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INTRODUCTION

HPLC science has always been moving to more specific and more sensitive detection modes. Advances made in LC-MS coupling (new interfacing techniques, more user friendly systems) make it easier for chromatographers to implement LC/MS.

Waters, the worldwide leader in HPLC, has a long experience of combining LC to MS. The acquisition of Micromass has created the opportunity for both companies to reinforce their structures and product offerings.

Both Waters and Micromass have a focus on innovative technologies and high performance products. Now, our new structure is offering to LC-MS users the most complete and homogenous product range for HPLC-MS coupling.

Every day, chromatographers make the decision to go for LC-MS, to get better information on their samples, to gain sensitivity and selectivity, or to cope with the need of a higher throughput.

When faced with a variety of techniques and instruments, making the correct choice of your LC/MS instrument is not an easy task.

We hope that this booklet will assist you in making the correct choice of an LC/MS instrument.

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FUNDAMENTALS:

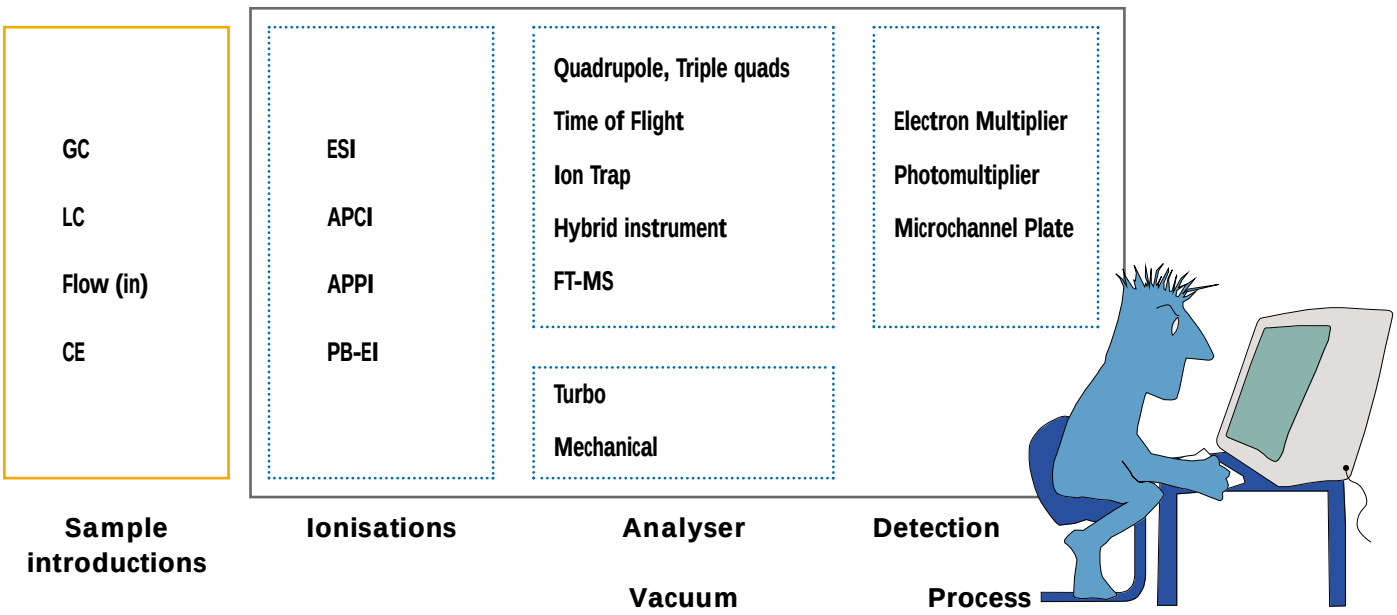
PRINCIPLE OF MASS SPECTROMETRY

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 analyser. This element separates the gas phase ions.

The analyser 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 analyser is operated under high vacuum, so that the ions can travel to the detector with a sufficient yield.

In addition to the analyser, 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

FUNDAMENTALS:

PRINCIPLE 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 ionisation.

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.

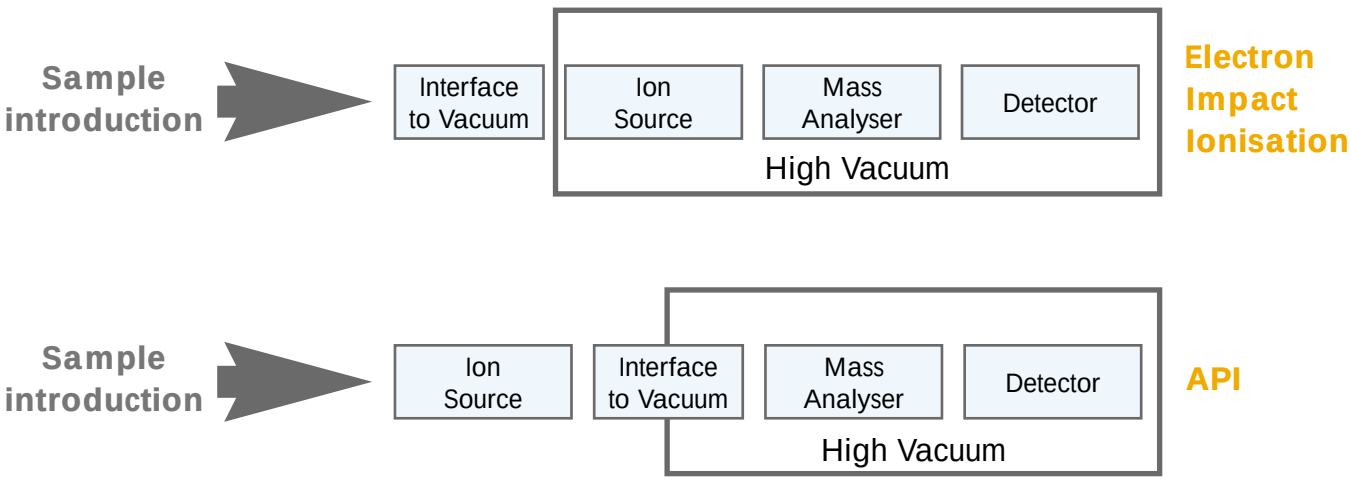
Most instruments now atmospheric pressure ionisation (API) technique where solvent elimination and ionisation steps are combined in the source and take place at atmospheric pressure.

When electron impact ionisation (EI) is the choice, the solvent elimination and ionisation 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 ionise 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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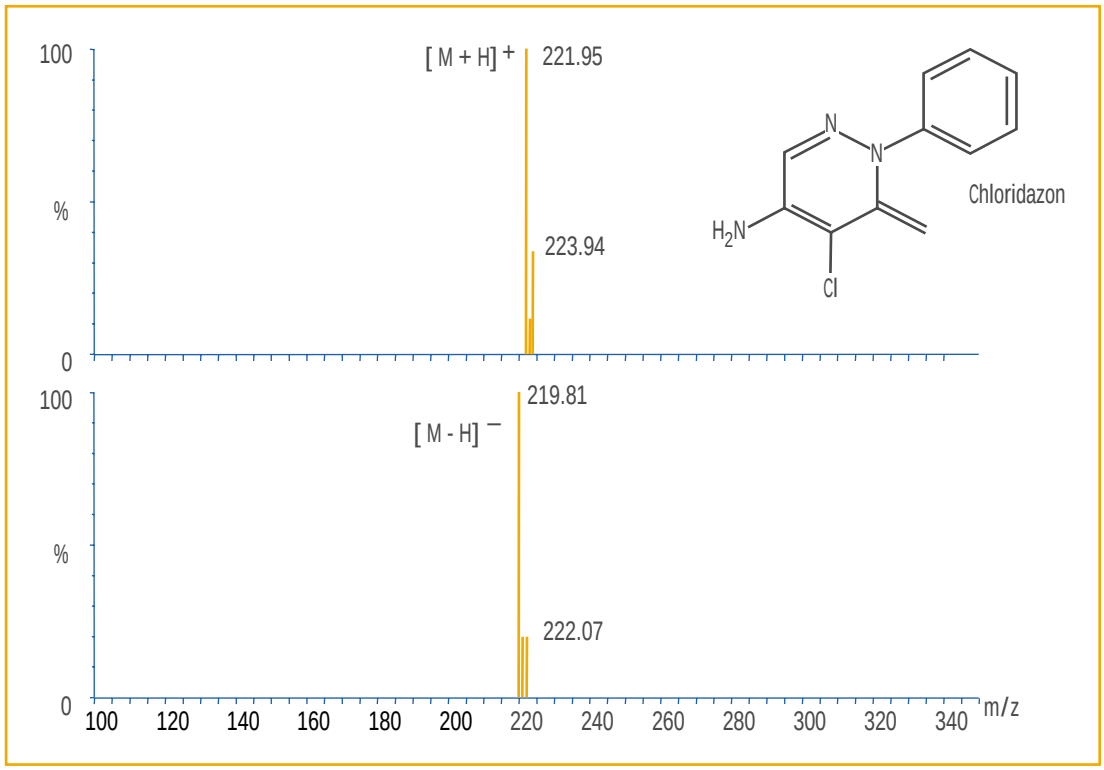
MASS DEFINITION



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

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 mass spectrometer measures the exact mass. Looking at the above 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.

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 C_{13} and Cl_{37} isotopes.

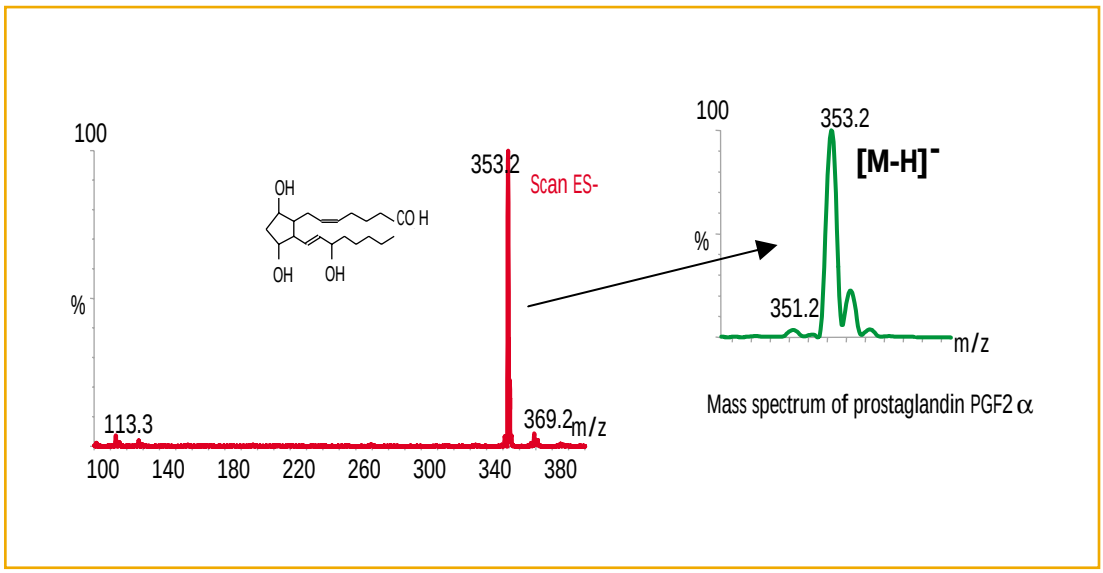
FUNDAMENTALS:

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.

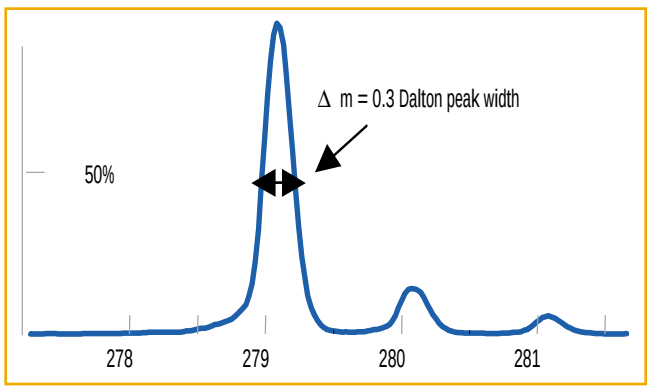
Resolution



Single Ion method

Full Width at Half Maximum (FWHM) or at 5% of the peak height

$$R = \frac{m}{\Delta m}$$



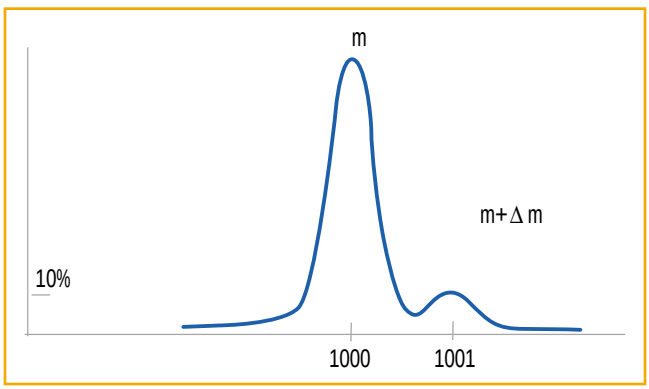
Resolution = m / (FWHM)

In that case R= 279 / 0.3 ~ 1000

Double Ion method

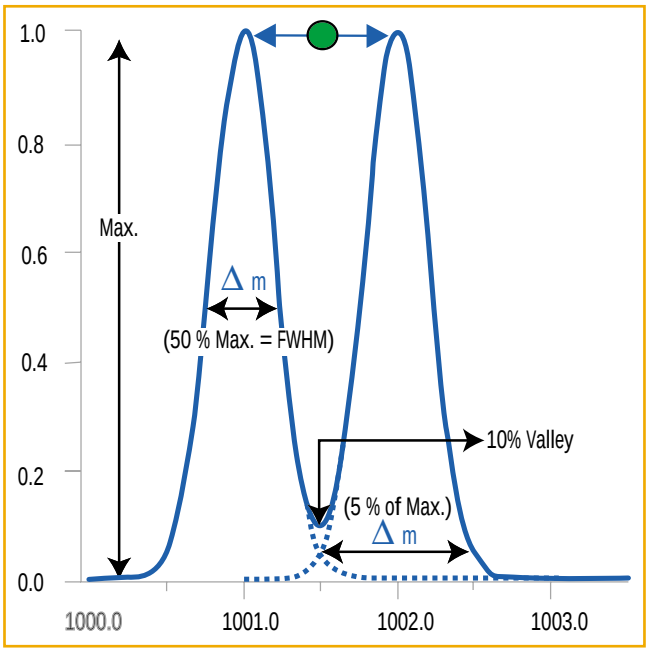
2 adjacent ion peaks with a 10% valley max

$$R = \frac{m}{\Delta mr}$$



In that case R= 1000 / 1 = 1000

MASS RESOLUTION, MASS ACCURACY



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

Accuracy

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

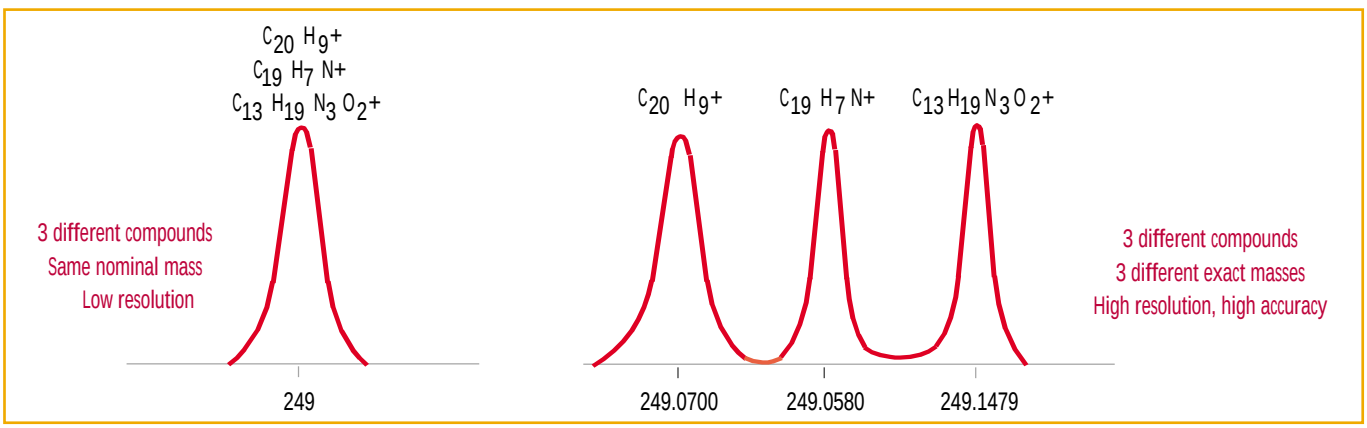
Dm accuracy = $m_{real} - m_{measured}$

It is often expressed in parts per million (ppm)

ppm = 10^6 * Δm 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.

THE MASS SPECTROMETER:

INTRODUCTION

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, QTofs.

We will not cover instruments like sectors, FTMS…, which are less commonly used in the LC/MS

application. Useful information on these instruments can be found on suppliers web sites,

THE MASS SPECTROMETER:

THE QUADRUPOLE ANALYSER

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.

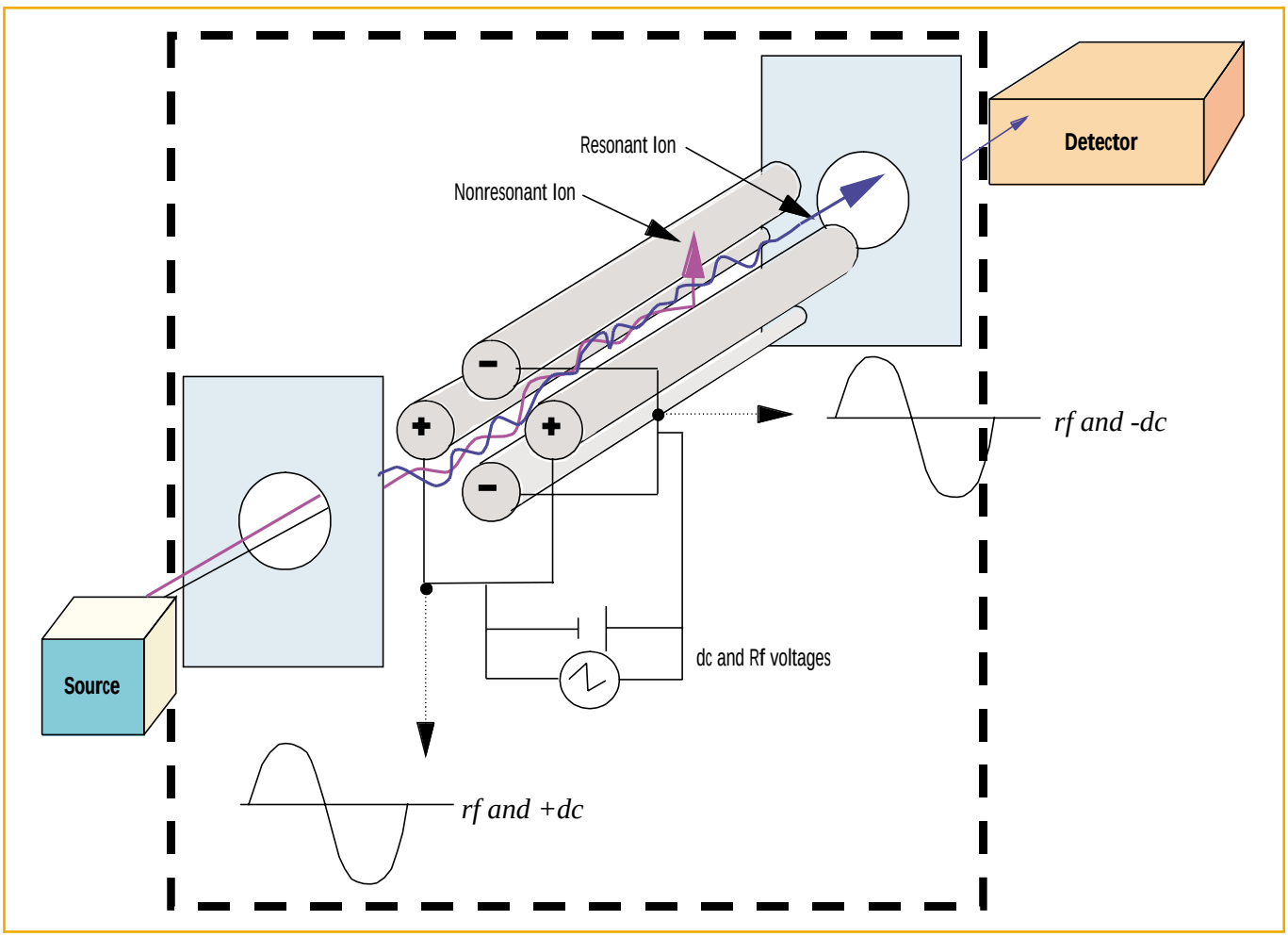
The main characteristics are:

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.

THE QUADRUPOLE ANALYSER:

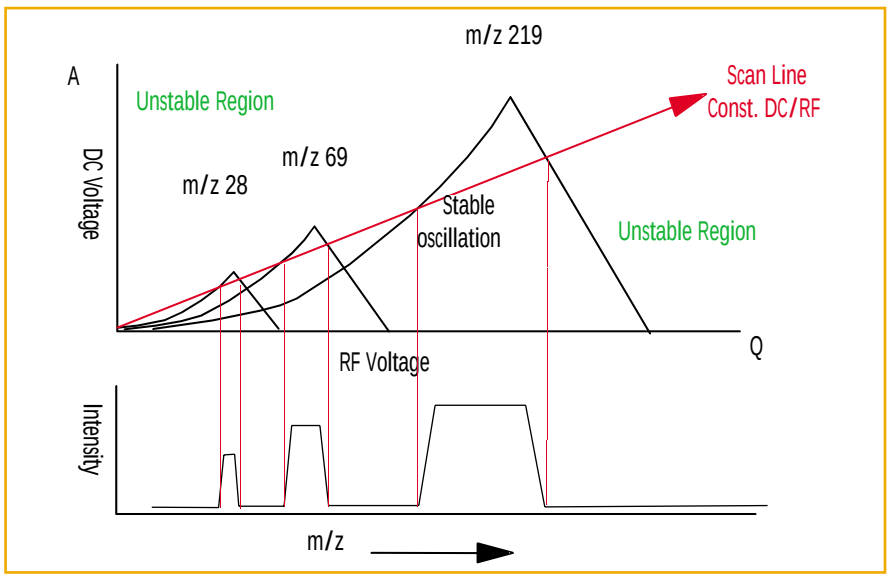
HOW IT WOKS

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 Q and A axis correspond to the following equations

$$Q = \frac{4\ e\ RF}{m r_o^2\ \omega^2}$$

$$A = \frac{8\ e\ DC}{m r_o^2\ \omega^2}$$

With:
e = charge, m = mass, ro = radius between the rods, w = RF frequency, RF = radio frequency voltage, DC = direct current voltage

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

THE QUADRUPOLE ANALYSER:

SCAN AND SIM

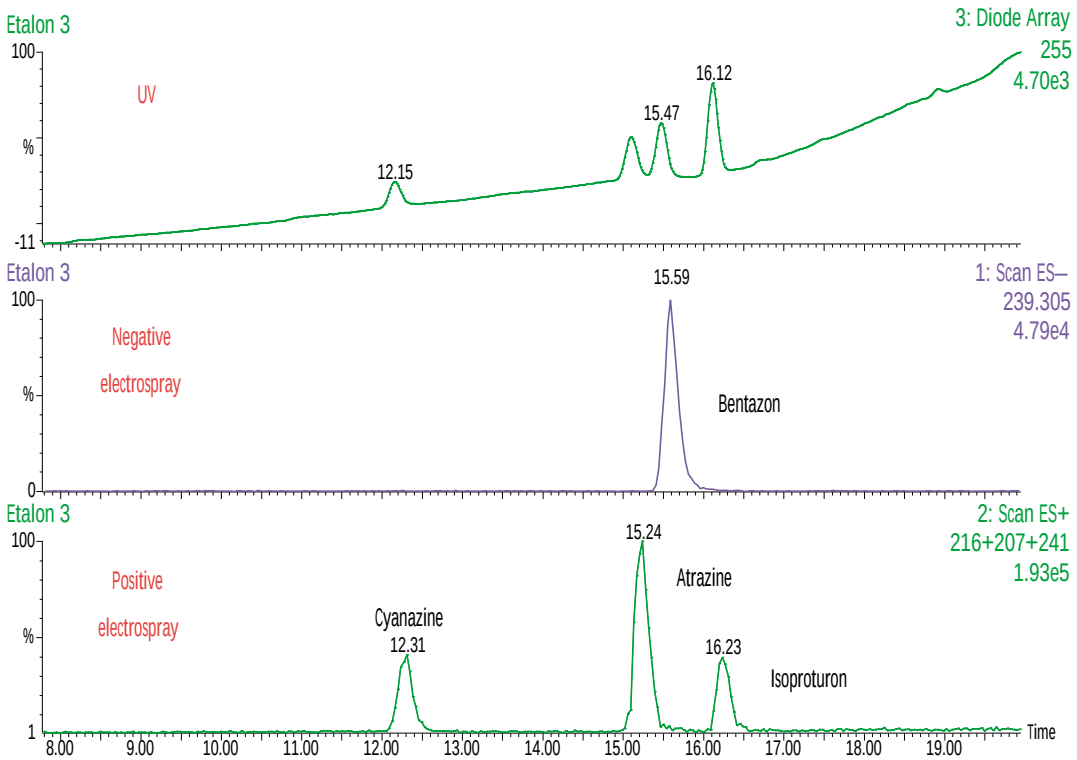
The quadrupole can be used in two modes: SIM (single ion monitoring) or Scan. The SIM mode is also called SIR (single ion recording)

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.

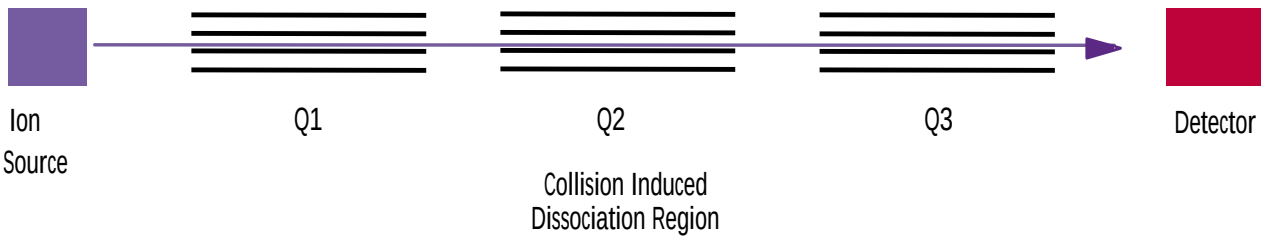


MS / MS WITH TRIPLE QUADRUPOLES

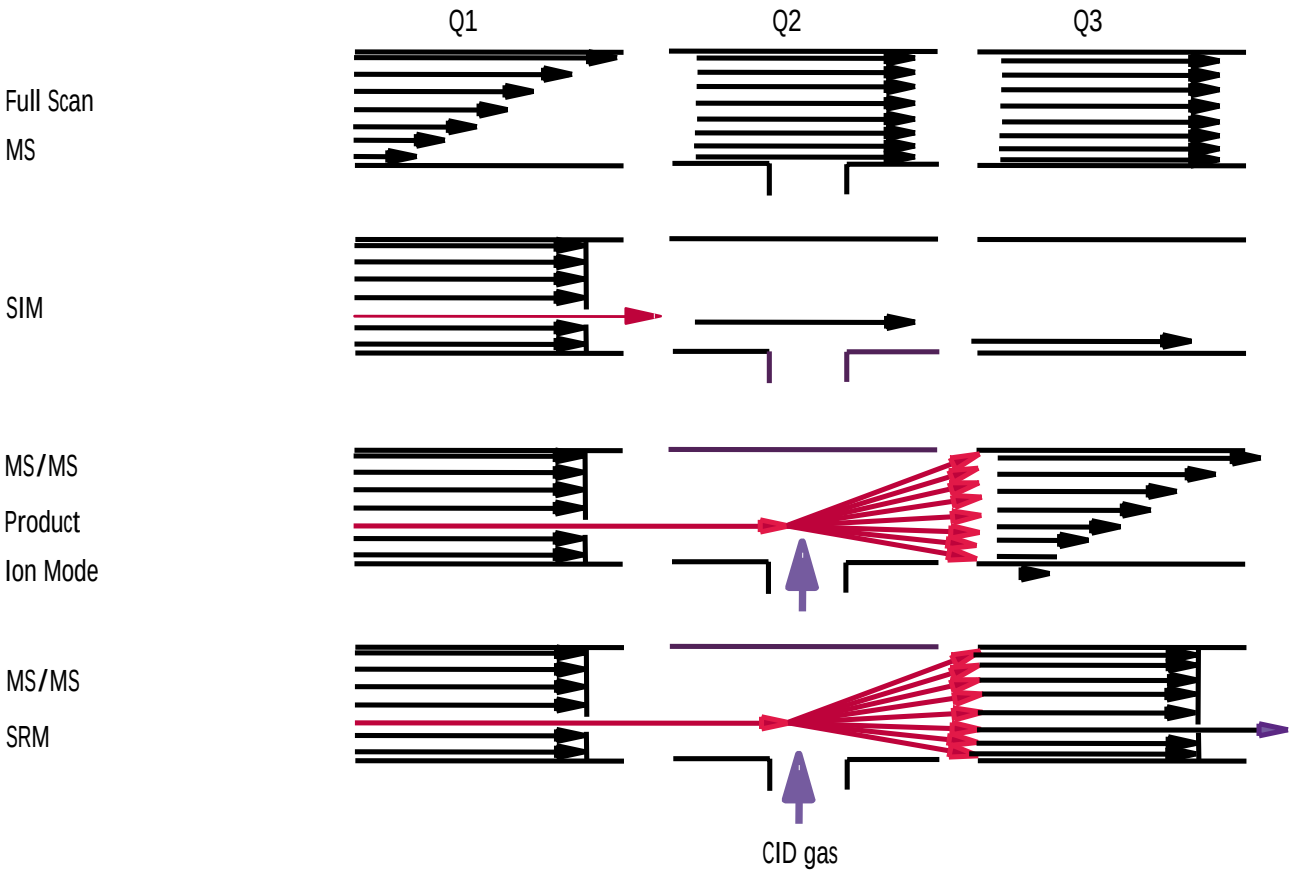
The analyser of a “triple quad” instrument consists in two quadrupoles, separated by a collision cell. Such a configuration is often referred as a "tandem in space" instrument.

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.



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.

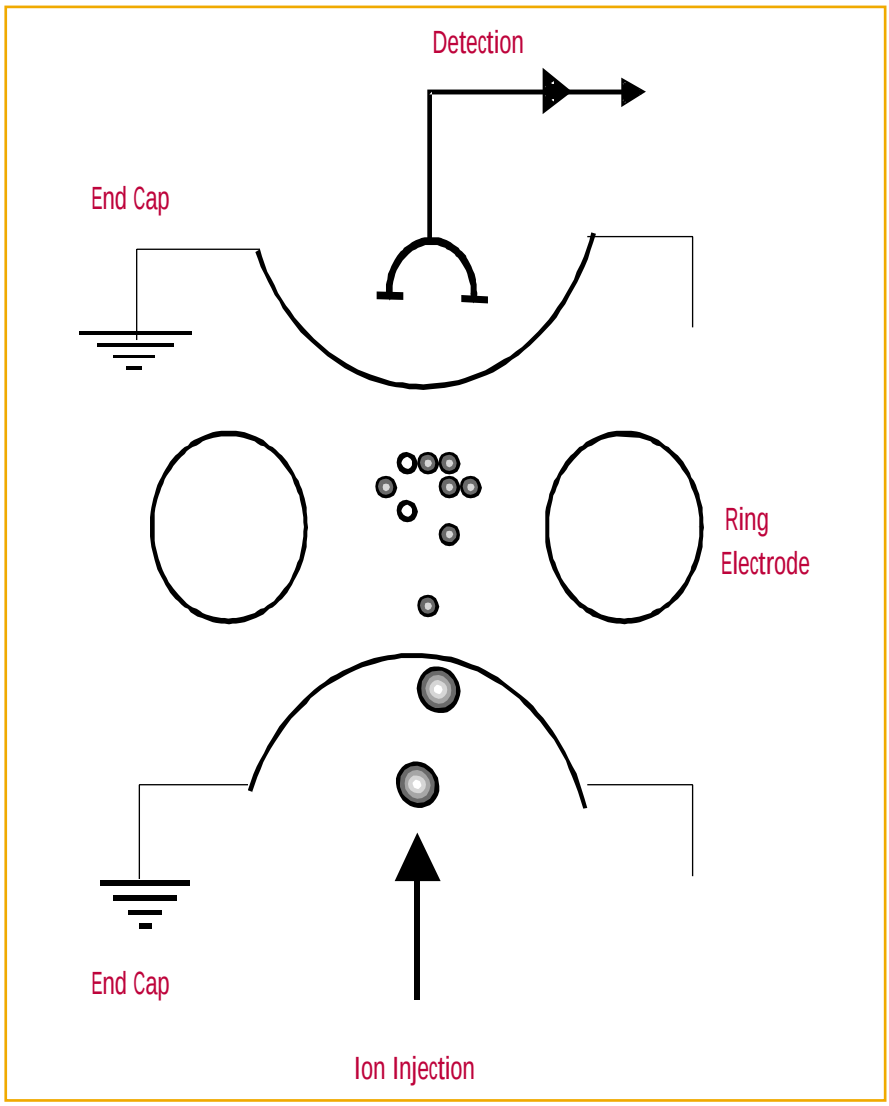
Having two analysers increases the selectivity. The ion signal is reduced during the transmission, but the chemical noise, which is a major limitation for complex samples, is also largely decreased, leading to an improvement of the signal to noise ratio. It is thus possible to do quantitative analysis 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.

THE ION TRAP ANALYSER

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.

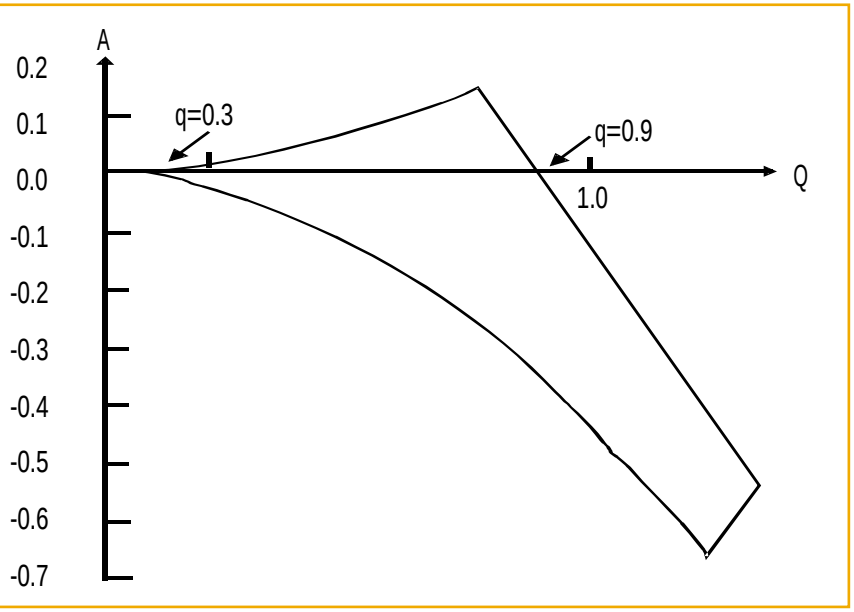


By ramping the RF voltage, or by applying supplementary voltages on the end cap electrodes, or by combination of both, it is possible to:

- 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

UNDERSTANDING THE TRAP

Understanding the trap: like for a quadrupole, the stability domain of the ions in the trap can be represented with the Mathieu stability diagram



$$Q = \frac{4\ e\ RF}{m r_o^2\ w^2}$$

With

e = charge = z, m = mass

r_o = radius between the rods

w = RF frequency

RF = radio frequency voltage

The ions are stable in the trap if the Q value lies between 0.3 and 0.9

0.3 < Q < 0.9.

This parameter has important consequences when doing fragmentation (in other words MS/MS) in a trap: the fragments which are smaller than about one third of the precursor ion will have a Q value > 0.9 and will be lost.

By increasing the RF voltage it is possible to extract an ion (m/z is fixed) from the trap. It is also possible to play with the voltage on the end cap electrodes, which affects the A value. By combining both actions, it is possible to eliminate from the trap the high masses and low masses, and keep in the trap only the desired ions. The process is more difficult if one wants to select a specific ion, in the presence of an excess of another ion having a close m/z value

Charge density in the trap: resolution and performance in an ion trap are dependent upon the charge density of ions in the trap. If too many ions are present at the same time in the trap, the electrical fields are distorted. Also, collisions between the ions may occur, leading to unexpected dissociation or chemical reactions. In this case, the spectra and the quantitation will be impacted.

This is why a short pre-scan is achieved automatically to determine the optimum ion sampling time for each of the acquisition point, so that enough, but not too many ions will be introduced. The optimum is to have between 300 and 1000 ions present in the trap. Consequently the sampling time varies along the chromatographic peak.

UNDERSTANDING THE TRAP

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

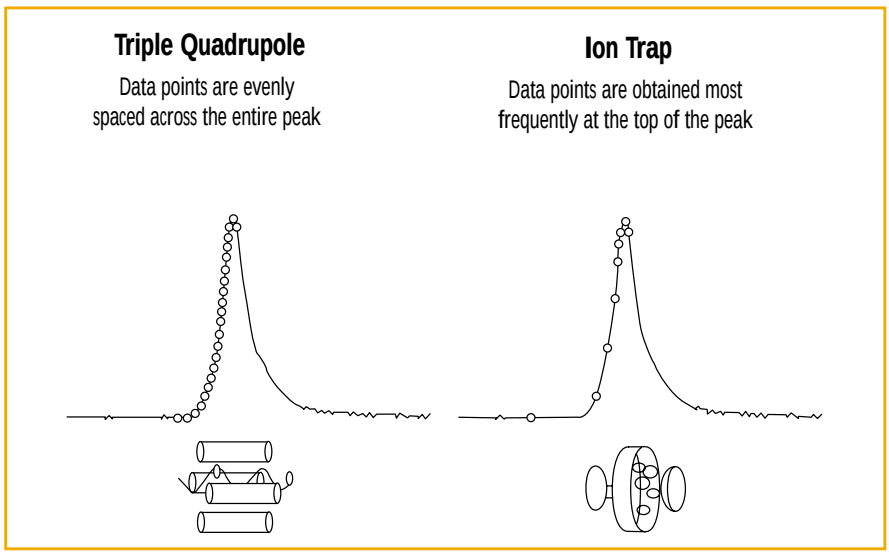
2: Ion injection: about 500 ms. This is the admission of the ions into the trap. The duration depends upon the signal intensity

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 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.

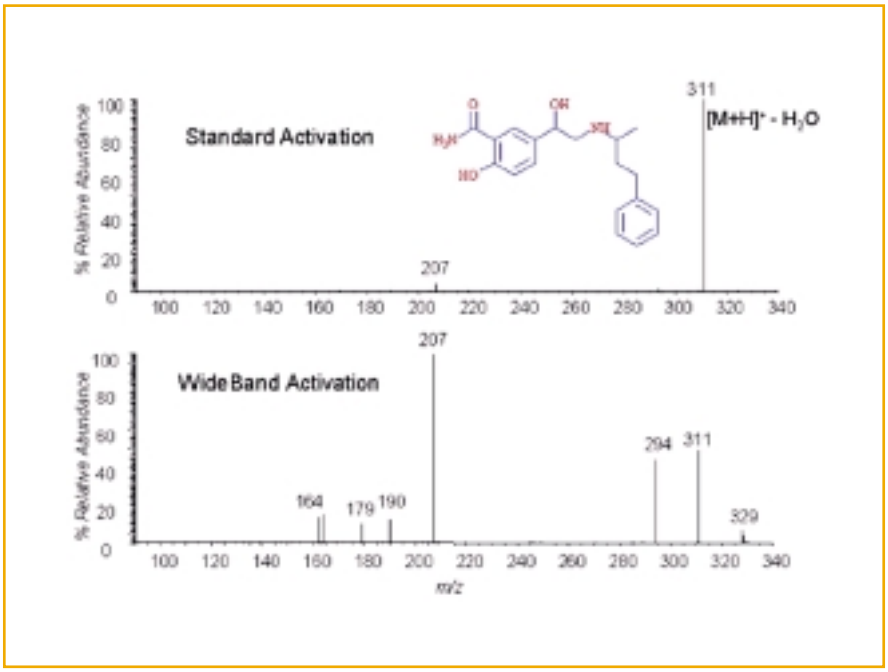
Generally quadrupole instruments used in SIM mode provide an order of magnitude better limit of quantitation with lower relative standard deviations for quantitative experiments than an ion trap, primarily due to integration effect (more data points to determine the peak start and end with a quadrupole).

MS/MS with ion traps:

The principle is to isolate only the ion of interest in the trap , fragment it and then scan the product ions.

Isolation is achieved by increasing the RF voltage to eliminate the low mass ions, then adjusting the voltage on the end cap electrodes to eliminate the high masses (see the Mathieu stability diagram)

Fragmentation: to fragment an ion , if is necessary to impart some energy. In the collision cell of a triple quad, or when doing in source fragmentation, this is obtained by applying an acceleration voltage. In an ion trap, this is obtained by "shaking" the ion, with an RF voltage. Each ion has it's own resonation frequency, so the product ions will normally not be fragmented, leading to limited structural information. In order to obtain richer spectra, a technique called wide band activation has been developed. It's possible to build libraries of ion trap spectra, but ion trap spectra are usually different from quadrupole or triple quad spectra.



The figure above compares fragmentation spectra obtained from an ion trap employing two modes of fragmentation on the protonated product ion of Labetalol (311 m/z). Top spectrum utilizes the standard mode of activation by isolating the product ion and applying a specific secular frequency.

The bottom spectrum was acquired via WideBand Activation™*.

** WideBand Activation™ is a trade mark of Thermo*

THE TIME OF FLIGHT ANALYSER

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 ($mv^2/2$) of the ion is given by equation (1).

$$\mathbf{mv^2/2 = z.e.E} \quad (1)$$

Equation (2) follows by simple rearrangement.

$$\mathbf{v = (2z.e.E/m)^{1/2}} \quad (2)$$

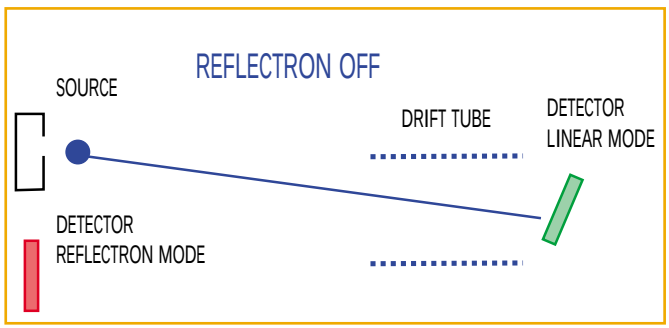
If the distance from the ion source to the detector is d, then the time (t) taken for an ion to traverse the drift tube is given by equation (3).

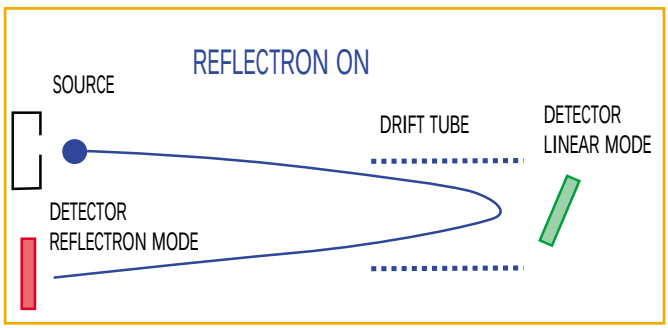
$$\mathbf{t = d/v = d/(2z.e.E/m)^{1/2} = d.[(m/z)/(2e.E)]^{1/2}} \quad (3)$$

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).

$$\mathbf{t = (m/z)^{1/2} \times a\ constant} \quad (4)$$

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

THE TIME OF FLIGHT ANALYSER

Characteristics of the time of flight analyser:

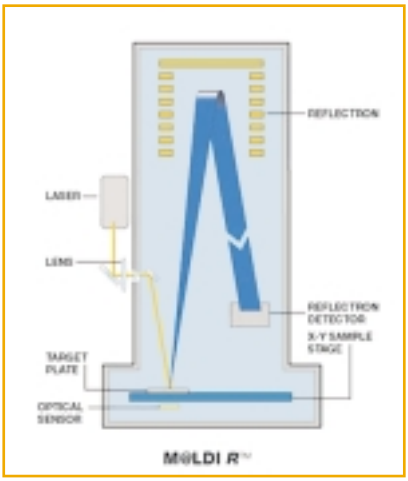
Mass range: there is no upper theoretical mass limitation; all ions can be made to proceed from source to detector and the the upper mass limit exceeds 500 kDa. In practice, there is a mass limitation, in that it becomes increasingly difficult to discriminate between times of arrival at the detector 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

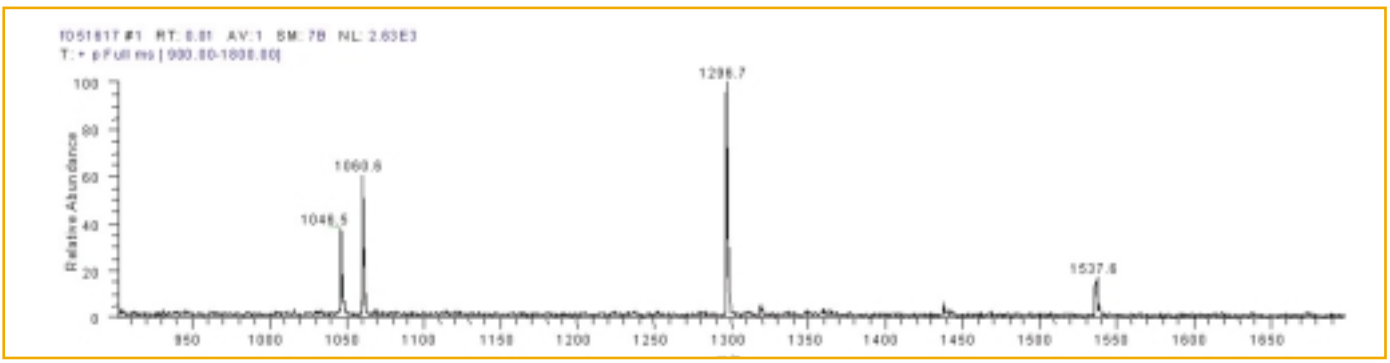
MALDI TOF, OA-TOF

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

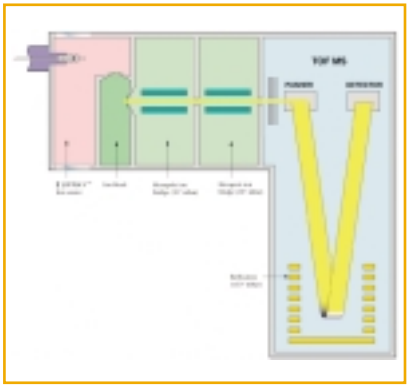


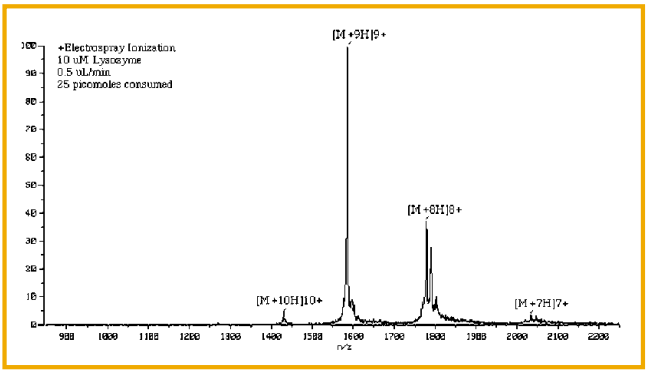
MALDI (Matrix Assisted Laser Desorption Ionisation): this is an off line technique. A laser beam hits the samples deposited in a matrix on a target plate. Samples are volatilised and ionised. The system measures the time of flight between the laser pulse and the detection.

MALDI TOF spectrum of a mix of Angiotensin I, Angiotensin II, Bradykinin, and Fibrinopeptide A (20 femtomoles of each peptide loaded). Each peak corresponds to a peptide.



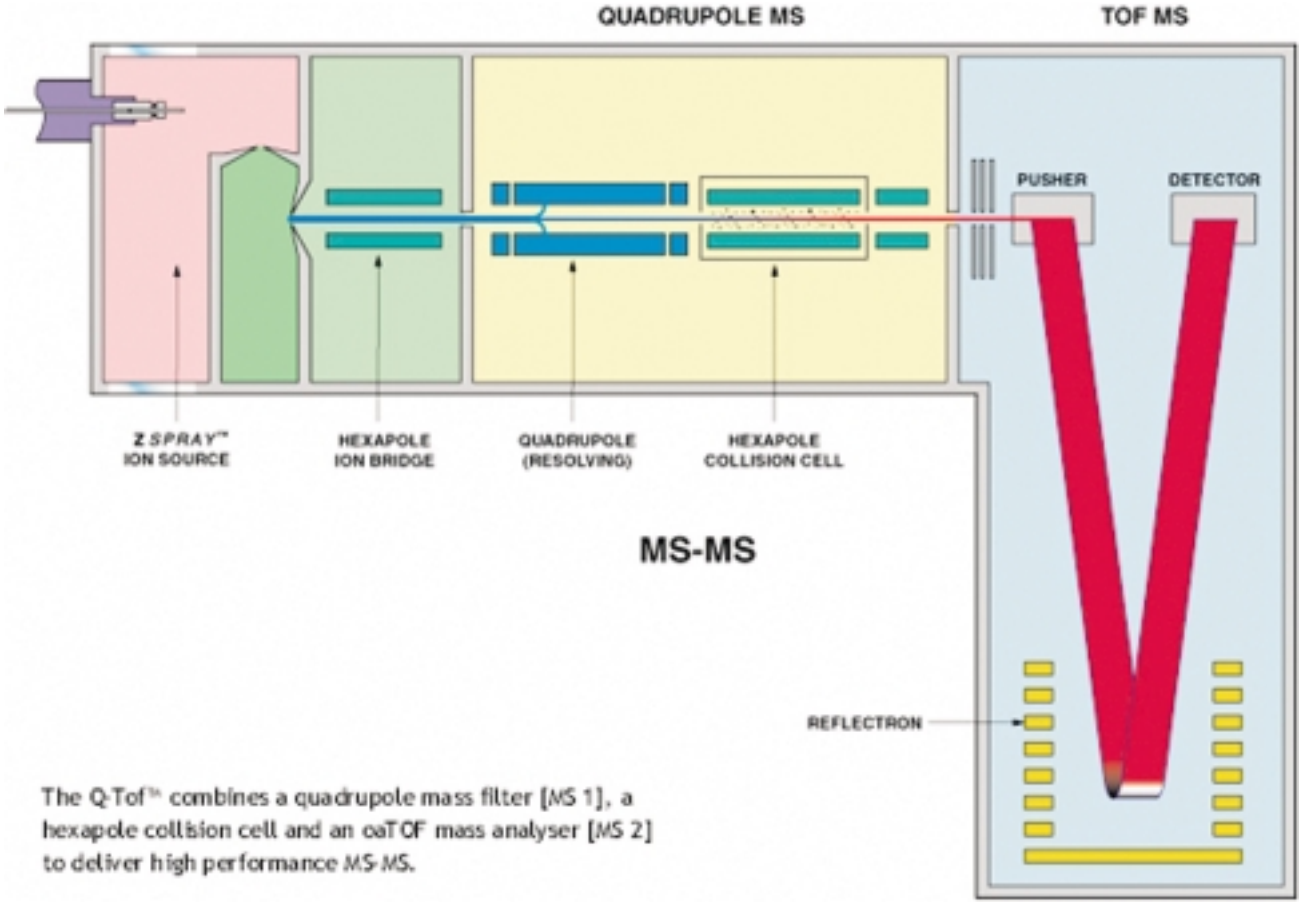
oa-TOF: this is for in line coupling. This configuration is used for LC / MS with API ionisation, and for GC / MS. The ions are transferred from the source to the analyser through a transfer optics. The "pusher" accelerates the ions to the same level of energy, and gives the start signal for timing the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



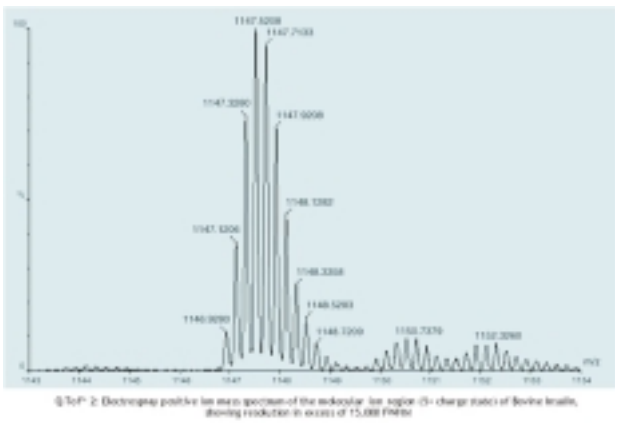


Electrospray TOF spectrum of Lysozyme. With electrospray ionisation, multiply charged ions are obtained. The peaks corresponds to various levels of charge.

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.



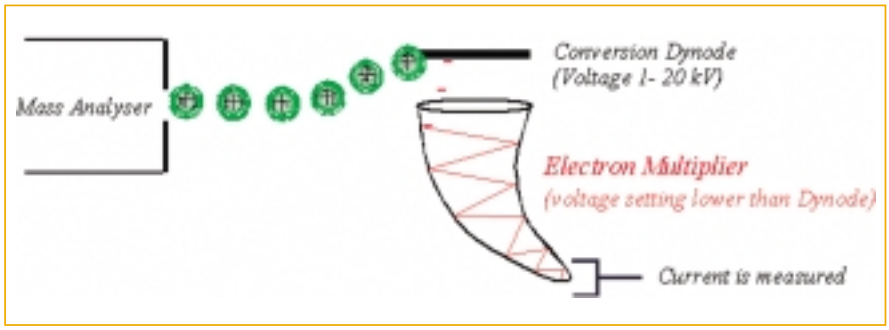
The Q-Tof™ combines a quadrupole mass filter [MS 1], a hexapole collision cell and an oaTOF mass analyser [MS 2] to deliver high performance MS-MS.



DETECTORS

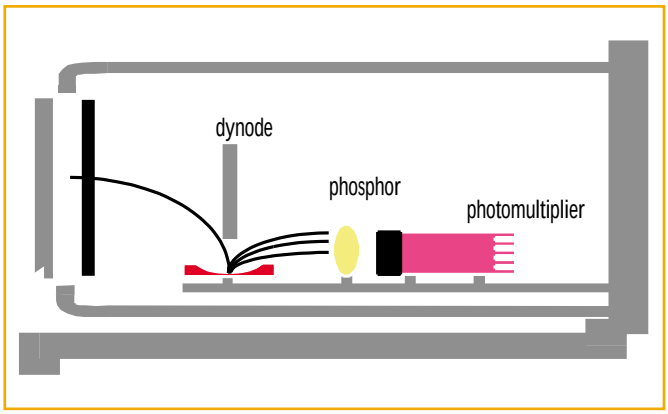
The detector is the device which detects the ions separated by the analyser.
3 different types of detector are used with the analysers described in the previous pages:
Electron multipliers, dynolyte photomultiplier, microchannel plates

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.

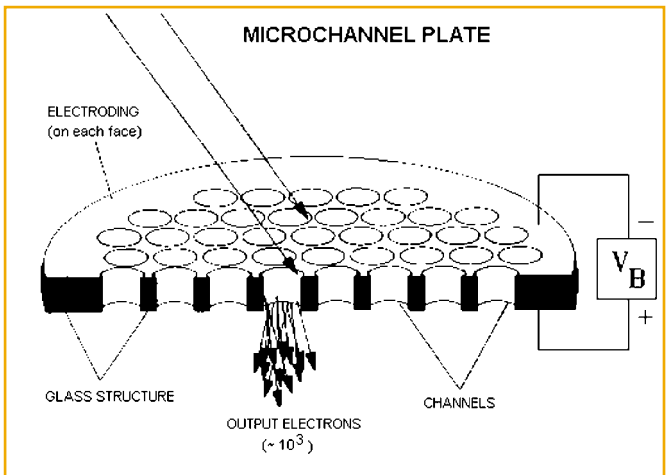
Dynolyte photomultiplier



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.

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.



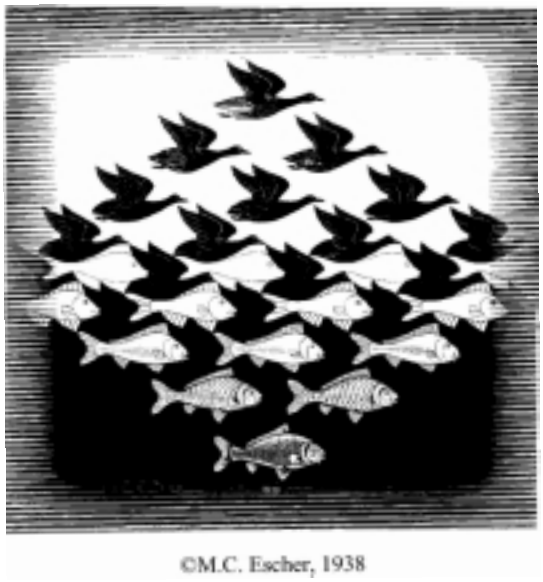
From http://hea-www.harvard.edu/HRC/mcp/mcp.html

INTERFACING HPLC AND MS

Interfacing a HPLC system with a mass spectrometer is not trivial.

The difficulty is to transform a solute into a gas phase ion. The challenge is to get rid of the solvent while maintaining adequate vacuum level in the mass spectrometer, and to generate the gas phase ions.

Since the early seventies, a number of approaches have been used. LC/MS became really popular with the introduction of of the thermospray interface and the particle beam interface. The next big improvement was the introduction of the electrospray and APCI techniques. The thermospray interface is no longer available on the market, but the particle beam is still available from Waters because it it the only method to provide electron impact spectra.



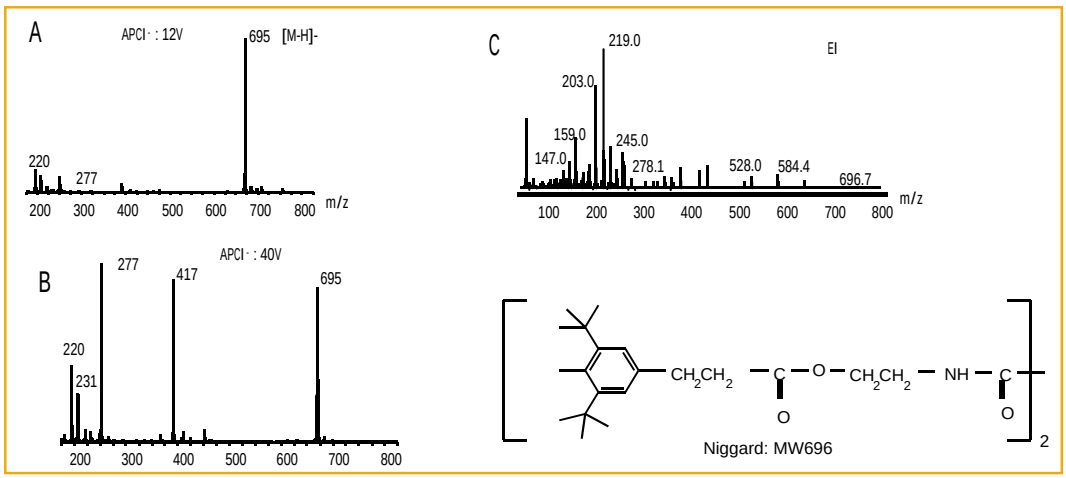
GET REPRO RIGHTS

Actually, the large majority of applications are done with electrospray and APCI ionisation. New techniques like APPI (atmospheric photo ionisation) are appearing, but are not yet largely used.

In the next pages, we will cover the principle and application of atmospheric pressure ionisation modes. We will not discuss older techniques, which are now mainly of historical or theoretical interest.

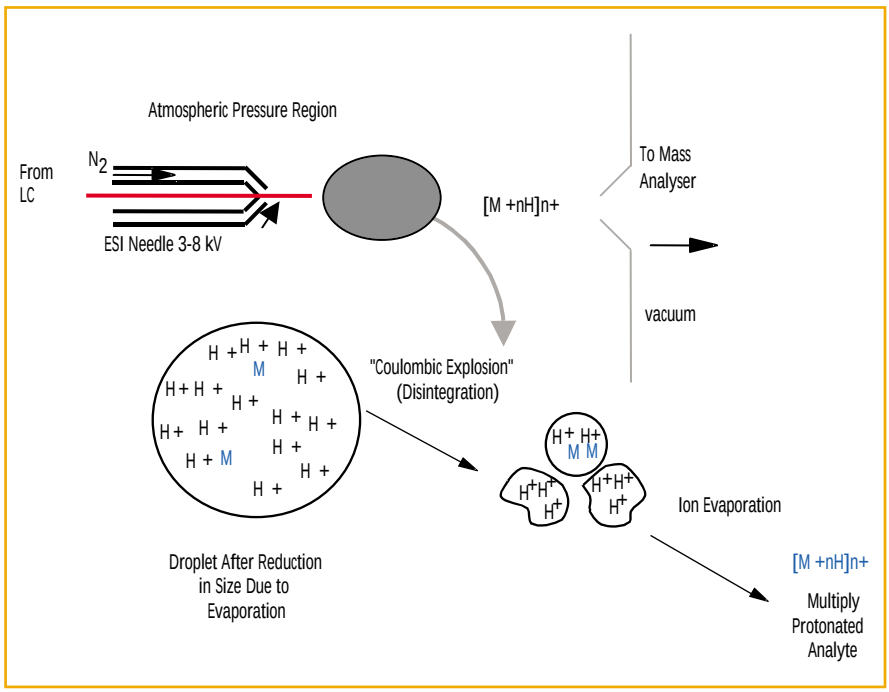
Electrospray and APCI are both API (atmospheric pressure ionisation) techniques. Ionisation takes place at atmospheric pressure and both are considered to be soft ionisation method. The mass spectrum provides mainly the molecular weight information, unless fragmentation techniques are used. The possible fragmentation techniques are in source CID (collision induced dissociation), CID in the collision cell of a tandem type instrument, fragmentation in an ion trap. This is very different from the spectra obtained with EI (electron impact ionisation).

Niggard spectra (polymer additive), obtained by negative APCI (A), negative APCI with in source fragmentation (B), electron impact ionisation (C).



ELECTROSPRAY IONISATION (ESI)

Description: the HPLC line is connected to the electrospray probe, which consists of a metallic capillary surrounded with a nitrogen flow. A voltage is applied between the probe tip and the sampling cone. In most instruments, the voltage is applied on the capillary, while the sampling cone is held at low voltage. First step is to create a spray. At very low flow rate (a few µl/mn), the difference in potential is sufficient to create the spray. At higher flow rate, a nitrogen flow is necessary to maintain a stable spray.



The API sources include a heating device, in order to speed up solvent evaporation.

A mandatory condition to work with electrospray is that the compound of interest must be ionized in solution.

If it is not compatible with the HPLC conditions (I.e. in case of normal phase chromatography), it is possible to use post column addition to get appropriate conditions.

In the electrical field, at the tip of the capillary, the surface of the droplets containing the ionized compound will get charged, either positively or negatively, depending on the voltage polarity . Due to the solvent evaporation, the size of the droplet reduces, and, consequently, the density of charges at the droplet surface increases. The repulsion forces between the charges increase until there is an explosion of the droplet. This process repeats until analyte ions evaporate from the droplet.

Multiply charged ions can be obtained depending on the chemical structure of the analyte. This is why ESI is the technique of choice for analyzing proteins and other biopolymers on quadrupole or ion trap analyzers.

Typical ions produced by electrospray ionisation:

Positive mode: [M+H]+ protonated molecule
[M+Na] +, [M+K] + … adducts
[M+CH3CN+H] + protonated, + solvent adducts

Negative mode: [M-H] - deprotonated molecule
[M+HCOO -] -, … adducts

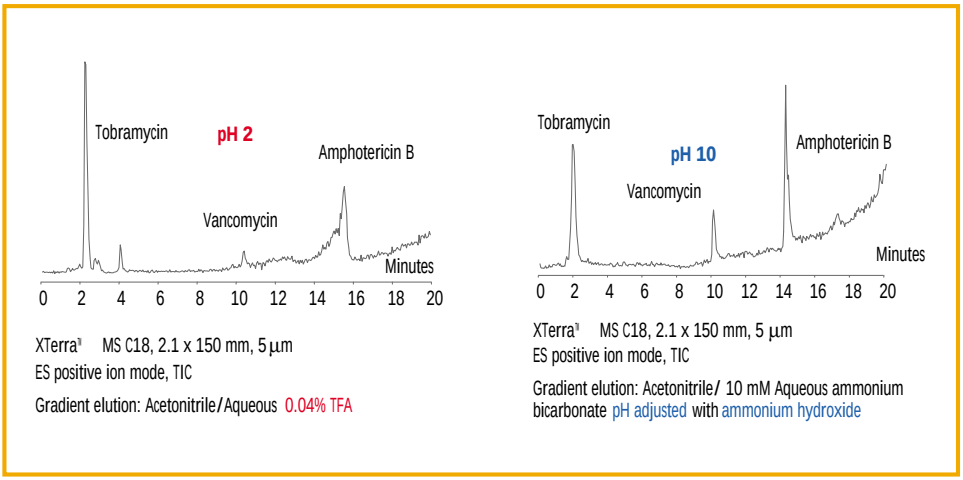
ELECTROSPRAY IONISATION (ESI) (2)

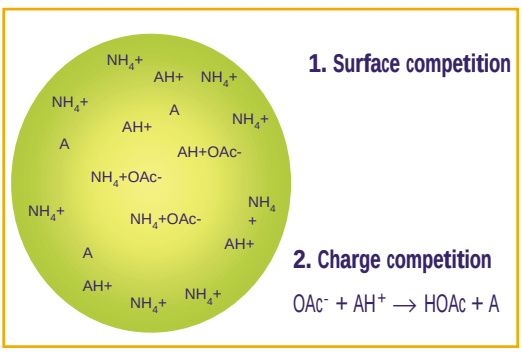
Operating conditions

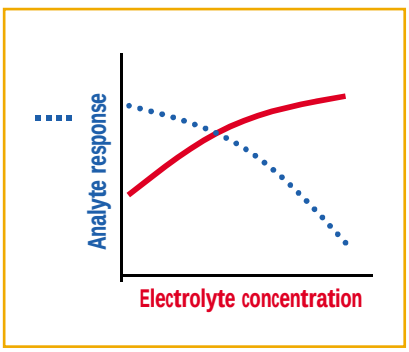
Flow rate: the best sensitivity is achieved at low flow rate. Working at 1 ml/mn or even higher is technically possible, but may cause a reduction in the signal to noise ratio.

Eluent pH: the mobile phase should have a pH such that the analytes will be ionized. An acidic mobile phase is suitable for the analysis of basic compounds, using positive ESI, while a basic pH will be chosen for analyzing acidic molecules. However, some exceptions exist to this general rule: positive ESI of basic compounds with a high pH mobile phase has been published.

Buffers: volatile buffers are to preferred for routine use. Operating the instrument with non-volatile buffers such as phosphate is technically possible, but the salt deposit in the source will have to be removed periodically. The concentration of the buffer, or acid or base used to adjust/control the pH should be as low as possible. If not, competition between analyte and electrolyte ions for conversion to gas-phase ions decreases the analyte response.This can be explained as follows: if a species is in large excess, it will cover the droplet surface and prevent other ions to access the surface, and thus to evaporate. A species in large excess will also catch all charges available and prevent the ionisation of other molecules present at much lower concentration.





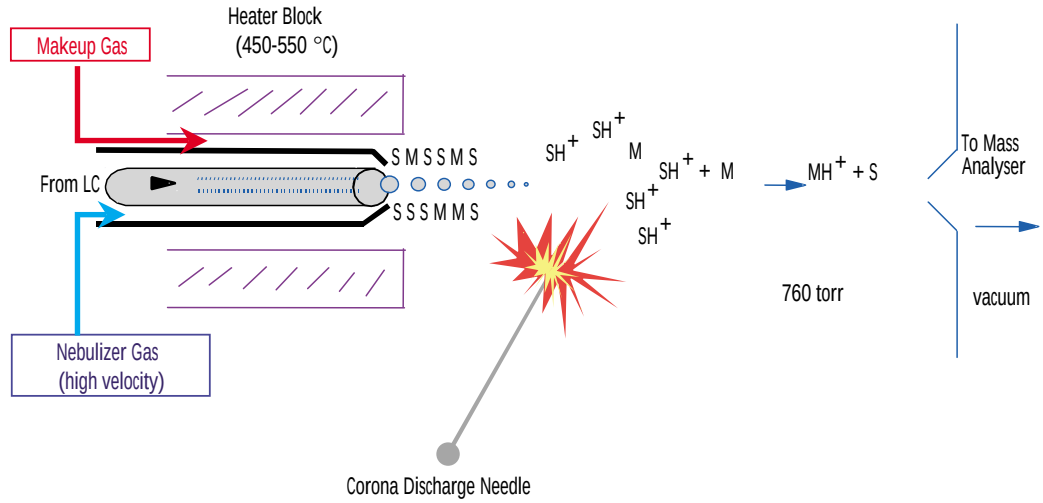


Ion pairing agents (sodium octanesulfonates…..) : these molecules have surfactant properties. The presence of surfactants in the mobile phase impacts the spray formation and droplet evaporation. There is also a surface competition mechanism phenomenon.

Matrix effects: when the sample contains high concentration of salts, or an excess of another analyte that can ionise in the operating condition, there might be a competition effect in the ionisation. This is called "ion suppression". The chromatographic separation must be developed to remove this effect, at least when doing quantitative analysis.

ATMOSPHERIC PRESSURE

CHEMICAL IONISATION (APCI)



Description:the HPLC line is is connected to the APCI probe which consists normally of a glass capillary surrounded with a nitrogen flow used for mobilization. Part of the APCI probe, or close to the probe tip are a heating device and an additional gas flow, to instantaneously volatilize the solvent and sample.

Close to the probe, there is a metallic needle, which is at potential of a few kilovolts. This is the "corona discharge electrode". The "corona effect" term describes the partial discharge around a conductor placed at a high potential. This leads to ionisation and electrical breakdown of the atmosphere immediately surrounding the conductor. This effect is known as corona discharge. St. Elmo's fire is an example of a naturally occurring corona. In the case of an APCI source, the atmosphere surrounding the corona electrode consists mainly in the vapour generated from the HPLC eluent, nitrogen, and the analyte molecules.

The eluent vapours are ionised by the corona effect, and react chemically with the analyte molecules in the gas phase.

The above figure shows the solvent molecules (S) being protonated by the corona (SH+), then reacting with the analyte molecule (M) to give the protonated form MH+.

The following conditions are required for APCI to work:

- the analyte must be volatile and thermally stable
- the mobile phase must be suitable for gas phase acid-base reactions
  - for working in positive mode, the proton affinity of the analyte must be higher than the proton affinity of the eluent (in other words, the analyte can catch a proton from the protonated solvent) SH+ + M _ S + MH+
  - for working in negative mode, the gas phase acidity of the analyte must be lower than the gas phase acidity of the eluent (in other words, the analyte can give a proton to the deprotonated solvent) [S – H] - + M _ S + [M – H] –

Thermodynamic values can be obtained from the internet (i.e. the NIST site http://webbook.nist.gov/chemistry/) or from handbooks of chemistry.

Solvent adducts or radical cations (M . +) can be observed

CHEMICAL IONISATION (APCI) (2)

Operating conditions.

Flow rate: APCI is usually used at higher flow rate than ESI. Optimum conditions are at a few hundreds µl/mn

Eluent: the mobile phase must be suitable for ionisation. If it is not the case, a small amount of modifier can be added, in order to generate the solvent gas phase ions.

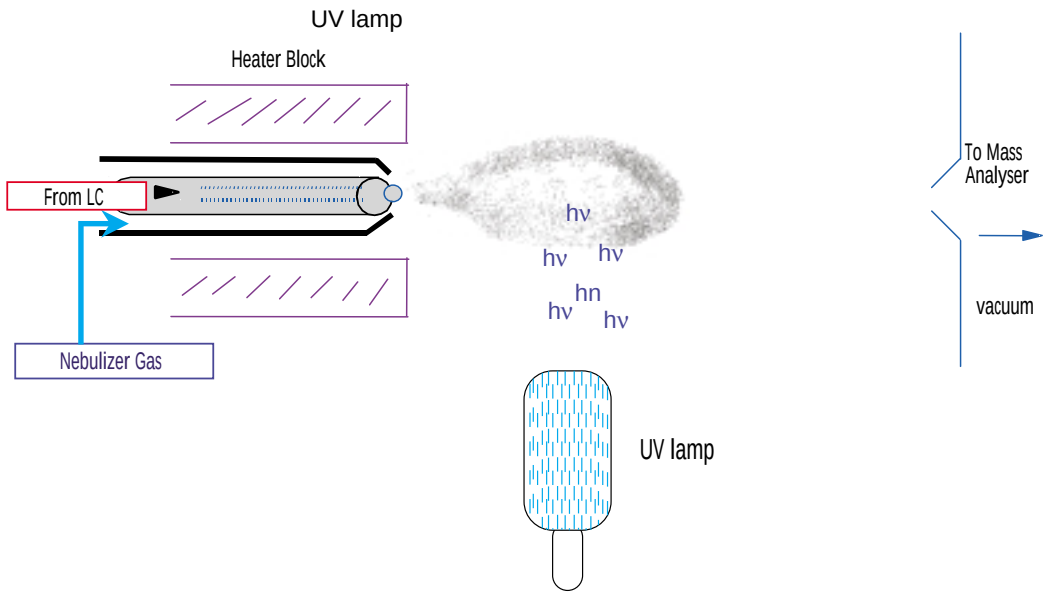
Buffers: buffers must be volatile.

ATMOSPHERIC PRESSURE

PHOTOIONISATION

Atmospheric pressure photo ionisation is a newly introduced technique. It was presented at ASMS 2000 by Andries Bruins from the University of Groningen, Netherlands. The principle is to use photons to ionise gas phase molecules.

The source is a modified APCI source, with the corona electrode replaced by an UV lamp



Ionisation mechanism: the ionisation can be obtained directly, or through a dopant (I.e acetone, or toluene)

Direct APPI: M + hv _M+

Dopant APPI: D + hv _D+ D+ + M _MH+ + D[-H]

Applicability: APPI is said to allow the ionisation of compounds which cannot be ionised with APCI or ESI, to be compatible with flow rates down to 100 µl/mn, and to be quantitative. Obviously, it is too early and there is not enough published work to estimate the range of applications. Is it of interest for a few specific cases, or will it be a significant complement to the traditional API techniques?

CHOOSING BETWEEN

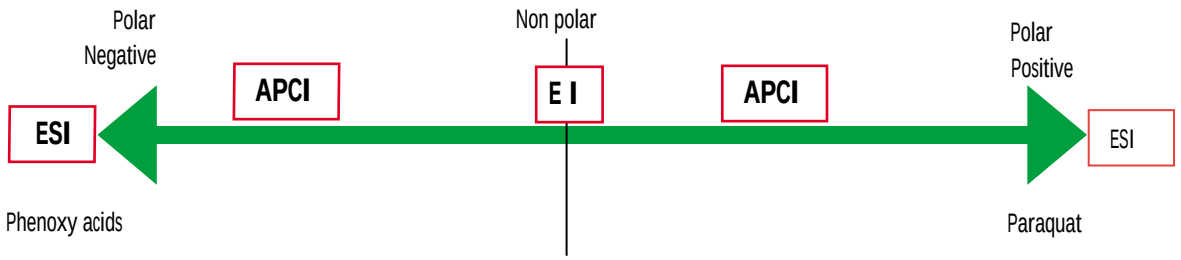
IONISATION TECHNIQUES

General rules can be given to chose between ESI, APCI, or EI

ESI is preferred for compounds which are ionic or very polar or thermo labile, or with masses higher than 1000…

APCI is preferred for compounds which are not very polar

If we take the example of pesticides, we can rule out that:
polar pesticides will be in ESI, less polar in APCI, volatiles in APCI, non polar better done with GC/EI….



But practical consideration are also important: changing from ESI to APCI cannot be done automatically during the separation. The instrument itself have an influence: paraquat and diquat are reported to work only in ESI on some instruments but also in APCI on other systems*. Depending on the supplier, some instruments might show better performance in APCI, while other ones will perform better in ESI.

So, when it comes to practice, and when the choice is not obvious, the best approach for determining the most suitable ionisation method is still to try to inject in various conditions, or to refer to existing work.

API SOURCE DESIGN

All MS suppliers are making a continuous effort to improve the design of the API sources, particularly as the source is a key element of the LC/MS instrument. A good source is essential for:

- clean MS spectra
- sensitive detection
- robustness and reliable operation
- easy use and maintenance

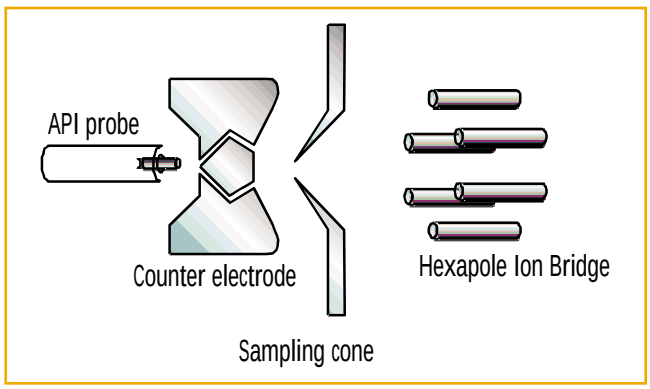
An API source always comprises

- the probe (ESI or APCI)
- the corona electrode (for APCI operation)
- Gas flows for mobilization, evaporation and desolvatation
- sampling cone
- transfer optics to the MS analyser

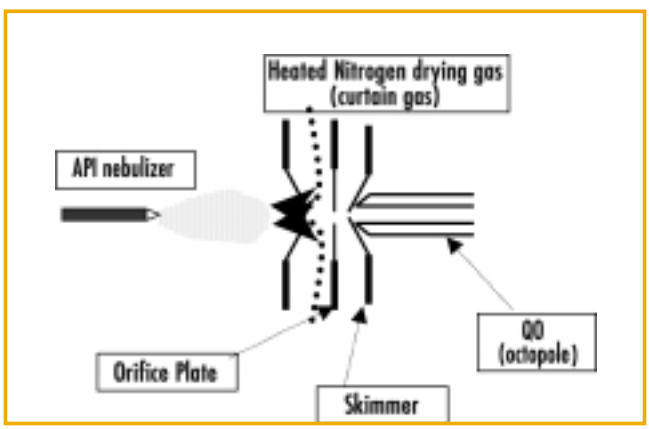
Trends in source design:

Looking back at the source design of various suppliers, we observe that, only a few years ago (1997-1998) all were using an axial design. The probe was in the axis of the optics. To limit the risk of plugging the sampling cone orifice with non volatiles from the eluent or from the sample, various approach were used.

Among the most efficient were the the "pepper pot" counter electrode from Micromass, and the "curtain gas" from PE-Sciex



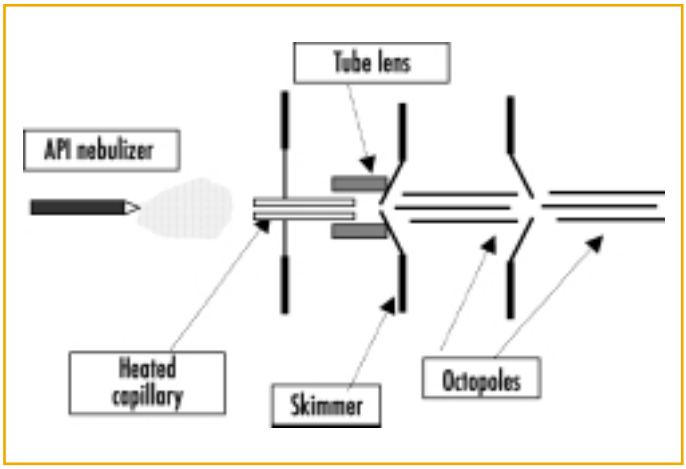
"Pepper pot" design: the ions have to travel through the channels of the metallic counter electrode, which is heated. The counter electrode protects the sampling cone orifice and helps in ion desolvatation



"Curtain gas" design: a flow of heated gas protects the orifice plate and helps in the desolvatation

API SOURCE DESIGN

Ion desolvatation is also an important aspect of source design. Some suppliers use a heated metallised transfer capillary.This approach was already available in the early electrospray source designs (Whitehouse, 1985) . Another approach is to use heated gas flows, which eliminates the risk of plugging the capillary, or of sample adsorption and degradation on the capillary walls.



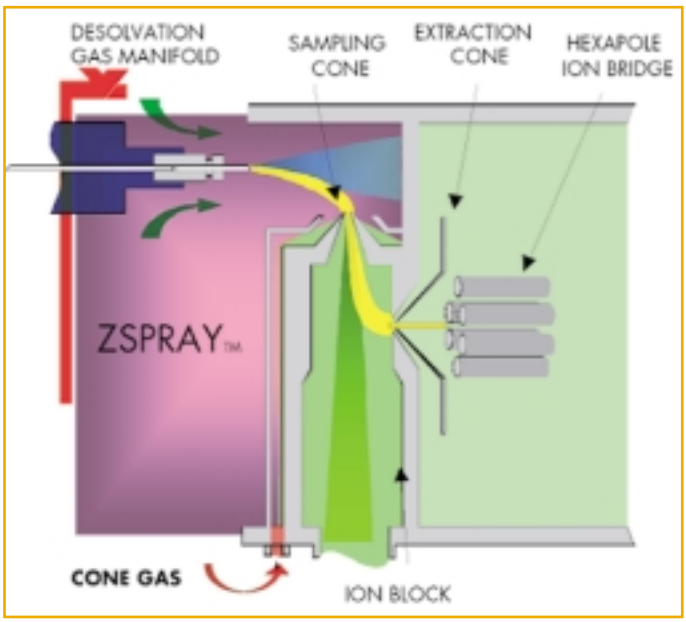
Axial source design with heated transfer capillary (first versions of the Finnigan LCQ). Similar design is still used on various instruments.

Design evolution: designs have changed, to give better protection of the sample orifice (gain in robustness), for introducing more ions into the optics (gain in sensitivity), for better and smoother desolvatation (gain in sensitivity, cleaner spectra, less thermal decomposition).

Those improvement are found on the MKII Zspray source, which is used in various versions on the Waters-Micromass LC/MS instruments. More than 2000 of these sources are in operation in the field.

MKII source design:

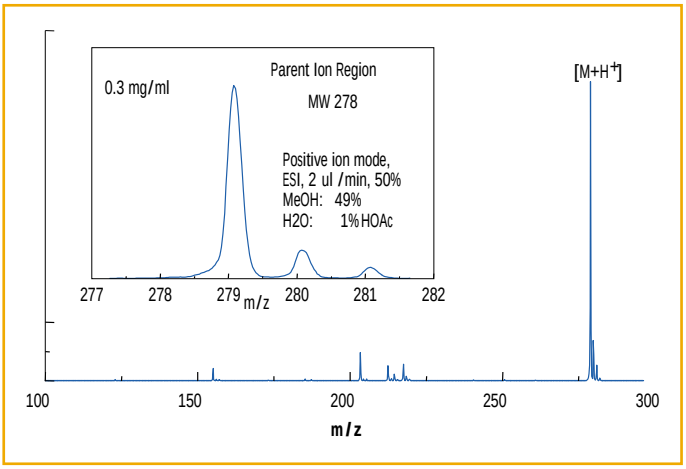
The probe is perpendicular to the sampling cone, which is protected by a "cone gas" flow. The second extraction cone is also perpendicular to the ion beam. This so-called "ZSpray" geometry protects very efficiently the optics against non-volatile material and non-ionised molecules. Consequently, cone orifices can be larger than in previous designs. The combination of larger orifices and noise reduction largely compensates for transmission losses due to the orthogonal geometry, giving a large gain in sensitivity.



Efficient desolvatation is provided by a heated nitrogen flow close to the probe and by the cone gas. The source block is also heated, for final desolvatation.

For easy maintenance, an isolation valve, is installed, so that it is possible to disassemble the sampling cone for cleaning without having to vent the system. A glass window gives the operator the possibility to observe the spray and the interior of the source during operation.

THE API MASS SPECTRUM



Both APCI and ESI are soft ionisation techniques. Thus, the MS spectra obtained with API ionisation will consist mainly of the "molecular" ions, unless fragmentation techniques are applied.

Looking at the sulfamethazine spectrum obtained by infusion of the sample, we observe the protonated molecule, and a few small ions. These small ions might be fragments, or more likely contaminants.

A closer look at the [M + H] + region shows the peak and isotopes. The acquisition is made in continuous (profile) mode and indicates a resolution of about 1000 (FWHM). The isotope pattern often provides useful information, since the well known rule of MS spectra interpretation can be applied to API spectra (nitrogen rule, double bond equivalent value). It is also possible to use the isotopic pattern to determine the number of carbon, chlorine, bromine…atoms

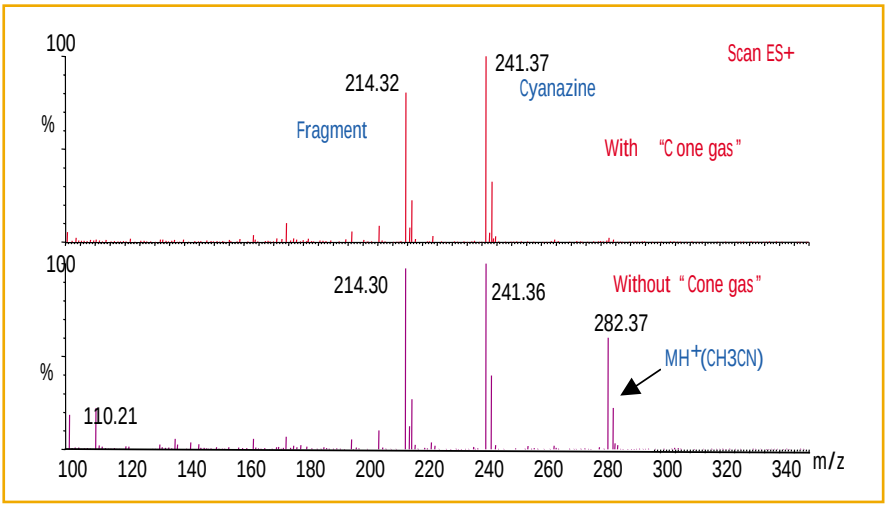
Adducts/clusters: the UPAC nomenclature is as follows:

"Adduct Ion - An ion formed by interaction of two species, usually an ion and a molecule, and often within the ion source, to form an ion containing all the constituent atoms of one species as well as an additional atom or atoms.

Cluster Ion - An ion formed by the combination of two or more molecules of a chemical species, often in association with a second species. For example, [(H2O)nH]+ is a cluster ion. "

Adducts and cationised or anionised molecules are often observed with API techniques.

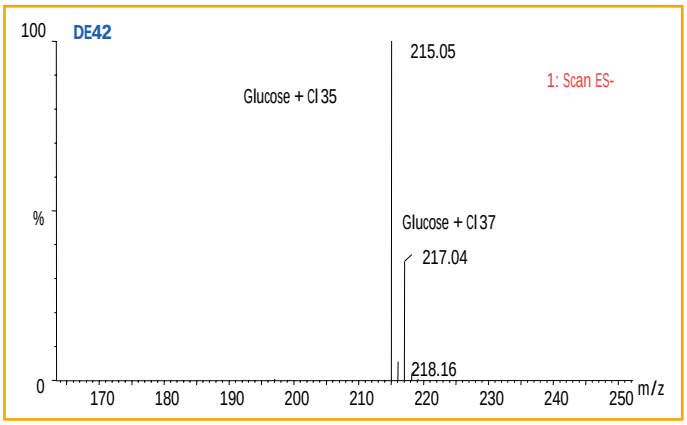
Those ions can be formed "accidentally", due for instance to the presence of sodium in the mobile phase or intentionally, for more specific or sensitive detection



Adduct formed with acetonitrile. By switching on the "cone gas", the desolvatation i improved, and the solvent adduct disappears.

The formation of adducts or clusters should be controlled when doing quantitative analysis, since the compound of interest should be in a well defined and stable form.

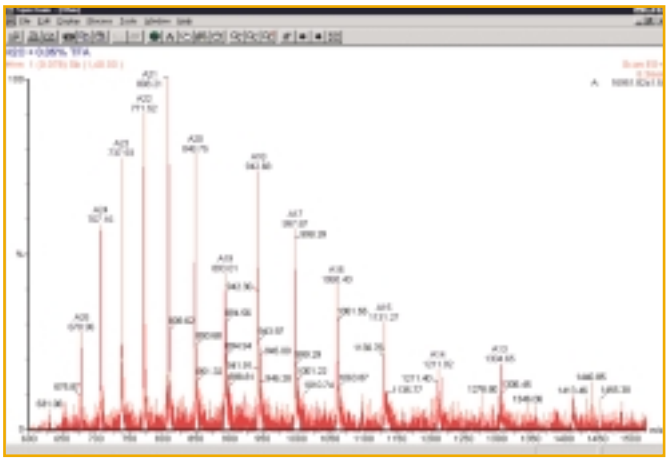
THE API MASS SPECTRUM



Negative ESI mass spectrum for glucose, with chloride adducts.

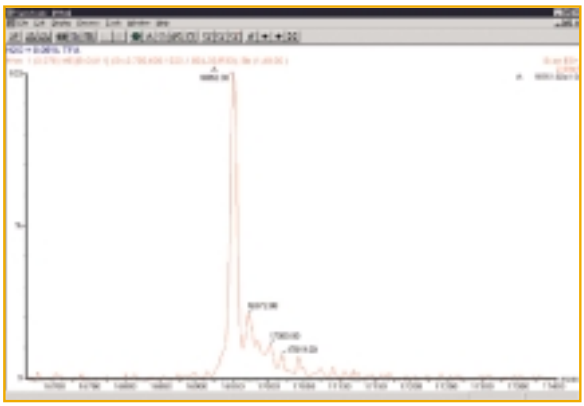
The gradient was 80% acetonitrile-water to 50% acetonitrile-water, both containing 0.001M LiCl. Chloride was added to form the sugar-chloride adduct, providing sensitive and selective detection. The characteristic Cl35/Cl37 isotope pattern makes carbohydrate identification easy.

Multicharged spectrum: with electrospray ionisation, multiply charged ions are obtained for proteins, peptides oligonucleotides, and in general, for any molecule which presents multiple ionisation sites. Various level of charge are present simultaneously, giving a spectrum with multiple peaks. The mass of the molecule can be obtained by calculation from the multiply charged spectrum. This can be done manually for small peptides, but de-convolution software options, available from instrument suppliers, are very useful for large molecules.

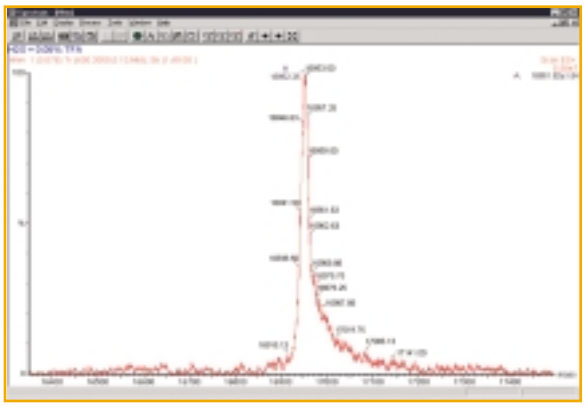


Each of the peaks of this mass spectrum represents a different charge level. The lower masses correspond to the larger number of charges. Deconvolution can be made by a mathematical approach (resolution of a system of equations) using the Transform software, or by a statistical approach, using the Waters/Micromass MaxEnt software.

Deconvolution
with Waters/Micromass MaxEnt

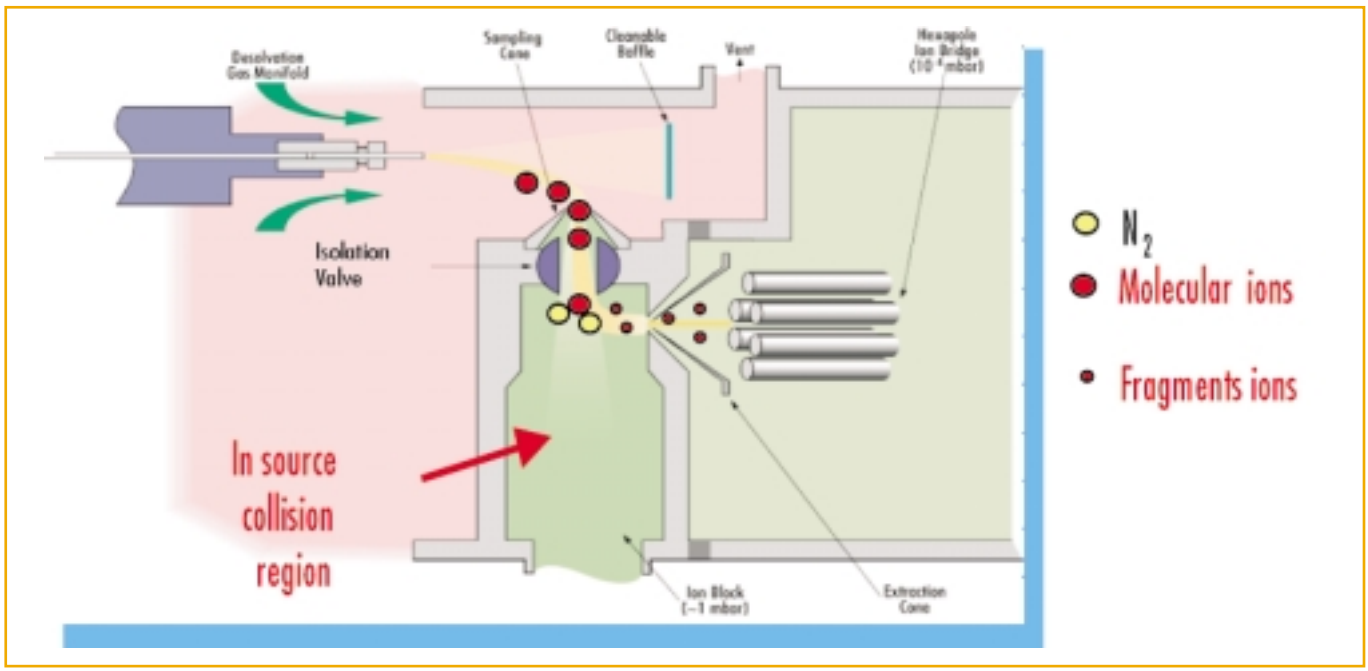


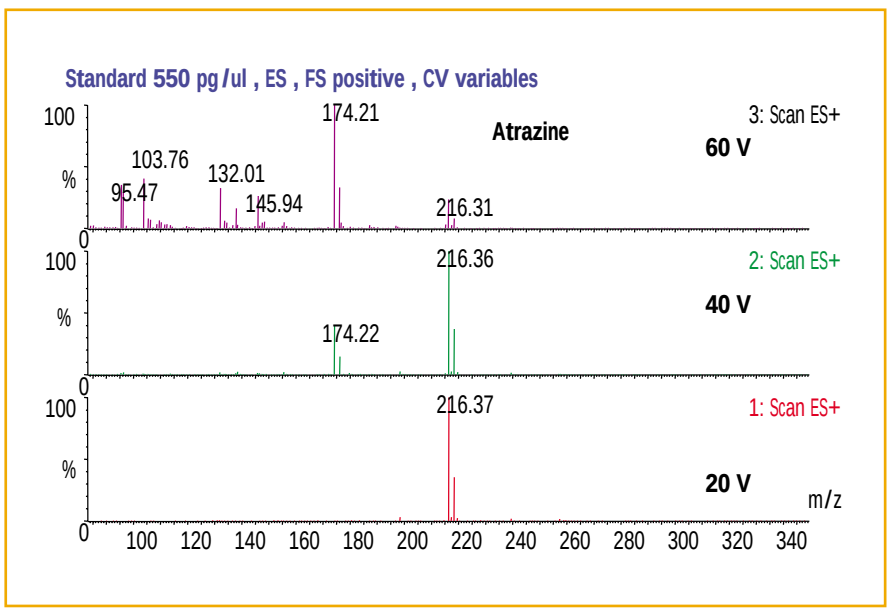
Deconvolution
with Waters/Micromass Transform



THE API MASS SPECTRUM

In source fragmentation: this is is obtained by accelerating the ions in the source, in a region where the pressure is in the millibar range. The acceleration is produced by applying a voltage (cone voltage) between the sampling cone and the next extraction lens.The ions collide with residual gas molecule, gain energy and fragment.





Increasing fragmentation with increasing cone voltage.

The fragmentation obtained by in source fragmentation is useful for confirming a peak identity, getting structure information, improving selectivity, building libraries….

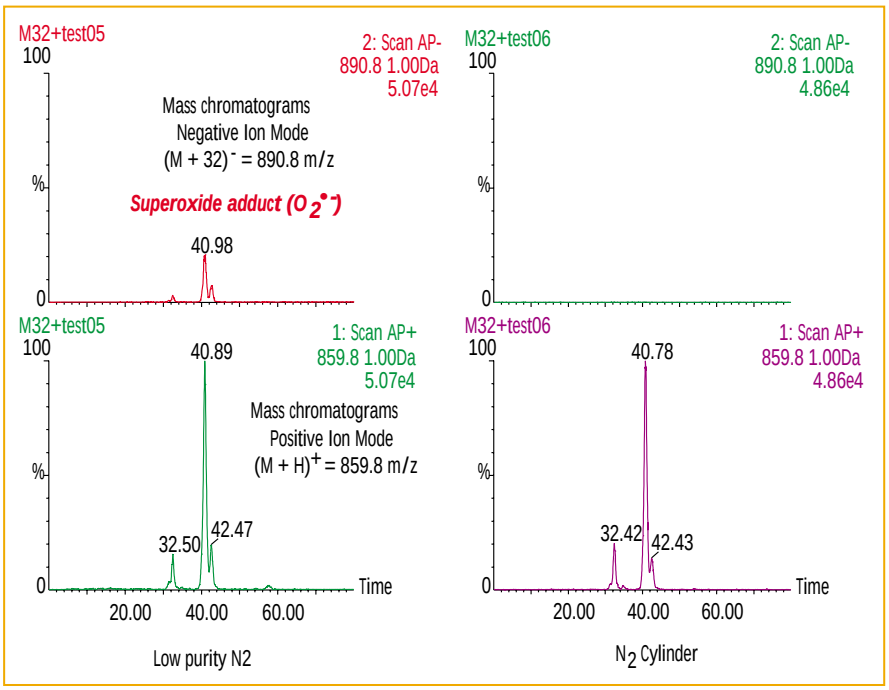
PRACTICAL ASPECTS OF USING LC/MS:

SYSTEM INSTALLATION

Site requirement:

When planning LC/MS equipment, it is necessary to make sure of the availability of a nitrogen supply. Possible options are nitrogen generators, liquid nitrogen and nitrogen cylinders.
An important point is that the gas must not contain organic impurities that would be visible on the mass spectra and could completely contaminate the mass spectrometer.
Severe contamination of the instrument due to an inappropriate gas installation are rather common. The main causes are the soldering flux (N2 tubing should be preferentially stainless steel, connected with Swagelock type connectors), and not maintained N2 filtration/purification devices.

Using compressed air in place of nitrogen is sometimes mentioned. Despite that we are not aware of any accident, there are potential safety issues, due to the presence of solvent vapours, high temperatures and high voltages in the source.
In addition, oxygen presence might be visible on the mass spectra. The figure below shows that the use of low purity nitrogen can lead to the formation of superoxyde adducts.
Thus, replacing nitrogen by air must be avoided.



Proper exhaust must be also available, to avoid the dispersion of solvent vapours (from the source) and oil vapours (from the vacuum pump) into the laboratory environment.
The lab temperature should be reasonably stable, to avoid mass drifts.

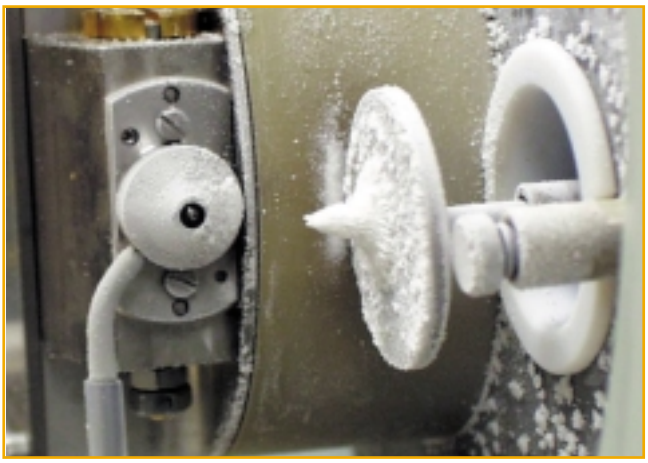
For long term successful operation, LC/MS needs a clean environment. This includes the lab environment, the quality of solvent and buffers, the quality of the columns, and sample preparation.
Laboratory environment: the lab must be ACAP (as clean as possible). Contaminations, which are not visible with a HPLC/UV system, may be a real headache with LC/MS! Traditional causes are solvent vapours, (i.e: DMSO), oil vapours (from not properly installed vacuum pumps). The HPLC system can also be a source of contamination. Traces from buffers, solvents or sample from previous runs are common. Traditional ones are surfactants (from ion pairing agents, TFA, THF, TEA….).

PRACTICAL ASPECTS OF USING LC/MS:

ROUTINE MAINTENANCE

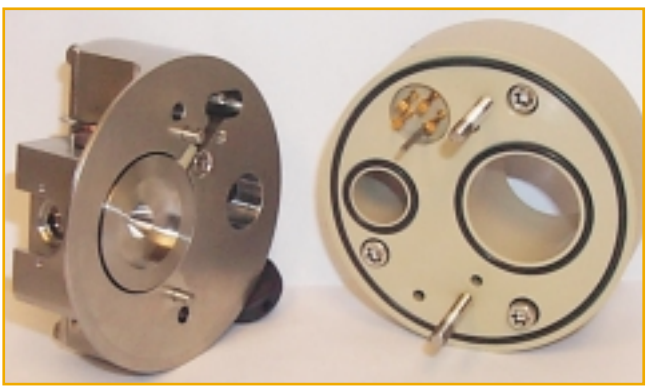
Instrument maintenance: proper maintenance of the LC/MS system is mandatory for successful longterm operation. This concerns both the HPLC part, and the MS.

Routine maintenance of the LC/MS consists mainly in cleaning the source. This includess the probe,the source enclosure, the sampling cone and eventually the transfer optics. When the source becomes dirty the quality of the signal degrades. This is due to contaminant ions and to a degradation of the ions transmission. Even without a visible blockage of lens orifices, a dirty surface affects the voltages and impacts the transmission.



Source enclosure after operation with phosphate buffer. The instrument is still operational, due to the cone gas which protects the sampling cone orifice (left photograph). However the phosphate deposit needs to be removed. After turning the isolation valve, the cone can be disassembled and cleaned without venting the system.

Cleaning of the source parts located after the sampling cone is sometimes needed. The cleaning frequency depends upon the system usage, nature of the samples, and source design. This operation can be made by the user, but is more or or less difficult to do, depending on source design. In any case, the system needs to be vented.



The Waters ZQ source, disassembled for maintenance. All parts are self aligning.

Vacuum system: the roughing pump (s) oil need to be replaced every few months. There is no user maintenance on the turbomolecular pumps.

Detector: if the detection system is an electron multiplier, it will need a replacement after about 2-3 years

Maintenance contracts: every supplier proposes maintenance contracts. The content of the contract is very variable. Opting for a preventive maintenance (like for a car) is the approach to minimize instrument downtime.

ELUENTS SELECTION

Using a mass detector brings some constraints upon the eluent selection. These constraint are different from what is to be considered with other detectors. For example, the eluent UV absorbance, which is of high importance with UV detection is irrelevant with MS detection.

Eluent selection criteria:

Ionisation: the eluent must be suitable for the ionisation. Thus the eluent must be selected depending on the Ionisation mode (ESI or APCI, positive or negative mode) and the analyte (pKa, gas phase acidity).

Eluent molecular weight: it is not possible to analyse compounds which MW is lower than the one of the eluent (or eluent additives

Volatility: for routine operation, it's easier to use volatile buffers.

Acids: HCl, H2SO4, methane sulfonic acid… might damage the instrument and should not be used. They must be replaced by volatile organic acids (TFA, formic, acetic).

Adduct formation: ions (Na, NH4, acetate …) from the eluent will trend to form adducts. In the case of phosphate, multiple adducts are observed, which produce complicated mass spectra. The formation of adduct is usually not a reason for avoiding an eluent, and at the opposite, adduct formation might be forced for analytical reasons.

Ion pairing reagents, surfactants: impact the spray formation, the droplet evaporation, and compete in term of ion formation

Buffer concentration: must be kept as low as possible (mM range). If the buffer concentration is too high, ion suppression occurs.

Common eluents for LC/MS: Methanol/water, acetonitrile/water (methanol usually gives a better sensitivity than acetonitrile) pH modifiers: formic, acetic acids, TFA, NH4, TEA, DEA, carbonates, ammonium formate, ammonium acetate, ammonium carbonates, ammonium phosphate (non volatile)…

HPLC column: the column must give a good separation without using high concentration of buffers, nor ion pairing reagents. The bonding must be stable, so that the column will not "bleed". Special MS versions of columns are available from suppliers.

A CLOSER VIEW AT A SINGLE QUADRUPOLE

INSTRUMENT: THE WATERS ZQ

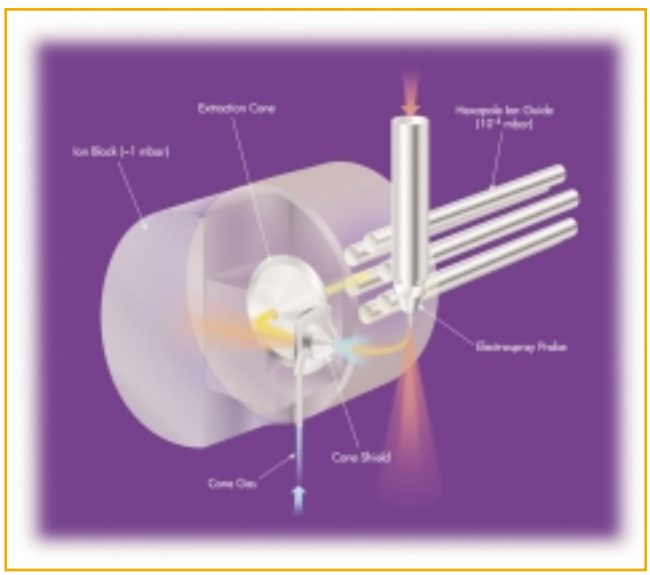
Instrument history: this instrument was introduced in theyear 2000 replacing the Micromass Platform and Waters ZMD family of single quadrupoles. It benefits from the experience gained with previous generations, but most of the components have been replaced with the latest technology, with the goal to reduce instrument size and gain in performance.

The instrument imbeds a syringe pump for infusion experiments and for calibration.

A switching valve is included, which can be, for example, used to discard the injection peak for a sample containing a high salt concentration.

Waters ZQ components: main sub assemblies are:

the source
the optics
the detector
the RF generator
the voltage supplies
the vacuum system
the electronics
the software



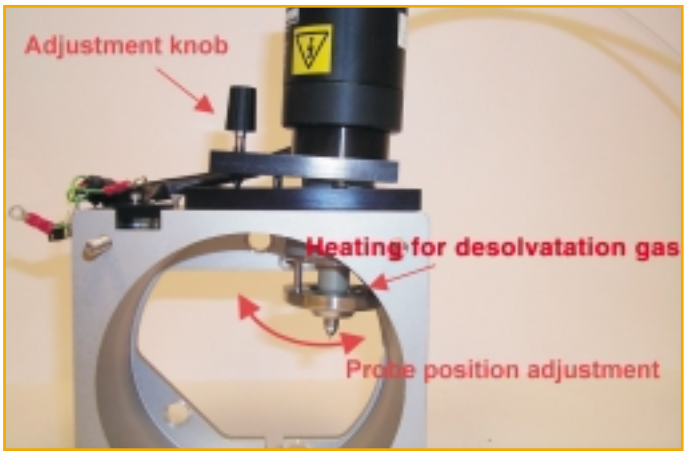
The source: it is a redesigned MKII type source, with the probe positioned vertically Having the probe placed vertically allows shorter connection to the cell of a detector (UV) placed on the top of the mass spectrometer. Optimised connections minimise peak broadening, and help in gaining sensitivity.

It also saves some lab or bench space.

Gas flows and source temperature are controlled from the software, to allow full documentation of experimental conditions.

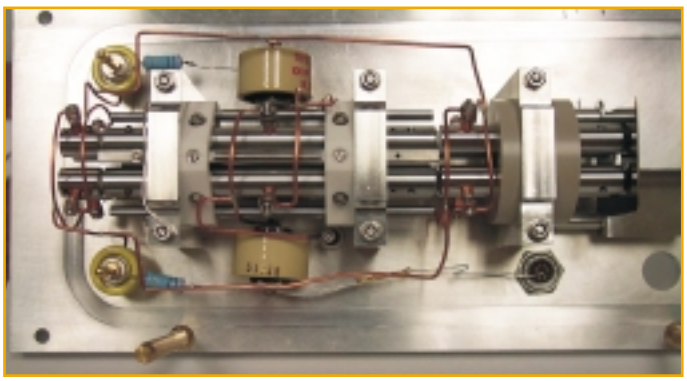
A CLOSER VIEW AT THE WATERS ZQ:

MAIN COMPONENTS

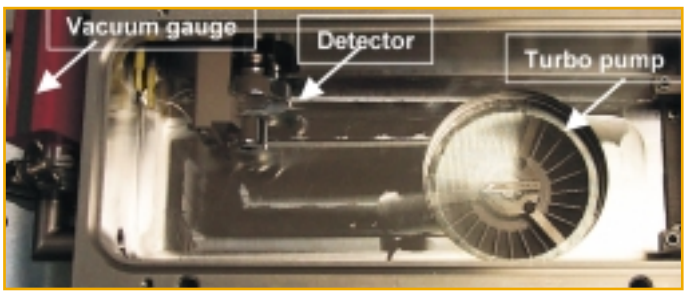


Depending on the flow rate the probe position can be adjusted to get the maximum sensitivity. If needed it is possible to lock the position of the probe.

The ion optics, consist of the extraction cone and lens (part of the source block), the transfer hexapole, the quadrupole, and the detector



The quadrupole is made of molybdenum rods, which are proven to provide excellent stability. For easier instrument manufacturing, and in the unlikely case of a cleaning need, all parts are self aligning and self connecting.



On this photograph, the quadrupole has been removed. The detector (dynolyte photo-multiplier) is placed perpendicular to the ion beam, leading to an improved signal to noise ratio, by reducing the noise from the neutrals.

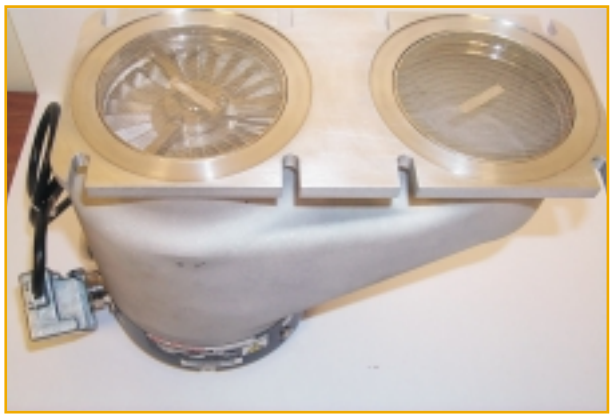
Also visible on the photo are the top of the turbo pump and a vacuum gauge.

A CLOSER VIEW AT THE WATERS ZQ:

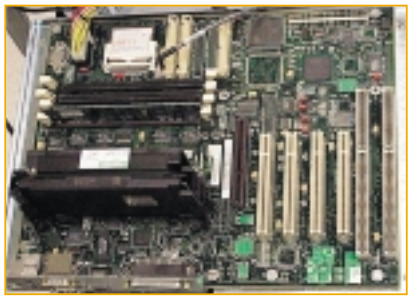
MAIN COMPONENTS

The vacuum system consist of an Edward roughing pump, and in an Edwards split flow turbo pump.

The pumping capacity is largely dimensioned, allowing very fast return to operation after venting the instrument.



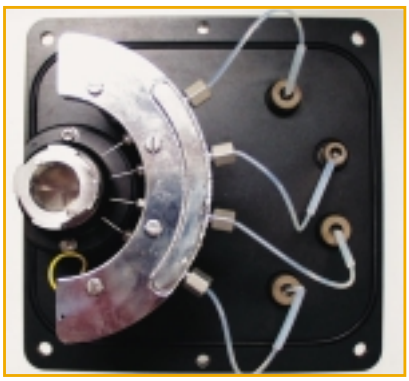
BOC Edwards EXT200/200Hi
190 l/s N2 at main inlet port
160 l/s N2 at side inlet port
Air Cooled
Only one pump and one controller



The electronics consist of a PC board and in an embedded PC

Using an embedded PC presents many advantages. The main one is fast signal processing. The ZQ is able to scan at 5000 Dalton/second, which is a real advantage for LC/MS applications. Fast scanning allows the acquisition of more data points per second, giving thus better peak shape, better integration and better reproducibility.

Other advantage is the possibility to do **multiplexing:**
Using the MUX source, up to four HPLC lines can be connected to the same Waters ZQ instrument for very high throughput applications, or for method development



Photographs: The ZQ equipped with the MUX source and close up view of the 4 ESI nebulizers. A rotor puts each nebulizer successively in line with the extraction cone. The signal is deconvoluted by the software, so that 4 independent data channels are acquired simultaneously.

A CLOSER VIEW AT THE WATERS ZQ

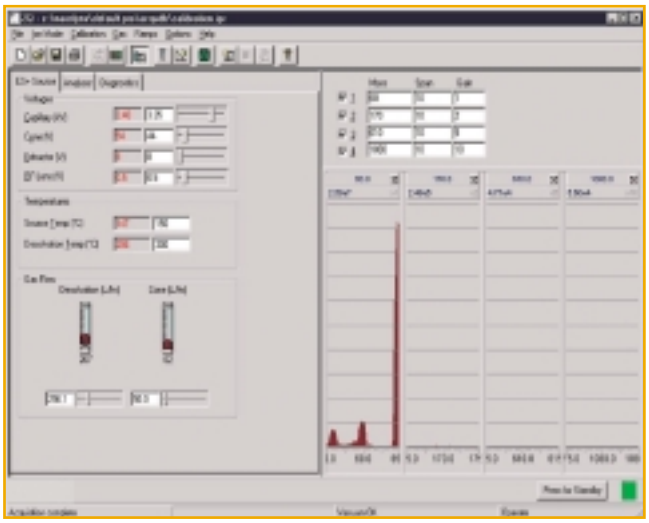
INSTRUMENT CONTROL

The software: the Waters ZQ is controlled either by MassLynx software, or by Millennium software.

MassLynx is the software developed by Micromass, a division of Waters Corporation, for the control of all Micromass instruments. MassLynx users benefit from a complete series of application managers, designed to fulfil specific needs, like Open Access to the instrument, screening of libraries from combinatorial chemistry, MS or UV triggered automated purification, protein applications…
Millennium is the Waters chromatography software. This software is used by more than 35000 users in the world, either for control and data acquisition from a single chromatograph, or in large network configurations. To fulfil Millennium users needs, Waters has implemented the control of the ZQ mass spectrometer into Millennium. Millennium users can easily benefit of MS detection by simply complementing their LC with a ZQ mass detector.

Software aspects: we will review only what is linked to the ZQ mass spectrometer. Information on HPLC methods (system control, signal acquisition and processing, reporting…) is available from Waters sales and support organisation.

The tune pages: these pages allow a direct control of the ZQ, for instrument fine tuning and diagnostics.

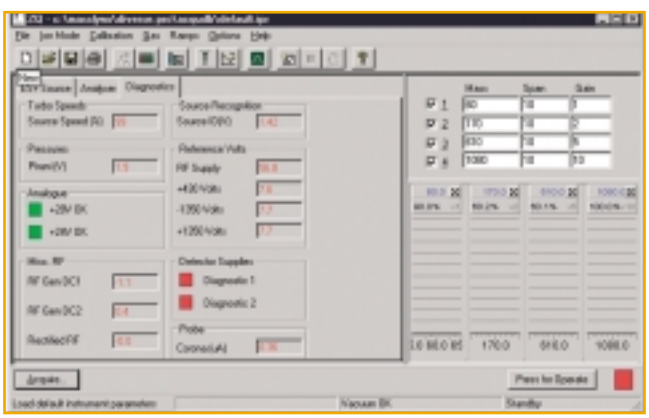


In the tune page, you can adjust the temperatures, gas flows, cone voltages, resolution, achieve the mass calibration of the instrument…

A real time display provides the visualisation of any modification. Acquisition can also be started from the tune page.

It is also possible to control a syringe pump for infusing the sample or calibration solution.

The tune page parameters are saved in a method which can be used for analysing sample series.



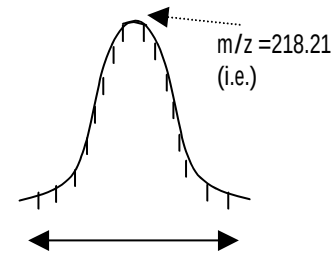
The diagnostic page provides a direct information on instrument "vital" functions.

For example, the rotation speed of the turbo pump is monitored. A vacuum leak will translate into an abnormal speed. Various voltages are also monitored.

SIGNAL ACQUISITION MODES

The Waters ZQ offers a large flexibility for choosing the signal acquisition parameters.

The raw signal from the photomultiplier is acquired in the form of data pairs (mass : intensity). The maximum resolution is 16 acquisition points per a.m.u. That value can be adjusted in the advanced parameters of the instrument method.



The information can be stored in this way. This is the **continuous acquisition** mode. To reduce data file size, it is also possible to compute immediately the signal, to provide a bar (or stick) representation of the signal. The bar height is proportional to the abundance of the ion. This is the **centroid mode,** which gives to much smaller files. It is possible to convert a spectrum from continuous to centroid, but a signal acquired in centroid cannot be converted into continuous.

A third acquisition mode is the **MCA mode** (Multi Channel Acquisition). This mode is adopted when infusing a sample into the MS with a syringe pump. The software accumulates the mass spectra acquired over a user defined time period. This is a way to enhance the quality of the signal.

SIM mode: In SIM mode [single ion monitoring or recording (SIR)], the instrument observes a specific mass, or a series of masses. The time spent to observe each ion is called the dwell time, and the observation window the "span". These parameters can be adjusted individually for each ion.

Scan mode: the mass range scan window, scan time, are the parameters to be defined

Positive / Negative mode: each of the above listed modes can be performed in positive or in negative polarity. Switching polarity is very fast and can be made during a single acquisition.

Acquisition function: an acquisition function is a line in the method, which sets the acquisition mode (continuous or centroid, SIM or Scan), and various other parameters (cone voltage, ESI or APCI voltages….). A method can combine up to 32 functions

A CLOSER VIEW AT THE WATERS ZQ

INSTRUMENT CONTROL

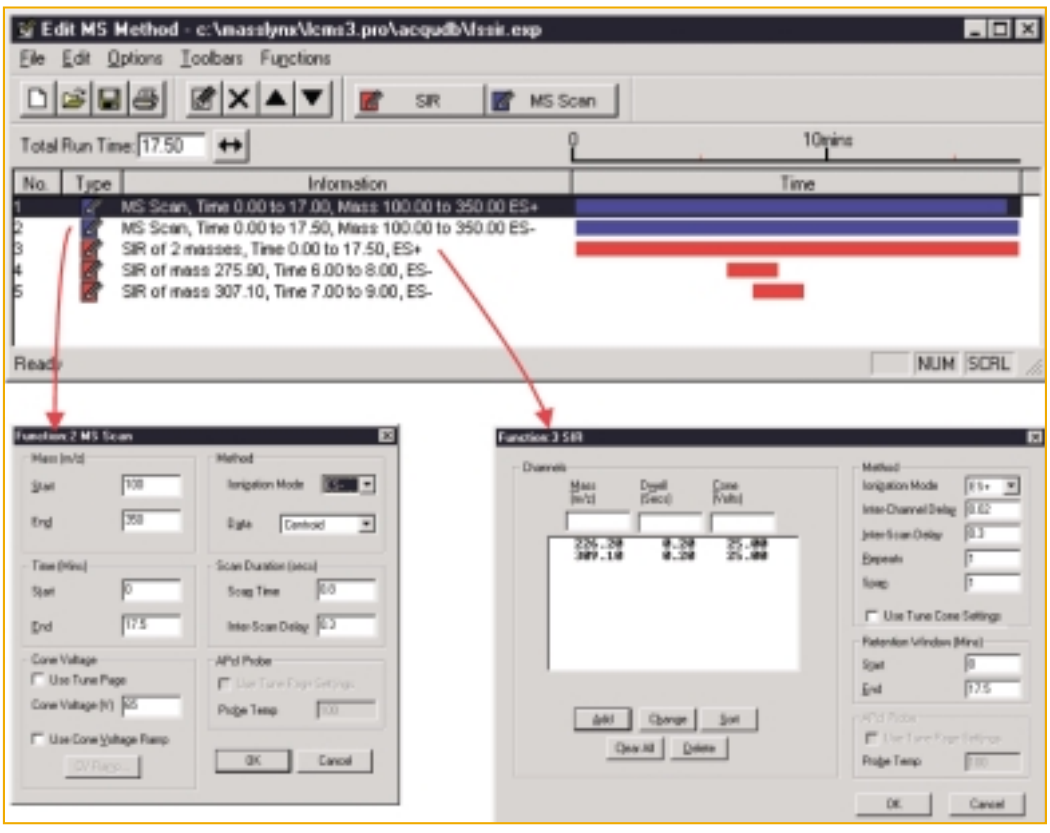
The MS instrument method: in this method, the user defines the acquisition parameters of the mass spectrometer. This can be compared to the method which is developed for using a traditional HPLC detector.

The MS instrument method will be one of the elements of the global LC/MS method used to run sample and generate results.

The MS instrument method consist of a series of lines, each line corresponding to a specific function (task) of the mass spectrometer. The ZQ method can combine up to 32 functions, allowing complete flexibility.

For example:
Function 1: from 0 to 17 minutes, acquisition in positive ESI, scan from 100 to 350 amu in 0.8 seconds,
Function 2: from 0 to 17.5 minutes, acquisition in negative ESI, scan from 100 to 350 amu in 0.8 seconds
Function 3: from 0 to 17 minutes, acquisition in positive ESI, SIR (same as SIM) on mass 226.2 and 309.1, dwell time 0.2 second, cone voltage at 25 volts
Function 4: from 6 to 8 minutes, acquisition in negative ESI, SIR on mass 275.9, dwell time 0.2 second, cone voltage at 25 volts
Function 5: from 6 to 9 minutes, acquisition in negative ESI, SIR on mass 307.1, dwell time 0.2 second, cone voltage at 25 volts

The instrument will go through all these functions and will generate 5 chromatograms simultaneously. The two scan function will provide a global view of the sample, while the 3 SIR function will be used to observe and quantitate targeted compounds.



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/Micromass 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.

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