Mass Spectrometry (MS) Primer

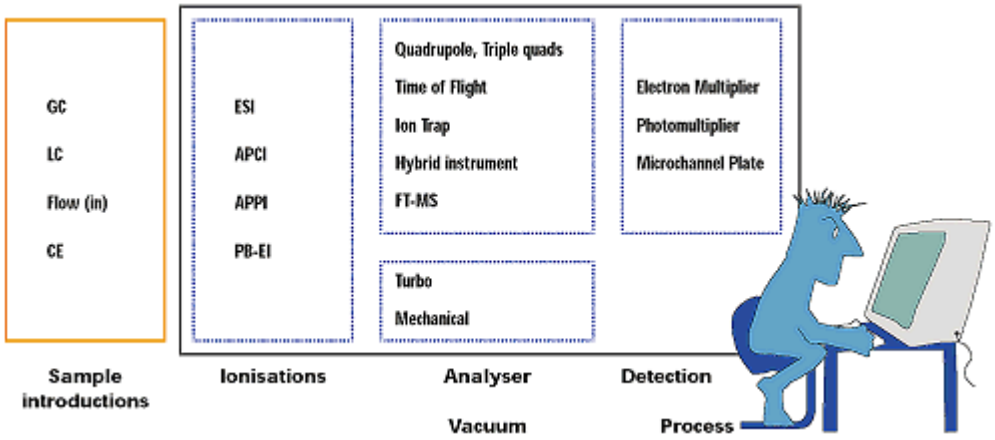
- Principles of Mass Spectrometry
- Principles of LC/MS
- Mass Definition
- Mass Resolution/Mass Accuracy

Principle Of Mass Spectrometry

The mass spectrometer is an instrument designed to separate gas phase ions according to their m/z (mass
to charge ratio) value.

The "heart" of the mass spectrometer is the analyzer. This element separates the gas phase ions.

The analyzer uses electrical or magnetic fields, or combination of both, to move the ions from the region where they are produced, to a detector, where they produce a signal which is amplified. Since the motion and separation of ions is based on electrical or magnetic fields, it is the mass to charge ratio, and not only the mass, which is of importance. The analyzer is operated under high vacuum, so that the ions can travel to the detector with a sufficient yield.



In addition to the analyzer, the mass spectrometer also includes

- A vacuum system
- Tools to introduce the sample (LC, GC …)
- Tools to produce the gas phase ions from the sample molecules

- Tools to fragment the ions, in order to obtain structural information, or to get more selective detection
- A detection system
- Software and computing

MS/MS is the combination of two or more MS experiments. The aim is either to get structure information by fragmenting the ions isolated during the first experiment, and/or to achieve better selectivity and sensitivity for quantitative analysis.

MS/MS is done:

- either by coupling multiple analysers (of the same or different kind)
- or, with an ion trap, by doing the various experiments within the trap

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Principles of LC/MS

LC/MS is a hyphenated technique, combining the separation power of HPLC, with the detection power of mass spectrometry. Even with a very sophisticated MS instrument, HPLC is still useful to remove the interferences from the sample that would impact the ionization.

Closely related to LC/MS are some other techniques, like flow injection/MS, CE or CEC/MS, capillary LC or nano LC/MS

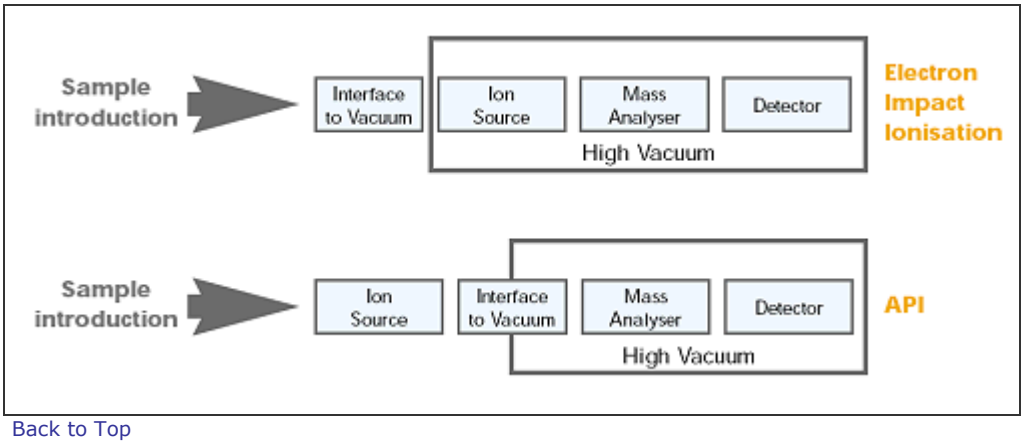
In all cases, there is the need for an interface that will eliminate the solvent and generate gas phase ions, then transferred to the optics of the mass spectrometer.

Most instruments now atmospheric pressure ionization (API) technique where solvent elimination and ionization steps are combined in the source and take place at atmospheric pressure.

When electron impact ionization (EI) is the choice, the solvent elimination and ionization steps are separate.

The interface is a particle beam type, which separates the sample from the solvent, and allows the introduction of the sample in the form of dry particles into the high vacuum region.

Electron impact is of interest for molecules which do not ionize with API technique, or when an electron impact spectrum is necessary, since it provides spectral information independent of the sample introduction technique (GC or LC, or direct introduction) and instrument supplier.



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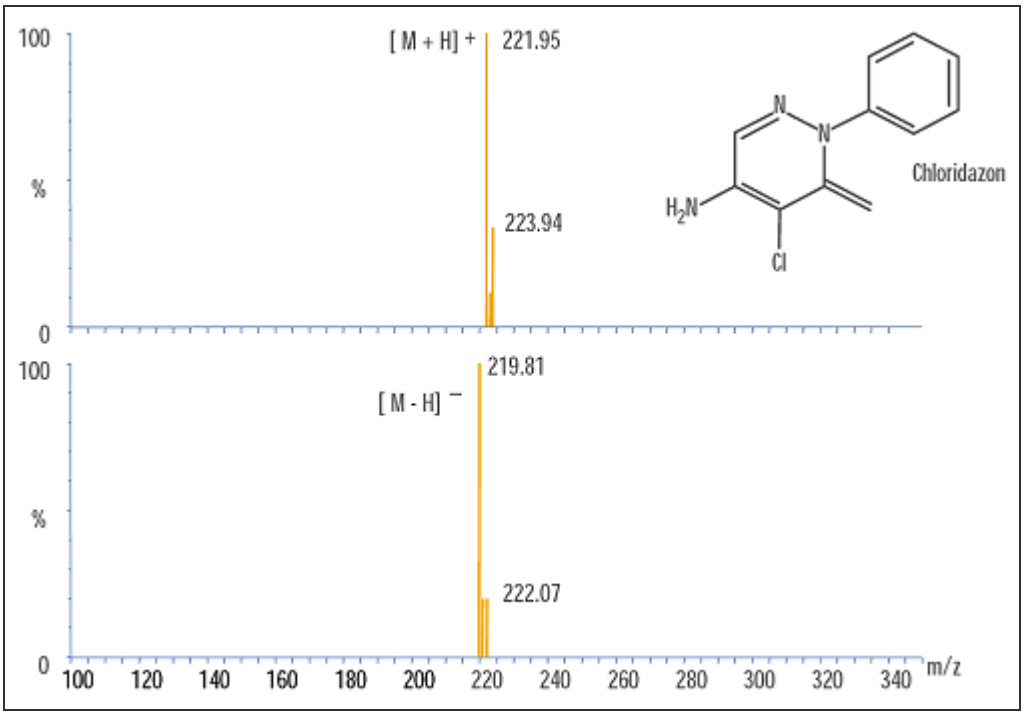
Mass Definition

The mass spectrometer measures the exact mass. Looking at the below mass spectra, the most abundant peak is at 221.95 (top) and 219.81 (bottom). These spectra are obtained with positive ionisation (top) and negative ionisation (bottom). The peaks correspond to the protonated or deprotonated molecule.



If we look for the molecular mass of the chloridazon pesticide, we can find various values in the spectra

- **221.6379:** this is the average mass. It is based on the average atomic masses.
- **221**: this is the nominal mass, calculated on the nominal mass of the most abundant isotopes
- **221.0278:** this is the exact (or monoisotopic) mass, based on the exact mass of the most abundant natural isotopes

The value is slightly different from the expected 222.0278 and 219.0278 because these spectra were obtained with a quadrupole instrument, which does not provide sufficient mass resolution and mass accuracy for obtaining the exact mass.

The next smaller peaks correspond to the C13 and Cl37 isotopes.

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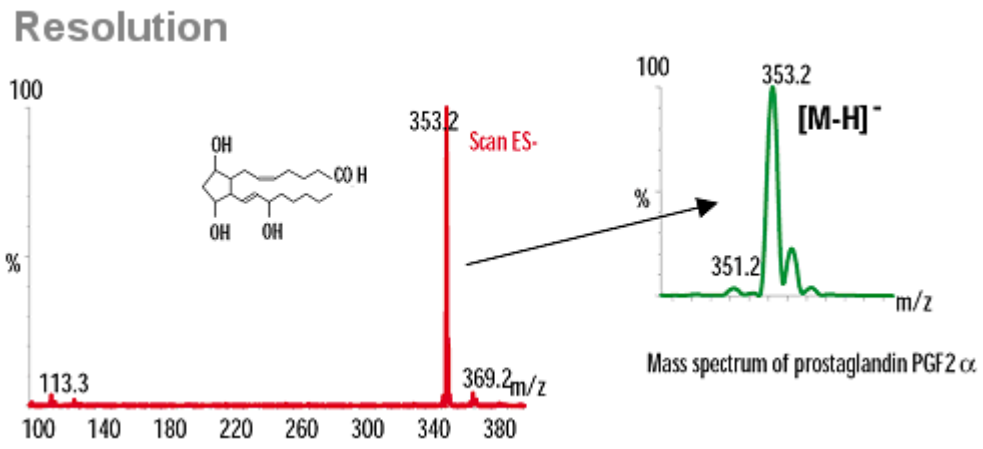
Mass Resolution/Mass Accuracy



Mass resolution: represents the ability to separate two adjacent masses. It measures the "sharpness" of the MS peak.

Mass accuracy: indicates the accuracy of the mass information provided by the mass spectrometer.

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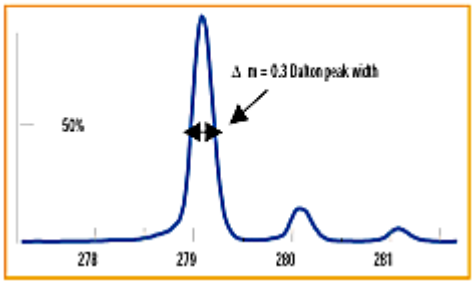
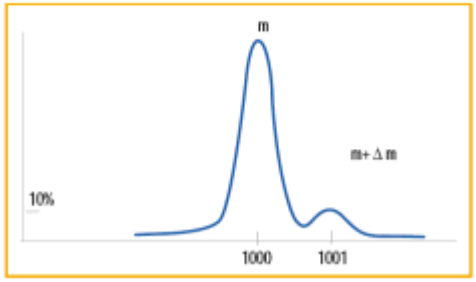
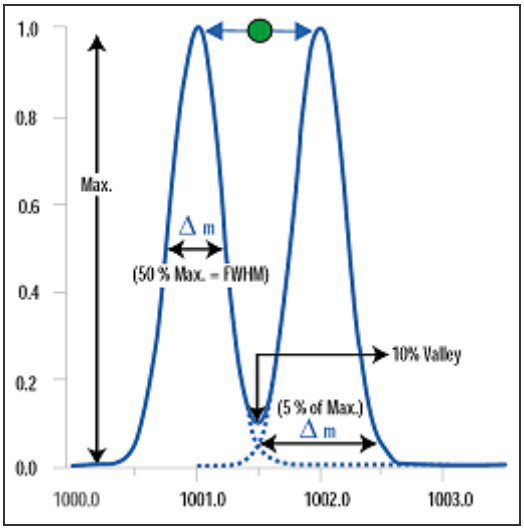
$R = \frac{m}{\Delta m}$ 	$R = \frac{m}{\Delta mr}$ 
Single Ion method Full Width at Half Maximum (FWHM) or at 5% of the peak height	**Double Ion method** 2 adjacent ion peaks with a 10% valley max

Resolution calculation: the above described methods can be used with various valley and Dm definitions, which are represented on the next figure.



The mass accuracy is the difference which is observed between the theoretical mass and the measured mass:

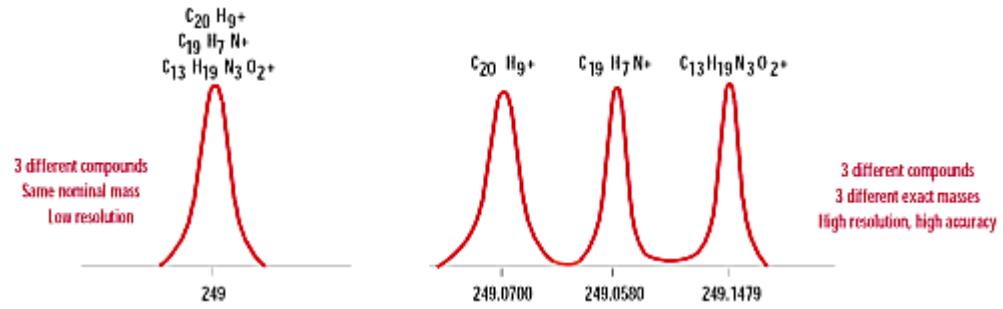
Dm accuracy = m_{real} – $m_{measured}$

It is often expressed in parts per million (ppm):

$ppm = 10^6 * \Delta m\ accuracy / m_{measured}$

i.e.: theoretical mass: 1000, measured mass: 999.9 error: 100 ppm

Mass accuracy is linked to the resolution. A low resolution instrument cannot provide a high accuracy



A high resolution instrument (time of flight, sector, FTMS…), properly used with a reference compound provides the mass information with an accuracy better than 5ppm, which is enough to unambiguously determine the elemental composition.

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Conclusion

The application field of LC/MS is extremely large and is covered by a wide range of instruments and
techniques.

Looking globally at the users, it is possible distinguish three groups, depending on how they use LC/MS

- Users for which the main useful information from the mass spectrometer is the mass information (molecular weight or fragments). The quantitative aspect is of no or little importance.

  Typically, these users wish to:
  - monitor or confirm an organic chemistry synthesis

    - or to trigger a fraction collector when the expected compound elute from the column or to check if a peak on a chromatogram is a metabolite or degradation product of a known parent compound

    - or to get molecular weight and structure information from their compound

- Users for which the main interest is getting a very selective and sensitive detection. These users are targeting specific molecules. The quantitative aspect is important, but the mass information is of secondary importance.

- Users targeting specific molecules, wanting the quantification and the confirmation of the identity. The molecular weight, and the presence of a few specific fragments which the expected abundance are as important as the sensitivity and selectivity.

Waters has developed instruments and software to address those various needs. Our MS specialists will be pleased to help you in selecting the instrument which is the best adapted to your need.

The Mass Spectrometer: Instrument Architectures and Main Characteristics

In this section, we will cover the mass spectrometers which are commonly used in LC/MS configurations.

The analysers used in these instruments are quadrupole, ion trap, time of flight, and combinations such as triple quadrupoles and QTofs. We will not cover instruments like sectors, FTMS…, which are less commonly used in the LC/MS application.

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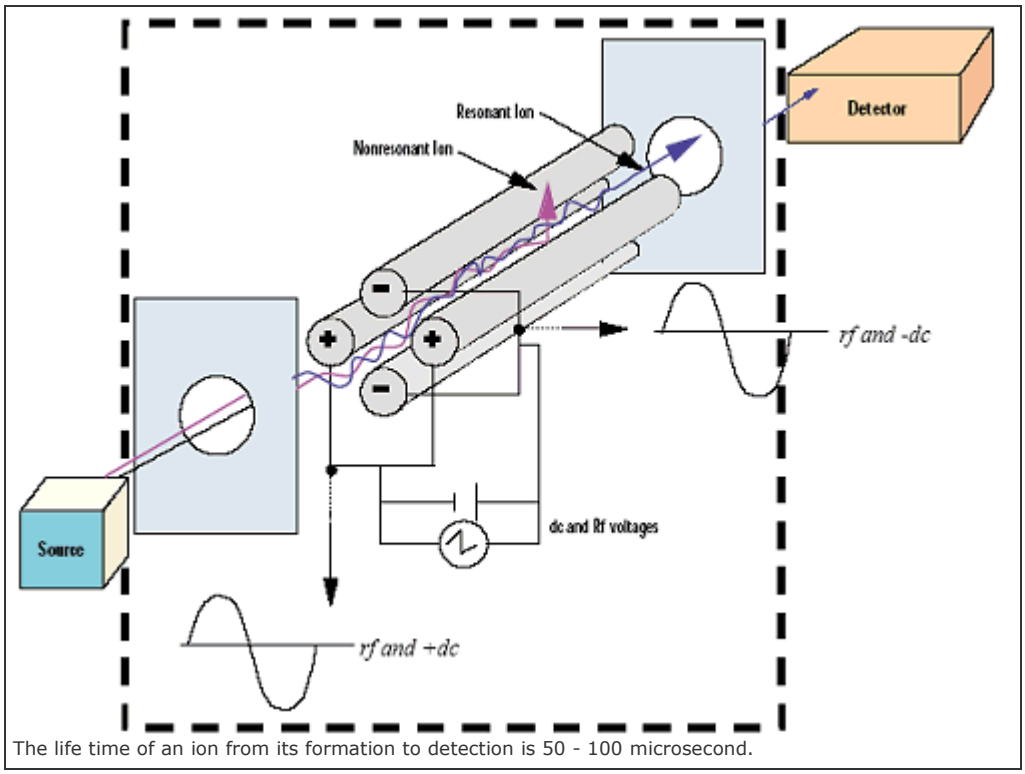
Quadropoles, triple quads

The Quadropole Analyzer

The quadrupole is the most widely used analyser due to its ease of use, mass range covered, good linearity for quantitative work, resolution and quality of mass spectra. All this for a relatively accessible price.

The main characteristics are:

- **Working mass range**: 10 to 4000 A.M.U.
- **Resolution**: usually operated at a resolution = 1000, but resolution can be reasonably pushed up to 4000
- **Mass accuracy**: 0.1 to 0.2 A.M.U.
- **Scan speed**: up to 5000 A.M.U per second



The life time of an ion from its formation to detection is 50 - 100 microsecond.

How it Works

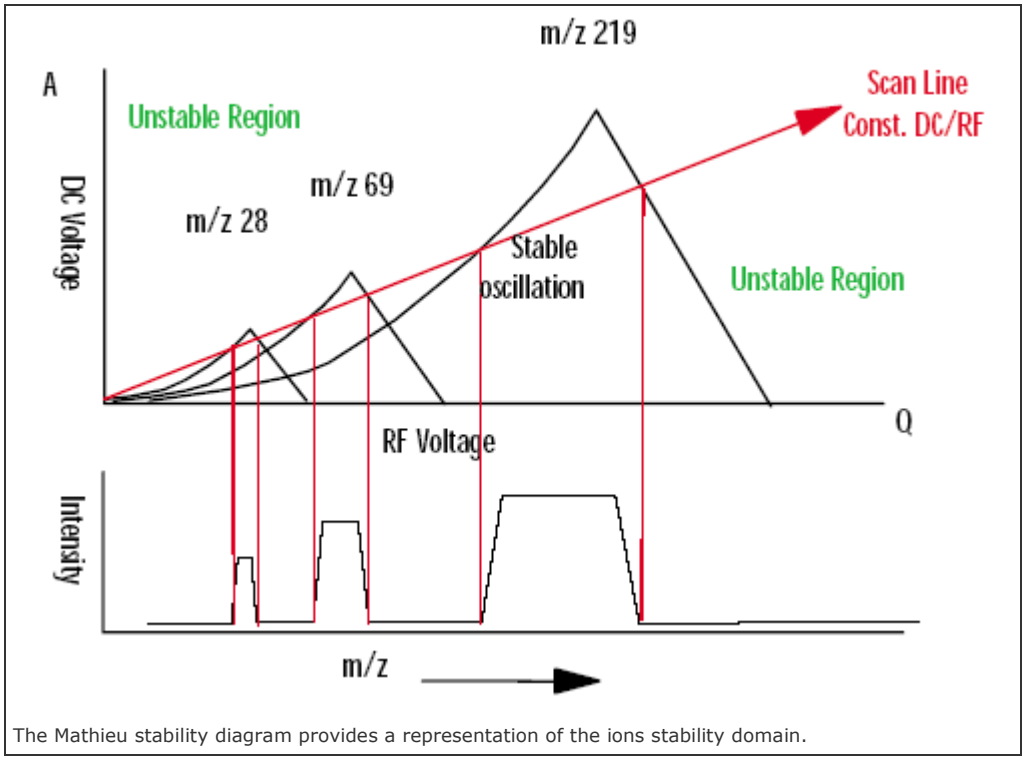
The quadrupole is composed of two pairs of metallic rods. One set of rod is at a positive electrical potential, and the other one at a negative potential. A combination of dc and rf (radio frequency) voltages is applied on each set .

$$V(t) = -V_{dc} - V_{rf}\cos\Omega t \qquad V(t) = V_{dc} + V_{rf}\cos\Omega t$$

The positive pair of rods is acting as a high mass filter, the other pair is acting as a low mass filter. The resolution depends on the dc value in relationship to the rf value. The quads are operated at constant resolution, which means that the rf/dc ratio is maintained constant.

For a given amplitude of the dc and rf voltages, only the ions of a given m/z (mass to charge) ratio will resonate, have a stable trajectory to pass the quadrupole and be detected. Other ions will be de-stabilized and hit the rods. The performance (i.e. ability to separate two adjacent masses across the applicable range) depends on the quad geometry, on the electronics, on the voltage settings and on the quality of the manufacturing. Increasing the

resolution means that fewer ions will reach the detector, and consequently impacts the sensitivity.



The Mathieu stability diagram provides a representation of the ions stability domain.

The quadrupole is scanned with A/Q = constant; the resolution depends on the slope of the scan line.

If the continuous voltage DC is switched off, the scan line is the Q axis: We have now a transfer only device like the hexapoles or octopoles used to transfer and focus the ions into the mass spectrometer optics.

SCAN and SIM

The quadrupole can be used in two modes: SIM (single ion monitoring) or Scan. The SIM mode is also called SIR (single ion recording).

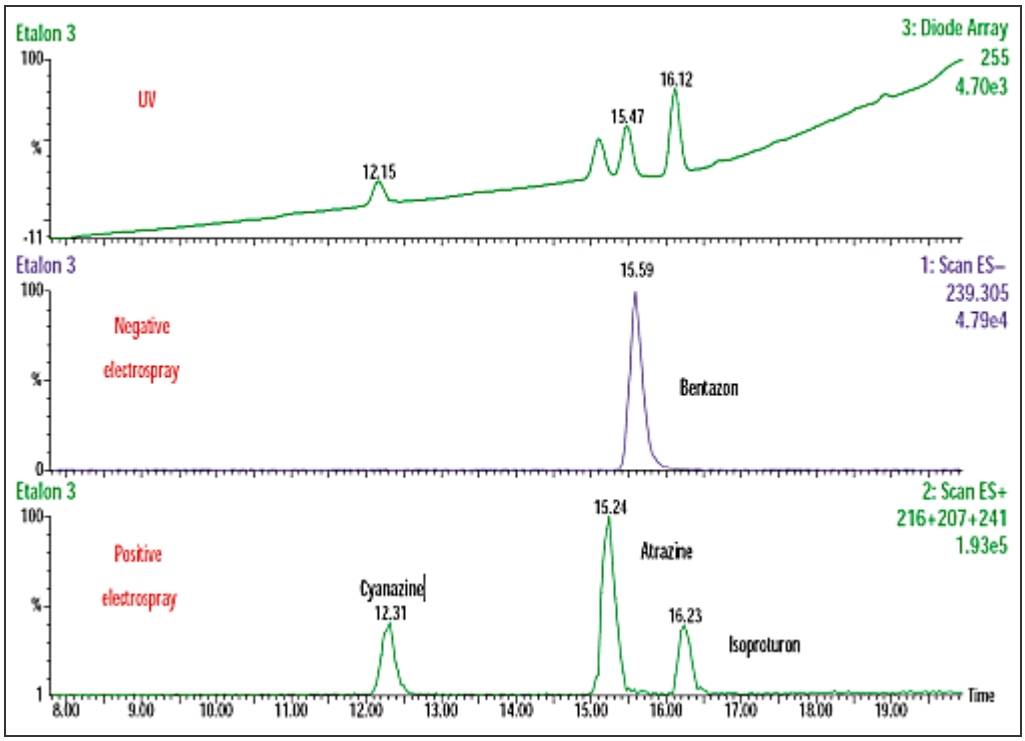
In SIM mode, the parameters (amplitude of the dc and rf voltages) are set to observe only a specific mass, or a selection of specific masses. This mode provides the highest sensitivity for

users interested in specific ions or fragments, since more time can be spent on each mass. That time can be adjusted; it is called the dwell time.

The mass window for observing an ion in SIM mode can be adjusted, in order to compensate small mass calibration shift. This is the span factor.

In Scan mode, the amplitude of the dc and rf voltages are ramped (while keeping a constant rf/dc ratio), to obtain the mass spectrum over the required mass range. The sensitivity is a function of the scanned mass range, san speed, and resolution.

With most LC/MS instrument, it is possible to do positive/negative switching, in order to analyse in the same run molecules that will ionise in positive and negative modes.

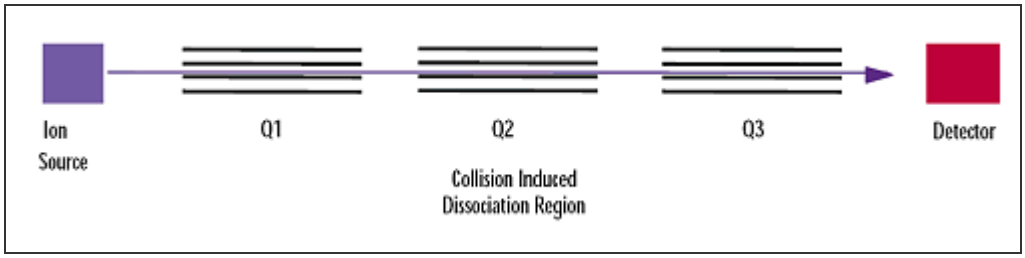


MS/MS with Triple Quadrupoles

The analyser of a “triple quad” instrument consists in two quadrupoles, separated by a collision cell. Such a configuration is often referred as a "tandem in space" instrument.

Precursor ions and product ions are created and analysed in different physical spaces.

Ions must be moved from “source” to analyser (different physical regions) where different functions take place.



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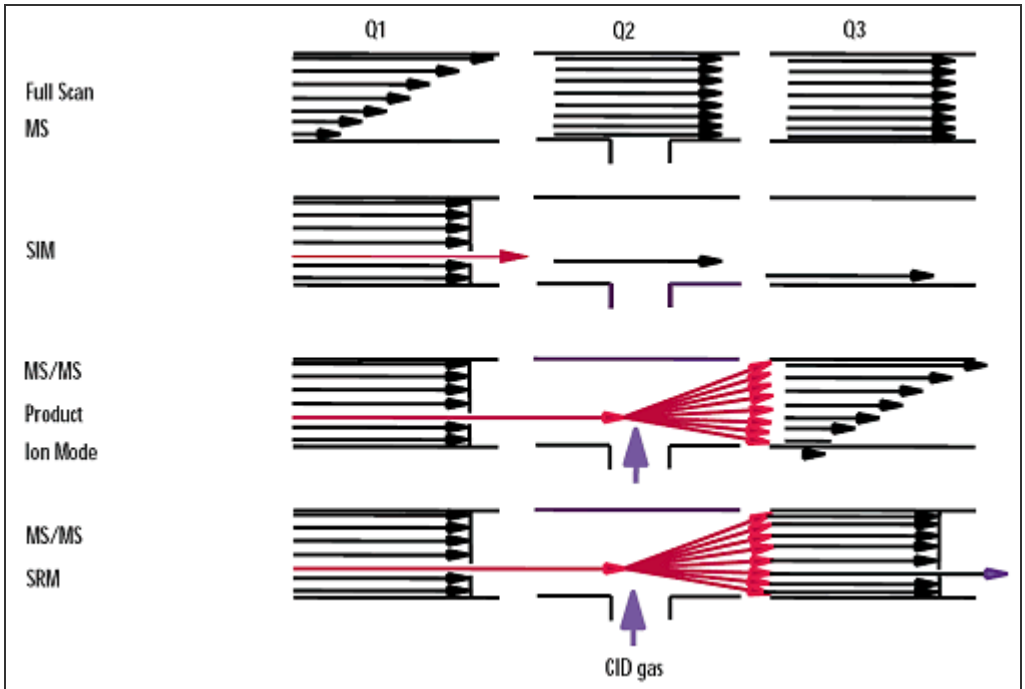

A triple quad instrument can be used in various ways, which are represented on the next figure. The firsts two experiments are in fact using the triple quad in a single quadrupole mode

The first quadrupole is used to select a first ion (precursor), which is fragmented in the collision cell. This is typically achieved in the collision cell by accelerating the ions in the presence of a collision gas (argon, helium…).

The energy of the collision with the gas can be varied to allow different degrees of fragmentaion. The resulting fragmentsare analysed by the second quadrupole, used either in SIM or in scan mode.

Study of mass spectral fragments can provide structural information. When using a single quadrupole instrument, it is possible to obtain fragmentation by using a technique called in source CID. The fragmentation takes place before the introduction of the ions into the optics of the mass spectrometer. This technique is useful if there is no chromatographic interference. With a triple quad system, the first quadrupole acts as a separation device, reducing the need for a perfect chromatographic separation.

The other use of a triple quad system is quantitation. The first analyser, used in SIM mode, selects the parent ion. The second analyser is also used in SIM mode to monitor a specific fragment.

Having two analysers increases the selectivity. The ion signal is reduced during the transmission, but the chemical noise, which is a major limitation for complex samples, is also largely decreased, leading to an improvement of the signal to noise ratio. It is thus possible to do quantitative analysis on complex samples like serum with a very short chromatographic separation, and even with no separation at all. This is the technique of choice for application such as pharmacology studies.

However, one should keep in mind that, when doing quantitation, the first important step is the ionisation, which takes place in the source.The presence of interfering compounds in the source might cause unexpected effects, like "ion suppression". Such effects impact the quantitation, whatever the MS analyser. Using an MS/MS system might reduce the problem, but does not eliminate it.

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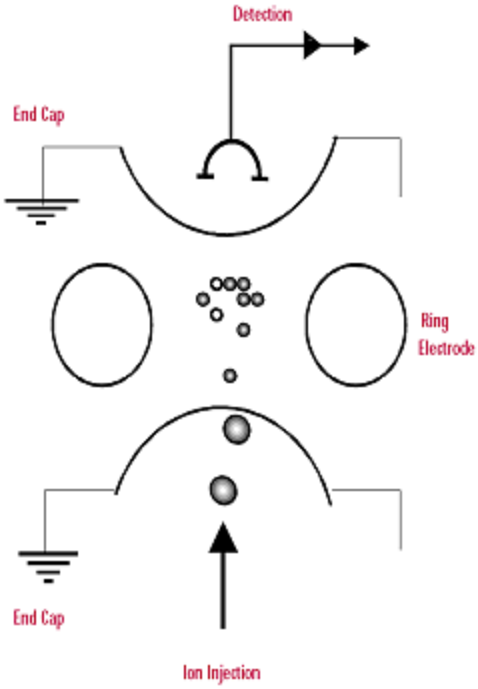
The Ion Trap Analyzer

This analyser is also known as the quadrupole ion trap analyser (QIT). It was first used on GC/MS instruments, then on LC/MS systems.

The principle of the trap is to store the ions in a device consisting of a ring electrode and two end cap electrodes. The ions are stabilized in the trap by applying a RF voltage on the ring electrode. Formaximum efficiency, the ions must be focussed near the centre where the trapping fields are closest to the ideal and the least distorted - maximizing resolution and sensitivity.This is achieved by introducing a damping gas (99.998% helium) that collisionally cools injected ions, damping down their oscillations until they stabilize.



By ramping the RF voltage, or by applying supplementary voltages on the end cap electrodes, or by combination of both, it is possible to:

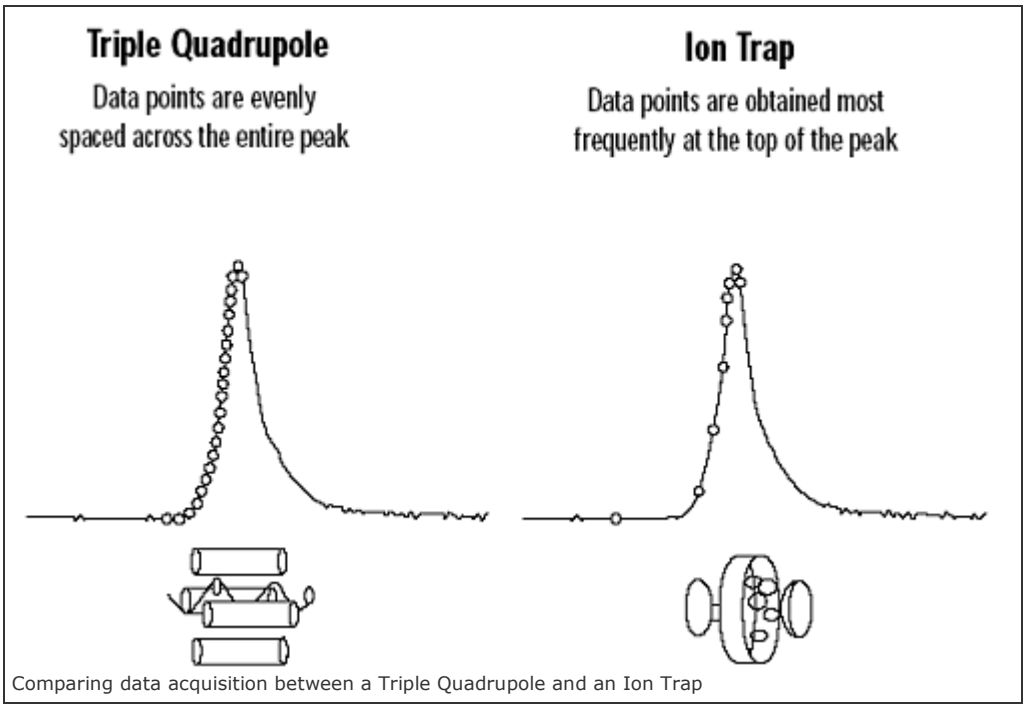
- destabilise the ions, and eject them progressively from the trap
- keep only one ion of a given m/z value in the trap, and then eject it to observe it specifically
- or keep only one ion in the trap, fragment it by inducing vibrations, and observe the fragments. This is MS/MS. Since everything takes place in the same place, but at a different time, that approach is called MS/MS "in time"
- repeat the last operation a few times to progressively fragment the ions. That is MS/MS^n

Resolution of the ion trap analyser: the resolution which is achievable with an ion trap depends upon the scan range and scan speed. When scanning over a few hundred Daltons in a fraction of a second, the typical resolution is similar to the resolution of a quadrupole. However, it is possible to increase the resolution by scanning at lower speed over a reduced mass range ("zoom scan"). In these conditions the resolution exceeds 5000 when scanning over a 10 Dalton window, which is sufficient to determine the number of charges of a multicharged small peptide.

Data acquisition: A typical acquisition cycle, for a trap used in MS mode, includes the following automated steps(from supplier literature)

1. Prescan: this is to determine the needed injection time : 60 ms
2. Ion injection: about 500 ms. This is the admission of the ions into the trap. The duration depends upon the signal intensity
3. Set the trap parameters for ion isolation, activation…: 80 ms
4. Mass Analysis: about 70 ms Additional steps are needed for MS/MS or MSn operation

Additional steps are needed for MS/MS or MS^n operation



Comparing data acquisition between a Triple Quadrupole and an Ion Trap

The ion trap is more sensitive in scan mode than in SIM mode.

Generally quadrupole instruments used in SIM mode provide an order of magnitude better limit of quantitation with lower relative standard deviations for quantitative experiments than an ion trap, primarily due to integration effect (more data points to determine the peak start and end with a quadrupole).

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Time of Flight (TOV) Anaylyzer

This analyser is commonly called the TOF. The TOF is used in single MS systems, with an LC introduction, with a GC introduction, or with MALDI ionisation. In MS/MS configuration, the TOF is associated to a quadrupole (QTof), or to another TOF (TOF-TOF) or to an Ion Trap (QIT/TOF).

Principle of the time of flight analyser: In a Time–Of–Flight (TOF) mass spectrometer, ions formed in an ion source are extracted and accelerated to a high velocity by an electric field into an analyser consisting of a long straight 'drift tube'. The ions pass along the tube until they reach a detector.

After the initial acceleration phase, the velocity reached by an ion is inversely proportional to its mass (strictly, inversely proportional to the square root of its m/z value).

Since the distance from the ion origin to the detector is fixed, the time taken for an ion to traverse the analyser in a straight line is inversely proportional to its velocity and hence proportional to its mass (strictly, proportional to the square root of its m/z value). Thus, each m/z value has its characteristic time–of–flight from the source to the detector.

Time of Flight equations: The first step is acceleration through an electric field (E volts). With the usual nomenclature (m = mass, z = number of charges on an ion, e = the charge on an electron, v = the final velocity reached on acceleration), the kinetic energy (mv /2) of the ion is given by equation (1).

$$mv/2 = z.e.E \quad (1)$$

Equation (2) follows by simple rearrangement.

$$v = (2z.e.E/m)1/2 \quad (2)$$

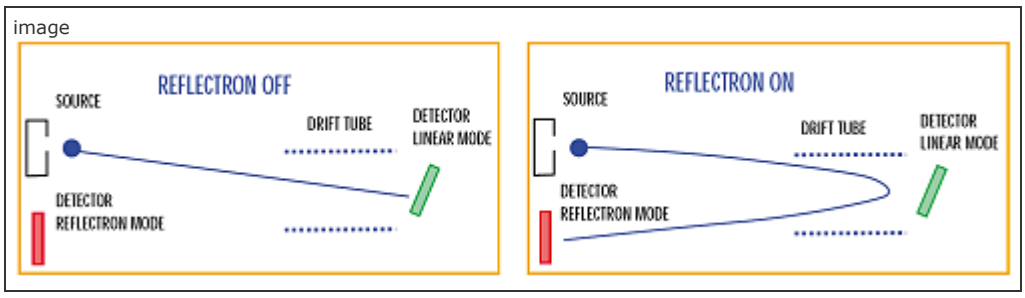
If the distance from the ion source to the detector is d, then the time (t) taken for an ion to traverse the drift tube is given by equation (3).

$$t = d/v = d/(2z.e.E/m)1/2 = d.[(m/z)/(2e.E)]\ 1/2 \quad (3)$$

In equation (3), d is fixed, E is held constant in the instrument and e is a universal constant. Thus, the flight time of an ion t is directly proportional to the square root of m/z (equation 4).

$$t = (m/z)\ 1/2\ x\ a\ constant \quad (4)$$

Equation (4) shows that an ion of m/z 100 will take twice as long to reach the detector as an ion of m/z 25:



In order to increase the resolution, the ion trajectory is bent by an electronic mirror, the reflectron. When going through the reflectron, the dispersion of ions of the same m/z value is minimized, leading to a great improvement of resolution

Characteristics of the time of flight analyser:

Mass range: there is no upper theoretical mass limitation; all ions can be made to proceed from source to detector and the the upper mass limit exceeds 500 kDa. In practice, there is a mass limitation, in that it becomes increasingly difficult to discriminate between times of arrival at the detector as the m/z value becomes large. Another limitation is that very large molecules are difficult to ionise. Using an ionisation technique which produces multiply charged ions, like electrospray ionisation, extends the working range of the TOF analyser

Resolution: with a TOF instrument, it is possible to obtain 10000 FWHM resolution

Mass accuracy: better than 5 ppm, using a reference mass; that allows unambiguous formula determination of small organic molecules

MALDI TOF, OA-TOF

Two different sample introduction/ionisation techniques are used with time of flight analysers: MALDI and orthogonal acceleration.

oa-TOF: this is for in line coupling. This configuration is used for LC/MS with API ionisation, and for GC/MS. The ions are transferred from the source to the analyser through a transfer optics. The "pusher" accelerates the ions to the same level of energy, and gives the start signal for timing the flight.

QTOF

The QTof is a hybrid MS/MS instrument combining a quadrupole with a Tof analyser. This combination provides the benefits of in space MS/MS (selectivity, flexibility for collision experiments) with the advantages of the Tof (sensitivity in scan mode, fast scan, accurate mass, resolution). This is an ideal combination for sophisticated applications.

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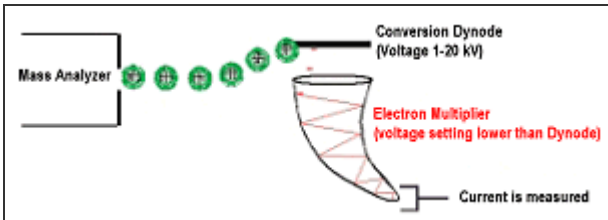
Detectors

The detector is the device which detects the ions separated by the analyser. 3 different types of detector are used with the analysers described in the previous pages: Electron multipliers, dynolyte photomultiplier, microchannel plates.

Electron multiplier

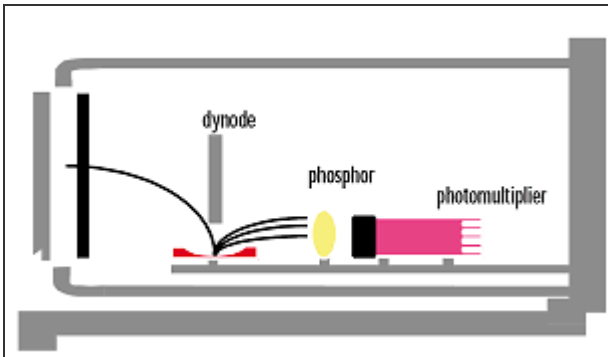
A conversion dynode is used to convert either negative or positive ions into electrons. These electrons are amplified by a cascade effect in a horn shape device, to produce a current. This device, also called channeltron, is widely used in quadrupole and ion trap instruments.



Electron muliplier

Dynolyte photomultiplier

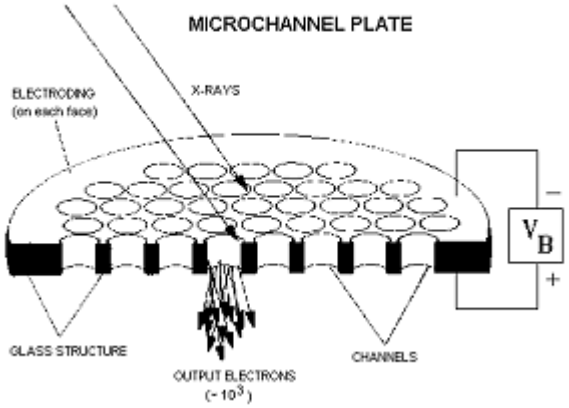
Ions exiting the quadrupole are converted to electrons by a conversion dynode.These electrons strike a phosphor which when excited, emit photons. The photons strike a photocathode at the front of the photomultiplier to produce electrons and the signal is amplified by the photomultiplier. The photomultiplier is sealed in glass and held under vacuum. This prevents contamination and allows the detector to maintain its performance for a considerably longer period than conventional electron multipliers.



Dynolyte photomultiplier

Microchannel plate

Most TOF spectrometers employ multichannel plate (mcp) detectors which have a time response < 1 ns and a high sensitivity (single ion signal > 50 mV). The large and plane detection area of mcp's results in a large acceptance volume of the spectrometer system. Only few mcp channels out of thousands are affected by the detection of a single ion i.e. it is possible to detect many ions at the same time which is important for laser ionisation where hundreds of ions can be created within a few nanoseconds. dynode phosphor photomultiplier.



Microchannel plate
Source: hea-www.harvard.edu/HRC/mcp/mcp.html