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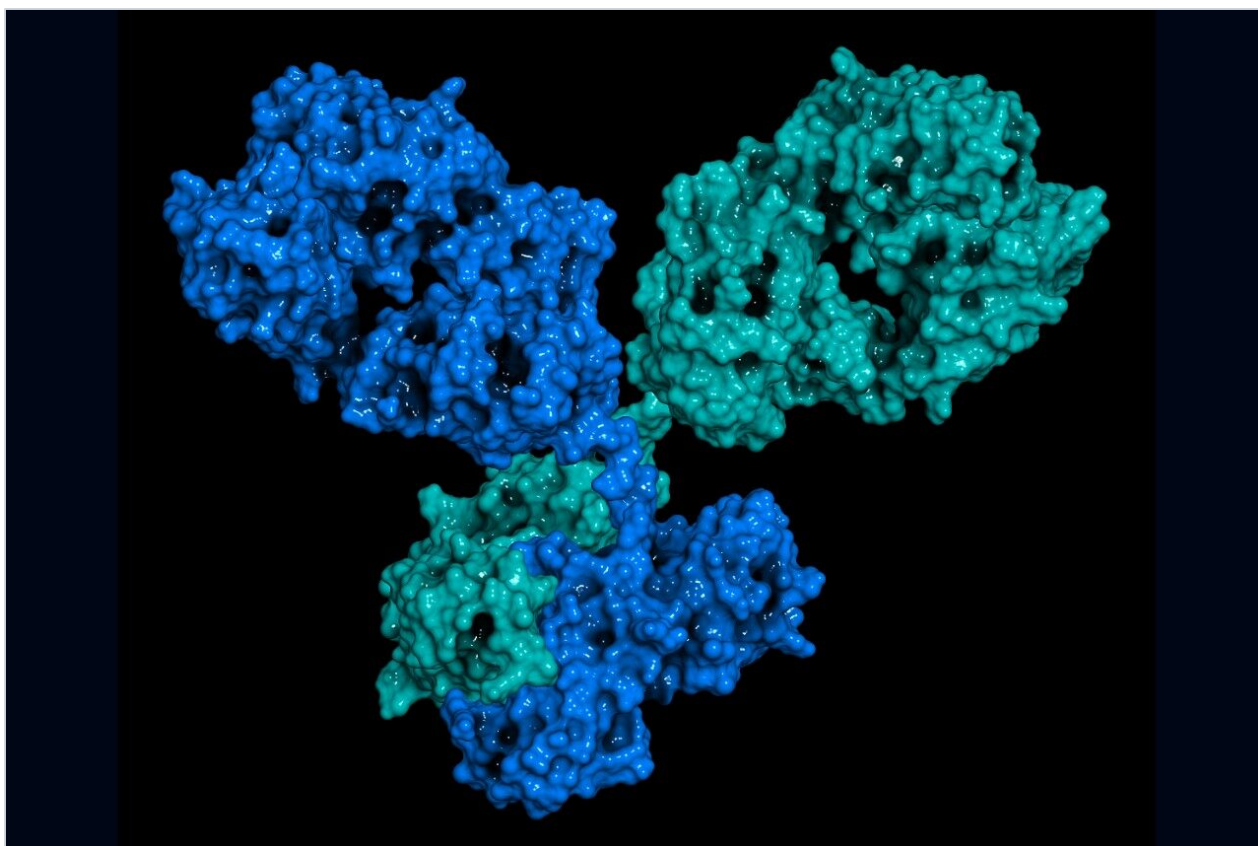
Biopharmaceutical System Solution with UNIFI: Intact Protein Characterization

Waters Corporation

This is an Application Brief and does not contain a detailed

Experimental section.

Abstract

This application brief demonstrates the capabilities of the integrated UPLC-MS analysis of an intact monoclonal antibody with a comprehensive platform, the Biopharmaceutical Platform Solution with UNIFI, for accurate mass measurement, data processing, and reporting with UNIFI Scientific Information System.

Benefits

This UNIFI-based platform addresses previous limitations with a comprehensively integrated platform for data acquisition by chromatography and mass spectrometry, with automated reporting.

Introduction

The growing biotherapeutic pipeline means that the efficient characterization of monoclonal antibodies (mAb) is of growing importance, both to regulatory authorities and to pharmaceutical companies. Being able to perform acquisition and processing within the same platform, complete with an audit trail, is an important goal for regulated environments.

Accurately identifying post-translational modifications such as protein glycosylation is required as part of guidelines as they play several key roles in biological systems. Fast and accurate analysis of the glycoproteins is required in order to ensure the safety and efficacy of the biotherapeutic.

The ACQUITY UPLC H-Class Bio's high-resolution bioseparations combined with high mass accuracy mass spectrometry detection with the Xevo G2 Tof provides routine UPLC-MS applications for biopharmaceutical laboratories.

There is a large set of data generated during each mAb analysis requiring interpretation of a variety of glycosylated forms and comprehensive characterization of the final product. This step sets productivity limits to otherwise high-throughput procedures and hinders automation of the process.

The UNIFI-based platform addresses these limitations with a comprehensively integrated platform for data acquisition by chromatography and mass spectrometry, with automated reporting.

Results and Discussion

To solve the problem of time-consuming data analysis and facilitate data processing of therapeutic mAb, the Biopharmaceutical Platform Solution with UNIFI was configured for the study of intact proteins. This represents a holistic approach of UPLC-MS data acquisition followed by automatic processing and annotation of the data in a high-throughput manner, which are further exported for data management.

UPLC-MS analysis of the mAb Trastuzumab was performed automatically. Aqueous solutions of 0.1% FA and 0.1% FA solution in acetonitrile were used as eluents A and B, respectively. Column temperature set to 80 °C is critical for successful chromatographic separation. The system included an ACQUITY UPLC H-Class Bio, an ACQUITY UPLC Protein BEH C_4 Column, and a Xevo G2 Tof. The UNIFI Scientific Information System for acquisition, data processing, and reporting completes this comprehensive Biopharmaceutical Platform Solution.

The intact protein analysis report demonstrates the report objects, which can be entirely configured by the user: TIC summarized chromatogram; raw, deconvoluted, and centroid mass spectra; and tabulated summary of the interpreted LC-MS data (Figure 1). This detailed view shows an example of a deconvoluted spectrum within a specified mass range and parameter settings defined in the method. Deconvolution reveals several core glycosylated species which match the number of glucose residues and level of fucosylation. Another report object is a table with mass measurement of the intact mAb and accurately assigned mAb glycan variants (Figure 2). Mass errors were reported for each Trastuzumab MS peak with a corresponding retention time entry from the TIC chromatogram.
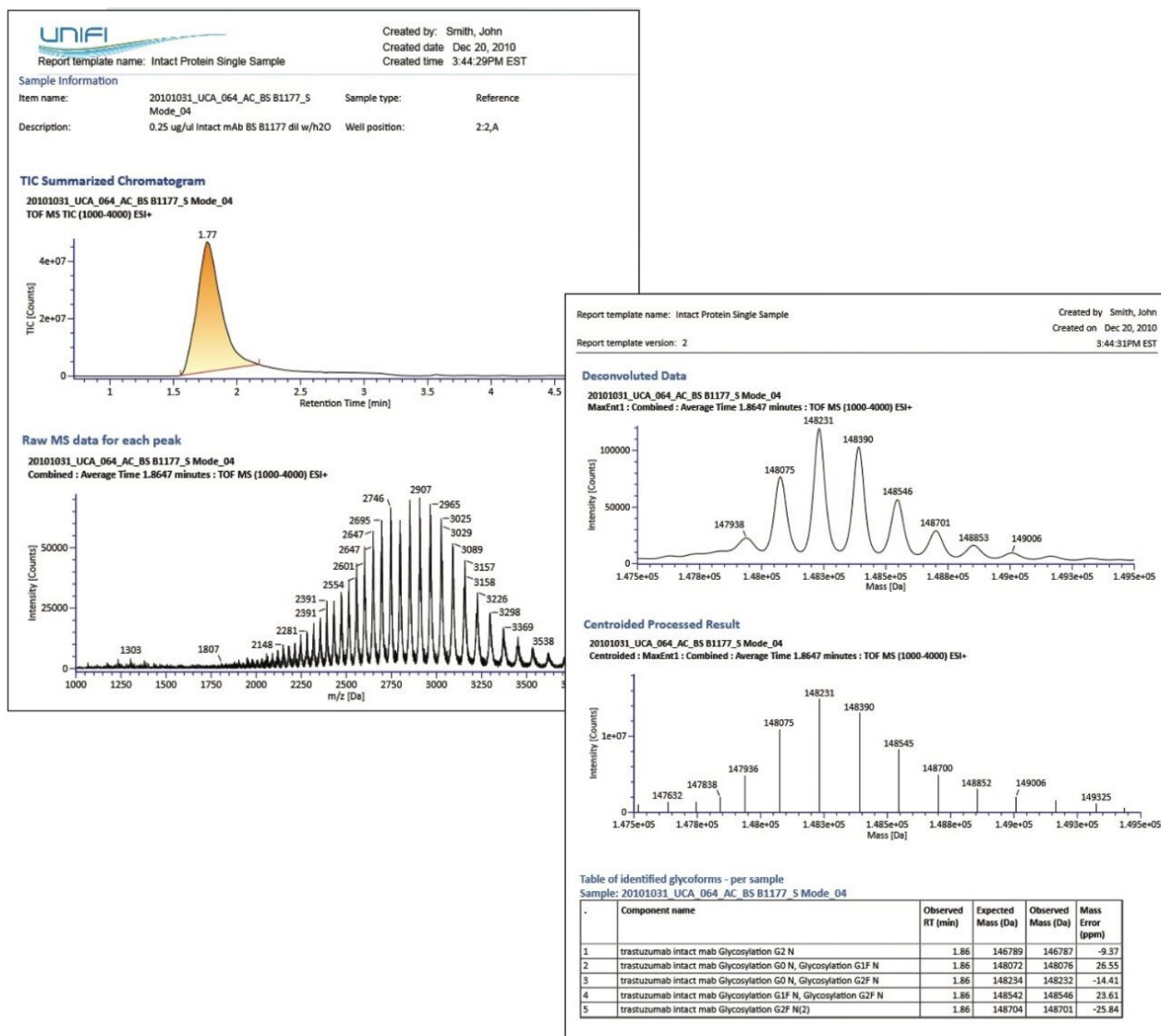
Figure 1. UNIFI's intact protein analysis report of an intact mAb, Trastuzumab. The report shows, from top to bottom, the integrated chromato-graphic peak, the charge state distribution, the continuum deconvoluted data, the integrated and processed data, and finally the summary table assigning major glycoforms according to mass.

Table of identified glycoforms - per sample
Sample: 20101031_UCA_064_AC_BS B1177_S Mode_04

.	Component name	Observed RT (min)	Expected Mass (Da)	Observed Mass (Da)	Mass Error (ppm)
1	trastuzumab intact mab Glycosylation G2 N	1.86	146789	146787	-9.37
2	trastuzumab intact mab Glycosylation G0 N, Glycosylation G1F N	1.86	148072	148076	26.55
3	trastuzumab intact mab Glycosylation G0 N, Glycosylation G2F N	1.86	148234	148232	-14.41
4	trastuzumab intact mab Glycosylation G1F N, Glycosylation G2F N	1.86	148542	148546	23.61
5	trastuzumab intact mab Glycosylation G2F N(2)	1.86	148704	148701	-25.84

Figure 2. Zoomed in view of the table in Figure 1 summarizing mass measurement of the intact mAb and accurately assigned mAb glycan variants.

Such an integrated LC-MS approach provides the user flexibility to work with both raw and processed data followed by quick and efficient data management.

Conclusion

The capabilities of the Biopharmaceutical Platform Solution with UNIFI have been successfully demonstrated with the example of an intact biotherapeutic mAb.

Modern instrumentation and evolving analytical techniques extend the limits of the biopharmaceutical industry and consequently impose strict control of manufacturing processes.

Highly efficient and cost-effective integrated UPLC-MS approaches with the UNIFI Scientific Information System for data processing and reporting satisfies regulatory requirements and facilitates intact protein characterization. This technology covers the range from detailed structural protein characterization to sophisticated data management with UPLC-MS platforms.

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UNIFI/nav.htm?locale=101&cid=10195515>

ACQUITY UPLC H-Class PLUS Bio System <https://www.waters.com/10166246>

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