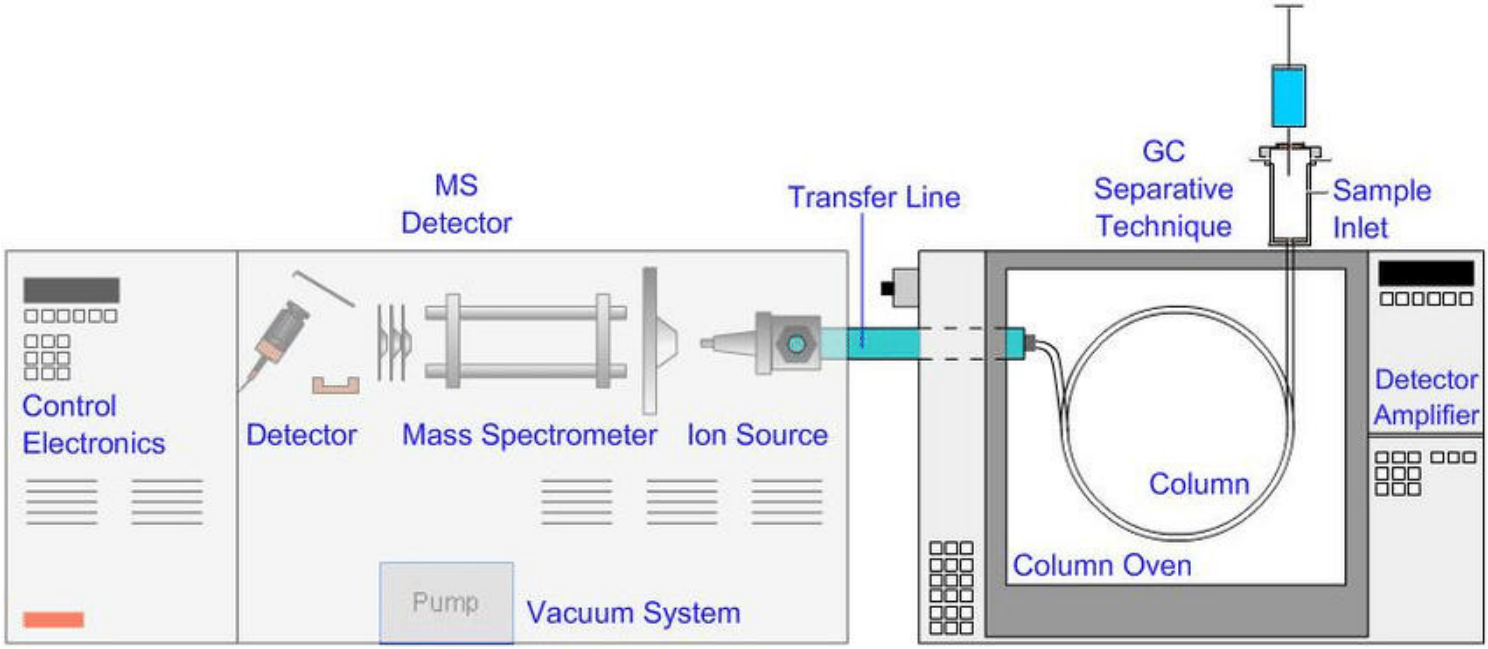
Mass Spectrometry
Fundamental GC-MS
Introduction



Aims and Objectives

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Aims

- Introduce fundamental GC/MS concepts.
- Explain the function of each major component of the GC/MS system.
- Indicate the major advantages of GC/MS and the application areas in which it is used.

Objectives

At the end of this Section you should be able to:

- Describe the function of the various elements that are present in a typical GC/MS system.
- List the most common interfaces for GC-MS making a clear difference between them.
- List the most common mass analysers currently used in the modern analytical laboratory.
- Understand the benefits and limitations of GC/MS analysis.
- Decide on the applicability of GC/MS for a particular analysis and the information likely to results from analysis in one of the two common ionisation modes.

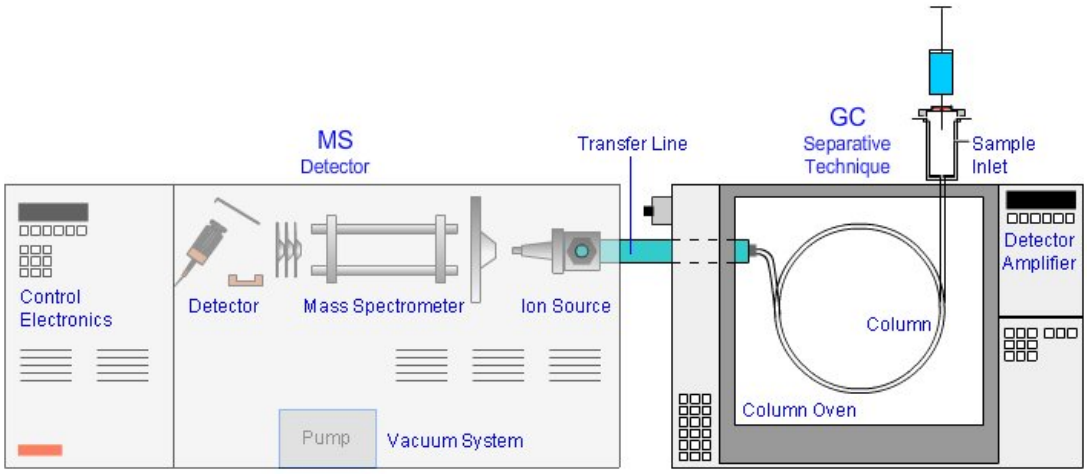
Content

Definitions

GC/MS is a hyphenated technique, which combines the separating power of Gas Chromatography (GC), with the detection power of mass spectrometry. Mass Spectrometry is a wide-ranging analytical technique, which involves the production and subsequent separation and identification of charged species according to their mass to charge (m/z) ratio.

The associated acronym, GC-MS (Gas Chromatography-Mass Spectrometry) covers a broad range of application areas. This module will explore the instrument acquisition methods used, and examine the type of data that can be produced from such systems.

For more information about GC you can refer to "Theory and Instrumentation of GC" from the GC Channel.[1]



GC/MS diagram

Instrument Fundamentals –GC

Gas Chromatography (GC) uses a **carrier gas** to transport sample components through either packed columns or hollow capillary columns containing the stationary phase. In most cases, GC columns have smaller internal diameter and are longer than HPLC columns.

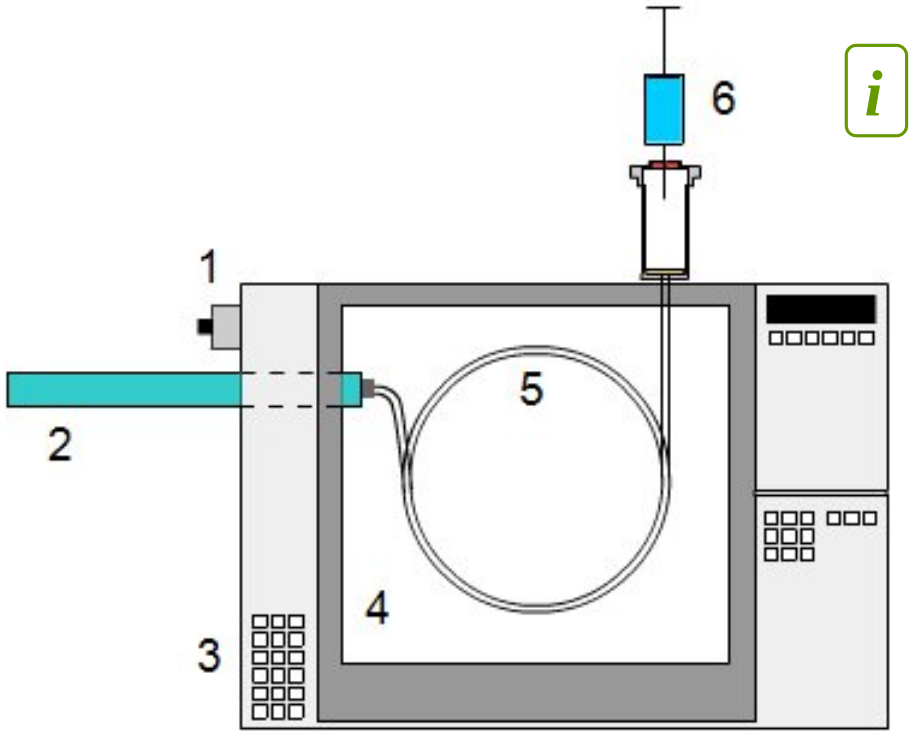
> **Carrier gas:** Alternative term for mobile phase; obviously, the term could only be used as such in gas chromatography.

GC has developed into a sophisticated technique since the pioneering work of Martin and Synge in 1952. GC is capable of separating very complex mixtures of volatile analytes.[2]

In Gas Chromatography, the mobile phase is a gas and the stationary phase is either:

- A solid, commonly termed "Gas solid chromatography (GSC)".
- An immobilised polymeric liquid, referred as "Gas Liquid Chromatography (GLC)".

Of these two types of GC, GLC is by far the most common. The main elements that are currently present in conventional GC systems are presented next.[3, 4, 5, 6]



Where:

1. Gas Supply:[6] Gas is fed from cylinders through regulators and tubing to the instrument. It is usual to filter gases to ensure high gas purity and the gas supply pressure. Required gases might include:

- Carrier – (H_2, He, N_2 – helium is most usual with MS detection)
- Make-up gas– (H_2, He, N_2 - if using an FID detector in parallel to the MS detector)
- Detector fuel gas – (H_2 & Air – if using an FID detector in parallel to the MS detector)

2. Interface: After separation in the GC system, analyte species have to be transported to the mass spectrometer to be ionised, mass filtered and detected. The interface in modern instruments is heated to prevent analyte condensation and in some instruments will contain a device to remove carrier gas molecules to allow analyte pre-concentration.

3. Pneumatic controls: The gas supply is regulated to the correct pressure (or flow) and then fed to the required part of the instrument. Modern GC instruments have Electronic Pneumatic pressure controllers –older instruments may have manual pressure control via regulators.

4. Oven:[5] Gas chromatography have ovens that are temperature programmable, the temperature of the gas chromatographic ovens typically range from 5°C to 400°C but can go as low as -25°C with cryogenic cooling.

5. Column:[4] Gas Chromatography uses a gaseous mobile phase to transport sample components through columns either packed with coated silica particles or hollow capillary columns containing the stationary phase coated onto the inner wall. Capillary GC columns are usually several meters long (10 – 120m is typical) with an internal diameter of 0.10 – 0.50mm, whilst packed GC columns tend be 1 – 5 meters in length with either 2 or 4mm internal diameter.

6. Injector:[3] Here the sample is volatilised and the resulting gas entrained into the carrier stream entering the GC column. Many inlet types exist including:

- **Split/Splitless** – a broadly applicable vapourising inlet
- **Programmed Thermal Vaporising (PTV)** – used to introduce thermally labile samples or for large volume injection for low concentration analytes.
- **Cool-on-column (COC)** - introduces the sample into the column as a liquid to avoid thermal decomposition or improve quantitative accuracy.
- **Headspace injection** – to introduce gas phase analytes volatilized from the sample.
- **Thermal desorption** – used to desorb tubes onto which volatile analytes have been trapped, typically used for environmental monitoring.

Weaknesses of GC:

- Requires the analyte to have significant vapour pressure between about 30 and 300^{o}C.
- Presents a lack of definitive proof of the nature of detected compounds. The identification process is based on retention time matching, which may be inaccurate or at worst misleading.

Instrument Fundamentals –MS

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

mass to charge ratio (m/z):

This represents the mass of a given particle (Da) to the number (z) of electrostatic charges (e) that the particle carries The term m/z is measured in Da/e.

Mass spectrometry involves the separation of charged species which are produced by a variety of ionisation methods. In GC/MS the most common ionisation methods are:[7]

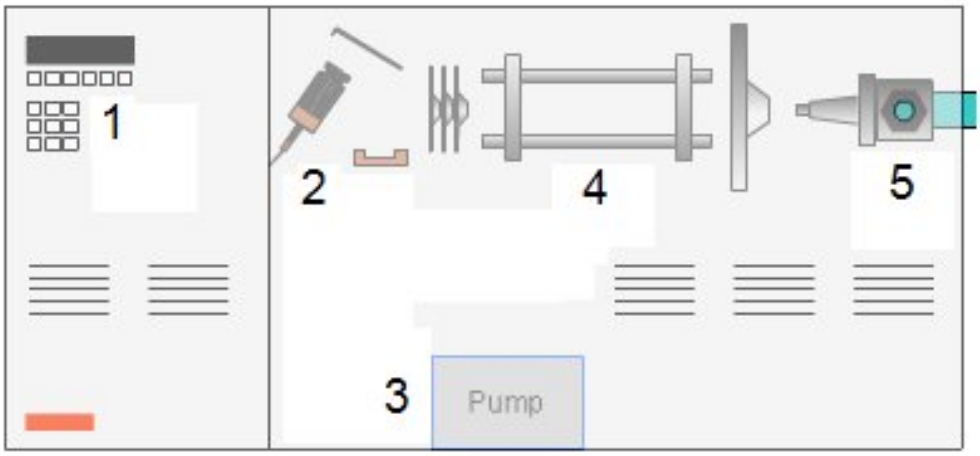
- Electron impact (EI)
- Chemical Ionisation (CI)

These ionisation methods will be explain in detail in a subsequent chapter.

The separation of the phase ions is achieved within the mass spectrometer using electrical and/or magnetic fields to differentiate ions.

Spectrometers are excellent for unambiguously identifying the structure of a single compound but much less when presented with mixtures of them.

In addition to the analyser, the mass spectrometer also includes an ionisation chamber, a vacuum system and a detector.



Where:

1. Control Electronics: The MS parameters can be selected and controlled from this panel. Modern instruments will also allow to control MS parameters from a computer by using specially designed software.

2. Detector: The ion beam that emerges from the mass analyzer, have to be detected and transformed into a usable signal.

The detector is an important element of the mass spectrometer that generates a signal from incident ions by either generating secondary electrons, which are further amplified, or by inducing a current (generated by moving charges).

3. Vacuum System: Mass analysers require high levels of vacuum in order to operate in a predictable and efficient way.

The vacuum systems of most modern LC-MS systems consist of a differentially pumped system, usually with a foreline pump establishing a 'rough' vacuum and a high vacuum pump or pumps situated on the analyser body to establish the high levels of vacuum required for effective mass to charge ratio measurement.

4. Mass Analyser: There are several very popular types of mass analyser associated with routine GC-MS analysis and all differ in the fundamental way in which they separate species on a mass-to-charge basis.

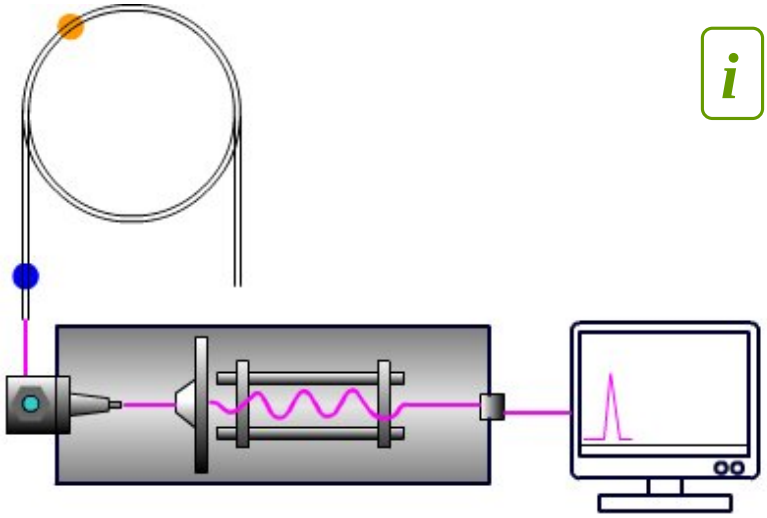
5. Ion Source: In the ion source, the products are ionized prior to analysis in the mass spectrometer.

Ionisation is the process whereby electrons are either removed or added to atoms or molecules to produce ions. In GC-MS charge may also be applied to the molecule via association with other charged molecules.

GC-MS Process

There are several discrete stages in GC/MS analysis, typically these include:[7]

- Sample components separation.
- Ionisation of sample components.
- Separation and detection of gas phase ions.



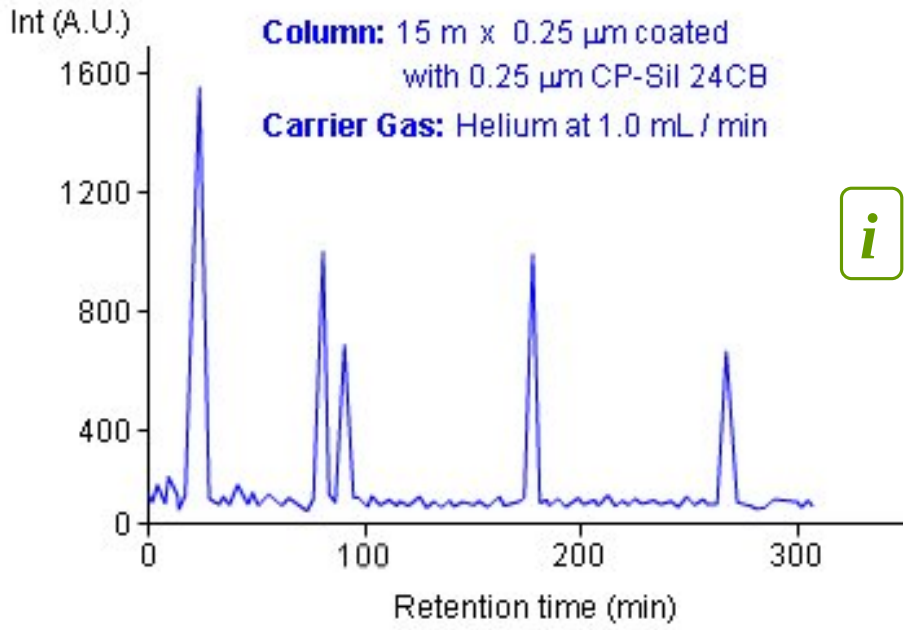
The mass analyser is used to sort ions according to their mass to charge ratio. Most popular analyser types include Quadrupole, Time of Flight, Ion Trap and Magnetic Sector. The mass analyser may be used to isolate ions of specific mass to charge ratio or to 'scan' over all ion m/z values present depending upon the nature of the analysis required.

The detector is used to 'count' the ions emergent from the mass analyser, and may also amplify the signal generated from each ion. Widely used detector types include: electron multiplier, dynode, photodiode and multi-channel plate.

All mass analysis and detection is carried out under high vacuum –established using a combination of foreline (roughing) and turbomolecular pumps.

Why and When to Use GC/MS

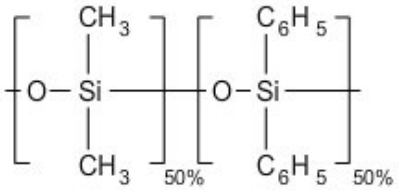
The use of GC-MS in many application areas within analytical science continues to grow almost exponentially. Listed below are some pointers as to the applicability of both GC as a separative technique and MS as a means of detecting analyte species.[4, 8, 9 10]

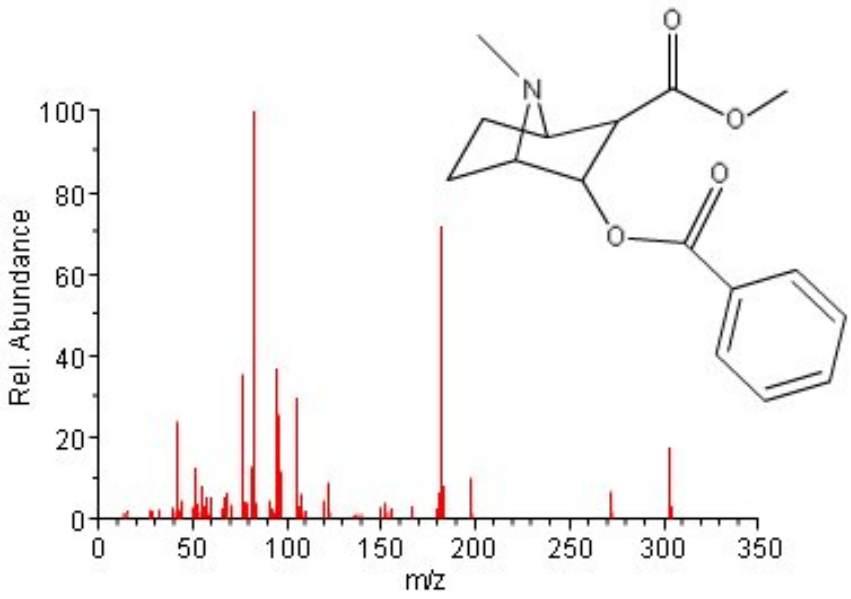


GC-MS analysis of urine, sample know to contain cocaine

CP-Sil 24 CB

This type of phase contains 50% dimethylsiloxane and 50% diphenylsiloxane monomer units, which is ideal for the separation of analytes such as drug molecules and pesticides.





MS spectrum of cocaine

GC Separations

- Produce fast analyses because of the highly efficient nature of the separations achieved
- By using a combination of oven temperature and stationary phase very difficult separations may also be undertaken
- Excellent for quantitative analysis with a range of sensitive and linear detectors to choose from (including the MS detector)
- Limited to the analysis of volatile samples. Some highly polar analytes can be 'derivatised' to impart a degree of volatility, but this is not always possible and quantitative errors may occur.
- A practical upper temperature limit for conventional GC columns is around 350-380^{o}C. In GC analysis, analyte boiling points rarely exceed 400^{o}C and the upper molecular weight is usually around 500 Da

MS Detection

- Allows specific compound identification. Structural elucidation via spectral interpretation combined with elemental composition from accurate mass analysers is possible.
- Allows high sensitivity detection - femto-gram amounts have been detected by certain mass analyzer types.
- Is highly selective, certain analyzer and experiment combinations can lead to highly selective and sensitive analysis of a wide range of analyte types.

The Coupling of GC/MS

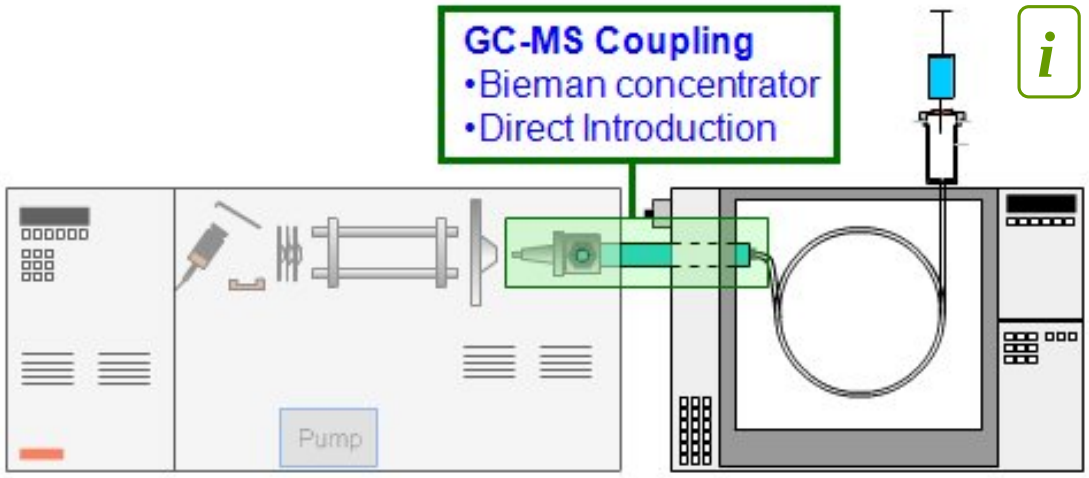
In comparison to liquid chromatography interfaced to mass spectrometry (LC-MS), GC-MS has the advantages of higher chromatographic resolution and higher peak capacity, a single mobile phase (helium), fewer issues with solubility and separations that can be adjusted by electronic controls such as temperature and flow programming.

The major hurdle to interfacing GC to MS is the volume of gas that has to be vented between the GC and MS stage in order not to compromise the vacuum under which the analysing device must operate. In the initial development of GC-MS only packed columns were commercially available, and thus the major problem was the elimination of very large amounts of carrier gas, this problem was solved by the use of vapour concentrator devices.

The simplest way to reduce eluent gas flow rate into the mass spectrometer is the use of carrier gas splitting (no sample enrichment occurs using this technique). This technique may be used when packed GC columns are used and where analyte concentrations are high.

Alternative momentum jet separating devices are also available where the carrier gas is vented in preference to the analyte where analyte enrichment will occur.

Today with the advent of capillary columns using relatively low carrier gas flow rates, the need for vapour concentrator devices has been eliminated. The mass spectrometer pumping system can easily deal with gas flows for direct injection into the mass spectrometer.[11,12]



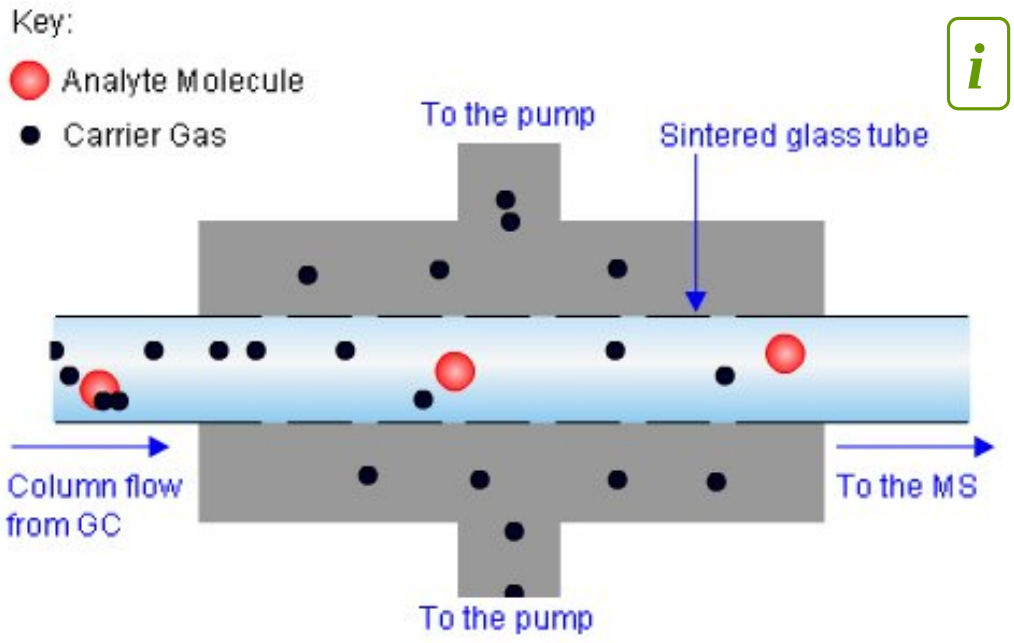
Vacuum System Considerations

The entire MS process must be carried out at very low pressures (~10^{-8} atm) and in order to meet this requirement a vacuum system is required.

It is difficult for packed GC columns to be interfaced to an MS detector because they have carrier gas flow rates that cannot be as successfully pumped away by normal vacuum pumps; however, capillary columns' carrier flow is 25 or 30 times less and therefore easier to "pump down." That said, GC/MS interfaces have been developed for packed column systems that allow for analyte molecules to be dynamically extracted from the carrier gas stream at the end of a packed column –See the Bieman concentrator below.

The Bieman Concentrator

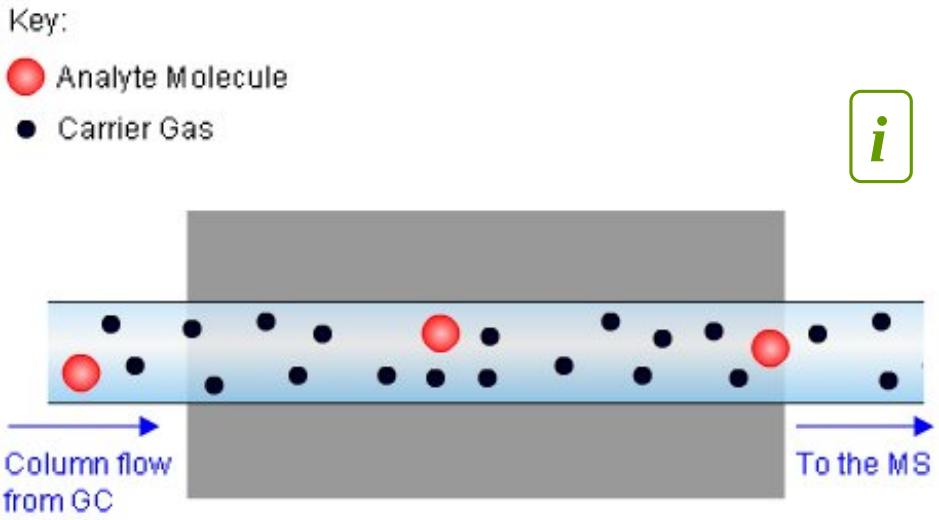
The Bieman concentrator device is used with packed columns or with wide bore capillary columns at higher flow rates. In this device the carrier gas is removed in preference to the analyte and hence analyte enrichment occurs.



Bieman Concentrator

Direct Introduction

Direct introduction is typically used with capillary GC columns and most modern instruments can easily cope with flow rates up to 2 mL/min.



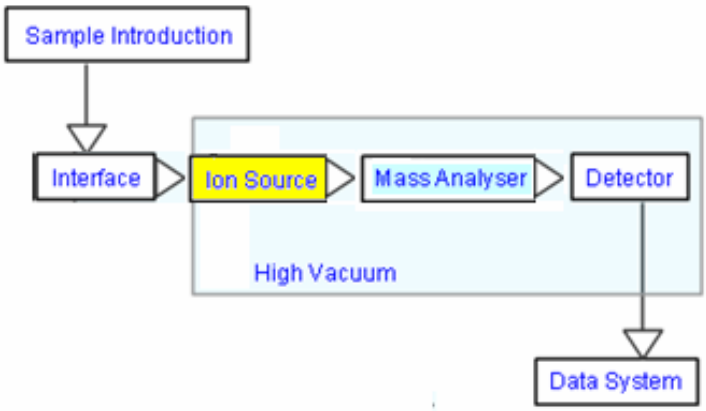
Direct Introduction

Ionisation

Overview

Ionisation is the process whereby electrons are either removed or added to atoms or molecules to produce ions. In GC-MS charge may also be applied to the molecule via association with other charged molecules.[11]

Such ions are produced in GC/MS systems by the use of strong electric fields in the vapour phase.



Mass spectrometry involves the separation of charged species which are produced by a variety of ionisation methods in GC-MS. These include:

- Electron impact Ionisation (EI) - where analyte molecules are directly ionised through collision with a bombarding electron stream resulting in the removal of an electron to form a radical cation species.
- Chemical Ionisation (CI) - where analyte molecules are charged through reaction processes with a charged reagent gas plasma producing either anion or cation species depending upon the analyte and analyser polarity.

Some representative reactions for electron impact and chemical ionisation are presented next.

In electron impact molecules are ionised by the interaction with electrons.

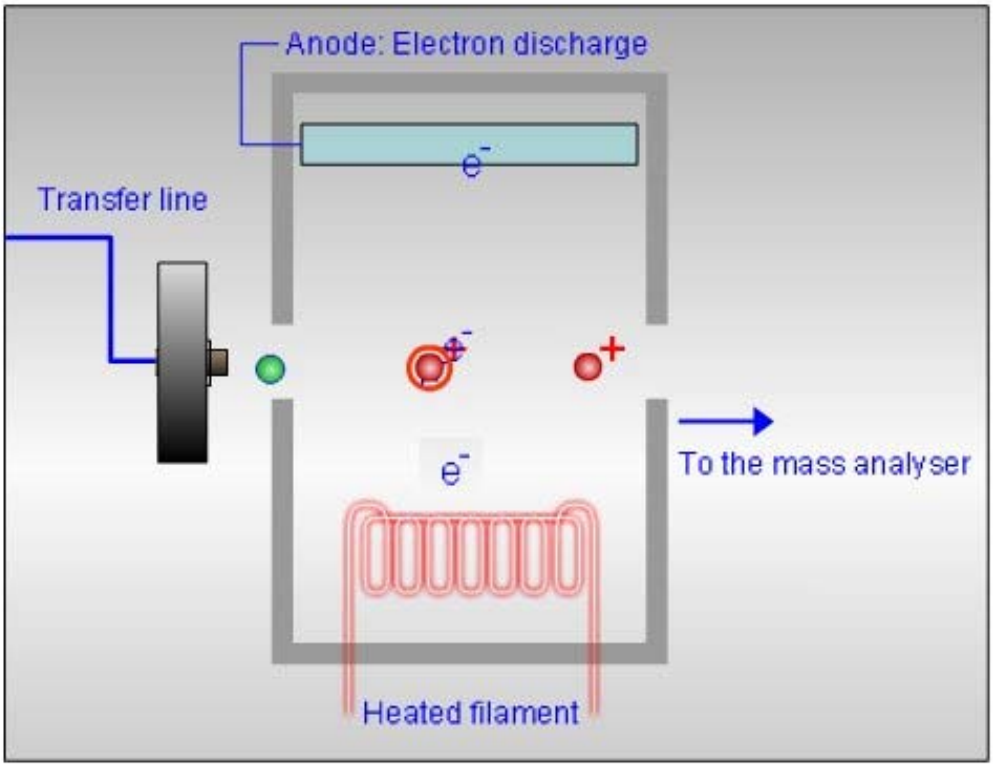
$$M_{(g)} + e^- \rightarrow M^+_{(g)} + 2e^-$$

In a chemical ionisation experiment experiment, ions are produced through the collision of the analyte with ions of a reagent gas that are present in the ion source. Some common reagent gases include: methane, ammonia, and isobutane. Let's consider ammonia chemical ionisation:

1. $n\ NH_3 + e^- \rightarrow NH_4^+ + 2e^-$
2. $NH_4^+ + M \rightarrow NH_3 + [M + H]^+$
3. $NH_4^+ + M \rightarrow [M + NH_4]^+$

Electron Impact (EI)

In the Electron Impact (EI) process, electrons are emitted from a heated filament (usually made of tungsten or rhenium) and are accelerated across the source by using an appropriate potential (5-100V) to achieve the required electron energy (sufficient to ionize the molecule).[7]



Electron Impact

The analyte is introduced into the mass spectrometer ion source, where it is impacted by this beam of ionizing electrons, leading to the formation of an analyte radical cation. The process can be described as follows:

$$M_{(g)} + e^- \rightarrow M^+_{(g)} + 2e^-$$

The process is a relatively harsh form of ionization and as a consequence, the parent molecule often breaks apart producing a variety of fragments with a relatively small amount of the parent ion remaining. In some circumstances, if the molecule is sufficiently

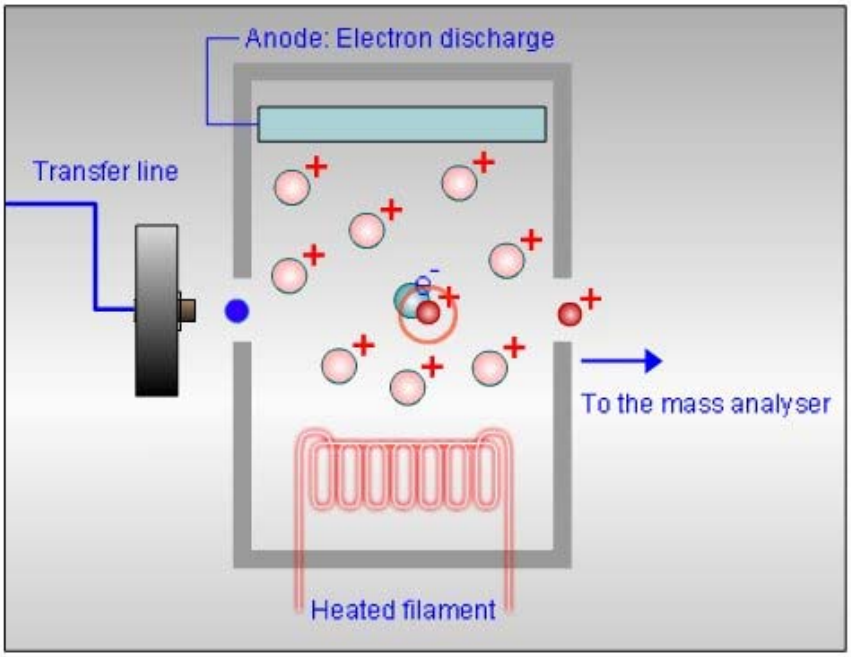
labile, no parent ion may be seen within the resulting spectrum. The degree of fragmentation depends upon the magnitude of the 1st ionisation potential of the analyte molecule and the energy of the impacting electrons.

Chemical Ionisation (CI)

Chemical ionisation involves the ionisation of a reagent gas, such as methane at relatively high pressure (~1 mbar) in a simple electron impact source.[7,13] Once produced, the reagent gas ions collide with the analyte molecules producing ions through gas phase reaction processes such as proton transfer.

1. $NH_4 + e^- \rightarrow NH_4^+ + 2e^-$
2. $NH_4^+ + M \rightarrow NH_3 + [M + H]^+$
3. $NH_4^+ + M \rightarrow [M + NH_4]^+$

Chemical ionisation is a soft process, because the energy of the reagent ions in general never exceeds 5eV, and as a consequence the spectrum produced by this technique usually shows little fragmentation.



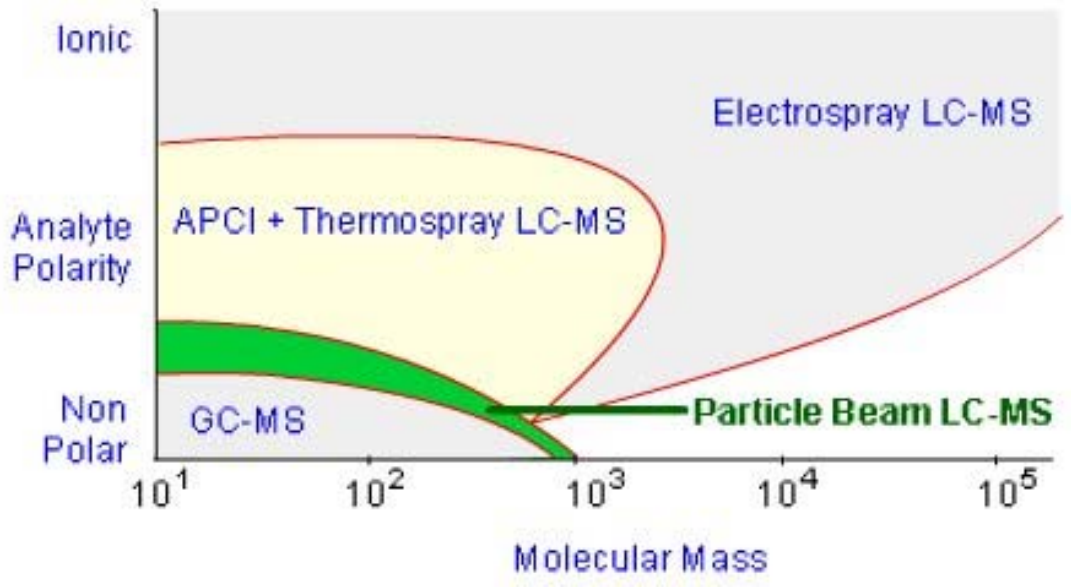
Chemical Ionisation

Under chemical ionisation conditions, the registered spectra will strongly depend upon the nature of the reagent used, and because of that different structural information can be obtained by choosing different reagent gases.

Commonly-used reagent gases are: methane, iso-butane, ammonia or combinations of these gases.

Suitable samples for GC/MS

Electron impact is the most commonly used method of ionization, and a great number of organic compounds are amenable to EI. To give an EI spectrum, the sample must be volatile enough to undergo GC analysis and may be solid, liquid, or gas. Since samples must usually be heated, thermally labile samples may be unsuitable or may require derivatistation. Unfortunately, some compounds will fragment completely and won't give molecular ions, however this is does not preclude these analyte types. Ionic samples generally do not work well by EI.[7]



Common ionisation techniques range of application.[14]

For compounds that do not work by EI, alternate methods of ionization have been developed, and among them chemical ionisation is the most widely used.

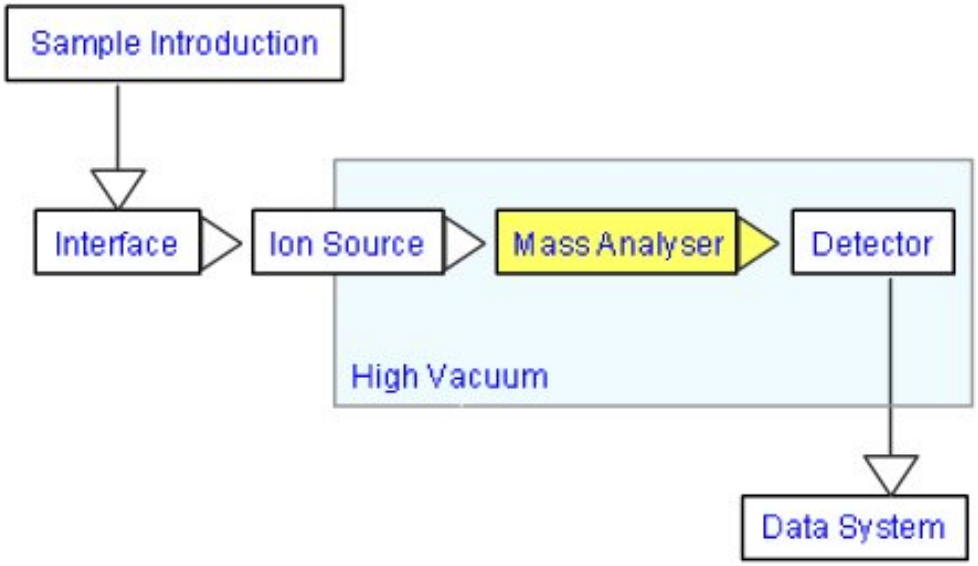
CI can produce molecular ions for some volatile compounds that do not give molecular ions in EI. CI is also particularly useful for highly sensitive quantitative analysis of halogenated compounds.

Mass Analysers

Overview

In its simplest form the process of mass analysis in GC-MS involves the separation or filtration of analyte ions or fragments of analyte ions created in the ion source.

There are several very popular types of mass analyser associated with routine gas chromatography mass-spectrometric analysis and all differ in the fundamental way in which they separate species on a mass-to-charge basis:[15]



Quadrupole and Ion Trap Mass analysers: ions are filtered using electrostatic potentials applied to the elements of the mass analysers which are used to 'select' ions according to their mass to charge ratio –non-selected ions are ejected from the mass analysing device and are not detected.

Time of Flight (TOF) mass analysers: use differences in flight times of accelerated ions through an extended flight path to separate ions.

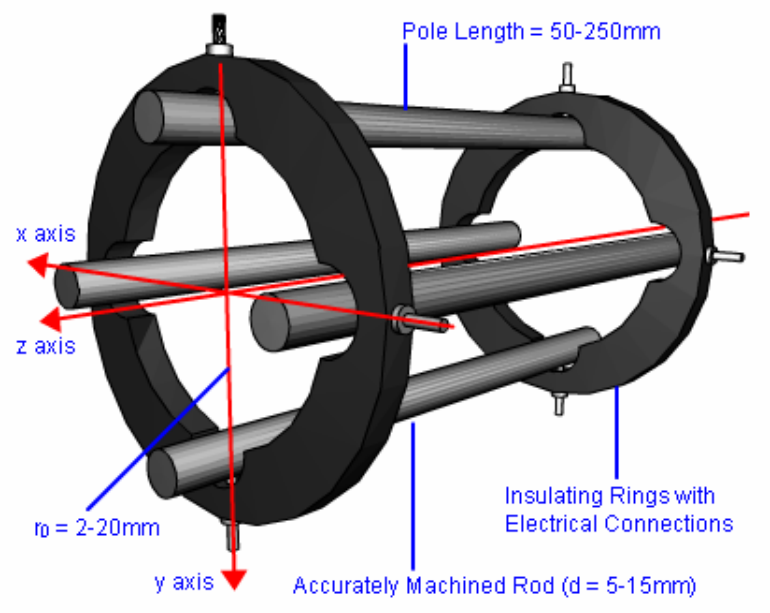
Magnetic Sector Mass Analysers: use magnetic fields to select ions by directing the beam of ions of interest towards the detector.

The analyte and fragment ions are plotted in terms of their mass-to-charge ratio (m/z) against the abundance of each mass to yield a mass spectrum of the analyte as shown.

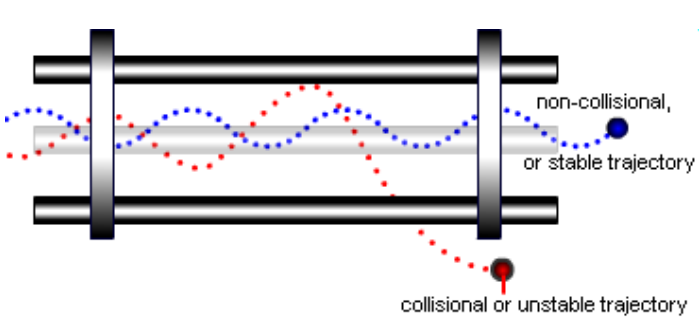
Quadrupole

In quadrople mass analysing devices, electric fields are used to separate ions according to their mass-to-charge ratio (m/z) as they pass along the central axis of four parallel equidistant rods (or poles). Ion separation is performed by using controlled voltages applied to the mass analyser rods which impart an electrostatic field inside the analysing device.[16]

As long as x and y, which determine the position of an ion from the centre of the rods, remains less than r_0, the ion will be able to pass through the quadrupole without touching the rods. This is known as a non-collisional or stable trajectory.



Where the ion is caused to oscillate with a trajectory whose amplitude exceeds r_0 it will collide with a rod, and become discharged and subsequently pumped to waste. This is known as an unstable or collisional trajectory.



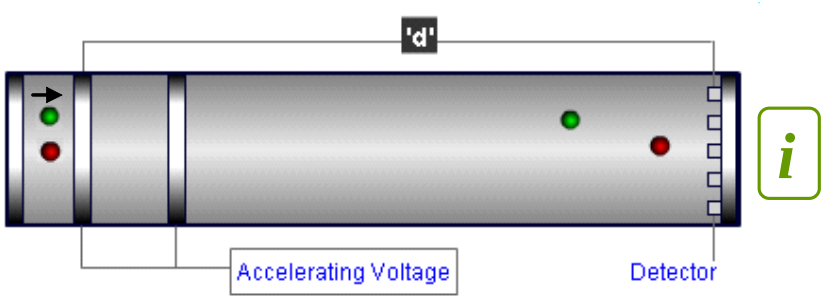
Quadrupole mass analyser

Advantages	Disadvantages
• Reproducibility • Low cost	• Low resolution • Mass discrimination. Peak height vs. mass response must be 'tuned'

Time-of-flight (TOF)

The basic principles of mass analysis using time-of-flight mass analysers are relatively straightforward in comparison to many of the other typical mass analysing devices.[17]

Ions are extracted (or produced) in short bursts or packets within the ion source and subjected to an accelerating voltage. The ions then 'drift' or 'fly' down an evacuated tube of a set length ('d'). Once free from the region of accelerating voltage the speed at which the ions travel down the tube is dependant upon their mass (m) and charge (z). This mass analyser is useful as all ions are detected (almost) simultaneously. Scanning the mass range of all ions is very rapid and as such the inherent sensitivity of the instrument is increased.



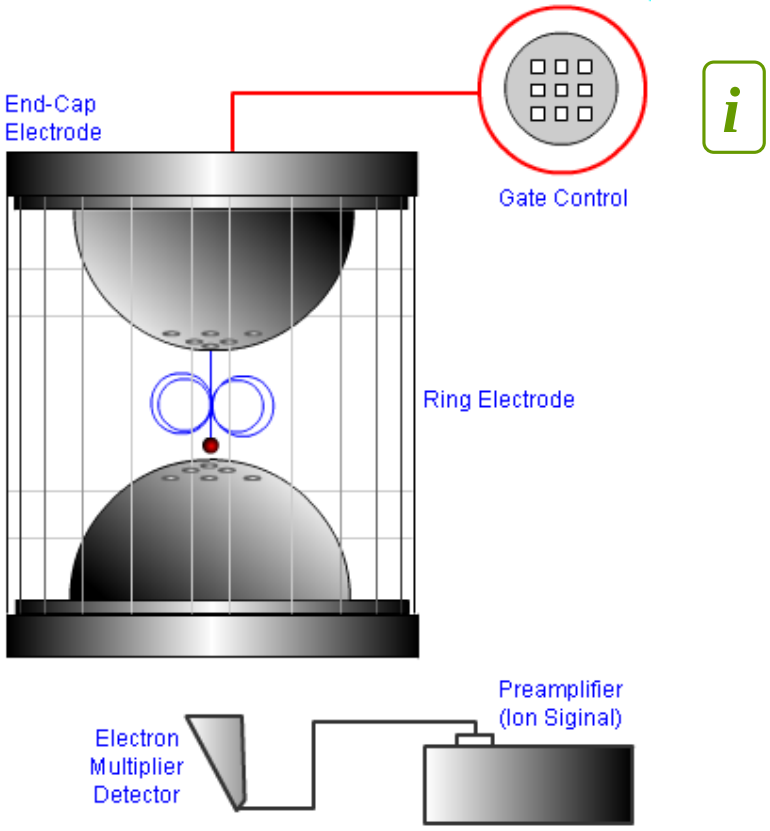
Time-of-flight (TOF)

Advantages	Disadvantages
• High ion transmission • Highest practical mass range of all MS analyzers • Detection limit	• Fast digitizers used in TOF can have limited dynamic range

Ion Trap Mass Analyser

Ion trap mass spectrometers work on the basis of storing ions in a "trap", and manipulating the ions by using applied DC and RF fields. The amplitude of the applied voltages enables the analyser to trap ions of specified mass to charge ratio within the analysing device. Non-selected ions are given a trajectory by the electrostatic field that causes them to exit the trap. By filling the trap with an inert gas fragmentation of selected ions is possible. This is useful when structural information is required.[18]

The system has some unique capabilities, including being able to perform, multiple product ion scans with very good sensitivity (MS^n). It should be noted that the spectra acquired with an ion trap mass analyser may be significantly different to those acquired from a triple quadrupole system due to the different collision regimes within the systems (collision energy/gas).



Ion trap mass analyser

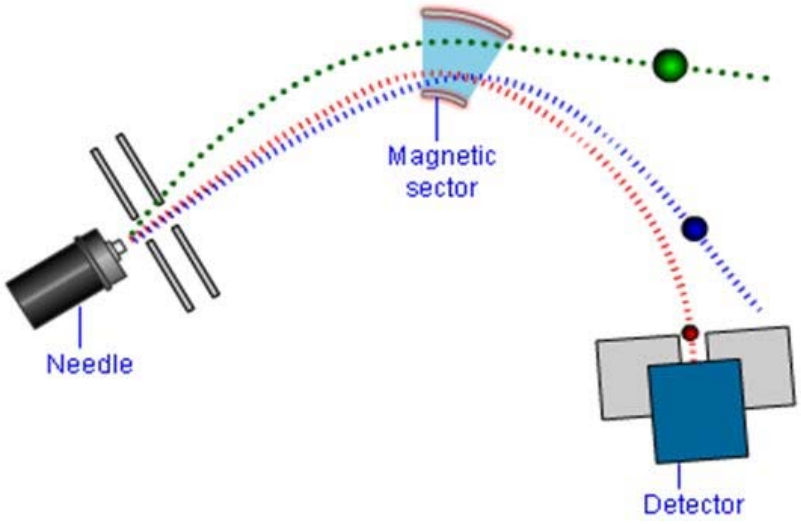
Ion trap mass analyser

Advantages	Disadvantages
• High sensitivity • Multiple Product Ion scan capability $(MS)^n$ • High resolution • Good for DDA analyses	• Produces very unusual spectra if the ions are stored in the trap too long. • Easily saturated • Poor for low mass work (below 100 Da) • Poor dynamic range (except the most modern devices) and hence may have limited quantitative use

Magnetic sector

Magnet / electric sector instruments are employed for mass analysis using the principle that charged species can be deflected in magnetic and electric fields. The degree of ion deflection in a magnetic field is proportional to the square root of their m/z ratio and the potential through which they are accelerated prior to mass analysis, making the measurements of mass-to-charge ratio very accurate when using this type of mass analyser.[18,19]

Electric fields are used in conjunction with magnetic fields to focus a fast moving beam of ions created in the source according to the kinetic energy of each ion, allowing each m/z value to be sharply focussed prior to deflection in the magnetic field. This focussing action helps to improve the resolution of the magnetic sector mass analyser so that measurements can be made between ions whose mass to charge ratio differs by only a few parts per million.



Magnetic sector

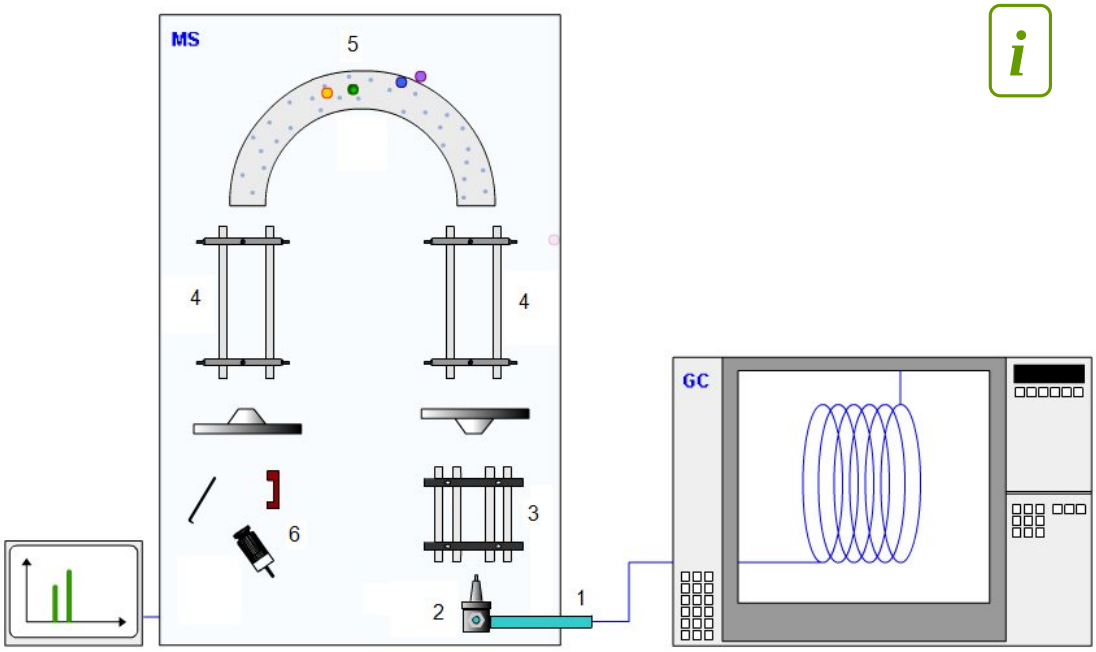
Tandem Mass Spectrometry (MS/MS)

MS/MS is the combination of two or more MS experiments. The aim is either to get structural information by fragmenting the ions isolated during the first experiment, and/or to achieve better selectivity and sensitivity for quantitative analysis by selecting representative ion transitions using both the first and second analysers.[20]

Product Ion MS/MS analysis can be achieved either by coupling multiple analysers (of the same or different kind) or, with an ion trap and carrying out successive fragmentations of trapped ions.

MS^n (should read MS to the n) is an acronym that refers to multiple ion production and filtering within a single instrument. Most common instruments use a combination of quadrupoles (as shown below) with a collision cell (usually a multi-pole device) between the analyzing devices in which the emergent ions from the first analyzer are fragmented

prior to secondary mass filtering. Other combinations of mass analysing devices such as quadrupoles and time of flight, or quadrupoles with magnetic sector instruments are possible.



GC-MS/MS

Where:

1. Transfer Line: The column's effluent is directed to the ion source

2. Ion Source: In the ion source, the products are ionized prior to analysis in the mass spectrometer.

3. Octapole: The ion beam that emerges from the source is focused prior to the first mass analyser.

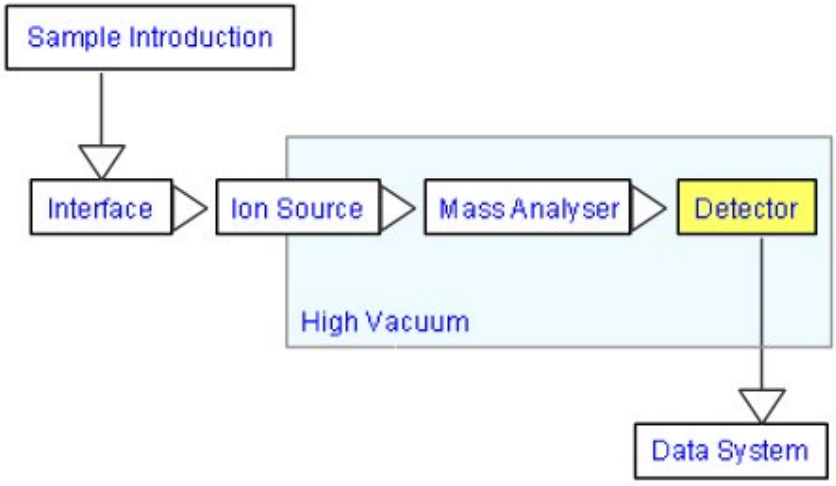
4. Quadrupole: In quadrople mass analysing devices, electric fields are used to separate ions according to their mass-to-charge ratio (m/z) as they pass along the central axis of four parallel equidistant rods (or poles).

5. Collision Cell: The function of a collision cell is to modify ions by either colliding into fragments, or to react them with other molecules.

6. Detector: The ion beam that emerges from the mass analyzer, have to be detected and transformed into a usable signal.

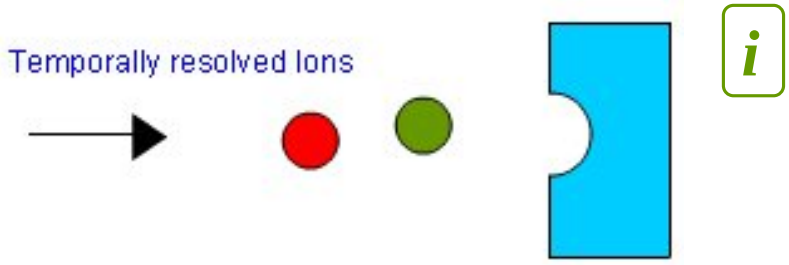
Detectors

Once the ions have passed the mass analyser they have to be detected and transformed into a usable signal.[21] The detector is an important element of the mass spectrometer that generates a signal from incident ions by either generating secondary electrons, which are further amplified, or by inducing a current (generated by moving charges).

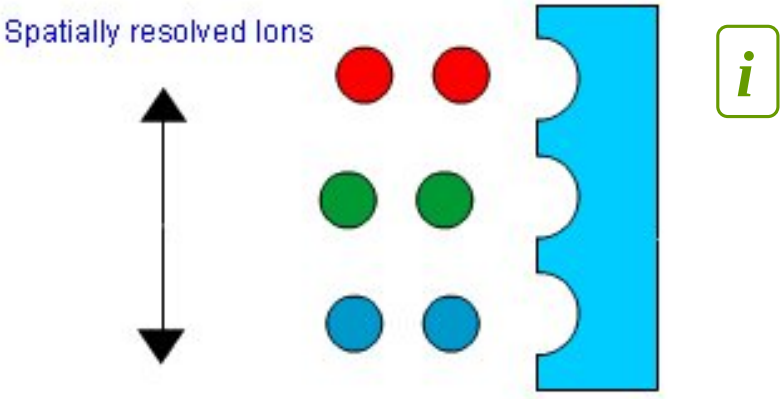


Ion detector systems fall into two main classes:

Point detectors: ions are not spatially resolved and sequentially impinge upon a detector situated at a single point within the spectrometer geometry.



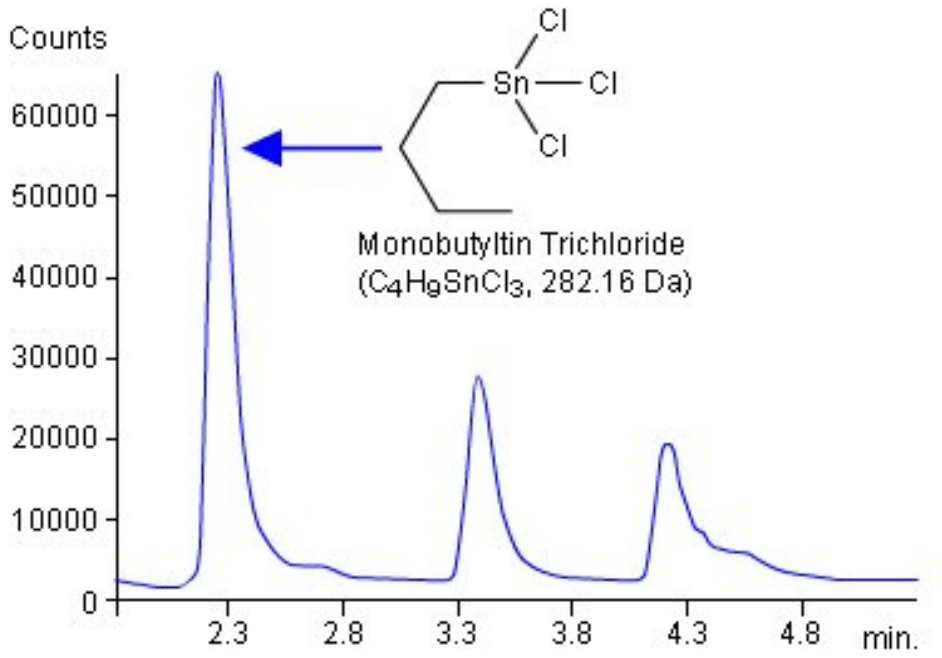
Array detectors: ions are spatially resolved and all ions arrive simultaneously (or near simultaneously) and are recorded along a plane using a bank of detectors.



Applications

To give a full list with the applications of GC/MS is simply impossible, its flexibility makes it applicable across many application areas. Examples of some interesting applications are listed below:

Inorganic Industrial Analysis:[22] Inorganic compounds with low molecular weights.

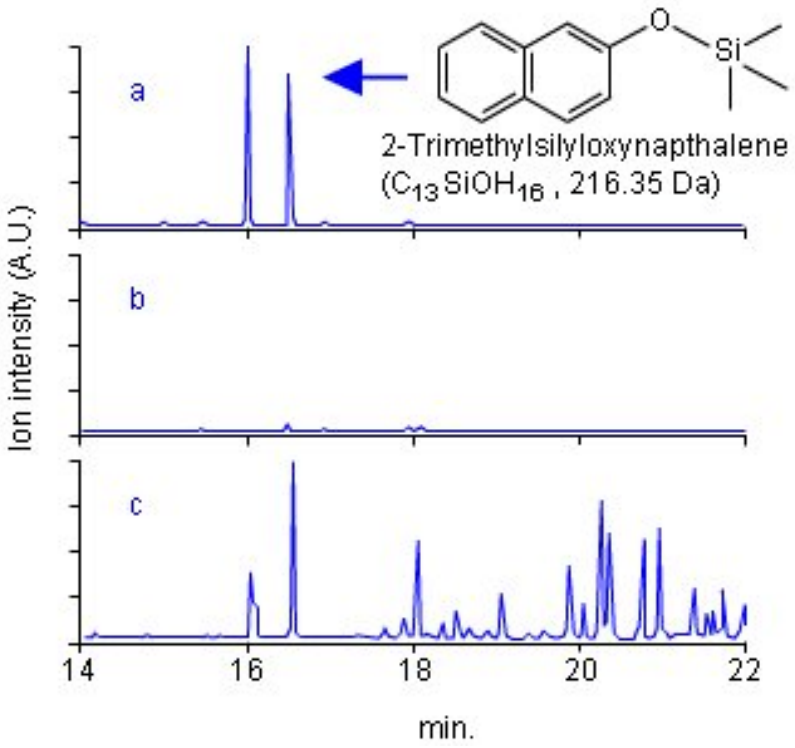


GC-MS analysis of monobutyltin trichloride (monobutyltin trichloride is currently employed in the glass industry. It forms thin film of tin-silicon bonds.)

Hewlett Packard gas chromatograph Model 6890 (quadrupole type) equipped with split/splitless injection. An HP-5 column (5% phenyl, 95% polydimethylsiloxane, 30 m length×0.25 mm id and 0.25 µm)

Environmental Sciences:[23] Analysis of a wide range of contaminants of low molecular weight.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants.

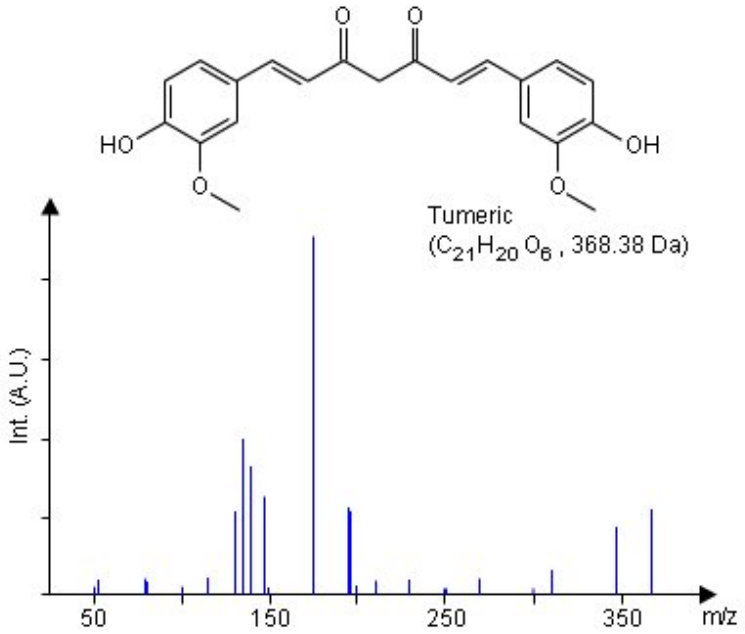


GC-MS spectra

a. Spectra from pure compound (<99%)
b. Bile sampled from plaice (Pleuronectes platessa)
c. Bile sampled from plaice (Pleuronectes platessa) exposed to crude oil

GC-MS system consisting of a HP5890 series II Gas chromatograph (triple quadrupole), Finnigan A200S autosampler and a Finnigan MAT SSQ7000 mass spectrometer (Thermo Finnigan, Huddinge, Sweden). Helium was used as carrier gas and the applied column was CP-Sil 8 CB-MS, 50 m × 0.25 mm and 0.25 µm.

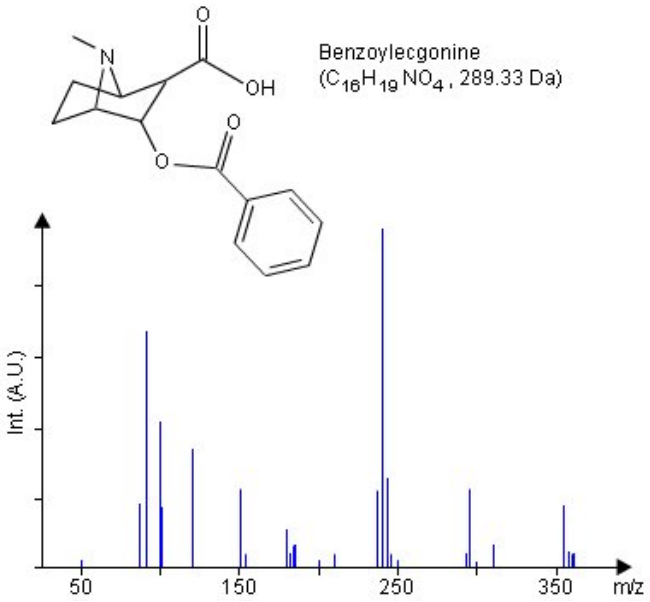
Food Analysis:[24] Analysis of molecules of importance in the food industry.



Electron impact chromatogram of turmeric (oversimplified)

GC was performed on a Varian gas chromatograph (quadrupole ion trap mass spectrometer), model cx-3400 using a capillary column Supelcowax-10, 30 m × 0.3 mm.

Pharmacochemistry:[8,9] Mainly focused in the analysis of molecules presenting drug activity.

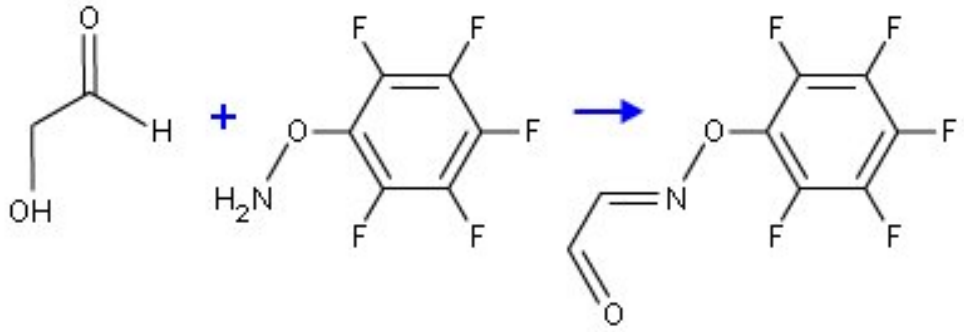


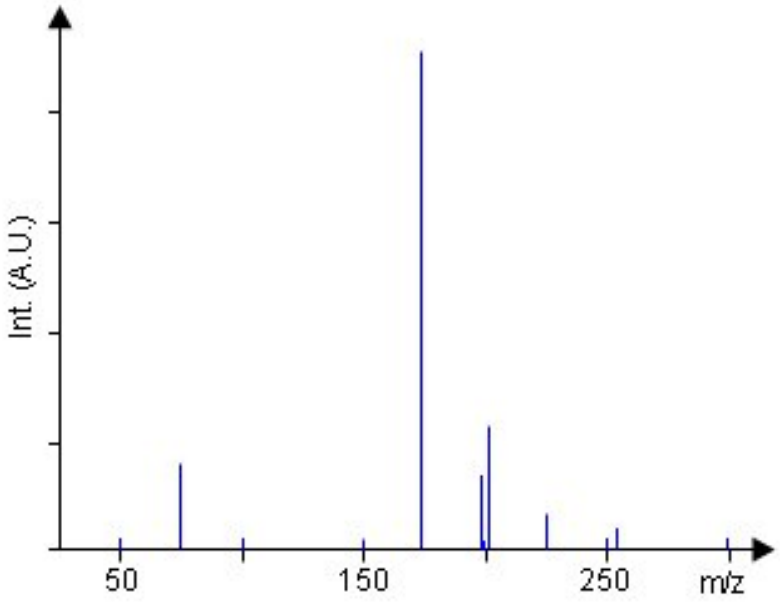
Benzoylecgonine (an alkaloid) GC-MS spectrum.

Column: 15 m x 0.25 mm coated with 0.25 µm CP-Sil 24
Carrier Gas: Helium at 1.0 mL/min
Mass spectrometer: Saturn Ion-trap GC/MS system

Bioanalysis:[25] which involves the quantification and identification of metabolites in biological fluids.

Glycolaldehyde is a remarkable molecule that is converted to acetyl coenzyme A. Glycolaldehyde can be derivatised with PFB hydroxylamine and then analysed with GC/MS:



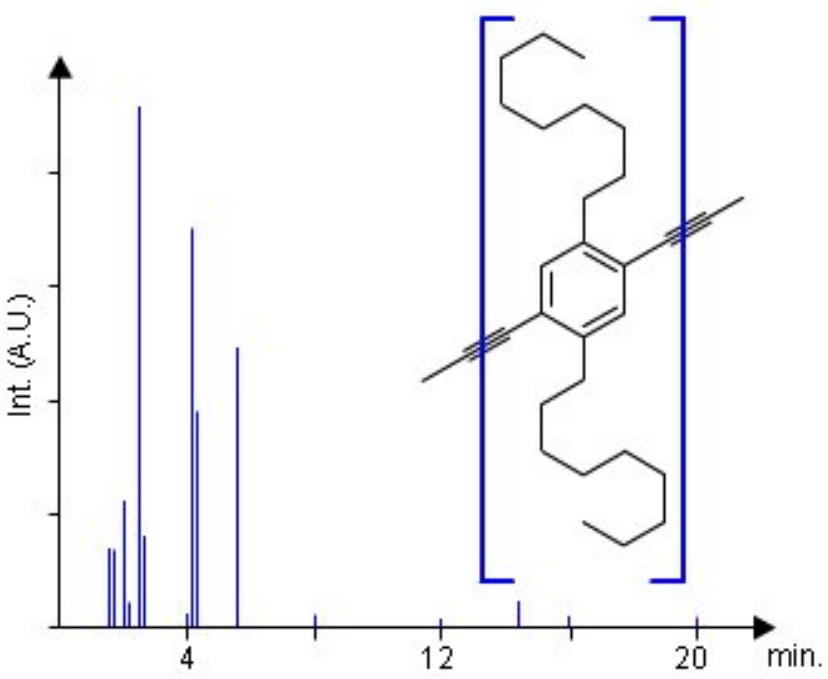


Derivatized glycolaldehyde was analyzed on a HP-1 capillary column (Hewlett-Packard Co., Palo Alto, CA; 12 m × 0.2-mm and 0.33 mm thickness)

The MS analysis was done in a Hewlett-Packard 5988A mass spectrometer in the negative-ion chemical ionization (NCI) mode with methane as the reagent gas.

Polymers:[26] The use of analytical pyrolysis with gas chromatography mass spectrometry (GC/MS) to study the structure of polymeric material must be based on an understanding of how these large molecules behave at elevated temperatures.

Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) has become an important technique for the analysis of synthetic macromolecules. Because the high molecular weight of polymers limits their volatility, polymers are commonly analyzed by chemical degradation to smaller fragments, followed by chemical derivatization to produce volatile components amenable to GC/MS.



Column: 5% phenyl–95% methyl polysiloxane coated capillary column 30 m × 0.25 mm ID × 0.25 µm (HP5-MS, Hewlett Packard).

The MS analysis was done in a Hewlett-Packard 5972 GCMSD (quadrupole type) mass spectrometer by using electron impact.

References

1. “Theory and Instrumentation of GC” from the GC Channel.
2. A. J. P. Martin and R. L. M. Synge. “A new form of chromatogram employing two liquid phases” Biochemical Journal. Volume 35, part 12 December 1941. 1358–1368.
3. “Sampling Techniques” and “Sample Introduction” from “Theory and Instrumentation of GC” -GC Channel.
4. “GC Columns” from the ‘Theory and Instrumentation of GC’. “Theory and Instrumentation of GC” -GC Channel.
5. “GC Temperature Programming” from the ‘Theory and Instrumentation of GC’. “Theory and Instrumentation of GC” -GC Channel.
6. “Gas Supply and Pressure Control” from the ‘Theory and Instrumentation of GC’. “Theory and Instrumentation of GC” -GC Channel.

Content

Introduction

Gas Chromatography uses a gaseous mobile phase (carrier gas) to transport sample components through either packed or hollow capillary columns containing the stationary phase. In most cases, GC columns have smaller internal diameter and are longer than HPLC columns

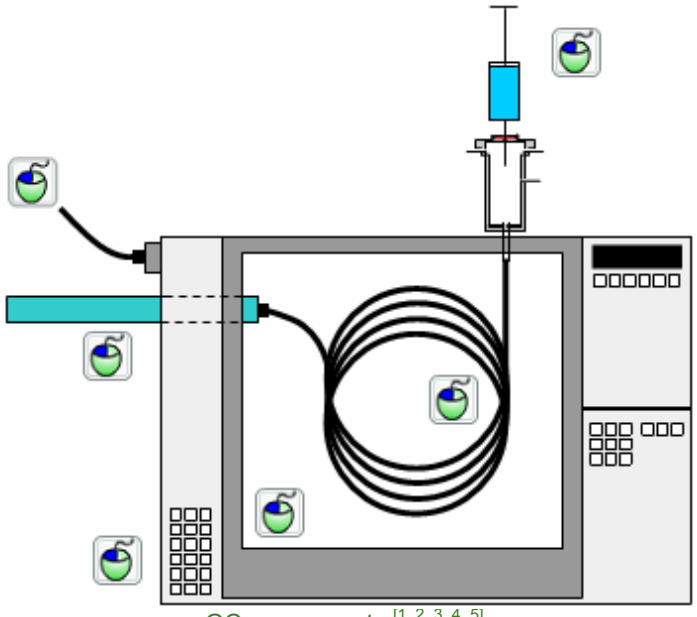
In Gas Chromatography (GC) the mobile phase is a gas and the stationary phase is either a solid, commonly termed "Gas solid chromatography (GSC)" or an immobilised polymeric liquid, referred as "Gas Liquid Chromatography (GLC)", of these two types of GC, GLC is by far the most common.[1]

The main elements that are present in conventional GC equipment are shown opposite.

Weaknesses:

- GC requires the analyte to have significant vapour pressure between about 30 and 300 °C.
- Lack of definitive proof of the nature of detected compounds, identification being based on retention time matching, which may be inaccurate or at worst misleading.

For more information about gas chromatography, please refer to the GC channel.[1]



GC components.[1, 2, 3, 4, 5]

Gas Inlets: Gas is fed from cylinders through supply piping to the instrument. It is usual to filter gases to ensure high gas purity and the gas supply pressure. Required gases might include:
•Carrier – (H_2, He, N_2)
•Make-up gas– (H_2, He, N_2)
•Detector fuel gas – (H_2 & Air/Ar or Ar and CH_3/N_2)

Interface: After separation in the GC system, analyte species have to be transported to the mass spectrometer to be detected.

Pneumatic controls: The gas supply is regulated to the correct pressure (or flow) and then fed to the required part of the instrument. Modern GC instruments have Electronic Pneumatic pressure controllers –older instruments may have manual pressure control via regulators.

Injector: Here the sample is volatilised and the resulting gas entrained into the carrier stream entering the GC column. Many inlet types exist including:

•Split/Splitless,
•Programmed Thermal Vaporising (PTV)
•Cool-on-column (COC), etc

The COC injector introduces the sample into the column as a liquid to avoid thermal decomposition or improve quantitative accuracy.

Column: Gas Chromatography uses a gaseous mobile phase to transport sample components through either packed columns or hollow capillary columns containing the stationary phase. In most cases, GC columns have smaller internal diameter and are longer than HPLC columns.

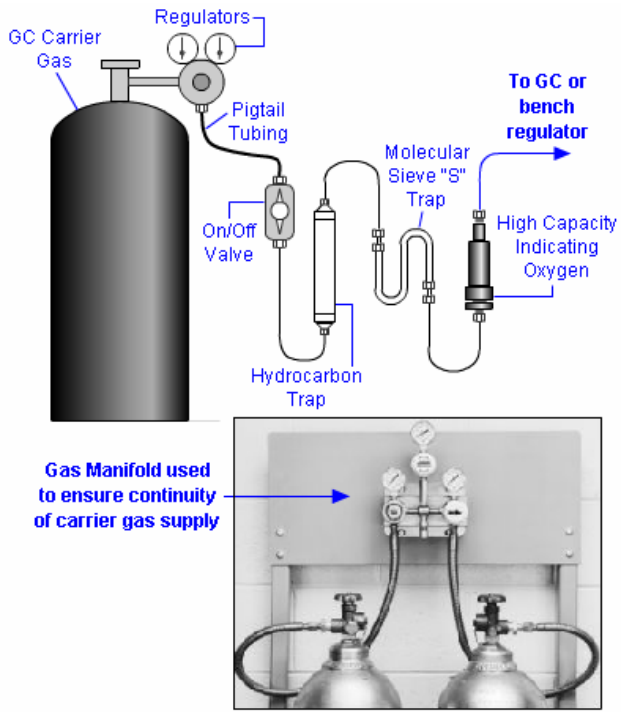
Oven: Both gas and liquid chromatography have ovens that are usually temperature programmable, the temperature of the gas chromatographic oven range from 5^{o}C to 350^{o}C and the liquid chromatography oven from about 5^{o}C to 120^{o}C.

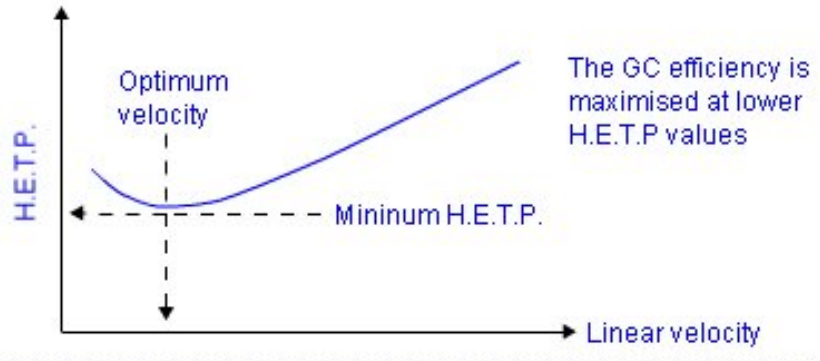
Carrier Gas

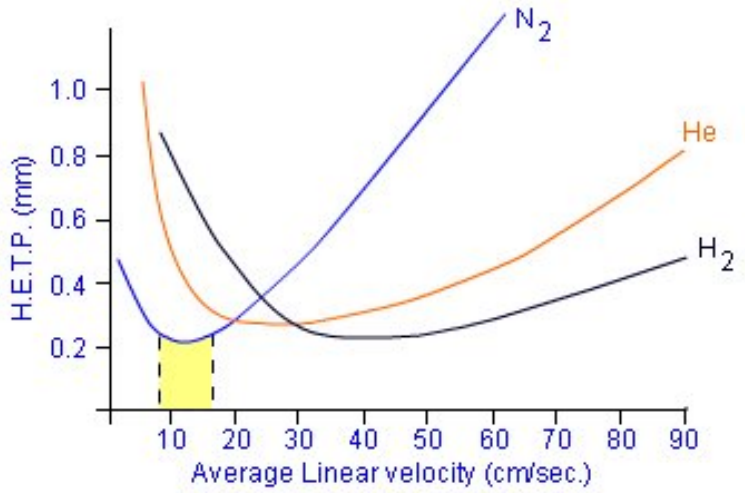
With capillary columns (typically used in modern GC-MS systems) linear velocities are high (in the order of 30 to 50 cm/sec) and hydrogen or helium would be the carrier gas of choice. When working at lower linear velocity (for example with packed GC columns) nitrogen would be the carrier of choice as separation efficiency is better for this gas at lower linear velocity (in the order 10-15 cm/sec).[1] Packed column GC-MS is very rare in modern times and care needs to be taken as flow rates (careful not to confuse flow rate with linear velocity) are high and special interface devices need to be used to reduce the column effluent flow.

When working with hydrogen special precautions need to be taken to avoid spark or ignition sources within the mass analyser to prevent explosion. Further, as the viscosity of hydrogen is relatively low, special high performance vacuum pumping equipment may be necessary in order to establish the required level of vacuum.

The supply and management of gases is of primary importance and a constant supply of high purity carrier gas is fundamental in GC-MS. Carrier gas of stated 99.999% purity should be used in all cases, to avoid degradation of chromatographic performance, stationary phase bleed and a high spectral background in the GC-MS. Air, water and hydrocarbons within the carrier are of particular concern. In general impurities should be kept below 1-2ppm.[1,2]


Regulators
GC Carrier Gas
To GC or bench regulator
Pigtail Tubing
Molecular Sieve "S" Trap
On/Off Valve
High Capacity Indicating Oxygen
Hydrocarbon Trap
Gas Manifold used to ensure continuity of carrier gas supply


Optimum velocity
The GC efficiency is maximised at lower H.E.T.P values
H.E.T.P.
Mininum H.E.T.P.
Linear velocity


N2
He
H2
1.0
0.8
0.6
0.4
0.2
H.E.T.P. (mm)
10 20 30 40 50 60 70 80 90
Average Linear velocity (cm/sec.)

The carrier gas flow rate is very important in GC-MS. Fluctuations in this parameter will have a major impact on the vacuum level of the GC system. In turn this can effect the filament lifetime – filaments will have shorter lifetimes at higher carrier gas flow rates. Further, the vacuum level can have an effect on the efficiency of the electron impact ionisation process and so may change the appearance of the spectrum.

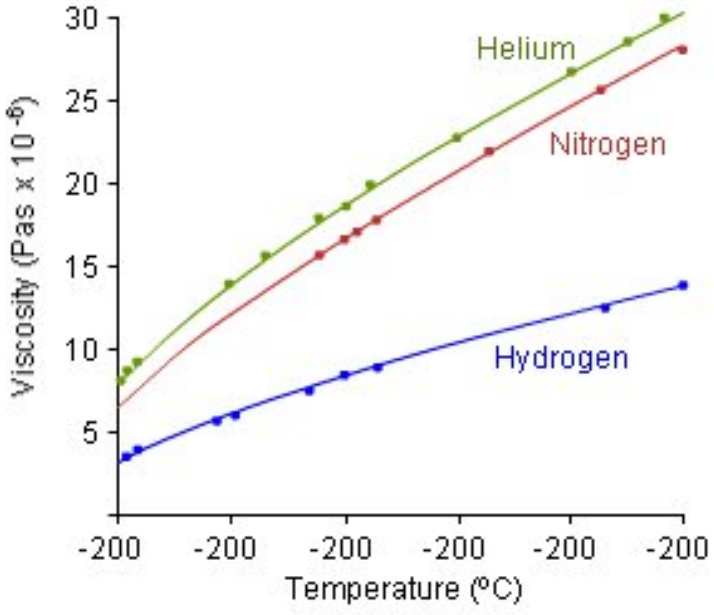
When the temperature of the carrier gas is increased, its **viscosity** is also **increased**. In a chromatographic system, with a constant pressure drop, an increase in viscosity results in a decrease in the linear velocity of the carrier gas, which may affect the efficiency of the ionisation process or the chromatographic separation.

Viscosity is the resistance of a liquid or gas to flow. The viscosity of a gas is determined by:

- Molecular weight
- Temperature

For more information see GC Channel / Theory and Instrumentation of GC / Gas Supply and Pressure Control / Pressure & Flow Programming

Some GC pneumatic systems maintain constant carrier gas mass flow - which will help to avoid changes in chromatographic efficiency or the efficiency of the ionisation process which may affect detector sensitivity or the appearance of the MS spectra obtained.

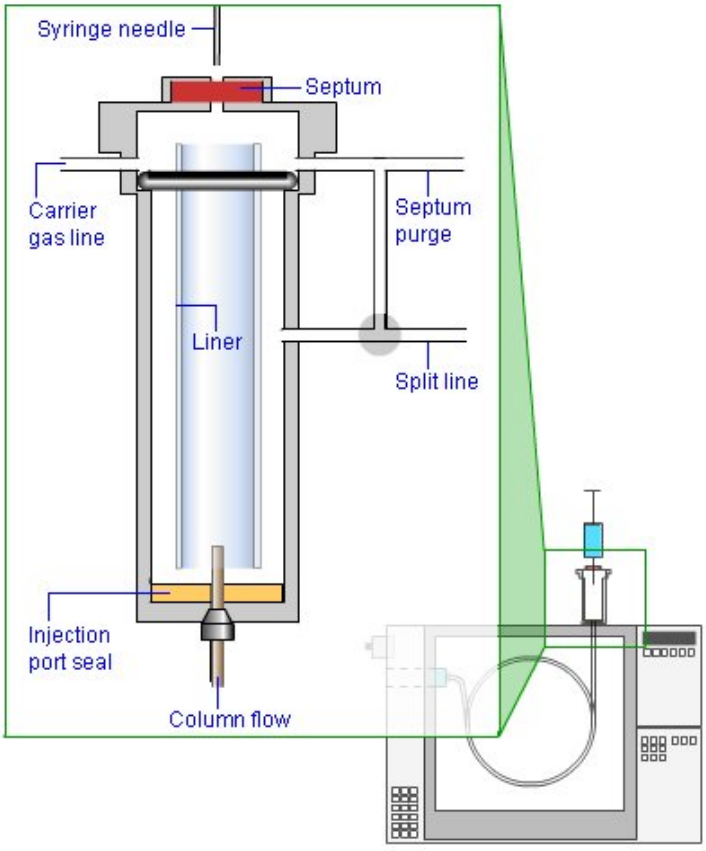


Temperature effects on the viscosity of common GC carrier gases.

Sample Introduction

Perhaps the most difficult step in any GC/MS analysis is the sample introduction. Solid or liquid samples need to be converted to the gas phase, and then efficiently transported onto the GC column.

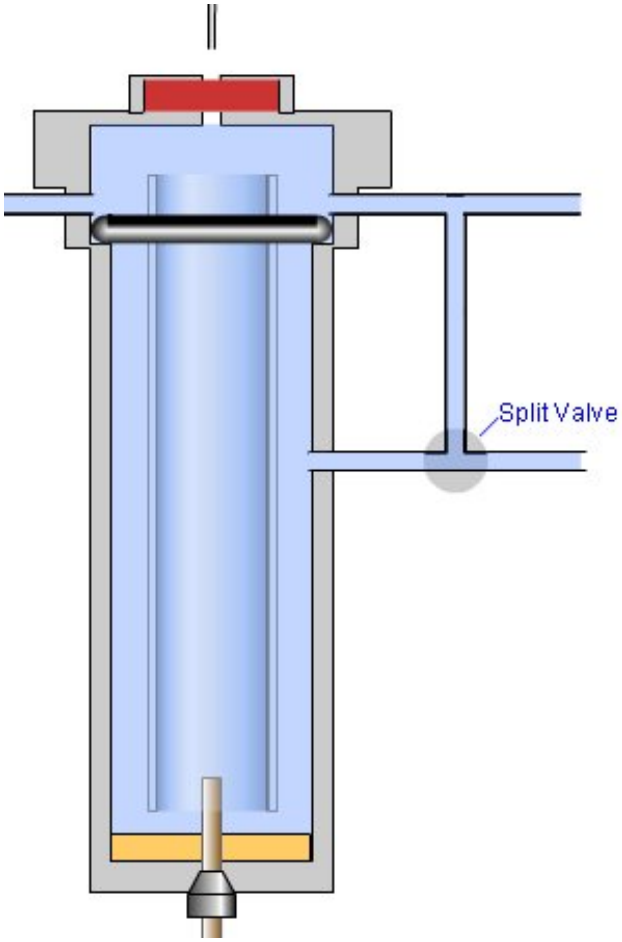
The primary aim with all sampling techniques is to ensure a representative and homogeneous gaseous aliquot of the sample under investigation is delivered to the GC column.



It is important to use the correct injection technique for the GC-MS application –usually splitless injection is used for samples where the analytes are capable of generating significant vapour pressures below about 250°C, are within reasonably clean matrices and are present at very low concentrations. Split injection is used for similar samples types but where the analyte concentration is higher. Modern instruments use common inlet hardware to perform both split and splitless injection.[1,3]

Splitless injection mode: is used when the entire sample needs to go onto the column (such as in trace analysis). The vent valve is closed, so that all rapidly vaporised sample is loaded onto the column.

Split injection mode: is used when only a fraction of the sample needs to go onto the column.



Split/splitless inlet

It is much less commonplace for solid sampling to be used in conjunction with GC analysis –unless being performed by special introduction techniques such as headspace sampling. Gas chromatography deals with volatile species in the liquid phase and as such solid samples must be dissolved in a suitably volatile solvent prior to injection.

For more information about split / splitless injection techniques in gas chromatography, please refer to the GC channel.[1]

Split Injection

Overview

In this injection mode the sample is introduced into the inlet liner where rapid volatilisation occurs. The sample vapour is then mixed with and diluted by the carrier gas flowing through the centre of the liner.

The diluted sample vapour then flows at high velocity past the column entrance where a small portion of it will enter the column. However, most of the diluted sample will flow past the column entrance and out of the inlet via the split line. The ratio of column flow to split flow will determine the ratio (or volume fraction) of sample entering the column to that leaving the inlet via the split line. The split flow rate may be altered to either increase or decrease the amount of sample reaching the column.

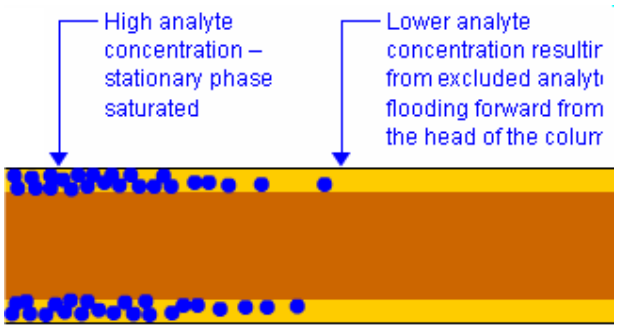
Split injection is conventionally used for analyses where the sample concentration is high and the user wishes to reduce the amount of analyte reaching the capillary column. As capillary columns have a limited sample capacity it is important that the column is not overloaded. A typical 25m GC column may contain only 10mg of stationary phase distributed over its entire length.

Split injection ensures that the sample is rapidly volatilised and transferred to the capillary column –hence ensuring a narrow analyte band. For this reason initial column temperatures for split injection tend to be higher than the boiling point of the sample solvent.[1]

Capillary columns have limited amounts of stationary phase onto which analyte and sample components may adsorb. Initially on injection, if the stationary phase at the head of the column becomes 'saturated' the analyte will flood down the column extended the band of analyte. A concentration gradient will form at the head of the column and this will reflect as a 'fronting' peak once the analyte band reaches the detector. This process is shown schematically below:

Table 1. Advantages and disadvantages of Split mode

Advantages	Disadvantages
• Simple to use • Rugged • Narrow analyte band on column • Protects column from involatile sample components • Easy to automate	• Not suitable for trace analysis • Suffers from discrimination • Dependent upon linear geometry • Analytes susceptible to thermal degradation



Setting Split Ratio

The 'Split Ratio' describes the ratio of gas flows between the capillary column and the split flow line – and effectively gives a measure of the volume fraction of the sample vapour that will enter the column.

The calculation of Split Flow is shown under the 'Equations' button opposite. Of course the magnitude of the split ratio will depend on the concentration of the sample injected and the capacity of the capillary column used.

The split ratio is usually adjusted empirically to obtain a good balance between analytical sensitivity and peak shape. If the split ratio is too low peak shape will be broad and may show the fronting behaviour associated with overloading. Of course if the split ratio is too high, too little sample will reach the column and the sensitivity of the analysis will decrease as peak areas decrease.

When using thick stationary phase film columns (>0.5 mm) or wide bore (0.533 mm i.d.) columns, the sample capacity increases and lower split ratios of 1:5 to 1:20 are typical. With very narrow GC columns (<100 mm i.d.) split ratios can be as high as 1:1000 or more.

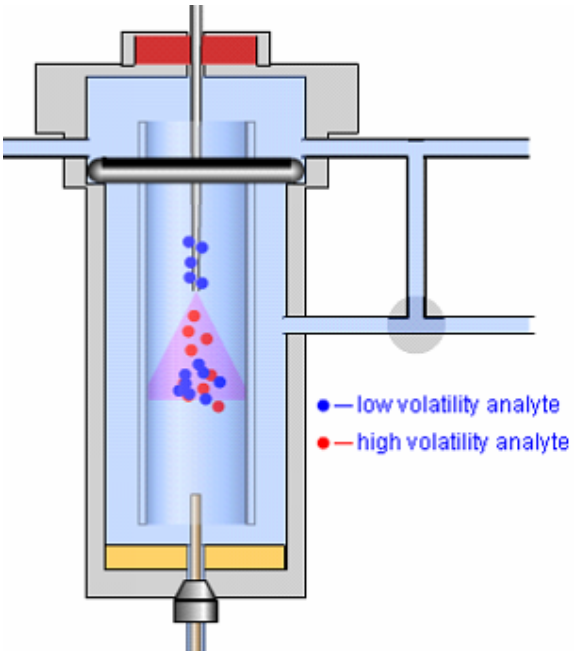
In most cases the split ratio should give an approximately linear relationship with analyte peak area, i.e. halving the split should halve the resultant peak area, however this is not recommended for calibrating the instrument response! Below a split ratio of around 1:15 reducing the split ratio may not give a linear relationship.[1]

Sample Discrimination

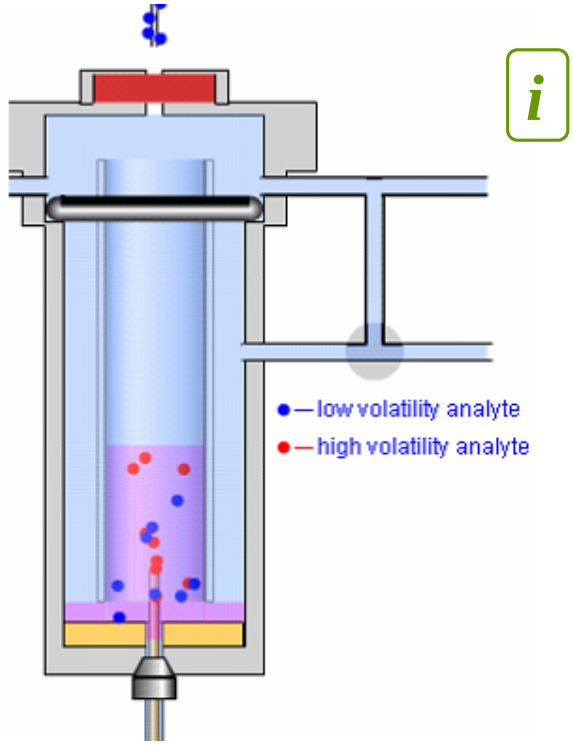
The phenomenon of sample discrimination leads to a non-representative sample entering the analytical column compared to the original sample. Sample discrimination is best described using the example on the 'Data' button opposite, which shows the detector response to an injection of n-alkanes at equal concentration. The normalised line shows the original sample composition, and the expected response for each of the n-alkanes. The more highly volatile n-alkanes show total recovery, however for C 25, only half of the analyte present in the sample is introduced into the column, and the recovery of C 37 is less than 25%.

For higher boiling (less volatile) analytes, the residence time of the syringe needle is too short. The analyte will condense on the cold inner and outer surfaces of the needle – prior to it being withdrawn from the inlet. Some less volatile analytes may never properly volatilise and the sample passes the split point (head of the capillary column) as a mixture of sample vapour and non-uniform liquid droplets. Several approaches to the problem have been postulated including:[1]

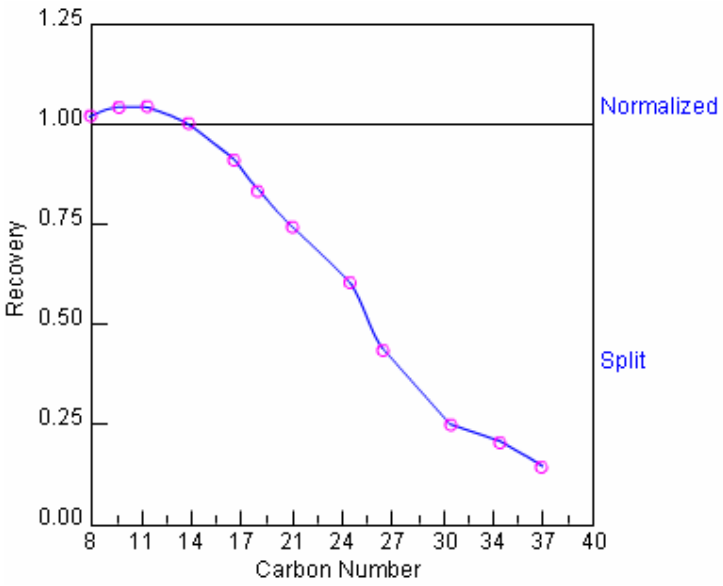
- Optimisation of liner geometry and packing materials to promote sample mixing and volatilisation.
- Optimising the injection routine (filled needle, hot needle, solvent flush, air flush, sandwich method etc.).
- Improved instrument design to reduce fluctuations in split flow.



Due to low inlet needle residence time, low volatility (high boiling point) analytes recondense onto the (relatively) cold injection syringe needle and are withdrawn from the inlet.



Low volatility analyte still held in non-uniform liquid droplets –carried past the column entrance (split point) and out of the inlet. High volatility analytes in the gas phase preferentially samples into the column.



Discrimination due to differences in boiling point: Hot split injection of solution containing equal amounts of normal alkanes in hexane

In general, the least amount of discrimination is obtained if the injection is performed as rapidly as possible. For this reason, fast autosamplers generally give less discrimination than manual injection.

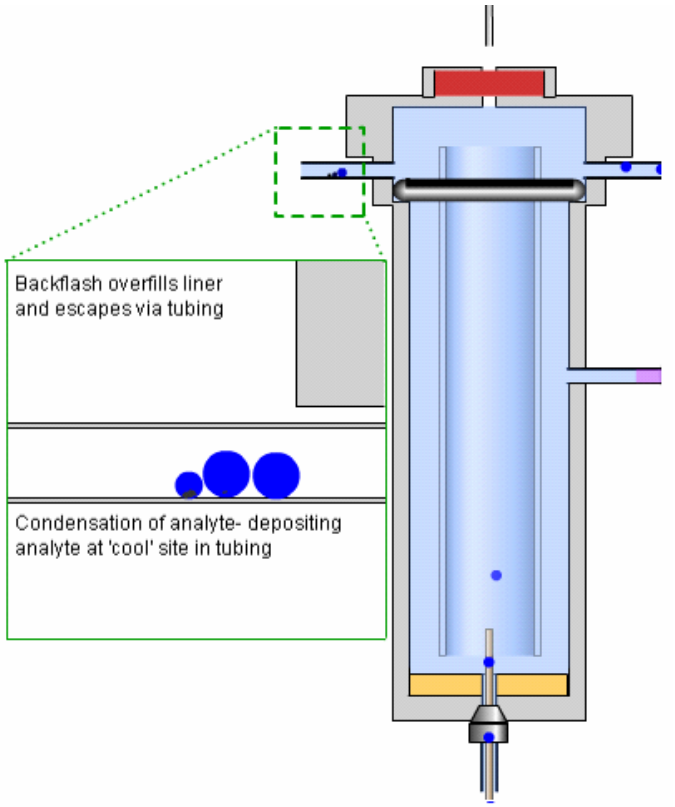
Injection Volume

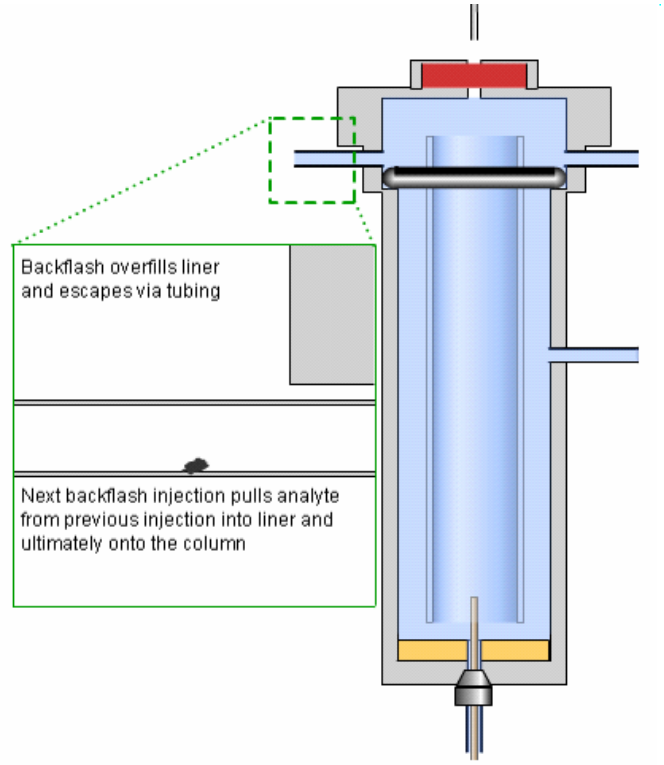
The nature and volume of the sample solvent injected into the split/splitless inlet will have a major effect on the accuracy and reproducibility of quantitative analysis and the chromatographic peak shape. As the injection is made, the sample solvent rapidly volatilises and expands into the gas phase. To avoid quantitative problems, the total volume of the gas should be able to be constrained within the volume of the inlet liner.

If this is not the case, then the excess gas will spill over into the inlet gas supply and septum purge lines. The temperature in these lines rapidly decreases, and it is possible for the sample solvent vapour (containing the analyte), to recondense, ultimately depositing analyte onto the inner walls of the tubing.

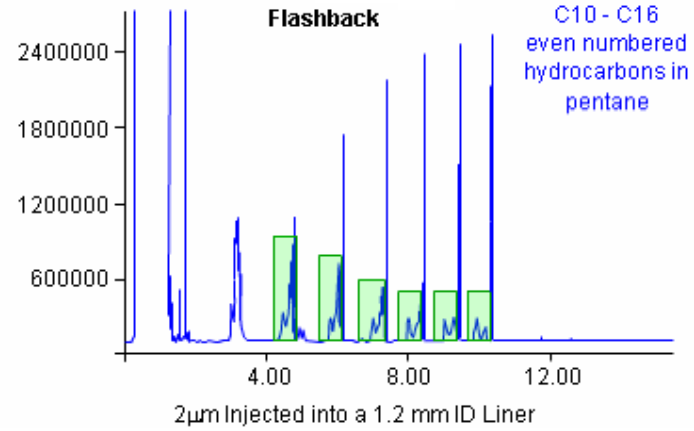
When the next 'overloaded' injection is made, the sample solvent from this injection will again 'backflash' into the gas lines. In this instance analyte deposited during the previous injection will be 'lapped' back into the inlet –ultimately finding its way onto the column. This will cause 'carry-over' and will reduce quantitative accuracy and reproducibility.

The expansion volume of the sample solvent is governed by the inlet pressure and temperature, as well as the natural expansion coefficient of the solvent. It is possible to predict the expansion volume and hence the volume of solvent that may be safely injected into an inlet liner of known volume, under set temperature and pressure conditions.[1]


Backflash overfills liner and escapes via tubing
Condensation of analyte- depositing analyte at 'cool' site in tubing


Backflash overfills liner and escapes via tubing
Next backflash injection pulls analyte from previous injection into liner and ultimately onto the column

Ghosts peaks.

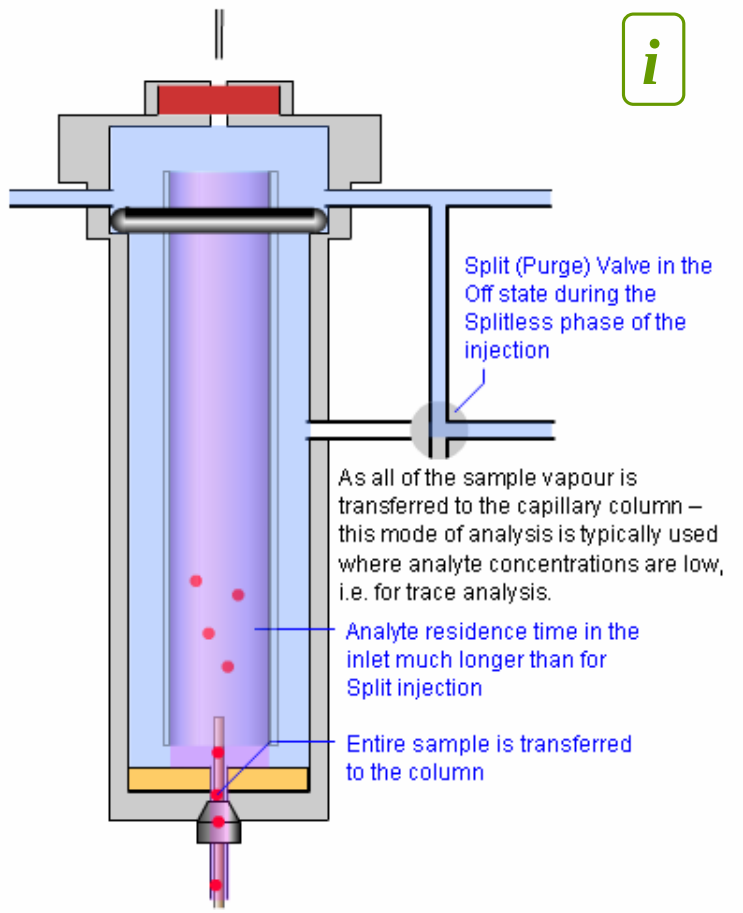


A technique known as 'pressure pulsed' injection may be used, in which the inlet pressure is raised during the sample injection cycle. This constrains the expansion of the solvent within the inlet liner and allows for large volume injections.

Splitless Injection

Splitless injection is analogous to split injection in many ways. The hardware used for splitless injection is almost identical to the split injector and most manufacturers will use the same inlet for both split and splitless injection – hence the term split/splitless injector. Just as with split injection, the sample is introduced into a hot inlet using a sample syringe where it is rapidly injected and volatilised. The splitless injector also belongs to the family of 'vaporising' injectors. Post injection, there are a number of differences in the way that the splitless injector works and a typical splitless injection routine is outlined below:

- The sample is introduced into the inlet, via the septum, using a syringe
- The sample is vaporised and is mixed with and diluted by the carrier gas
- Initially the split line is turned off using a valve in the split line to prevent the escape of the sample vapour and carrier gas
- ALL OF THE SAMPLE is transferred to the capillary column by the carrier gas during this initial SPLITLESS phase of the injection
- The transfer of the sample vapour (diluted with carrier gas) from the inlet is much slower compared to split injection
- The sample vapours are trapped (condensed) on the head of the analytical column using a low initial oven temperature
- At an optimised time the split line is turned on to clear the inlet of any residual vapours
- The oven temperature is programmed to elute the analytes from the column



Advantages:

- Rugged
- Excellent for trace analysis ~(0.5ppm with FID should be easily achievable)
- Easily Automated
- Highly reproducible with optimised inlet settings

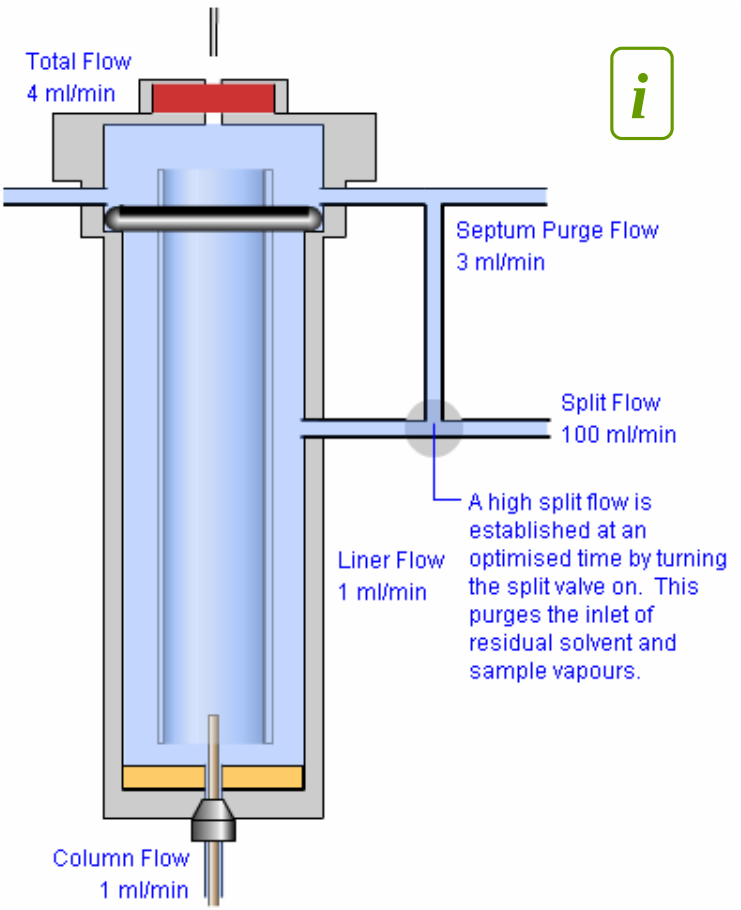
Disadvantages:

- More complex than split injection –more parameters to optimise.
- Suffers from discrimination.
- Analytes susceptible to thermal degradation –more so than with split injection due to longer analyte residence time in the inlet.
- Column contamination possible –all sample components introduced into the column.

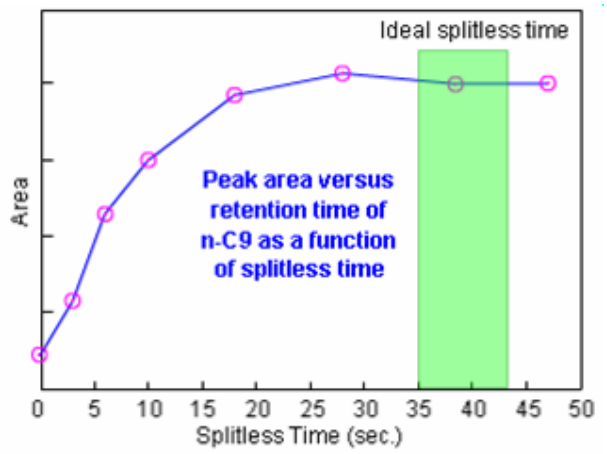
Optimising Splitless Injection

Purging the inlet

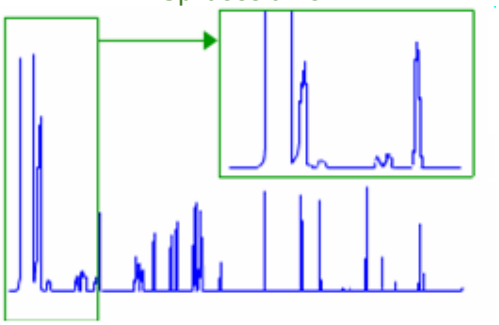
During splitless injection, it is of vital importance that the inlet is purged of residual vapours once the analyte has been transferred to the capillary column. If this is not done, the solvent peak will show a high degree of tailing and the GC baseline signal may be noisy and rise markedly as the analysis progresses. This is due to the slow bleed of excess solvent and sample (not analyte) components from the inlet into the capillary column.



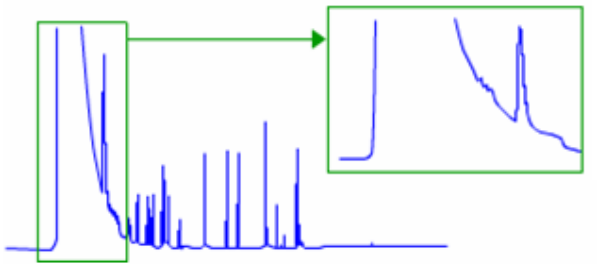
The inlet purge is achieved by actuating the Split (Purge) Valve that allows a high split flow through the liner, which quickly purges the residual vapours from the inlet. The split flow is high as the aim is to quickly purge the inlet, split flows of 100-200 ml/min. are typical. The time from the beginning of the injection to the time at which the split line is turned on is known as the splitless time. It is vital that the splitless time is optimised for each application. Too short a splitless time will mean that analyte still resident in the liner will be discarded via the split line. This may lead to poor analytical sensitivity and reproducibility. Too long a splitless time will lead to badly tailing solvent peaks, extraneous peaks and a rising baseline, making reproducible integration difficult.



Spiltless time



Spiltless time too SHORT –loss of higher boiling analytes



Spiltless time too LONG –broad solvent peak and rising baseline

The splitless time is usually empirically optimised by monitoring the peak area of a mid-eluting peak in the chromatogram. The peak area is plotted against the splitless time and a plot of the form shown under the Data Optimisation button opposite is dervived. For reproducible analysis the splitless time should be chosen just onto the plateau of the area response curve as indicated. Typical splitless times lie in the region 20 – 90 seconds.

Analyte Focussing

The analyte is slowly introduced from the inlet during the whole of the splitless time (the inlet volume may be exchanged as few as two times during this whole splitless period).

This slow sample vapour transfer would result in the analyte band entering the column over a period of 30 – 60 secs. or so depending upon the exact analytical conditions. This would entirely negate any efficiency gained through the use of capillary columns and the resulting chromatographic peaks would be unacceptably broad.

To overcome these problems Focussing techniques are used, which usually involves setting the initial oven temperature at a suitably low value ensuring that condensation and reconcentration takes place in the column.

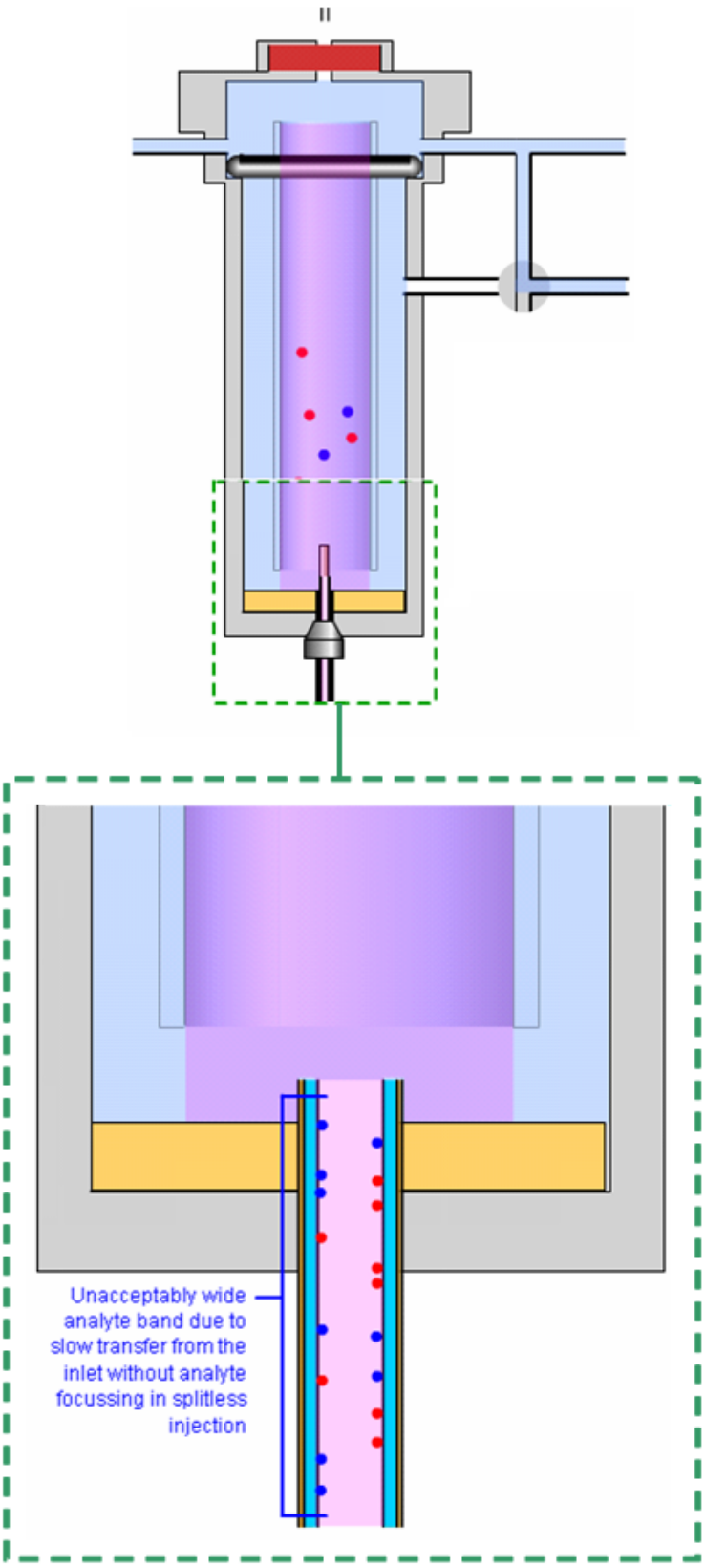
Two discrete focussing mechanisms can be identified:

Cold trapping: higher boiling analytes are condensed in a tight band in the temperature gradient between the inlet (~250 ^{o}C) and the column oven (~40 ^{o}C)

Solvent effect: low boiling (more highly volatile) components remain dissolved in the solvent, which also condenses on the inner wall of the GC column at low initial oven temperatures. The solvent slowly evaporates to give a thin concentrated band of analyte.

For volatile analytes (whose boiling point is close to the sample solvent), a different focussing mechanism exists. If a suitably low initial oven temperature is used, the sample solvent will condense as a film on the surface of the column bonded phase. This film will contain the volatile analytes in a disperse form. The flow of the carrier reduces the vapour pressure within the column and the solvent band will evaporate slowly from the inlet end. The band will evaporate to a much lower solvent volume, containing a concentrated, narrow band of the analyte –effectively overcoming the band broadening incurred in the slow sample vapour transfer from the inlet.

This mechanism works most effectively when the initial oven temperature is at least 20^{o}C below the boiling point of the sample solvent.

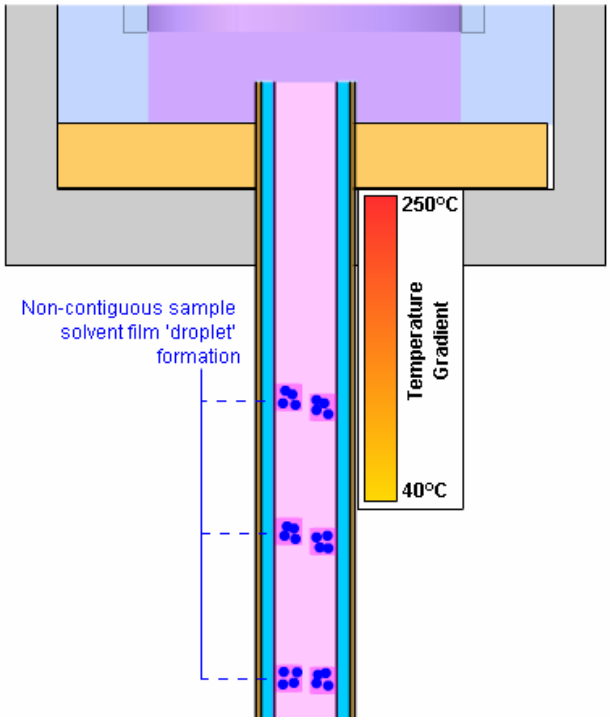

Unacceptably wide analyte band due to slow transfer from the inlet without analyte focussing in splitless injection

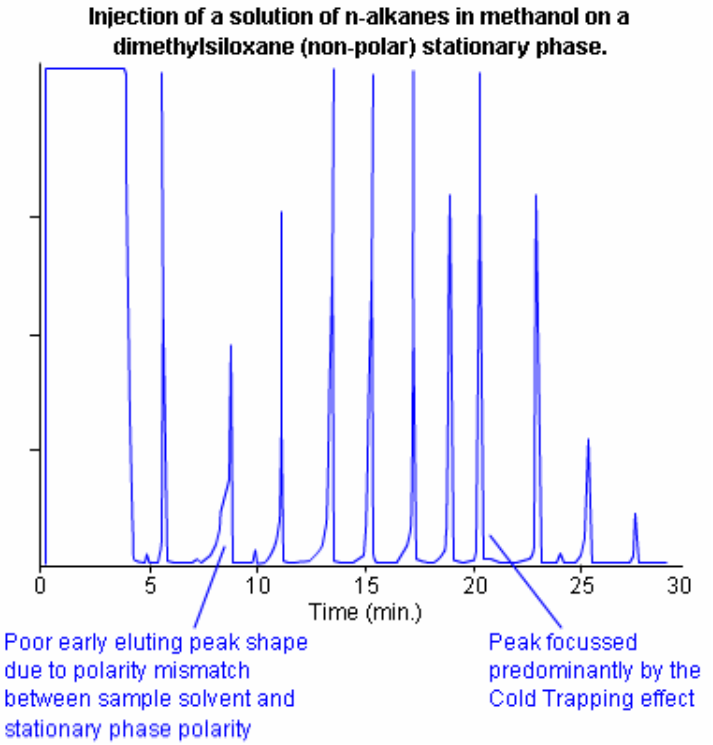
Solvent Choice

All of the comments regarding solvent injection volume are also true for splitless injection. The allowable solvent volume does not differ greatly between split and splitless injection and in each case the allowable volume may be calculated from a combination of the liner dimensions, solvent type and inlet head pressure. However, with splitless injection there is one further constraint on the sample solvent. The nature, or more specifically the polarity, of the sample solvent MUST match the polarity of the stationary phase used in the GC column.

The solvent effect relies on the formation of a single contiguous film coating the inside wall of the capillary column. This will only occur if the solvent polarity is matched to that of the stationary phase. If this is not the case (e.g. using methanol as the sample solvent with a methyl silicone stationary phase), the solvent will not condense as a film, rather droplets of solvent will form, each acting with an individual solvent effect. This will lead to broad, split or fronting & tailing peaks (the latter being known as the 'Christmas Tree' effect due to the triangular peak appearance).

These issues are generally most prevalent for earlier eluting (more volatile) analytes as later eluting analytes tend to be focussed via the cold trapping mechanism. Solvent droplet formation usually only occurs when a critical solvent injection volume is exceeded (usually between 1 and 2 mL). If a mismatch between the sample solvent and column stationary phase is required (due to sample solubility characteristics), then a retention gap may be used. This is a short piece of capillary column (0.5 – 3m) which is coated using a phase which matches the sample solvent polarity.





PTV Inlets

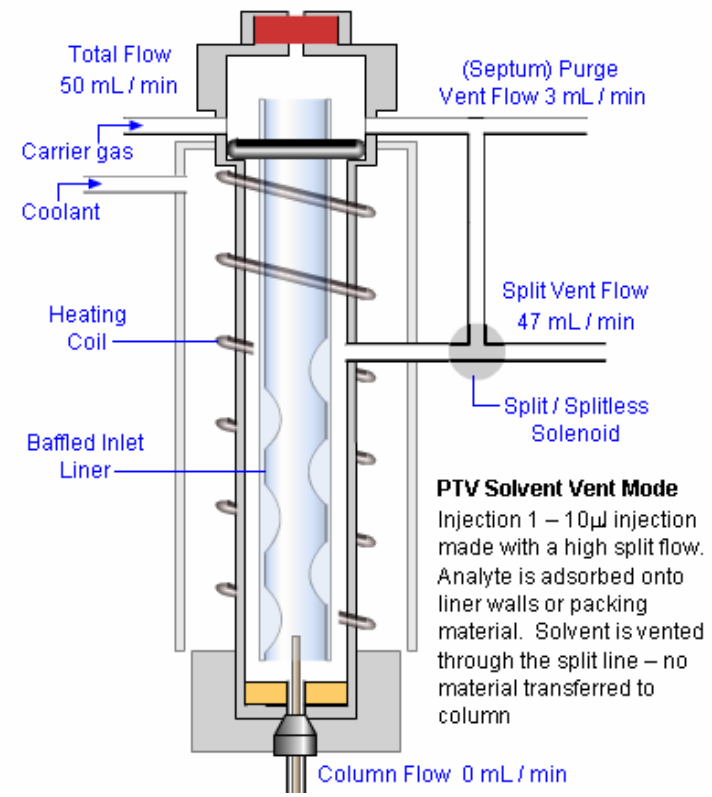
Programmed temperature sample introduction was first described by Vogt in 1979.[4] Programmed temperature vaporising injectors (PTV) closely resemble split/splitless inlets but differ in two very important ways:

- The inlet is kept cool during sample introduction –allowing the analyte to condense inside the liner, whilst the solvent is vented via the split line
- The inlet has a very low thermal mass, which allows rapid heating to transfer the analyte to the GC column after solvent venting has taken place

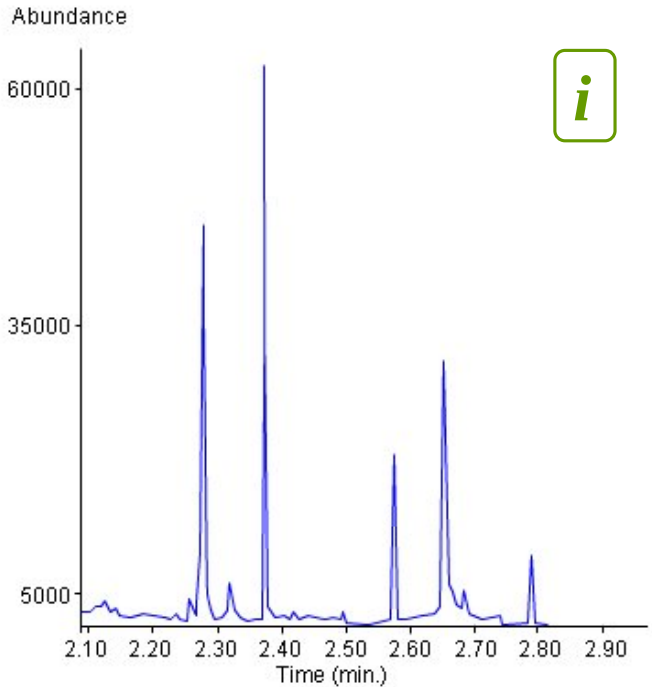
These two important differences in inlet design give the PTV several important advantages:

- Large volumes may be injected at controlled speeds into the inlet allowing the introduction of very large sample volumes
- Discrimination due to differences in analyte boiling point can be eliminated

PTV injection has been used to introduce large volumes of samples and even solids onto the column of gas chromatographs, to reduce discrimination effects and to enhance sensitivity.[5]



Application Example –Determination of explosives.



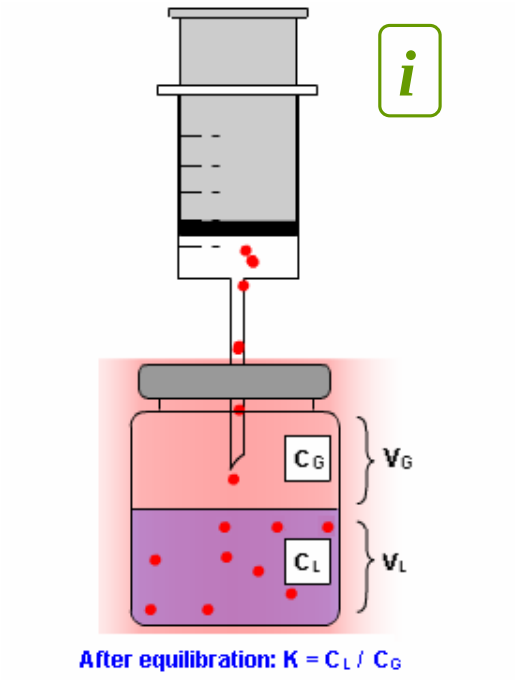
SIM chromatogram of explosives. The analysis was performed on a GC equipped with a PTV inlet and mass selective detection.

Headspace Sampling

Headspace sampling is the analysis of the vapours above a sample contained in a sealed vial. Volatile compounds in almost any matrix can be analysed without the need for extractions, dissolving samples or dilutions.

Because headspace relies on volatilisation of the sample to extract the analyte of interest, extraction, clean-up and preconcentration are not necessary. Headspace avoids problems due to non-volatile materials being carried into the detector or onto the column.

The combination of headspace sample introduction and gas chromatography with mass selective detection provides the analyst with a powerful, fully automated technique for the determination of volatile compounds.



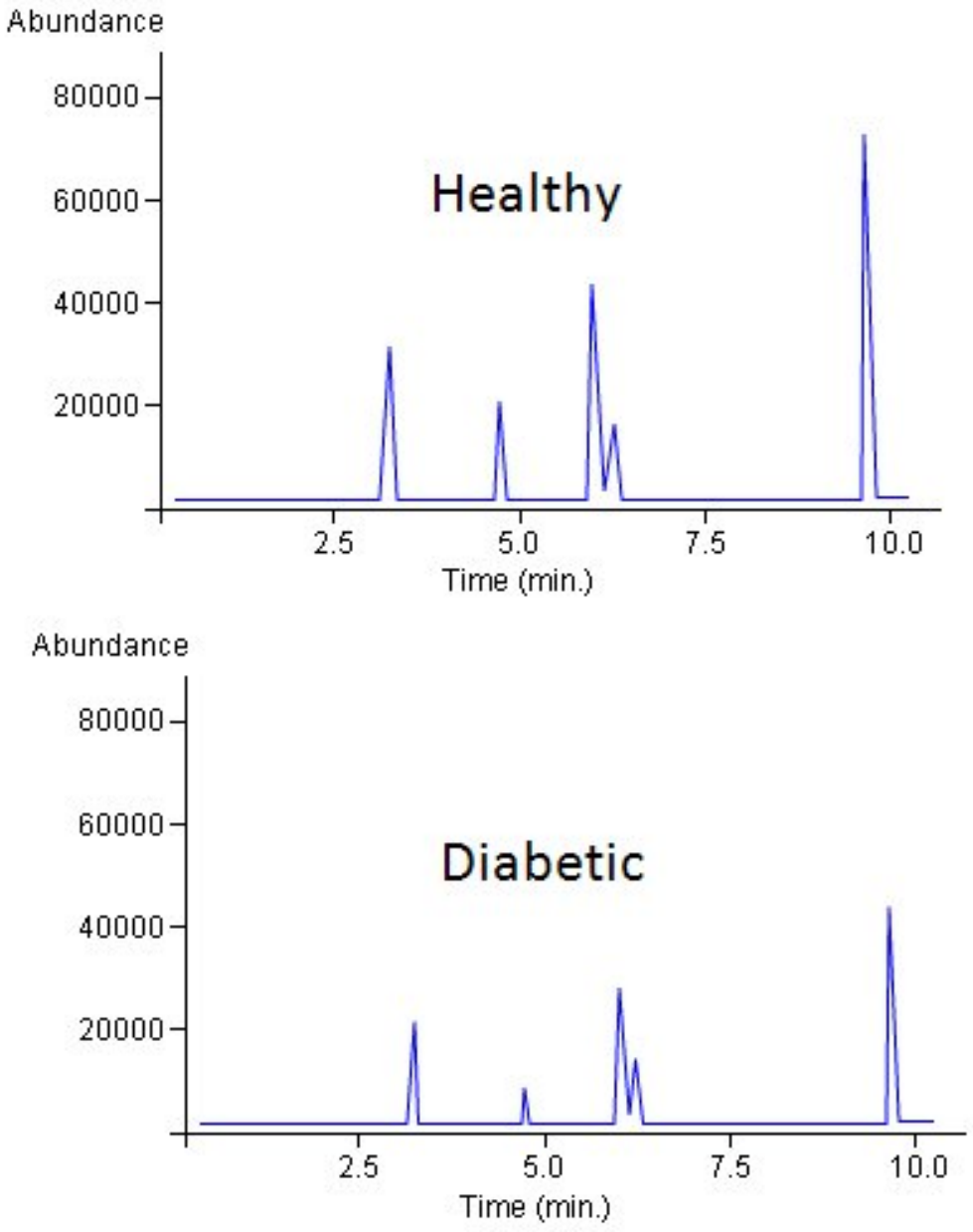
After equilibration: $K = C_L / C_G$

The sample / headspace partition co-efficient
C_L = analyte concentration in the liquid sample
C_G = analyte concentration in the headspace gas

The concentration of analytes in the headspace gas is proportional to the concentration in the original sample.

Typical examples of headspace analysis include:[7,8,9,10]

- Volatile Organic Compounds (VOC) from wastewater and contaminated land samples.
- Residual solvents in packaging and pharmaceuticals.
- Blood alcohol and toxicology screening.
- Aroma components from food and beverages.
- Diagnostic gas analysis from oils.



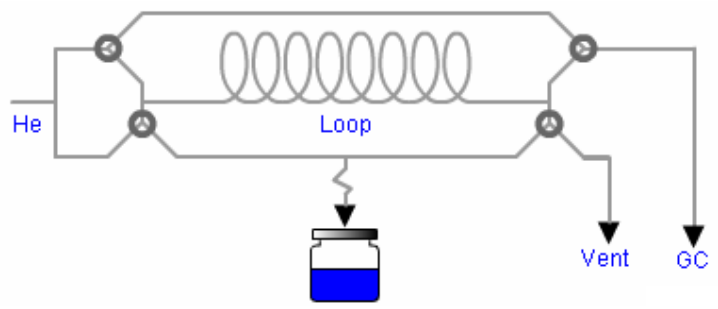
GC-MS SIM chromatogram of non acidified urine samples.

Headspace Autosamplers

Headspace GC techniques are amenable to automation using gas sampling loops. Most commercially available systems operate on the cycle described below:

- Standby –the sample is heated and agitated in a small oven enclosed within the instrument
- Pressurisation –a concentric needle arrangement is used to introduce an inert gas into the vial headspace to increase the vapour pressure
- Loop filling / Venting –the headspace gas is allowed to flow through a gas sampling loop to vent –loop filling time and closed loop pressure equilibration times are of great importance
- Injection –valves are altered to allow the carrier to flow through the gas loop and sweep the contents through an inert, heated transfer line into a split/splitless inlet and onto the GC column. A small split flow is often maintained to ensure efficient transfer onto the column and ensure sharp peaks. Cryogenic cold trapping / focussing of analytes at the column head is also possible to ensure good peak shape.

When using headspace auotsamplers there are several parameters that need to be optimised. Variables such as column flow, split flow and initial oven temperature may differ substantially from those used in conventional analysis. These parameters are discussed in greater detail next.



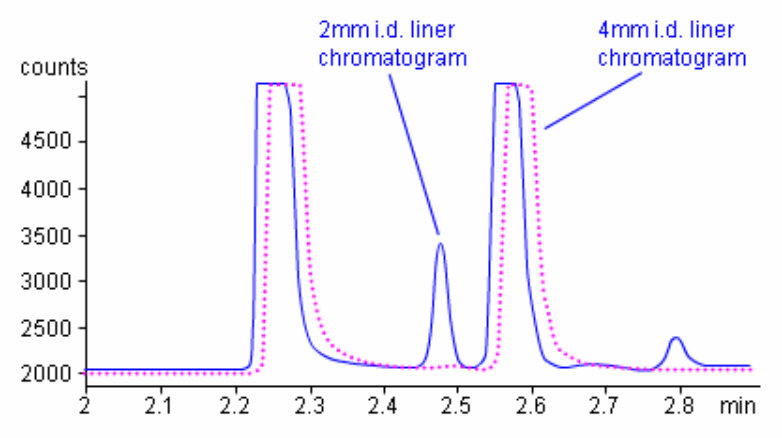
Headspace GC techniques automation by using gas sampling loops

Table 2. Critical parameters for optimisation in headspace analysis

GC	Headspace
• Inlet (liner) • Flow • Start temperature • GC Cycle time	• Equilibration time • Temperatures • Injection times and volumes • Pressure (pressurization) • Time (loop filling time) • Vial (sample amount) • Loop

Inlet (Liner)

As with SPME techniques, using a smaller internal diameter liner can considerabley sharpen peaks. This is particularly noticeable with trace level analytes.



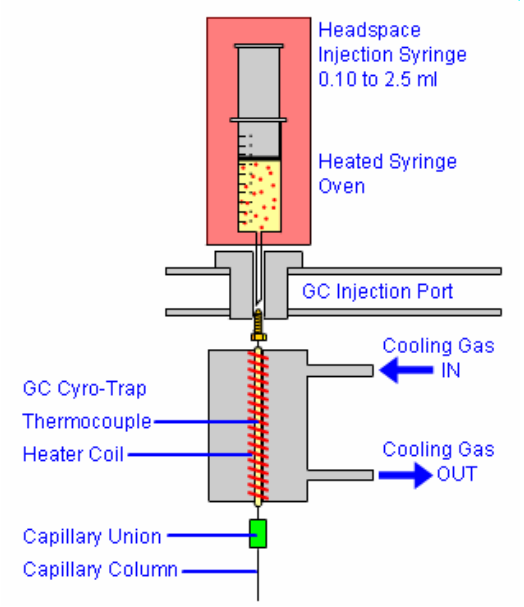
Inlet internal diameter effect

Flow

Headspace GC analysis often requires a higher than conventional carrier gas flow (50-100mL/min.). Higher flow rates ensure tha the gas loop is emptied and the analytes transferred on to the column effciently. For this reason wider internal diameter column (0.53mm) are often used, as they create smaller back pressure at high flow.

Start temperature

Low initial oven temperatures are often used in Headspace GC to ensure that analytes are thermally focussed at the head of the analytical column. It is also possible to have cryo-trapping of analytes using conventional cryogenic column adapters as shown.



GC with a conventional cryogenic column adapter

GC Cycle Time

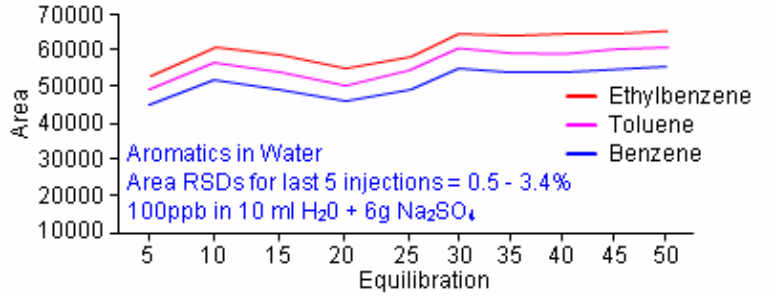
The GC cycle time must include the time required for headspace sample preparation (including sample incubation). Most modern instruments have communication between the headspace instrument and the GC to enable synchronisation. It is also possible to begin sample incubation whilst the GC separation of the previous sample is occurring, so reducing overall injection to injection cycle time.

Equilibration Time

As equilibration time is increased, the partition of the samples in the vapour phase rises, and reaches a plateau.

The higher the equilibration temperature, the longer the equilibration time needs to be.

It is not necessary to use over-long equilibration times, just long enough for the partitioning to equilibrate.



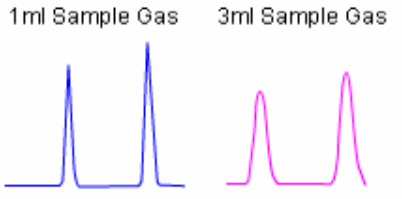
Example

Temperatures

See comments in equilibration time above. As the equlibration temperature increases – the equilibration time increases.

Injection Time and Volumes

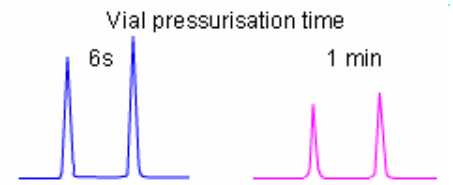
Increasing the injection volume at low transfer (carrier gas) flow rates can lead to peak broadening, even to loss of separation, especially for highly volatile compounds.



Example

Pressure (Pressurization Time, Loop Filling Time)

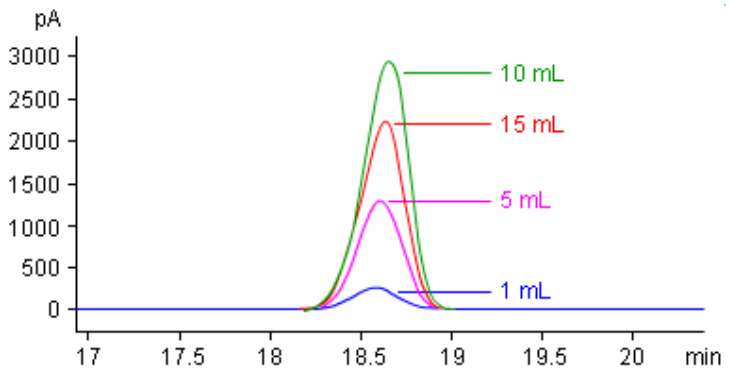
The vial pressurisation is of great importance. Too high a vial pressure (usually adjusted by altering pressurisation time), will risk loss of analyte via the vial cap seal, septum – needle seal or by overfilling the sample loop.



Example

Vial (Sample amount)

Increasing the sample amount (relative to a fixed headspace volume) will bring an increasde in sensitivity. Note in the graphic though that the 15ml sample volume results in a smaller peak area than expecetd. This is mainly due to the equilibration time being too short for the increased sample volume –sample volume and equilibration time are directly linked.



Toluene in water (oven temperature 80°C, time 15 min, 20mL vial)

Loop

It is usually possible to change loop sizes to increase or decrease the volume of gas injected into the GC. Care should be taken with larger gas sample volumes to preserve peak efficiency during transfer to the GC column.

Columns

Most GC-MS columns are of the Wall Coated Open Tubular (WCOT) design. These are of fused silica construction and are coated with polyamide to give them flexibility and strength. The stationary phases are bonded to the inner wall of the silica tube in various thicknesses silica.[11,12] Remember, the column geometry has a major impact on the GC-MS separation:

1. Film Thickness (d_f)

- Thicker films increase retention times.
- Thicker films increase sample capacity
- Thicker films minimise overloading but increase inherent column bleed – which needs to be strenuously avoided in GC-MS

2. Column Radius (r) (or Internal Diameter (i.d.))

- Increasing internal diameter reduces retention times
- Increasing internal diameter (keeping film thickness) increases phase ratio
- Halving the internal diameter (whilst keeping the phase ratio constant) doubles resolution whilst keeping analysis time constant!

Phase ratio (ß): is a measure of the stationary to mobile phase ratio at any point in the column and is given by:

$$\beta = \frac{r}{2d_f}$$

Where:

r = Column radius (mm)
d_f = Film thickness (µm)

Increasing the phase ratio can also reduce the sample capacity (if the film thickness remains constant)

3. Column length: Increasing the column length increases resolution

- Generally increasing the column length increases the resolving power of the column – doubling column length increases resolution by a factor of about 1.4 but also doubles analysis time and usually column cost!
- Long columns are used when the sample contains large numbers of sample components.

Typical column gas flow rates

Gas	Flow Rate (mL/min)
Hydrogen	0.6
Helium	0.3
Nitrogen	0.2

Typical column dimensions

Length (m)	Film Thickness (µm)					
12	0.33					
17	0.10					
25	0.11	0.33	0.50			
50	0.11	0.33	0.50			

0.2 | 0.25 | 0.32 | 0.53

Column Internal Diameter (mm)

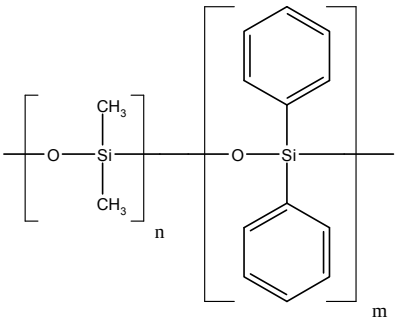
Stationary Phases

Polysiloxanes are the most common stationary phases for GC-MS. They are available in the greatest variety of chemistries and are the most stable, robust and versatile. Standard polysiloxanes are characterized by the repeating siloxane backbone. Each silicon atom contains two functional groups. The type and amount of the substituent groups distinguish each stationary phase and its properties.[14,15]

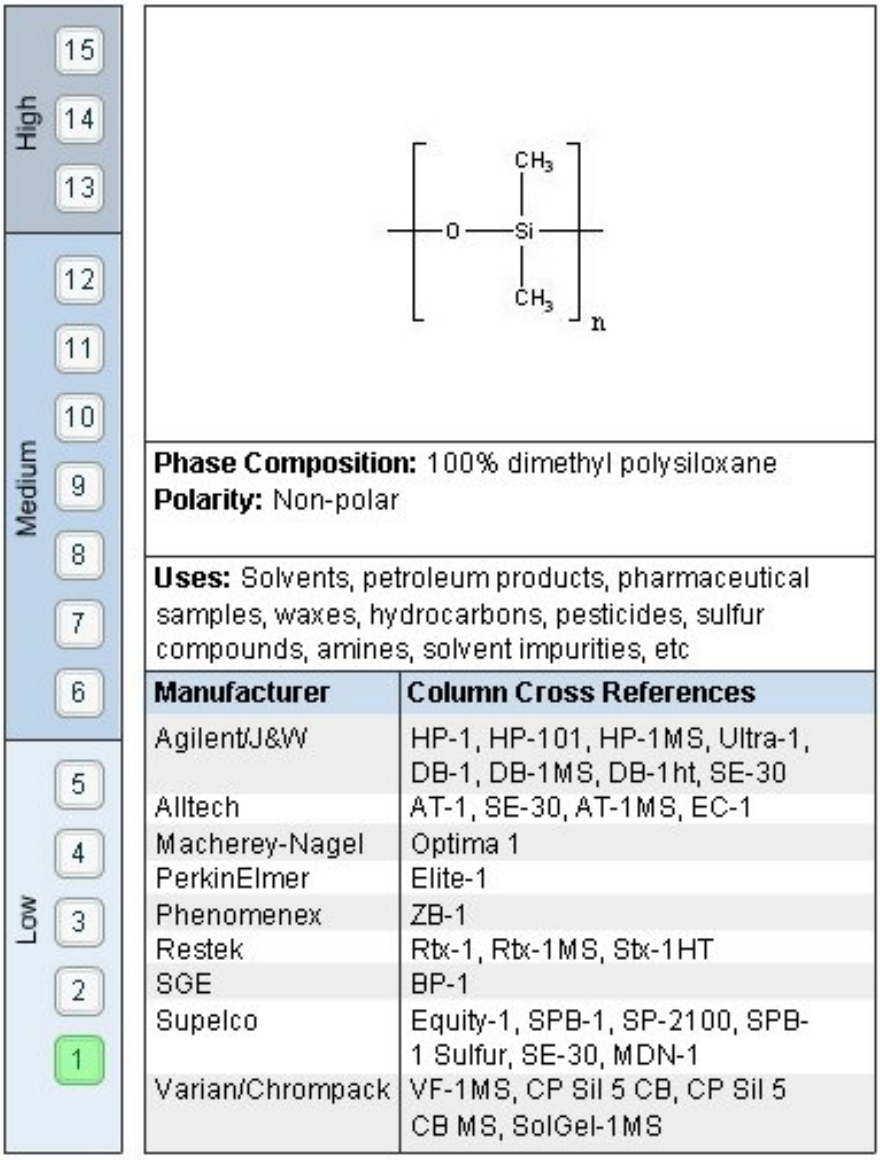
The most basic polysiloxane is the 100% methyl substituted. When other groups are present, the amount is indicated as the percent of the total number of groups. For example, a 5% diphenyl-95% dimethyl polysiloxane contains 5% phenyl groups and 95% methyl groups. The "di-" prefix indicates that each silicon atom contains two of that particular group. Sometimes this prefix is omitted even though two identical groups are present.

Traditional polysiloxane-type GC stationary phases degrade at elevated temperature,[16] and consists of the thermal rearrangement of the siloxane backbone to produce cyclic groups. These groups are volatile and elute from the column as “column bleed.”

Modern ‘MS’ designated ‘phenyl type’ columns are designed with the functional phenyl groups within the polymer backbone (siliphenylene moieties) restricts the formation of the cyclic degradation products and the bleed process is decreased. Shown opposite are some typical GC-MS stationary phases and an indication of their application areas.[17,18]



Example of a GC-MS low bleed stationary phase presenting siliphenylene moieties



Phase Composition: 100% dimethyl polysiloxane
Polarity: Non-polar

Uses: Solvents, petroleum products, pharmaceutical samples, waxes, hydrocarbons, pesticides, sulfur compounds, amines, solvent impurities, etc

Manufacturer	Column Cross References
Agilent/J&W	HP-1, HP-101, HP-1MS, Ultra-1, DB-1, DB-1MS, DB-1ht, SE-30
Alltech	AT-1, SE-30, AT-1MS, EC-1
Macherey-Nagel	Optima 1
PerkinElmer	Elite-1
Phenomenex	ZB-1
Restek	Rtx-1, Rtx-1MS, Stx-1HT
SGE	BP-1
Supelco	Equity-1, SPB-1, SP-2100, SPB-1 Sulfur, SE-30, MDN-1
Varian/Chrompack	VF-1MS, CP Sil 5 CB, CP Sil 5 CB MS, SolGel-1MS

GC-MS Column Selection

Most stationary phases can be used with a GC/MS system. However, it is a good idea to choose a phase for your application that has the lowest amount of column bleed possible and the lowest linear velocity of carrier into the vacuum region of the MS device.[1,11,12,14] There are a few simple rules for choosing GC/MS columns:[15]

1. Choose a low-bleed phase for your application. Several low-bleed versions of the most common GC stationary phases are currently available for GC/MS, these columns also have the advantage of an increased temperature upper limit.

2. If a low-bleed column is not available, choose a low-polarity column with a moderate film thickness. The amount of bleed will rise with increases in polarity, film thickness, and length.

3. Column length and internal diameter combinations are restricted to provide the appropriate GC/MS flow rate.

- Narrow-bore columns (0.25mm i.d. and smaller) can be directly coupled to the GC interface.
- Wide-bore columns (0.32 mm i.d. and 30m or longer) can be directly coupled to the interface. If using a short length column (less than 30m), an effluent splitter or jet separator should be used.
- Columns with internal diameters greater than 0.45mm should not be directly coupled to the GC interface, an effluent splitter or jet separator should be installed

For more information about GC columns, please refer to the GC channel.[1]

Table 3. Column outlet flows for columns of varying internal diameter at optimum linear velocity and 100°C

Carrier gas.	Column length (m)	Flow rate (mL/min)			
		0.20 mm column I.D.	0.25 mm column I.D.	0.32 mm column I.D.	0.53 mm column I.D.
Hydrogen	15	0.77	1.1	1.73	4.34
	30	0.98	1.29	1.9	4.5
	60	1.43	1.72	2.27	4.93
Nitrogen	15	0.21	0.31	0.49	1.24
	30	0.24	0.34	0.51	1.38
	60	0.31	0.41	0.58	1.38
Helium	15	0.66	0.91	1.35	3.35
	30	1.01	1.14	1.56	3.54
	60	1.47	1.66	2.04	3.97

The column selection requires good knowledge of the separation to undertake. According to the chemical nature of the sample it is possible to have an idea of the correct column (column dimensions and stationary phase) for the separation to take place. It is not possible to predict the correct column for each application, but it is possible to make an educated guess to start. It is important to consult column manufacturers, they are the ones that best know their own columns and are able to put you in the correct direction.

Table 4. Some stationary phase typical applications

Stationary Phase	Typical Applications
100% dimethyl polysiloxane	Petroleum products, waxes, hydrocarbons, pesticides, sulfur compounds, amines, solvent impurities, etc
95% dimethyl / 5% diphenyl polysilarylene	Flavors, environmental, pesticides, PCBs, aromatic hydrocarbons
80% dimethyl / 20% diphenyl polysiloxane	Volatile compounds, alcohols
6% cyanopropylphenyl / 94% dimethyl polysiloxane	Insecticides, carbohydrates, fatty acids
65% dimethyl / 35% diphenyl polysiloxane	Pesticides, PCBs, amines, nitrogen-containing herbicides
14% cyanopropylphenyl / 86% dimethyl polysiloxane	Pesticides, PCBs, alcohols, oxygenates
Trifluoropropylmethyl polysiloxane	Freon gases, drugs, ketones, alcohols
65% diphenyl / 35% dimethyl polysiloxane	Triglycerides, rosin acids, free fatty acids
Carbowax (polyethylene glycol –PEG)	Acids, amines, solvents, xylene isomers

Fittings

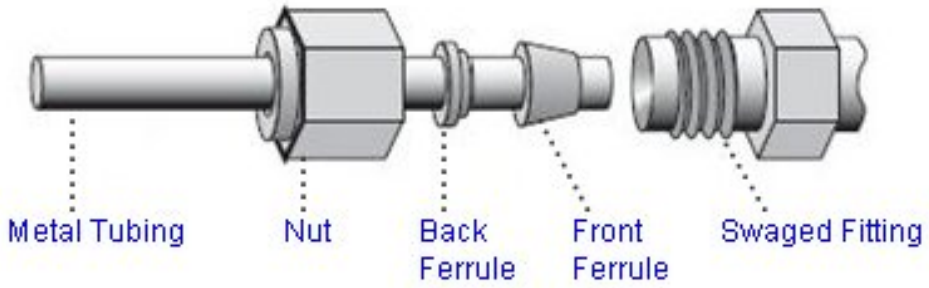
In order to get optimum performance, GC-MS systems require the use of appropriate fittings. Proper fittings help to ensure best separation and detection performance are achieved while getting the most out of gas tanks and filters.

GC-MS connections have specialized requirements and assembly procedures that must be followed. Not doing so risks wasting analysis gases and exhausting in-line filters prematurely, as well as causing excessive band broadening and peak distortion, shortened column life, and poor MS detector performance. The correct way of using some GC-MS fittings is presented opposite.

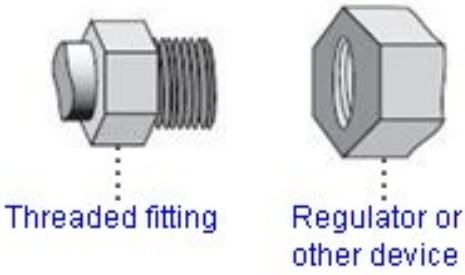
Leaks cause a number of problems, including poor retention time and peak area reproducibility, loss of accuracy, drifting detector baselines, high detector noise, shortened column life, and increased gas consumption.

A leak is not just a wasteful one-way path from the high pressures inside the tubing to the atmosphere; oxygen can diffuse from the atmosphere back into areas where its partial pressure is lower, which is the case with GC-MS. High oxygen levels in the carrier gas can cause excessive stationary phase bleed and greatly reduced column life, as well as increased detector or mass spectrometry (MS) noise and background signal levels.[19,20]

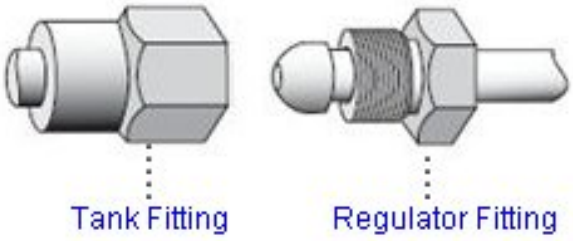
Swaged fitting for metal tubing



Swaged fitting for metal tubing



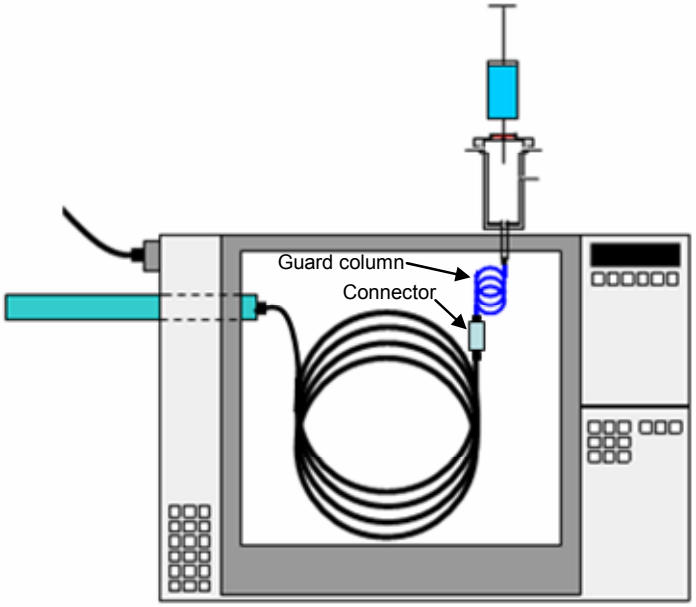
Threaded pipe fitting



Regulator fitting

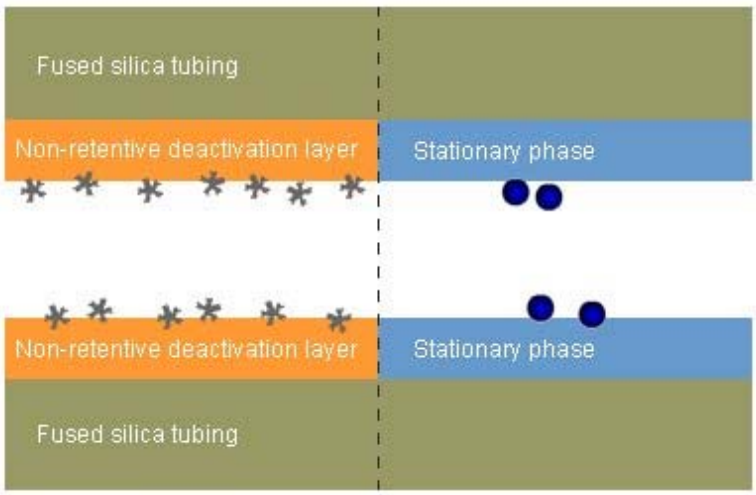
Guard columns (Retention Gap)

A guard column is a short length of deactivated, uncoated fused silica that is placed between the injection port and the analytical column. Guard columns are coupled to the analytical column by using connectors or by using columns that incorporate both, guard and analytical column in a continuous length of tube.[21,22,23]



Guard column (Retention Gap)

Guard columns prevent non-volatile residues from collecting at the head of the analytical column. The alternative to guard columns is packed inlets, but in general, they only remove a portion of the residue. Non-volatile residues may be high molecular weight organic compounds, inorganic salts, or particulate materials. If these contaminants enter the analytical column, they can cause adsorption of active compounds, loss of resolution, and poor peak symmetry. When this contamination begins to affect sample analysis, a small section of the analytical column must be removed to restore proper performance.

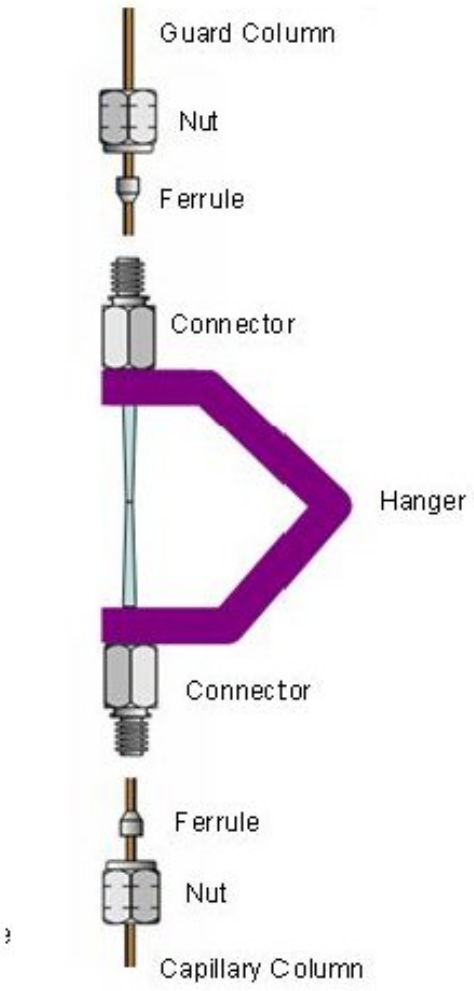


Guard column protection.

Each time a section of the analytical column is removed, the retention time is altered and resolution is decreased, eventually resulting in a useless column. By removing contaminated loops from the guard column, the inertness and length of the analytical column remains intact.

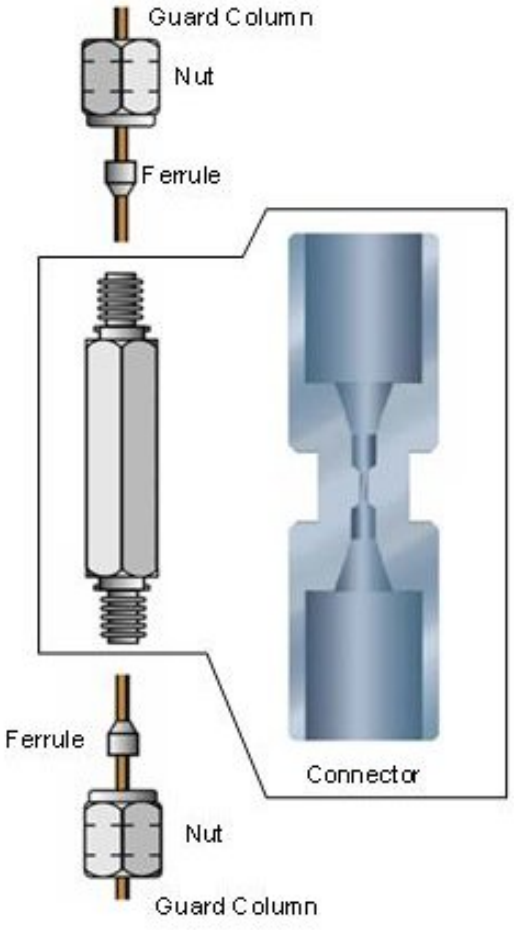
The amount of time the sample spends in the guard column is minimized since there is no stationary phase. This reduces the interaction between sample components and contamination from non-volatile residues. Guard columns allow more injections to be made before contamination interferes with analytical results.

Table 5. Connectors in GC and GC-MS.



GC Connectors.

IMPORTANT: Not all connectors in GC are suitable for GC/MS applications



GC/MS Connectors

IMPORTANT: Certain manufacturers offer columns that incorporate both, guard and analytical columns in a continuous length of tube (leak free), eliminating connection associated problems.

Air leaks

The connections between a GC column injector and detector are points at which leaks can develop.

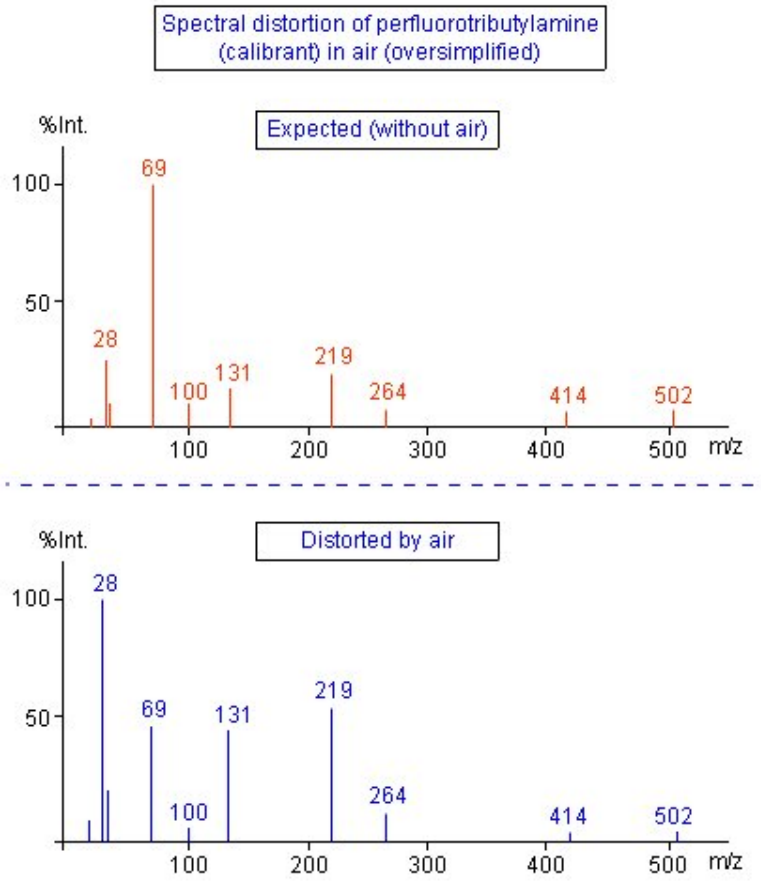
Air leaks are a problem for any instrument that requires a vacuum to operate. Leaks are generally caused by vacuum seals that are damaged or not fastened correctly.

Air leaks also allow contaminants entering into the system, adversely affecting the analysis.

Symptoms of leaks include:

- Higher than normal vacuum manifold pressure or foreline pressure.
- Higher than normal background.
- Peaks characteristic of air (m/z 18, 28, 32, and 44 or m/z 14 and 16).
- Poor sensitivity.
- Low relative abundance of m/z 502 (this varies with the MS used).

The proper column nut and ferrule combination are critical for a leak-tight seal. The proper ferrule will be dependent on column outer diameter. The ferrule should only be slightly larger than the column outer diameter.[15]



Ferrules

Overview

Despite their importance, ferrules are sometimes underestimated; however, without ferrules the airtight sealing that is required at the MS detector and injector of a GC system would be impossible to achieve.

The connections between a GC column injector and detector are points at which leaks can develop. Mass spectrometers are particularly prone to air leaks that can also draw contaminants from the atmosphere into the instrument. While all unions are potential leak points, the most problematic is the seal at the transfer line interface of the mass spectrometer.

Ferrules for GC-MS are used to seal the connections between the column and the injection and detection systems.[16] The ideal ferrule will provide seal avoiding leaks, must not stick to the column and must tolerate temperature changes during programming.

The choice of ferrule is largely a personal preference; however, the severe GC-MS working conditions demand specially designed ferrules.

Under the analysis conditions, ferrules should not fragment or allow oxygen to permeate into the system.[24] Until recently Vespel/Graphite ferrules dominated GC-MS applications. Nowadays metallic ferrules are also a very important option.

Graphite Ferrules.

Graphite ferrules

Graphite is the best material to work at high temperature. As graphite is a very soft material, these ferrules are easily destroyed or deformed.

Advantages:

- They perfectly seal in fused silica and glass columns.
- Endure high temperatures (up to 450^{o}C).
- Ideal for FID and NPD detectors.
- Easy to remove.

Disadvantages:

- They are easily deformed and can only be reused if they are not tighten in excess.
- Porous to oxygen. Not recommended for oxygen sensitive detectors **–not recommended for GC-MS applications.**

Vespel Ferrules.

Vespel ferrules

The composition of this mechanically robust ferrule is 100% polyamide. It is a reusable ferrule ideal for glass and metal columns. Upper temperature limit of 350°C

Advantages:

- Ideal for applications with isotherm temperature.
- Mechanically robust.

Disadvantages:

- Must be frequently retightened.
- Leaks in case of temperature programming.
- At high temperatures may adhere to glass or metal.
- **Not recommended for GC-MS applications.**

Metallic Ferrules

Metallic (aluminium) ferrules

Metallic ferrules together with the metal nut have the same coefficient of thermal expansion; consequently, they expand and contract at the same rate eliminating any chance of annoying leaks. Hence, the nut does not need to be re-tightened after initial temperature cycles.

Fused-silica columns cannot use conventional metallic ferrules made of aluminium, steel or brass because the column will be crushed.

Advantages:

- No need for retightening
- Suitable for temperature programmed applications
- Resist high temperatures
- **Recommended for GC-MS applications**

Disadvantages:

- Not suitable for all type of columns

PTFE Ferrules

PTFE (Teflon) ferrules

PTFE ferrules are completely inert and an economical choice. They have an upper temperature limit of 250°C. These ferrules conform well to the shape of the column upon compression and can be reused if handled carefully.

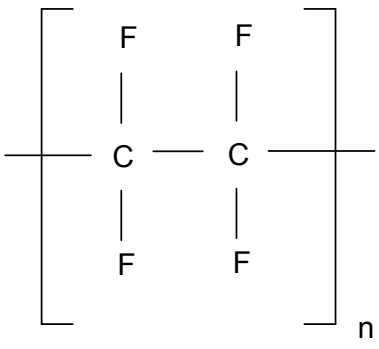
Advantages:

- Reusable but must be carefully handled
- Ideal for glass columns
- It is the most inert ferrule in the market

Disadvantages:

- Must be frequently retightened
- Not suitable for temperature programming applications
- Do not resist high temperatures
- **Not recommended for GC-MS applications**

PTFE: poly(tetrafluoroethene) or poly(tetrafluoroethylene) (PTFE) is a synthetic fluoropolymer which finds numerous applications. PTFE's most well known trademark in the industry is the DuPont brand name Teflon.



Vespel/Graphite Ferrules.

Vespel/Graphite ferrules

Composites of Vespel and Graphite combine the advantages of the two materials. They are less likely to adhere to the column than Vespel but are more durable than graphite. Ferrules are easy to reuse and stable at temperatures to 350°C. Two types dominate the market:

- 85% Vespel / 15% Graphite for GC-MS applications.
- 60% Vespel / 40% graphite for all-purpose applications.

Advantages:

- Ideal for applications with isotherm temperature
- Mechanically robust
- **Recommended for GC-MS applications**

Disadvantages:

- Cannot be reused
- Must be frequently retightened
- Do not resist high temperatures

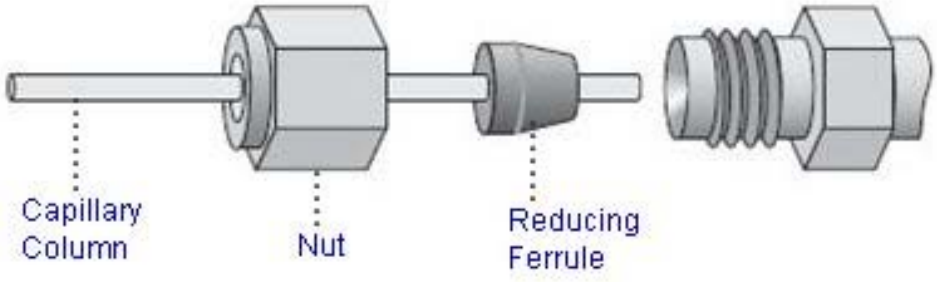
Practicalities

1. When selecting a ferrule consider:
   - Injector temperature
   - Type and sensitivity of the detector
   - Type of material that provides perfect seal to avoid leaks

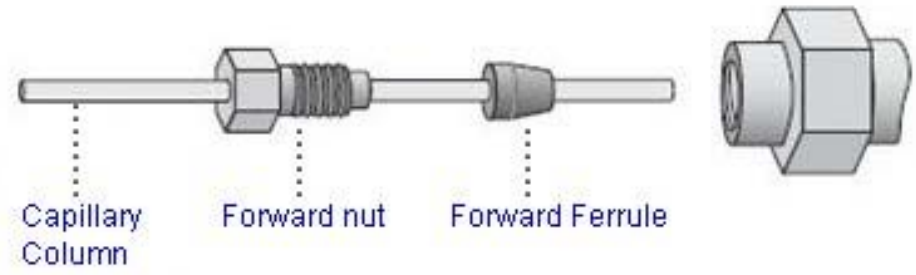
2. How to avoid problems:
   - Change the ferrules on installing a new column
   - Avoid all type of fingers' grease and other contaminants
   - Do not over tighten the ferrules
   - Observe if the reusable ferrules are damaged before using them again

3. When is necessary to change the ferrules:
   - When some changes are observed in retention times
   - In case of baseline drift caused by the entrance of oxygen and possible reaction with the stationary phase
   - When sample loss is observed
   - Increase of the detector background signal.

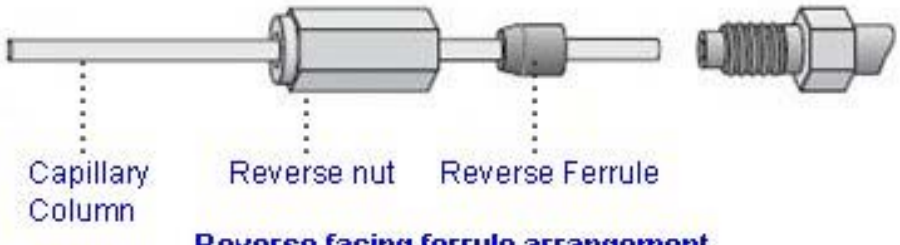
4. There is ONE way of correctly using a ferrule, in case of doubt ask!!! The correct way of using some common ferrules is presented next.[16]



Reducing ferrule arrangement



Forward facing ferrule arrangement



Reverse facing ferrule arrangement

Septum

Overview

The septum isolates the inlet from atmospheric pressure (allowing the inlet to be pressurised). The septum is pierced with the injection syringe needle to allow the sample to be injected.[1] There are many types of septa available. Care should be taken to use the correct septum and syringe combination for optimum performance.

Size: The diameter and depth of the septum is important to achieve a good fit in the upper part of the inlet as well as the correct compression characteristics when the septum nut is tightened. Check with your manufacturer for the correct size.

Material: The material of construction generally dictates the temperature profile and the level of bleed seen from the septum in routine use.

Sandwich: Some septa are manufactured using 'layers' of different material, usually a softer upper layer to minimised 'coring' and a temperature optimised lower layer to give less bleed. Septa may have up to three discrete layers.

PTFE Faced: The upper and/or lower face of the septum may be covered with a thin layer of PTFE. This performs two functions –on the upper surface it will help to reduce septum coring. On the lower face it will also help to reduce septum bleed.

Pre-drilled: Many septa are available with a counter-sun k pre-drilled hole through to act as a needle guide and to extend the number of injections possible with the septum.

Table 5. Some common types of septum.

Septum.	Information
	**Rubber/PTFE** • Routine analysis. • Moderate resealing. • Excellent chemical inertness. • Not recommended for multiple. • injections or storage of samples. • Least expensive.
	**Silicone/PTFE** • Excellent resealing. • Resists coring. • Good for multiple injections.
	**High Pressure** • General purpose septa. • Available in various formulations including 100% silicone, and PTFE- or polyimide-coated silicone.
	**PTF/Silicone/PTFE** • Used in trace analysis applications. • Above average resealing. • Most resistant to coring.

Selection

There are many types of septa available. The most successful designs include a septum core (made of one or more different materials) which can be coated or sandwiched between different layers.

Care should be taken to use the correct septum and syringe combination for optimum performance.[13]

Table 6. Some common types of septum currently used in GC-MS applications.

Septum Material	Compatible with	Incompatible with	Max. Temp.
Rubber (Natural/Butyl)	ACN, acetone, DMF, alcohols, diethylamine, DMSO, phenols	Chlorinated solvents, aromatics, hydrocarbons, carbon disulfide	100 °C
PTFE/Natural or Butyl Rubber	PTFE resistance until punctured, then septa or liner will have compatibility of rubber	---	100 °C
Silicone/Silicone Rubber	Alcohol, acetone, ether, DMF, DMSO	ACN, THF, benzene chloroform, pyridine, toluene, hexane, heptane	200 °C
PTFE/Silicone, PTFE/Silicone/PTFE	PTFE resistance until punctured, then septa will have compatibility of silicone	---	200 °C
VITON	Chlorinated solvents, benzene, toluene, alcohols, hexane, heptane	DMF, DMSO, ACN, THF, pyridine, dioxane, methanol, acetone	260 °C

PTFE: also known as poly(tetrafluoroethene) or poly(tetrafluoroethylene) is a synthetic fluoropolymer which finds numerous applications. PTFE's most well known trademark in the industry is the DuPont brand name Teflon.

Natural Rubber: Natural rubber is an elastic hydrocarbon polymer that naturally occurs as a milky colloidal suspension, or latex, in the sap of some plants. It can also be synthesized.

Butyl Rubber: Synthetic rubber that is made by the polymerization of isoprene and isobutylene; provides good resistance to weathering, and high levels of moisture.

Silicone: Silicones are largely inert compounds with a wide variety of forms and uses. Typically heat-resistant, nonstick, and rubberlike, they are frequently used in cookware, medical applications, sealants, lubricants, and insulation. Silicones are polymers that include silicon together with carbon, hydrogen, oxygen, and sometimes other chemical elements.

VITON: is a brand of synthetic rubber and fluoropolymer elastomer commonly used in O-rings and other molded or extruded goods. The name is a registered trademark of DuPont.

Considerations

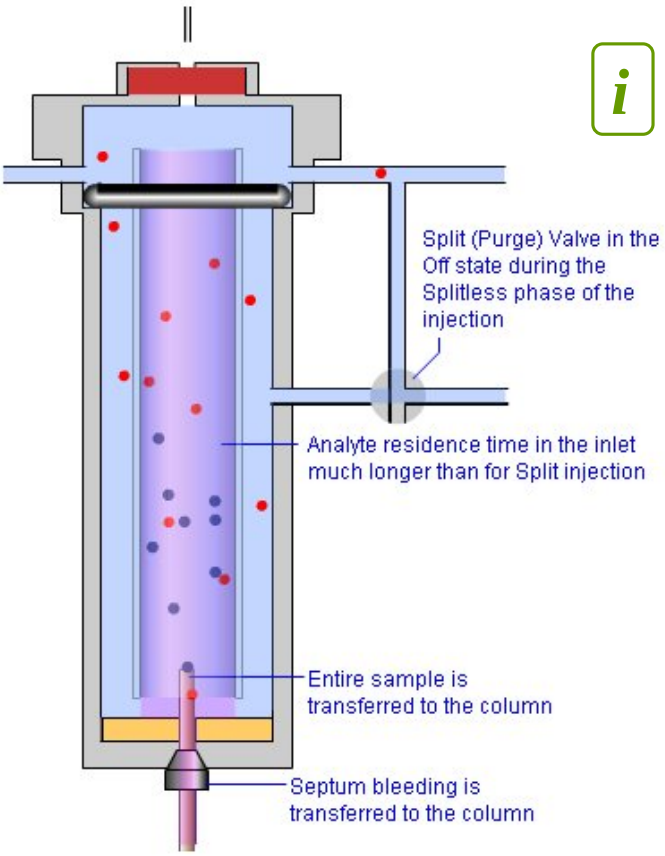
The septum is 'plastic' in nature (i.e. it is deformable), and is held under mechanical pressure with a retaining nut, allowing it to seal around the injection syringe needle and maintain inlet pressure during the injection phase.[1]

Care is required with the torque applied to the septum nut. Over tightening the nut will compress that septum and may promote splitting. Under tightening the nut may cause leaks and pressure failures. Many instrument manufacturers recommend having the septum nut 'fingertight'.

The materials used to plasticise the septum bleed continuously (phthalates etc.). In capillary GC the bleed products may give rise to discrete noise peaks and may also result in a rising baseline as shown opposite. The septum purge flow of the inlet helps to reduce these effects, however correct septum choice with regards to inlet temperature is important.

Following a basic preventative schedule can prevent most septum related problems:

- Change septa regularly
- Ensure that the GC 'Septum Purge' is at the correct flow rate
- Use the correct injection syringe
- Ensure that the septum nut is correctly tight
- Ensure that the injection liner is clean



Bleeding process.

Contamination

Contamination is usually identified by excessive background in the mass spectra. It can come from the GC or from the MS component of the equipment.

The source of the contamination can sometimes be determined by identifying the contaminants. Some contaminants are much more likely to originate in the GC, some others in the MS port.

Contamination sources in the GC port:

- Column or septum bleed.
- Dirty injection port.
- Injection port liner.
- Contaminated syringe.
- Poor quality carrier gas.
- Dirty carrier gas tubing.
- Fingerprints.
- Air leaks.
- Cleaning solvents and materials.

The action required to remove the contamination depends on the type and level of contamination. Minor contamination by water or solvents can usually be removed by allowing the system to pump (with a flow of clean carrier gas). Serious contamination by rough pump oil, diffusion pump fluid or fingerprints is much more difficult to remove; it may require extensive cleaning.[15]

Table 7. Common GC-MS contaminants

Ions (m/z)	Compound	Possible Source
13,14,15,16	Methane	CI gas
18, 28, 32, 44 or 14, 16	H_2O, N_2, O_2, CO_2 or N, O	Residual air and water, air leaks
31, 51, 69, 100, 119, 131, 169, 181, 214, 219, 264, 376, 414, 426, 464, 502, 576, 614	PFTBA and related ions	PFTBA (tuning compound)
31	Methanol	Cleaning solvent
43, 58	Acetone	Cleaning solvent
78	Benzene	Cleaning solvent
91, 92	Toluene or xylene	Cleaning solvent
105, 106	Xylene	Cleaning solvent
151, 153	Trichloroethane	Cleaning solvent
69	Foreline pump fluid or PFTBA	Foreline pump oil vapor or calibration valve leak
73, 147, 207, 221, 281, 295, 355, 429	Dimethylpolysiloxane	Septum bleed or methyl silicone column coating
77, 94, 115, 141, 168, 170, 262, 354, 446	Diffusion pump fluid	Diffusion pump fluid and related ions
149	Plasticizer (phthalates)	Vacuum seals (O-rings) damaged by high temperatures, use of vinyl or plastic gloves
Peaks spaced 14 Da apart	Hydrocarbons	Fingerprints, foreline pump oil