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Adding MS Detection to the Analysis of Small Biotherapeutics Using Waters ACQUITY QDa Detector

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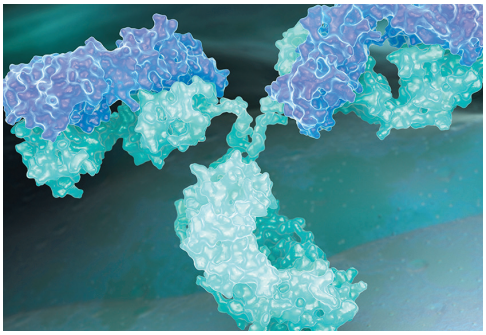

GOAL

To demonstrate the applicability of the Waters® ACQUITY® QDa® Detector in providing orthogonal mass data for a diverse sample set of small biotherapeutics.

BACKGROUND

As part of a sound pharmaceutical quality system, the evaluation of new and innovative technologies is encouraged to identify opportunities to improve and expand the body of knowledge surrounding a product. Conventional LC-UV based workflows represent one area that could benefit from the implementation of new technology to enhance product knowledge. To ensure products are safe and efficacious, LC-UV based methods are often complemented with orthogonal techniques which can impact productivity. Mass spectrometry (MS)-based techniques, which offer enhanced sensitivity and specificity for measuring product quality attributes, have increasingly been deployed in analyses throughout the product lifecycle to address these challenges. High resolution MS instrumentation, however, often requires expert users for instrument operation and data interpretation. Therefore, it is highly valuable to employ orthogonal detection techniques, such as mass detection, that allows scientists without extensive MS training to operate and can be readily adapted to existing LC-UV workflows to increase confidence in data interpretation for improved productivity in biotherapeutic analyses.

The ACQUITY QDa Detector enables the acquisition of orthogonal mass data across a wide range of small biotherapeutics as an in-line mass detector.

Figure 1. The ACQUITY QDa Detector. The compact footprint of the ACQUITY QDa Detector allows for easy integration into existing instrument stacks. Its plug-and-play design allows for the implementation of complementary orthogonal detection techniques with minimal effort into a single integrated system and workflow.

THE SOLUTION

The ACQUITY QDa Detector's compact design (Figure 1) and straightforward instrument interface allows for simultaneous acquisition of optical and MS data in LC-UV-based workflows for enhanced assay performance and improved productivity. The ACQUITY QDa Detector has been established as a fit-for-purpose mass detector with successful deployment in regulated environments for the monitoring of critical quality attributes associated with biotherapeutics such as monoclonal antibodies.

To demonstrate its applicability to a broader set of biotherapeutics: insulin, interferon, and (HGH) were selected as model analytes that span a molecular weight range from 5 kDa to 22 kDa. The separations were

performed and optimized on an ACQUITY UPLC® H-Class Bio System equipped with a Tunable Ultra-Violet (TUV) detector followed by the ACQUITY QDa Detector. Formic acid (0.1% v/v) was used as mobile phase modifier due to its compatibility with MS detection. As shown in Figure 2, insulin and HGH were resolved from their low abundance impurities, while interferon appeared as one peak with the TUV detection. The increased sensitivity afforded by the ACQUITY QDa Detector is apparent in the increased detector response of the low abundance impurity peak of insulin.

As shown in the zoomed-in chromatogram, the SNR was increased from 13.8 to 60.2 using the ACQUITY QDa Detector. The implementation of the ACQUITY QDa Detector as an orthogonal detection technique allows for the determination of the molecular weight for analytes and adds a level of confidence in data interpretation. Although the mass range of the ACQUITY QDa Detector is only up to 1,250 amu, which does not cover the ions with lower charge states, the observed *m/z* values within the mass window are sufficient for the calculation of molecular weights. The mass of insulin, interferon, and HGH were calculated to be 5,807 Da, 16,466 Da, and 22,120 Da, respectively, confirming the mass of the three biotherapeutics. The mass accuracy was within 0.03% for all three samples. Collectively, these results demonstrate that the ACQUITY QDa Detector provides an efficient solution to improve assay sensitivity and specificity; while providing orthogonal data that expands process knowledge and increase confidence in the product quality of small biotherapeutics.

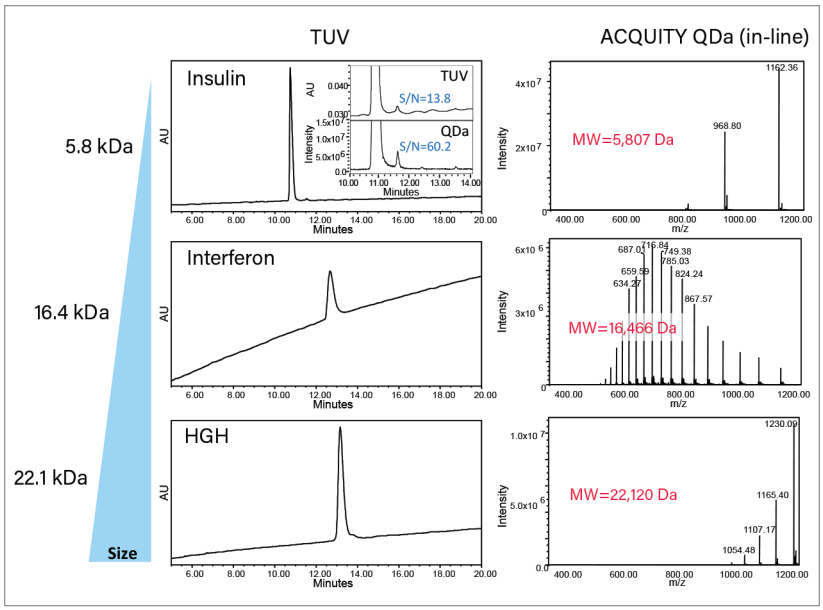

Figure 2. Optimized separation for three representative therapeutics: insulin, interferon, and HGH with the masses of 5.8 kDa, 16.4 kDa, and 22.1 kDa. Column: ACQUITY Peptide CSH™ C_{18} Column (100 × 2.1 mm, 130 Å) for insulin, ACQUITY BEH™ C_4 Column (100×2.1 mm, 300 Å) for interferon and HGH.

SUMMARY

Orthogonal techniques that add value and can be employed with minimal cost and effort are highly desirable in the pharmaceutical industry. This technical brief has demonstrated how new technologies, such as the ACQUITY QDa Detector, can be incorporated into existing workflows for improved product knowledge, which is in accordance with ICH Q10. As an orthogonal detection technique, the ACQUITY QDa Detector improved assay sensitivity and specificity of biotherapeutics that ranged in molecular weight from 5 kDa to 22 kDa. The ACQUITY QDa Detector as an in-line detector enables the simultaneous acquisition of MS information with optical data in LC-UV based workflows for deeper product understanding without compromising productivity.

References

1. ICH Q10, Pharmaceutical Quality System. Continual Improvement of Process Performance and Product Quality. April 2009.

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