MSVision

Dedicated to Mass Spectrometry

èMSION

ECD for the Masses

Electron Capture Dissociation and Ion Mobility

ECD of highly charged precursors

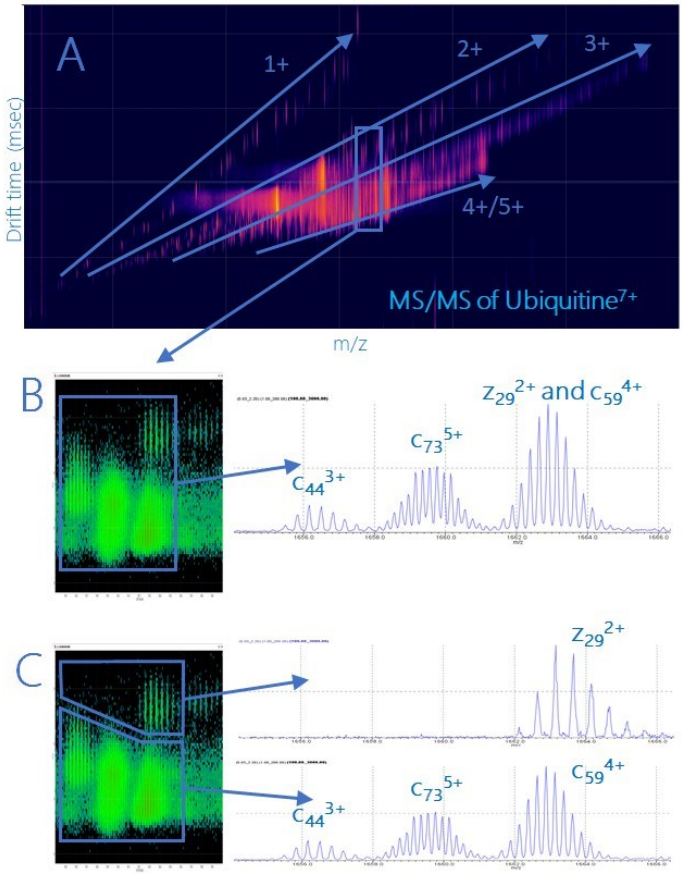
Electron-capture dissociation is a technique ideally suited to fragment intact proteins. The reason for this is the nature of the fragmentation mechanism. First, the interaction between highly positively-charged, intact proteins and negative electrons is intense. Second, ECD is a process involving charge reduction. Thus when an electron is absorbed by the precursor, one charge is taken away. If this happens with a singly-charged precursor it results in two neutral fragments which cannot be detected. If a doubly-charged precursor is fragmented, it results in one singly-charged and one neutral fragment, thus statistically half of the fragment ions are lost.

Only when we do the fragmentation on triply charged precursors will we on average get two charged fragments. And here the magic starts… While a doubly charged precursor results in a pair of a neutral and a charged fragment, the two may still stick together by electrostatic forces (ECnoD). To separate and detect them properly, additional vibrational activation is required. When a triply-charged precursor is fragmented, resulting in two charged fragments, these are driven apart by electrostatic repulsion and the fragmentation becomes much more efficient.

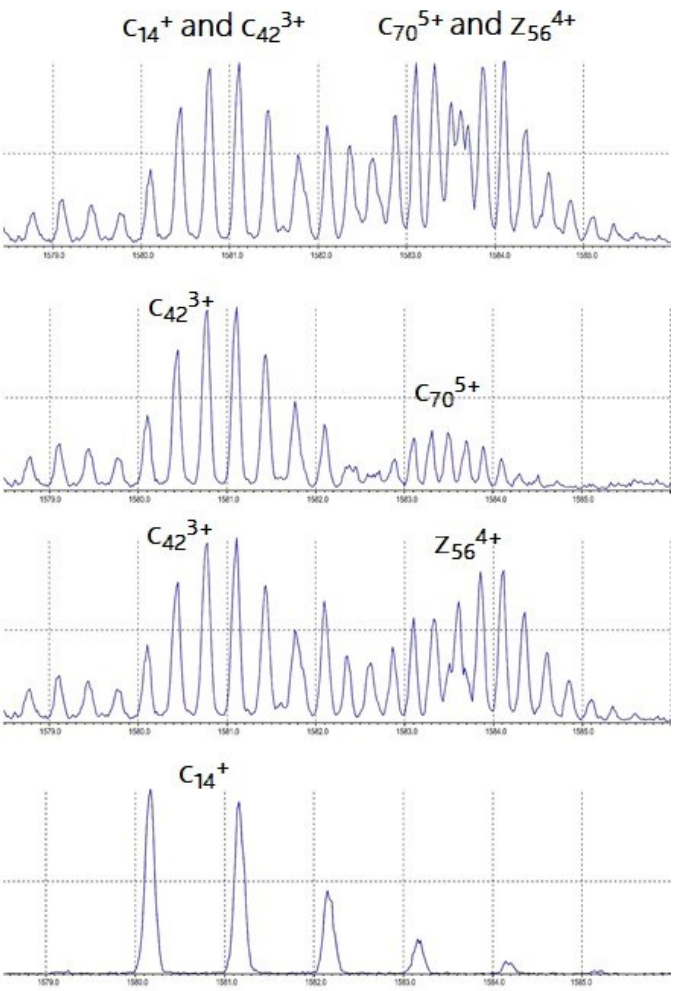
So, high precursor charges are favourable. Yet when starting with a highly-charged precursor and fragmenting it to large number of highly-charged fragments with mixed charge states, spectra will get very crowded, making deconvolution potentially difficult. This has been observed as a major bottleneck for the implementation of ECD or ETD (electron-transfer dissociation) on QTOF systems in the past. The way to overcome this is to either increase resolution (e.g. by going from QTOF to Orbitrap or FT-ICR based systems) or to use an additional separation step to simplify the fragmentation spectra.

ECD/IMS allows for spectra simplification

As mentioned, the IMS-based separation of ECD fragments with different charges allows one to simplify the spectra, to assist in deconvolution and to assign more fragment ions which are otherwise hidden. In the TWAVE-based IMS implementation, it is easily possible to separate 1+ - 4+ charge states and to simplify higher charges' state spectra by removing the lower charge states. An example is given with the fragmentation of the 7+ charged precursor of Ubiquitin. It produces fragment ions with charges between 1+ and 5+, that are all mixed and overlapping in the MS/MS spectrum.



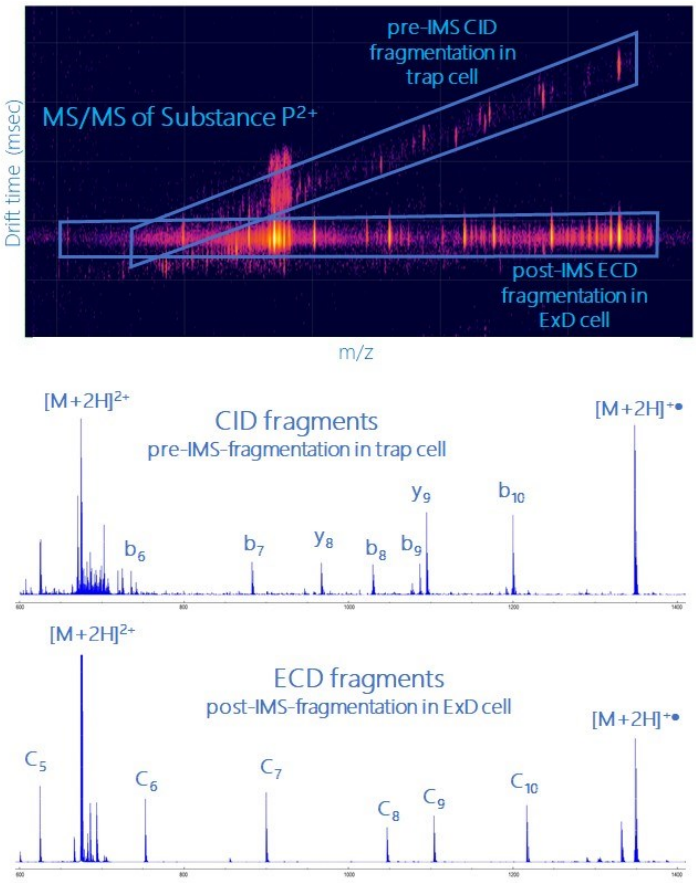
The IMS-based charge separation allows for the separation of the z_{29}^{2+} ion from the c_{59}^{4+} ion resulting in clear assignment of both ions. Another example is given below, where two overlaps of 1+/3+ and 4+/5+ can be resolved.



The IMS based charge separation of the ECD fragments thus dramatically increases the separation space, adds clarity in assignments, and reveals fragment ions otherwise hidden due to lower ion abundance. In this case, the additional possibilities of IMS make ECD much more useful than an ECD implementation on a conventional QTOF without IMS capability.

ECD and CID in combination with IMS

Another application is to combine classical CID fragmentation in the Synapt's trap region (before IMS) with ECD after the IMS region. This requires the ECD cell to be behind the IMS cell. This combination allows for unique types of experiments where both types of fragmentation may be applied in parallel:



While the singly-charged CID fragments can be separated by IMS, the singly-charged fragments generated by ECD show a nice and clean spectrum without interference of the CID-generated ions. This can help to focus on labile modifications which are lost in CID fragmentation, such as Lys, His or Arg phosphorylation or sulfatation, due to differences between CID and ECD fragments being revealed in a single analysis.

The IMS data were processed with mineXpert software (Rusconi et al, JASM 2021,32, 1138-1141)