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Interpreting MS Spectra Acquired Within Empower 3 Using the ACQUITY QDa Mass Detector

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates to how the export functionality within the Empower CDS can be leveraged in the interpretation of complex mass spectra data.

Benefits

Empower 3 supports the ability to directly export MS spectral data for increased flexibility in data interpretation.

Introduction

Interpretation of mass spectral data acquired within the Empower CDS can be straightforward when analyzing small analytes that produce relatively "simple" mass spectrum with a limited number of charge states as shown in Figures 1A and 1B. Albeit a manual process, nominal mass of the analyte can be determined from the Empower MS spectra using the formula for mass per charge (m/z), or $m/z = (M + zA)/z$, which can be applied to the individual m/z charge states in the spectrum. For routine monitoring or samples that consistently produce the same charge states, custom fields can be programmed to automate this calculation to determine nominal mass. While this approach works for simple or moderately simple molecules, more complex samples such as the one shown Figure 1C would benefit from deconvolution algorithms to efficiently process MS spectra. Conveniently, Empower 3 supports the ability to directly export spectral data for increased flexibility in the interpretation of MS data for improved productivity in the lab.
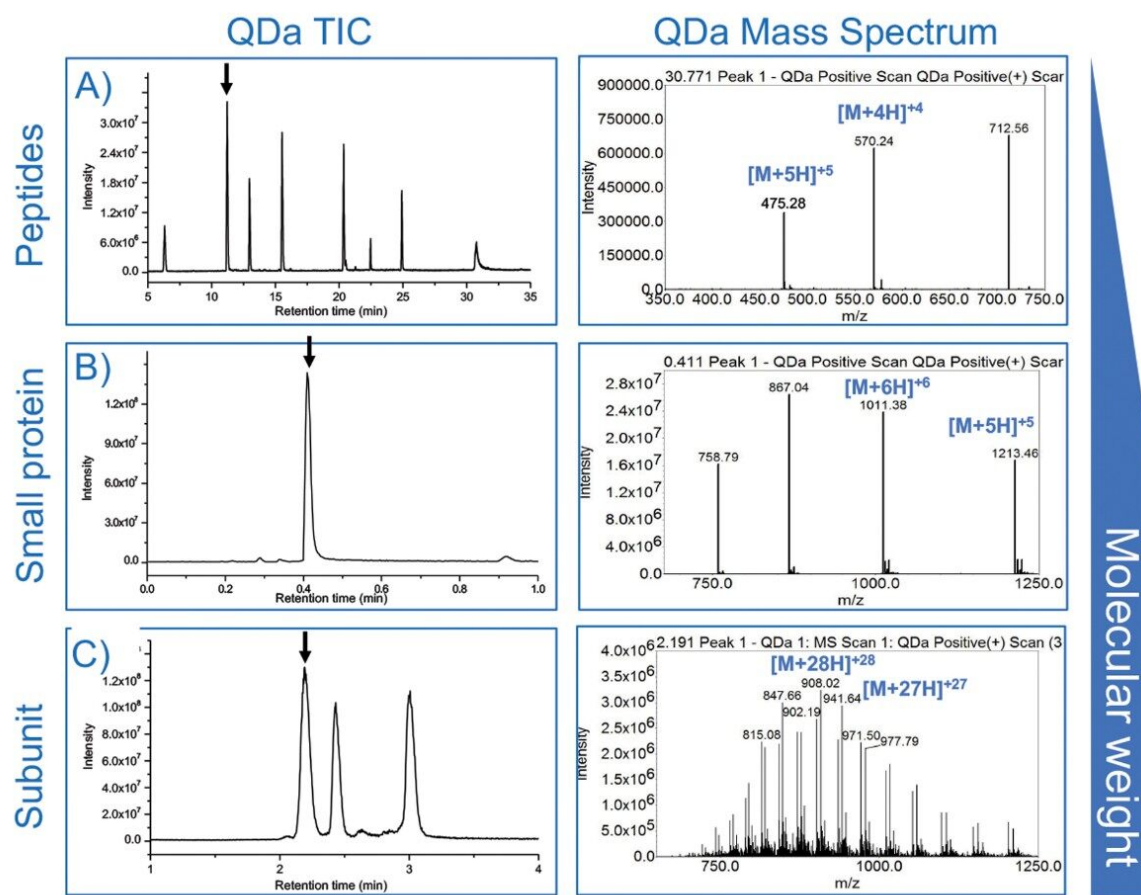
Figure 1. Sample selection. The samples evaluated included (A) Waters MassPREP Peptide Standard, (B) insulin glargine, and (C) reduced NIST mAb subunits. These samples were chosen to give a range of molecular weights from 899.0 Da to 25.0 kDa.

Results and Discussion

Empower 3 allows for the direct export of MS spectral data via the integrated spectrum review window within Empower for increased flexibility in data interpretation. As shown in Figure 2, once mass spectra data is acquired, spectral information such as individual m/z and associated intensities can be accessed via the "Spectrum Points" tab. Using the Window's "copy" keyboard shortcut, "Ctrl+C," the spectral data can be exported and saved as text or in a spreadsheet for further processing and interpretation.
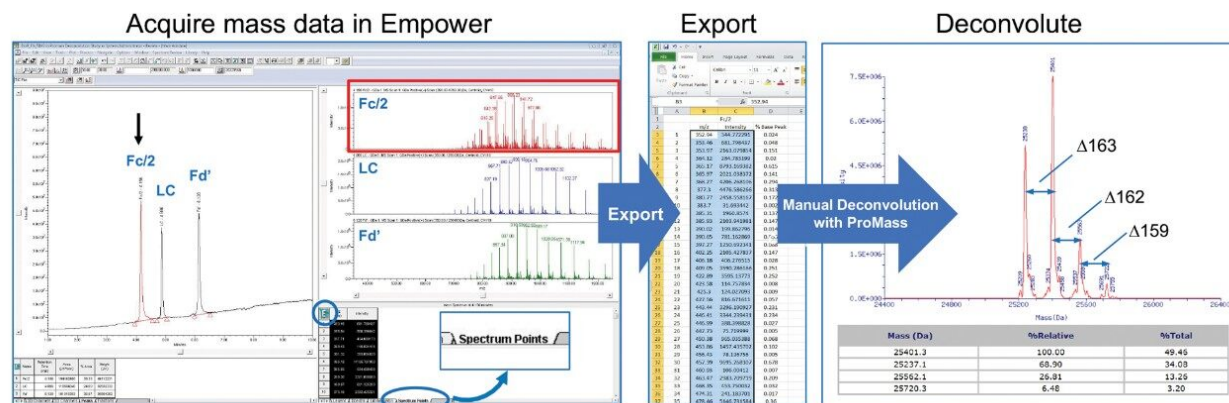
Figure 2. Empower3 direct export. Mass spectrum data acquired using either the ACQUITY QDa or ACQUITY SQD2 under the control of the Empower 3 CDS Software can be directly exported for manual deconvolution. In this example, mass spectrum data from the NIST mAb reduced subunits (Fc/2, LC, and Fd') were acquired using Empower 3 on a ACQUITY QDa Mass Detector. The data was manually exported from Empower 3 to a spreadsheet, from where the m/z and spectrum intensity data was exported to a third-party software for manual deconvolution.

As shown in the far-right panel of Figure 2, MS data acquired using the ACQUITY QDa was deconvoluted using the ProMass software by Novatia to determine the relative abundance and nominal mass of the main glycoforms of the Fc/2 form of a reduced mAb subunit sample. In addition to being able to process complex spectra, ProMass is capable of deconvoluting relatively simple spectra as well as the ones generated from peptides or small proteins such as insulin. Given this, the Waters MassPREP Peptide Standard, insulin glargine, and IDES digested NIST mAb (Figure 1) were chosen to assess the applicability and accuracy of the method over a wide range of molecular weights with MS spectra of varying complexity. As shown in Table 1, the deconvoluted mass data produced by manually exporting spectra to ProMass was within instrument specification of +/- 0.2Da × z, with the larger NIST mAb subunits exhibiting higher mass difference due to ionizing at higher charge states (z).

Compound	Expected mass (Da)	Observed mass (Da)	Mass (Da)
Angio. Frag. 1-7	899.0	898.9	0.1
Bradykinin	1060.2	1060.1	0.1
Angiotensin II	1046.2	1046.3	-0.1
Angiotensin I	1296.5	1296.4	0.1
Renin	1759.0	1759.0	0.0
Enolase T35	1873.0	1873.0	0.0
Melitin	2846.5	2846.6	-0.1
Insulin	6062.9	6063.0	-0.1
Fc/2 : FA1	25236.0	25237.1	-1.1
Fc/2 : FA2G1	25398.1	25401.0	-2.9
Fc/2 : FA2G2	25560.3	25562.1	-1.8
Light Chain (LC)	23127.5	23126.0	1.5
Fd'	25688.9	25687.0	1.9

Table 1. Deconvoluted mass comparison. Mass spectra acquired under Empower 3 were manually deconvoluted using third-party software as illustrated in Figure 2. All deconvoluted masses were within instrument specification.

Conclusion

Empower 3 allows for the direct export of MS spectral data via the integrated spectrum review window within Empower for increased flexibility in data interpretation. This functionality is useful in LC-MS workflows, particularly for applications involving critical peaks with complex mass spectra, where information about the presence and relative quantity of a peak's glycoforms is not discernable from mass spectra alone.

Featured Products

Empower 3 Chromatography Data Software <https://www.waters.com/10190669>

ACQUITY QDa Mass Detector <https://www.waters.com/134761404>

SQ Detector 2 <https://www.waters.com/134631584>