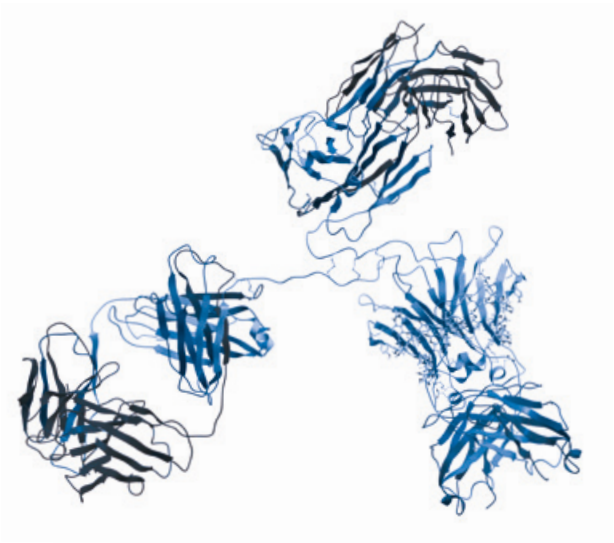
maXis II for Biopharma Analysis

- mAb Development made easy

Innovation with Integrity

BioPharma

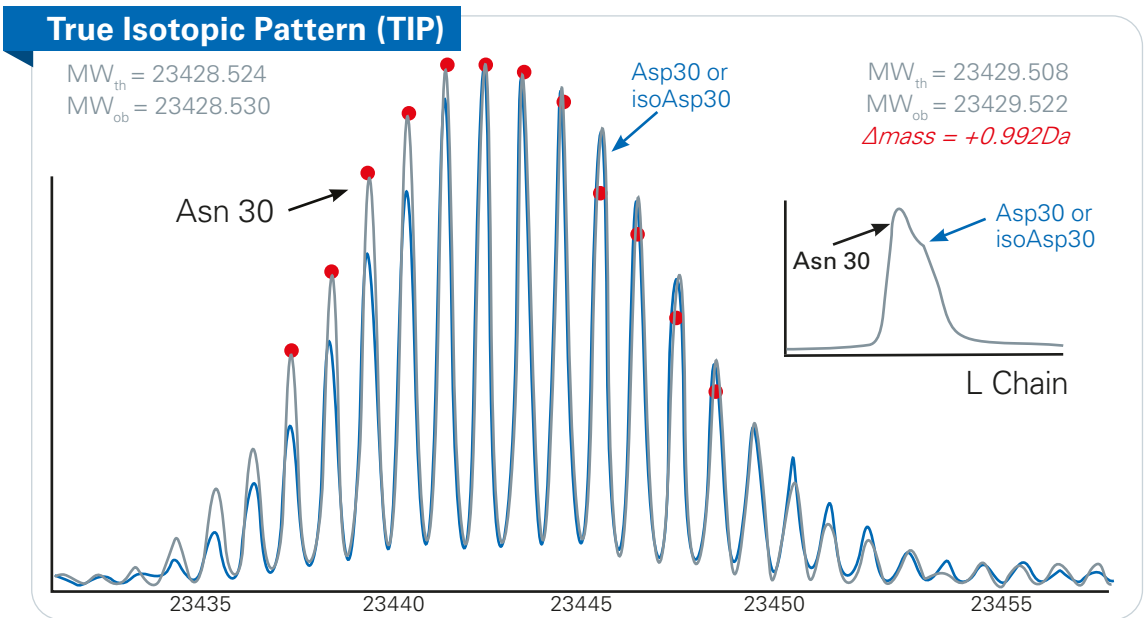
maXis II: Unmatched Performance



Determining the monoisotopic mass for large molecules (> 25 kDa) and related variants is essential for mAb developability assessments during early stage development.

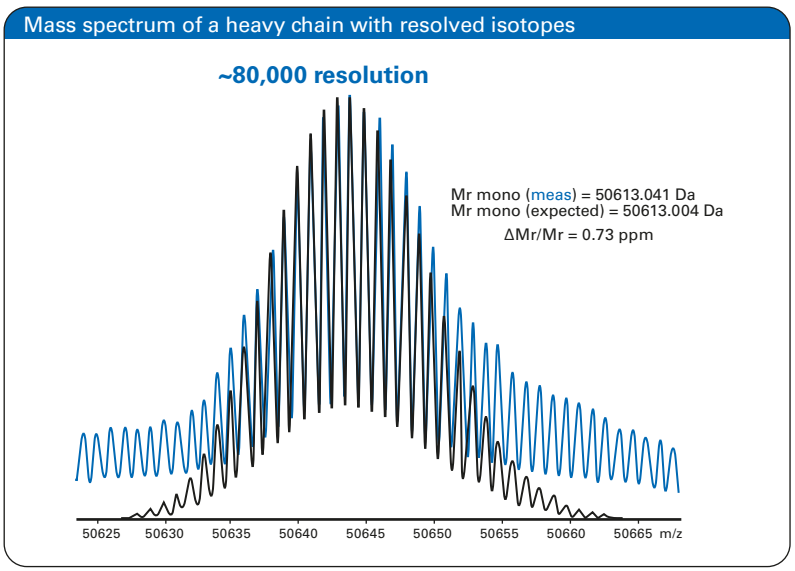
The maXis II UHR-QTOF mass spectrometer together with the SNAP-II deconvolution algorithm possesses an intrinsic edge for the rapid profiling of large proteins and their heterogeneities.

Sub-unit characterization (e.g. de-amidation) using the power of True Isotopic Pattern (TIP) raw data

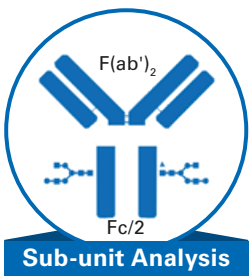


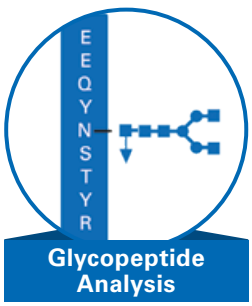
The maXis II acquires True Isotopic Pattern (TIP) raw data that allows for rapid determination of modifications such as deamidation of the light chain (LC). Coupled to SNAP-II algorithm, these subtle modifications can be routinely characterized at the sub-unit level.

The ‘de facto’ standard for biologics



MaxEnt deconvolution spectrum measured in blue. Simulated mass spectrum in black.





Increase speed to clinic with the maXis II:

- Understand and predict PK properties of antibodies by thorough characterization of glycans
- Accurately determine deamidation
- Rapid drug-antibody ratio (DAR) analysis
- Reduce downstream manufacturing risks
- Monitor batch to batch variability

The maXis II comes with electron transfer dissociation (ETD) capability as well as a high-mass option (HMO) for native MS applications.

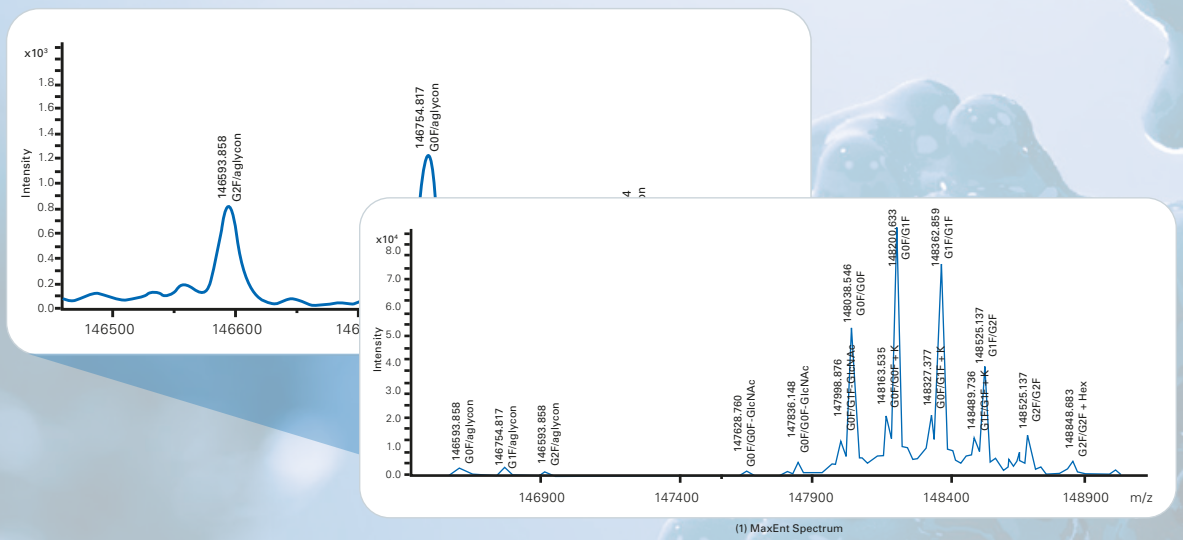
Superior Glycan Profiling Performance

Glycosylation is a crucial quality attribute for many biopharmaceuticals, as small changes in glycosylation profiles can have significant impact on stability, efficacy and/or safety.

Identifying, monitoring and controlling the glycosylation levels is key for successful biopharmaceutical development and the maXis II confidently addresses these key challenges in many pharmaceutical labs today at the intact, subunit, glycopeptide and released glycan level.

Confidently determine:

- **Relative abundances** (The most glycan IDs and 1-sigma groups in NISTmAb round robin study)*
- **Composition** (glycopeptide or released)**
- **Localization** within protein sequence (glycopeptide)



Protein	Form	Annotation	Mr Ref	Mr Sample	Δ Mr [ppm]	Δ Mr [Da]	Int. [a.u.]	Rel. Int. Ref [%]	Rel. Int. Samp...	Rt Ref [min]	Rt Sample [m...	Δ Rt [min]	Confirmed
NIST mAb	G0F/ aglycon	G0F/ aglycon	146591.8000	146593.4060	10.96	1.6060	4.866E+02	0.0	0.2	7.40	7.43	0.03	Yes
NIST mAb	G1F/ aglycon	G1F/ aglycon	146754.0000	146754.8958	6.10	0.8958	7.168E+02	0.0	0.4	7.40	7.43	0.03	Yes
NIST mAb	G2F/ aglycon	G2F/ aglycon	146916.1000	146918.9621	19.48	2.8621	2.445E+02	0.0	0.1	7.40	7.43	0.03	Yes
NIST mAb	G0F/G0F - 2G...	G0F/G0F - 2GlcNAc	147630.8000	147628.0991	-18.30	-2.7009	3.615E+02	0.0	0.2	7.40	7.43	0.03	Yes
NIST mAb	G0F/G0F - Gl...	G0F/G0F - GlcNAc	147834.0000	147836.1872	14.79	2.1872	3.034E+03	1.7	1.5	7.40	7.43	0.03	Yes
NIST mAb	G0F/G1F - Gl...	G0F/G1F - GlcNAc	147996.1000	147998.8855	18.82	2.7855	7.225E+03	3.7	3.6	7.40	7.43	0.03	Yes
NIST mAb	G0F/G0F	G0F/G0F	148037.2000	148038.4855	8.68	1.2855	2.939E+04	14.3	14.4	7.40	7.43	0.03	Yes
NIST mAb	G0F/G0F + K	G0F/G0F + K	148165.3000	148163.6050	-11.44	-1.6950	1.201E+04	6.1	5.9	7.40	7.43	0.03	Yes
NIST mAb	G0F/G1F	G0F/G1F	148199.3000	148200.6541	9.14	1.3541	4.924E+04	23.6	24.2	7.40	7.43	0.03	Yes
NIST mAb	G0F/G1F + K	G0F/G1F + K	148327.5000	148327.3263	-1.17	-0.1737	1.234E+04	6.3	6.1	7.40	7.43	0.03	Yes
NIST mAb	G1F/G1F	G1F/G1F	148361.4000	148362.8452	9.74	1.4452	4.228E+04	20.6	20.8	7.40	7.43	0.03	Yes
NIST mAb	G1F/G1F + K	G1F/G1F + K	148489.6000	148489.7867	1.26	0.1867	7.961E+03	4.2	3.9	7.40	7.43	0.03	Yes
NIST mAb	G1F/G2F	G1F/G2F	148523.6000	148525.1205	10.24	1.5205	2.091E+04	10.3	10.3	7.40	7.43	0.03	Yes
NIST mAb	G1F/G1F + 2K	G1F/G1F + 2K	148617.8000	148623.3378	37.26	5.5378	2.187E+03	1.3	1.1	7.40	7.43	0.03	Yes
NIST mAb	G1F/G2F + K	G1F/G2F + K	148651.8000	148652.7697	6.52	0.9697	3.543E+03	2.0	1.7	7.40	7.43	0.03	Yes
NIST mAb	G2F/G2F	G2F/G2F	148685.7000	148687.2332	10.31	1.5332	8.345E+03	4.3	4.1	7.40	7.43	0.03	Yes
NIST mAb	G2F/G2F + Hex	G2F/G2F + Hex	148847.7000	148848.7602	7.12	1.0602	3.106E+03	1.7	1.5	7.40	7.43	0.03	Yes

Profiling at the intact level usually has a mass accuracy of around 10 ppm accuracy, as illustrated above. With resolved isotopes (< 50 kDa) it drops under 1 ppm. The maXis II UHR-QTOF sets the performance standard, with an unrivaled dynamic range for large proteins and glycoforms.

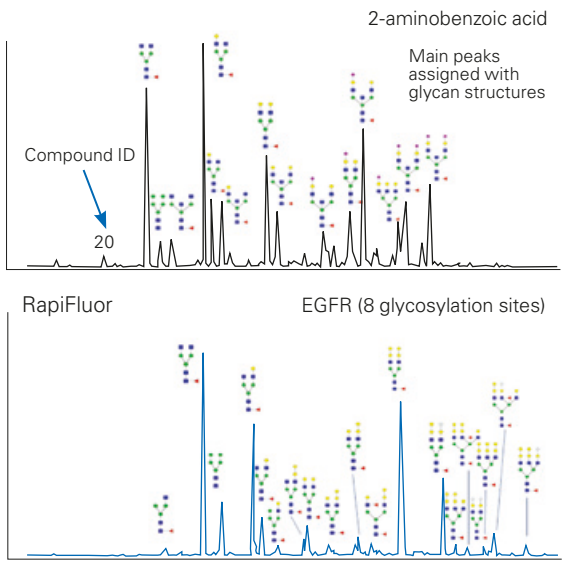
*Maria Lorna A. De Leoz et al: NIST Interlaboratory Study on the Glycosylation of NISTmAb, NIST Pubs(2017), DOI: 10.6028/NIST.IR.8186

**H. Hinneburg et al.: The Art of Glycopeptide Destruction, J. Am. Soc. Mass Spectrom. (2015), DOI: 10.1007/s13361-015-1308-6

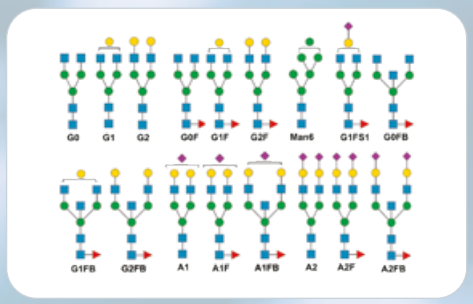
Increased Confidence in Released Glycan Analysis

- Empirical spectra library comprising accurate mass and MS/MS spectra of 2AB and RapiFluor® labeled glycans **(25 glycoproteins including most IgG subtypes and over 15 cell lines)**
- Optimized LC and MS acquisition method
- Data processing with built-in expert knowledge. Scoring based on fragment intensities and **quantitation of coeluting glycans**
- Report generation including MS and fluorescence based data with minimized user intervention
- **Workflow can be applied to regulated environments**

GlycoFiler™ Results



Extract from GlycoFiler reports: Fluorescence chromatograms for an IgG1 and EGFR released glycans annotated with the main identified structures.



GlycoFiler™ Workflow

Sample Preparation

- Enzymatic release of glycans from the protein
- Fluorescence labeling
- HILIC-UPLC-FLR-CID-MS/MS

Measurement
HILIC-UPLC-FLR-CID-MS-MS

FLR Detection

MS Detection
ESI-QTOF-MS/MS

Data Processing
Automated

Integration
of FLR Peaks

Identification
Match with Glycan Spectra Library

Data Processing
Automated

FLR-MS Data Hybridization
Table of glycan structures correlated to relative fluorescence peak areas

Reporting

Certificate of Analysis
Calculation of biologically relevant parameters

Dr. Sven Bahrke, Glycotype GmbH, Germany

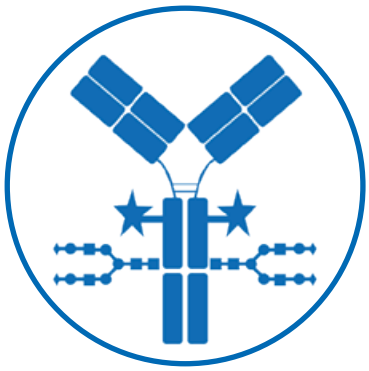
"GlycoFiler ™ improved our lab productivity by reducing the time spent on evaluating data, especially in the case of complex glycan mixtures with coeluting peaks. In addition the ability to unambiguously identify and precisely quantify even low abundant structures reduces the non-annotated peak area to a minimum as well as the risk of false identifications. Integrated and automated quality control of the analyses as well as straightforward comparison of results between products round out the GlycoFiler ™ workflow."

Rapidly Characterize Drug-Antibody Conjugates under GLP

The maXis II easily detects and quantifies micro-heterogeneities directly on intact antibodies including under native conditions.

This makes the maXis II the perfect platform to rapidly develop assays for drug distribution and average drug loading (DAR) without waiting for HPLC methods to be established.

Comparability with orthogonal methods and robustness makes it possible to validate these methods for GLP operations.



Method Reproducibility Assessment

Validation of drug distribution and DAR assay under native conditions on the maXis II UHR-QTOF

	%Peak Area			
	0-Drug%	1-Drug%	2-Drug%	DAR
Prep 1	0.000	3.889	96.111	**1.96**
Prep 2	0.000	3.619	96.381	**1.96**
Prep 3	0.000	4.177	95.823	**1.96**
Prep 4	0.000	4.199	95.801	**1.96**
Prep 5	0.000	3.820	96.180	**1.96**
Prep6	0.000	4.192	95.808	**1.96**
Mean	0.000	3.983	96.017	1.96
SD	*0.000*	*0.206*	*0.206*	*0.00*
%RSD	*0.000*	*5.162*	*0.214*	*0.00*

Analyst	%Peak Area			
	% 0-Drug	% 1-Drug	% 2-Drug	DAR
Analyst 1 - Day 1	0.000	4.177	95.823	1.96
Analyst 2 - Day 1	0.000	3.500	96.500	1.96
Analyst 1 - Day 2	0.000	3.883	96.117	1.96
Mean	0.000	3.853	96.147	1.96
SD	*0.000*	*0.277*	*0.277*	*0.00*
RSD	*0.000*	*7.193*	*0.288*	*0.00*

- Interday
- Intersite
- Under GLP

7 Reasons the maXis II makes mAb development easy

- 80,000 FSR (Full Sensitivity Resolution) with high intra-scan dynamic range
- True Isotopic Pattern (TIP) not limited by space charge effects for accurate DAR analysis
- SNAP-II algorithm for accurate monoisotopic mass determination for large biologics
- Supports 21 CFR Part 11
- Electron Transfer Dissociation
- Native mass spectrometry enabled
- Glycan analysis with Glycofiler library

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Bruker Daltonics GmbH & Co. KG

Bremen · Germany
Phone +49 (0)421-2205-0

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com - www.bruker.com

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