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Asp/isoAsp-Differentiation in Biologics

Why Asp/isoAsp differentiation?

The isomerization of asparagine to aspartic or isoaspartic residues is a common process during peptide or protein aging in aqueous solutions. The isomerzation takes place at higher rates at elevated temperature, thus forced aging studies can reveal which residues are prone to isomerization. The isomerization itself involves a cyclic intermediate aminosuccinyl structure which can open in water to either form an aspartic acid or isoaspartic acid residue from the asparagine.

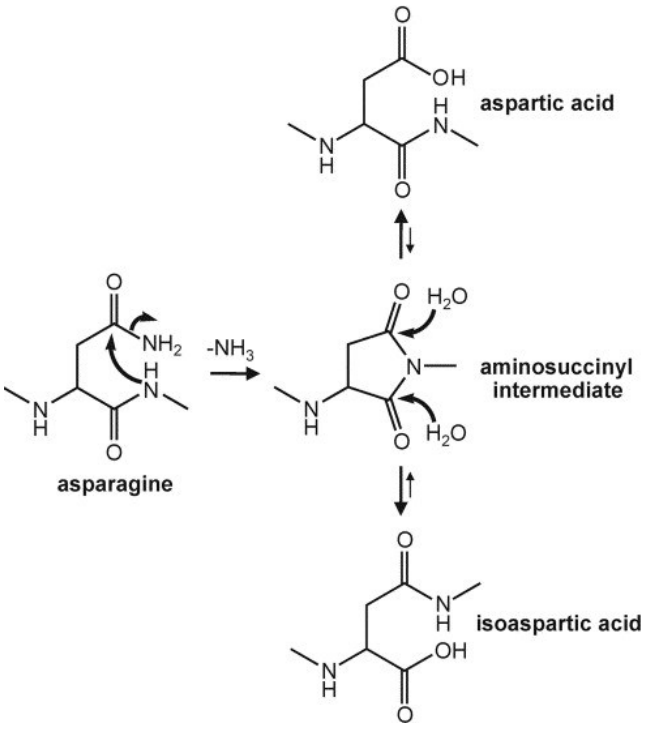

Figure from Cournoyer, J. J.; Lin, C.; Bowman, M. J.; O'Connor, P. B. Quantitating the Relative Abundance of Isoaspartyl Residues in Deamidated Proteins by Electron Capture Dissociation J. Am. Soc. Mass Spectrom. 2007, 18, 48.

The Asp/isoAsp isomerization leads to different structures which can have entirely different properties. The structural change can lead to precipitation, changes in immunogenicity or other significant attributes. Thus it is regarded as a critical quality attribuite (CQA) to be characterized during the cause of stability and forced aging tests according to ICH Q6B 6.2.2

Why ECD for Asp/isoAsp differentiation?

The problem with this isomerization is that it is easy to spot the deamidation using peptide mapping due to the mass change of +1 Da on the peptide level. However, the question whether Asp or isoAsp is formed is much harder to answer. While the two forms can be chromatographically separated, CID-based MS/MS can not answer which form is actually being present as the b- and y-type fragments generated by both forms are identical in mass. Currently this distinction has to be made based on custom synthesized reference peptides.

Another way of differentiating Asp and IsoAsp is using electron-capture dissociation or ECD. ECD fragmentation forms characteristic side chain fragment ions which can be used to unambiguously differentiate between the two residues:

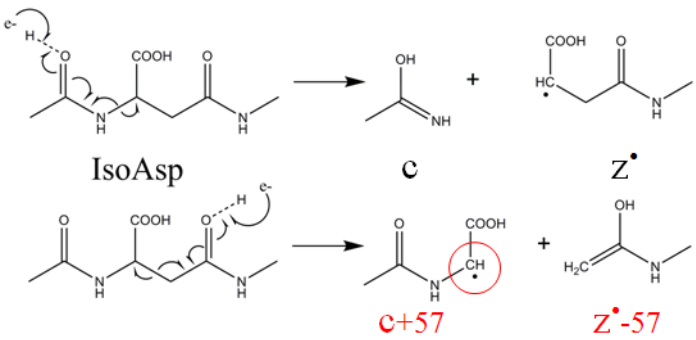

Figure from www.bumc.bu.edu/ftms/research/isoaspartome/

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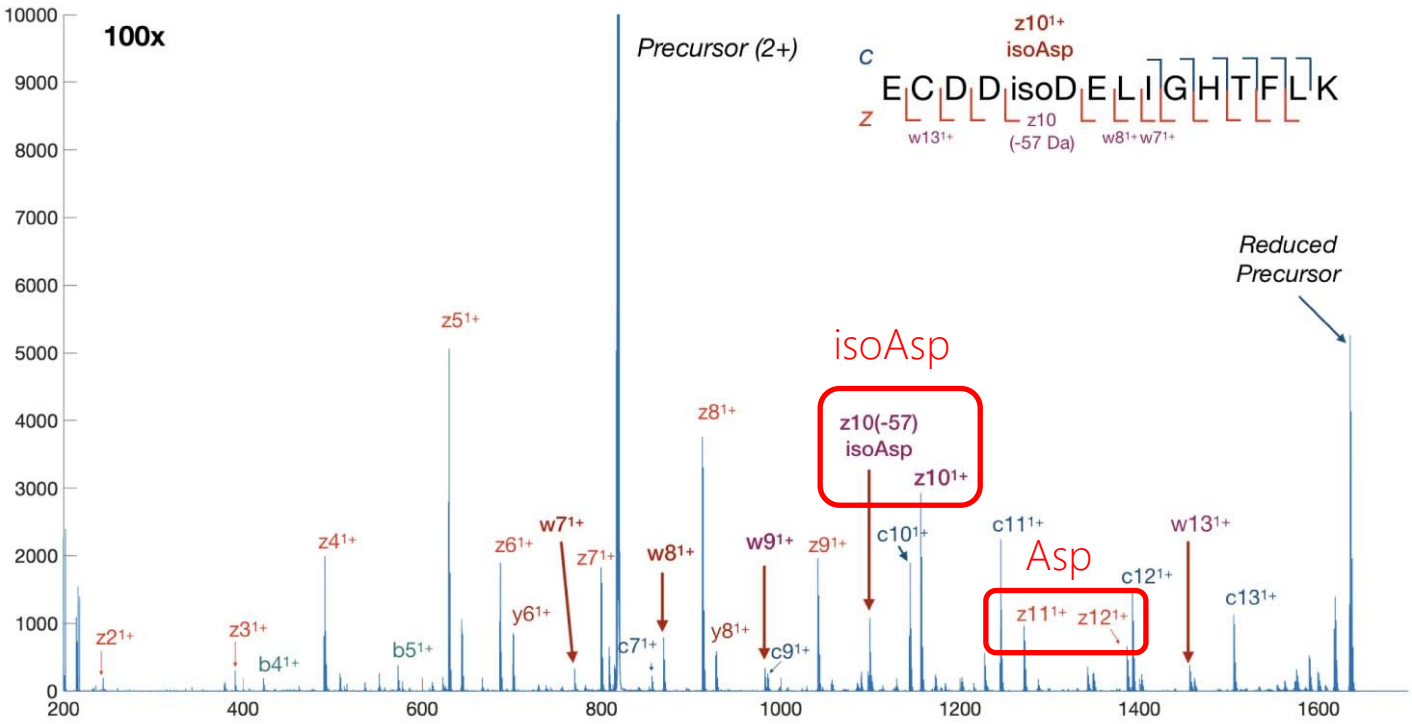
The differentiation between Asp and isoAsp is demonstrated using a synthetic model peptide containing two Asp and as well as one isoAsp residue. ECD fragmentation allows to unambiguously assign all three:



Literature

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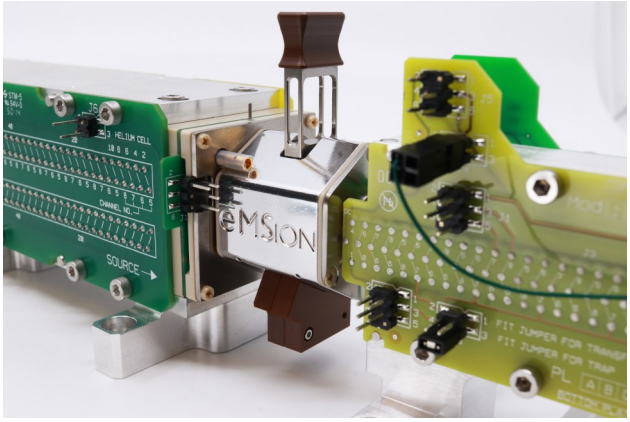
In collaboration with e-MSion, MS Vision offers ECD upgrades for existing Waters Synapt G2 and ThermoFisher QExactive systems. Ask us for details and pricing!



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