Analytical Science

A course (in 15 Chapters) developed as an Open Educational Resource, designed for use at 2nd year England & Wales undergraduate level and as a CPD training resource

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Description	This chapter considers the important technique of mass spectroscopy from the viewpoint of the analytical scientist rather than that of the interpretative spectroscopist. Topics covered include fundamentals of MS, ionisation modes, brief descriptions of instrumentation and the use of MS as a detector in other analytical technologies – hyphenated techniques.
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Chapter 13 – Mass spectroscopic techniques

Contents

Mass Spectrometry – a universal analytical technique

Mass spectrometry (ms) is very probably the most important of all techniques in modern analytical science. Recent developments in methodology have expanded its range of applications enormously. For example it is now an important weapon in the fight against terrorism, and is used on the widest scale in studies of the size and structure of protein molecules – two areas of application undreamt of only a relatively short time ago.

The originator of ms was Francis W Aston (1877 – 1945), whose research career included a period as an assistant to JJ Thomson, the discoverer of the electron. In 1919 Aston built his first "mass spectrograph" to study positively charged ions in the gas phase. This instrument could separate two ions with a mass difference of <1%, and with it Aston immediately showed that neon has two isotopes (^{20}Ne and ^{22}Ne). In 1922 he was awarded the Nobel Prize for Chemistry, and he later built another instrument with a better mass resolution. With it he discovered over 200 naturally occurring isotopes (there are 281 in all). Modern ms systems differ from Aston's in almost every way, but the basic principles of the method remain the same: by one means or other a sample is converted to ions, and the gaseous ions are separated on the basis of their mass-to-charge (*m/z*) ratios. This fairly simple idea needs much technical ingenuity to put into practice, but a very large range of instruments for a variety of applications is now available.

Mass Spectrometry: major applications

The applications of ms are so numerous and diverse – and require such a range of instrument types – that it is convenient to list the main ones straight away:

- Determination of the molecular masses and structures of organic and inorganic compounds: controlled fragmentation of the molecular ion of a compound is often crucial in this area.
- Separation and analysis of mixtures of macromolecules such as proteins; further structural studies of such molecules, e.g. amino-acid sequencing.
- Using **isotope ratio** ms to determine [e.g.] $^{12}C/^{13}C$ ratios provides important data on the nature and history of foodstuffs, forensic and archaeological samples, etc.
- Ion mobility ms is used for detecting traces of volatile materials, for example in chemical warfare, and in the detection of drugs, explosives and environmental hazards. In this area miniaturised instruments are now very important.
- Detection and determination of (bio-)organic materials separated by gas or liquid chromatography – this is perhaps the commonest current application of ms.
- Detection and determination of samples studied by inductively coupled plasma (ICP) spectrometry – the combined method is called ICP-MS.

Principles of Mass Spectrometry

The principal components of a mass spectrometry system are shown in the block diagram, figure (13.1) below.

In broad terms a sample, generated from a variety of sources, is converted to [usually] positive gas-phase ions by one of several techniques, and these ions are separated in a high vacuum on the basis of their *m/z* ratios, again by one of a number of different methods. The ions are detected by means of an electron multiplier device. Each ion reaching the detector cathode triggers the release of electrons, and a series of dynodes amplifies the electron numbers by a factor of 10^5 or so before they arrive at the detector anode and are recorded as a current. This is a similar process to that occurring in a photomultiplier tube as used in optical spectroscopy, and can easily be modified to detect negative instead of positive ions.

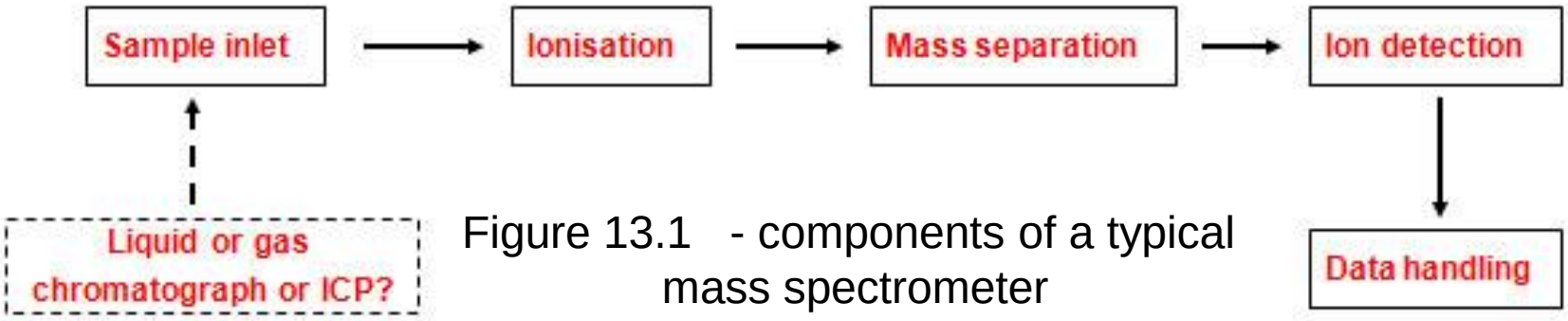


Figure 13.1 - components of a typical mass spectrometer

Electron impact mass spectrometry

If the sample molecules are reasonably volatile (i.e. with a boiling point below about 500° C) and of modest molecular weight (<ca. 1000), then **electron impact (EI)** ionisation is a relatively simple method of producing positive ions from them. The electrons are emitted by a hot filament and accelerated to ca. 70 eV before colliding with the gaseous sample. About 0.01% of the sample molecules interact with one of these energetic electrons and absorb about 20% of its energy, with the loss of another (low energy) electron, i.e.

$$M + e^- \xrightarrow{\sim 70\ eV} M^{\cdot +} + e^- + e^- \quad (\sim 55\ eV,\ \sim 0.1\ eV)$$

The resulting species $M^{\cdot +}$ is a **radical ion** with the same molecular weight as the original molecule M, and is called the **molecular ion**.

The molecular ion acquires ca. 15 eV of energy, more than is normally required to expel an electron, so the EI method is called a **hard ionisation** method. It often results in the further fragmentation of the sample molecule. (This is a situation not dissimilar to the one arising in optical spectroscopy, where the absorption of an energetic photon can lead to photodecomposition). Sometimes EI produces a mixture of molecular ions and smaller fragments, while in others, fragmentation is more extensive and the molecular ion is hard or impossible to detect.

Fragmentation in electron impact mass spectrometry

The discussion that follows refers to a mass spectrum shown on the next slide as figure (13.2). This is a simple example of an electron impact mass spectrum of methanol, which has a molecular mass of 32. Important features of the spectrum are:

- Aliphatic molecules such as methanol are especially prone to fragmentation, and its mass spectrum actually has its most intense peak at the *m/z* value 31. This is obviously due to the CH_2OH^+ ion. The *m/z* = 31 peak is called the **base peak** and is said to have a *relative abundance* of 100%.
- The **molecular ion** peak at *m/z* = 32 has a relative abundance of about 70%, and the peak at *m/z* = 29, due to CHO^+, a relative abundance of 40%. Several quite small fragments down to C^+ (*m/z* = 12) are formed, including CH_3^+ (*m/z* = 15) with a relative abundance of 12%.
- Close examination of the spectrum shows that, in addition to the CH_3OH^+ peak at *m/z* = 32, there is a small peak at *m/z* = 33. This arises from the ca 1% level of the ^{13}C isotope in the sample. (Other isotopes of O and H are also present, but in very much lower amounts).

Note: Such isotopic effects often complicate mass spectra (for example bromine occurs naturally as the two isotopes ^{79}Br and ^{81}Br in almost equal amounts), but may also help in their interpretation. Molecular spectra as presented normally use whole mass numbers, even though the actual molecular masses are usually not exact whole numbers.

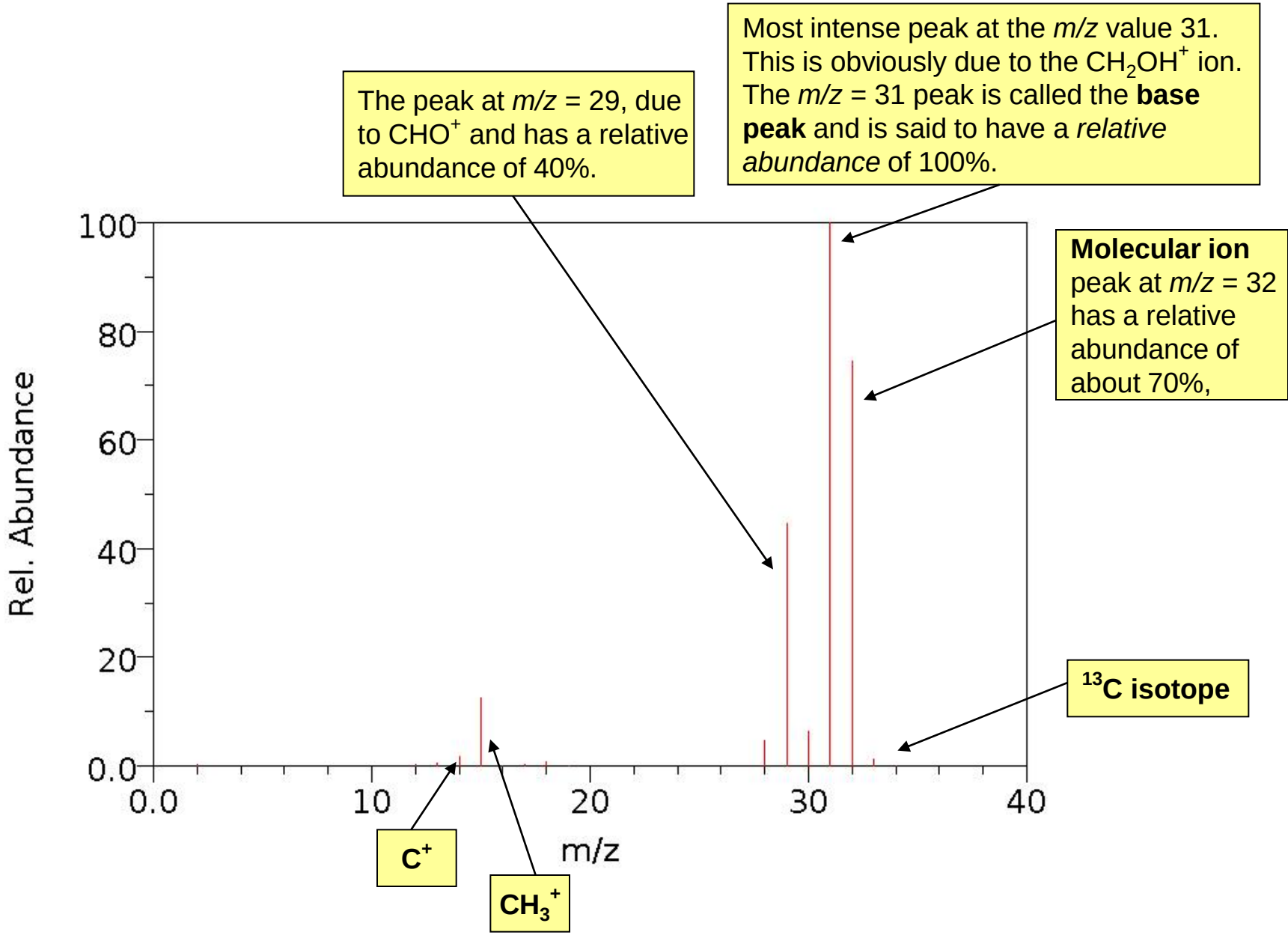


Figure 13.2 – mass spectrum of methanol, copied by permission of NIST

Chemical ionisation mass spectrometry

Chemical ionisation (CI) is a **softer** (less energetic) method for producing ions from volatile molecules. It uses electron impacts with energies of 100–200 eV. The ionisation chamber contains the sample **and** a large excess of a reagent gas such as methane, ammonia or isobutane.

Taking methane as the reagent gas for example, the first results of electron impact expected, is the formation of the CH_4^+ radical ion, but this ion then provides further products, e.g.

$$CH_4^{\cdot +} + CH_4 \rightarrow CH_5^+ + CH_3^{\cdot}$$
$$CH_4^{\cdot +} \rightarrow CH_3^+ + H^{\cdot}$$
$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$$

The ions highlighted in red then ionise the sample molecules, M. The CH_5^+ ions react with M to give CH_4 and MH^+, i.e. an ion with a mass one unit higher than that of the parent molecule (**the "M + 1" peak in the mass spectrum**). The $C_2H_5^+$ ions may produce a similar **proton transfer** result, generating MH^+ and C_2H_4. But it may also participate in a **hydride transfer** reaction, giving the $(M - 1)^+$ ion and ethane. These ions allow the molecular mass of M to be found with ease. In each case fragmentation may also occur, but it is usually less than in the EI case, and if ammonia is used as the reagent gas, polar and basic sample molecules are hardly fragmented at all (non-polar molecules do not ionise with this reagent).

Other MS ionisation methods

In the last 20 years, methods have been developed for the ionisation of proteins and other large or non-volatile materials. They have made major contributions to recent advances in several biosciences. Three of them are summarised here.

- **Electrospray Ionisation** occurs at atmospheric pressure and temperatures. Liquid samples emerge at a few *μ*l per minute from a steel needle at a high potential. A charged spray of fine droplets results, and charged and desolvated molecules are formed in a desolvating capillary. There is often little fragmentation, and the ions produced are multiply charged (a typical protein might carry dozens of positive charges) so can be detected in conventional systems with *m/z* ranges up to 1500.
- **Fast Atom Bombardment (FAB)** ionises molecules using argon or xenon *atoms* at several keV in a high vacuum system. Protein samples are usually dissolved in a protective glycerol matrix; low mass but non-volatile materials are also studied. Both positive and negative ions are formed, and fragmentation is again limited.
- In **Matrix Assisted Laser Desorption Ionisation (MALDI)** the sample is mixed with an excess of a compound that absorbs the laser radiation and evaporated on to a metal probe. Laser pulses produce ions suitable for study in a time of flight mass spectrometer (see slide xx). No fragmentation occurs; doubly or triply charged ions may be formed.

Mass Spectrometry: Separating the Ions

Once the sample molecules have been converted into ions, the latter need to be separated according to their *m/z* ratios. The classical method of doing this, still used in high resolution ms studies, is achieved by accelerating the ions with an electric field, then passing them through a magnetic field at 90° to the direction of travel of the ions. Figure (13.3) illustrates the essential features of a sector type mass spectrometer.

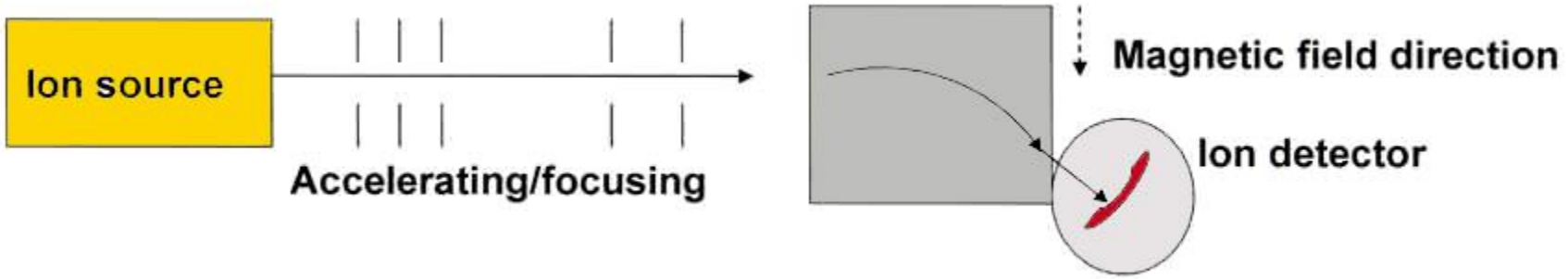


Figure 13.3 – components of a sector type mass spectrometer

Equations to show how a mass spectral separation occurs are shown on the next slide.

If an ion with mass ***m*** and charge ***z*** is accelerated by a potential difference ***V*** its potential energy will be ***zeV***, where ***e*** is the electronic charge.
Its velocity (***v)*** can be found by equating this potential energy with its kinetic energy, $\frac{1}{2}mv^2$, so ***v*** is given by:

$$v = \sqrt{(2zeV/m)} \qquad \text{Equation (13.1)}$$

Inside the magnetic sector in a magnetic field of strength ***B****,* the ion is deflected through a circular path of radius ***r***, given by equating the force provided by the field, ***zevB***, and the centripetal force mv^2/r. This gives:

$$v = zeBr/m. \qquad \text{Equation (13.2)}$$

Comparing these two results for ***v*** we can see that:

$\sqrt{(2zeV/m)} = zeBr/m$, which can be rearranged to give:

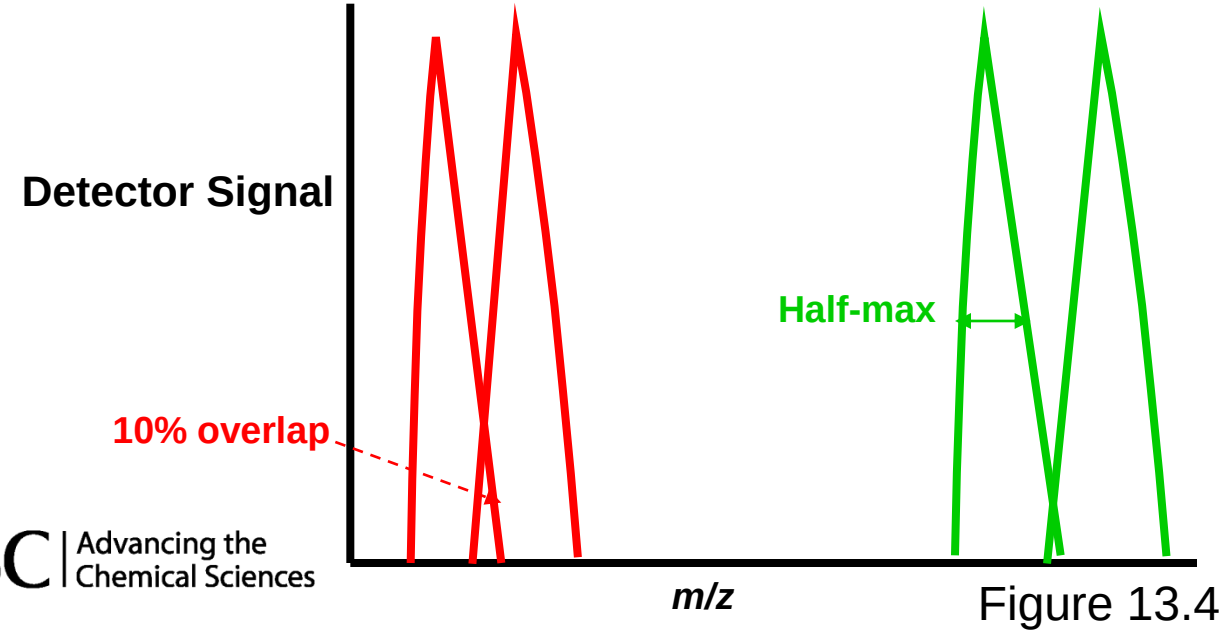
$$m/z = eB^2r^2/2V. \qquad \text{Equation (13.3)}$$

This equation shows how ***m/z*** values are related to the radius of the ion trajectory. In practice the latter is fixed by the instrument layout, including the fixed detector position. Ions with different ***m/z*** values are thus resolved by scanning the value of ***B****,* the magnetic field strength.

Resolution in Mass Spectrometry

As in all analytical methods in which molecules are separated, the resolution (*R*) of a mass spectrometry system is an important characteristic, as it defines the ability of the system to resolve two ions with closely similar *m/z* values. If the resolution of the instrument is insufficient, the peaks from two such ions may merge to give a single peak. Rather surprisingly, two methods for measuring *R* are in common use, and it is evidently essential to specify which one is adopted in any given experiment. The equation for *R* is simple:

R* = *m*/Δ*m, where ***m*** is the lower *m/z* value of two adjacent peaks in the spectrum, and Δ*m* is a measure of their separation – see figure (13.4)



In some cases **Δ*m*** is defined as the peak separation when they overlap by 10% at their bases, while in other cases **Δ*m*** if given by the peak width at half-maximum intensity.

Figure 13.4 – resolution in MS

Other approaches to *m/z* separation

A typical magnetic sector ms instrument might provide a resolution of about 1000. This is adequate for many purposes, but some applications need higher *R* values. In **double-focusing** ms instruments the ions are passed between two charged metal plates to separate them electro-statically before they enter the magnetic sector: this more complex method can provide resolutions up to ~100,000.

A number of other types of ms system have become extremely popular in recent years, mostly in connection with the use of ms as a detector for chromatographic or spectroscopic analyses. The three types of instrument that are currently important are:

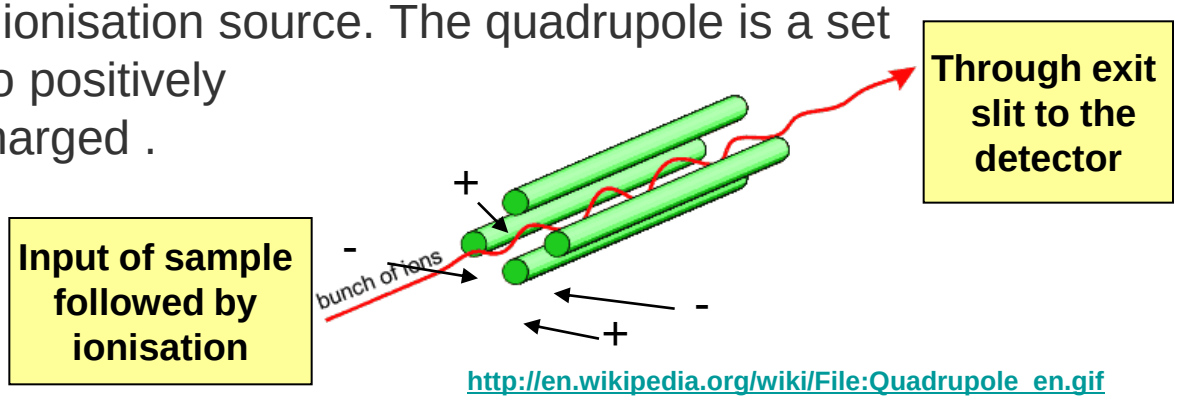
- The quadrupole mass analyser;
- The time-of-flight mass spectrometer;
- Ion-trap mass spectrometer.

The quadrupole mass analyser

Probably the best-known of the alternative mass spectral analysers is the **quadrupole mass analyser**, a relatively simple, compact and efficient device that can be inter-faced with a gas or liquid chromatographic system.

It has a high-vacuum electron ionisation source. The quadrupole is a set of four parallel metal rods, two positively charged and two negatively charged .

Figure 13.5 – quadrupole mass analyser



http://en.wikipedia.org/wiki/File:Quadrupole_en.gif

A dc voltage and an oscillating radiofrequency (rf) ac voltage is applied to them so that their polarities alternate at the radiofrequency. The effect of the applied voltages is that only ions with specific *m/z* ratios that resonate with the rf field can pass completely through the channel between the rods. Other ions are deflected on to the rods and so are not detected. Changes in the applied voltages allow different ions to reach the detector: several complete mass spectra per second can be recorded, with *m/z* values up to 4,000. Such a system is obviously suitable for the detection of peaks, only a fraction of a second wide, from a gas chromatograph.

Continued on the next slide

Figure 13.6 – Varian double quad MS detector/analyser fro HPLC

Figure (13.6) shows a typical layout of a modern LC-MS analysis system. The first quadrupole detector is tuned to select an individual target ion which if possible is specific to the target analyte. This ion (Parent ion) is then passed into the collision cell where It undergoes further breakdown to produce a range of 'Daughter ions'. The 2nd quadrupole analyser then selects one of these daughter ions which is unique to the target analyte to create a specific Identification together with quantification if required.

For additional information on quadrupole MS see:
http://www.chm.bris.ac.uk/ms/theory/quad-massspec.html

Time of Flight mass spectrometry

Very simple physical principles allow the separation of a group of ions which all have the same *kinetic energy*. This energy is provided electro-statically by a repeller plate in the region where the ions are produced. This plate is raised to a voltage of several kV several thousands of times per second to expel the ions from the **source region** into the **drift region**. Here the lighter ions (smaller *m/z*) travel faster than the heavier ones through an evacuated tube to the detector: hence the name of the method – time of flight (TOF) ms. A schematic diagram of A **Reflectron type instrument**, is shown in figure (13.7).

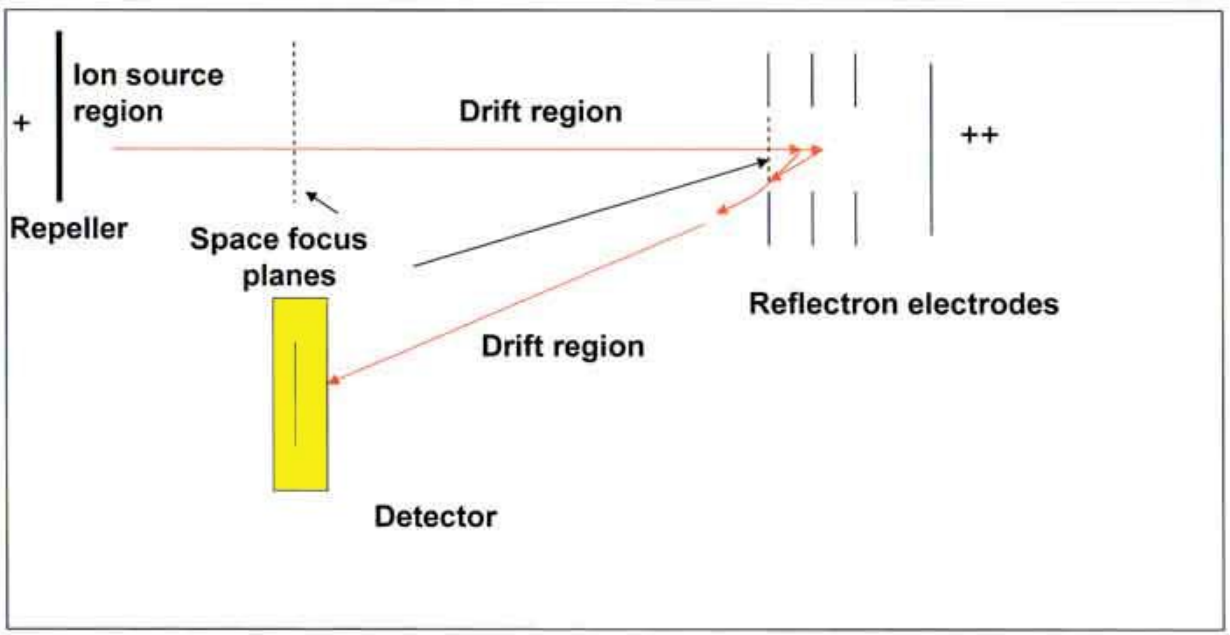
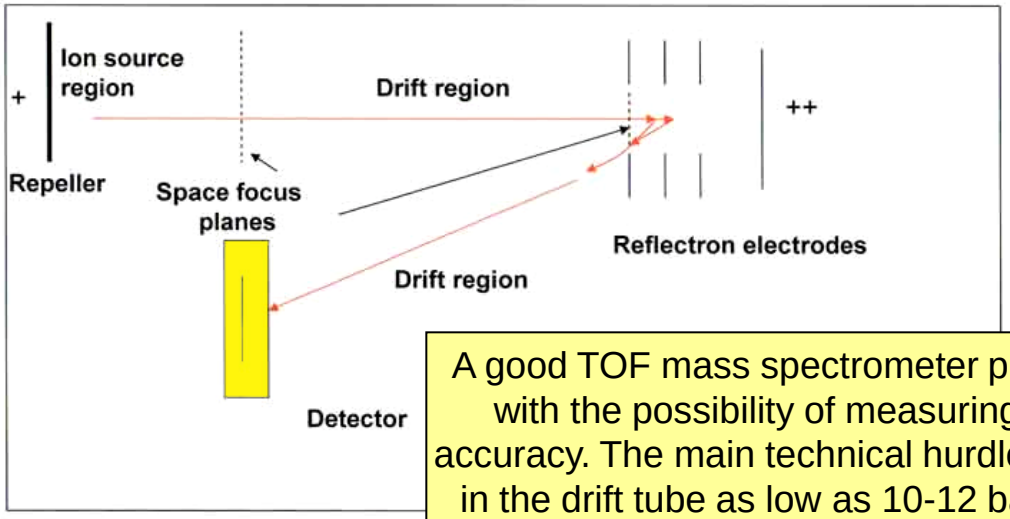


Figure 13.7 - Reflectron TOF mass spectrometer

Continued on the next slide

In practice it is not possible to provide all the ions with exactly the same kinetic energy, as they are formed at slightly different distances from the repeller plate. An ion formed close to the plate will have higher energy than one formed further away, but the former ion will leave the ionisation area slightly later than the latter.

In time the more energetic ion, (faster), catches up the slower one at the **space focus plane**. All ions of a given mass reach this position simultaneously, but then start to separate again, with the faster ones now overtaking the slower ones. Without further measures the resolving power of this type of system is quite poor. In more advanced instruments the ions enter a *reflectron*, in which they are slowed to a halt and reflected in the reverse direction by a set of ring electrodes at increasingly positive potentials, culminating in one which has a higher potential than the original repeller plate. The reflectron produces a new space focus plane, all ions of a given mass reaching it simultaneously. They then enter a second drift region before reaching the detector.

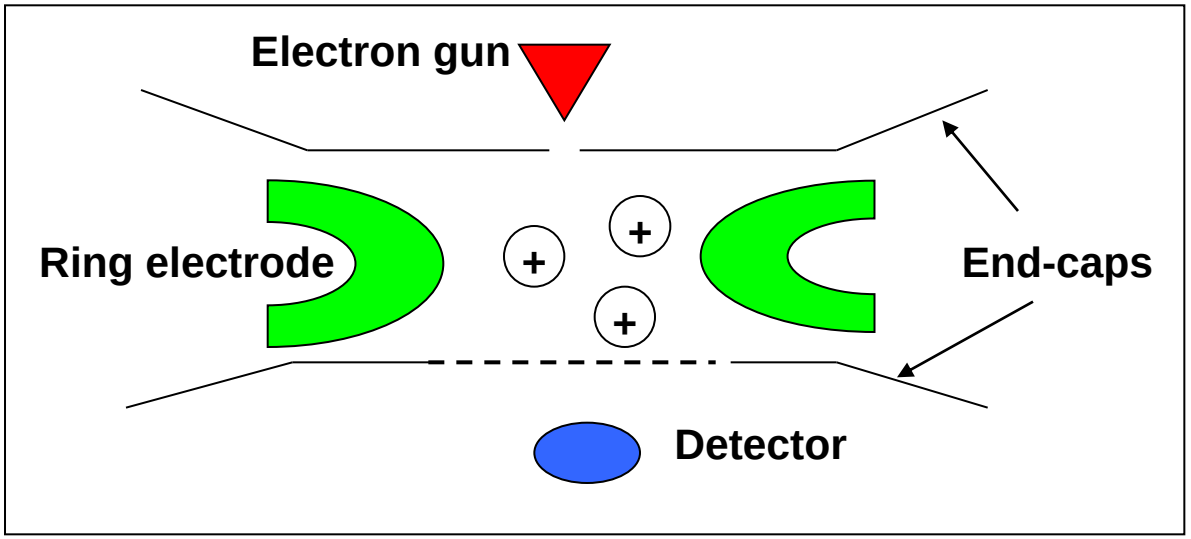


A good TOF mass spectrometer provides up to 100 spectra per second, with the possibility of measuring very high masses (~10^6) with high accuracy. The main technical hurdle is the provision of a very high vacuum in the drift tube as low as 10-12 bar. TOF instruments are often used in conjunction with MALDI ion sources.

Ion trap mass spectrometry

An ion trap mass spectrometer is a very simple, compact and rugged device, again well suited to chromatography detection. As shown in figure (13.8) below, it contains a doughnut-shaped ring electrode, and electrically isolated end-cap electrodes. One end-cap has a grid through which ionising electrons or CI gases are admitted; the other has openings through which ions reach the detector. A radiofrequency voltage applied to the ring electrode provides stable orbits in which ions of varying *m/z* values can move. Increasing the frequency by controlled increments expels from the ion trap ions with selected *m/z* values for detection by an electron multiplier. Ion traps can provide several scans per second for ions of moderate size: they will handle *m/z* values up to a few thousand, and they detect a much higher proportion of the ions generated than other detectors, giving them picogram sensitivities.

Figure 13.8 diagram of the components of an ion-trap ms



Mass spectrometry in elemental analysis

In the last 25 years, mass spectrometry has been increasingly used as a major detection method for elemental analysis, in addition to its numerous applications in the study of the structures of large and small organic molecules, and as a very versatile detection technique in chromatography. In one sense this represents a return to the original purpose of mass spectrometry, the study of atoms and isotopes, and indeed the study of isotopic ratios is now an important application.

Several different methods for producing atomic samples for mass spectrometry have been described. The most important of these is **inductively coupled plasma mass spectrometry (ICPMS)**, in which an ICP torch provides the atoms and ions, liquid samples being introduced into it by a nebuliser device. Solid samples can also be vaporised by the use of high power pulsed laser beams. This **laser ablation** approach has been shown to work well even for refractory minerals, glasses, soil samples and so on, and the laser can focused on areas of only a few square micrometers. **The principles of ICP spectrometry are covered in Chapter 12 of this teaching and learning programme (see slides 36 -51).** In ICPMS it is usual to use a quadrupole system as the mass analyser, operating at a pressure of ca. 10^{-5} – 10^{-4} torr. Interfacing these two components is clearly the key to the successful operation of the method as a whole.

Interfacing in ICPMS

In a typical ICPMS interface the plasma gas from the ICP torch is passed through a minute hole in the vertex of a water-cooled metal cone, into a zone pumped to a pressure of about 1 torr. Rapid expansion and cooling of the gas occurs.

A small hole in a second (“skimmer”) cone allows a fraction of this cooled gas to pass into a chamber held at the mass spectrometer pressure. Here, application of a negative potential separates and accelerates the positive ions which are focused by a magnetic lens on to the entrance of a quadrupole detector.

In the analysis of metal ions a mass range up to ca. 300 is sufficient, with a resolution of m/z = 1. Modern instruments can analyse most elements rapidly (a few seconds) at the ppb level and below, and have an excellent dynamic range, the instrument response being proportional to concentration over several orders of magnitude. Since multi-element analyses are routinely available, this method is extremely powerful and has found many applications across diverse fields, from geology and environmental chemistry to biochemistry and molecular biology.

Since many elements have isotopes with the same m/z values, e.g. $^{40}Ar^{+}$ and $^{40}Ca^{+}$, quantitative analyses may involve using less abundant isotopes (e.g. $^{44}Ca^{+}$, with an abundance of 2.1%), or isotope ratios. The software provided with modern instruments readily handles such problems.

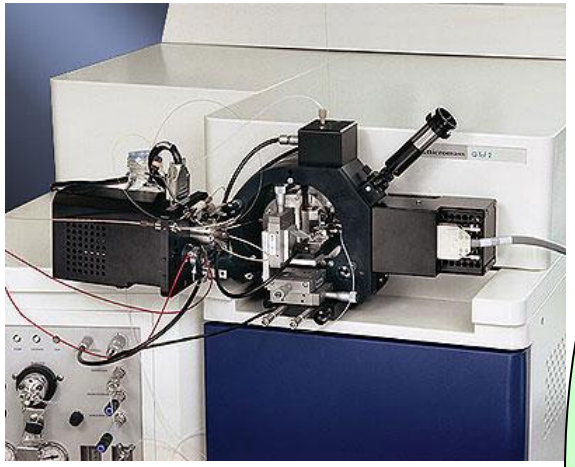
Hyphenated techniques

It has been shown in previous slides, that although mass spectrometry may be used as a technique in its own right, it is increasingly used by non specialist analytical scientists, as a technique in tandem with other analytical techniques. Such combinations are referred to as **hyphenated** or **tandem** analytical systems. The main reason for combining techniques together, is to increase:

- Sensitivity of detection
- Selectivity of separation
- Improve reliability of identification

This latter usage is of particular importance is the analysis of forensic samples, where evidence is to be presented in court or in cases of suspected drug abuse.

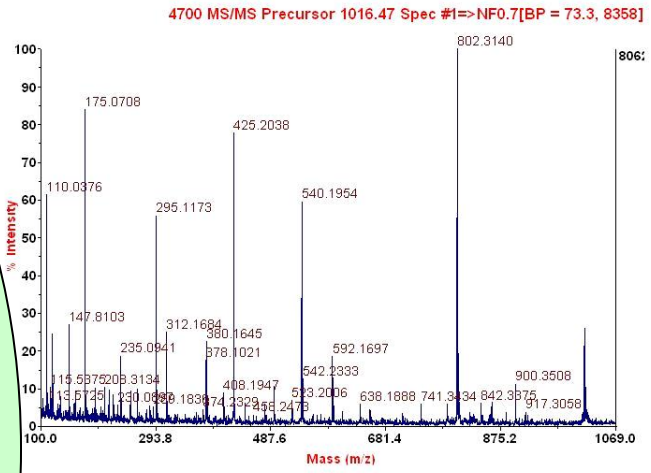
Techniques frequently referred to under this umbrella of **hyphenated** are Illustrated as figure (13.8) on the next slide.



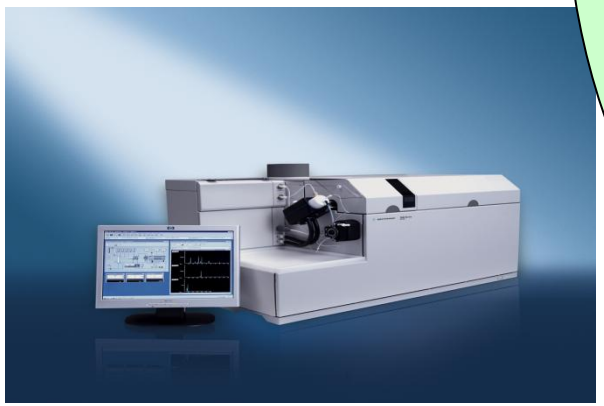
LC-MS

Hyphenated techniques refer to systems where two or more analytical techniques are combined together in a tandem arrangement in order to increase analytical specificity. Examples include:

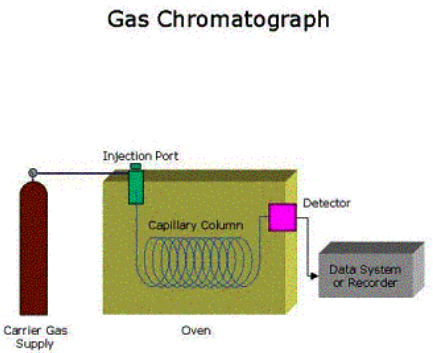
MS-MS
GC-MS
LC-MS
ICP-MS



Protein MS-MS



ICP-MS



Schematic of a GC-MS

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Figure 13.9 – hyphenated techniques

Where the analyte is in a sample with other analytes and/or possible interfering substances, then a separation technique may be required prior to analysis. In these applications, chromatographic techniques will be used for sample clean up and separation prior to characterisation by mass spectrometry. [**See Chapter 6 in this teaching and learning programme to revisit the introduction to chromatography**].

By combining the efficient separation capabilities of modern gas chromatography (GC) or liquid chromatography (LC) you have a very powerful analytical technique capable of **unambiguous (specific) identification and quantification of analytes in a complex mixture or matrix.** The order of instrumental components in a GC/MS or LC/MS are shown in figure (13.10) below

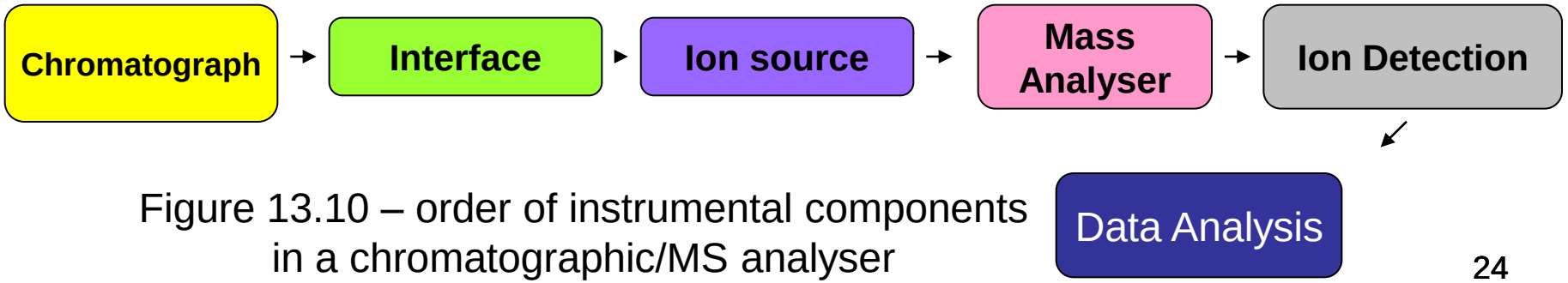

Figure 13.10 – order of instrumental components in a chromatographic/MS analyser

Terminology

Descriptions of Hyphenated Techniques using at least two sophisticated analytical techniques and an array of analytical strategies can often become difficult to read as they are often littered with jargon and acronyms, often necessary to describe the complex processes involved.

Table (13.1) below, summarises some of the popular mass spectroscopic technologies used in hyphenated procedures.

Table (13.1) – mass spectroscopic techniques used to support hyphenated technologies

Ionisation	Mass Analyser
ESI **(Electrospray interface)**	TOF **(Time of flight)**
APCI **(Atomospheric pressure chemical ionisation)**	ITMS **(Ion trap mass spectrometer)**
MALDI	*Quadrople*
Chemical Ionisation	*Ion Trap*
Electron ionisation	*Fourier Transform*

Total ion *versus* selective ion monitoring

Total ion monitoring – this is the sum of the currents, resulting from all of the fragment ions from a molecule, plotted as the ions pass through the detector. This results in a familiar chromatogram as illustrated by figure (13.11) below.

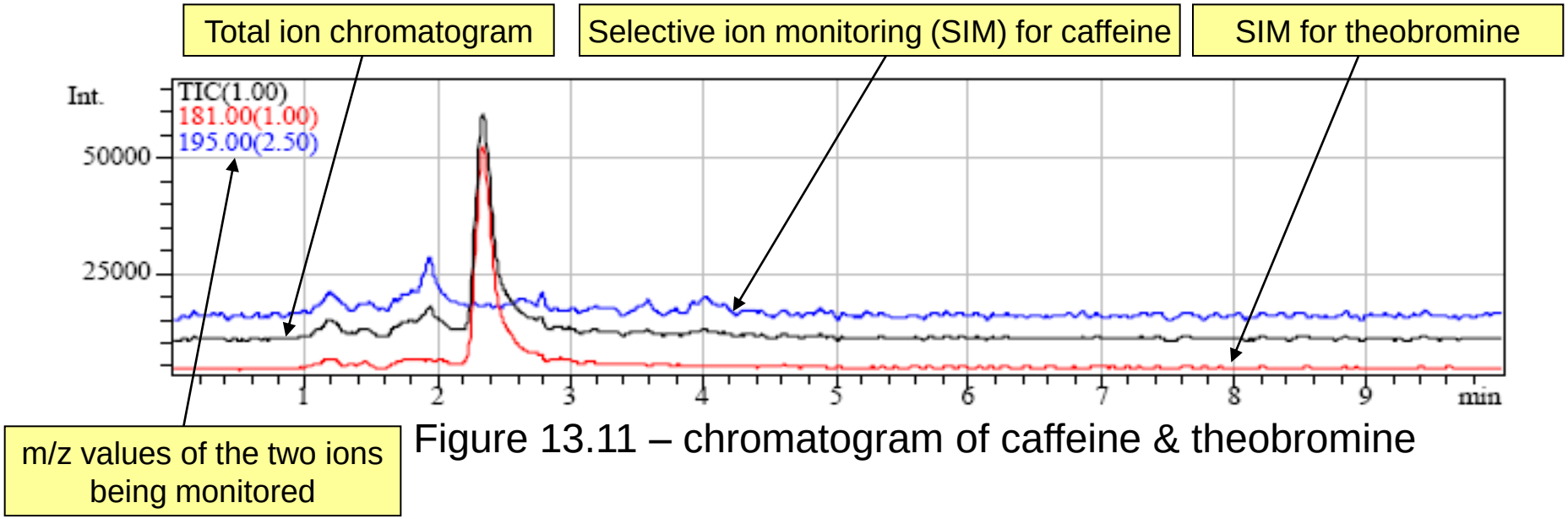

Figure 13.11 – chromatogram of caffeine & theobromine

If the analyst is interested in a particular m/z ratio then **selective ion monitoring** is used. A specific m/z is monitored, and only molecules containing a fragment of that ratio will be detected. The mass spectrum of each ion may also be retrieved

Isotope dilution

Isotope dilution mass spectrometry, IDMS, can be described as an absolute method involving an analytical standard. The standard is an **enriched analyte isotope**, i.e. an isotopically labelled (or heavy version of) the analyte is used as an **internal standard**. The mass is **traceable** back to SI units, and total sample recovery is not required as the sample is spiked with a known amount of standard. Isotope ratios (**R**) for the sample (**s**), the standard (**st**) and the spiked sample (**s+st**) are measured.

The amount of analyte present in the sample:

$$\mathbf{m_a = (R_{st}-R_{s+st})/(R_{s+st}-R_s) \times (1+R_s)/(1+R_{st}) \times m_{st}}$$

This is an important quantitative strategy, (deemed to be definitive), and is used in HPLC-IDMS, GC-IDMS and ICP-IDMS. Figure (13.12) below shows the available isotopes of carbon.

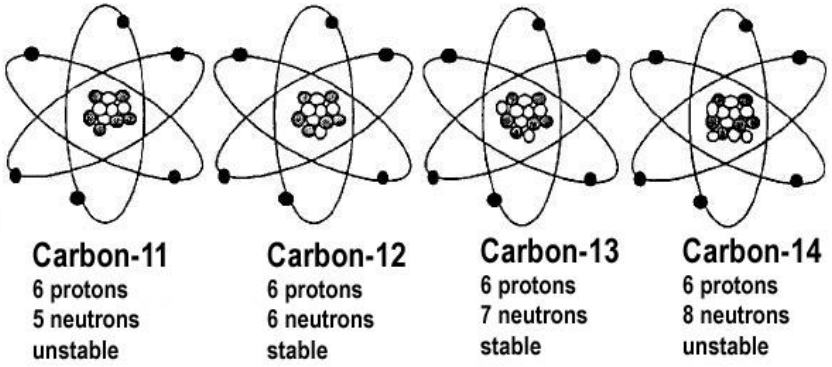

Figure 13.12 – isotopes of carbon

Tandem Mass Spectrometry

Tandem Mass Spectrometry, referred to as **MS-MS** or **MS^n** is a method used to find individual ions in a mixture. The ions of interest are identified by their characteristic m/z ratio, these ions are then introduced, one at a time, into a second mass spectrometer where they are fragmented and produce a series of mass spectra, one for each molecular ion produced by the first mass spectrometer.

The **molecular ion** produced by the first MS is called the **precursor**, or **parent ion:** **T**his ion is isolated and further fragmented to produce **product,** or **daughter, ions** and neutral fragments. The product ions are analysed in the second spectrometer. [see figure (13.13) below].

Tandem mass spectrometry can use various combinations of ion sources and detectors, to describe these are beyond the scope of this unit. This technique is **selective**, reducing signals from matrix components and other interferents.

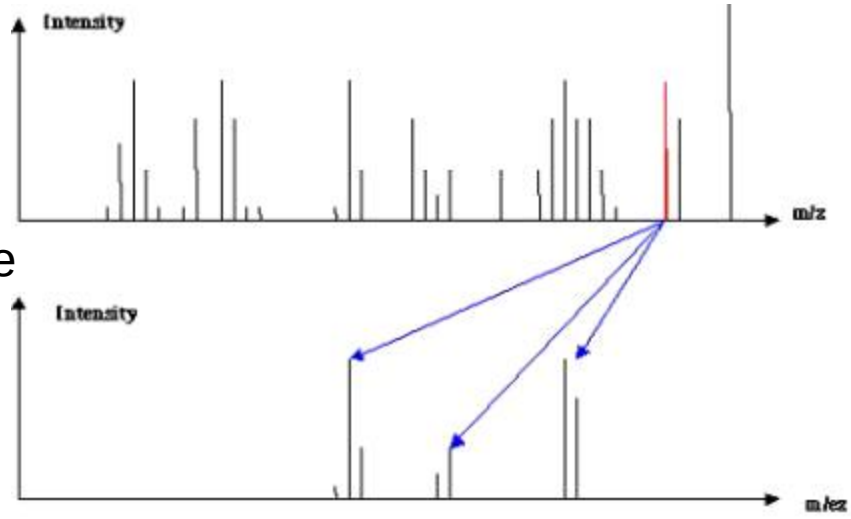

Figure 13.13 – mass spectrum showing production of daughter ions

Gas Chromatography-Mass Spectrometry (GC-MS)

Gas Chromatography Mass Spectrometry, known as **GC-MS**, was developed in the 1950's and is probably the most routine hyphenated technique involving MS to date. To be suitable for separation by gas chromatography the analytes should be volatile, or capable of being **derivitised** to a volatile form. To review the fundamentals of gas chromatography please refer to **Chapter 7 of this teaching and learning programme.**

The high resolution separation of complex mixtures in the gas phase is ideally suited for further analysis by mass spectrometry. The main components of a typical GC-MS are shown in figure (13.14) below

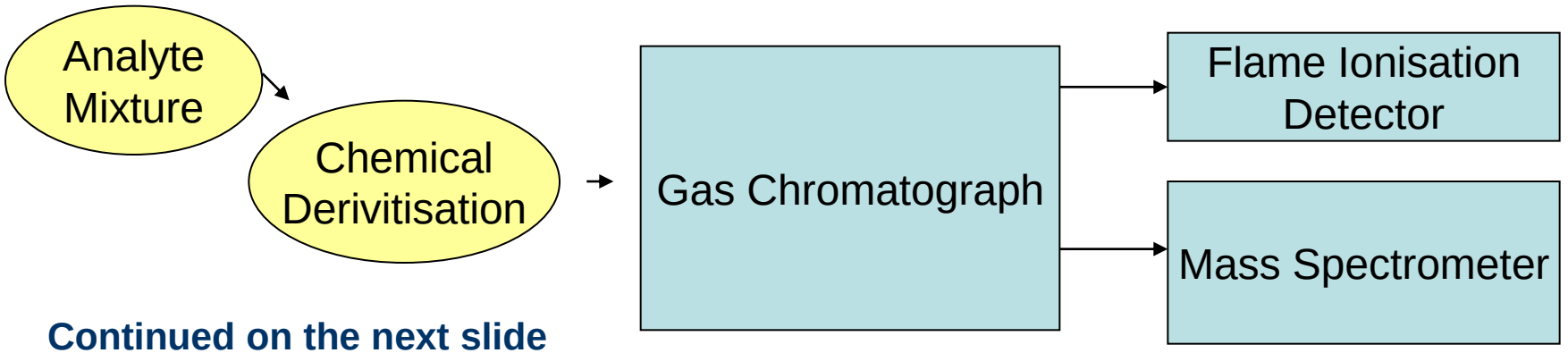

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Figure 13.14 – main components of a GC-MS

The tandem technique of GC-MS is both **qualitative** and **quantitative**. In figure (13.12) shown on the previous slide, the flame ionisation detector is used for quantitative analysis and the mass spectrometer is used to identify the components of the mixture - qualitative analysis.

In GC-MS the **full scan mode** (or **total ion monitoring**) can be used for qualitative analysis. In this mode the instrument is set up to target analytes of m/z 50 to m/z 400. Any lower than m/z 50 may pick up interferents from the GC mobile phase such as nitrogen at m/z 28, or CO_2. In this mode the technique is not very sensitive, as it is scanning over a wide range of fragments.

For quantitative analysis, **selective ion monitoring** (see slide 26), is more sensitive. More scans are run per second and as only a small number of mass fragments are being monitored, interferences from the matrix can be avoided.

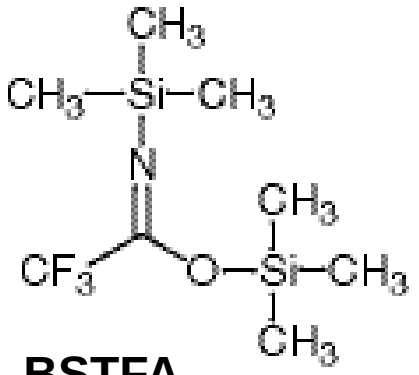
Also the **stable isotope dilution** method can be used for very accurate quantitative analysis of metabolites in biological fluids. This method differs from the **IDMS** (described in slide 27), because in **stable isotope dilution** the isotopes are non-radioactive.

Sample preparation

To be effectively analysed by GC-MS the analyte must be both volatile and thermally stable, typically between 50 to 300^{o}C. Some samples may need to be derivitised prior to analysis and / or cleaned up, often by solvent extraction.

Derivitisation may be used to increase volatility and reduce polarity. It may also be used to increase the weight of some smaller volatile substances, this leads to a more complex mass spectrum and therefore a greater confidence in the substance identification. Excess derivitising agent should be removed prior to separation and analysis.

BSTFA, N,O-bis-(trimethylsilyl)-trifluoroacetamide is a common derivitising agent for polar drugs, such as those found in urine. It produces trimethylsilyl (TMS) ethers, esters or amides. It does not need to be removed as it is suitable for injection into the GC-MS. The derivitivised samples are also stable for storage, without refrigeration.



BSTFA

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Monitoring for illicit drug use has become routine in some sectors e.g. transport, military and sport. Drug metabolites are rapidly excreted in urine, but can also be monitored in sweat, hair, oral fluids and even the meconium of newborns. The samples are usually screened by an immunoassay such as ELISA [Enzyme Linked Immunosorbent Assay (*http://en.wikipedia.org/wiki/ELISA*)] and those with a positive result are analysed by GC-MS for confirmation. Derivitisation is usually required for these analysis. As with all analysis the sample preparation and selection of an appropriate derivitising reagent is vitally important. The derivitive should produce at least three fragments not found in the sample matrix.

The TMS amide derivitives of amphetamines produces a small number of ions, including m/z 91, which is found in most matrices, therefore BSTFA is not a suitable reagent for this analysis. An alternative such as 4-carbethoxyhexafluorobutyryl should be used. Derivitisation of morphine and codeine with BSFTA results in the di- and mono-TMS ether respectively, which can be easily quantified by GC-MS.

Figure 13.15 – sample of urine for drug testing

Instrumentation

GC-MS instruments are becoming increasingly more portable, useful for airport security, on site analysis at crime scenes, and aboard space probes.

Figures 13.16 & 13.17 - portable GC-MS instrumentation

A challenge for GC-MS instruments is the interface, although the analytes have been separated and are in the vapour phase, depending on the instrument there can be a significant pressure difference between the **GC outlet** and the **ion source of the MS**. For packed column GC, the **jet separator** exploits the differential diffusion of the analyte and carrier gas molecules. The GC eluant enters a vacuum chamber through a small orifice, the stream of gas is focussed on the inlet of the MS. On entering the chamber the lighter carrier gas molecules diffuse out into a cone, leaving the heavier analyte molecules to continue on the straight path to the MS. Capillary GC, with fused silica columns can often be interfaced directly to the ion source.

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A common configuration for GC-MS is an electron impact source and quadrupole mass filter [figure (13.18)]. The quadrupole, the time of flight and Fourier transform mass analysers all have sufficiently fast scanning speeds for GC-MS. Some modern instruments can achieve a scanning speed of 100 scans per second in **single ion monitoring** mode. Fast scanning allows useful definition of narrow peaks and improved signal to noise ratio which in turn improves detection limits.

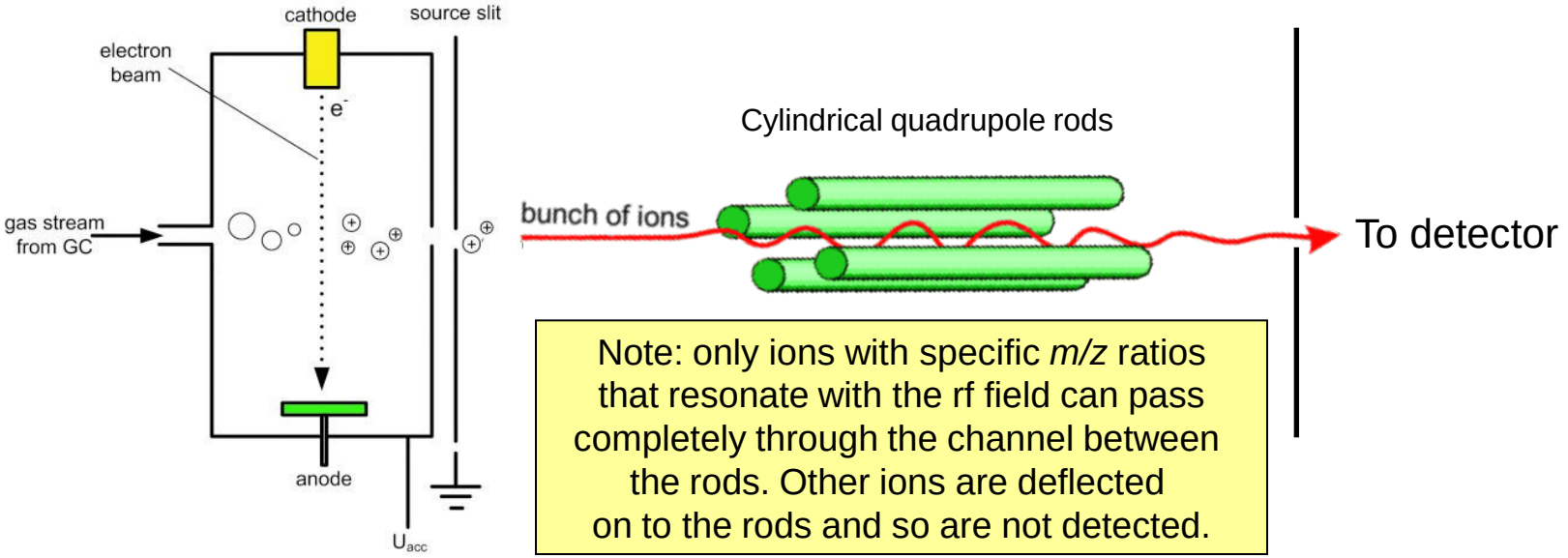

Figure 13.18– electron impact source and quadrupole mass filter

Applications of GC-MS

The applications of GC-MS are hugely varied, from clinical, to forensic to archaeological to environmental. Materials range from biological samples, drugs, oils and polymers.

In forensic science GC-MS has been described as the gold standard and is considered to be a **specific** analytical technique. In a GC two substances can co-elute, in MS two similar substances can have very similar spectra, by using both techniques it is extremely unlikely that two substances will have both identical GC and MS profiles. Therefore the technique can **positively identify** the presence of a particular substance in a mixture.

Volatile organic compounds, (VOCs) range from attractive odours such as essential oils, to odours leading to less pleasant sensations such as ammonia. GC-MS is routinely used in the odour management branch of environmental analysis. Portable air sampling devices incorporating solid phases such as Tenax (**see Chapter 2 of this teaching and learning programme**) are used to trap the 'odours' which can then be transferred to the GC-MS by **thermal desorption**. The results of the chemical analysis can be compared to the perceptions of human testing panels.

Liquid Chromatography- Mass Spectrometry (LC-MS)

Liquid Chromatography-Mass Spectrometry is a useful technique particularly in the area of bioanalysis, where the separation and subsequent analysis of large charged molecules, from proteins to drugs to metabolites is a requirement. The selectivity of the technique is particularly important when detecting and identifying metabolites. **Xenobiotic matabolism**, the metabolism of foreign compounds, from drugs, pollutants to pesticides, relies on a selective technique, capable of achieving low limits of detection and with the capacity to deal with a number of different compounds. Figure (13.19) shows the structure of Cortisone, a compound which could be analysed by this technology.

Liquid chromatography (HPLC), separates non-volatile, polar compounds, which may also be **labile**.

Conventional MS detectors were initially unsuitable, but the introduction of **soft ionisation techniques** has made the analysis of these compounds feasible.

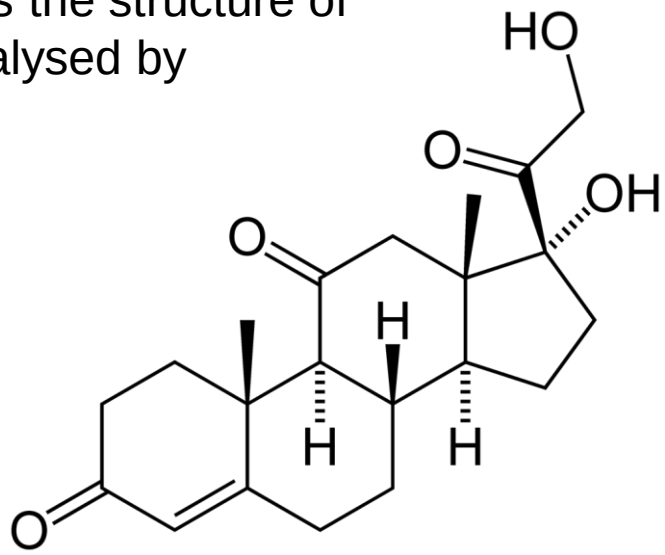

Figure 13.19 – Cortisone, a steroid hormone

Introduction to the technology

As with all hyphenated techniques the coupling of two, often very different, technologies is the first challenge. With LC-MS the analyte passes through a high pressure environment, at a fast flow rate into a vacuum. Typical mobile phases used with LC are aqueous/organic solvent mixtures often incorporating inorganic buffers. The mobile phase would evaporate on reaching the vacuum necessitating the removal of large volumes of gas. **This makes the choice of an appropriate interface a key decision.**

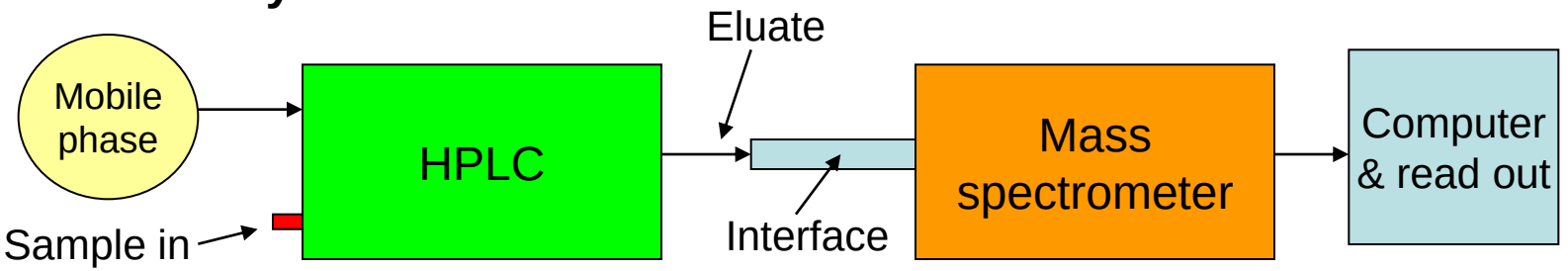

Figure 13.20 – block diagram of an LCMS tandem analyser

Originally a **Thermospray** interface was used. The volatile chromatographic eluant is heated in a capillary and sprayed into the vacuum chamber. As the solvent evaporates, very small, charged, solid particles, containing the analyte as core, are dissociated in the vacuum. The sensitivity of this technique, however, was limited, and the degree of fragmentation was small, limiting the structural information.

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The alternative to **Thermospray** was the **Particle Beam interface**. The eluant is pumped to a nebuliser, the resulting cloud of droplets are scattered in a flow of helium and pass through a heated, low pressure desolvation chamber, where they are partially 'desolvated'. The process is illustrated diagrammatically in figure (13.21). Most of the gas is swept away at the separator, leaving a narrow beam of High speed aprticles.

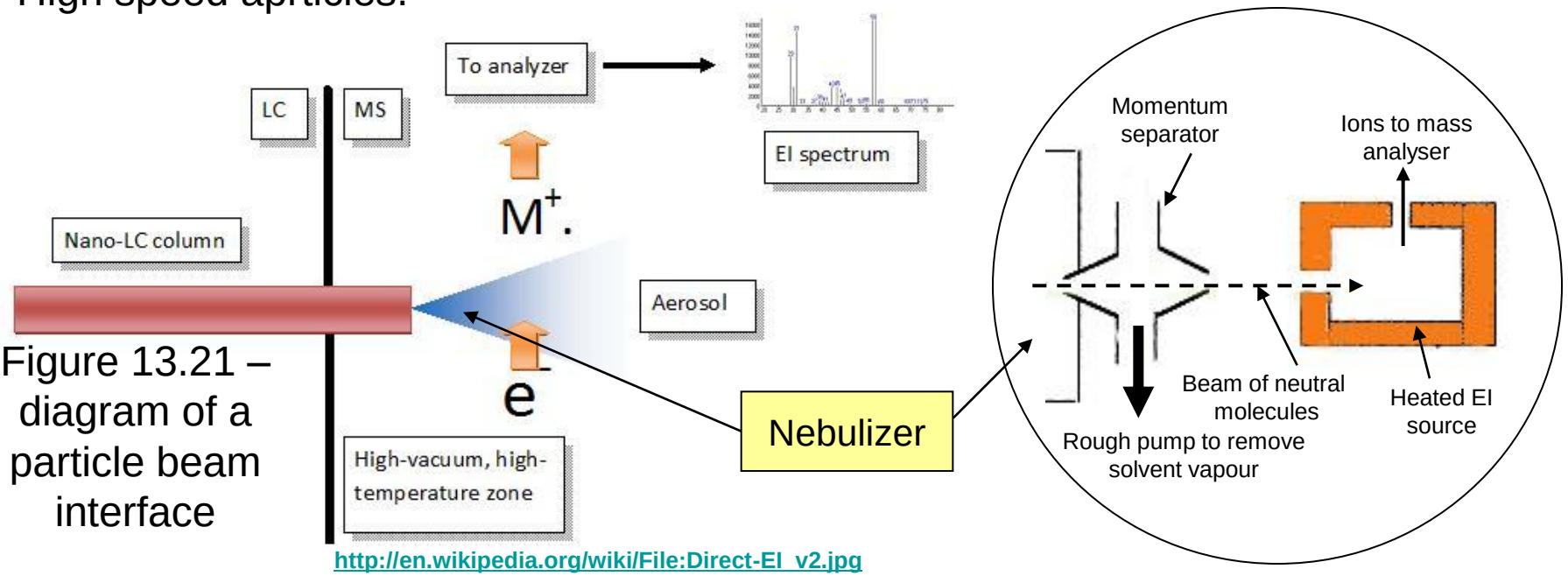

Figure 13.21 – diagram of a particle beam interface

http://en.wikipedia.org/wiki/File:Direct-EI_v2.jpg

The lighter solvent and helium molecules diffuse from the centre of the beam, leaving a concentrated beam of analyte particles to pass into the ion source to become ionised.

Ionisation methods

More sensitive methods are now required and modern LC-MS exploit less destructive methods of ionisation, i.e. soft ionisation techniques:

Electrospray interface (ESI):

The liquid eluant from the LC is forced through a narrow capillary and subjected to a strong electric field, charged droplets of analyte in solvent are produced, the solvent is removed by evaporation. As the solvent is removed the analyte molecules are forced closer together until they repel and 'explode' (**coulombic fission**) into ions, which are directed into the mass analyser.

Atmospheric Pressure Chemical Ionisation (APCI):

The eluant from a standard bore LC is directed into a nebuliser where it is subject to a high speed flow of nitrogen. The analyte/solvent droplets pass through a heated quartz tube, so that when the hot gas arrives at the reaction site of the source, it has been chemically ionised by proton transfer. The mobile phase acts as the ionising gas $(M + H)^{+\cdot}$. Figure (13.22) shown below, shows this process diagrammatically.

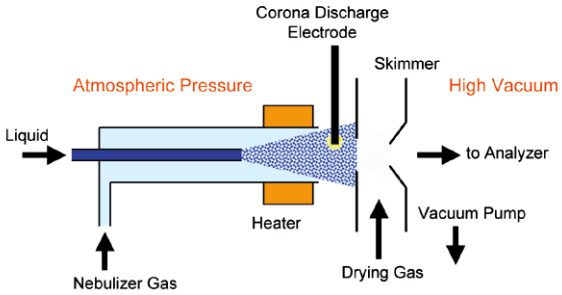

Figure 13.22 – diagram of APCI ionisation

http://en.wikipedia.org/wiki/APCI

UPLC-MS-MS

UPLC is a Waters trade name for a high speed liquid chromatographic separation, referred to as **Ultra High Performance Liquid Chromatography**. Figure (13.23) shows a typical UPLC instrument.

UPLC uses very small particle sizes (ca. 1.7 μm) for the column packing. Based on the Van Deemter equation [see Chapter 6 of this teaching and learning programme], it is shown that smaller particle sizes increases efficiency without compromising flow rates or linear velocity.
Ultra-performance is associated with:

- Increased capacity;
- Improved resolution at higher speeds.

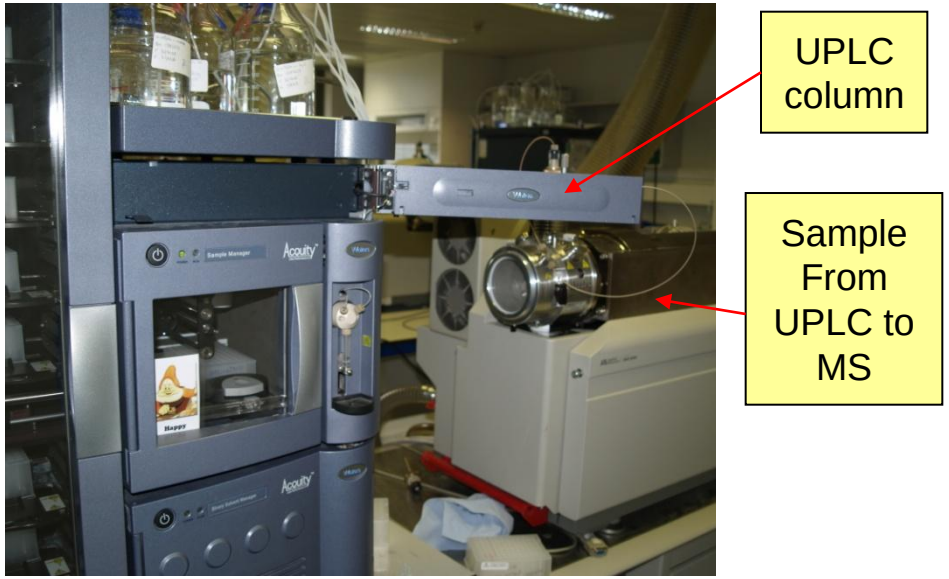

Figure 13.23 – typical UPLC instrument

Instrumentation also evolved to cope with the demands of the more efficient columns - detector volumes were significantly reduced to preserve the separation efficiency. With a fast detector response the sensitivity is also enhanced. All this making it a natural candidate for coupling with Mass Spectrometry or even MS-S.

Metabonomics

Detecting and identifying metabolites, at high throughput is of great importance to the pharmaceutical industry. The separation is challenging as there are potentially many compounds in the mixture.

A matrix affect often encountered in LC-MS is **ion suppression**. Co-eluting compounds, similar to the ion of interest may cause the metabolite to ionise, often in the LC-MS interface, before it gets to the MS detector resulting in a reduced signal, hence the term **ion suppression**. Various mechanisms have been proposed, they depend on the ionisation used, and are beyond the scope of this program. However the phenomenon maybe avoided by:

- Careful sample clean up, to remove contaminants;
- Improved resolution to avoid co-elution;
- Tandem MS techniques to improve selectivity.

Similarly an increase in detector response may also be observed for similar reasons.

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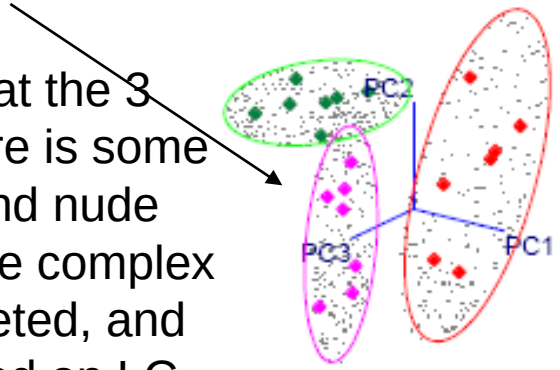
The use of increasingly sophisticated and fast techniques, generates a lot of useful data. Managing it can be challenging. This case exploits **chemometrics** to manage the data obtained and to recognise patterns that emerge from the analysis. Three species of mouse were studied, black, white and 'nude'. The 'endogenous metabolic profile' of their urine was analysed by LC-MS. Data from the LC-MS profile was saved as a **peak value**, associated with a **retention time** and **mass**. All peaks as a **time-mass pair** can be represented graphically, and **principal component analysis** (PCA) carried out.

It can be seen clearly that the 3 strains are different, there is some overlap with the black and nude strains, but in general the complex data can now be interpreted, and the strain predicted based on LC-MS analysis of metabolic profile.



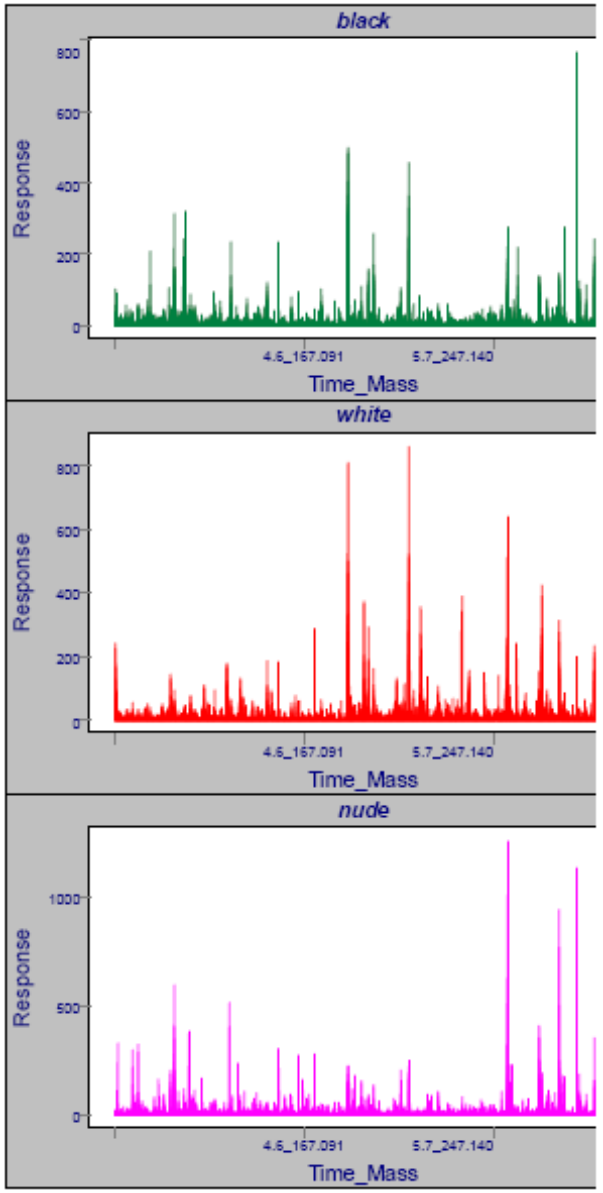
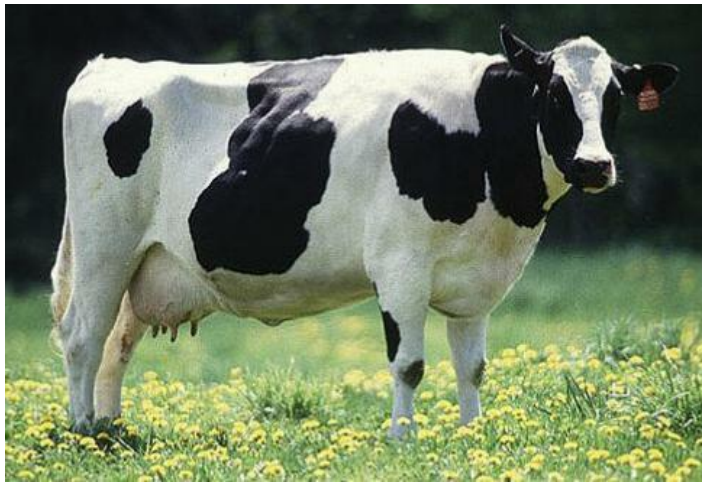
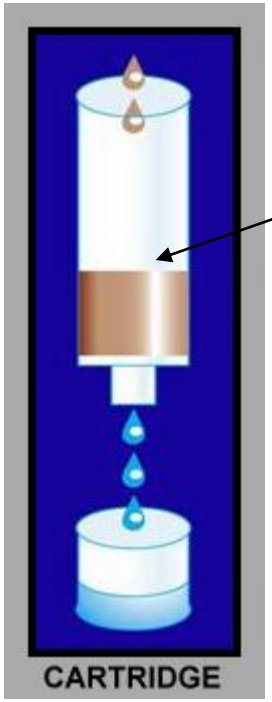


Figure (13.24) – The metabolic profiles of the 3 strains of mice. Spectra reproduced by permission of Infometrix.com

Case Study – Veterinary Residues in Animal Products

The analytical problem in this case study can be simply described as the identification and determination of a variety of veterinary residues in animal products. The residues come from a variety of classes of chemicals, growth promoters and hormones such as steroids, antibacterial agents and antiparasitic drugs, even sedatives.





In this case the sample matrix is the liver. Sample clean-up is usually achieved by **Solid Phase Extraction** [**see Chapter 3 of this teaching and learning programme**].

Method Transfer - the Approach: selecting separation parameters

This involves transferring an existing LC-MS method for veterinary residues to a new UPLC-MS method with subsequent method validation.

After an initial literature survey, to search for similar analytical problems, a series of samples and standards should be selected. It is important to understand the chemical properties of the substances you are attempting to separate and identify, in order to select the appropriate chromatographic conditions.

The starting point of this transfer is the chromatography. An ideal situation would be to use the same or similar separation parameters such as the packing material and mobile phase. Practical considerations such as the challenges imposed by smaller particle sizes of the separating column packing material needs to be addressed. The column may get blocked easily, so the mobile phase should be filtered. Selection of the filter size will be informed by the pore size of the **frits** at the ends of the separating column.

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Keeping the mobile phase simple is critical in UPLC-MS-MS, as components of some buffers, such as sodium salts, can contribute to **ion suppression**, see above.
Sample preparation is also important. As much of the matrix as possible is removed to avoid both ion suppression and causing a blockage in the column. The sample preparation step should also be validated to ensure the extraction efficiency is constant. Transferring the analysis to UPLC requires filtering the prepared sample solution with an appropriate filter, see above.

Choice of Mass Analyser:

- **Time of Flight (TOF)** mass spectrometers are compatible with LC, as they are capable of high scan rates and have an almost unlimited mass range.

- **Quadrupole ion trap mass spectrometers (ITMS)** are also used with LC, and are particularly useful for biochemical analysis. [**See slides 14 – 19**]

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Use of a triple quadrupole tandem MS

A typical instrument is illustrated in figure (13.25) below:

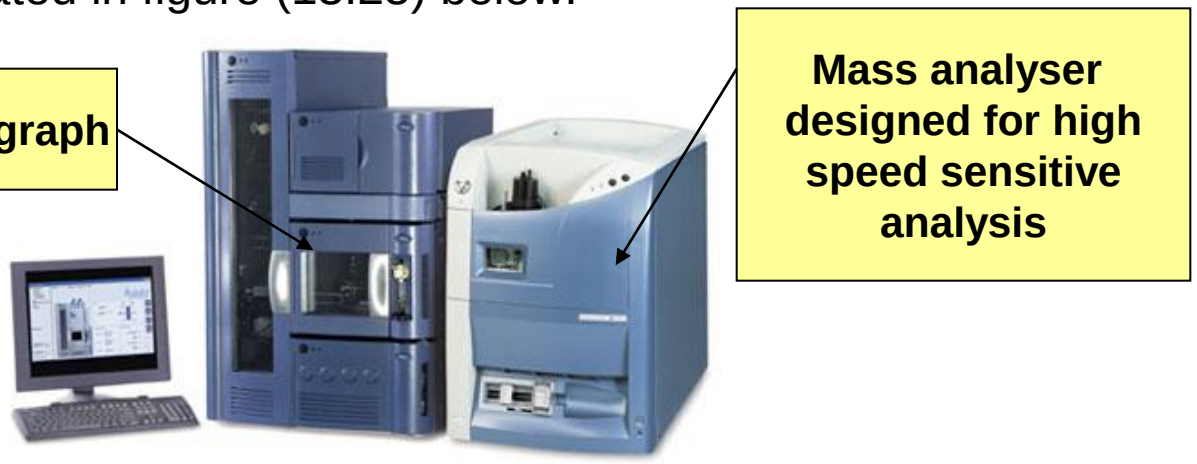

Figure 13.25 – typical LC-tandem MS-MS instrument

Dilute standards are infused into the mass spectrometer to confirm the presence of a molecular ion. If the signal is low the solution composition (the mobile phase) may be optimised. This process is referred to as **tuning**. To tune for MS-MS a collision gas is introduced to the collision cell, varying the collision energy produces different fragments, the production of a fragment from a parent ion is referred to as a **transition**. At least two transitions should be selected for each compound to be analysed. Tuning for each compound is necessary to ensure a **sensitive** quantitative analysis.

Method Transfer – the measurement

Once the instrument has been tuned for each analyte and the chromatographic conditions have been optimised for maximum sensitivity, the mixtures are then tested. In real samples the optimum conditions for the different target analytes will not be the same. Some analytes may break down during the ionisation process or some will form adducts (a sodium adduct for example will not fragment).

The instrument is then programmed to recognise each transition and thus the parent ion. Then the instrument can be programmed to look for certain analytes under different sets of conditions, conditions that best suit that analyte's chemistry. Optimisation of these sophisticated techniques can be challenging and requires a thorough understanding of the chemistry involved. Once validated these strategies can provide very sensitive quantitative analysis.

Inductively Coupled Plasma - Mass Spectrometry (ICP-MS)

Inductively Coupled Plasma - Mass Spectrometry, ICP-MS, combines the elemental analysis capability of ICP, with the ability to determine individual isotopes by using mass spectrometry. ICP-MS can perform **isotope ratio** and **isotope dilution** measurements. [**Refer to Chapter 12 of this teaching and learning programme, to review ICP-Optical Emission Spectrometry**]. The instrument comprises an ICP torch, which atomises and ionises the sample, interfaced to the mass spectrometer. The interface transports the sample, now ionised, from the ICP at high temperature and atmospheric pressure into the high vacuum and ambient temperature of the mass spectrometer, for analysis. [**refer also to slide 21 earlier in this chapter**]

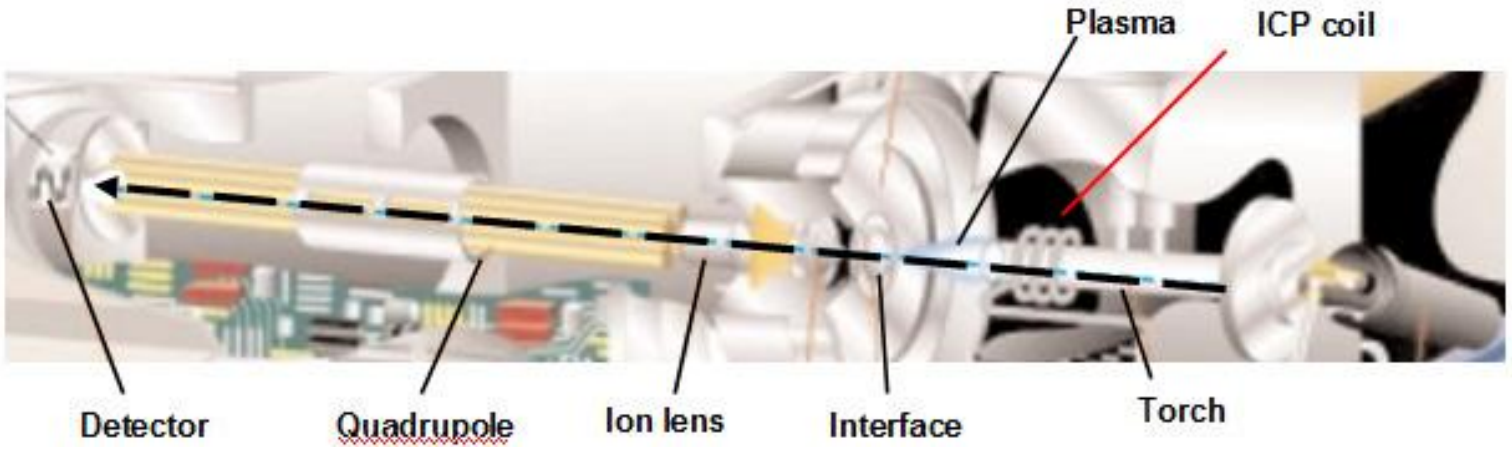

Figure 13.26 – diagram of ICP-MS

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The main distinction between ICP-MS and either GC-MS or LC-MS is the high temperature of the plasma, typically 6000K. The plasma destroys the molecules in the sample, leaving only the elemental ions to be detected by the MS. The technique is capable of analysing solid, liquid and gas samples. Gases can be introduced directly into the ICP torch, liquids are introduced via a nebuliser which produces an aerosol, in the same manner as conventional ICP. Solid samples are introduced via **electrothermal vaporisation**, (graphite furnace) or by **laser ablation** (later).

The impressive limits of detection of this technique make it particularly useful for the determination of trace element contamination in the semiconductor industry. [see figure (13.27) – a 'clean' laboratory within the semiconductor industry]

The technique is applied to raw materials, substrates/wafers, and clean-room air. It can also be used to monitor processes, such as chemical vapour deposition.

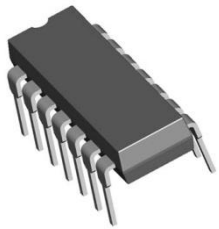

Figure 13.27 – a 'clean' laboratory within the semi-conductor industry

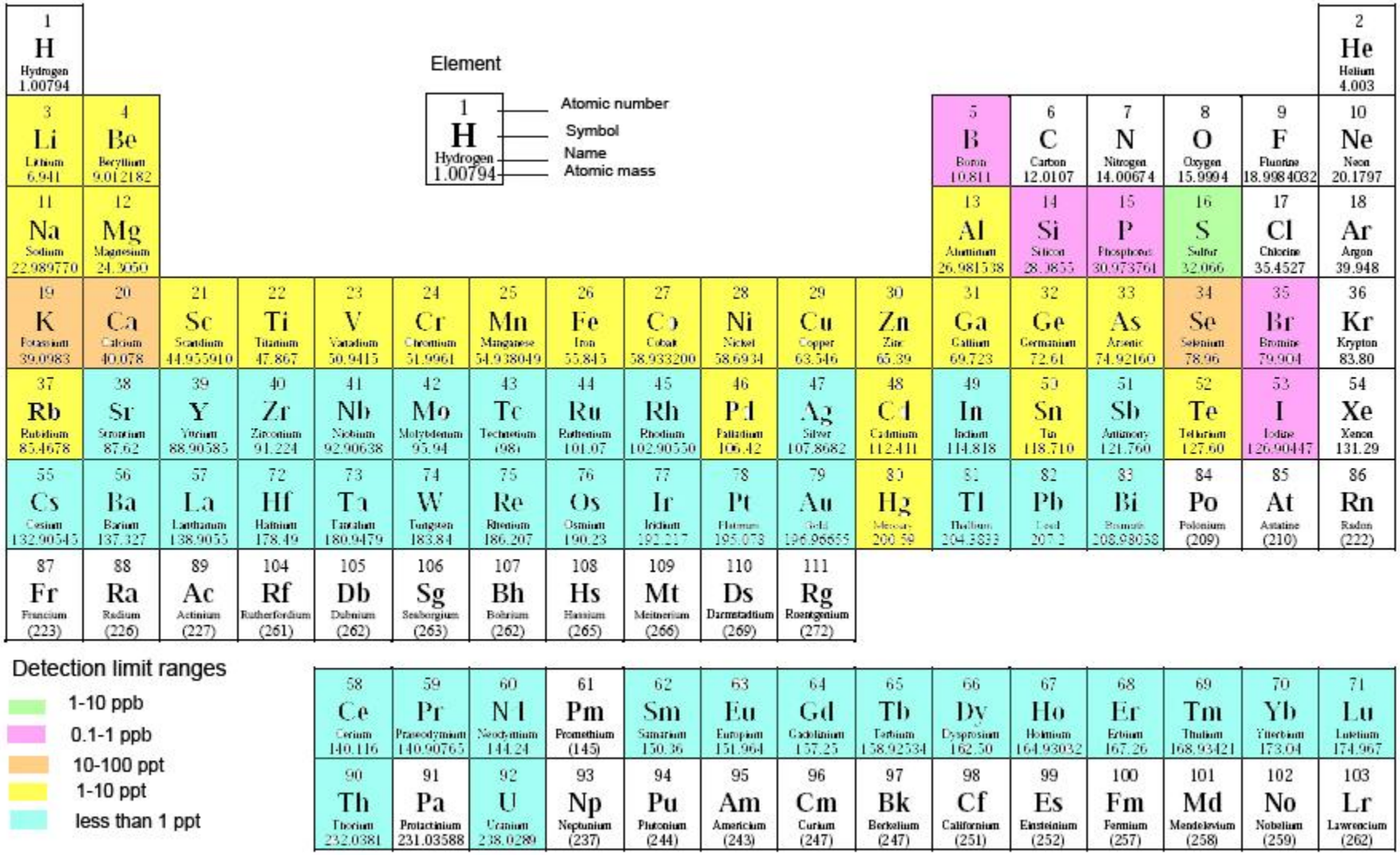

Figure 13.28 – periodic table showing elements analysable by ICP-MS

Figure (13.28) - This table represents the elements that can be analysed by ICP-MS, the detection limits are regularly in the order of ppt (parts per trillion, equivalent to ng/dm^3).

Sampling Techniques for ICP-MS

As sample introduction is one of the major sources of uncertainty in ICP-MS it is worth considering modern sampling strategies at this stage.

Laser ablation is a very powerful sampling technique, the development of which is closely associated with ICP-MS. Solid samples can be analysed directly, applications include geochemical samples, coatings and solid state technology. These techniques can have LOD's in the order of μg/g and very good spatial resolution.

A powerful laser is focussed onto the sample in an argon (or other inert gas such as helium) atmosphere. A tiny portion of the solid is vaporised, and the resulting sample-containing aerosol is swept into the ICP where it is atomised and ionised, and the ions are analysed by the mass spectrometer.

The laser can be focussed on different areas of the solid sample, and thus used for spatial characterisation of heterogeneous solids (mapping) and depth profiling.

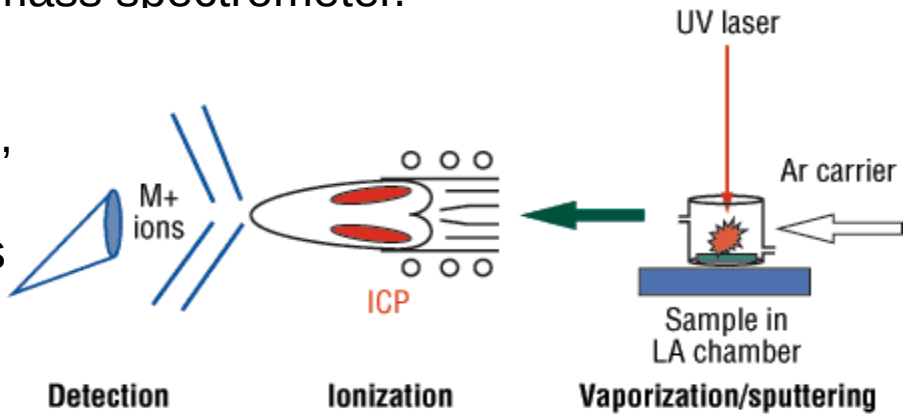

Figure 13.29 - Laser Ablation ICP-MS

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There are challenges when analysing solid samples directly, for example standardisation approaches such as the use of internal standards are more difficult, usually a similar matrix with a known amount of analyte is used as a reference material.

Quantitative analysis is challenging because the laser can cause **elemental fractionation**, which is the variation of measured isotope ratios with time. Due to the ablation process, it is said to be dependent on wavelength and pulse duration. These effects can be overcome by using very short laser pulses, i.e. in the order of femtoseconds, 10^{-15} s, or shorter wavelengths.

The technique although destructive, can be described as minimally invasive because of the small size of the sample crater, sometimes less than100μm in diameter. This makes it a promising technique in conservation and forensic science. Detecting art forgery by looking at the elemental composition of the paint, the substrate material and binders and comparing them to known materials, is one such application.

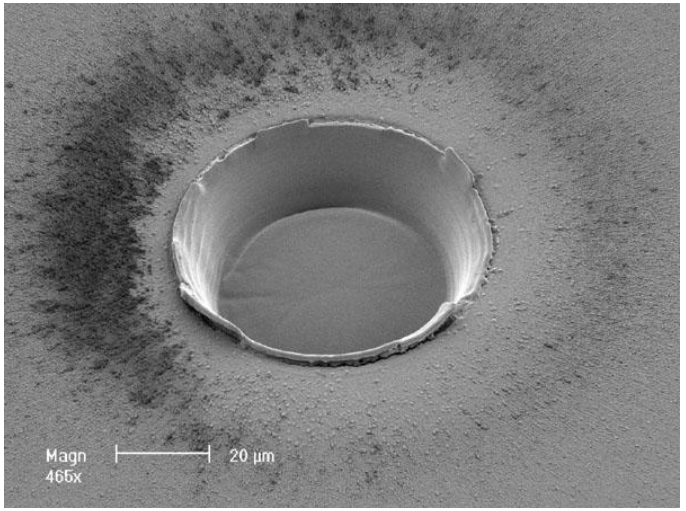


Figure 13.30 a laser ablation crater

Sampling Techniques – ultrasonic nebulization

Organic solvents, if introduced into the ICP-MS, can destabilise the plasma, carbon can also act as an interferant. If the sample has undergone digestion prior to analysis it may contain traces of hydrofluoric acid, or other caustic substances. Ideally the solvent should be removed prior to introduction into the plasma torch (**desolvation**). This can be achieved using the sampling technique, **ultrasonic nebulisation**.

This method, which in addition to removing potentially interfering substances, also pre-concentrates the sample, resulting in improved detection limits

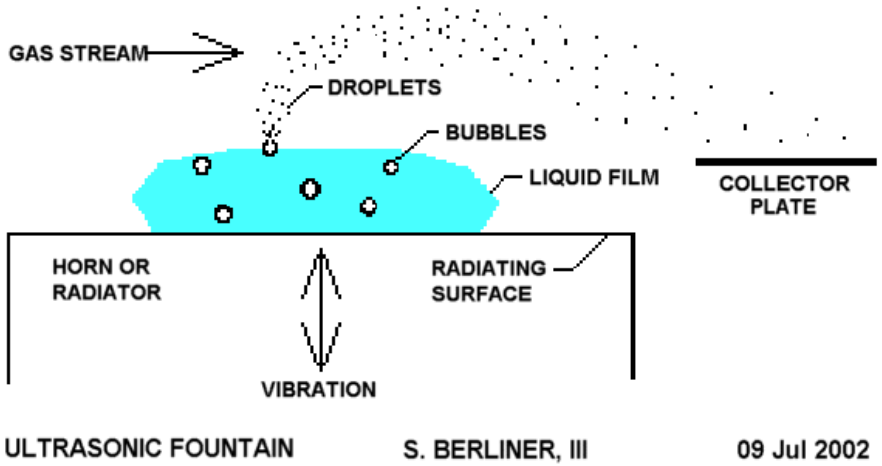
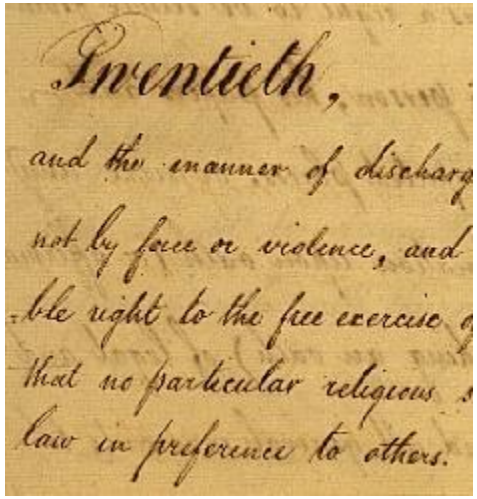


Figure 13.31` – Ultrasonic nebulisation

The nebuliser uses an ultrasonic generator to 'drive' a piezo-electric crystal at a frequency ranging from 200 kHz to 10 MHz. The pressure disrupts the liquid/air interface and the resulting aerosol is swept into the ICP torch by a stream of inert gas, where it is ionised and subsequently analysed by MS.

Case Study ICP-MS

Conservation science requires non-destructive, sensitive trace analysis. In this example, samples of old manuscripts were analysed to determine the composition of the paper and the iron gall inks used. The iron in the ink can accelerate the breakdown of the inks and damage the manuscripts; copper and other elemental impurities have also been implicated and need to be precisely determined to aid the selection of an appropriate conservation treatment.



To calibrate the LA-ICP-MS technique, synthetic reference manuscripts were prepared and the elemental analysis was determined by ICP-MS after an appropriate acid digestion.

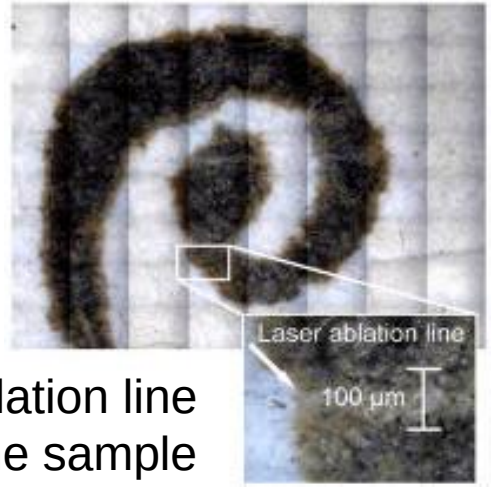


Figure 13.32 – a soft laser ablation line resulting in minimal damage to the sample

Question 13.1 Carbon occurs naturally in two isotopic forms: ^{12}C with an abundance of 98.9% and ^{13}C with an abundance of 1.1%. How will this isotope pattern affect the mass spectrum of the molecular ion of ethane, C_2H_6?

Question 13.2(a) What resolution is required by a mass spectrometer to identify two differing by one mass unit.? The ions are 4500 and 4501 respectively

Question 13.2(b) If an instrument has a resolving power of 5000, and one ion has a value of 50 amu, what ions can it be resolved from?

Question 13.2(c) One of the characteristics of a suitable mass analyser for GC-MS is a high scanning speed. Why is this important?

Question 13.3 Locate the original research paper on the LC-MS analysis of ancient Mayan Cacao usage and use it to interpret the SIM on slide 26. The paper can be found on *www.nature.com.* The authors will include Thomas R Hester

Outline answer to question 13.1

Help with this question may be found on slides 3 - 8

- Assuming that the two isotopes occur in the molecules at random, we shall find molecules where both the carbon atoms are ^{12}C, some where both carbon atoms are ^{13}C, and some where there is one carbon atom of each isotope. So when we look at the molecular ion from ethane, $C_2H_5^+$, in a mass spectrometer we shall see *m/z* values of 29, 30 and 31.

- The probability of finding an ethane molecule containing two ^{12}C atoms (*m/z* = 29) is evidently $(0.989)^2 = 0.978$, while the probability of getting two ^{13}C atoms (*m/z* = 31) is similarly $(0.011)^2 = 0.000121$ (almost negligible, and perhaps quite hard to detect). So the probability of getting one atom of each isotope (*m/z* = 30) is then ca. 0.022. (Note that we have ignored hydrogen isotope effects here, as the abundance of deuterium, ^2H, is so low).

- Isotope effects of this kind are very common, and it would seem that they might make mass spectra harder to interpret. But in practice they can be extremely valuable. For example chlorine occurs as two isotopes, ^{35}Cl and ^{37}Cl, with abundances of ca. 75.8 and 24.2% respectively. As a result, chlorine containing compounds give characteristic and readily observable mass spectral patterns (e.g. with two peaks separated by *m/z* = 2 if only one Cl atom is present) which facilitate the interpretation of the spectra.

Outline answer to question 13.2(a) & (b)

Please refer to slide 13

(a) Answer : *4500/(4500-4501) = 4500*

(b) Answer: *An ion of 50.00 amu can be resolved from an ion of 50.01 amu, or 100 from 100.02 or 1000 from 1001.*

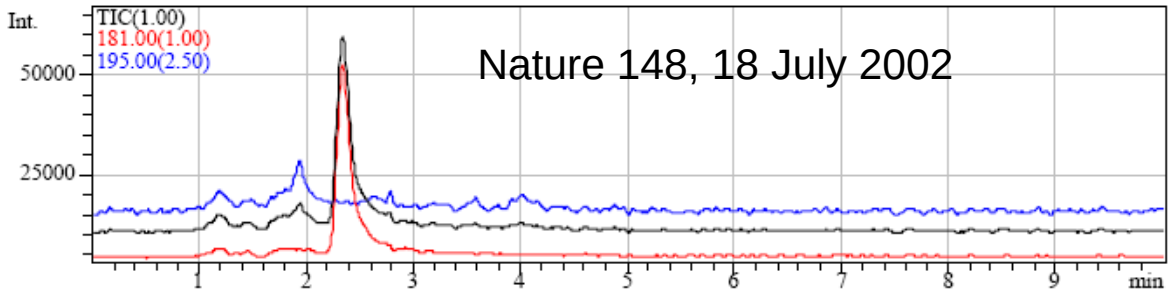
Outline answer to question 13.2(c)

For qualitative analysis you want a good mass spectrum of each component as it elutes. Therefore the more scans you can take of a peak before it elutes the better. Also modern capillary GC instruments have high capacity columns and relatively fast elution which facilitates the separation of complex mixtures, therefore high speed scanning is vital.

For quantitative analysis, the more scans per peak the more sensitive the technique.

Outline answer to question 4c.2

Cacao usage by the earliest Maya civilization: Residues from ceramic vessels were analysed using LC-APCI MS. *Theobroma Cacao* (chocolate) from as early as 600 BC was identified. Chocolate has a complex chemical composition of over 500 compounds, theobromine was used as a marker to confirm the presence of *cacao*.



The LC-MS probe was set to positive ion mode (not covered in this unit) to monitor for theobromine at m/z 181 and caffeine at m/z 195.

The **Total Ion Chromatogram** shows a small peak for Caffeine at 1.9 min, and a larger peak for Theobromine at approx 2.4 min. **Selected Ion Monitoring** set at **m/z 181** for theobromine shows the large theobromine peak but does not detect the caffeine, whereas the **Selected Ion Monitoring** set at **m/z 195**, detects the caffeine and not theobromine. Illustrating how the technique can be 'tuned' to select certain marker ions in complex mixtures. The early peak at approx 1.2 min is due to solvent.