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Application Note

Enhanced In-Spectrum Dynamic Range of the Xevo G2 QTof: Improving Peptide Quantitation and Identification in LC/MS Peptide Map Analysis

Waters Corporation

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

Abstract

Demonstrate enhanced in-spectrum dynamic range and accurate mass measurements that are achievable using the Xevo™ G2 QTof for peptides spanning four orders of magnitude in concentration during routine UPLC/MS peptide mapping analysis.

Benefits

Using automated data processing and informatics to recognize the low-level and coeluting labeled peptide with high mass accuracy demonstrates the power of the Xevo G2 QTof for routine characterization of protein therapeutics

Introduction

Peptide mapping using LC/MS is a powerful methodology that is routinely applied by the biopharmaceutical industry to detect and identify minute variations in biotherapeutic proteins. Information acquired from peptide mapping is useful to demonstrate product quality and consistency, and can further be applied to identify new product-related impurities that arise during development. The ability to identify and quantify these product impurities relies on proper mass spectrometric detection and data processing for peptides and modified peptides that vary widely in concentration and ionization efficiency. Such analyses require simultaneous quantitative and qualitative analysis of high-abundance product peptides and the low-level impurity peptide variants arising from imperfections in the biology of product production or post-translational chemical modifications of the biotherapeutic. The complexity and dynamic range of peptide variants are further coupled with challenges introduced by the ESI-MS process. In-spectrum dynamic range becomes critically important to provide accurate mass measurements not only for peptides with highest MS response, but also for peptides that ionize with 1.0% or even 0.1% efficiency of the best ionizing peptides. Without sufficient in-spectrum dynamic range, the instrument will fail to routinely detect these critical quality-indicating or stability-indicating peptides

Results and Discussion

The Xevo G2 QTof is capable of accurately measuring the mass of a peptide, within a monoclonal antibody LC/MS peptide map, spanning four orders of magnitude in concentration. An isotopically-labeled peptide analog that bears an exact sequence match to a tryptic peptide (IYPTNGYTR) within a therapeutic antibody was synthesized and serially spiked in to the digested antibody (50 pmol/µL) at levels spanning four orders of magnitude lower than the unlabeled peak (5 fmol/µL). The spiked digest was separated on an ACQUITY UPLC® BEH 300 C_{18} Column with a 90-min gradient, and LC/MS^E peptide mass and fragmentation data were acquired on a Xevo G2 QTof in the ESI positive resolution mode of the instrument, using GFP as a lockmass reference compound. Due to the identical sequences, these two peptides coelute during chromatographic separation but differ by about 10 Da (5 m/z for the 2^+ charge state) from the stable isoptope-labeled Arg on the spiked-in peptide. Data processing was accomplished using manual analysis in MassLynx™ Software or automated analysis using BiopharmaLynx™, a MassLynx Application Manager supporting automated peptide map and intact protein LC/MS analysis workflows.

Figure 1 shows the MassLynx summed spectrum for the UPLC/MS peak corresponding to the peptide, IYPTNGYTR, highlighting the 2^+ charge state of the unlabeled and the spiked-in stable isotope labeled synthetic

analog. The results clearly indicate that high mass accuracy was maintained despite the vast range of peptide intensities. More importantly, both peptides were properly identified with a common retention time and superior mass accuracies for both versions of the peptide (Figure 2) using the advanced processing capabilities of BiopharmaLynx.

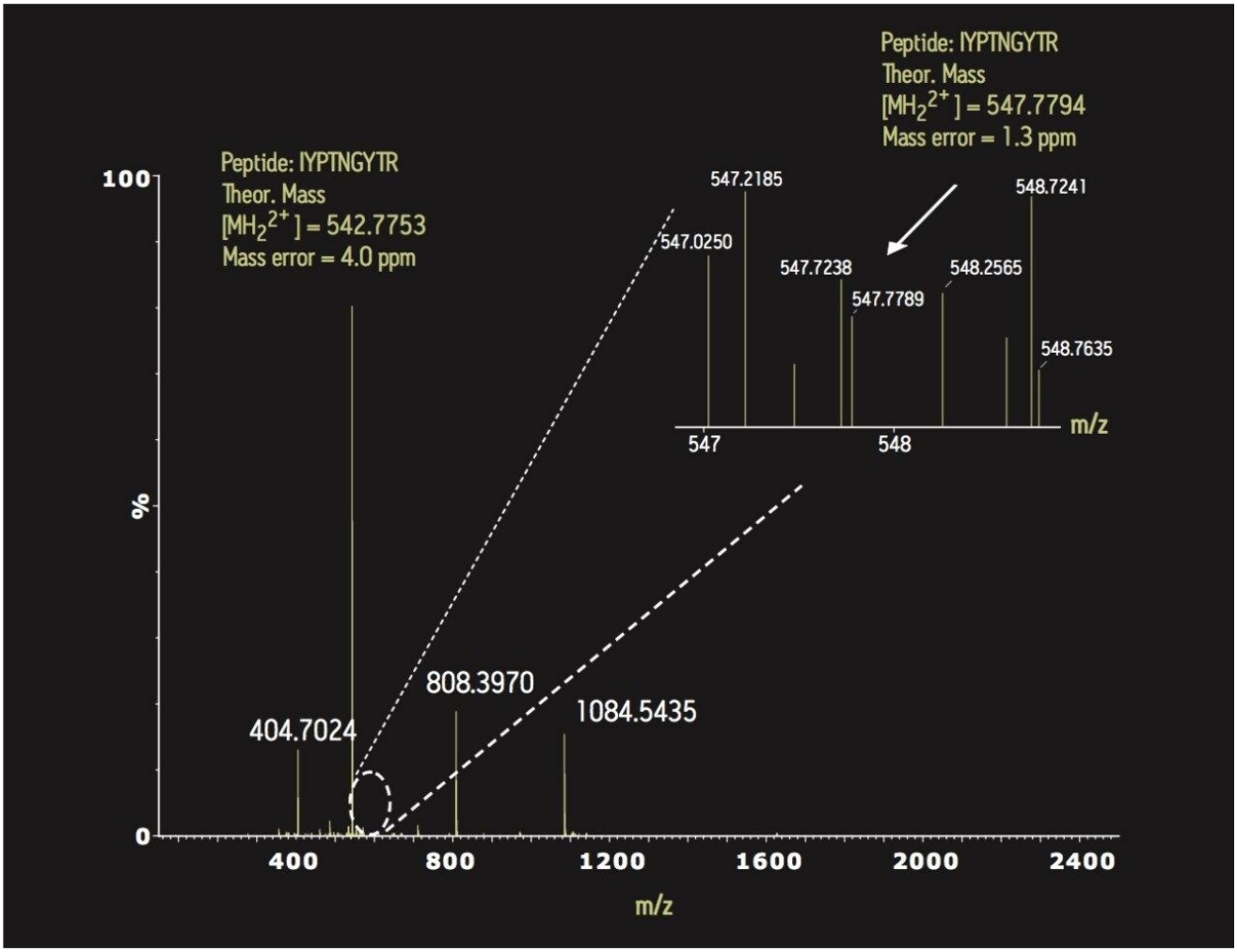

Figure 1. Summed spectrum for the UPLC/MS peak corresponding to the peptide, IYPTNGYTR, highlighting the 2 $^{+}$ charge state of the unlabeled and the spiked-in stable isotope labeled synthetic analog.

	Protein	Peptide	Fragm...	Start	End	Modifiers	▲1 ...	Control Inte...	Control Mass Error (ppm)
1	Trastuzumab Heavy Chain	IYPTNGYTR	1:T6*	51	59	Isotopically Labeled Arg(1)	14.3	353.0	-2.4
2	Trastuzumab Heavy Chain	IYPTNGYTR	1:T6	51	59		14.3	1101177.0	-0.2
3	Trastuzumab Heavy Chain	IYPTNGYTR	1:T6*	51	59	Deamidation N(1)	15.2	613769.0	1.8
4	Trastuzumab Heavy Chain	IYPTNGYTR	1:T6*	51	59	Deamidation N(1)	16.5	160632.0	1.6

Figure 2. BiopharmaLynx processes and assigns identity to lowest level map components.

The ability to use an automated data processing and informatics workflow to recognize the low-level and coeluting labeled peptide with such high mass accuracy demonstrates the enhanced utility of the Xevo G2 QTof and its QuanTof™ hybrid ADC mass detector for routine characterization of protein therapeutics. This enables organizations to maximize their investment in innovative technology and reduces the average time scientists spend analyzing peptide maps for biotherapeutic proteins by days to weeks.

Conclusion

The enhanced in-spectrum dynamic range of Xevo G2 QTof mass spectrometer enables the system to analyze a digested protein sample that contains components across a wide dynamic range of concentrations. When coupled with the appropriate informatics tools, such as BiopharmaLynx, the Xevo G2 QTof offers a powerful solution for comprehensive biotherapeutic protein characterization.

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