CUSTOMER APPLICATION NOTE 73898

Seamless LC-MS method transfer in a biopharmaceutical development laboratory

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Application benefits

- Achieve consistent high-quality data when switching methods from an existing hybrid quadrupole Thermo Scientific™ Orbitrap™ mass spectrometry platform to a new platform
- Accomplish seamless adoption of new LC-MS technology in a biopharmaceutical development laboratory with the effortless platform transfer operation
- Perform faster acquisition with no sample preparation using a new generation hybrid quadrupole Orbitrap MS platform, which offers higher resolution and improved sensitivity while maintaining reproducibility
- Save laboratory space moving to new generation Orbitrap-based mass spectrometry platform

Introduction

During fast-paced biopharmaceutical development, innovative analytical technologies are needed to provide unique structural insights previously unachievable just a few years ago. This often requires updating an existing mass spectrometer (MS) technology, to more modern and sensitive platforms. However, while updating to a new system offers performance improvements, consistency in data generation between different platforms needs to be assured.

At Symphogen, Denmark, Intact Mass analysis of therapeutic proteins under non-denaturing conditions ("Native MS") has been performed using an LC-MS system comprising a Thermo Scientific™ Vanquish™ Horizon Duo UHPLC and Thermo Scientific™ Q Exactive™ Plus hybrid quadrupole-Orbitrap mass spectrometer equipped with BioPharma option for several years. This system combines chromatographic versatility and robustness with mass spectrometry performance with exceptional spectral clarity. The platform has become an indispensable tool in biopharmaceutical development and lead selection studies at Symphogen, as demonstrated in an earlier application note[1].

The Q Exactive Plus MS at Symphogen has been a successful workhorse system analyzing thousands of mAb samples. In the previously published application note[1] the utility of SEC-MS was demonstrated with the following benefits:

- Reduction of analysis time per sample using a tandem LC-MS configuration
- Obtaining independent information on different product quality attributes (PQAs), such as aggregation and intact MS in one analysis
- Platform robustness and superior data quality

Symphogen develops complex therapeutic proteins and protein mixtures. This complexity drives the need for ever more sophisticated and powerful mass spectrometric techniques. When Symphogen chose to add additional and higher-performance LC-MS capabilities into their laboratory, they installed a new generation benchtop Orbitrap-based high-resolution accurate mass (HRAM) mass spectrometer, the Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer. The Orbitrap Exploris 480 MS has been demonstrated as a high-performance, flexible system capable of the complete characterization of monoclonal antibodies (mAbs) under native and denaturing conditions[2].

Consequently, the execution of the native mass analysis measurements hyphenated to size exclusion chromatography (SEC-MS) for lead selection studies has been transferred by Symphogen to the new Vanquish Horizon Duo UHPLC system for Tandem LC-MS coupled to the Orbitrap Exploris 480 MS system.

The new MS platform offers several improvements:

- Completely redesigned hardware delivering increased robustness
- Higher resolution and acquisition rate
- Improved sensitivity for intact protein analysis
- Predefined method templates for three major protein analytical workflows
- Significantly smaller footprint
- Simplified system setup, calibration, and maintenance routines

For data quality comparison and consistency assessment, the performance of the same SEC-MS method was evaluated using both mass spectrometers. The simple workflow schematic of the experimental setup using both instruments is shown in Figure 1. The Vanquish Horizon Duo UHPLC system for Tandem LC-MS, which was previously connected to the Q Exactive Plus MS system, is now coupled to the Orbitrap Exploris MS system, to allow it to be continuously leveraged for increased throughput when used for LC-MS analyses. After method transfer, the Orbitrap Exploris 480 MS has become the primary system for lead selection studies. For each lead selection study, hundreds of samples are analyzed now with the Orbitrap Exploris 480 MS, and for each, a system suitability test antibody is analyzed several times.

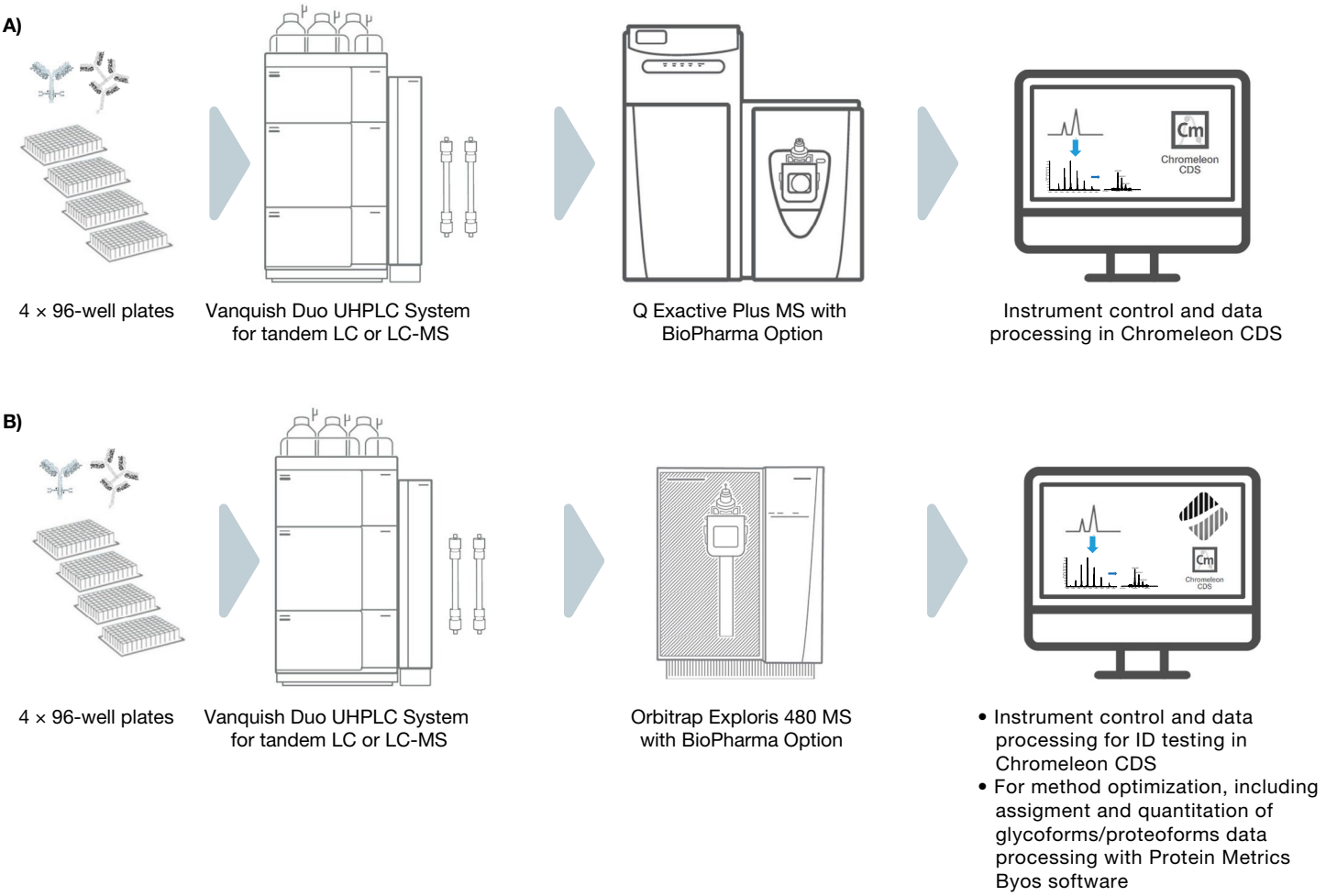

Figure 1. SEC-MS workflow setup for lead selection studies at Symphogen; A) using the Q Exactive Plus MS with BioPharma option and B) using the Orbitrap Exploris 480 MS with BioPharma Option for MS analysis, Protein Metrics Byos™ software for data analysis during method development

Experimental

Consumables and instrumentation used in the current study are summarized in Table 1 and Table 2, respectively. When conducting lead selection studies, a total of 384 lead candidates are typically consecutively analyzed in one sequence. A system suitability test (SST) reference is analyzed initially, then once after every 24th sample, and finally after the last sample. Typically, a total of 17 SST runs are performed in one lead selection study. Results presented here are based on the SST runs performed as part of Symphogen's lead selection studies.

Table 1. Consumables

Recommended consumables	Part number
Honeywell Riedel-de Haën™ CHROMASOLV™ Water LC-MS grade	39253-1L
Fisher Chemical™ Optima™ Ammonium acetate LC/MS grade	A114-50
Sigma-Aldrich™ Glacial acetic acid	27225-1L-M
Commercially available SEC Column, 4.6 × 150 mm, 1.7 µm	

Table 2. Instrumentation

Instrumentation	Part Number
Thermo Scientific Vanquish Horizon Duo system consisting of:	
Vanquish System Base	VH-S01-A
Vanquish Duo for Tandem LC Kit	6036.2020
2x Vanquish Binary Pump H	VH-P10-A
Vanquish Sampler HT	VH-A10-A
Vanquish Column Compartment H	VH-C10-A-03
Vanquish VWD detector HL	VF-D40-A
Vanquish MS Connection Kit	6720.0174
Thermo Scientific Q Exactive Plus mass spectrometer with Biopharma option	0726060
Thermo Scientific Orbitrap Exploris 480 mass spectrometer with BioPharma option	BRE725539

LC configuration

LC settings are summarized in Table 3. Solvent A is prepared by adding ammonium acetate (1.93 g) and acetic acid (220 µL) directly to the LC-MS grade water bottle (1 L).

Table 3. LC settings

Parameter	Value
Mobile phase	25 mM ammonium acetate pH 5.4
Column storage solution	20 mM MES, 0.1% (w/v) sodium azide, pH 6.5
Injection wash solvent	20% ethanol
Sample load*	10 µg (2–20 µg).
Flow	0.3 mL/min
Column temperature	Setpoint: 20.0 °C, Acceptable range: 18.0–22.0 °C
Thermostatting mode	Still air
Pre-inject wash	100 s
Post-inject wash	100 s
Max. column pressure	220 bar (3190 psi) and 300 bars (4350 psi)
Autosampler temperature	Setpoint: 5.0 °C
UV detection primary wavelength (reporting)	280 nm
UV detection secondary wavelength (characterization)	214 nm
Data collection rate	4.0 Hz
Response time	1.00 s
Narrowest peak width	0.100 min
Length of MS data acquisition	Single column: 8 min Tandem LC setup: 4.7 min

* Injection volume depended on sample concentration (≤ 20 µL).

For both platforms, the Vanquish Duo UHPLC system is set up for tandem LC-MS. With this LC configuration, the analysis time is 4.7 min per sample, and data acquisition on the MS is active all the time. To eliminate time spent on loading samples and washing steps taking up otherwise utilizable acquisition time, a “PrepareNextInjection” command is executed 3.5 min into the run for the tandem LC column setup. More details on the tandem LC-MS setup using the Vanquish Duo system can be found in the previously published customer application note[1].

MS configuration

Platform 1. Native MS data was acquired on a Q Exactive Plus mass spectrometer using the settings shown in Table 4. For a detailed comparison of settings for the analysis under denaturing and native-like conditions please refer to the earlier application note[3]. The Vanquish Duo tandem column configuration allows for 100% MS utilization by continuously acquiring data while sample flow is resulting from alternating between columns.

Table 4. MS settings for Q Exactive Plus system

Scan parameter	Setting
Scan type	HMR – Full MS
Scan range (*m/z*)	2,500 to 8,000
Source fragmentation (V)	130
Resolution (at *m/z* 200)	35,000
Polarity	Positive
Microscans	10
Lock masses	Off
AGC target	3e6
Maximum inject time (ms)	200
H-ESI source	
Sheath gas flow rate	25
Aux gas flow rate	5
Sweep gas flow rate	0
Spray voltage (kV)	4.20
Capillary temp. (°C)	275
S-lens RF level (%)	200
Aux gas heater temp. (°C)	175

Platform 2. Native MS data was acquired on the Orbitrap Exploris 480 mass spectrometer equipped with the Biopharma option, which offers the utilization of the Intact Protein mode and enables mass detection up to *m/z* 8,000. Detailed MS settings are shown in Table 5.

Table 5. MS settings for Orbitrap Exploris 480 system

Scan parameter	Setting
Application mode	Intact protein
Pressure mode	High
Scan type	Full MS
Scan range (*m/z*)	2,500 to 8,000
Source fragmentation (V)	120
Resolution (at *m/z* 200)	45,000
Polarity	Positive
Microscans	10
Lock masses	Off
AGC target	3e6
Maximum inject time (ms)	200
H-ESI source	
Sheath gas flow rate	25
Aux gas flow rate	5
Sweep gas flow rate	0
Spray voltage (V)	4,200
Capillary temp. (°C)	275
S-lens RF level (%)	200
Aux gas heater temp. (°C)	175

Chromatography Data System

The Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, was used for data acquisition and data processing of the large datasets produced for the lead selection studies.

For data analysis supporting method development, data processing and reporting were performed using Byos™ software (Protein Metrics Inc.); settings used for intact protein deconvolution are shown in Table 6.

Results and discussion

Native SEC MS method optimization on the Orbitrap Exploris 480 MS

The native SEC-MS method using the Q Exactive Plus MS platform was performed with resolution setting 35,000 (at *m/z* 200) and with a consumed sample amount of 10 µg.

This sample consumption was lower compared to the amount needed for two separate analytical workflows for aggregate analysis and Intact Mass analysis (which was altogether 15 µg: 10 µg for SEC and 5 µg for intact MS).

To set up the new Orbitrap Exploris 480 MS for data acquisition and to generate comparable intact protein spectra on the Q Exactive Plus MS platform, different MS system settings needed to be evaluated, and methods with resolution settings of 7,500–60,000 (at *m/z* 200) were tested. For this the acquired maximum ion intensity (total ion chromatogram) and the resolution of the different peaks in the averaged raw data spectra, resulted in different resolution settings, were compared (Figure 2).

Table 6. Intact Mass deconvolution settings applied using the Protein Metrics Byos platform

Parameter	Setting
Basic	
Mass range	143,000–151,000
m/z range	3,000–6,500
Min difference between mass peaks (Da)	10
Max number of mass peaks	10
Peak sharpening	Disable sharpening
Spread function width (Da)	10.00
Advanced	
Deconvolution	
Charge vectors spacing	0.6
Baseline radius (*m/z*)	15
Smoothing sigma (*m/z*)	0.02
Spacing (*m/z*)	0.04
Mass smoothing sigma	3
Mass spacing	0.5
Iteration max	10
Charge range	5–40
Sharpening	
Blur skewness	1.10
Range	8.00
Blur type	Gaussian

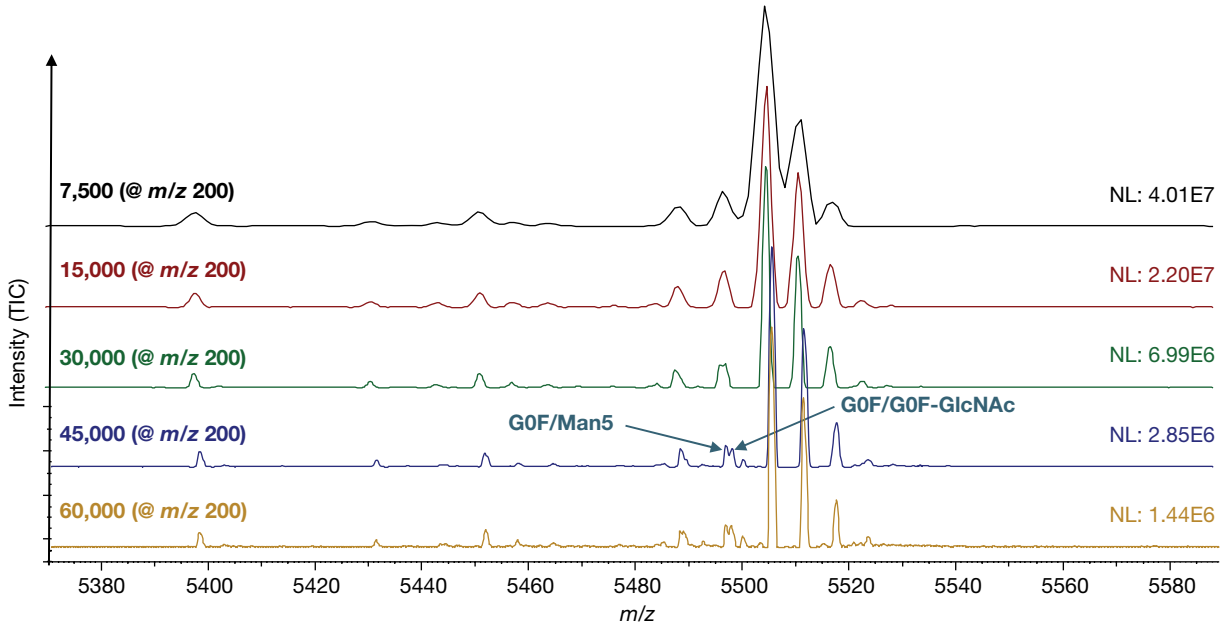

Figure 2. Intact protein MS data acquired at different MS resolution settings using the Orbitrap Exploris 480 platform. A zoomed spectrum of a single charge state (z = +27) of antibody used for SST is shown.

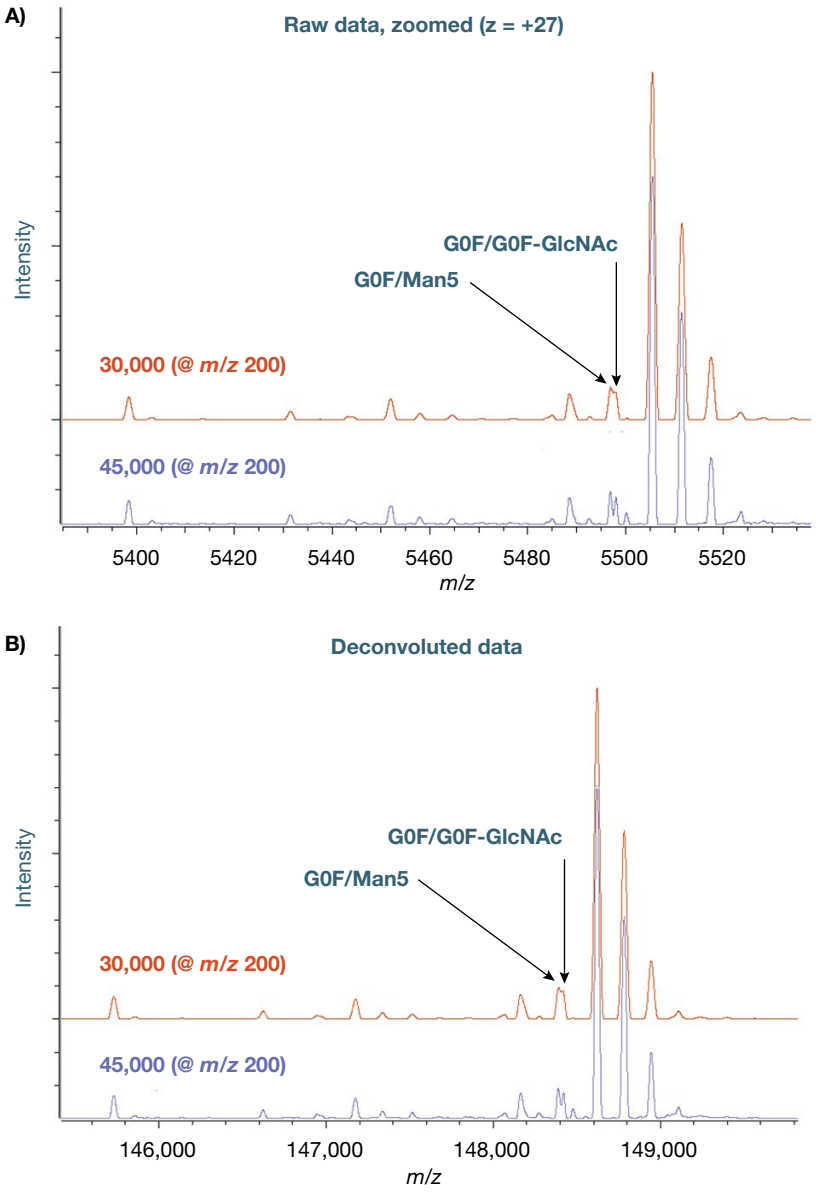

Figure 3. Intact protein MS raw (A) and deconvoluted (B) data acquired at MS resolution settings for 30,000 and 45,000 (at *m/z* 200 using the Orbitrap Exploris 480 MS). 45,000 resolution gives the best combination of spectral resolution and signal-to-noise ratio.

Analysis at resolution ≤30,000 (at *m/z* 200) did not provide sufficient spectral resolution for two glycoforms that are very close in mass and overlapping isotopic distribution (G0F/Man5 and G0F/G0F-GlcNAc, mass difference 25 Da). However, when using a resolution setting of 45,000 (at *m/z* 200), optimal spectral resolution was achieved. Further increasing the resolution to 60,000, however, did not improve spectral resolution of these glycoforms (due to the natural overlap in isotopic distribution), and a resolution setting of 45,000 was chosen for further analysis. The direct comparison of the raw and deconvoluted mass spectra (Figure 3) for the 30,000 and 45,000 resolution settings clearly shows that the 45,000 resolution provides the best combination of spectral resolution and signal-to-noise ratio, and also the clear assignment of the glycoforms is assured for both raw data and deconvoluted data.

As the follow-up step, the optimal sample injection amount and its impact on data quality were investigated. For SST runs, the antibody was injected with protein levels ranging from 1 to 10 µg of sample loaded on to the column. As shown in Figure 4A the data quality is consistently high. All data were deconvoluted and the relative quantitation of the different glycoforms compared between measurements. No trends are observed for the relative levels at different injection amounts, reliably consistent results are generated regardless of the sample amount (Figure 4B).

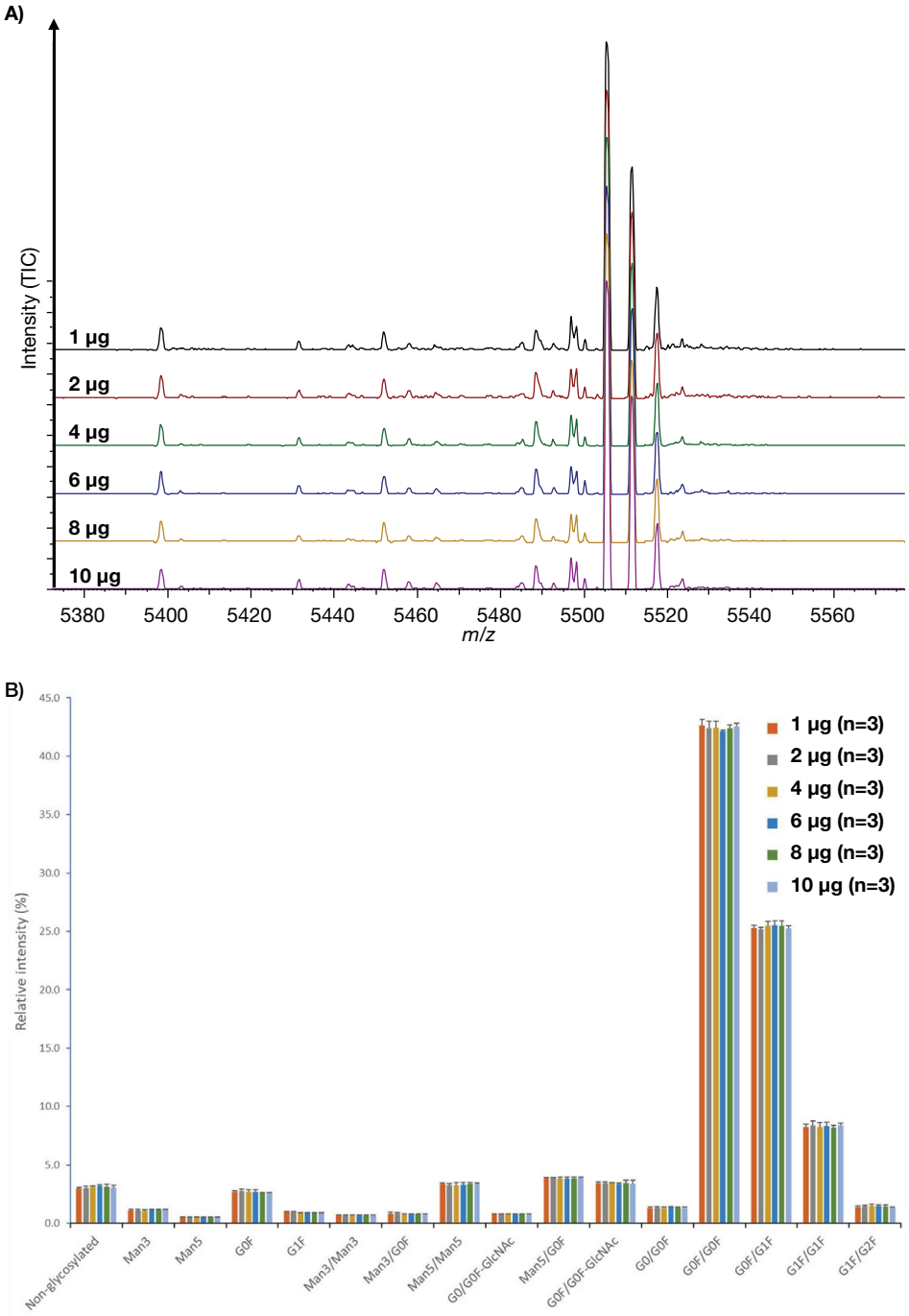

Figure 4. Intact protein MS data acquired with different sample injections using the Orbitrap Exploris 480 MS.
A) Zoomed spectrum of a single charge state (z = +27) for the different loading sample amounts of antibody used for SST loaded on column (1–10 µg). B) average relative quantities (n = 3) determined for the different glycoforms of the SST antibody.

Samples received for lead selection studies are provided with varying concentrations, often with concentrations of 0.1–1.0 µg/µL. For consistent sample injection on the Vanquish Horizon Duo UHPLC system taking sample concentration into account 4 µg was chosen as the target sample amount for most analyzed samples, as well as for the analysis of the antibody used for SST.

The antibody used for SST was analyzed using both the Q Exactive Plus MS, and the Orbitrap Exploris 480 MS, and the relative quantities of the glycoforms determined from the deconvoluted data were compared. The results are shown in Figure 5. The injection amount on the Q Exactive Plus MS platform was 10 µg, while on the Orbitrap Exploris 480 MS it was 4 µg, and the applied resolution settings 35,000 and 45,000 (at *m/z* 200), respectively. The relative glycoform levels are highly comparable on both systems, as shown in Table 7.

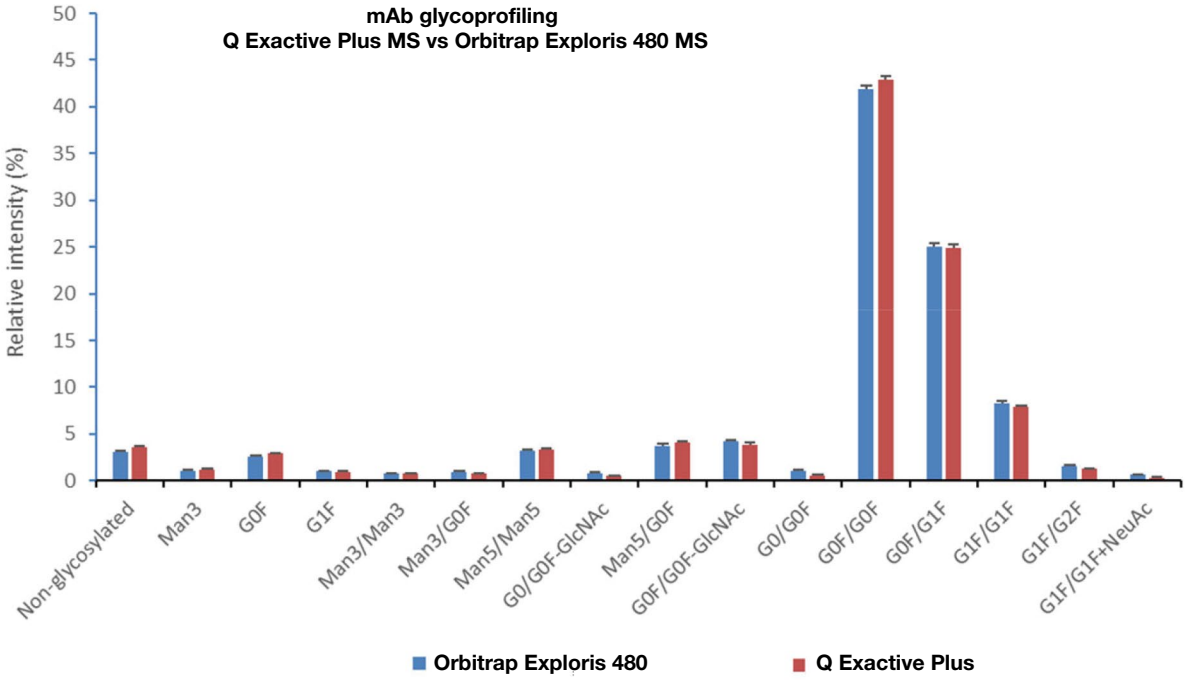

Figure 5. Results for relative quantitation of glycoforms from the Q Exactive Plus and Orbitrap Exploris 480 mass spectrometers were compared based on an average of three triplicates.

Table 7. Results for relative intensities of the different glycoforms as determined by using the two different MS platforms

	Q Exactive Plus MS	Orbitrap Exploris 480 MS
Load	10 µg	4 µg
Resolution (at *m/z* 200)	35,000	45,000
Acquistion date	January 2019	April 2020
Glycoform	**Relative intensity %**	**Relative intensity %**
Non-glycosylated	3.1	3.6
Man 3	1.1	1.2
G0F	2.6	2.9
G1F	1.0	0.9
Man3/Man3	0.8	0.8
Man3/G0F	1.0	0.8
Man5/Man5	3.3	3.4
G0/G0F-GlcNAc	0.8	0.5
Man5/G0F	3.8	4.1
G0F/G0F-GlcNAc	4.2	3.9
G0/G0F	1.1	0.5
G0F/G0F	41.9	42.9
G0F/G1F	25.0	24.9
G1F/G1F	8.2	7.9
G1F/G2F	1.6	1.3
G1F/G1F+NeuAc	0.6	0.3

Excellent reproducibility and platform robustness were demonstrated using both hybrid quadrupole Orbitrap platforms. The antibody for system suitability test is injected after at least every 24 mAb samples during each lead selection study analyzing hundreds of different antibodies (varies from 300 to 400 candidate mAb molecules per study). As shown in Figure 6, the results assessed for platform robustness show exceptionally high similarity between the MS platforms, with very low variability and %CV values demonstrated.

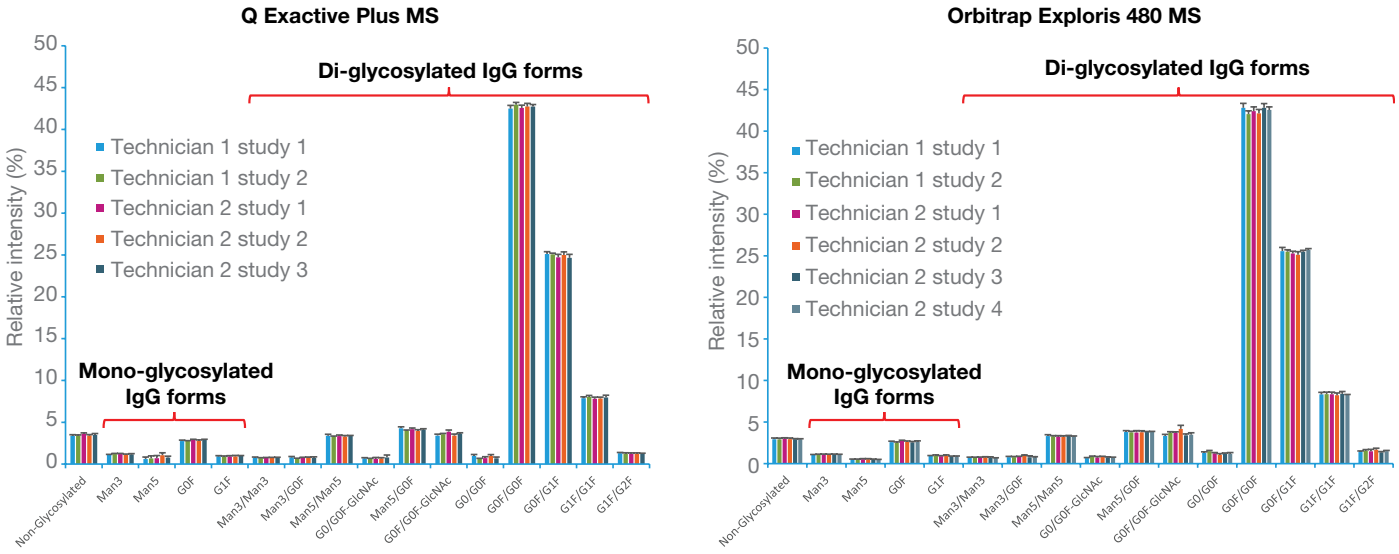

Figure 6. Assessment of robustness through investigation of glycoform level determination reproducibility. A) Q Exactive Plus MS data collected from 5 independent SEC MS sequences over a 6-month period (January – June 2019). Experiments were performed by two technicians. B) Orbitrap Exploris 480 MS data collected from 6 independent SEC MS sequences over a 7-month period (March – September 2020). Experiments were performed by two technicians.

Conclusions

At Symphogen, the implementation of native SEC MS using Vanquish Duo UHPLC has significantly reduced analysis time during early lead selection studies. Simultaneously, intact MS data of excellent spectral quality were obtained on the Q Exactive Plus MS system. When Symphogen upgraded to a new generation hybrid quadrupole Orbitrap MS platform, the Orbitrap Exploris 480 mass spectrometer, method parameters were systematically evaluated and optimized to generate the same high-quality data allowing the transfer of the execution of lead selection studies to the next generation platform. Here seamless method transfer was presented between these platforms, which both were used for the analysis of several hundred samples.

- Consistent high-quality data were acquired on both platforms, with consistently reliable and reproducible relative quantitation of glycoforms of the reference antibody.
- Method transfer between platforms was effortless and consistent: the same data acquisition software was used, Chromeleon CDS, and at the method optimization stage the same data processing software Protein Metrics' Intact Mass workflow was used, thereby avoiding system-specific, or user-induced errors.
- The new system offers increased resolution, increased sensitivity with lower sample injection amount requirements on the smallest Orbitrap-based benchtop HRAM MS system.
- The new platform when used for the analysis of hundreds of different antibodies in each lead selection study demonstrated the same system robustness as previously achieved on the Q Exactive Plus mass spectrometer.

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Acknowledgments

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