Poster Note PN-16

Rapid Detection of Deamidation in Monoclonal Antibodies using Ultrahigh-Resolution QTOF Mass Spectrometry

Introduction

Characterizing the heterogeneity occurring in therapeutic antibodies (mAbs) is a fundamental task of all product development and commercialization phases. Asn deamidation is a particularly important source of heterogeneity, however challenging to detect on intact proteins given the +0.984 Da mass addition, and currently is characterized with bottom-up approaches. As proteolytic digests take considerable time and can introduce additional method-related deamidation, we developed a rapid method that utilizes middle-up antibody fragment isotopic distributions to detect, identify, and profile the degree of antibody deamidation while minimizing risks of artificial deamidation. The method was developed and tested on a model system for deamidation and then applied to a partially deamidated antibody light chain (LC).

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Methods

A model system for deamidation of biologics was generated in the following way:
Adalimumab (Abbvie) was partially reduced with DTT – only interchain disulfide bonds are cleaved.

- Sample was split into two aliquots – one aliquot alkylated with iodoacetamide (IAM) the other with iodoacetic acid (IAA).
- The LC of the two aliquots (LC-IAM and LC-IAA)) differ by 1 Da thus modeling deamidation. LC-IAM represents the unmodified form, LC-IAA, the deamidated protein.
- LC-IAM : LC-IAA were mixed in different ratios:
  - 95:5, 90:10, 75:25, 60:40 and 50:50
  - and compared to the pure aliquots.
- LC-MS was performed using standard reverse-phase chromatography coupled to a maXis II UHR Q-TOF (Bruker) providing 80,000 resolution and isotopic resolution of light and heavy chains.
- Automated data processing with Biopharma Compass included MS raw spectrum generation, followed by Maximum Entropy deconvolution and monoisotopic peak picking using the SNAP algorithm (Bruker).

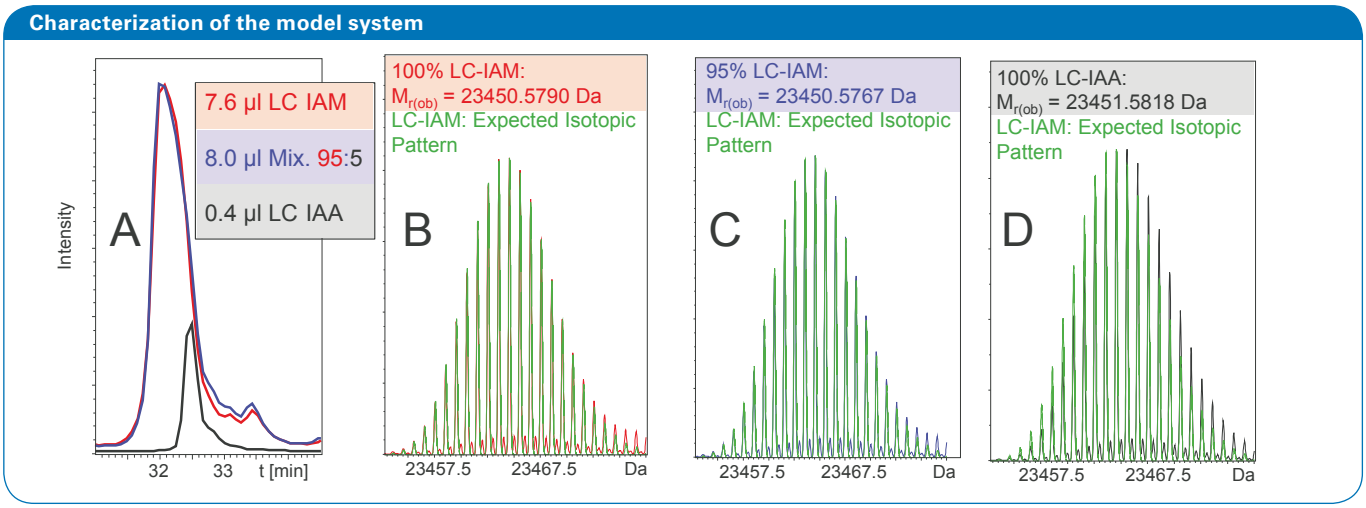

Figure 1: Overlaid TICs from 3 different samples. The amount on column of LC-IAM and LC-IAA in the mixture and in the pure samples are the same (A). (B-D) show the derived Max. Ent. spectra and the comparison to the expected isotopic pattern of LC-IAM.

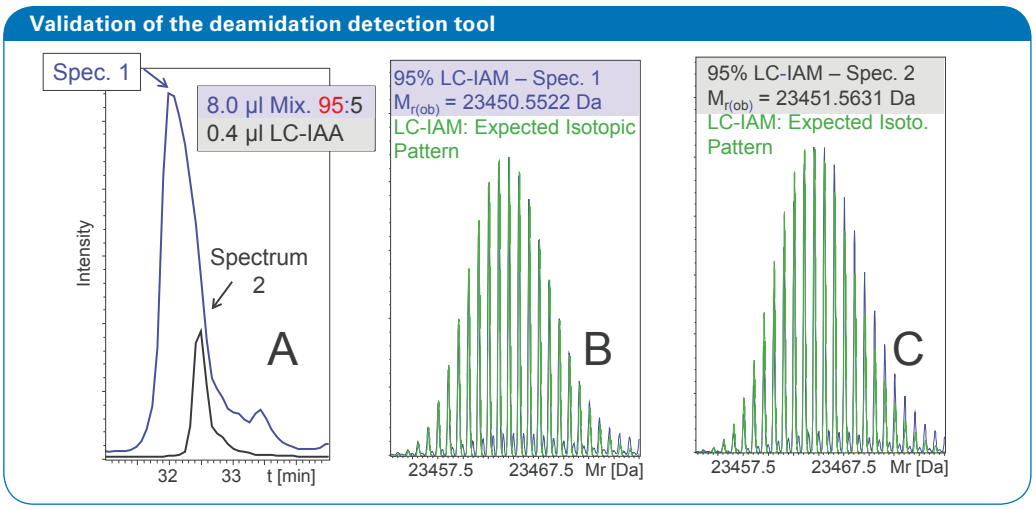

Figure 2: Overlaid TICs from the 95:5 mixture and pure LC-IAA in the same amount as contained in the mixture (A). Two spectra acquired at different R times are shown and compared to the expected isotopic pattern of LC-IAM (B, C): green. While spectrum 1 shows a perfect match (B), spectrum 2 shows a + 1Da mass shift indicating the presence of the deamidated species (C); The Mr of LC-IAA is determined in spectrum 2 as well.

Results

- Fig. 1 shows the total ion chromatogram (TIC) for mixing ratio LC-IAM:LC-IAA 95:5 together with the TICs of the pure samples constraining the same amount of LC-IAM and LC-IAA as the mixture (A). The derived spectra are compared to the expected isotopic pattern of LC-IAM. The pure LC-IAM spectrum results in a perfect match (B), the 95% mixture shows small deviations (C), while a clear +1 Da shift is apparent for pure LC-IAA (D).
- The mass of the most abundant species in the TIC compound spectra is reliably detected with mass accuracy of 1 ppm and better.

Mixing Ratio LC-IAM : LC-IAA	Deamid. Evidence	Deamid. Percent. / %
pure LC-IAM	FALSE	0
95 : 5	TRUE	17
90 : 10	TRUE	26
75 : 25	TRUE	40
60 : 40	TRUE	51
50 : 50	TRUE	63

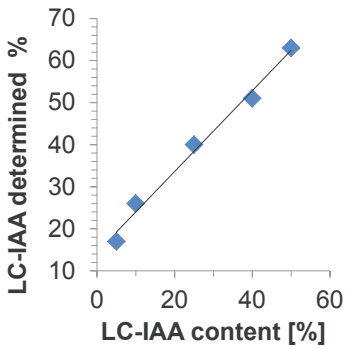

Table 1 and Figure 3: Result of the automated processing of different mixing ratios compared to the pure sample. Deamidations percentage are reliably detected.

- The new method for rapid deamidation detection on middle-up level makes use of the small retention time shift (Fig. 1) and the reliable detection of the dominant species: For each MS spectrum of the chromatographic peak the Mr is calculated. If a 1 Da mass shift is observed an evidence for deamidation is reported. The intensities of the MS spectra are used for profiling.
- Fig. 2 compares two spectra of different retention times (RT) for the 95:5 mixture (A). During elution the isotopic pattern and the determined Mr changes (B, C).
- Tab. 1 and Fig. 3 summarizes the application of the method to all samples of the model system with LC-IAM: LC-IAA rations from 50 : 50 to 100 : 0. The pure LC-IAM samples do not show changes in Mr as indicated in the "Deamid. Evidence" column. For all mixtures in the study the deamidation percentage was reliably determined.
- The "Deamid. Percentage" derived from the intensities of the deconv. MS spectra overestimates the content of LC-IAA, probably due to chromatographic saturation effects. However, it is a good semi-quantitative measure of the impurity amount (Fig. 2).
- Fig. 4 displays the automatically generated report indicating detection of deamidation, while Fig. 5 shows the application of the method to a partially deamidated LC of a mAb.

Automated deamidation report in BioPharma Compass

Compound MS Peak Lists

Compound Name	ID	Mr (Ana.)	Mr (Ref)	Delta Mr [ppm]	Deamid. Evid.	Deamid. Perc. [%]
Cmpd 1; 32.0 min						
Cmpd 1; 32.0 min	LC-IAM	23450.5780	23450.5994	-0.91	True	17

Figure 4: Excerpt from the report for the 95:5 mixture which is automatically generated under BioPharma Compass, indicating that deamidation was observable and its percentage.

Detection of deamidation of an antibody Light Chain

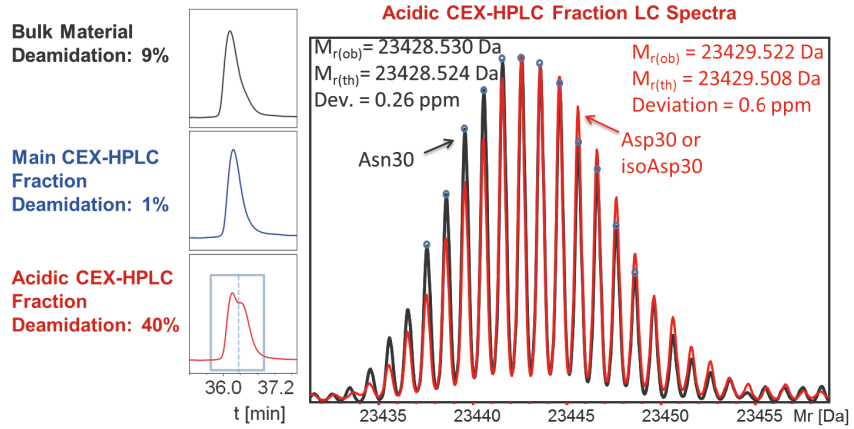

Figure 5: Application of the new method to an mAb LC with deamidation. Using only RP-HPLC a deamidation of 9% was detected. This was confirmed by cation exchange chromatography (CEX). Running HPLC on the main and on the acidic CEX fraction resulted in detection of a 40% deamidation and an observable Rt shift in the acidic fraction.

Conclusion

- Latest UHR-QTOF technology allows for determination of monoisotopic masses of antibody subunits with accuracies of 1 ppm and better with precisely matching isotope patterns.
- The new Middle-Up deamidation detection method determines the Mr of the isotopic peak pattern across the chromatographic peak. A change in Mr caused by deamidation is reported and quantified.
- Mixing ratios up to 95:5 were reliably and automatically detected for the deamidation model system and successfully applied to a deamidated mAb.

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