

The development of the ion trap as a detector for Liquid Chromatography

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Since its commercial introduction as a detector for LC in 1995 the ion trap has arguably been more significant than any other mass analyser in making mass spectrometry accessible to a broad range of chromatographers. The ion trap itself has undergone more technical innovations in its short lifetime than any other detector for LC.

HPLC coupled to the atmospheric pressure ionisation (API) source, with its production of ions external to the mass analyser made this a very robust, sensitive and easy to use LC/MS interface. Before the introduction of the ion trap, detectors based upon the quadrupole were the most widely used for LC/MS. Whilst the triple stage quadrupole (TSQ) was, and still is, the workhorse for quantitative analysis they suffered from a lack of sensitivity for qualitative work which required full scan information. The API techniques generated molecular weight information but tandem MS experiments were

needed to obtain structural information. The introduction of the ion trap suddenly made it possible for anyone, even with very little experience, to obtain MS/MS spectra from low level chromatographic components. The sorts of experiments which had previously been the domain of the "expert" mass spectrometrist were now available to all. The ion trap also had the unique feature of being able to generate multiple stages of MS/MS known as MSⁿ. This opened up new areas of structural work that could be undertaken with LC/MS analysis.

often said to be mass spectrometry in the time domain. (compared with tandem MS in space.)

The helium bath gas serves two purposes. Ions injected into the trap have excess kinetic energy and following a series of collisions with the He, lose energy, become focused in the center of the trap, and are said to have become cooled. The helium also serves as the collision gas when conducting MS/MS experiments.

Application of ion trap technology

Whilst the ion trap found its way into many different laboratories two applications are of particular note. The newly born field of proteomics was continually looking to increase the throughput of peptides sequenced. The ion trap's ability to automatically generate MS/MS spectra of peptides as they eluted with no intervention from an operator meant that complex tryptic digests could now be analysed by LC/MS/MS in a routine manner. The highly sensitive, reproducible and information rich nature of ion trap MS/MS spectra (see Figure 2, an example peptide MS/MS spectrum.) lead to their widespread use coupled with protein identification software tools. Many labs around the world setup "peptide sequencing factories" based upon LC/MS with ion trap detectors.^[1]

In the world of pharmaceutical research, more emphasis was being placed upon detecting and identifying drug metabolites at earlier stages of the discovery process. The ion trap became used widely thanks to the ability of its data dependent experiments to automatically generate MS, MS/MS and MSⁿ spectra of all components eluting from the column. Post

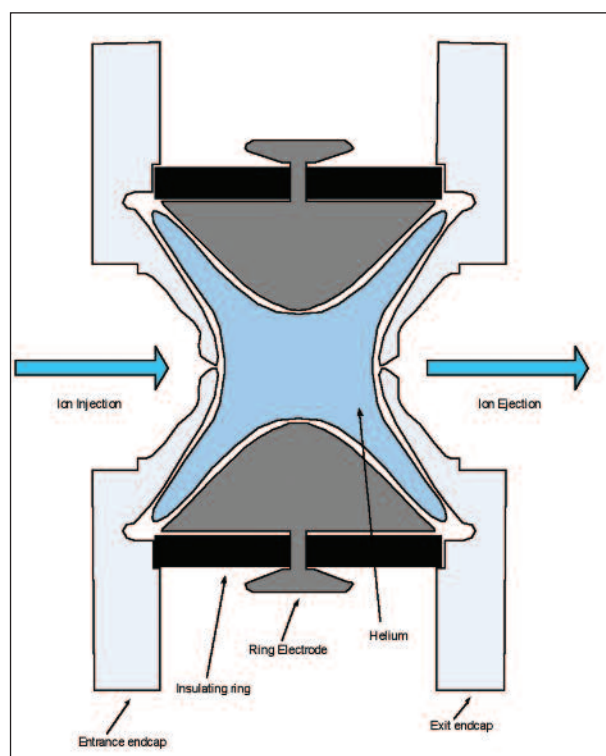


Figure 1. schematic diagram of a quadrupole ion trap

Ion trap design

Quadrupole ion traps are constructed from two end cap electrodes and a central ring electrode separated by insulating rings. (See Figure 1) A pressure of approx. 1 mTorr helium is maintained in the trap. RF and DC voltages are applied to the electrodes, their nature and amplitude are dependent upon the experiment being carried out. To acquire a mass spectrum from an ion trap requires several steps. These include ion injection, trapping, and ejection. In addition for MSⁿ experiments isolation and activation steps are needed. Due to the fact that these steps take place in the same space and follow one after the other, the ion trap is

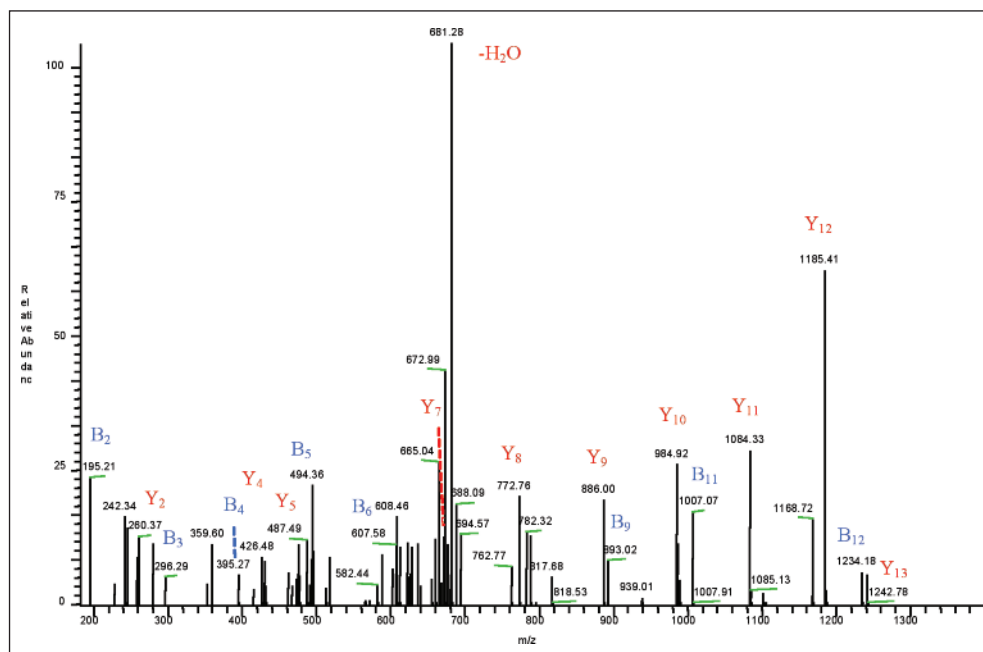


Figure 2. MS/MS spectrum of peptide HGVTVLTALGGILK, from a 40 amol digest of myoglobin

acquisition software tools were then used to pull out and identify metabolites from the endogenous compounds. The fact that MSn spectra were acquired throughout the LC run meant that it was often possible to determine the position of the bio transformations without the need to reinject the sample.

Recent advances

With the advent and adoption of the faster UPLC technique, the 3D trap began to be seen as scanning too slowly to take advantage of narrow chromatographic peaks afforded by UPLC. The advent of the linear or 2D ion trap answered a lot of the criticisms. In construction, the linear ion trap resembled a quadrupole mass analysers but had two fundamental advantages, better sensitivity and a faster duty cycle. These came as a result of a higher storage capacity and better trapping and ejection efficiencies^[2]. Whereas the 3D trap could collect MS/MS spectra at 1-

2 Hz, the linear trap could collect 4-5 unique spectra per second.

For the biochemists, this meant the ability to sequence more peptides within a chromatogram and with greater confidence in the search results. For the metabolism chemist they could delve deeper into the structure of their metabolites.

The continuous drive for improvements mean that some of the latest design changes to the ion trap have helped chromatographers achieve more information from fast separations. It had long been known that the pressure of helium bath gas inside the ion trap was a compromise. For efficient trapping and fragmentation a higher pressure was desirable, whereas for fast ejection of ions a lower pressure was needed. The latest dual cell ion trap effectively handles this by decoupling these processes. Ions are trapped, isolated

and fragmented in a higher pressure cell. Then they are transferred to a lower pressure cell before being scanned out and the spectrum recorded. Using this device it is possible to collect 10 full scan mass spectra per second.

Recent advances have utilised ion traps as introduction devices for high resolution mass analysers capable of measuring masses of sufficient accuracy to unambiguously determine empirical formulae. In particular when coupled to another type of ion trap, the Orbitrap,^[3] in the same way that the quadrupole ion trap made it possible for non experts to generate MS/MS information, these hybrid mass spectrometers have now made it possible for everyone to generate reliable accurate mass information in real time across a chromatographic run.

Conclusions

The desire for ever greater speed and sensitivity in many analytical fields shows no signs of diminishing. In the same way that the ion trap has evolved to meet the challenges of the past 15 years it is likely that instrumental research will keep the ion trap as a mainstay of the LC/MS world for the foreseeable future.

References

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