

Note d'application

## Simplified Routine QC Monitoring of Glycans Using ACQUITY™ QDa™ II Mass Detector

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Ce document est une note d'application et ne contient pas de section détaillée concernant l'expérimentation.

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### Abstract

Routine monitoring of glycans is crucial to pharmaceutical development, ensuring product quality, consistency, regulatory compliance, and patient safety. The ACQUITY QDa II Mass Detector provides a robust, comprehensive, and reliable alternative to traditional testing methods. In this overview, a simple, cost-effective analytical solution for routine QC monitoring of glycans was tested, demonstrating adequate performance within a challenging sample matrix.

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### Introduction

Glycans are complex carbohydrate structures, which when covalently linked to proteins and lipids can play an integral role in therapeutic and diagnostic processes. Precise and reliable analysis of glycan profiles is essential in these contexts, often requiring a comprehensive approach to fully elucidate both their structural and functional properties.

Typically, these workflows involve multi-step processes that leverage advanced mass spectrometry techniques;

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high-resolution mass spectrometry (HRMS) and tandem-quadrupole mass spectrometry (MS/MS) are commonly used for their selectivity and sensitivity advantages. These techniques can enable more detailed glycans characterization, providing important insights into their molecular composition and potential chemical modifications.

Advanced Mass spectrometry techniques are often associated with high operational costs and complexity, which can make routine monitoring challenging. Consequently, there is a growing interest in exploring cost-effective alternatives for these workflows, such as the ACQUITY QDa II Mass Detector, that may be better suited to high-throughput, robust applications, particularly in QC testing laboratories.

Ensuring consistency in QC workflows is necessary to determine therapeutic efficacy, regulatory compliance, and ultimately, patient safety. A cost-effective and reliable instrument, the ACQUITY QDa II Mass Detector (see Figure 1) offers an extended mass range of 30–1500  $m/z$ , as well as the sensitivity and robustness required for such routine pharmaceutical applications. Automated resolution, calibration, and tuning on start-up allow for simplicity and ease-of-use, reducing the burden on the analyst and facilitating more sustained instrument uptime.



*Figure 1. The ACQUITY QDa II Mass Detector and ACQUITY Premier UPLC configuration.*

In this study, a glycan fragment was monitored and quantified using the ACQUITY QDa II Mass Detector, using intentional in-source fragmentation to target a fragment ion with a mass of 844 Da. This targeted fragmentation approach offers a unique and reliable method for fingerprinting and monitoring larger glycan structures, making it well-suited for routine QC testing in a cost-effective manner. The workflow was evaluated for sensitivity, linearity, compound carryover, and overall method performance.

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## Results and Discussion

A method was developed using an unnamed 20 µg/mL glycan sample. The instrument was operated in positive

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electrospray ionization (ESI+) mode with an optimized cone voltage of 35 V, a capillary voltage of 1.2 kV, and a desolvation temperature of 600 °C. The fragment ion of interest was monitored at  $m/z$  845 using selected ion recording (SIR) mode. Simultaneously, a full scan acquisition (total ion current, TIC) from  $m/z$  150 to 1500 was performed to evaluate the overall elution profile of the protein structure. An example of the data from these two monitoring channels is shown in Figure 2.

## Chromatographic Separation

An ACQUITY Premier Protein BEH™ C<sub>4</sub> 300 Å 1.7 μm, 2.1 x 50 mm Column ([186010326 < https://www.waters.com/nextgen/global/shop/columns/186010326-acquity-premier-protein-beh-c4-300-a-17--m-21-x-50-mm-1-pk.html >](https://www.waters.com/nextgen/global/shop/columns/186010326-acquity-premier-protein-beh-c4-300-a-17--m-21-x-50-mm-1-pk.html) ) was selected and a 30 °C column temperature was found to be optimal using a 1 μL injection. An ACQUITY Premier LC System was also used in this workflow, with the FTN (flow-through needle) design helping to reduce sample dispersion and carryover.

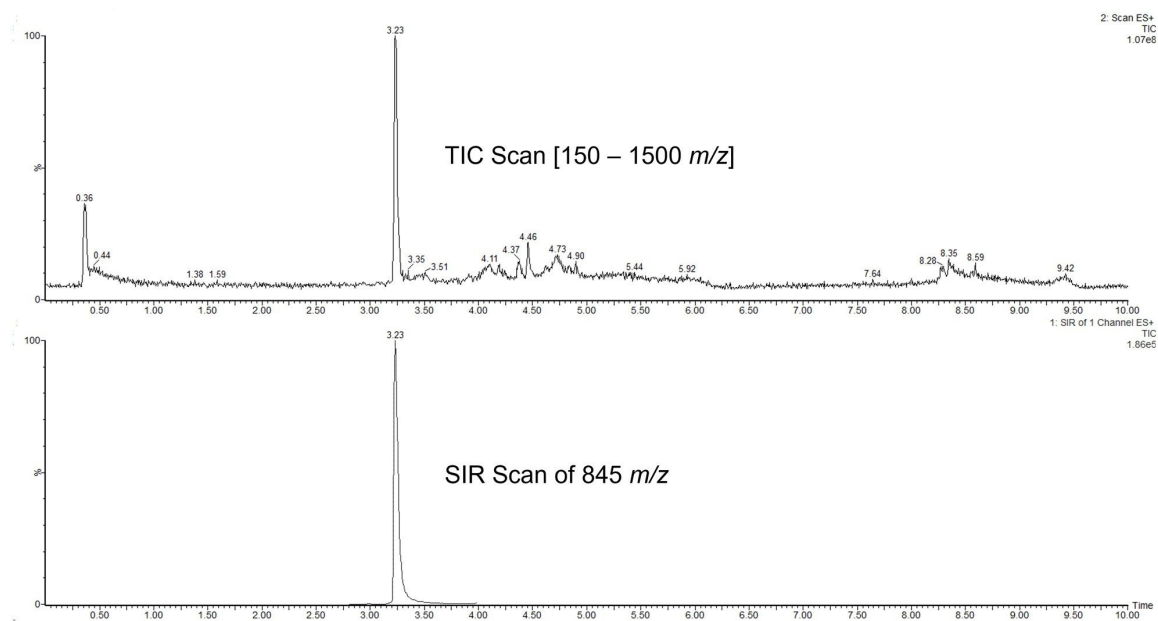


Figure 2. Stacked chromatogram plots showing a TIC Scan from  $m/z$  150–1500 and an SIR channel monitoring  $m/z$  845 in positive (+ve) electrospray ionization mode, for the same injection of a 20 μg/mL glycan sample.

## Carry-over Assessment

Reducing carry-over was a critical aspect of this work. To address this, fragment ion carry-over was assessed, with the method modified to minimize carry-over in subsequent blank injections. Incorporating Isopropyl alcohol (IPA) into the composition of the mobile phase was pivotal in this solution, reducing carry-over in solvent injections immediately following the sample to less than 1%, while maintaining the instrument's response to the compound (see Figure 3).

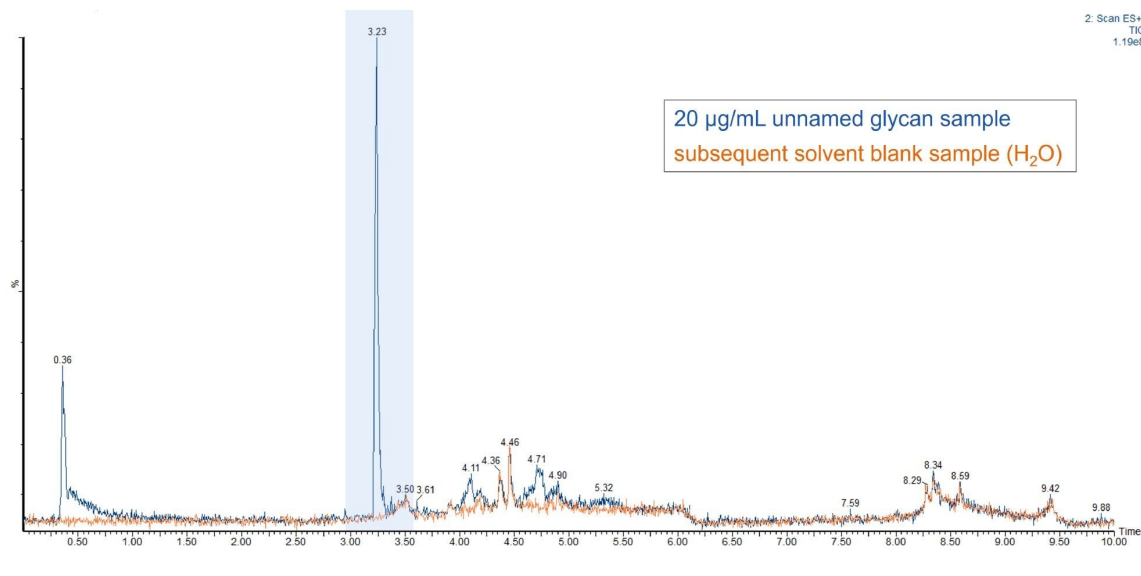


Figure 3. Overlaid TIC chromatograms of a 20 µg/mL injection of an unnamed glycan sample (blue) and the subsequent solvent blank injection (orange).

## Linearity

The peak area response of the fragment ion of  $m/z$  845 was linear over a calibration range of 2–20 µg/mL (corresponding to 2–20 ng of glycan sample on column) with a coefficient of determination  $R^2 = 0.99$ . No internal standard correction was used. The corresponding residual values for any given datapoint did not exceed 12%, as shown in Figure 4. Precise and reliable quantitation offers the possibility of accurate routine determination of this fragment ion.

Compound name:  
Correlation coefficient:  $r = 0.993711$ ,  $r^2 = 0.987462$   
Calibration curve:  $418.759 * x + -593.705$   
Response type: External Std, Area  
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

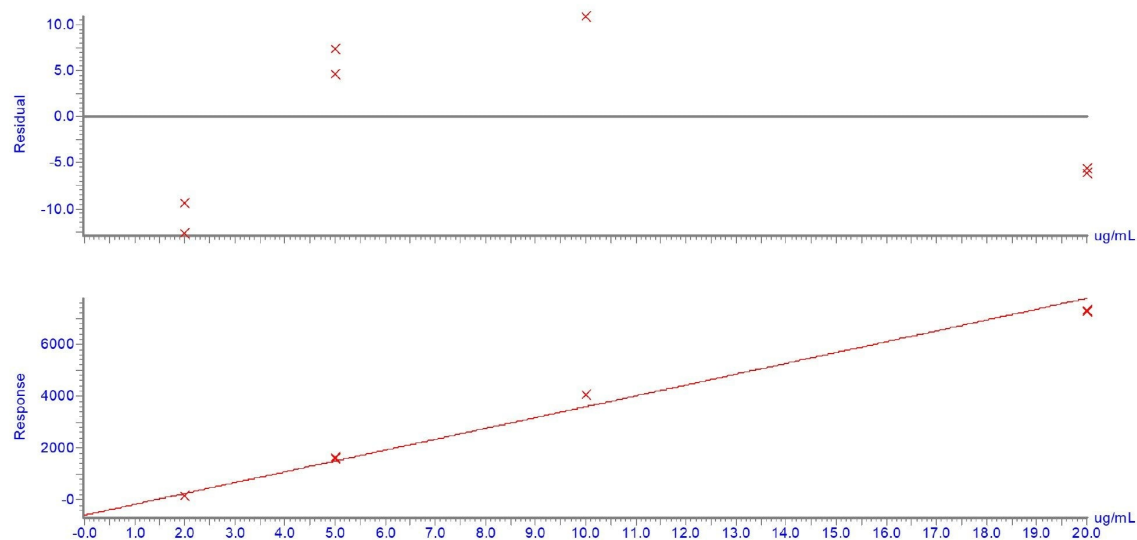


Figure 4. Calibration response of the  $m/z$  845 fragment ion over a concentration range of 2–20  $\mu\text{g/mL}$ . Residual values (top) as well as calibration curve (bottom) are shown.

## Quantitation

A sample was also assessed at 1  $\mu\text{g/mL}$  (corresponding to 1 ng on-column) giving a signal-to-noise ratio of 102:1 (S/N as peak to peak) which can be seen in Figure 5.

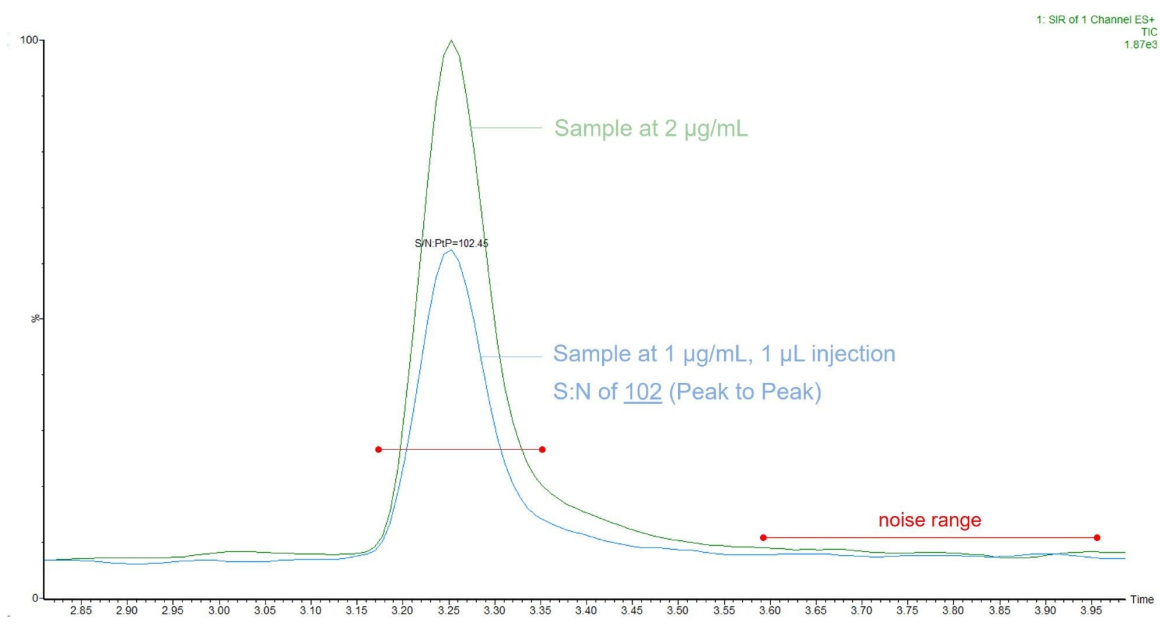


Figure 5. An SIR ( $m/z$  845) chromatogram of a  $1 \mu\text{g/mL}$  glycan sample with a  $S/N$  (peak to peak) of 102, with signal and noise regions selected shown in red.

## Conclusion

The ACQUITY QDa II Mass Detector is a compact and stackable device designed for seamless integration with LC systems. It is user-friendly, robust, and cost-effective, making it an ideal choice for routine glycan monitoring. It offers:

- Adequate sensitivity, detecting glycan fragments ions at  $1 \text{ ng}$  on-column in challenging matrices
- Robust performance, handling challenging matrices with reduced carryover and ensuring accurate quantitation
- Linear response for glycan fragment ion over a concentration range of  $2$  to  $20 \mu\text{g/mL}$
- High-throughput capability to support efficient quality control for complex proteins, reducing the need for more expensive equipment

- Reduced carryover and accurate quantitation of challenging matrices by employing the ACQUITY Premier Protein BEH C<sub>4</sub> Column, and ACQUITY Premier LC System
- Compatibility with both MassLynx™ and Empower™ Software for seamless integration into QC labs

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