In-Depth Versatile Characterization of Aspartic Acid Isomers via a Cyclic Ion Mobility Mass Spectrometry (cIMS)

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Introduction

Challenge:

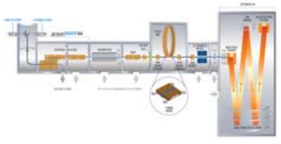
- Protein isomerization may have detrimental effects on efficacy and safety of a biotherapeutic drug, and therefore must be thoroughly characterized.
- Peptides containing isobaric isomers are often a challenge to characterize via LC-MS since they cannot be distinguished by mass alone.

Solution:

- Ion mobility can offer additional separation capability to resolve the isomerized peptides based on their gas-phase conformation.
- The versatile design of the SELECT SERIES™ Cyclic™ Ion Mobility MS (cIMS) system offers additional capabilities for characterization, which are demonstrated using peptides of biotherapeutic relevance containing aspartic (Asp) acid and isoaspartic (isoAsp) acid residues. Differences between native and isomeric species at precursor and fragment levels were investigated.

Experimental

- Samples: a) Synthetic beta amyloid 1-16 & Trastuzumab (TmAb) HC T12 isomeric peptide pairs (0.5 µM in 10 mM ammonium acetate for infusion); b) Reduced, alkylated, and tryptic digested sample of TmAb
- LC-MS System: ACQUITY™ H-Class coupled to SELECT SERIES Cyclic IMS
- Infusion Experiments: IMS separation via multi-pass cIMS followed by IMS^n or post-IMS ECD fragmentation
- Online LC/MS: DIA with ion mobility (HDMS^E) and targeted MS/MS with ion mobility (HDMS/MS)



Results

Single vs Multi-Pass cIMS: Beta Amyloid 1-16 Peptide Isomers

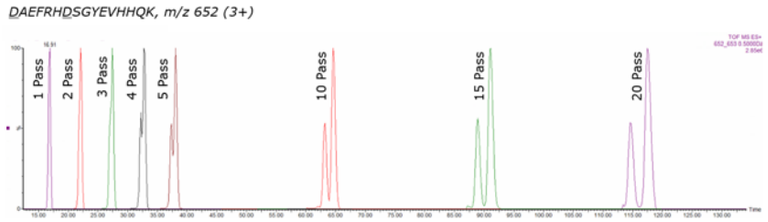


Figure 1. Mobiligram overlays for infused beta amyloid peptide isomer pairs, comparing single vs multi-pass IMS separations. No separation is observed in single pass, while baseline resolution is achieved with 15-20 passes.

IMS^n on IMS-Separated Beta Amyloid 1-16 Peptide Isomers

The cIMS instrument allows the user to select IM-separated species via "slicing" (sending the species to the pre-store array) and reinjecting them into the cyclic device for further separation, with the option of applying energy to generate CID fragments upon reinjection. The beta amyloid peptide in the example above were sliced out after 20 passes and generated fragments were reinjected. The mobility of each fragment for Asp & IsoAsp species were compared to identify site of isomerization.

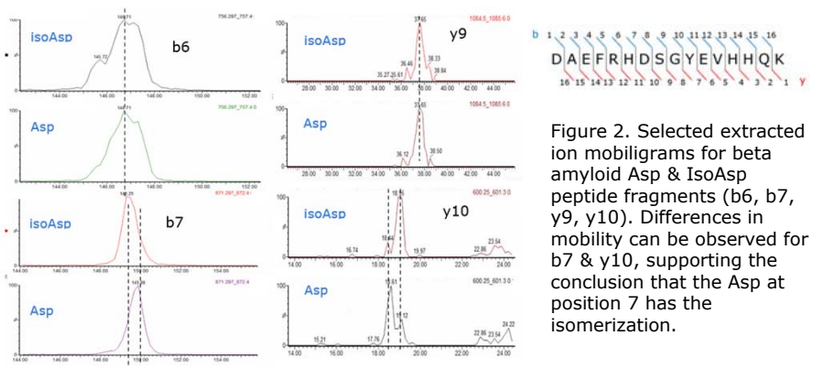


Figure 2. Selected extracted ion mobiligrams for beta amyloid Asp & IsoAsp peptide fragments (b6, b7, y9, y10). Differences in mobility can be observed for b7 & y10, supporting the conclusion that the Asp at position 7 has the isomerization.

TmAb HC T12 Isomer Investigation (Asp/IsoAsp)

*WGG**D**GFYAMDYWGQGTLVTVSSASTK*

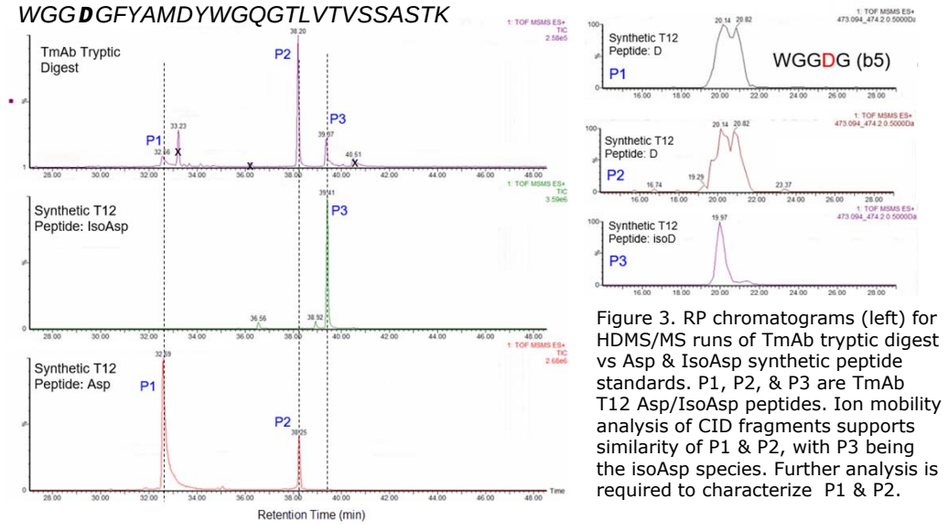


Figure 3. RP chromatograms (left) for HDMS/MS runs of TmAb tryptic digest vs Asp & IsoAsp synthetic peptide standards. P1, P2, & P3 are TmAb T12 Asp/IsoAsp peptides. Ion mobility analysis of CID fragments supports similarity of P1 & P2, with P3 being the isoAsp species. Further analysis is required to characterize P1 & P2.

ECD Fragmentation for Isomerization Site Identification: TmAb HC T12

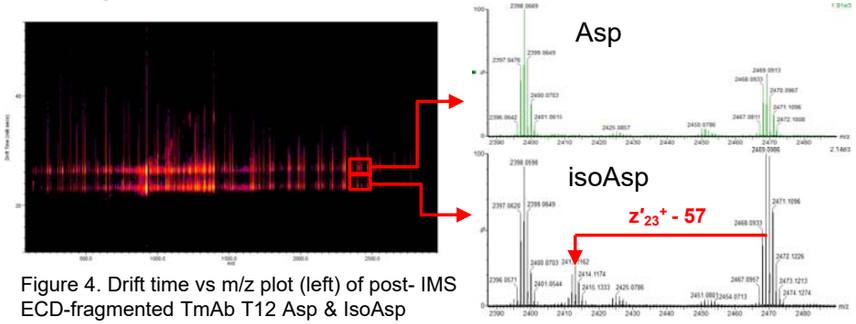


Figure 4. Drift time vs m/z plot (left) of post- IMS ECD-fragmented TmAb T12 Asp & IsoAsp peptides, and corresponding MS spectra (right).

Conclusions

The unique and flexible design of the cyclic IMS instrument supports innovative characterization of Asp and IsoAsp peptide isomer species, such as beta amyloid and TmAb peptides. Future investigations for TmAb T12 isomerization species is currently underway.

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1. Zheng, X; *Chem Commun (Camb).* **2017.** *53 (56): 7913-7916*
2. Yang, H; *J Proteome Res.* **2009.** *8 (10): 4615-4621*