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Identification of Isomeric Amino Acids In The Antigen Binding Region Of A Monoclonal Antibody By Electron Capture Dissociation

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Introduction

Peptide mapping using liquid chromatography-mass spectrometry (LC/MS) is a key analytical tool in the biopharmaceutical industry for studying the primary structure of monoclonal antibodies (mAbs), including sequence and posttranslational modifications (PTMs). Molecular fragmentation by tandem mass spectrometry (MS/MS) using collision induced dissociation (CID) is widely applied in these analyses, yet this approach is limited by its inability to distinguish between isomeric residues. In this work, electron capture dissociation (ECD) was used to differentiate isomeric amino acids such as leucine/isoleucine and aspartate/isoaspartate in the complementarity-determining region (CDR) of a mAb. ECD is a softer fragmentation technique where ions capture low-energy electrons, leading to radical-driven peptide back bone cleavage. This mainly produces c- and z-type of ions instead of b- and y-type ions as is the case for CID. In addition, side chain fragmentation leads to the formation of w-type ions that enable differentiation of isomeric amino acids.

Experimental

Sample preparation: The mAb was denatured using 3 M guanidine-HCl in 100 mM Tris-HCl (pH 7.5), reduced with 5 mM dithiothreitol (DTT) for 30 minutes at 60 °C, and alkylated for 1 hour at 37 °C using 10 mM iodoacetamide (IAA). Afterwards protein was digested for 4 hours at 37°C by adding trypsin.

LC conditions: Tryptic peptides were separated by reversed phase liquid chromatography on an AdvanceBio Peptide Mapping C18 column (2.1 x 150 mm, 2.7 μ m) using an [Agilent 1290 Infinity II Bio LC](#) system. Mobile phases consisted of water and acetonitrile containing 0.1% formic acid.

MS conditions: Mass spectra were obtained using an [Agilent 6545XT AdvanceBio LC/Q-TOF](#) equipped with an Agilent Dual Jet Stream ESI source and an [Agilent ExD cell](#). Data processing was performed with [Agilent MassHunter Qualitative Analysis](#) and [Agilent ExDViewer](#).

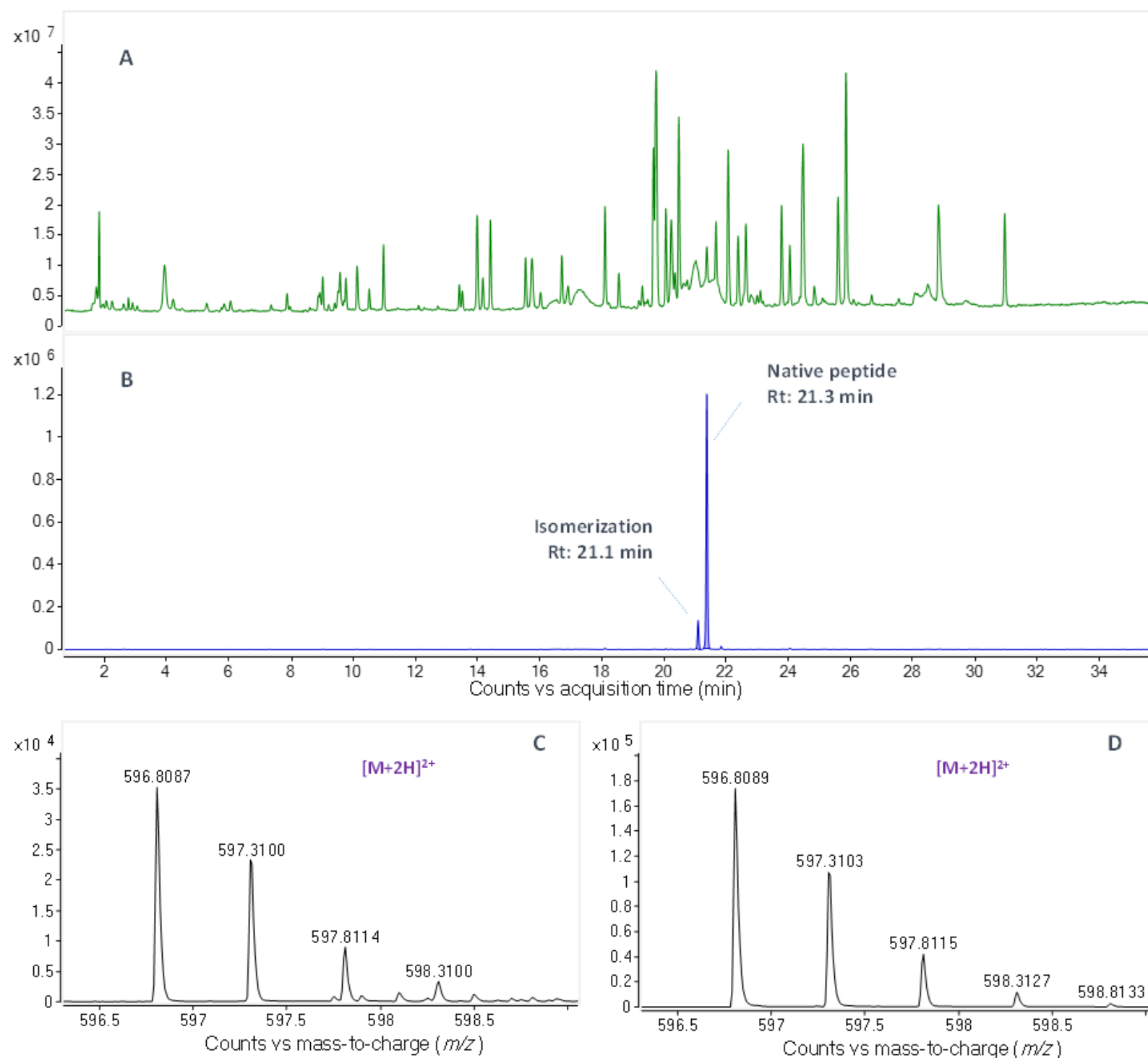


Figure 1. (A) TIC profile of the tryptic digest and (B) EIC of the target tryptic peptide located in the CDR of the mAb, extracted at 20 ppm mass accuracy. The isotopic patterns of the doubly charged tryptic peptides for the (C) isomeric and (D) native peptides are also shown.

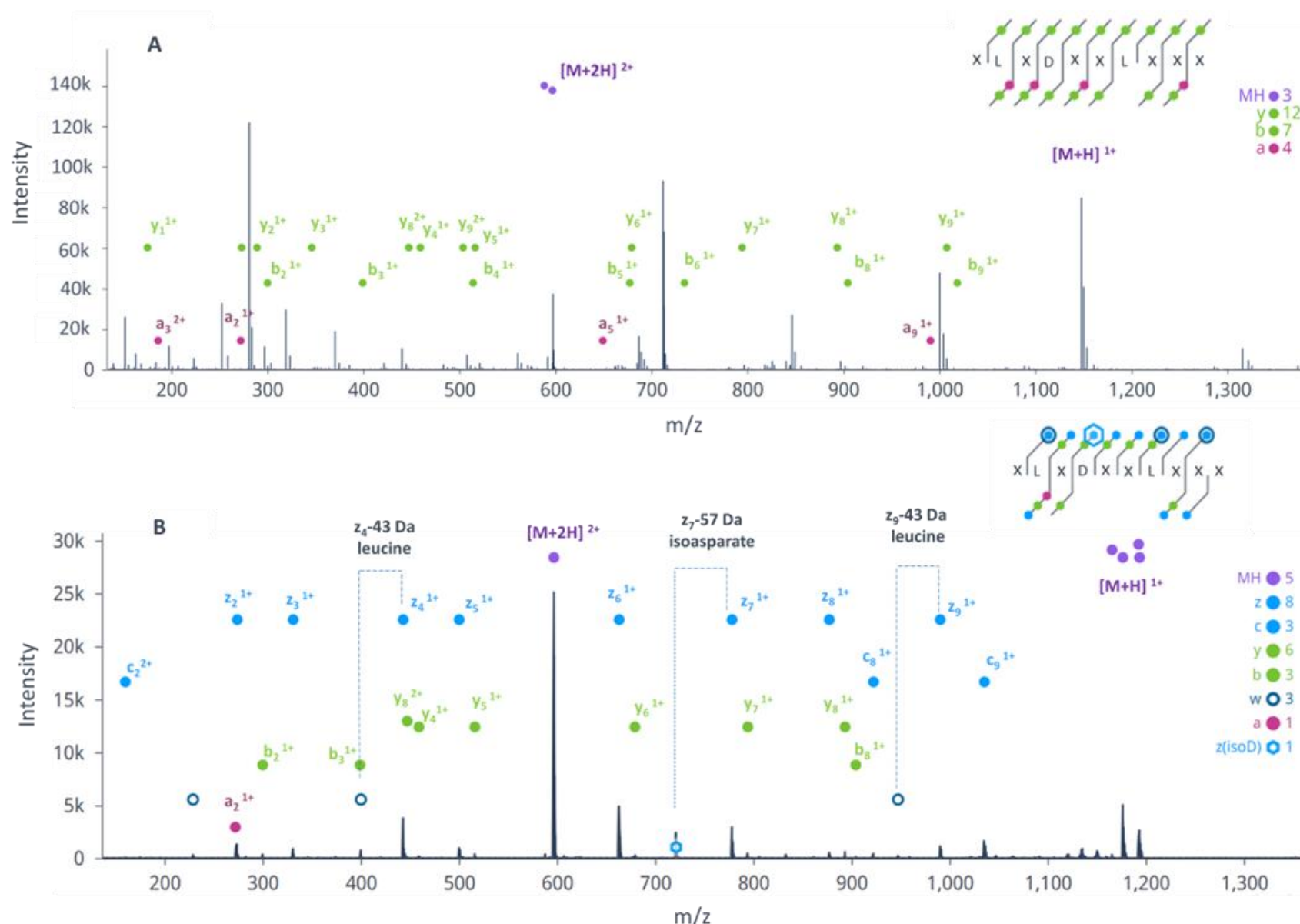


Figure 2. MS/MS spectra acquired after (A) CID and (B) ECD for the doubly charged precursor ion at retention time 21.1 minutes. CID- and ECD-type ions are labeled as green and blue, respectively, while precursor and a-ions are labeled as purple and magenta, respectively.

Results

The total ion current (TIC) and extracted ion chromatogram (EIC) for the tryptic peptides associated with the CDR region and containing potential isomeric amino acids are shown in Figure 1. Two peaks are found in the EIC with identical mass at Rt 21.1 and 21.3 min. The CID and ECD MS/MS fragmentation spectra for the peptide present at 21.1 min in the digest are shown in Figure 2. The relative abundances of the various ion types (c-, z-, b-, y-, a- or precursor ions) present in the CID and ECD spectra are illustrated in Figure 3.

Studying the fragmentation spectra in more detail reveals the clear presence of two leucine residues in the peptide (Figure 4 A and B). A leucine will generate a secondary w-ion by the radical loss of an isopropyl group (z-ion -43 amu), while an isoleucine involves the loss of an ethyl radical (z-ion -29 amu). The presence of isoaspartate in the peptide at Rt 21.1 min is also clearly demonstrated by a shift of 57 amu relative to the corresponding z-ion for aspartate in the ECD spectra (z-ion -57 amu; Figure 4 C). In contrast, in the native peptide at Rt 21.3 min, a neutral loss of 44 amu is observed, indicating the presence of aspartate (Figure 4 D).

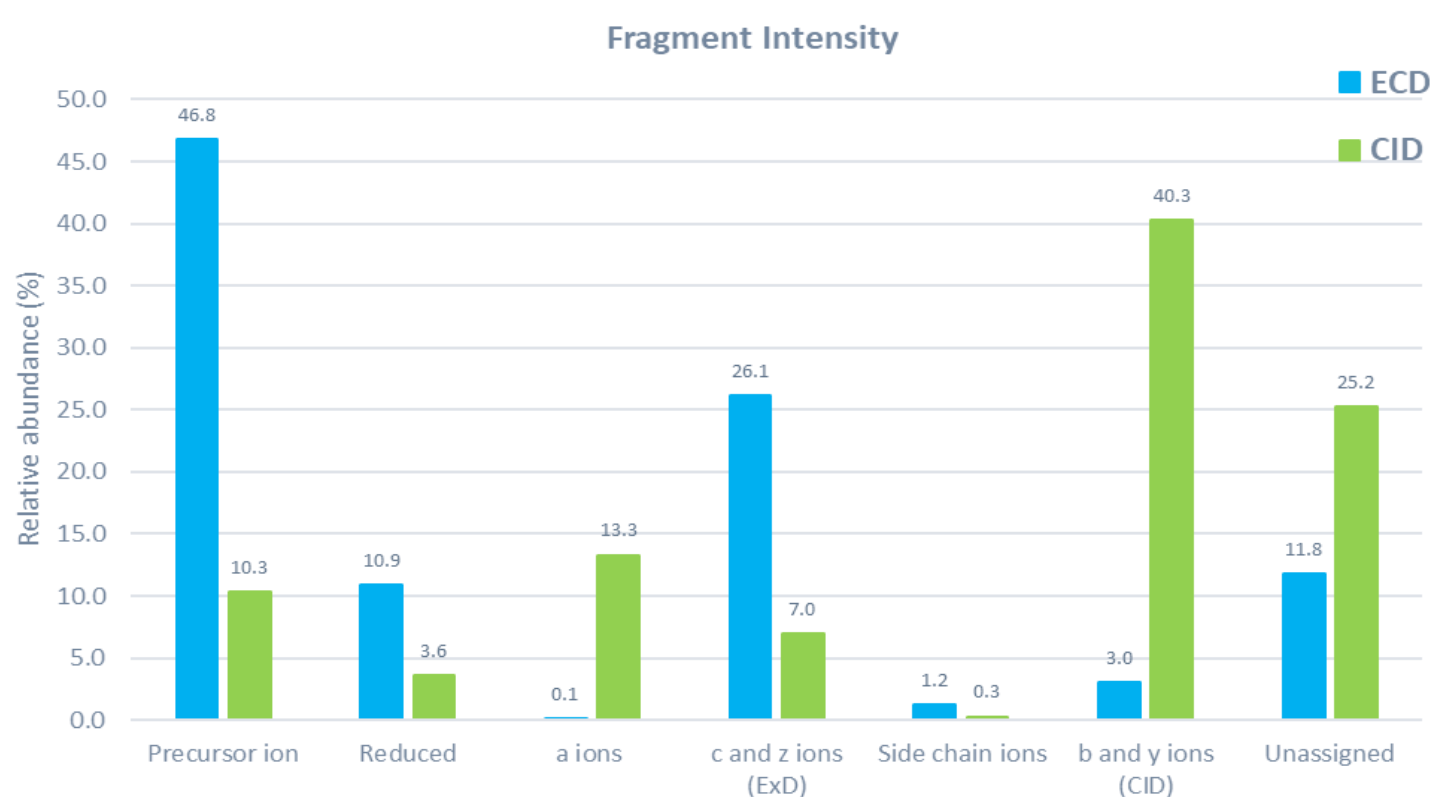


Figure 3. Relative abundances of the various ions observed in the CID and ECD spectra of the doubly charged target ion.

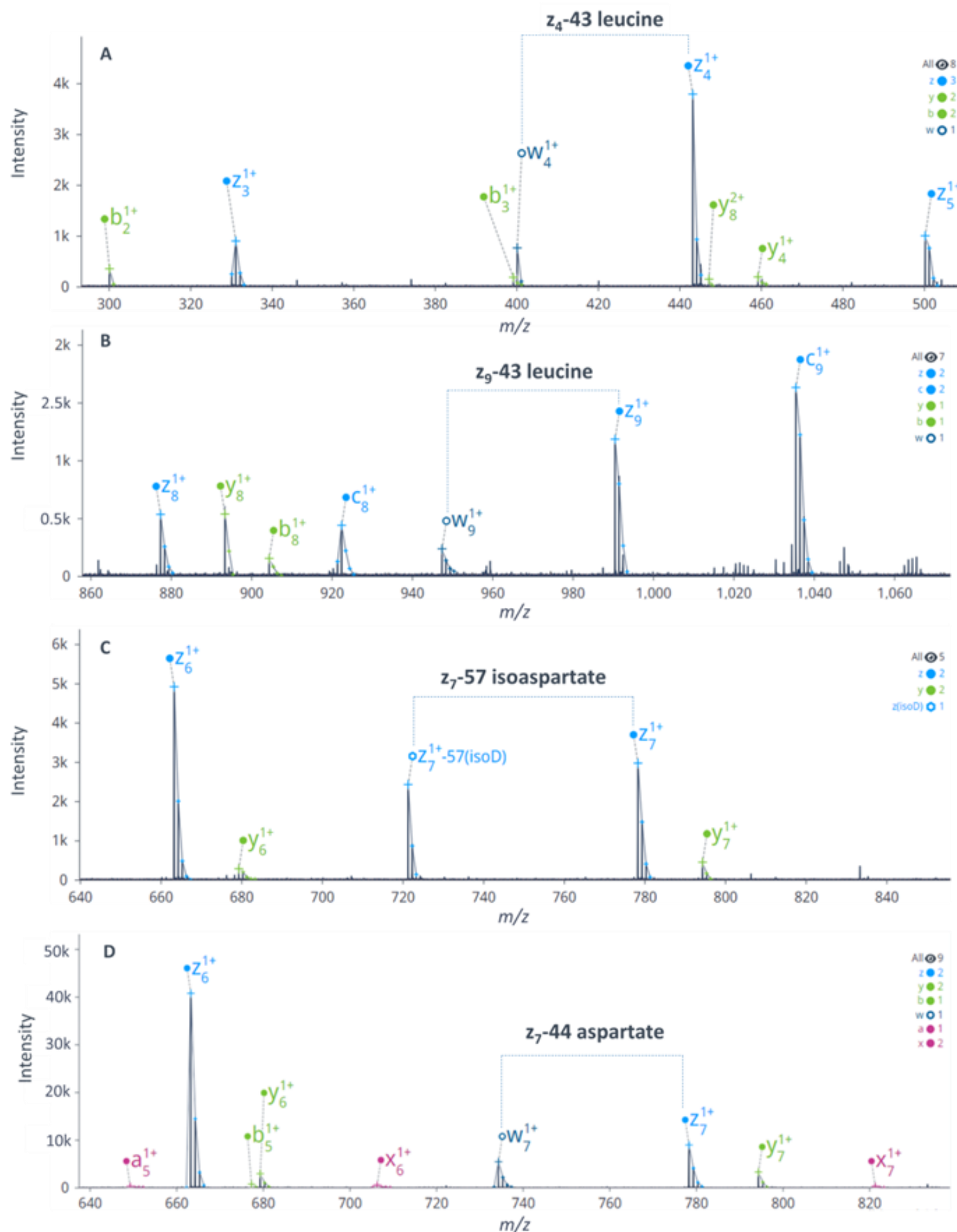


Figure 4. Zoomed-in ECD spectra highlighting the characteristic w-ions related to leucine at (A) position 7 and (B) position 2, as well as the characteristic w-ion (or z-57 amu) for (C) isoaspartate at position 4. The latter ion is absent in the ECD spectrum of the (D) native peptide.

Conclusions

MS/MS fragmentation by ECD of tryptic peptides was successfully applied to differentiate between the isomeric amino acids leucine/isoleucine and aspartate/isoaspartate in the CDR region of a therapeutic mAb. The unambiguous identification of an isoaspartate residue is particularly relevant because isomerization may alter binding affinity and therefore represents a critical quality risk during development of therapeutic mAbs. In addition, monitoring isoaspartate formation is of prime importance during stability studies used for shelf-life determination. Overall, ECD-based MS/MS fragmentation was shown to be a powerful analytical tool for mAb characterization and development.

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