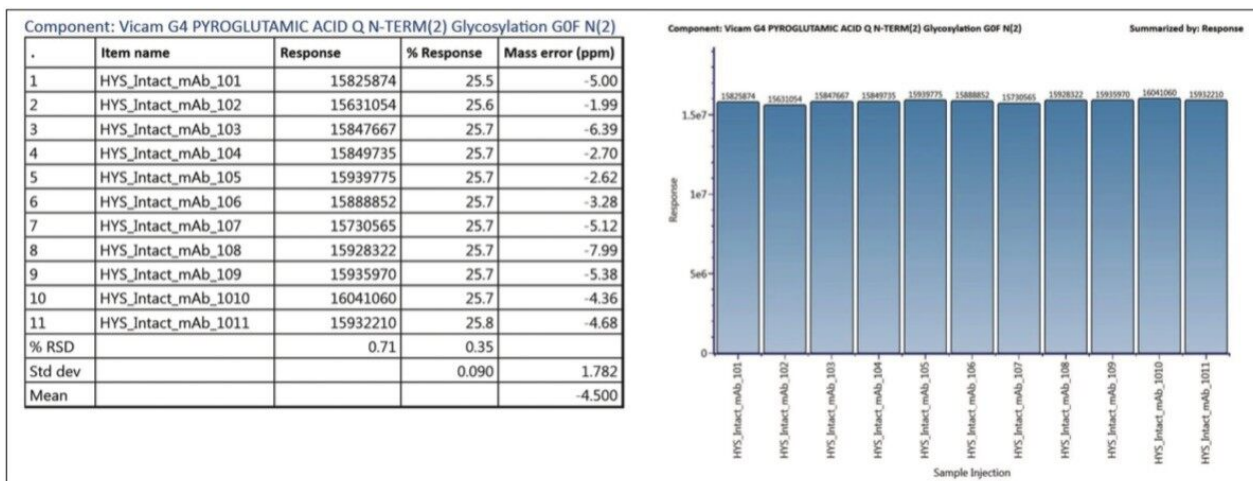


Figure 7. The report object summarizes MS response and mass accuracy/precision across all injections within the sample set in table and bar chart formats.

Conclusion

The intact mass analysis workflows within the Biopharmaceutical Platform Solution with UNIFI enable automated data acquisition, processing, and reporting of a typical method validation sample set. This demonstrates UNIFI Software's ability to facilitate robust glycoform profiling of a recombinant mAb, removes the necessity of manual data processing, and improves the process of data review and reporting. The implementation of such highly automated workflows should enable biotherapeutic development and quality organizations to handle larger volumes of sample requests with the same resources, while improving the quality of the information they provide.

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<https://www.waters.com/waters/partDetail.htm?partNumber=186004495>>

Intact mAb Mass Check Standard <
<https://www.waters.com/waters/partDetail.htm?partNumber=186006552>>

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