MSVision

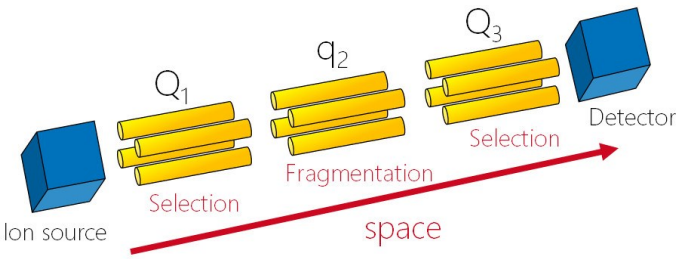
Dedicated to Mass Spectrometry

Understanding differences in CID fragmentation in a mass spectrometer

There are currently a number of different fragmentation techniques for mass spectrometry on the market. EI and CI for GC-MS, CID, HCD, ECD. ETD, IRMPD, SID and others for LC-MS. To properly interpret your data, it is crucial to have some understanding on how the instrument setup and the fragmentation technique will influence your result.

MS/MS in space

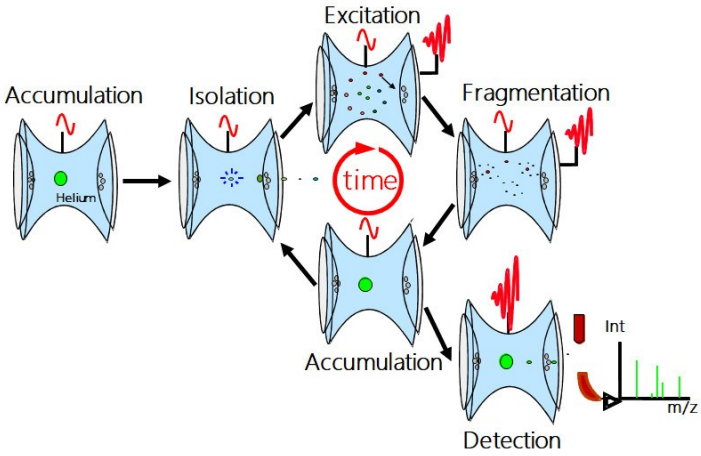
It is important to understand that there are two significantly different approaches to MS/MS: MS/MS in space (most common nowadays) and MS/MS in time. In MS/MS in space, selection of precursor, fragmentation, fragment selection and detection take place at different places as the ions travel through the instrument along the ion path:



This setup is typical for TripleQuad instruments but also for Q-TOF and Q-FT/Orbitrap setups. The fragmentation takes place in a quadrupole or hexapole collision cell where the ions collide with a neutral collision gas. The faster they move (the steeper the DC gradient along the collision cell is), the more energy is transferred onto the analytes during collision.

MS/MS in time

The second variant, MS/MS in time is typical for ion traps. Accumulation, isolation, fragmentation and detection all takes place in the ion trap but one step after the other:



In an ion trap there is no DC gradient during activation. Ions are excited by a frequency on the endcaps with increasing amplitudes. When the amplitude/excitation is strong enough to induce fragmentation by collision with the Helium in the trap the ion fragments and the product ions are formed.

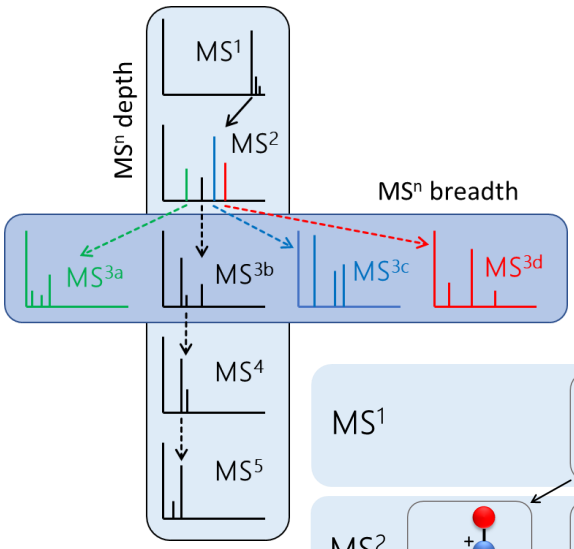
The important difference between those two setups is the following:

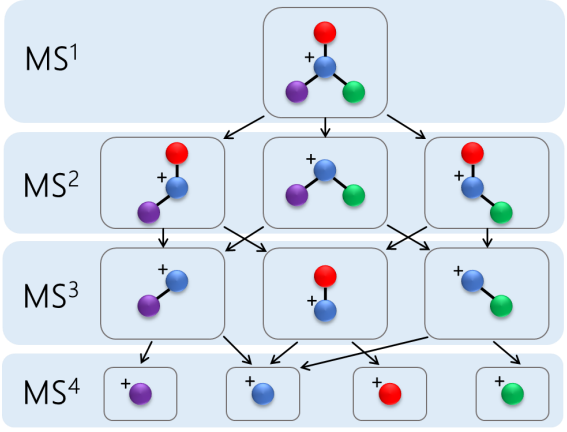
- In a quadrupole collision cell ions move from start to end. On their way they collide with gas molecules and can undergo fragmentation. BUT: this can happen multiple times during the passage! So, from the spectrum at the end you don't know whether the fragment is the product of a single or multiple collisions.
- In an ion trap only a single m/z value (or range) is activated. That means that only a specific m/z value undergoes fragmentation while the product ions don't undergo further fragmentation.

When is this relevant? In peptide analysis you typically would like to get broad fragmentation to cover the entire sequence. In ion traps the fragmentation usually occurs at the weakest site. Typically this is next to proline residues or C-terminal of Asp residues.

Ion trap peptide MS/MS spectra usually have a quite different appearance than e.g. QTOF spectra with more distinct intensity differences between signals. Quadrupole-generated peptide MS/MS spectra show a more even distribution of fragment intensities. While the latter often provides better coverage and higher search scores, the ion trap spectra may provide more insight into the chemistry going on.

The difference becomes much more relevant when it comes for structural analysis of small molecules. As discussed, in quadrupole fragmentation spectra you don't know whether the fragment arises from a single or multiple fragmentations. In ion trap spectra this is well known. Every fragment ion in an ion trap spectrum is the result of exactly one single fragmentation event and originates directly from the selected precursor. This allows to create fragmentation trees which can be very helpful in structural analysis of e.g. natural compounds. The following figure adapted from the paper "Using fragmentation trees and mass spectral trees for identifying unknown compounds in metabolomics" from Arpana Vaniya and Oliver Fiehn (TrAC Vol 69, June 2015, Pages 52-61) illustrates this nicely:





Linear ion traps are somewhere in between both techniques. The fragmentation is not a pure ion trap fragmentation as activation also takes place by dragging ions forward and backward in the linear traps between the two trapping lenses. So this is not solely m/z-specific anymore. However, it is also not a classical quadrupole based fragmentation which is the reason why Thermo implemented the HCD cell in addition to the linear trap on newer LTQ-Orbitrap generations.

And be aware that an Orbitrap, while technically being an ion trap, is ONLY a detector! It is not capable of isolating ions nor of fragmenting them as in particular the latter requires gas, which should not be present in the Orbitrap (the lower the pressure, the better it works). So, do not mix up classical ion traps and Orbitraps. As Orbitraps are only the detector, they are usually used in combination with classical ion trap (LTQ-Orbitrap) or Qq-frontend (QExactive) to create a full blown hybrid instrument.

The question is now: when to use what? Nowadays peptide fragmentation is mostly performed using quadrupole based fragmentation on QTOF or Q-Orbitrap based systems. For quantitation it doesn't really matter which technique is used as long as the fragment ions monitored are specific and free of matrix interference. MS^3 as in QTraps can help here but sensitivity drops also with every further fragmentation step.

For small molecule structural analysis ion trap based fragmentation is preferable. Either using an ion trap alone or in combination with an additional detector such as an Orbitrap or TOF for high mass accuracy measurements.

When planning your experiments in mass spec, know your instrument. Be aware what it can do and what not! No system is ideally suited for any type of experiment.

Learn what the pro's and con's of your system are to fully exploit its capabilities.product ions don't undergo further fragmentation.