

Applying Improved TOF Mass Resolving Capability to Enhance the Characterization of Therapeutic Antibodies in Middle-Up and –Down Workflows



CASSS AT Europe 2015, P-126

W. Jabs¹, A. Resemann¹, A. Wiechmann¹, W. Imhoff¹, W. Evers¹, C. Evans², G. Tremintin³, R. Hartmer¹, D. Suckau¹, E. Wagner-Rousset⁴, A. Beck⁴

¹ Bruker, Bremen, Germany

² Bruker, Coventry, UK

³ Bruker, Fremont, CA

⁴ Pierre Fabre, Saint Julien en Genevois, France

Introduction

Reducing the inter-chain disulfide bonds of a mAb frees the light (LC) and heavy chains (HC) so their molecular weight and their amino-acid sequences can be analyzed independently by intact mass analysis and by Middle-Down (MD) sequencing. This analysis is routinely carried out to detect amino-acid sequence variations and post-translational modifications (PTMs). Here we extend the specificity of this workflow by deploying MD MALDI-MSD for high quality identification of sequence variations in combination with a new ultrahigh resolution (UHR)-ESI QTOF providing monoisotopic mass determinations of HCs with no speed or dynamic range compromise.

Methods

- Four mAbs covering the IgG subclasses on the market were investigated: adalimumab, cetuximab (IgG1), panitumumab (IgG2), and natalizumab (IgG4)
- mAbs were reduced with TCEP and measured using standard chromatography coupled to a maXis II (Bruker) providing a mass resolution of 80,000
- LC and HC raw spectra were deconvoluted using Maximum Entropy deconvolution. From the deconvoluted spectra monoisotopic masses were automatically determined using the SNAP algorithm
- MALDI Top-Down Sequencing (TDS) with an ultrafleXtreme (Bruker) was applied to localize sequence errors or PTMs detected in the middle-up experiments

Results

- Fig. 1 and Fig. 2 show that measured masses and isotopic pattern for the HCs of adalimumab, cetuximab and panitumumab are in perfect agreement with the expectations resulting from their amino-acid sequences

Fig. 4 MD-MALDI spectrum of the natalizumab HC. The continuous c-ion series up to residue 133 is unequivocally providing a match to the Wang sequence.

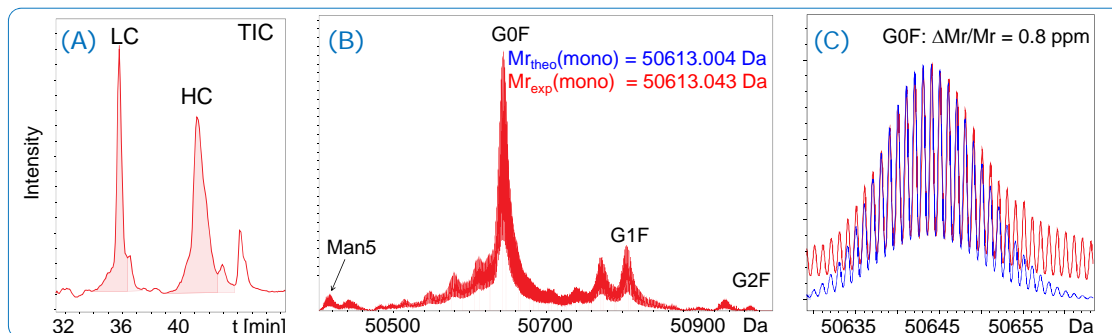


Fig. 1 Adalimumab: (A) Total Ion Chromatogram (TIC), (B) deconvoluted HC spectrum showing the glycosylation profile and the theoretical and measured monoisotopic masses of the G0F glycoform, (C) zoom-in of the G0F glycoform with measured (red trace) and theoretical (blue trace) isotopic patterns.

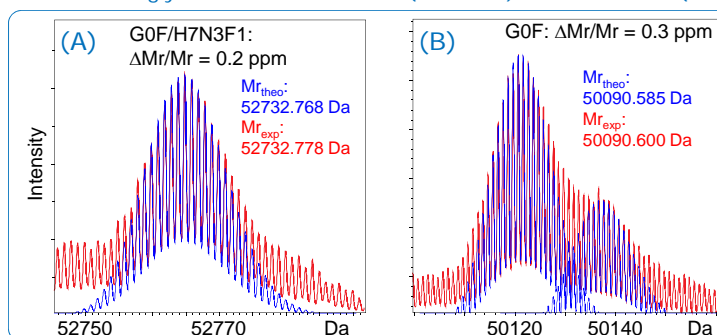


Fig. 2: Cetuximab HC: (A) G0F/H7N3F1 glycoform; (B) Panitumumab HC: G0F glycoform. Measured (red trace) and theoretical (blue trace) isotopic patterns are shown for both glycoforms. For panitumumab oxidation is observed as indicated by the second theoretical peak pattern.

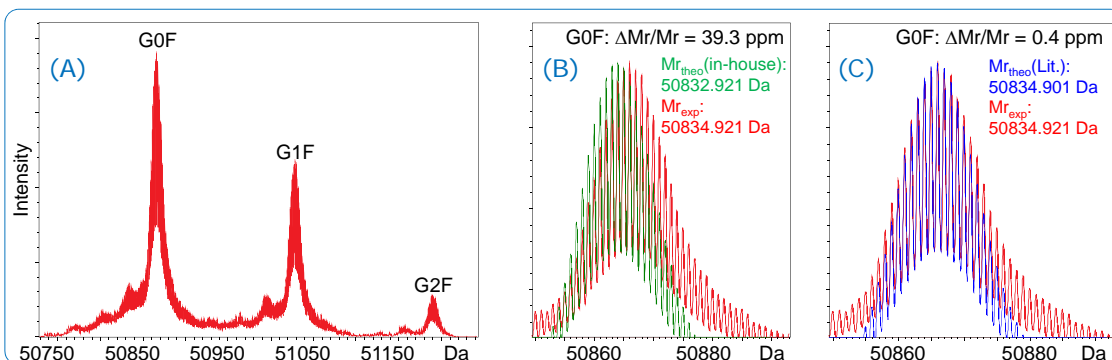
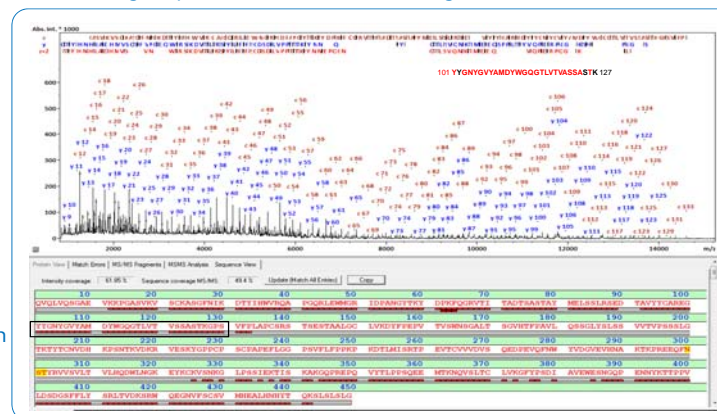


Fig. 3: Natalizumab: (A) HC spectrum with glycosylation profile. (B), (C) Zoom-in of the G0F glycoform with measured (red trace) and expected isotopic patterns. The expected pattern derived from an in-house sequence (green trace, B) is shown in comparison with the expected pattern derived from a sequence published by Wang et al. (blue trace, C). The high mass accuracy and perfect match of the isotopic patterns for the Wang sequence allowed the unambiguous confirmation of the latter sequence.



Results (cont.)

- For natalizumab an in-house determined sequence was used for theoretical mass and isotopic pattern calculation resulting in significant deviations (Fig. 3B)
- Using an alternative natalizumab sequence published by Wang et al. (mAbs 1(3), 254-67, 2009) results in the same perfect match of expected mass and isotopic pattern as observed for the other three mAbs (Fig. 3C)
- MD-MALDI analysis further validated the Wang sequence as correct sequence (Fig. 4)

Conclusions

- Latest TOF technology allows for isotopic resolution of HCs in routine LC-MS workflows
- Using four representative therapeutic antibodies, an average mass deviation of 0.4 ppm was obtained for the monoisotopic MWs of HCs
- This accuracy allows to confidently distinguish sequence candidates and PTMs on reduced mAb level

Accurate MW UHR-QTOF MD-MALDI Sequencing