Fundamentals of Gas Chromatography: Theory

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Introduction

In analytical chemistry, scientists use **gas chromatography (GC)** to separate and analyze compounds that can be vaporized without decomposition. They often use GC to test the purity of a particular substance, or to separate the components of a mixture to determine the relative amounts of each.

Scientists use GC for both qualitative and quantitative analysis of volatile analytes.

The instrument, called a gas chromatograph, employs a mobile phase and a stationary phase. That is, a moving gas carries the sample across a stationary support (a piece of glass or metal tubing called a column) inside the instrument.

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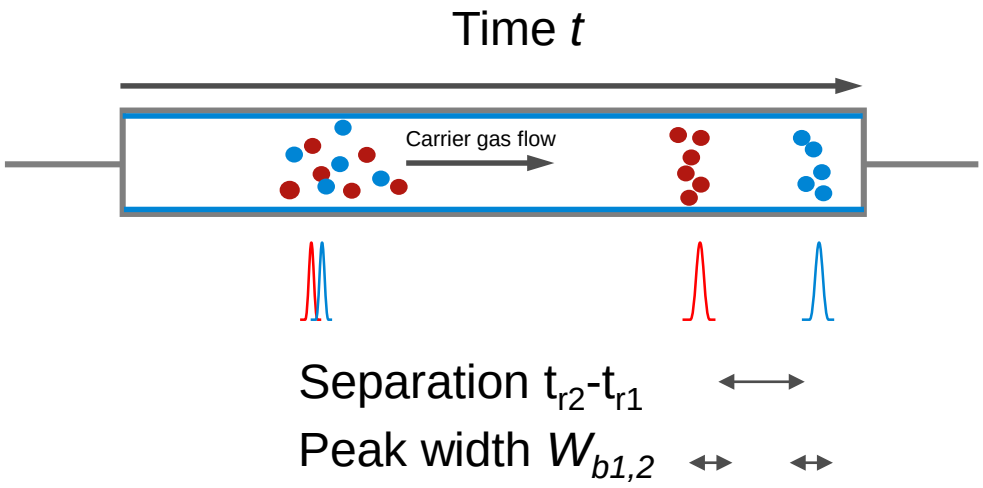
Introduction
Compound Separation



Compounds are separated by their different affinities to the column during the stationary phase. Compounds with less affinity will elute from the column sooner; compounds with greater affinity will elute later.

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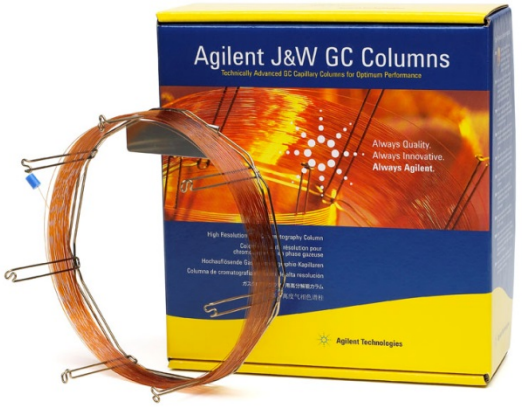
Introduction

What Happens Inside the Column?

Gas chromatography uses a gaseous mobile phase to transport the sample through the column, which can be packed or coated on its inside surface. In most cases, GC columns have smaller internal diameters and are longer than HPLC columns.

As the GC column is heated, the compounds begin to separate based on boiling point. Changing the column to polar stationary phase will change the separation capabilities. Compounds will separate by both boiling point and polarity characteristics.



GC columns

HPLC columns

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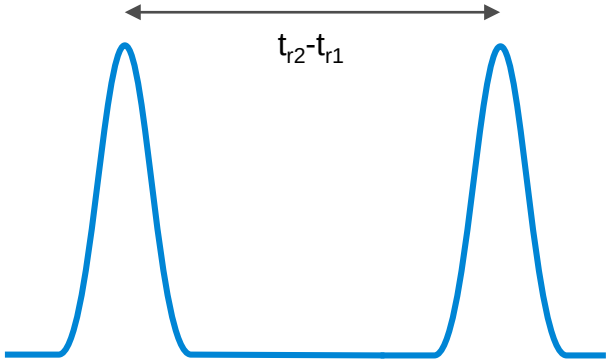
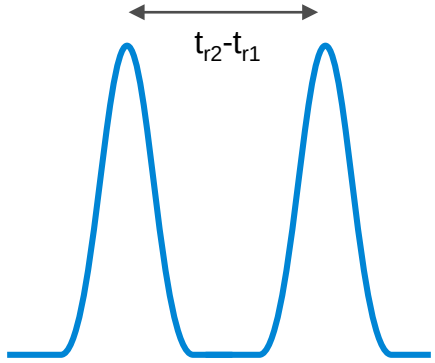
Introduction

What Happens Inside the Column?

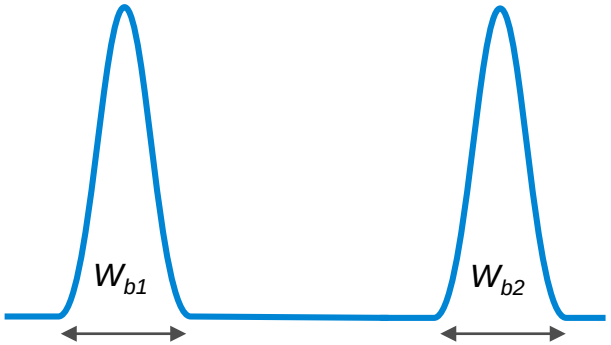


Superior separation

vs

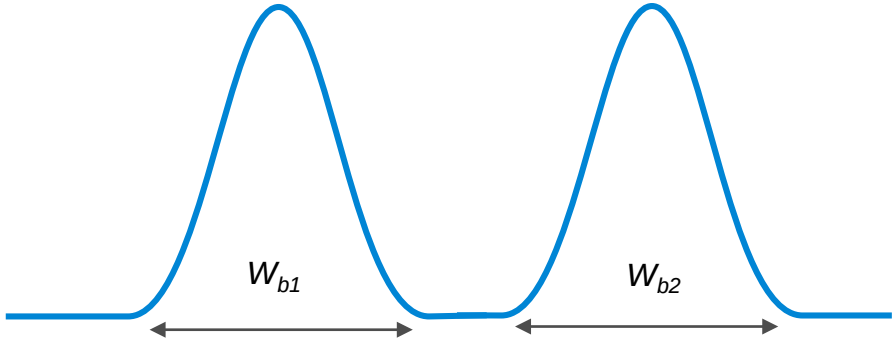


Inferior separation



Superior separation

vs



Inferior separation

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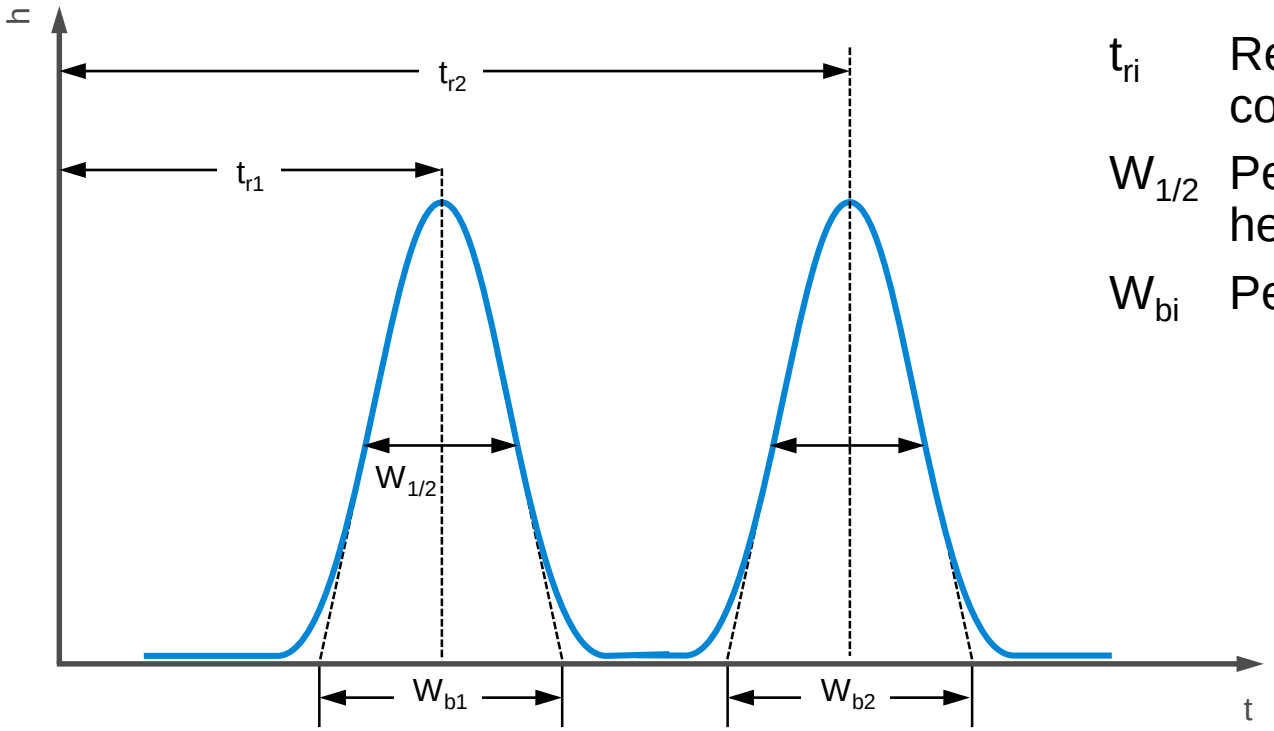
Key Parameters

Retention Time and Peak Width



t_{ri}	Retention time compound i
$W_{1/2}$	Peak width at half height
W_{bi}	Peak width at baseline

The retention time of an unretained compound (t_M or t_0) is also known as hold-up time. Unretained solute molecules travel down the column at the same rate as the carrier gas.

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Key Parameters

Retention Factor (k`)

$$k` = \left(\frac{t_r - t_M}{t_M} \right) = \frac{t'_R}{t_M}$$

t_r Retention time

t_M Retention time of unretained peak

The retention factor (also known as partition ratio or capacity factor) is the ratio between the time a solute spends in the stationary and mobile phases. It is calculated by dividing the retention time by the time for an unretained peak (t_M). An unretained compound has k = 0.

Since all solutes spend the same amount of time in the mobile phase, the retention factor is a measure of retention by the stationary phase.

Parameter influencing retention factor:

- Stationary phase

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Key Parameters

Selectivity or Separation Factor (α)

$$\alpha = \frac{k_2}{k_1}$$

α	Selectivity
k_1	Retention factor of first peak
k_2	Retention factor of second peak

Selectivity is a measure of the time or distance between two peaks.

If α = 1, the two peaks have the same retention time and co-elute.

Selectivity is defined as the ratio in capacity factors.

Parameters influencing retention factor:

- Stationary phase
- Mobile phase
- Temperature

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Key Parameters

Efficiency or Number of Theoretical Plates (N)

$$N = 16 \cdot \left(\frac{t_r}{W_b} \right)^2 \qquad N = 5.54 \cdot \left(\frac{t_r}{W_{1/2}} \right)^2$$

Column efficiency is used to compare the performance of different columns. It is expressed as the theoretical plate number, N.

Columns with high plate numbers are more efficient. A column with a high N will lead to narrower peak at a given retention time than a column with a lower N.

Parameters influencing column efficiency:

- Column length (increasing colum length increases efficiency)
- Particle size (decreasing particle size increases efficiency)

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Key Parameters

Height Equivalent to a Theoretical Plate (H)

$$H = \frac{L}{N}$$

L	Length of column (mm)
N	Number of theoretical plates

Another measure of column efficiency is the height equivalent to a theoretical plate, denoted as H. It is usually reported in millimeters.

The shorter each theoretical plate, the more plates are “contained” in any length of column. This translates to more plates per meter and higher column efficiency.

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Key Parameters

Utilization of Theoretical Efficiency (UTE)

$$UTE\% = \left(\frac{H_{actual}}{H_{theoretical}} \right) \cdot 100$$

Coating efficiency (CE%) is a historical term that compares the measured column efficiency (H_{actual})and its theoretical maximum efficiency ($H_{theoretical}$).

Historically, $H_{theoretical}$ was usually so heavily impacted by heterogeneities in the stationary phase film that extra-column contributions to H_{actual} could be ignored (such as injection anomalies or mechanical and electronic lag times).

Because of improvements to coating efficiency this is no longer the case. H_{actual} is usually more heavily impacted by extra-column contributions than the column itself. The term “utilization of theoretical efficiency”, or UTE, takes these factors into account.

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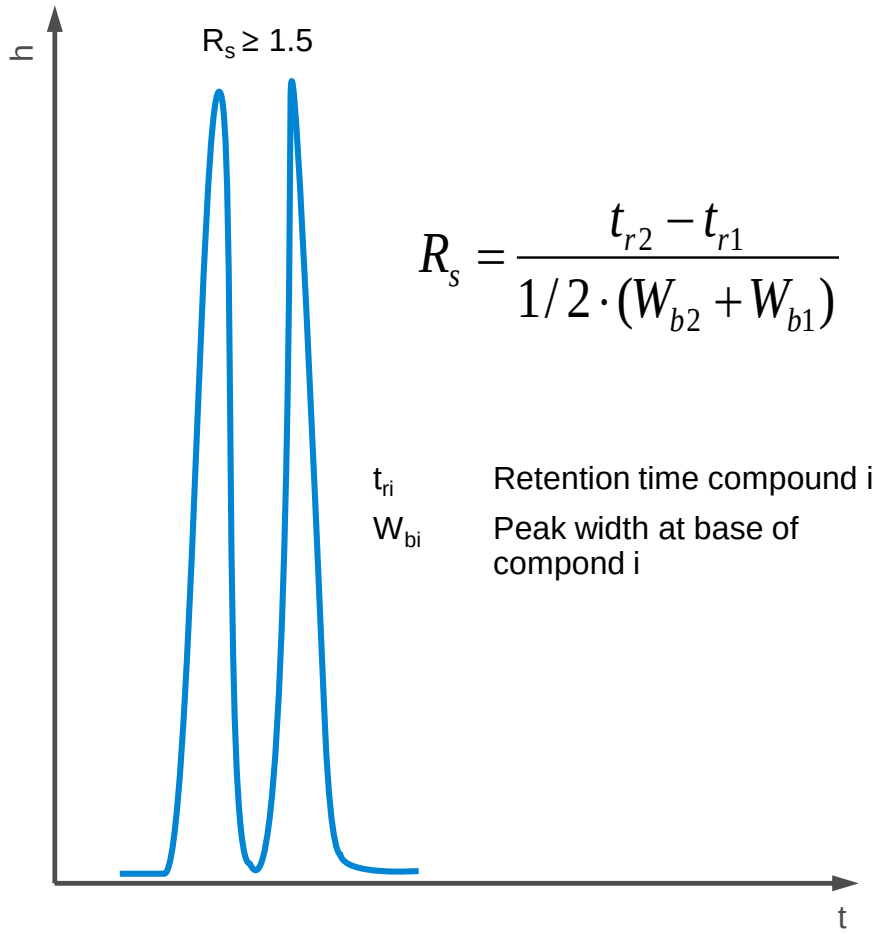
Key Parameters

Resolution – Baseline Separation

Resolution describes the ability of a column to separate the peaks of interest.

Resolution takes into consideration efficiency (N), selectivity (α) and retention (k).

- A value of 1 is the minimum for measurable separation and adequate quantitation.
- A value of 0.6 is required to discern a valley between two equal-height peaks.
- Values of 1.7 or greater generally are desirable for rugged methods.
- A value of 1.6 is considered a baseline separation and ensures the most accurate quantitative result.



$$R_s = \frac{t_{r2} - t_{r1}}{1/2 \cdot (W_{b2} + W_{b1})}$$

t_{ri} Retention time compound i

W_{bi} Peak width at base of compond i

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Key Parameters

Resolution – The Fundamental Equation

$$R_s = \frac{1}{4}\sqrt{N} \cdot \left(\frac{\alpha - 1}{\alpha}\right) \cdot \left(\frac{k}{1+k}\right)$$

Efficiency **Selectivity** **Retention**

One can improve resolution by improving any of these parameters:

- **Selectivity** has the highest influence on the resolution. Small changes in selectivity lead to big changes in resolution.
- **Retention** has a significant influence at small k-values.
- **Efficiency** describes the separation power of the column.

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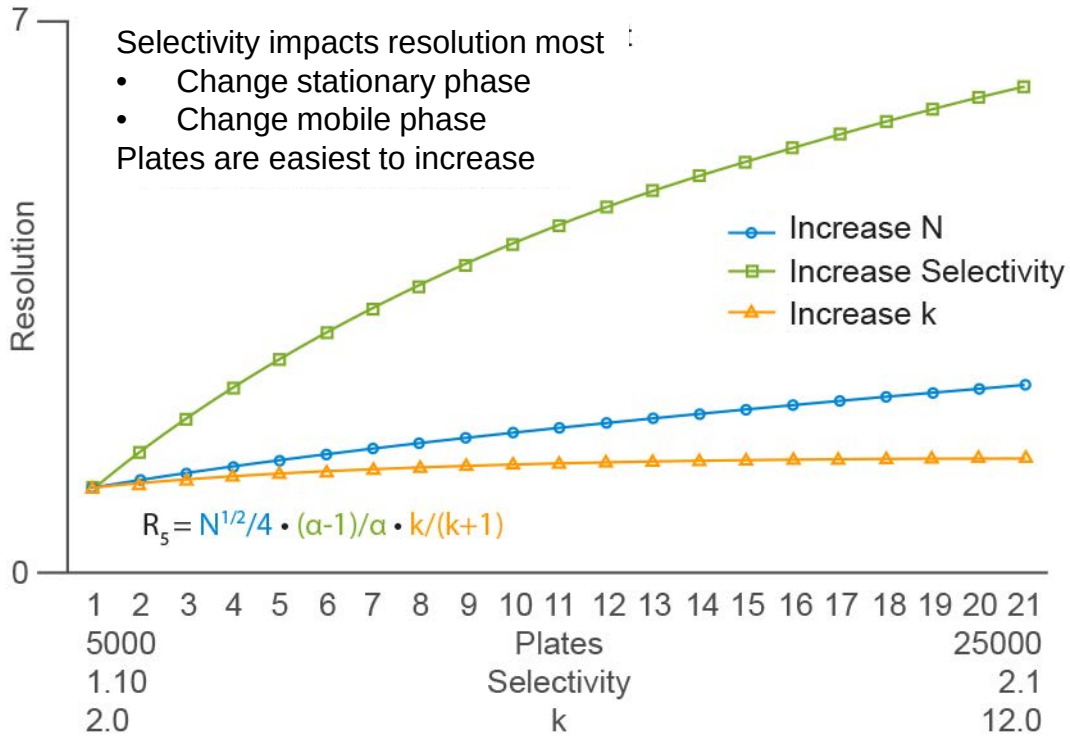
Key Parameters

Influence of N, α, and k on Resolution



The figure shows resolution as a function of selectivity, column efficiency, and retention.

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How to Influence Separations

What is a “Plate” in Chromatography?

$$R_s \sim \frac{1}{4}\sqrt{N} \qquad R_s \sim \frac{1}{4}\sqrt{\frac{L_c}{H}} \sim \frac{1}{4}\sqrt{\frac{L_c}{h \cdot d_p}}$$

L_C	Column length
d_p	Particle size
h	Reduced height of a theoretical plate

A theoretical plate is the hypothetical stage in which two phases of a substance (its liquid and vapor phase) establish an equilibrium.

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How to Influence Separations

High plate number (*N*) provides:

- Sharp and narrow peaks
- Better detection
- Peak capacity to resolve complex samples

But resolution increases only with the square root of the plate number.

- $R_S \sim \sqrt{N}$

Plate number increase is limited by experimental conditions

- Analysis time, pressure

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How to Influence Separations

Bringing It Together – Peak Width and Reduced Height of a Theoretical Plate

$$R_s = \frac{t_{r1} - t_{r2}}{1/2 \cdot (W_{b2} + W_{b1})} \qquad R_s \sim 1/4 \sqrt{\frac{L_c}{h \cdot d_p}}$$

$$h = f(w)$$

$$h = f(w_{eddy} + w_{ax} + w_C)$$

h: reduced height of a theoretical plate

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Van Deemter Equation

The Van Deemter equation relates the variances per unit length of a separation column to the linear mobile phase velocity by considering physical, kinetic, and thermodynamic properties of a separation (Wikipedia).

$$h = f(w_{eddy} + w_{ax} + w_C)$$

$$h = A + B/u + C\,u$$

- Eddy diffusion
- Diffusion coefficient
- Resistance to mass transfer

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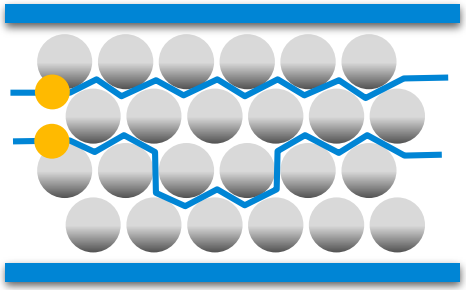
Van Deemter Equation

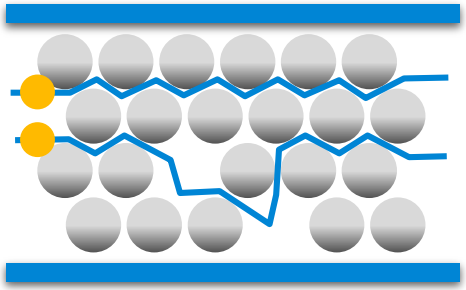
Eddy Diffusion

$w_{eddy} \sim \lambda\, d_p$ λ: Quality of column packing

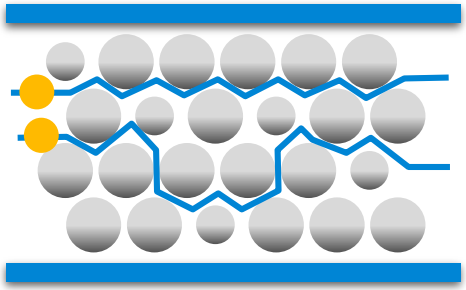
Differences in diffusion paths due to:



Different paths



Poor column packing



Broad particle size distribution

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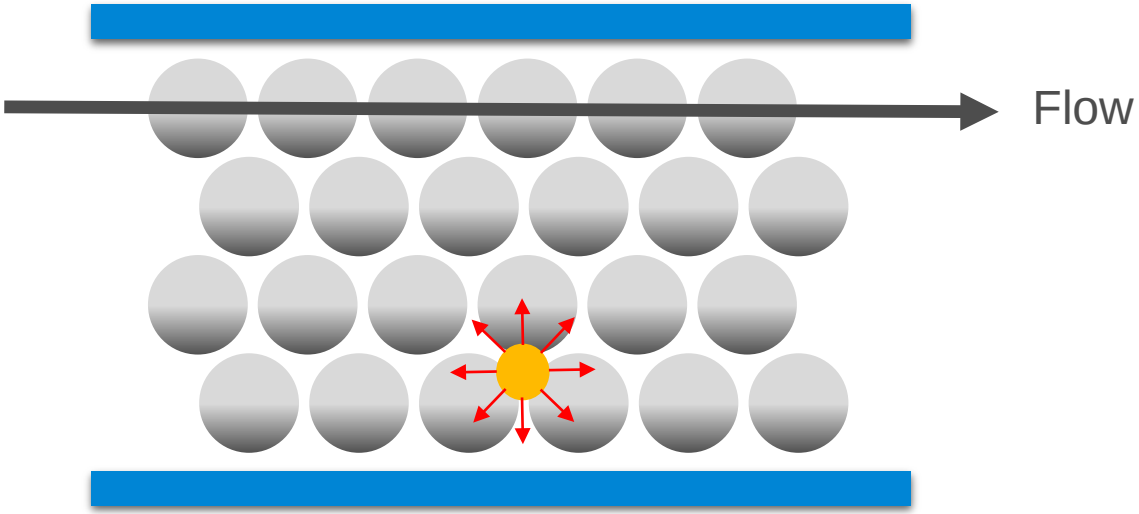
Van Deemter Equation

Axial or Longitudinal Diffusion

Increase in peak width due to self-diffusion of the analyte

At low flow, the analyte remains in the mobile phase for a long time

- High increase in peak width
- Increased height of a theoretical plate



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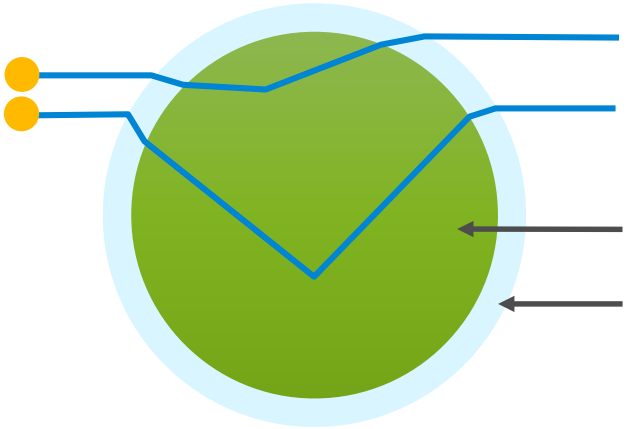
Van Deemter Equation

Resistance to Mass Transfer

$$w_C \sim d_p^2$$



Different diffusion paths

Porous particle

Stationary layer of mobile phase

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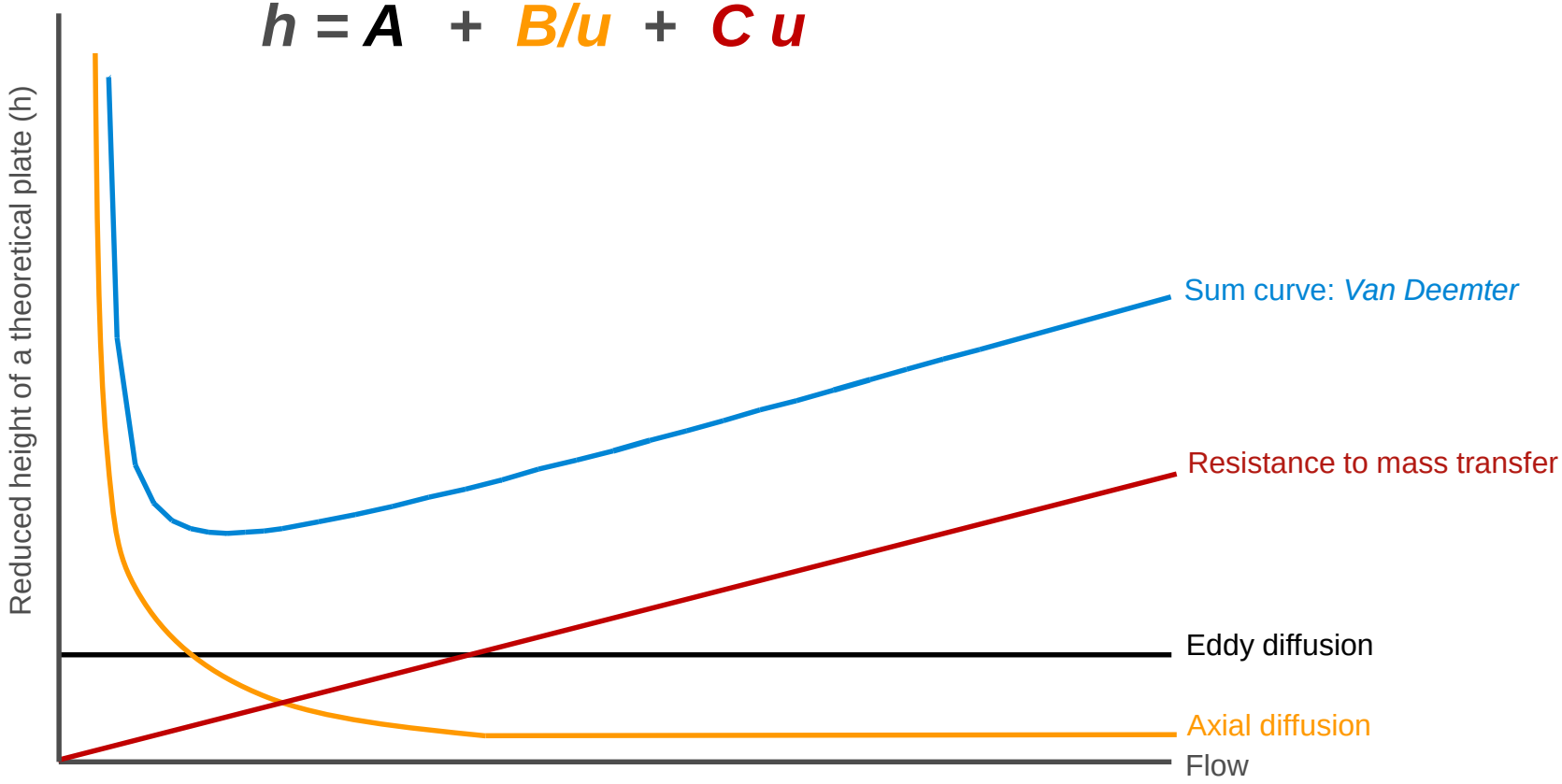
Van Deemter Equation

$$h = A + B/u + C\,u$$



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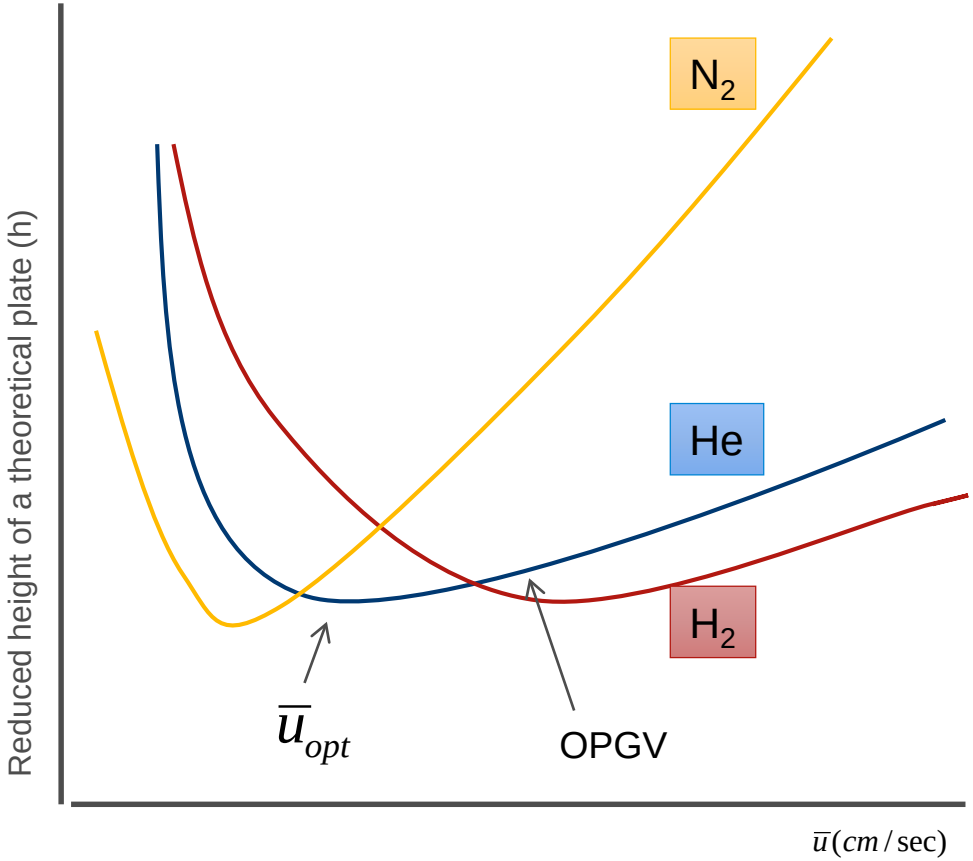
Van Deemter Equation
Carrier gas



Linear velocities (and flow rates) of the carrier gas depend on column temperature. At a constant head pressure, linear velocities decrease as column temperature increases. The effect of carrier gas average linear velocity (u) on efficiency is best illustrated using a van Deemter curve.

$U_{opt:}$ naximum efficiency

OPGV: optimal practical gas velocity

See notes for details

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Publication	Title	Pub. No.
Primer	Fundamentals of Gas Chromatography	G1176-90000
Video	Fundamentals of Gas Chromatography (14 min)	
Guide	Agilent J&W GC Column Selection Guide	5990-9867EN
Web	CHROMacademy – free access for students and university staff to online courses	
Application compendium	A compilation of Application Notes (22MB)	5991-3592EN

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Abbreviations

Abbreviation	Definition
α	selectivity
d_p	particle size
ΔΦ	gradient range
F	flow rate
h	reduced height of a theoretical plate (a measure of the resolving power of a column)
H	height equivalent to a theoretical plate
k	retention factor (also known as capacity factor (k') or partition ratio)
L, L_c	length (column length)
λ	quality of column packing
N	efficiency or column plate number
R	resolution

Abbreviation	Definition
t	time
t_r	retention time
T_0 or t_m	column dead-time, hold-up time
t_G	gradient time
UTE%	utilization of theoretical efficiency
V_m	colum volume
w	peak width
$W_{1/2}$	peak width at half height
W_{bi}	peak width at baseline
w_{eddy}	eddy diffusion
w_{ax}	axial or longitudinal diffusion
w_C	resistance to mass transfer
w_{av}	average peak width

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Fundamentals of Gas Chromatography: Hardware

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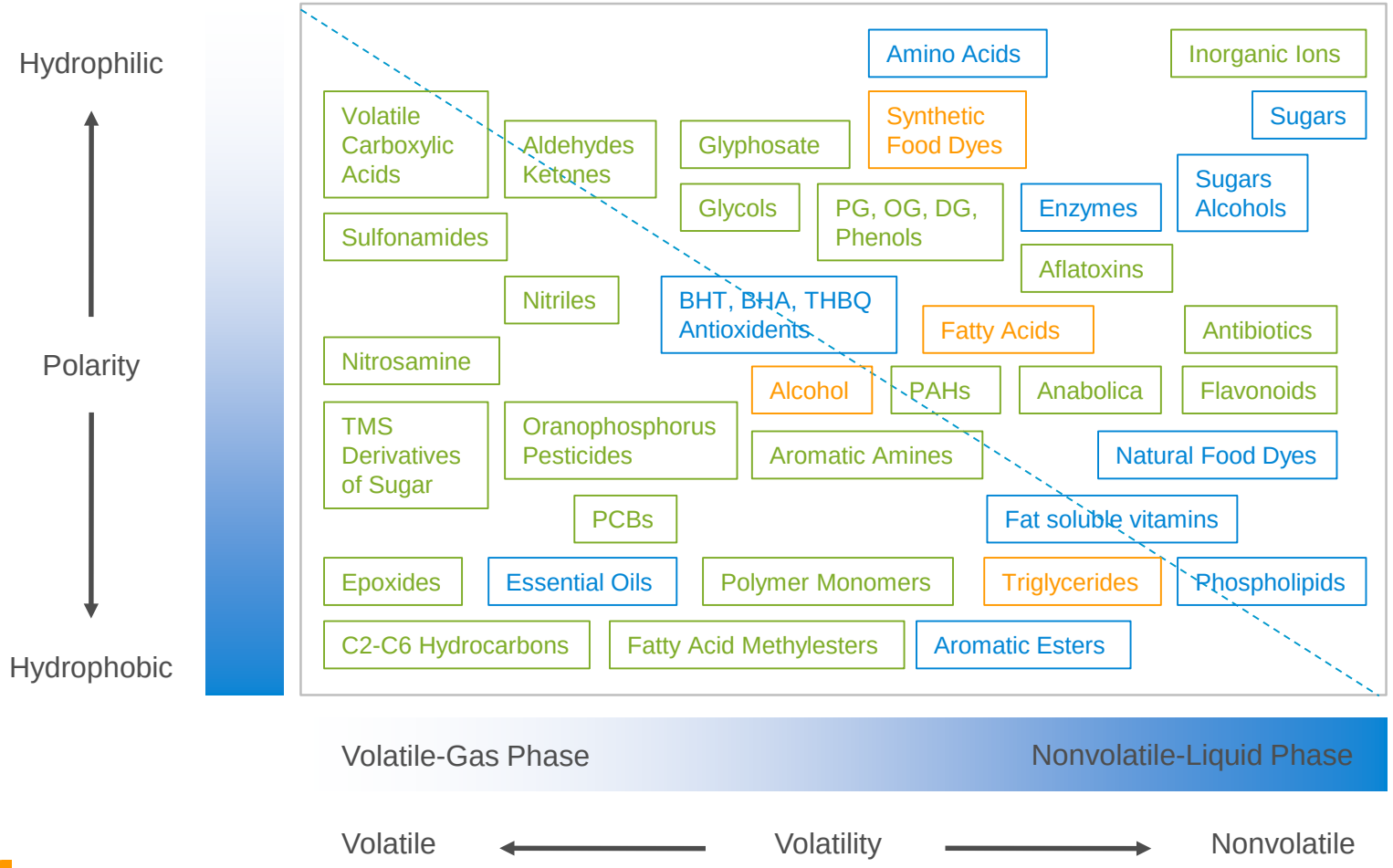
Which Separation Technique for Which Compound?



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Introduction

What Is Gas Chromatography?

Gas chromatography (GC) is a technique to separate the individual components of a given mixture so that each can be identified and quantified.

To be suitable for GC analysis a compound must have sufficient volatility and thermal stability. If all or some of the components of a sample are volatile at around 400°C or below, and do not decompose at these temperatures, the compound can probably be analyzed using a gas chromatograph.

The instrument vaporizes a sample of the compound and transports it via a carrier gas into a column. The components of the sample travel through the column at varying rates depending on their physical properties.

The eluted components enter a heated detector that generates an electronic signal based on its interaction with the component. A data system records the size of the signal and plots it against elapsed time to produce a chromatogram.

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Introduction

What Is GC Used For?

GC is used to separate polar and nonpolar compounds that are volatile.

Typical applications:

- Food and flavor analysis
- Environmental analysis (PAH, pesticide, herbicides, benzene)
- Industrial chemical analysis (alcohol, halogenated hydrocarbons, aromatic solvents, phenols)
- Petroleum industry analysis (gasoline, volatile sulfur compounds, refinery gases)

If a compound is nonvolatile (for example, proteins, salts, polymers), then liquid chromatography is a better separation technique.

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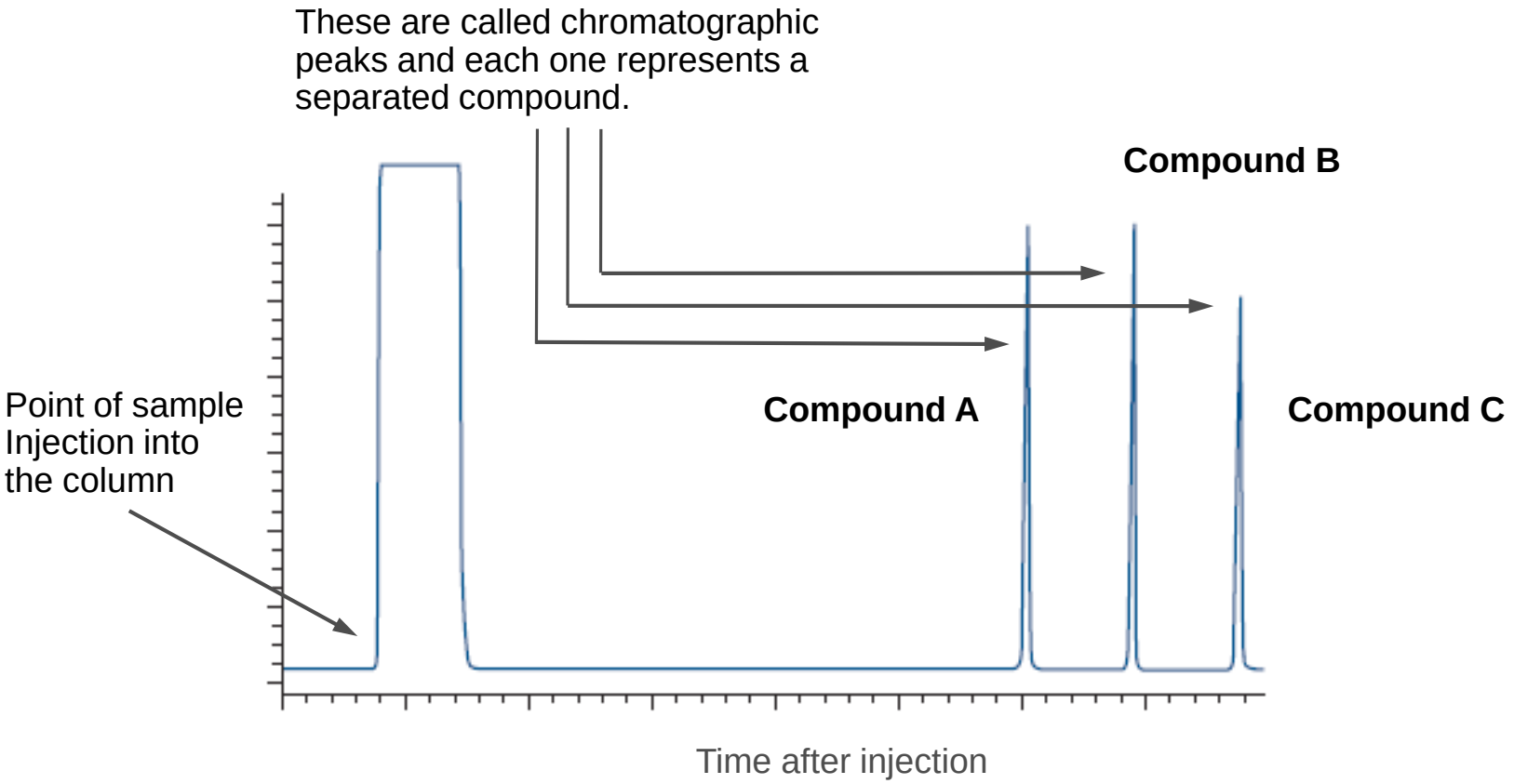
What Does a Chromatogram Look Like?



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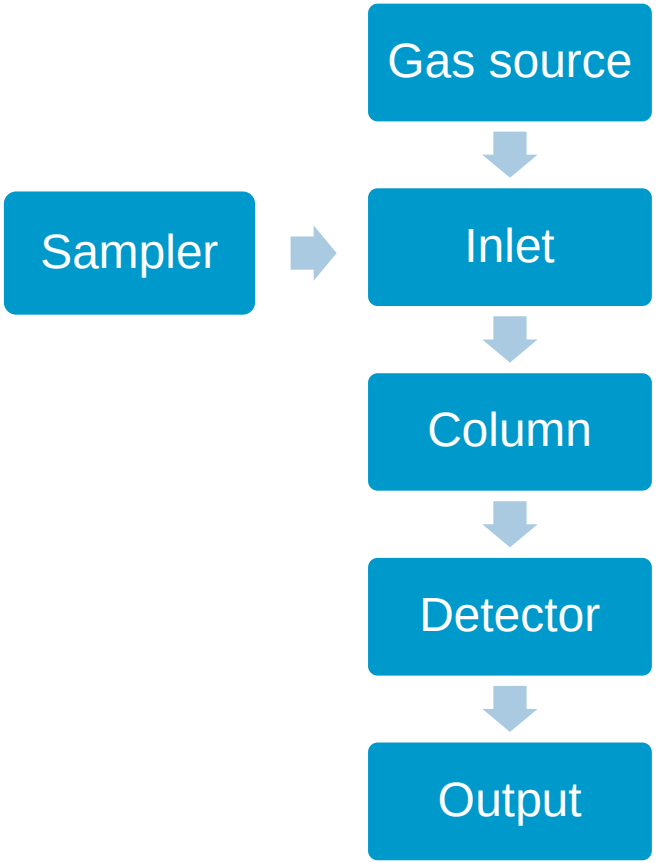
Configuration of a GC System
General Overview

A gas chromatograph consists of

- A regulated and purified carrier gas source, which moves the sample through the instrument
- An inlet, which also acts as a vaporizer for liquid samples
- A column, in which the time separation occurs
- A detector, which responds to the components as they elute from the column by changing its electrical output
- Output: Data interpretation of some sort



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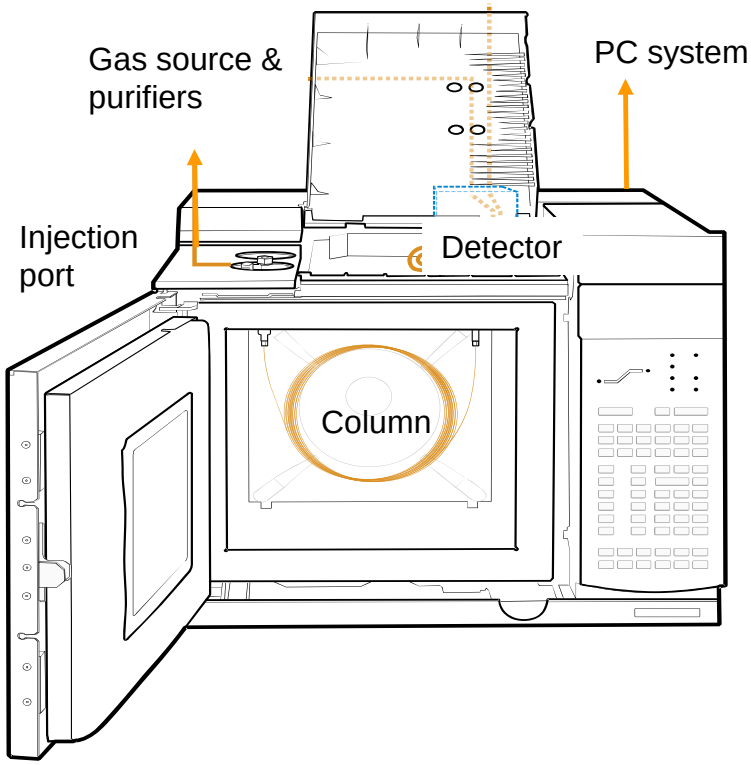
Configuration of a GC System



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Configuring a GC System

The Gas Source

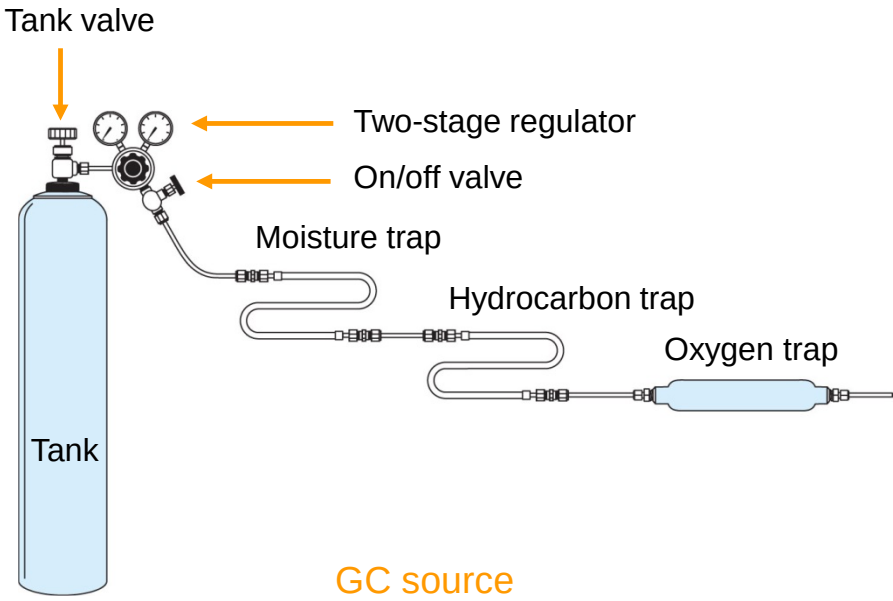
The carrier gas such as helium, nitrogen, hydrogen, or a mixture of argon and methane must be pure (>99.9995%). Contaminants may react with the sample and the column, create spurious peaks, load the detector and raise the baseline, and so on.

The function of the carrier gas is to transport the sample through the system.

A high-purity gas with traps for water, hydrocarbons, and oxygen is recommended.

Specific detector gases support certain detectors (FID, for example).

Compressed gas cylinders or gas generators supply the gas.



GC source

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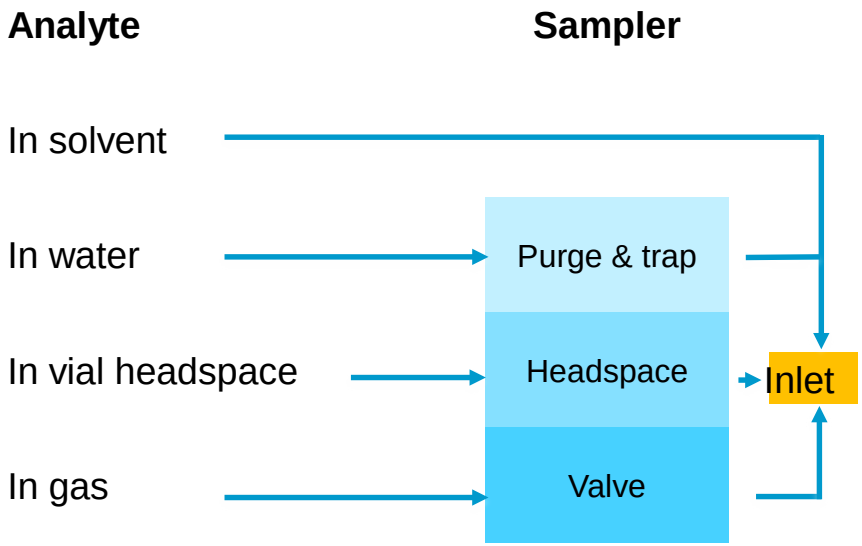
Configuring a GC System
The Sampler

The choice of the sampler depends on the analyte matrix.



GC autosampler

GC headspace sampler

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Configuring a GC System
The Inlet

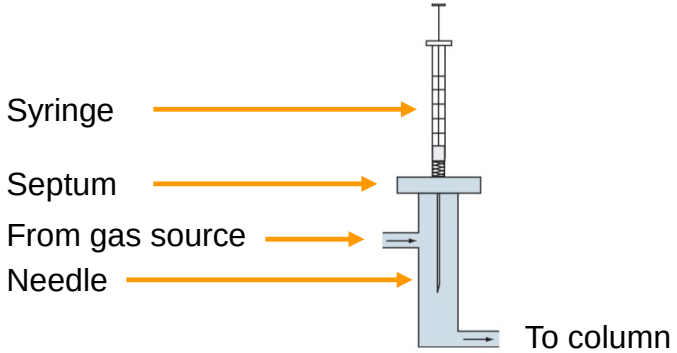
The inlet introduces the vaporized sample into the carrier gas stream. The most common inlets are injection ports and sampling valves.

- Injection ports
  - Handle gas or liquid samples
  - Often heated to vaporize liquid samples

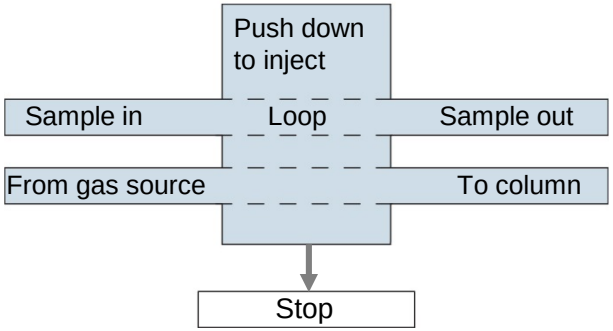
Liquid or gas syringes are used to insert the sample through a septum into the carrier gas stream.

- Sampling valves

The sample is flushed from a loop that is mechanically inserted into the carrier gas stream. Different valves are used for liquids and gases due to different sample volumes



Scheme of injection port



Scheme of sampling valves

Source: Fundamentals of Gas Chromatography
Publication #: G1176-90000

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Configuring a GC System

The Different Inlet Types

Split / Splitless

- This is the most common inlet
- In splitless mode, all the sample goes on column
- Inlet heated to vaporize sample

Cool-on-Column

- Whole sample introduced directly into column
- High precision
- Eliminates sample discrimination
- Eliminates sample degradation

Programmable Temperature

- Sample injected into cool liner
- Inlet heated to vaporize sample

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Configuring a GC System

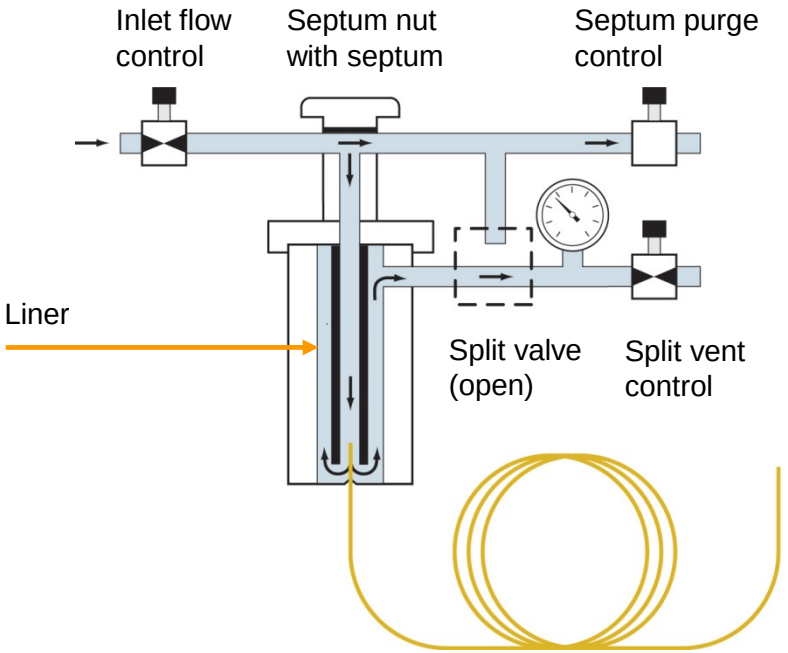
The Different Inlet Types – Split/Splitless Port

Split mode

Capillary columns have low sample capacities. Small sample sizes (µl) must be used to avoid overloading the column.

The split mode provides a way to inject a larger sample, vaporize it, and then transfer only a part of it to the column. The rest is vented as waste.

The split valve remains open. The sample is injected into the liner, where it vaporizes. The vaporized sample divides between the column and the split vent.



A typical split/splitless port in split mode.

Source: Fundamentals of Gas Chromatography
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Configuring GC System

The Different Inlet Types – Split/Splitless Port

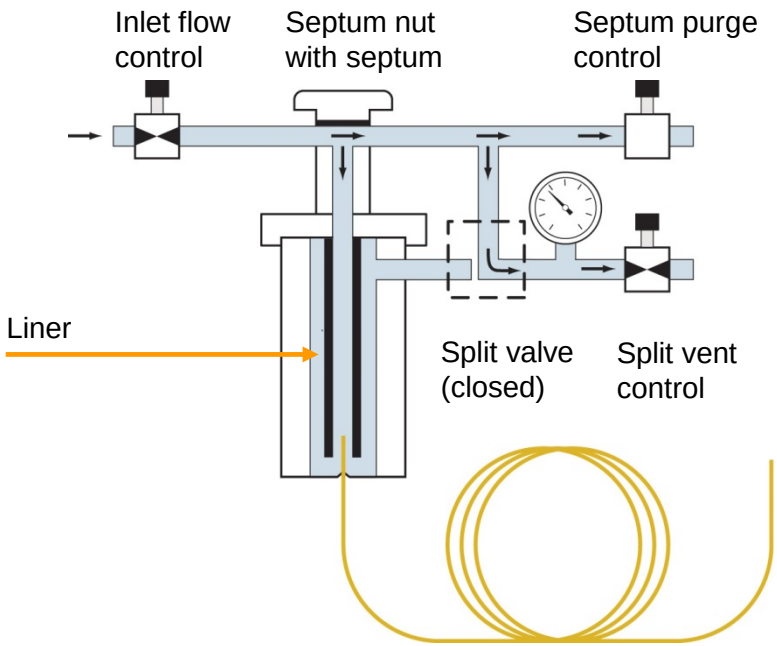
Splitless mode

This mode is well suited to low concentration samples. It traps the sample at the head of the column while venting residual solvent vapor.

Step 1: Split valve closed, sample injected. The solvent (the major component) creates a saturated zone at the head of the column, which traps the sample components.

Step 2: Once the sample is trapped on column, open the split valve. The residual vapor in the inlet, now mostly solvent, is swept out the vent.

The flows are now the same as in the split mode.



Splitless mode in injection.

Source: Fundamentals of Gas Chromatography
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Configuring a GC System
The Column

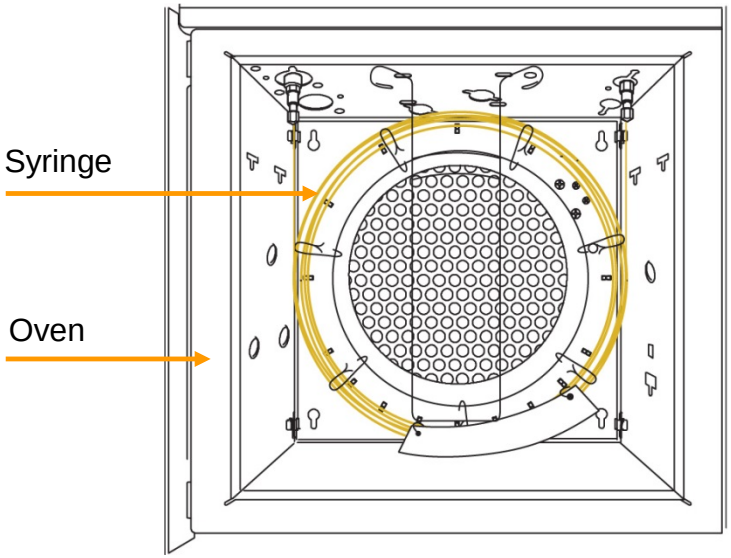
The separation happens here.

Most separations are highly temperature-dependent, so the column is placed in a well-controlled oven.

The sample vapor is directed into a column by a carrier gas. Compounds selectively partition between stationary phase (coating) and mobile phase (carrier gas).

The oven temperature may be ramped to elute all compounds.

- Isothermal: temperature stays the same for run
- Ramped: temperature is raised during run



Column and oven

Source: Fundamentals of Gas Chromatography
Publication #: G1176-90000

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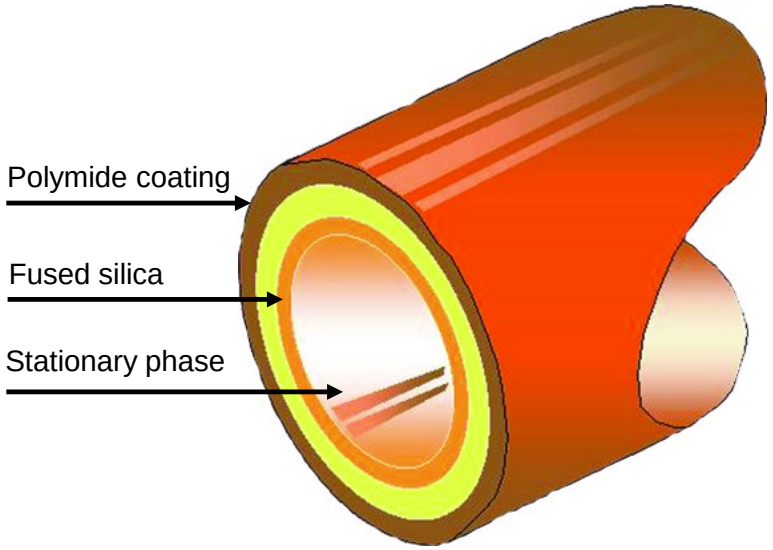
Configuring a GC System
Inside a Capillary Column

A capillary GC column is composed of narrow tubing (0.05 to 0.53 mm ID) with a thin polymer coating (0.1 – 10.0 µm) inside.

Selecting the right capillary column is critical and depends on factors such as selectivity, polarity, and phenyl content.

Column diameter influences efficiency, solute retention, head pressure, and carrier gas flow rate.

Column length affects solute retention, head pressure, bleeding, and costs).



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Configuring a GC System
Column Selection Summary

1. If no information is available about which column to use, start with a DB-1 or DB-5.
2. Low bleed (“ms”) columns are usually more inert and have higher temperature limits.
3. Use the least polar stationary phase that provides satisfactory resolution and analysis times. Non-polar stationary phases have superior lifetimes compared to polar phases.
4. Use a stationary phase with a polarity similar to that of the solutes. This approach works more times than not; however, the best stationary phase is not always found using this technique.
5. If poorly separated solutes possess different dipoles or hydrogen bonding strengths, change to a stationary phase with a different amount of the dipole or hydrogen bonding interaction. Other co-elutions may occur upon changing the stationary phase, thus the new stationary phase may not provide better overall resolution.
6. If possible, avoid using a stationary phase that contains a functionality that generates a large response with a selective detector. For example, cyanopropyl containing stationary phases exhibit a disproportionately large baseline rise (due to column bleed) with NPDs.
7. A DB-1 or DB-5, DB-1701, DB-17, and DB-WAX cover the widest range of selectivities with the smallest number columns.
8. PLOT columns are used for the analysis of gaseous samples at above ambient column temperatures.

Source: Agilent J&W GC Column Selection Guide
Publication #: 5990-9867EN
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Configuring a GC System

The Detector

The gas stream from the column, which contains the separated compounds, passes through a detector. The output from the detector becomes the chromatogram.

Several detector types are available but all perform the same tasks:

- Produce a stable electronic signal (the baseline) when pure carrier gas (no components) is in the detector
- Produce a different signal when a component is passing through the detector.

GC detector

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Configuring a GC System

Common Detectors

Thermal conductivity detector	• Detects compounds with thermal conductivity that differs from carrier gas
Flame ionization detector	• Detects compounds that burn or ionize in a flame
Electron capture detector	• Detects electron-capturing compounds (for example, halogenated compounds)
Nitrogen-phosphorus detector	• Detects compounds that contain nitrogen and phosphorus
Flame photometric detector	• Detects compounds that contain sulfur and phosphorus
Atomic emission detector	• Tunable to many elements
Mass selective detector	• Identifies components from mass spectra (when combined with GC, the most powerful identification tool available)

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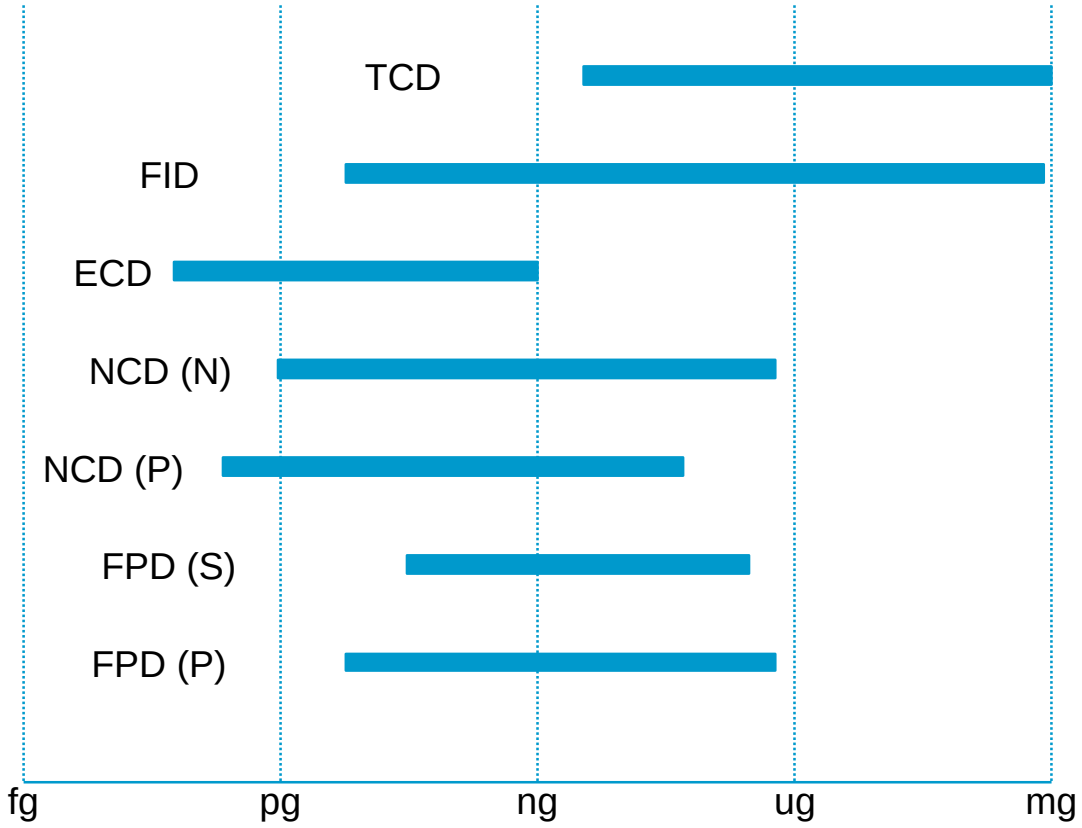
Configuring a GC System

Detector Sensitivity



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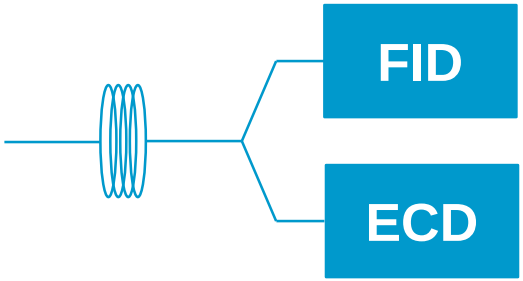
Configuring a GC System

Detector Arrangement

Serial

Place non-destructive detector before other detector



Parallel

Split column effluent to different detectors

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Configuring a GC System

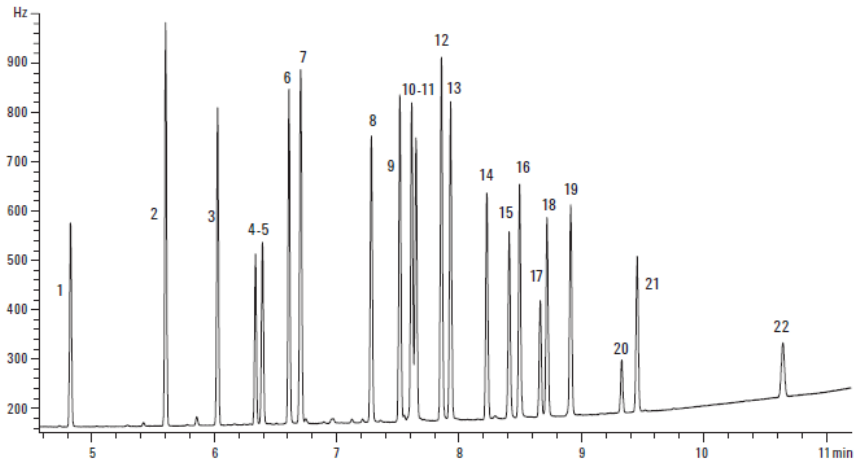
GC Output

The chromatogram plots abundance against time.

Peak size corresponds to the amount of compound in the ample. As the compound`s concentration increases, a larger peak is obtained.

Retention time (t_R) is the time it takes of a compound to travel through the column.

If the column and all operating conditions are kept constant, a given compound will always have the same retention time.



1. Tetrachloro-m-xylene (surrogate standard)
2. α-BHC
3. γ-BHC
4. β-BHC
5. Heptachlor
6. δ-BHC
7. Aldrin
8. Heptachlor epoxide
9. γ-chlordane
10. α-chlordane
11. Endosulfan I
12. Dieldrin
13. p,p-DDE
14. Endrin
15. p,p-DDD
16. Endosulfan II
17. p,p-DDT
18. Endrin aldehyde
19. Endosulfan sulfate
20. Methoxychlor
21. Endrin ketone
22. Decachlorobiphenyl (surrogate standard)

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The Capabilities of GC

Key Points to Remember

Strengths

- Easy to use
- Robust
- Many detectors
- Low cost

Limitations

- Lack of confirming data other than retention time, except for mass spectrometer detection
- Compounds must be thermally stable

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Publication	Title	Pub. No.
Primer	Fundamentals of Gas Chromatography	G1176-90000
Video	Fundamentals of Gas Chromatography (14 min)	
Guide	Agilent J&W GC Column Selection Guide For Research Use Only. Not for diagnostic procedures.	5990-9867EN
Web	CHROMacademy – free access for students and university staff to online courses	
Application compendium	A compilation of Application Notes (22MB)	5991-3592EN

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THANK YOU

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Publication number 5991-5423EN

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Mass Spectrometry Fundamentals – Theory

BUILDING
BETTER SCIENCE

AGILENT AND YOU

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Introduction

Mass spectrometry (**MS**) is an instrumental method in analytical chemistry that helps identify the type and amount of chemicals present in a sample according to the mass-to-charge ratio and abundance of gas-phase ions.

A mass spectrum (plural *spectra*) is a pattern of the ion signal generated in a mass spectrometer as a function of the mass-to-charge (m/z) ratio. The masses of the molecular ions and fragments are used to determine the elemental composition or isotopic signature of a compound. Depending on the information obtained the chemical structures of molecules can be determined.

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Introduction

Nobel Prize Winning Technology

John Fenn and Koichi Tanaka won the Nobel Prize in Chemistry in 2002 for the development of two soft ionization technologies:

- Electrospray technology, Dr. Fenn
- Soft laser desorption, Dr. Tanaka

Kurt Wüthrich was the third winner of the Nobel Prize in Chemistry in 2002 for the development of methods for identification and structure analysis of biological marcomolecules.

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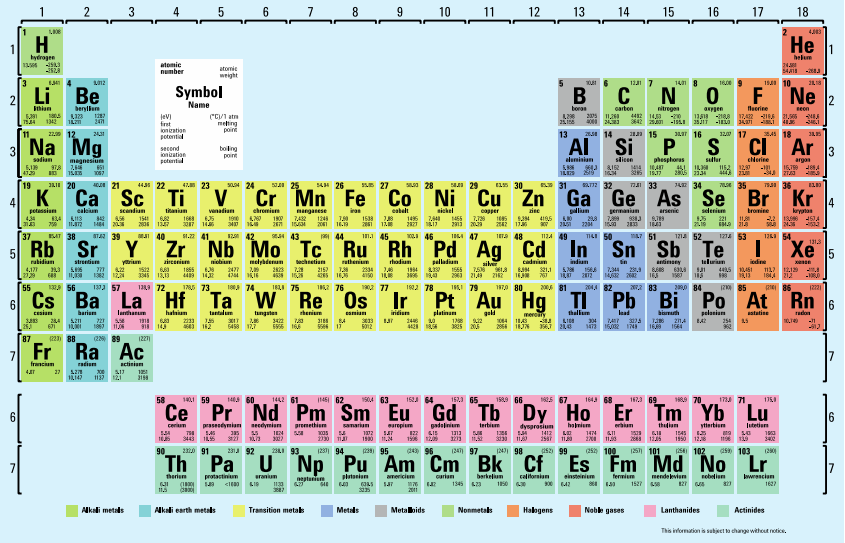
Introduction

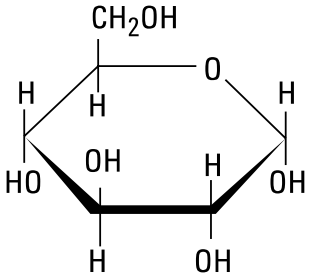
Basic Considerations

Elements can be uniquely identified by their mass. Mass Spectrometry is an analytical method to measure molecular or atomic weight.

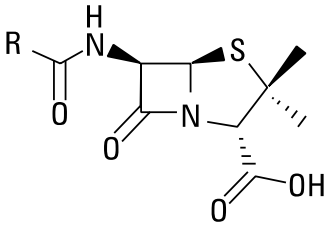


Source: Periodic table, poster SI-0186

Compounds, consisting of different elements, can be distinguished by their mass:



Glucose $C_6H_{12}O_6$
MW: 180,1559 g/mol



Penicillin $C_{16}H_{18}N_2O_4S$
MW: 334,39 g/mol

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Introduction

Masses in Mass Spectrometry

The **average mass** of a molecule is obtained by summing the average atomic masses of the constituent elements.

Average mass of water (H_2O): 1.00794 + 1.00794 + 15.9994 = 18.01528 Da

The **monoisotopic mass** is the sum of the masses of the atoms in a molecule using the unbound, ground-state, rest mass of the most abundant isotope for each element instead of the isotopic average mass. Monoisotopic mass is typically expressed in unified atomic mass units or daltons (Da).

The **accurate mass** (more appropriately, the measured accurate mass) is an experimentally determined mass that allows the elemental composition to be determined. For molecules with mass below 200 u, 5 ppm accuracy is often sufficient to uniquely determine the elemental composition.

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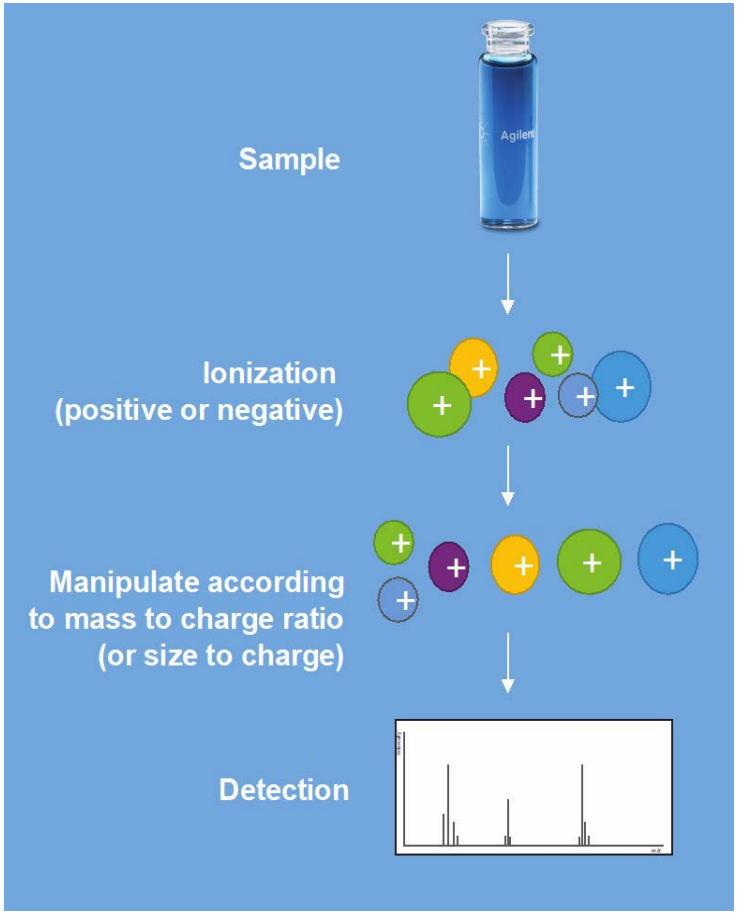
Introduction
Fundamental Steps

Typical MS procedure:

- Sample (solid, liquid, gas) is ionized
- Sample's molecules might break into charged fragments during ionization
- Ions are separated according to their mass-to-charge ratio (m/z)
- Ions are detected by a mechanism capable of detecting charged particles (e.g. electron multiplier)
- Results are displayed as spectra of the relative abundance as a function of m/z ratio
- Identification is done by correlating known masses to the identified masses or through a characteristic fragmentation pattern



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How It Works
Ionization

Before the sample can be mass analyzed, it must be ionized in the ion source.

Gaseous Sample Introduction:

- Electron Ionization (EI)
- Chemical Ionization (CI)

Liquid Sample Introduction:

- Electrospray Ionization (ESI)
- Atmospheric Pressure Chemical Ionization (APCI)
- Atmospheric Pressure Photo Ionization (APPI)
- Multimode Ionization (MMI)
- Matrix Assisted Laser Desorption Ionization (MALDI)
- Inductively Coupled Plasma (ICP)

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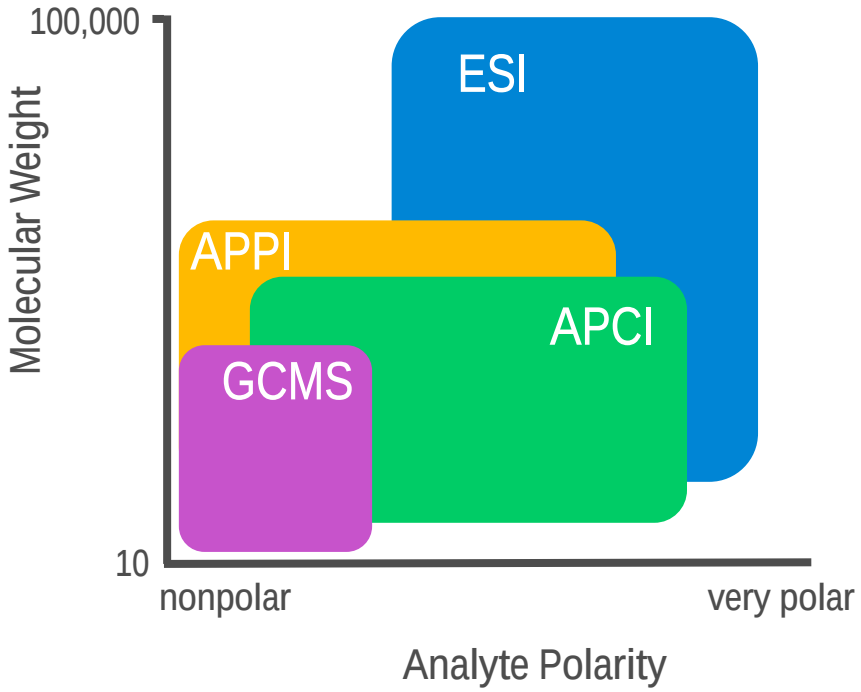
How It Works
Ionization

Polarity of analytes determines the ionization source.



ESI	Electrospray ionization
APPI	Atmospheric pressure photo ionization
APCI	Atmospheric pressure chemical ionization
GC/MS	Gas chromatography / Mass spectrometry

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How It Works

Ionization – Electron Ionization (EI)

Electron Ionization (EI) is well established, and is the most common method of ionization in Gas Chromatography (GC).

The molecules exiting the gas chromatograph are bombarded by an electron beam (70 eV) which removes an electron from the molecule resulting in a charged ion.

$$CH_3OH + 1\ electron \rightarrow \quad CH_3OH^{+\bullet} + 2e^-$$

Molecular ion

EI typically produces single charged molecular ions and fragment ions (smaller parts of the original molecules) which are used for structure elucidation.

$$CH_3OH^{+\bullet} \rightarrow \quad CH_2OH^+ + H^\bullet \text{ or } CH_3OH^{+\bullet} \rightarrow \quad CH_3^+ + OH^\bullet$$

Fragment ion

An electron or photomultiplier detects the separated ions.
The generated mass spectrum plots the signal intensity at a given m/z ratio.

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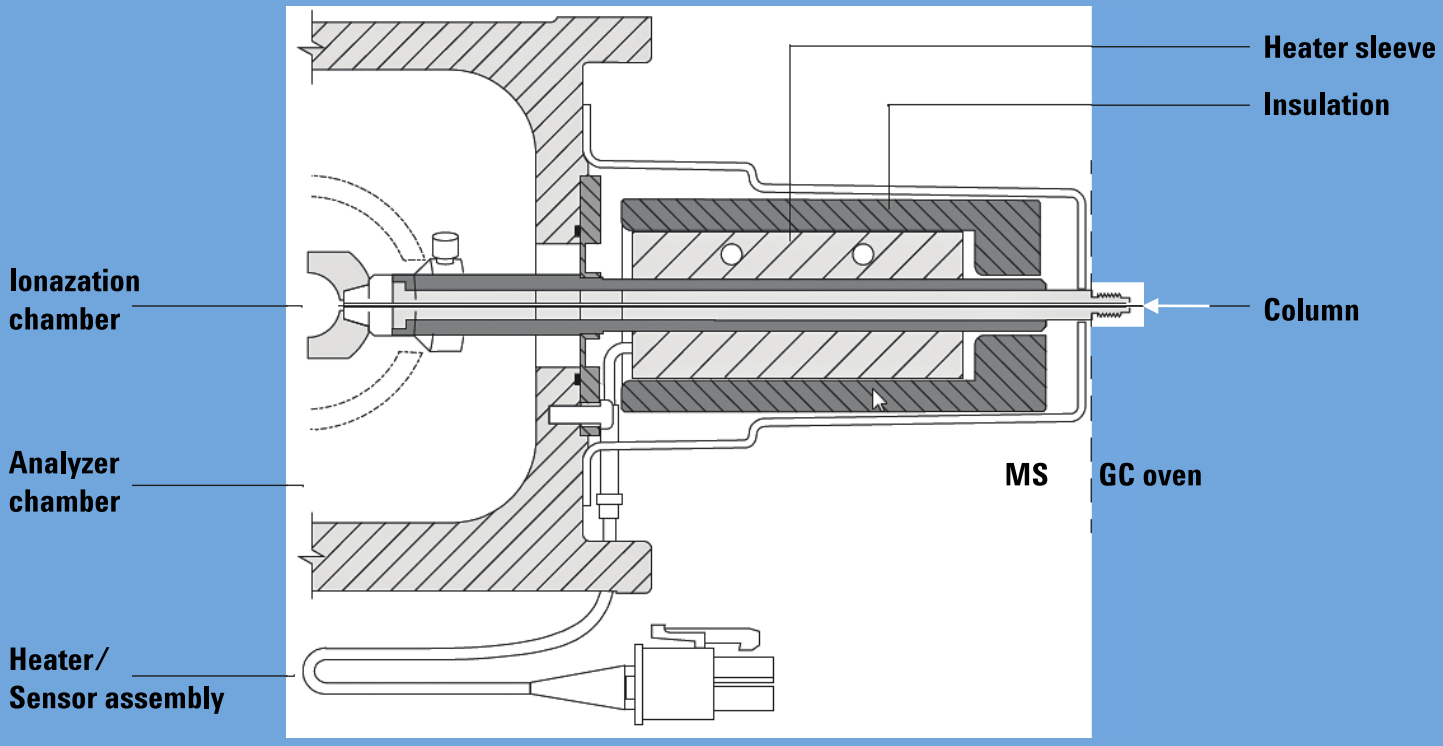
How It Works

Ionization – Electron Ionization (EI)

The GC/MS interface operates at high temperatures.



The EI GC/MS Interface. Source: Agilent 7000 Series Triple Quad GC/MS Operation Manual (p 46)

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How It Works

Ionization – Chemical Ionization (CI)

EI is a direct energy transfer process with electron kinetic energy deposited directly into an analyte molecule.

CI is an indirect process involving an intermediate chemical agent. This is particularly true in positive chemical ionization (PCI). In PCI, the ion source is filled with a reagent gas which is ionized to create reagent ions which react with the analyte.

Most frequently used reagent gases: **methane**, **iso-butane** and **ammonia**.

The applied reagent gas determines the ionization and fragmentation behavior of the analyte.

The principal methane reactions are:

$CH_4 + e^- \rightarrow CH_4^+, CH_3^+, CH_2^+$ The reagent gas is ionized by electrons entering the ionization source.

$CH_4 + CH_4^+ \rightarrow CH_5^+, CH_3^\bullet$

$CH_2^+ + CH_4 \rightarrow C_2H_4^+ + H_2$

$CH_2^+ + CH_4 \rightarrow C_2H_3^+ + H_2^+H^\bullet$

$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$

$C_2H_3^+ + CH_4 \rightarrow C_3H_5^+ + H_2$

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See notes for details

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How It Works

Ionization – Sample Considerations (LC/MS)

ESI	APCI	APPI
Volatility not required	Some volatility required	Some volatility required
Preferred technique for thermally labile analytes	Analyte must be thermally stable	Analyte must be thermally stable
Ions formed in solution	Ions formed in gas phase	Ions formed in gas phase
Can form multiply charged ions	Forms singly charged ions only	Forms singly charged ions only

Many compounds will ionize well using all three sources. APCI / APPI can ionize molecules that are too non-polar for ESI to ionize.

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How It Works

Ionization – Sample Considerations (LC/MS)

ESI

- **Ions in solution** e.g. catecholamine, sulfate conjugates, quaternary amines
- **Compounds containing heteroatoms** e.g. carbamates, benzodiazepines
- **Compounds that multiply charge in solution** e.g. proteins, peptides, oligonucleotides

APCI

- **Compounds of intermediate MW and polarity** e.g. PAHs, PCBs, fatty acids, phthalates, alcohols
- **Compounds containing heteroatoms** e.g. carbamates, benzodiazepines
- **Compounds that are too non-polar for ESI response**

APPI

- **Compounds of intermediate MW and intermediate to low polarity** e.g. PAHs, PCBs, fatty acids, phthalates, alcohols
- **Compounds containing heteroatoms** e.g. carbamates, benzodiazepines
- **Compounds that are too non-polar for ESI response**

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How It Works

Ionization – Electrospray (ESI)

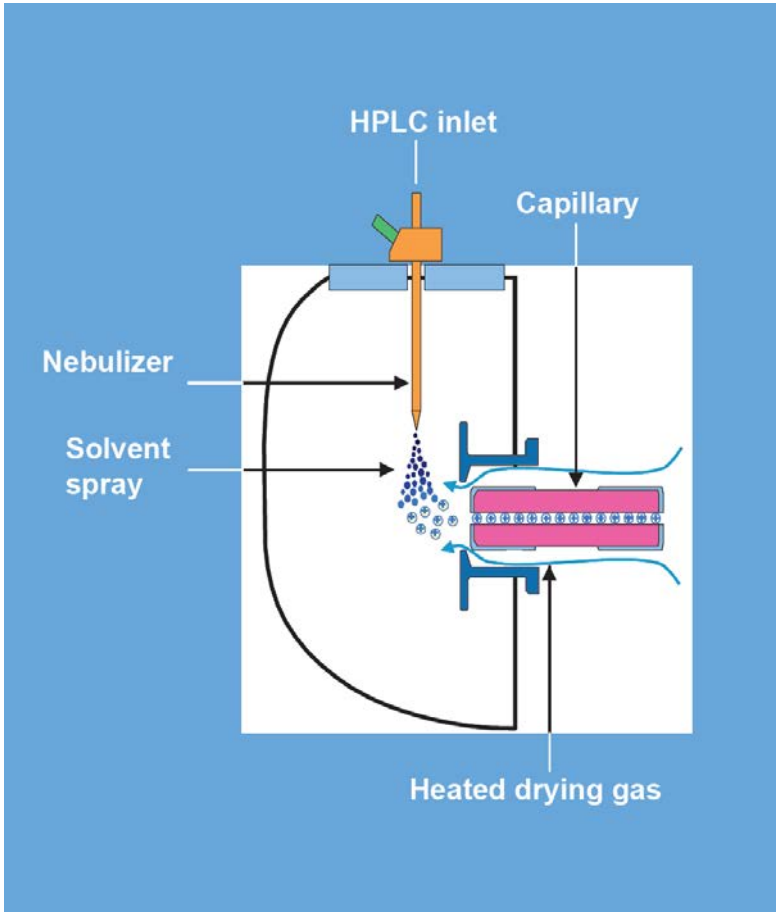
Electrospray ionization (ESI) is a soft ionization technique.

LC eluent is sprayed (nebulized) into a spray chamber at atmospheric pressure in the presence of a strong electrostatic field and heated drying gas. The electrostatic field occurs between the nebulizer, which is at ground in this design, and the capillary, which is at high voltage.

Suitable molecules:

- Small molecules (glucose) and large biomolecules (proteins, oligonucleotides)

Multiple charging is the phenomena in ESI that allows analysis of larger molecules (-> Deconvolution)



Electrospray ion source
Source: LC/MS concept guides (p 22)

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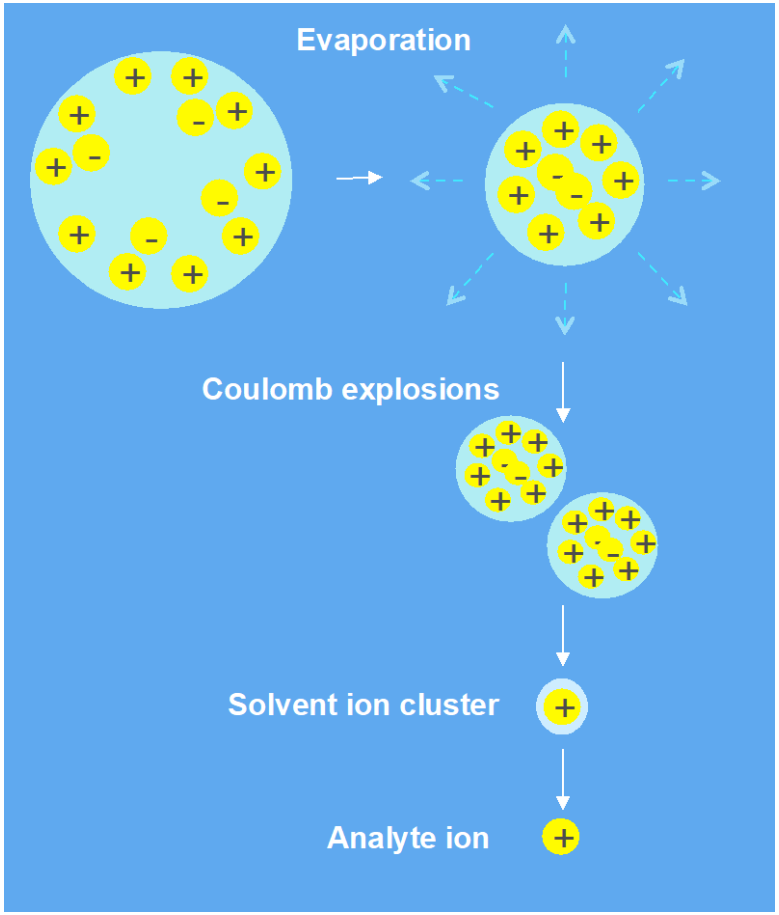
How It Works

Ionization – ESI Process

From charged droplets to analyte ions

The nebulizer produces a uniform droplet size.
The charged droplets are attracted toward the dieletric capillary. The heated nitrogen stream surrounding the capillary shrinks the droplets. This process is called **desolvation**.

The droplets continue to shrink until the repulsive electrostatic (Coulombic) forces exceed the droplet cohesive forces, leading to droplet explosions.
This process is repeated until analyte ions are ultimately desorbed into the gas phase, driven by strong electric fields on the surface of the micro droplets. This process is called **ion evaporation**.



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How It Works

Ionization – Atmospheric Pressure Chemical Ionization (APCI)

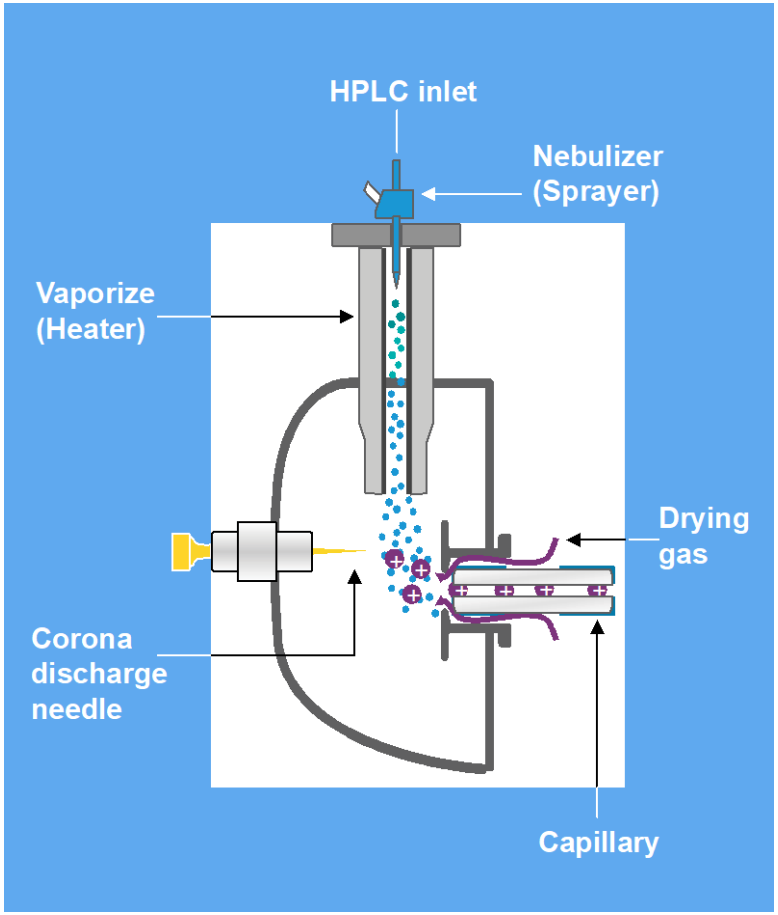
APCI is a gas-phase chemical ionization process. Therefore, the analyte needs to be in the gas phase for ionization.

LC eluent passes a nebulizing needle, which creates a fine spray.

The droplets are fully vaporized in a heated ceramic tube (~ 400 to 500°C).

Suitable molecules:

- Molecules < 1,500 u
- Less polar and non-polar compounds (typically analyzed by normal-phase chromatography)



Atmospheric pressure chemical ionization source
Source: LC/MS concept guides (p 27)

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How It Works

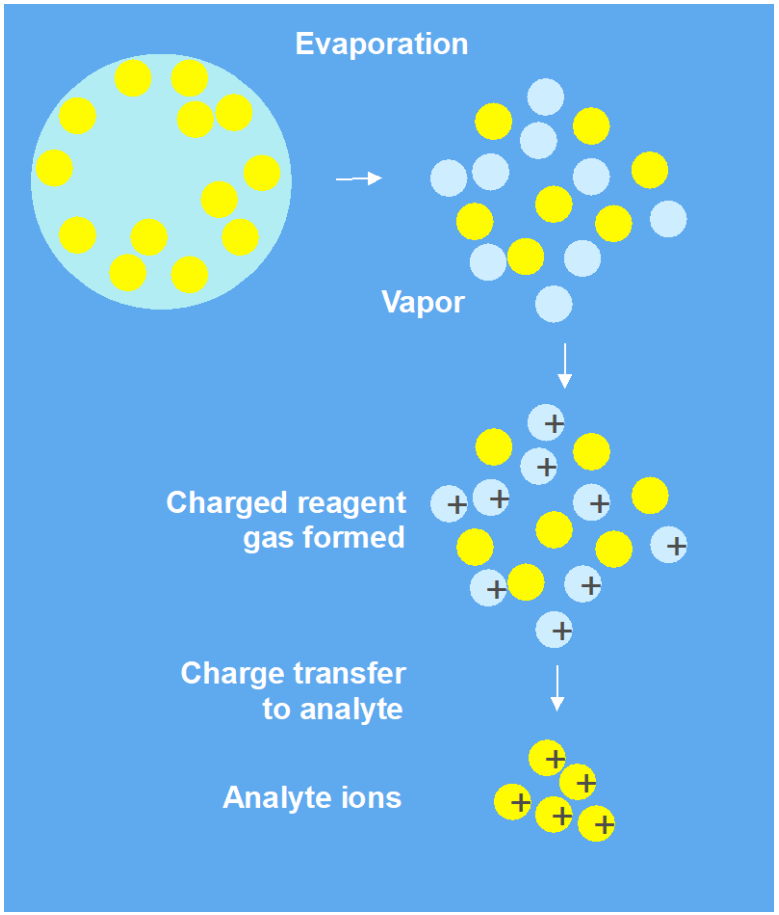
Ionization – APCI Process

This shows the evaporation and ionization processes of APCI.

Note that the analyte is not ionized until after evaporation and after the reagent gas is ionized.

The reagent gas then transfers a charge to the analyte.

Typically APCI generates just singly charged ions, however, it is possible to get doubly charged ions where the charge sites are held apart (usually by a hydrophobic region).



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How It Works

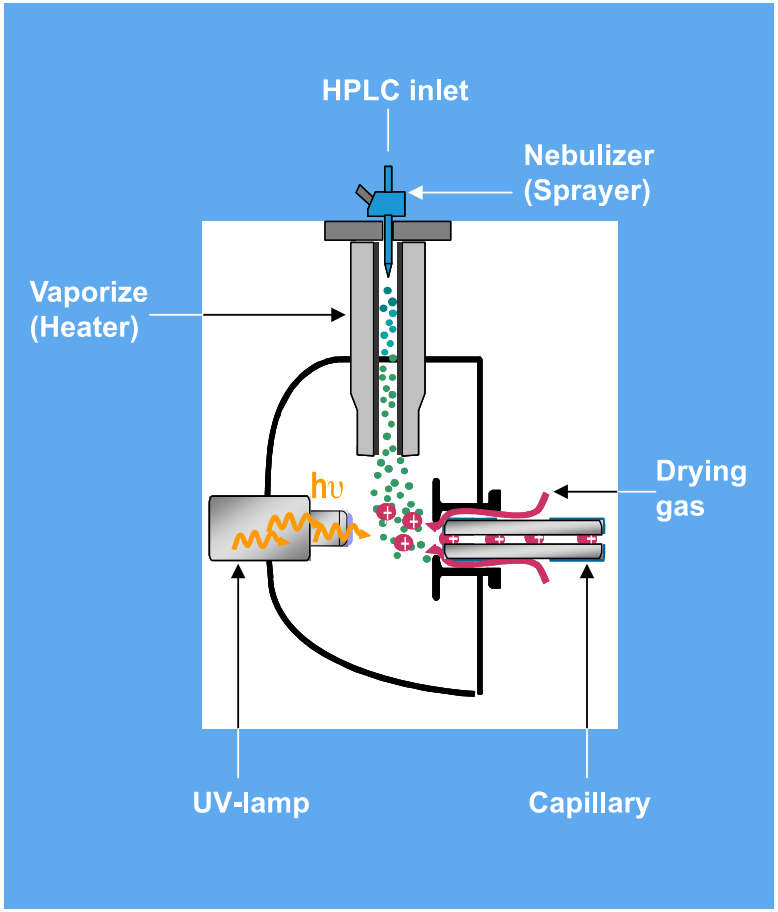
Ionization – Atmospheric Pressure Photo Ionization (APPI)

With the APPI technique, LC eluent passes through a nebulizing needle to create a fine spray.

Droplets are fully vaporized in a heated ceramic tube.

The gas/vapor mixture passes through the ultraviolet light of a krypton lamp to ionize the sample molecules. The sample ions are then introduced into the capillary.

APPI is applicable to many of the same compounds that are typically analyzed by APCI. APPI has proven particularly valuable for analysis of non-polar, aromatic compounds.



Atmospheric pressure photoionization source
Source: LC/MS concept guides (p 29)

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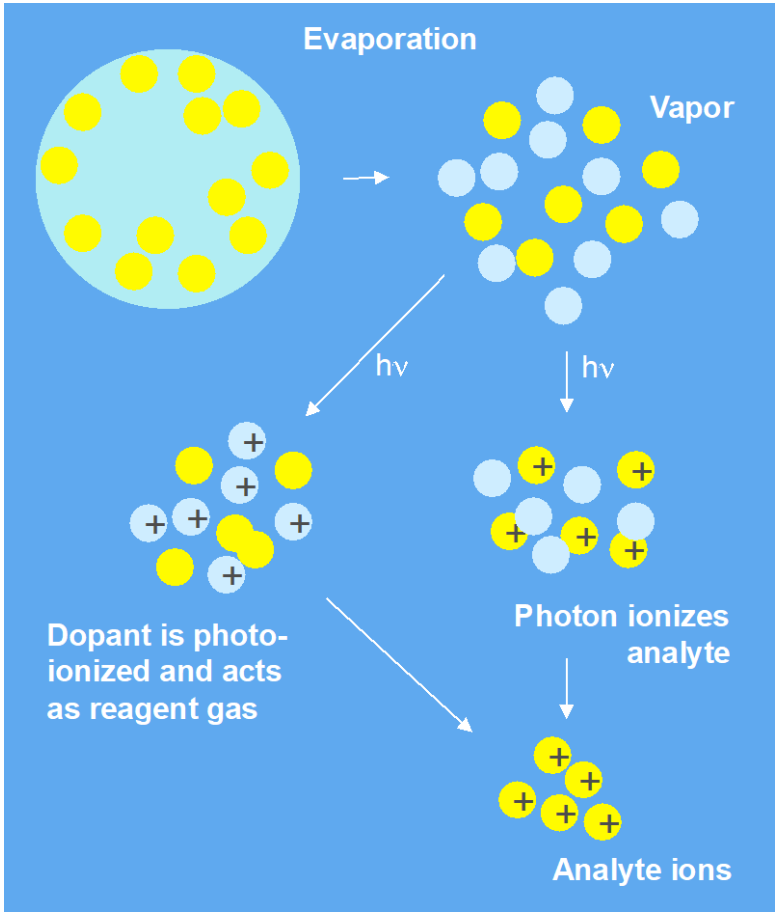
How It Works

Ionization – APPI Process

This shows the evaporation and ionization processes of photoionization.

APPI and APCI are similar, with APPI substituting a lamp for the corona needle for ionization. APPI often also uses an additional solvent or mobile phase modifier, called a “dopant” (*D*), to assist with the photoionization process.



Direct APPI:

$$M + h\upsilon \rightarrow M^{\bullet +} + e^{-}$$

$$M^{\bullet +} + SH \rightarrow [M + H]^{+} + S^{\bullet}$$

Dopant APPI:

$$D + h\upsilon \rightarrow D^{\bullet +} + e^{-}$$

$$D^{\bullet +} + M \rightarrow [M + H]^{+} + D$$

$$D^{\bullet +} + M \rightarrow M^{\bullet +} + D$$

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How It Works

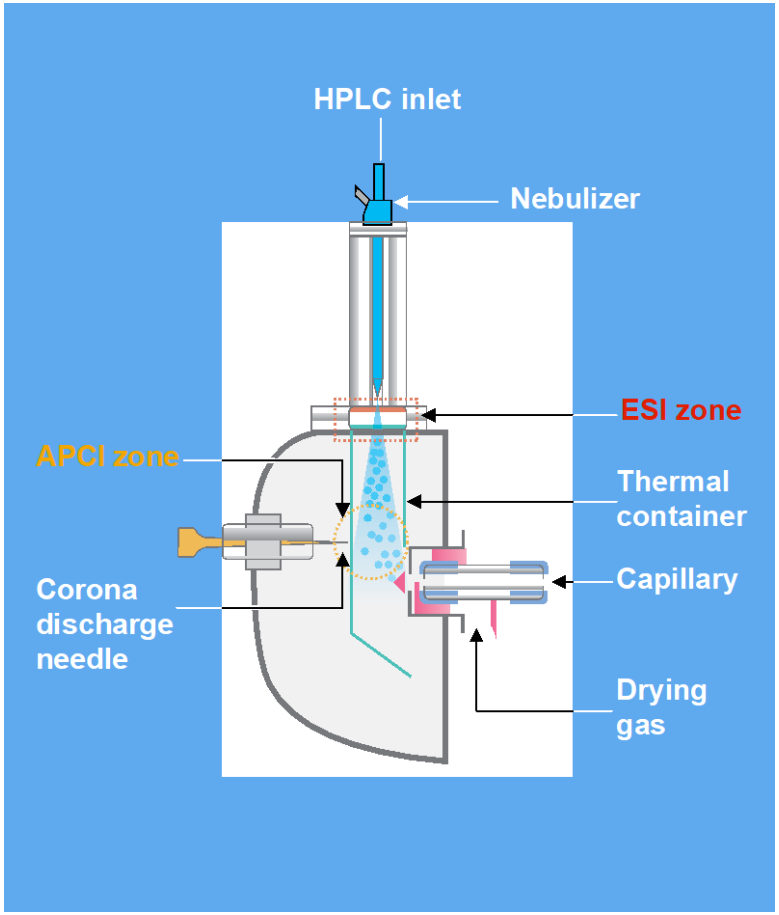
Ionization – Multi Mode Ionization (MMI)

The multimode source is an ion source that can operate in three different modes:

- APCI
- ESI
- Simultaneous APCI/ESI

It incorporates two electrically separated, optimized zones – one for ESI and one for APCI. During simultaneous APCI/ESI, ions from both ionization modes enter the capillary and are analyzed simultaneously by the mass spectrometer.

MMI is useful for screening of unknowns, or whenever samples contain a mixture of compounds where some respond by ESI and some respond by APCI.



Multimode source
Source: LC/MS concept guides (p 30)

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How It Works

Ionization – Matrix-Assisted Laser Desorption/Ionization (MALDI)

Matrix-assisted laser desorption/ionization (MALDI) is a soft ionization technique.

Sample is mixed with matrix and applied to a metal plate.

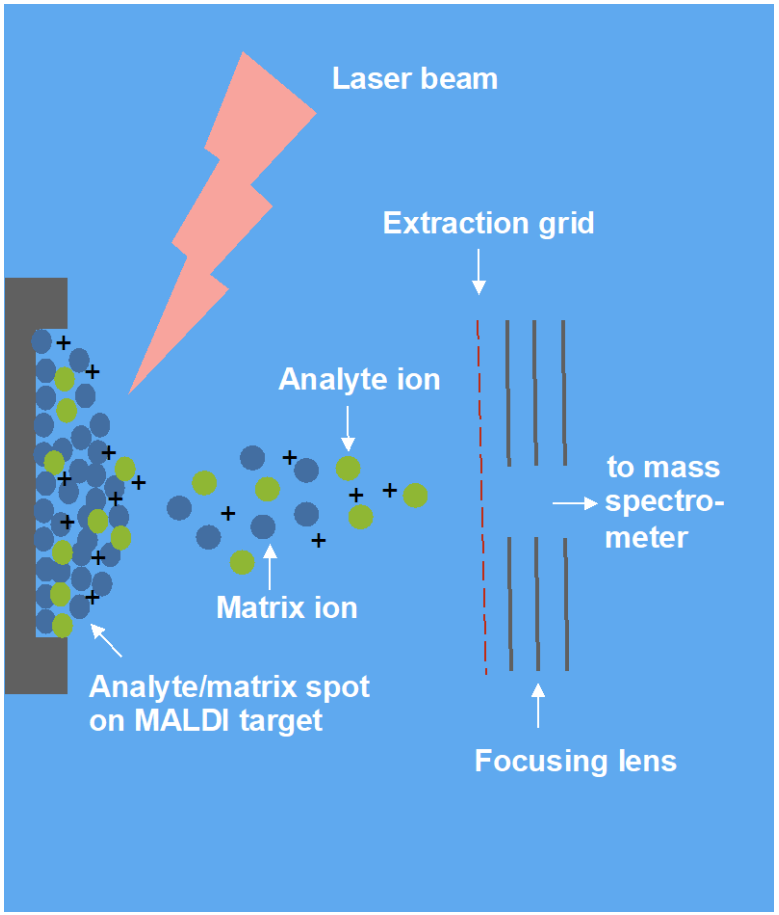
A pulsed laser irradiates the sample, triggering ablation and desorption.

The analyte molecules are ionized in the hot plume of ablated gases.

Ions are accelerated into the mass spectrometer.

Suitable molecules:

- Biomolecules (DNA, proteins, sugars)
- Large organic molecules (polymers)



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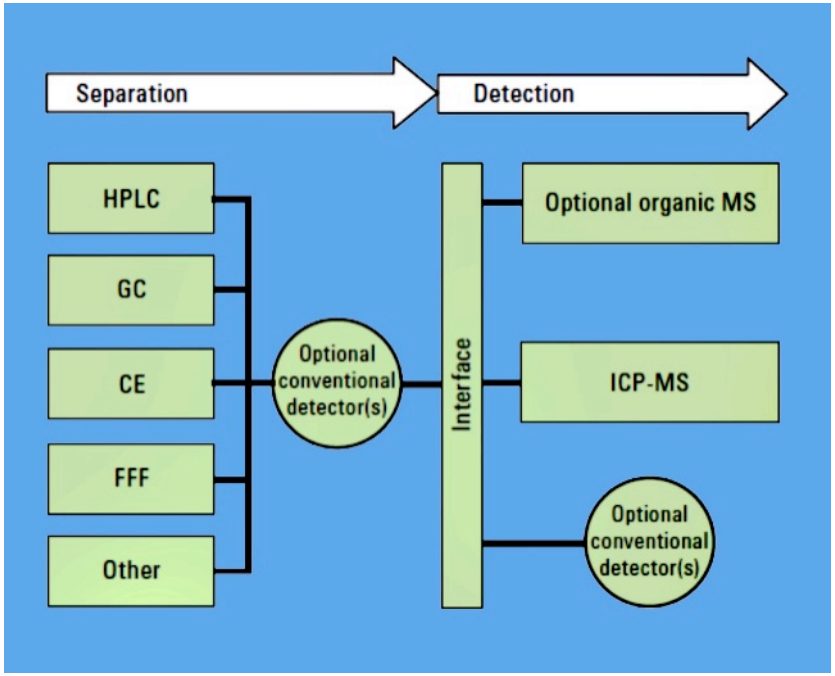
How It Works

Ionization – Inductively Coupled Plasma (ICP)

An inductively coupled plasma (ICP) instrument uses a plasma source in which the energy is supplied by electric currents which are produced by electro-magnetic induction, that is, by time varying magnetic fields. The plasma is so energetic it reduces molecules to ionized elements.

There are different types of ICP geometries available that can be coupled to different technologies:

- ICP-AES Atomic Emission Spectroscopy
- ICP-OES Optical Emission Spectroscopy
- ICP-MS Mass Spectrometry
- ICP-RIE Reactive-Ion Etching



Schematic diagram showing the interrelationships of the various components in a hyphenated ICP-MS system

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How It Works
Mass Analyzer

After ionization and ion transport, analytes enter the mass analyzer.
The mass spectrometer measures the ion signals resulting in a mass spectra, which can provide valuable information about the molecular weight, structure, identity, and quantity of a compound.

There are different types of mass analyzers:

- Single Quadrupole (SQ)
- Triple Quadrupole (QQQ)
- Time-of-Flight (TOF)
- Ion Trap (IT)

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How It Works

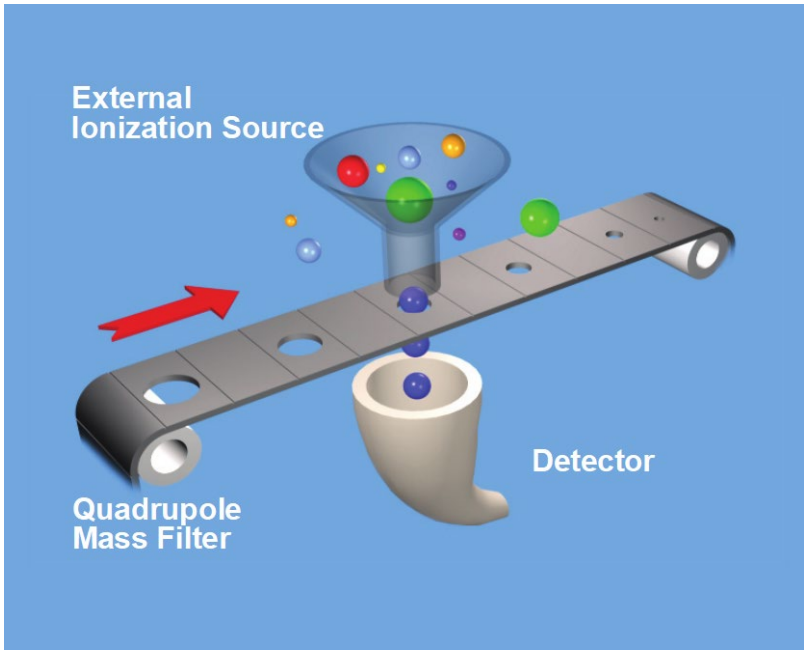
Mass Analyzer – Single Quadrupole (SQ)

Charged ions generated in the ion source enter the mass analyzer.
The quadrupole mass analyzer is scanned sequentially such that only a single ion m/z may be passed at one time. All other ions are lost.

***m/z* - mass-to-charge ratio:**

Mass of an ion (Daltons or u) divided by the number of charges on the ion

Information received: **MS only**



Conceptual model – Single quadrupole

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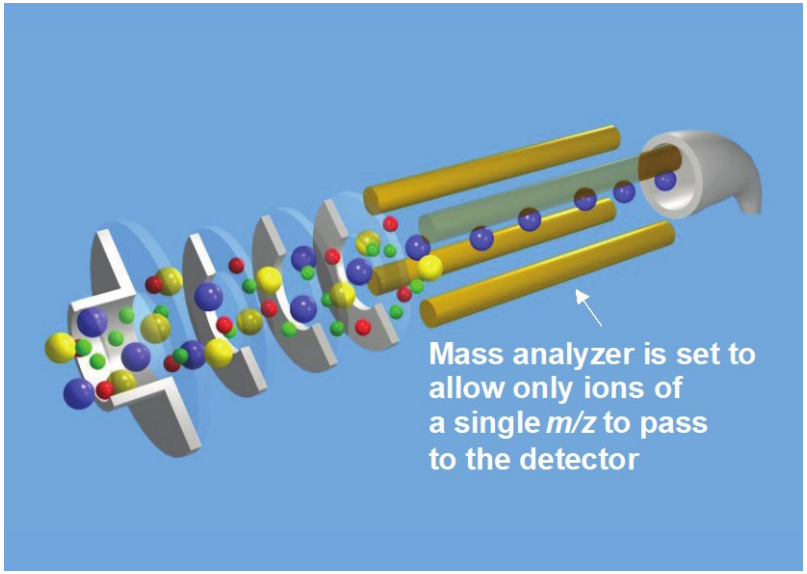
How It Works

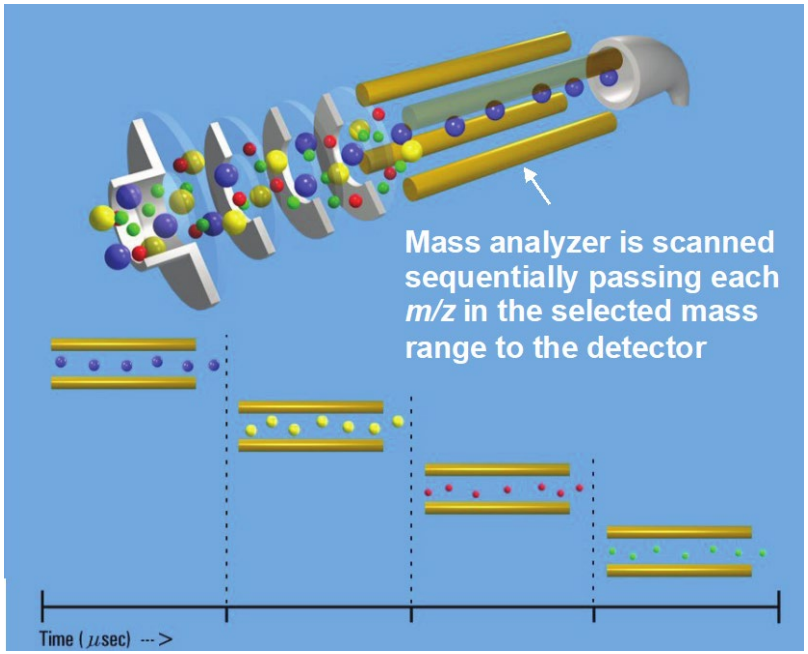
Mass Analyzer – Single Quadrupole (SQ)

Single Ion Monitoring (SIM)



A target ion with specific *m/z* is monitored. SIM on a single quad permits the best sensitivity for quantitation, however it lacks specificity.

Scan Mode



In Scan MS mode, the quadrupole mass analyzer is scanned sequentially allowing only 1 *m/z* at a time to pass to the detector.

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How It Works

Mass Analyzer – Triple Quadrupole (QQQ)

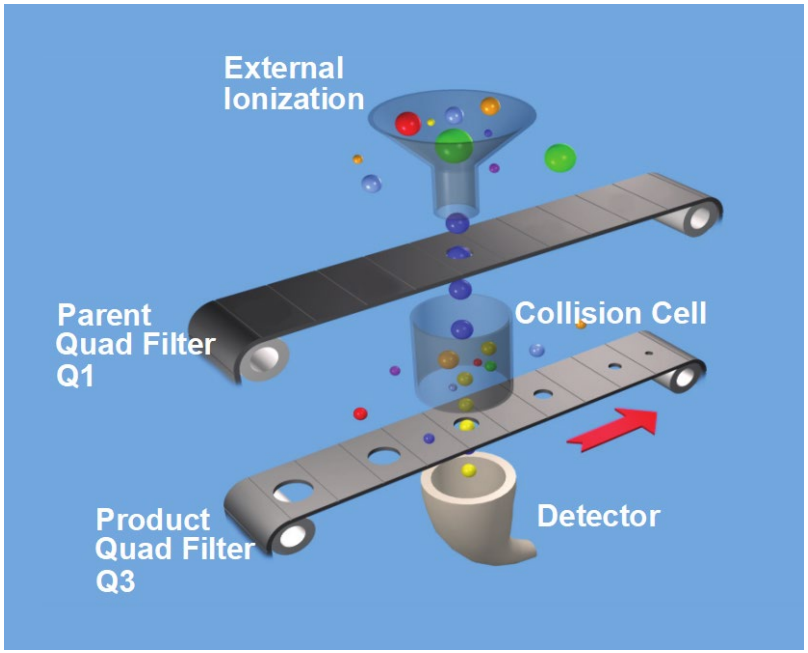
Charged ions generated in the ion source enter the mass analyzer.

The analyzer consists of three quadrupoles (Q1-Q3) and therefore several modes of operation resulting in different information.

A common set is the following:

- Q1: used as a filter for specific *m/z* (precursor ion)
- Q2: used as collision cell to fragment the precursor ion and generate product ions
- Q3: set to specific *m/z* (SRM or MRM) or scan mode (product ion scan)

Information received: **MS and MS/MS**



Conceptual model – Triple quadrupole
Schematic shows SRM mode

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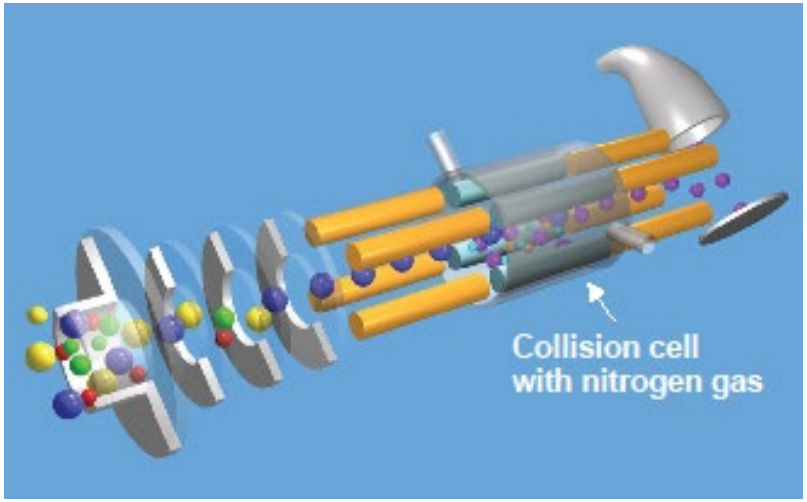
How It Works

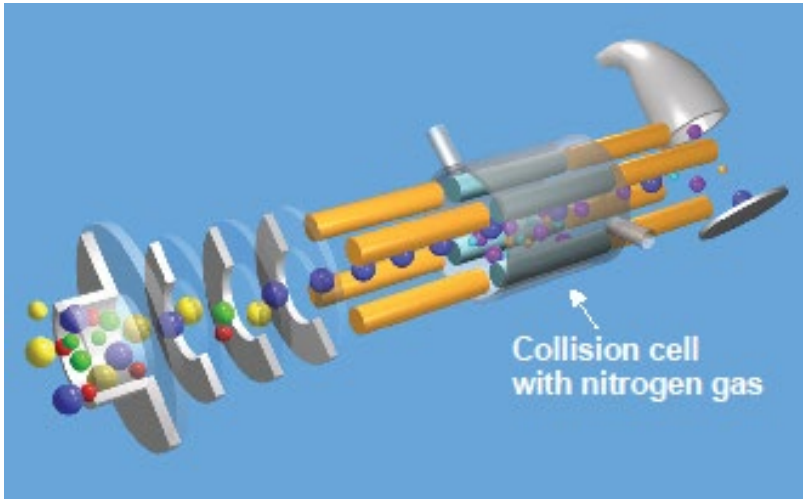
Mass Analyzer – Triple Quadrupole (QQQ)

Multiple Reaction Monitoring (MRM)



Precursor ions with single *m/z* are passing to collision cell. Fragment ions are generated by collision with nitrogen molecules. Q3 is set to single *m/z* of specific fragment ion. This is a very sensitive method and used for quantitation.

Full Scan MS/MS Mode



The difference in full scan mode compared to SRM/MRM is the scanning function. Q3 is scanned sequentially allowing only 1 *m/z* at a time to pass to the detector. A product ion spectrum is generated. This mode of operation is less sensitive compared to SRM/MRM.

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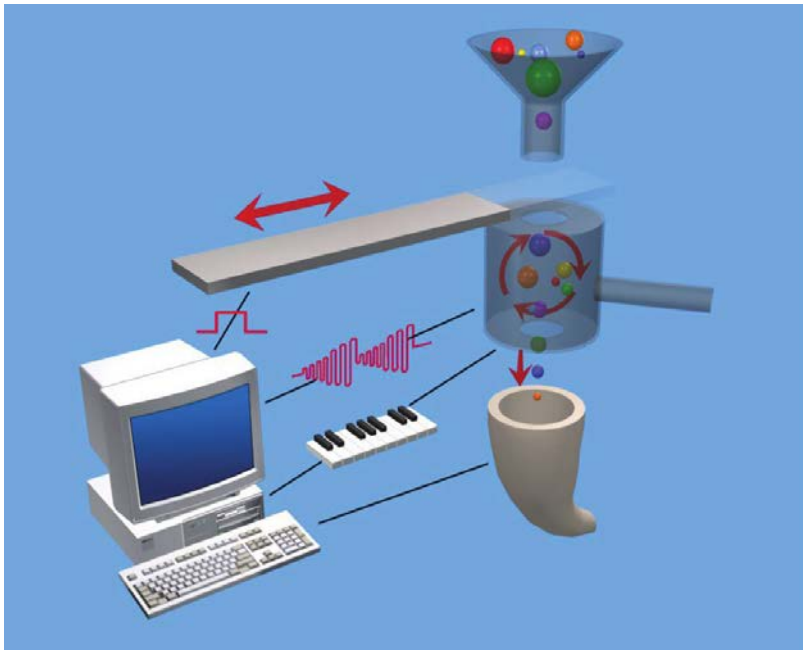
How It Works

Mass Analyzer – Ion Trap (IT)

Charged ions generated in the ion source enter the mass analyzer. All ions of the selected polarity over the selected mass range can be stored at once in the trap. The ions can be manipulated in the ion trap mass analyzer – performing multiple isolation and fragmentation stages – until time to detect.

Instead of four parallel rods, the ion trap consists of a circular ring electrode plus two end caps that form a “trap”.

Information received: **MS and MS/MS**



Conceptual model – Ion Trap

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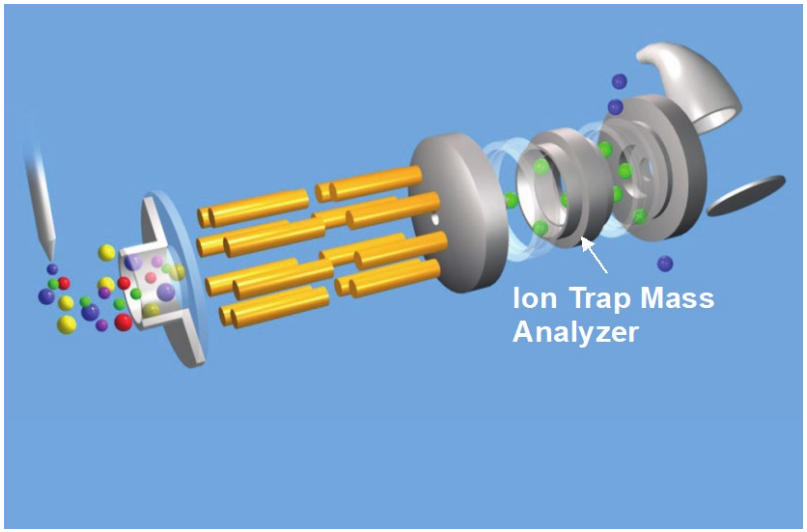
How It Works

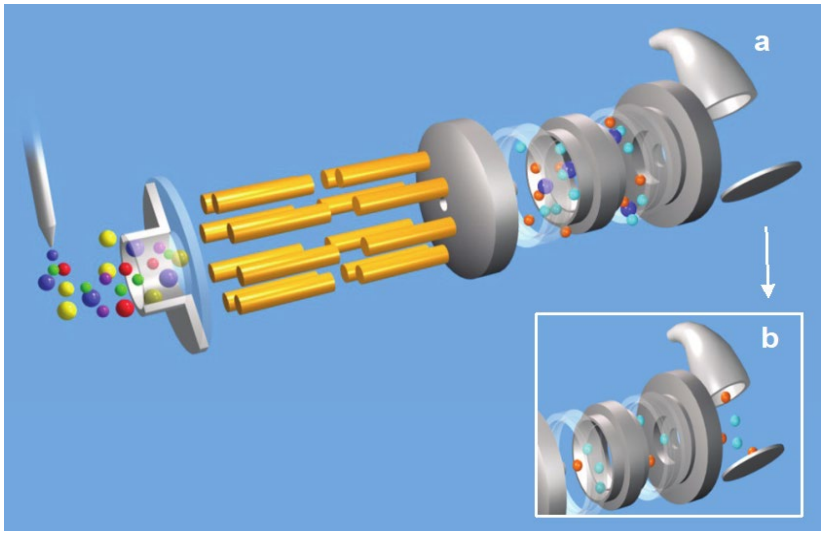
Mass Analyzer – Ion Trap (IT)

Step 1: Isolation of Precursor Ion



Once ion injection and accumulation are complete, the ion gate closes and ions are no longer injected into the mass analyzer. Waveforms are applied to eject masses above and below the precursor ion.

Step 2: Fragmentation of Precursor ion



Resonance excitation of the precursor ion causes collision induced dissociation (CID) and product ions are generated (a). The full scan product ions are ejected to the detector (b).

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How It Works

Mass Analyzer – Time-of-Flight (TOF)

Charged ions generated in the ion source enter the mass analyzer.

Analyzer components:

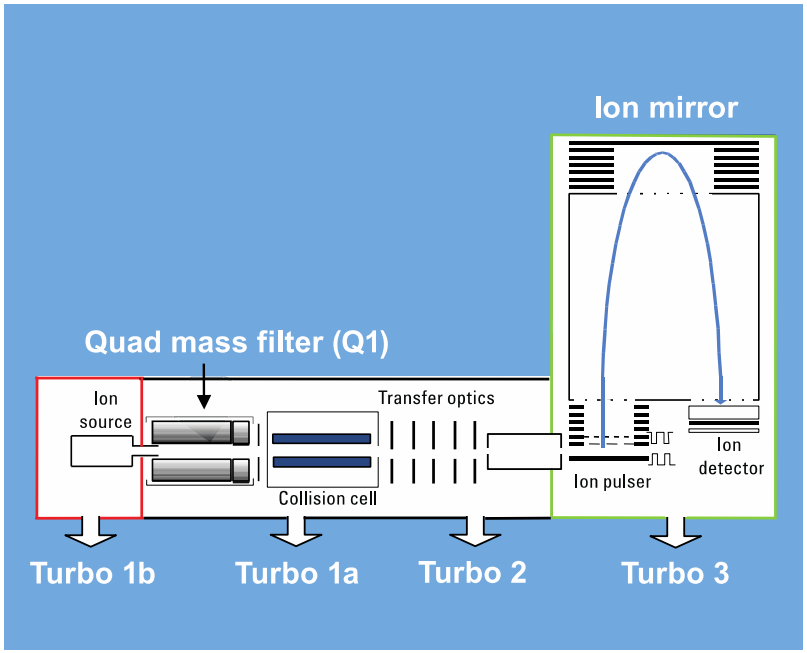
- Mass filter (Q1), optional
- Flight tube
- Collision cell (Q-TOF)

After ions have passed the quadrupole or collision cell they arrive at the ion pulser. A high voltage pulse is applied which accelerates the ions into the flight tube. An ion mirror at the end of the tube reflects the ions and sends them to the detector that records their time of arrival.

Information received:

TOF: **MS only**

Q-TOF: **MS and MS/MS**



Schematic of Time-of-Flight mass spectrometer.
Source: Time-of-Flight Mass Spectrometry
Graphic shows a Q-TOF

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How It Works

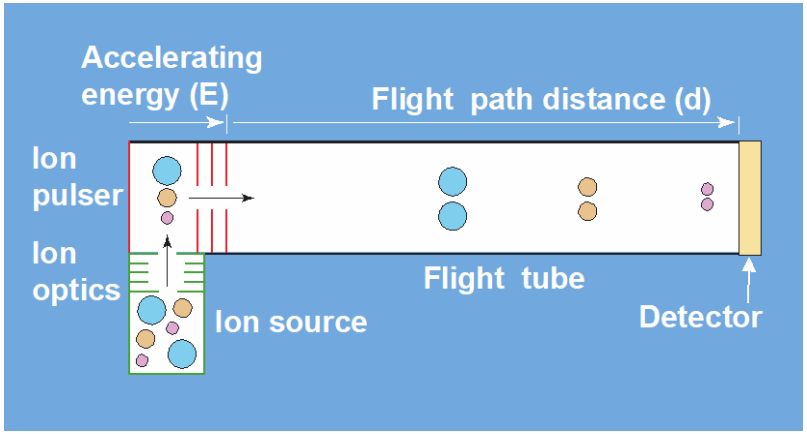
Mass Analyzer – Time-of-Flight (TOF)

The flight time (*t*) for each mass is unique and is determined by the energy (*E*) to which an ion is accelerated, the distance (*d*) it has to travel, and m/*z.*

$E = 1/2mv^2$ which is solved for *m* looks like:

$m = 2E/v^2$ and solved for *v* looks like:

$v = \sqrt{(2E/m)}$ equation 1



The equation says that for a given kinetic energy, *E*, smaller masses will have greater velocities than larger masses. Ions with lower masses arrive at the detector earlier.

Velocity is determined (and consequently the mass) by measuring the time it takes an ion to reach the detector.

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How It Works

Mass Analyzer – Time-of-Flight (TOF)

The second equation is the familiar velocity (v) equals distance (d) divided by time (t): $v = d / t$

Combing equation 1 and 2 yields: $m = (2E / d^2)t^2$

For a given energy (*E*) and distance, the mass is proportional to the square of the flight time of the ion. *E* and *d* are kept constant and summarized in variable A which leads to a simplified equation: $m = A \cdot t^2$

To be really precise, a time delay for applying the high voltage needs to be considered as well: $t = t_m - t_0$

This results in the final equation: $m = A(t_m - t_0)^2$

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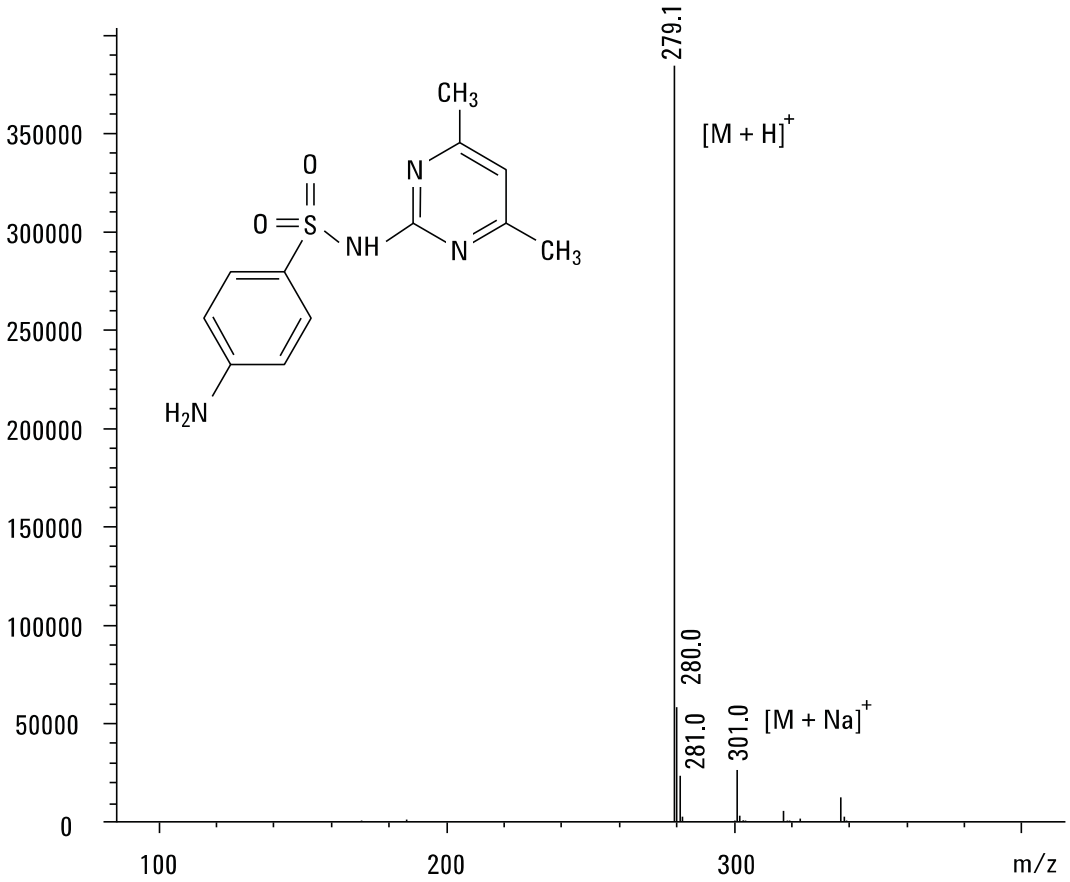
Results

Example 1



Mass spectrum of sulfmethazine analyzed with a single quadrupole mass analyzer

Molecular Formula: $C_{12}H_{14}N_4O_2S$
$[M+H]^+$: 279.33

Mass spectrum of sulfmethazine.
Source: Agilent 6100 Series Quadrupole LC/MS Systems (p 17)

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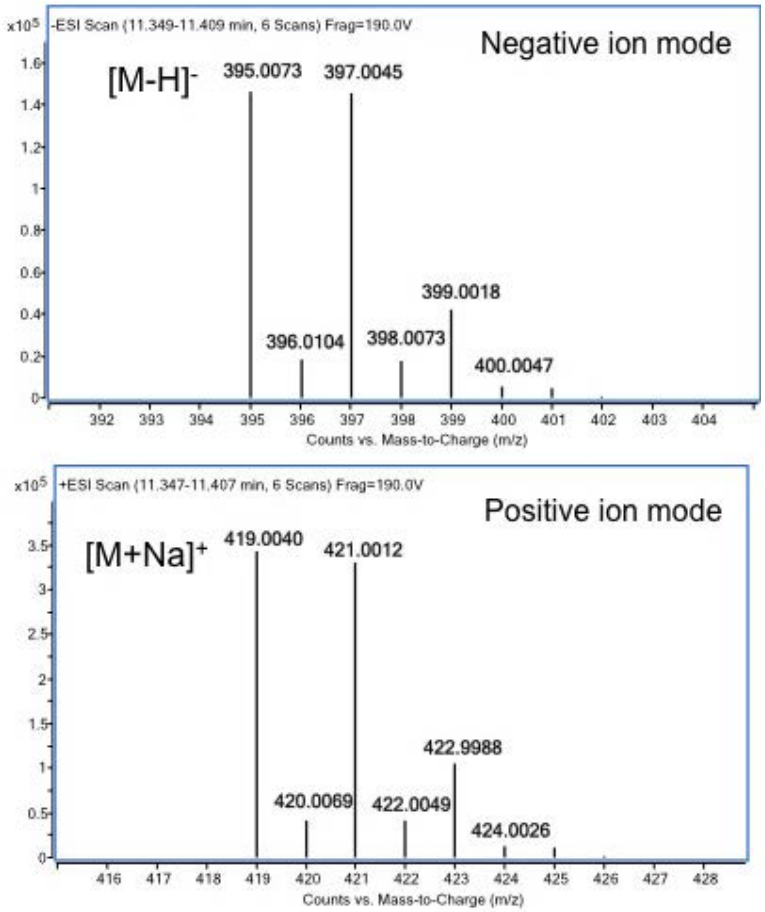
Results

Example 2



Mass spectrum of sucralose with a Q-TOF mass analyzer

Molecular Formula: $C_{12}H_{19}Cl_3O_8$
Molar mass: 397.64 g/mol

Mass spectrum of sucralose in (A) negative ion mode and (B) positive ion mode.
Source: Identification and Fragmentation of Sucralose Using Accurate-Mass Q-TOF LC/MS and Molecular Structure Correlator Software (Fig 1, p 3)

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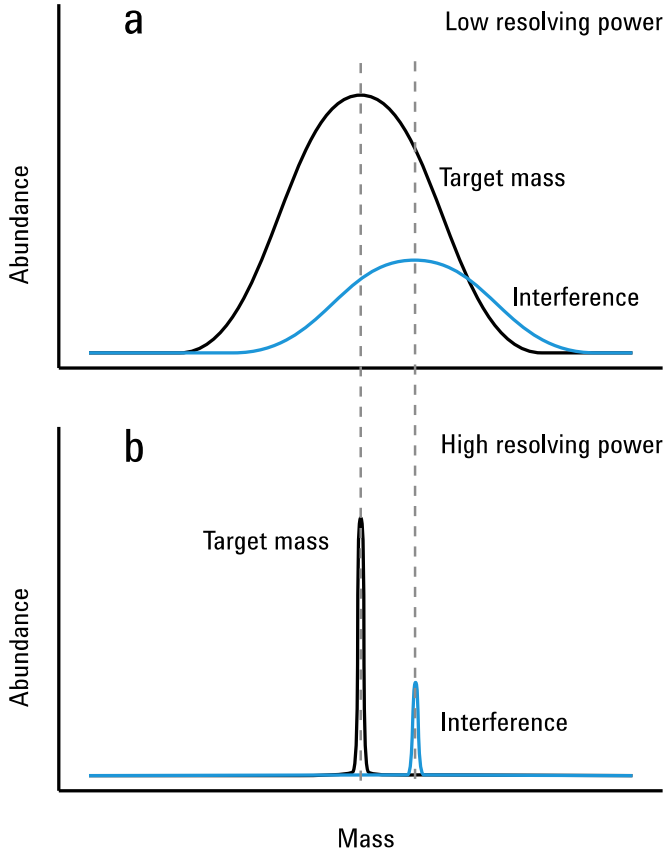
Results

Single Quad vs. High Resolution TOF

The analysis with a Single (Triple) quadrupole delivers nominal mass information (low resolving power), Time-of-Flight instruments can deliver accurate mass information (high resolving power).

Continuous calibration of a TOF system is needed for time-of-flight analysis to ensure best possible mass accuracy. Measurements typically deviate by only a few parts per million (ppm).

With sufficient mass resolution and mass accuracy, a TOF mass spectrometer can positively confirm elemental composition.



Resolving power of Single quadrupole (a) versus Time-of-Flight (b); Source: Agilent data

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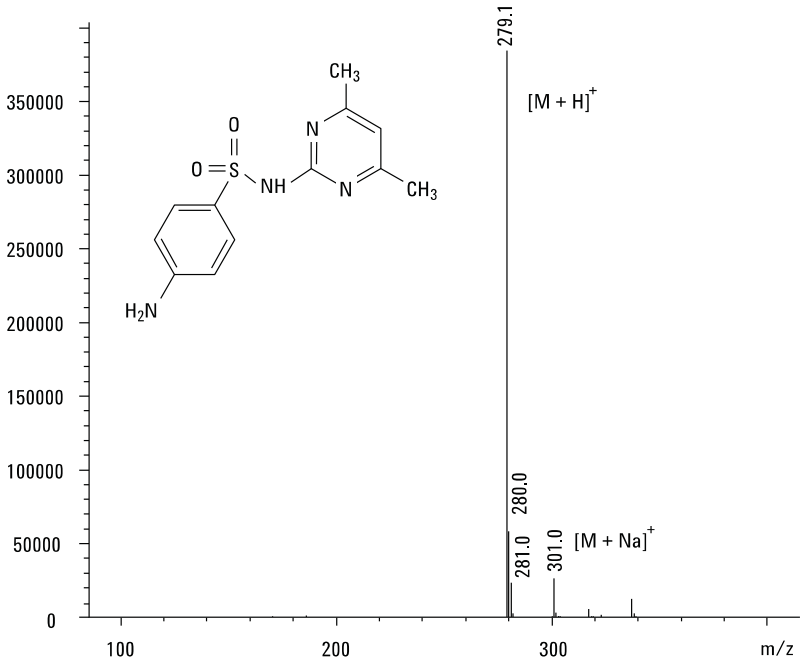
Results

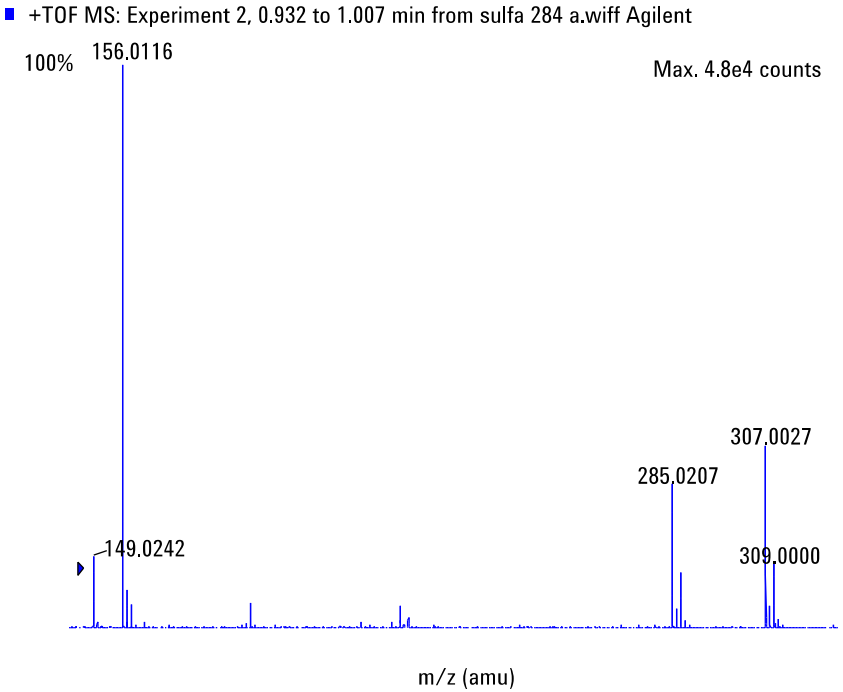
Single Quad vs. TOF

Typical Single Quadrupole mass spectrum



Mass spectrum of sulfamethazine. Source: G1960-90083 (p 17)

Typical TOF mass spectrum



Mass spectrum of sulfachloropyridazine with adduct and fragment ions. Source: Agilent data

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Results

Multiply Charged Ions and Deconvolution

Depending on the analyzed molecule and the ionization technique, multiple charged ions can be generated.

Small molecules and APCI delivers single charged molecules:

The measured *m/z* corresponds to the molecular weight after subtracting (positive ion) or adding (negative ion) the charge carrier.

For large molecules (peptides, proteins) ionized with ESI, more than one potential charge site (for protonation or deprotonation) is available which can result in multiply charged ions:

This makes large molecules like antibodies (> 1 Mio Da) accessible to mass spectrometry since the measured ions are shifted to a more readily measure *m/z* range.

A mathematic algorithm is needed to determine the real molecular weight from the measured *m/z*. This process is known as **deconvolution**.

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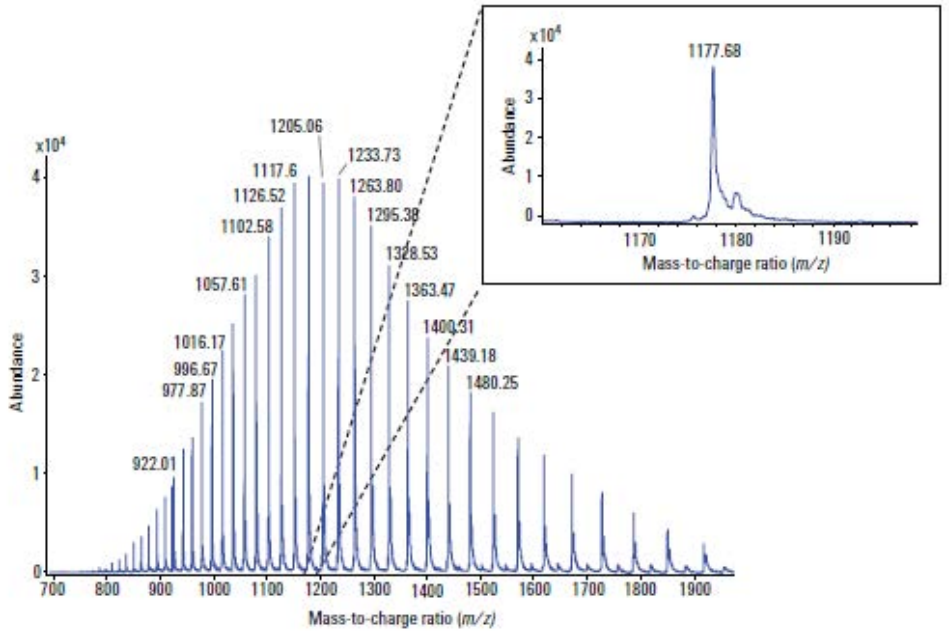
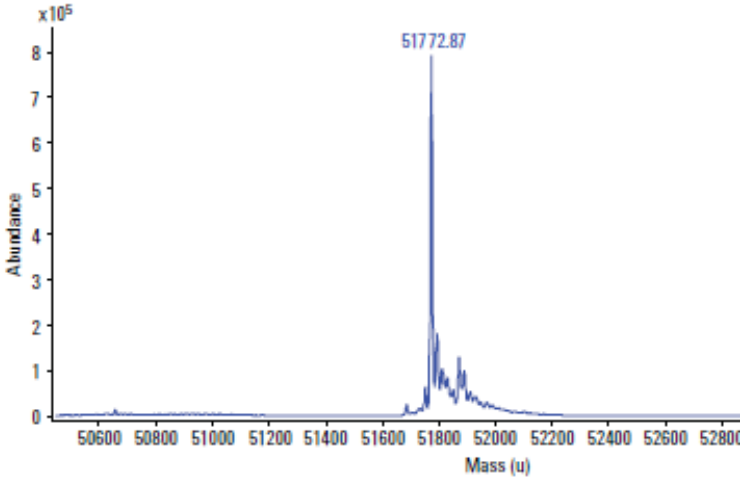
Results

Multiply Charged Ions and Deconvolution – Example



Mass spectrum of expressed glutamine synthetase.

Expected mass of unmodified glutamine synthetase: 51,772.7 u



Deconvoluted mass spectrum of expressed glutamine synthetase.

Source: Accurate-Mass LC/TOF-MS for Molecular Weight Confirmation of Intact Proteins (Fig 1, p 4)

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Publication	Title	Pub. No.
Manual	Agilent 7000 Series Triple Quad GC/MS Operation Manual	G7000-90044
Guide	Agilent 6100 Series Quadruple LC/MS systems – Concepts Guide	G1960-90083
Technical Overview	Time-of-Flight Mass Spectrometry	5990-9207EN
Application	Identification and Fragmentation of Sucralose Using Accurate-Mass Q-TOF LC/MS and Molecular Structure Correlator Software	5991-4066EN
Application	Accurate-Mass LC/TOF-MS for Molecular Weight Confirmation of Intact Proteins For Research Use Only. Not for use in diagnostic procedures.	5989-7406EN
Videos	www.agilent.com/chem/teachingresources	
Images	www.agilent.com/chem/teachingresources	

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Publication number 5991-5857EN

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Abbreviations

Abbreviation	Definition
APCI	Atmospheric Pressure Chemical Ionization
APPI	Atmospheric Pressure Photo Ionization
CI	Chemical Ionization
CID	Collision Induced Dissociation
D	Dopant (APPI)
Da	Dalton
EI	Electron Impact
ESI	Electrospray Ionization
GC	Gas Chromatography
GC/MS	Gas Chromatography Mass Spectrometry
ICP	Inductively Coupled Plasma
IT	Ion Trap

Abbreviation	Definition
LC/MS	Liquid Chromatography Mass Spectrometry
M	Molecule Ion
MALDI	Matrix Assisted Laser Desorption Ionization
MMI	Multimode Ionization
MS	Mass Spectrometry
m/z	Mass to Charge Ratio
QQQ	Triple Quadrupole
SIM	Single Ion Monitoring
SH	Solvent Molecules
SQ	Single Quadrupole
MRM	Multiple Reaction Monitoring
(Q) - TOF	Time-of-Flight

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Fundamentals of Mass Spectrometry: Hardware

BUILDING
BETTER SCIENCE

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Table of Contents (ToC)

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Introduction

Mass spectrometry (**MS**) is an instrumental method in analytical chemistry that helps identify the type and amount of chemicals present in a sample according to the mass-to-charge ratio and abundance of gas-phase ions.

A mass spectrum (plural *spectra*) is a pattern of the ion signal generated in a mass spectrometer as a function of the mass-to-charge (m/z) ratio. The masses of the molecular ions and fragments are used to determine the elemental composition or isotopic signature of a compound. Depending on the information obtained the chemical structures of molecules can be determined.

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Introduction

What Is MS Used For?

Mass spectrometry is a technique to qualitatively or quantitatively analyze and detect ions. It is often combined with Liquid or Gas chromatography

The applications are broad – here are some examples:

- Food and flavor analysis (mycotoxines, antibiotics, food profiling)
- Environmental analysis (PAH, pesticide, herbicides, benzene)
- Pharma applications (Bioanalysis, DMPK, drug discovery)
- Veterinary drug analysis
- And many more

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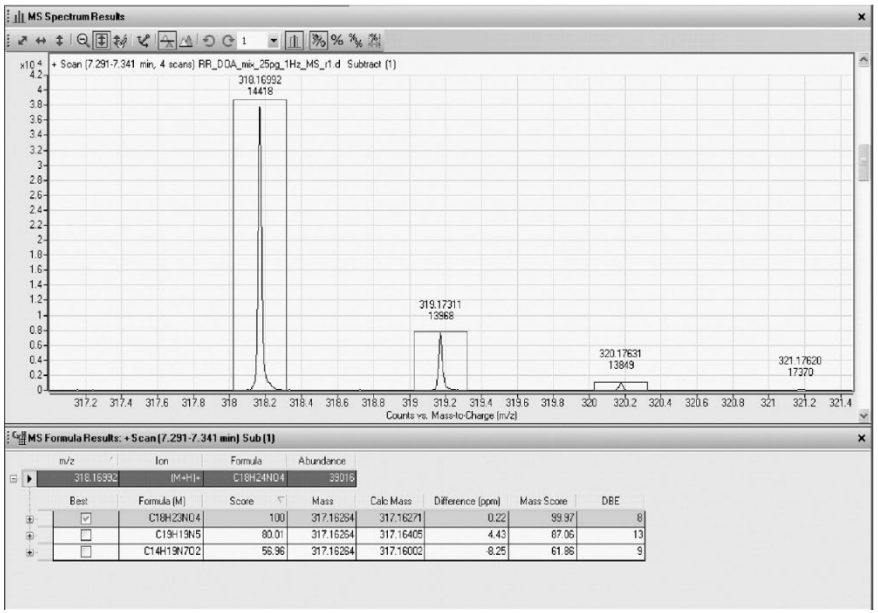
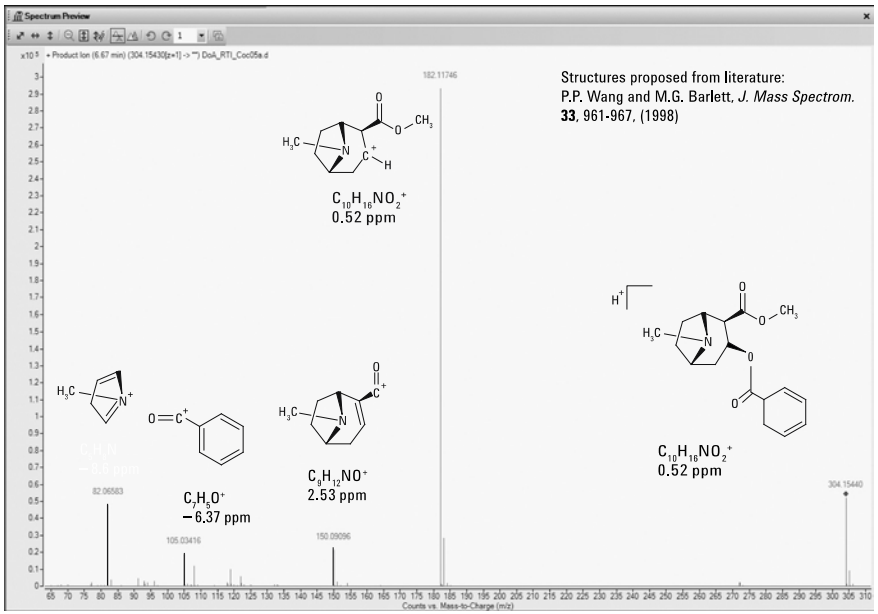
Introduction

What Does a Mass Spectrum Look Like?

ESI mass spectrum of cocaethylene



ESI MS/MS spectrum of cocaine



Source: A comparison of several LC/MS techniques for use in toxicology (Fig 36 & 37) For Forensic Use

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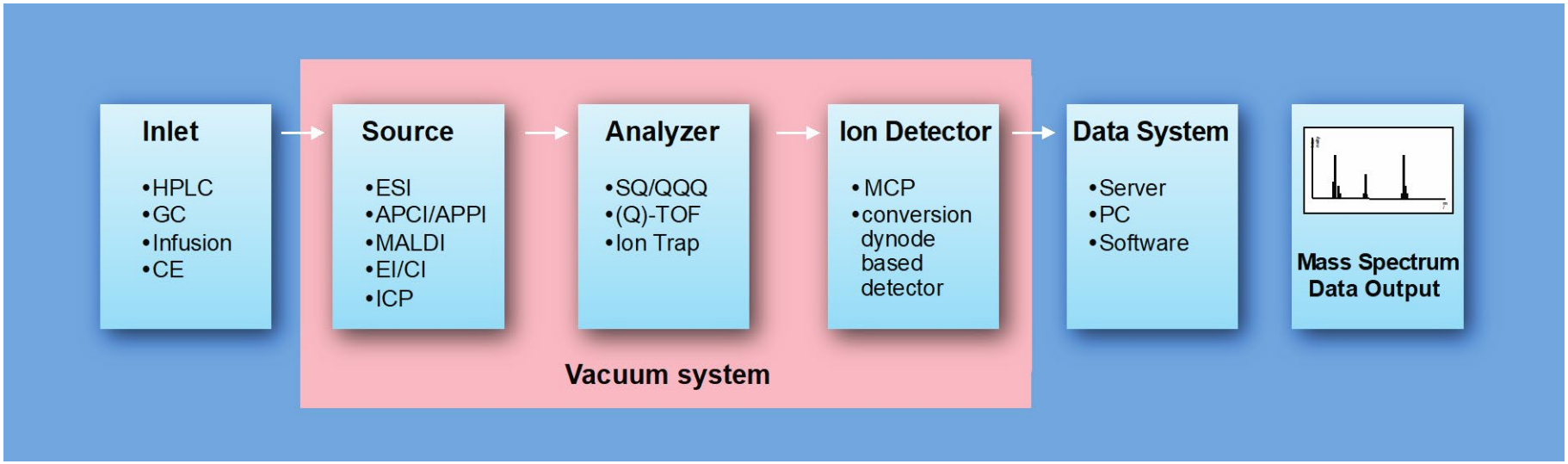
Introduction

Components of a Mass Spectrometer



The **ion source** produces ions of the analytes.
The **analyzer** manipulates the ions according to their mass-to-charge (m/z) ratio.
The **detector system** detects the ions and records the relative abundance of the ion species.

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Configuration of a MS System

Source

Before the sample can be mass analyzed, it must be ionized in the ion source.

Gaseous Sample Introduction:

- Electron Ionization (EI)
- Chemical Ionization (CI)

Liquid Sample Introduction:

- Electrospray Ionization (ESI)
- Atmospheric Pressure Chemical Ionization (APCI)
- Atmospheric Pressure Photo Ionization (APPI)
- Multimode Ionization (MMI)
- Matrix Assisted Laser Desorption Ionization (MALDI)
- Inductively Coupled Plasma (ICP)

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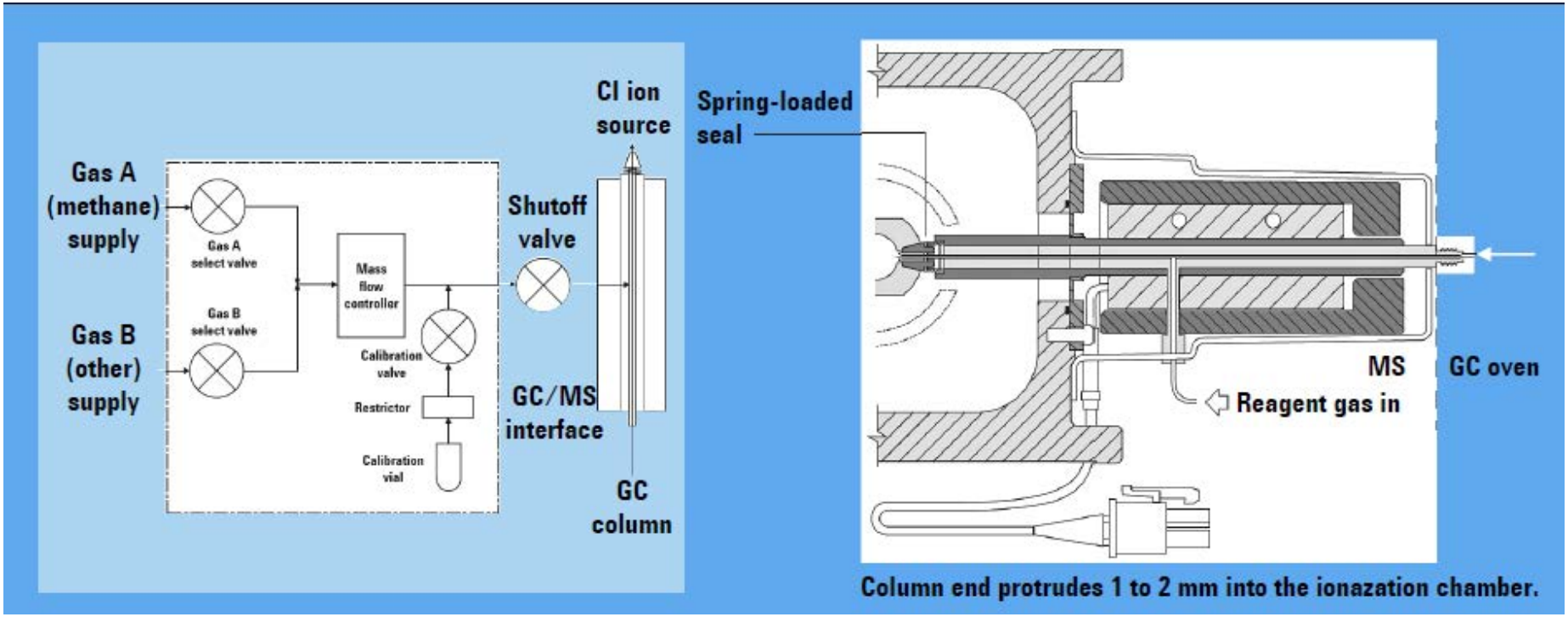
Configuration of a MS System

Source – Chemical Ionization

The CI reagent gas flow control module regulates the flow of reagent gas into the CI GC/MS interface. The flow module consists of a mass flow controller (MFC), gas select valves, CI calibration valve, shutoff valve, control electronics, and plumbing.



The CI GC/MS Interface. Source: Agilent 7000 Series Triple Quad GC/MS Operation Manual (p 79)

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Configuration of a MS System

Source – Electrospray

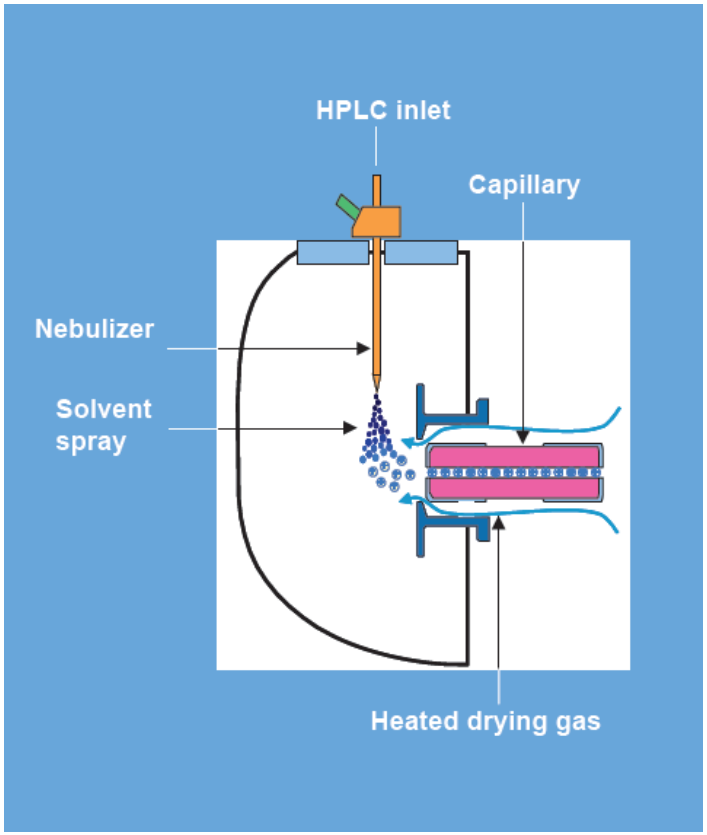
Electrospray ionization (ESI) is a soft ionization technique.

LC eluent is sprayed (nebulized) into a spray chamber at atmospheric pressure in the presence of a strong electrostatic field and heated drying gas. The electrostatic field occurs between the nebulizer, which is at ground in this design, and the capillary, which is at high voltage.

Suitable molecules:

- Small molecules (glucose) and large biomolecules (proteins, oligonucleotides)

Multiple charging is the phenomena in ESI that allows analysis of larger molecules (-> Deconvolution).



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Configuration of a MS System

Source – General Considerations LC/MS

When adapting an LC method for LC/MS, there are 3 key points that should be considered:

1. Compatibility of mobile phase
2. Compatibility of flow rate and column
3. Ionizability of analytes
   - Is the analyte volatile?
   - Is the analyte thermally labile?
   - Does the analyte have heteroatoms that can accept (N_2 > O_2) or lose (O_2 >> N_2) a proton?
     - accepts a proton - use positive ion mode
     - loses a proton - use negative ion mode

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Configuration of a MS System

Source – General Mobile Phase Considerations

Metal ion buffers interfere with ionization

Surfactants interfere with evaporation

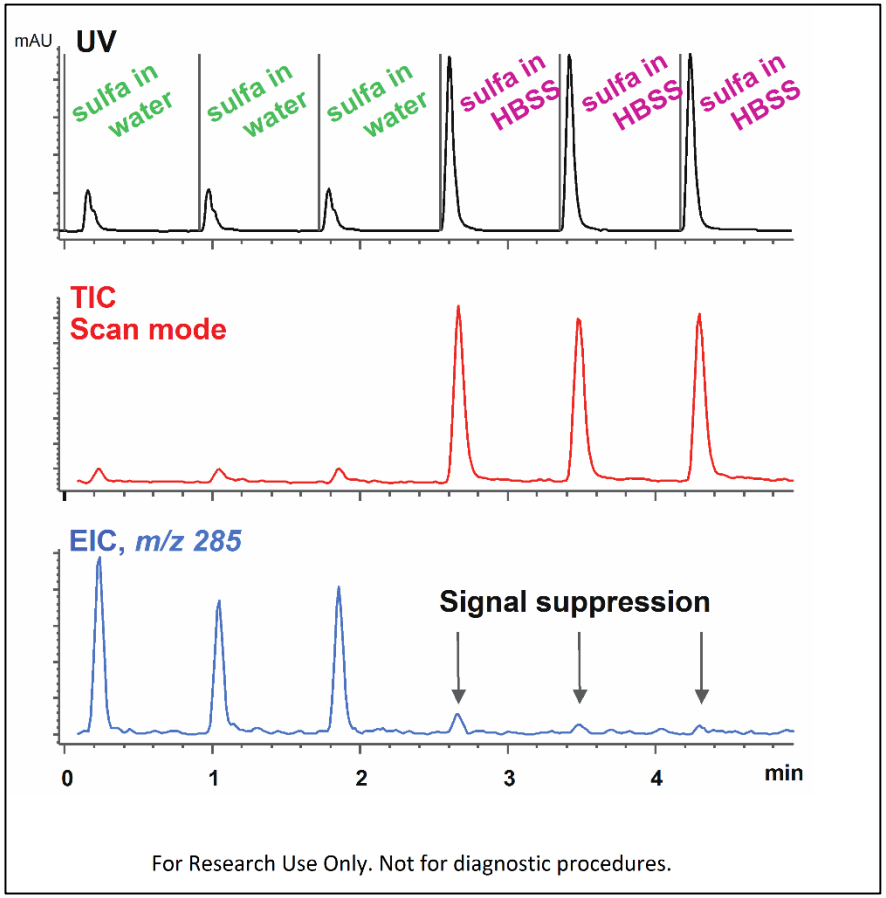
Ion pairing reagents can ionize and create a high background

Strong ion pairing with an analyte can prevent the analyte from ionizing

These interactions are commonly described as "**ion suppression**".

Some mobile phase additives will cause persistent background problems:

- TEA interferes in positive ion mode (*m/z* 102)
- TFA interferes in negative ion mode (*m/z* 113)



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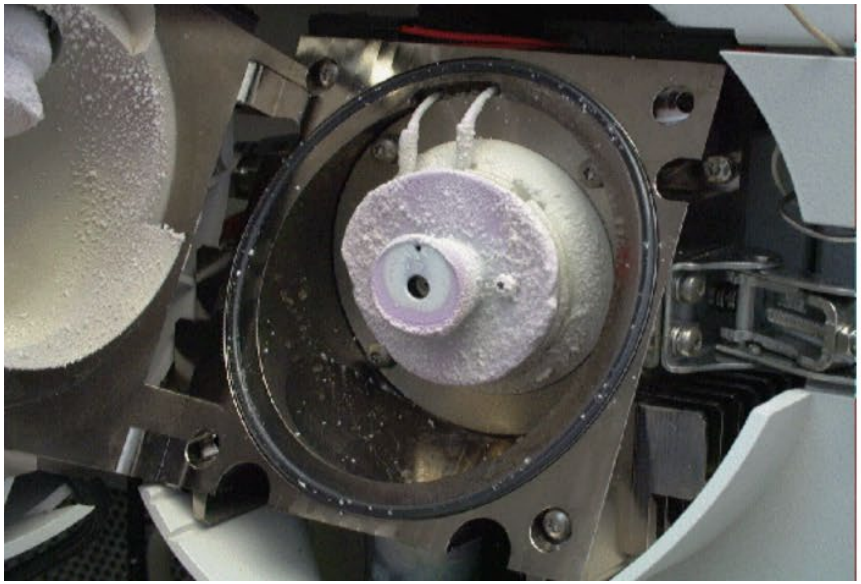
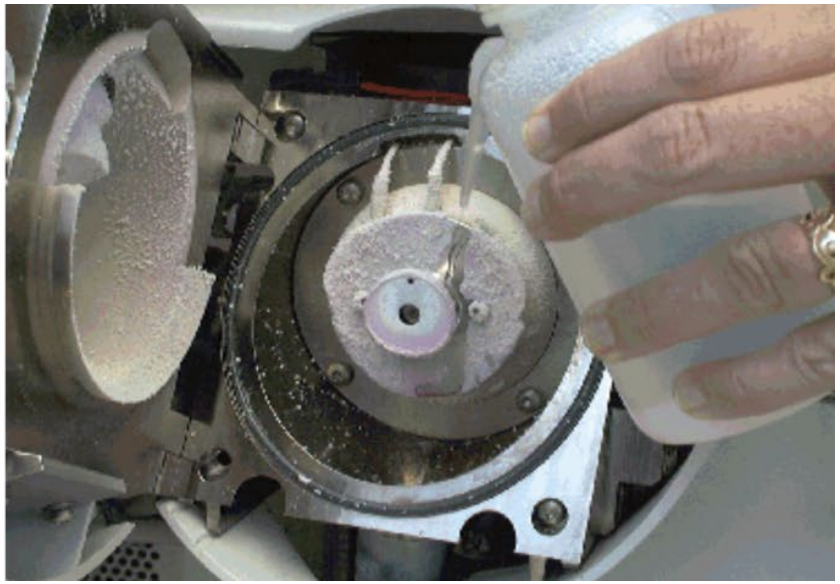
Configuration of a MS System

Source – General Mobile Phase Considerations

A well-designed ion source is robust and tolerates non-volatile components; however, the ionization process is effected by the concentration and type of salt/buffer (see next slide).



APCI Spray Chamber after 635 Injections of sample in HBSS



Cleaning the spray chamber

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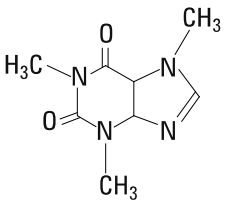
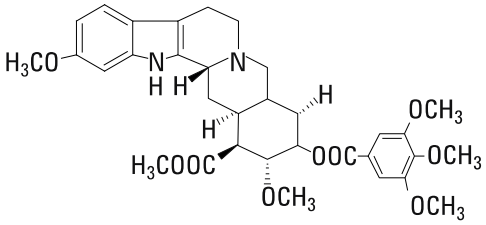
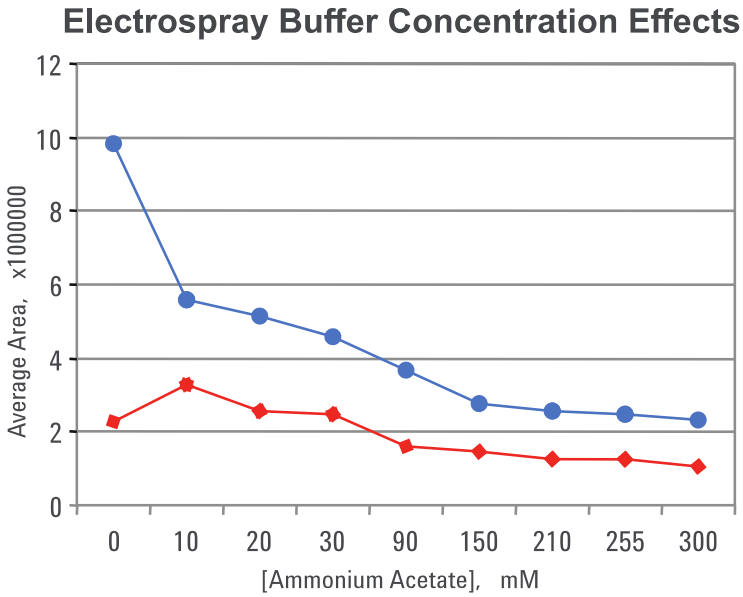
Configuration of a MS System

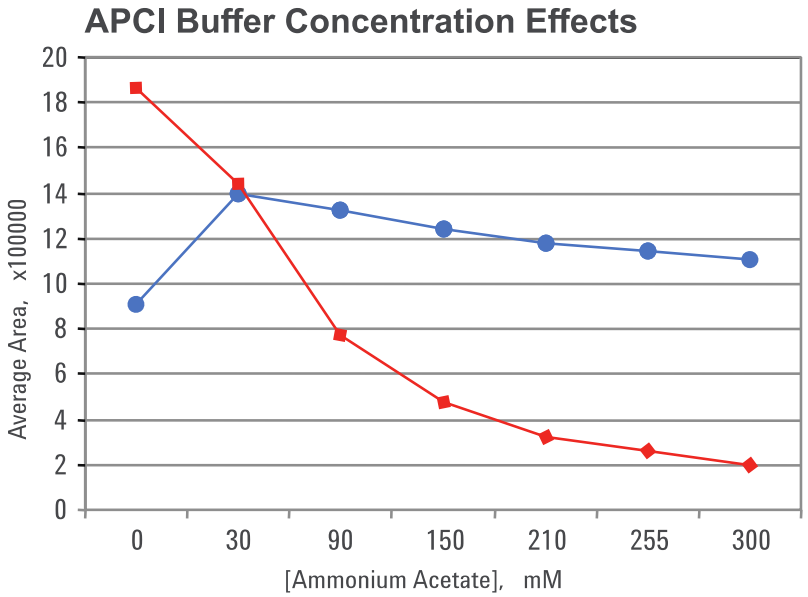
Effect of Volatile Buffer Concentration on Signal





Caffeine **Reserpine**





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Configuration of a MS System

Source – Sample Considerations LC/MS

ESI	APCI	APPI
Volatility not required	Some volatility required	Some volatility required
Preferred technique for thermally labile analytes	Analyte must be thermally stable	Analyte must be thermally stable
Ions formed in solution	Ions formed in gas phase	Ions formed in gas phase
Can form multiply charged ions	Forms singly charged ions only	Forms singly charged ions only

Many compounds will ionize well using all three sources.
APCI / APPI can ionize molecules that are too non-polar for ESI to ionize.

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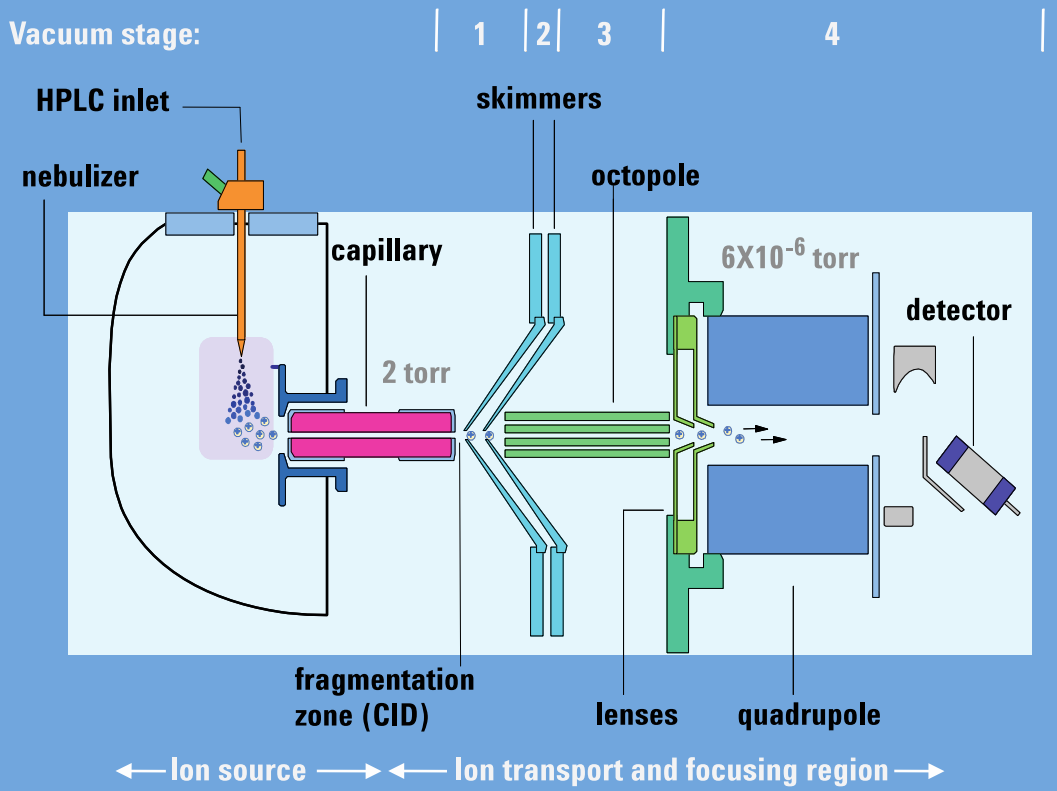
Configuration of a MS System

Analyzer – Single Quad (SQ)

Key Applications:

- Confirmation of synthesis
- Impurity analysis
- Confirmation of product uniformity
- Verify low levels of impurities



Ion path for Agilent 6120 Quadrupole LC/MS system

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Configuration of a MS System

Analyzer – Single Quad (SQ)

API – atmospheric pressure ionization, main steps:

- Ion source forms sample ions and transfers them into the vacuum system
- Various ion-optic elements focus and guide the ions through a series of vacuum stages
- A quadrupole mass analyzer filters the ions by *m/z*
- A detector records the selected *m/z* ions as the intensity for that *m/z* value

Ion transport and fragmentation (first vacuum stage)

- Ions are electrostatically drawn through a drying gas and a heated sampling capillary into the first stage of the vacuum system. Near the exit of the capillary is a metal skimmer with a small hole
- A combination of electric fields and ion momentum allow analytes to pass through the skimmer aperture
- Most of the uncharged, lighter drying gas (nitrogen) molecules are deflected by the skimmer and pumped away by a vacuum pump
- The ions that pass through the skimmer move into the second stage of the vacuum system

Atmospheric pressure ionization techniques are all relatively “soft” techniques. They generate primarily:

- Molecular ions M^+ or M^-
- Simple adduct ions $[M + Na]^+$
- Protonated molecules $[M + H]^+$
- Ions representing simple losses, such as the loss of a water molecule $[M + H - H_2O]^+$

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Configuration of a MS System

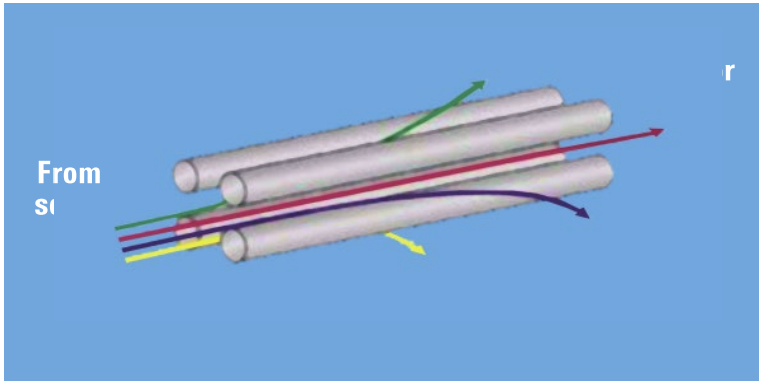
Analyzer – Single Quad (SQ)

Ion transport (second and third vacuum stages)

- Neutral gas molecules are removed and ions are confined by an octopole ion guide that traverses two vacuum stages. Radio-frequency voltage is applied to the octapole rods which focuses the ions to the center of the rods while gas molecules are pumped away
- After exiting the octapole the ions pass through two focusing lenses into the fourth stage of the vacuum system

Ion separation and detection (fourth vacuum stage)

- Ions are separated by the m/z ratio in the quadrupole mass analyzer
- The quadrupole mass analyzer consists of four parallel rods to which specific direct-current (DC) and radio-frequency (RF) voltages are applied
- An electric field generated by applying voltages determines which m/z ratio ion can pass through the filter at a given time
- The ions that pass through are focused on the detector
- An electron multiplier then detects the ions



Quadrupole mass analyzer

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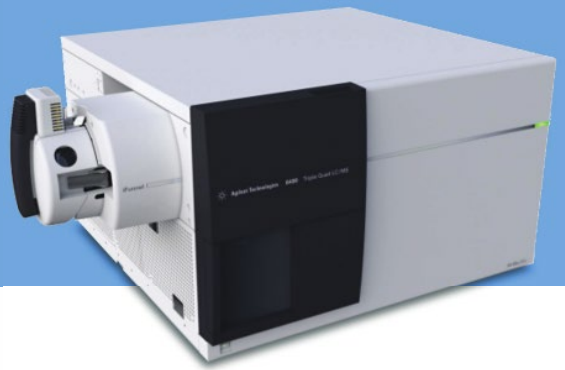
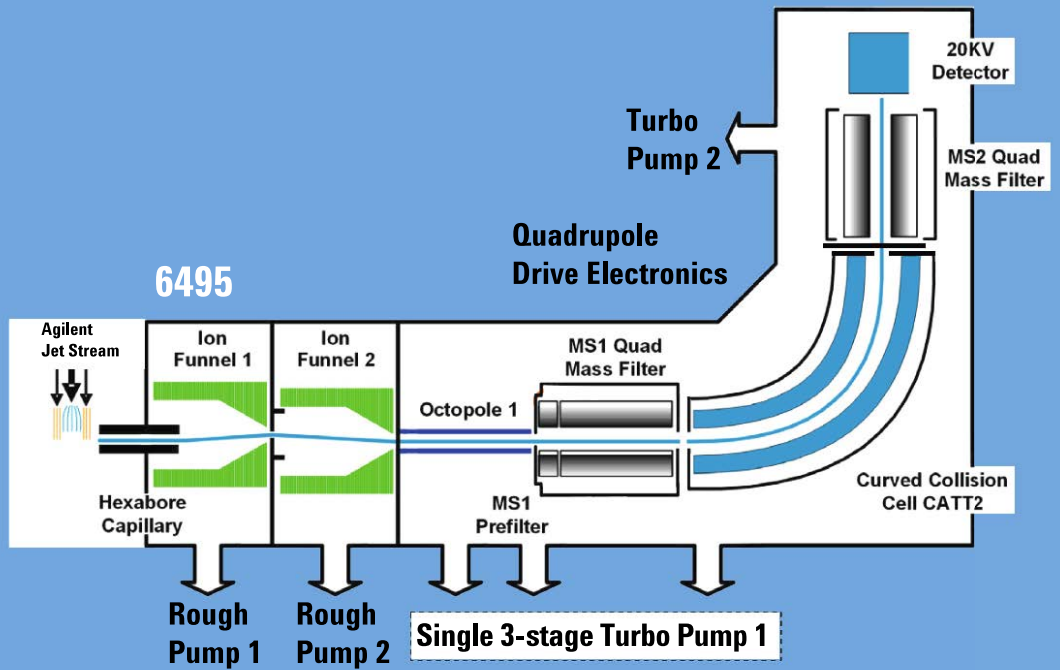
Configuration of a MS System

Analyzer – Triple Quad (QQQ)



Key Applications:

- Quantitative analysis of small molecules and peptides
- Targeted analysis
- Trace analysis in complex matrices such as Food safety / environmental studies, Drug discovery, Bioanalysis…



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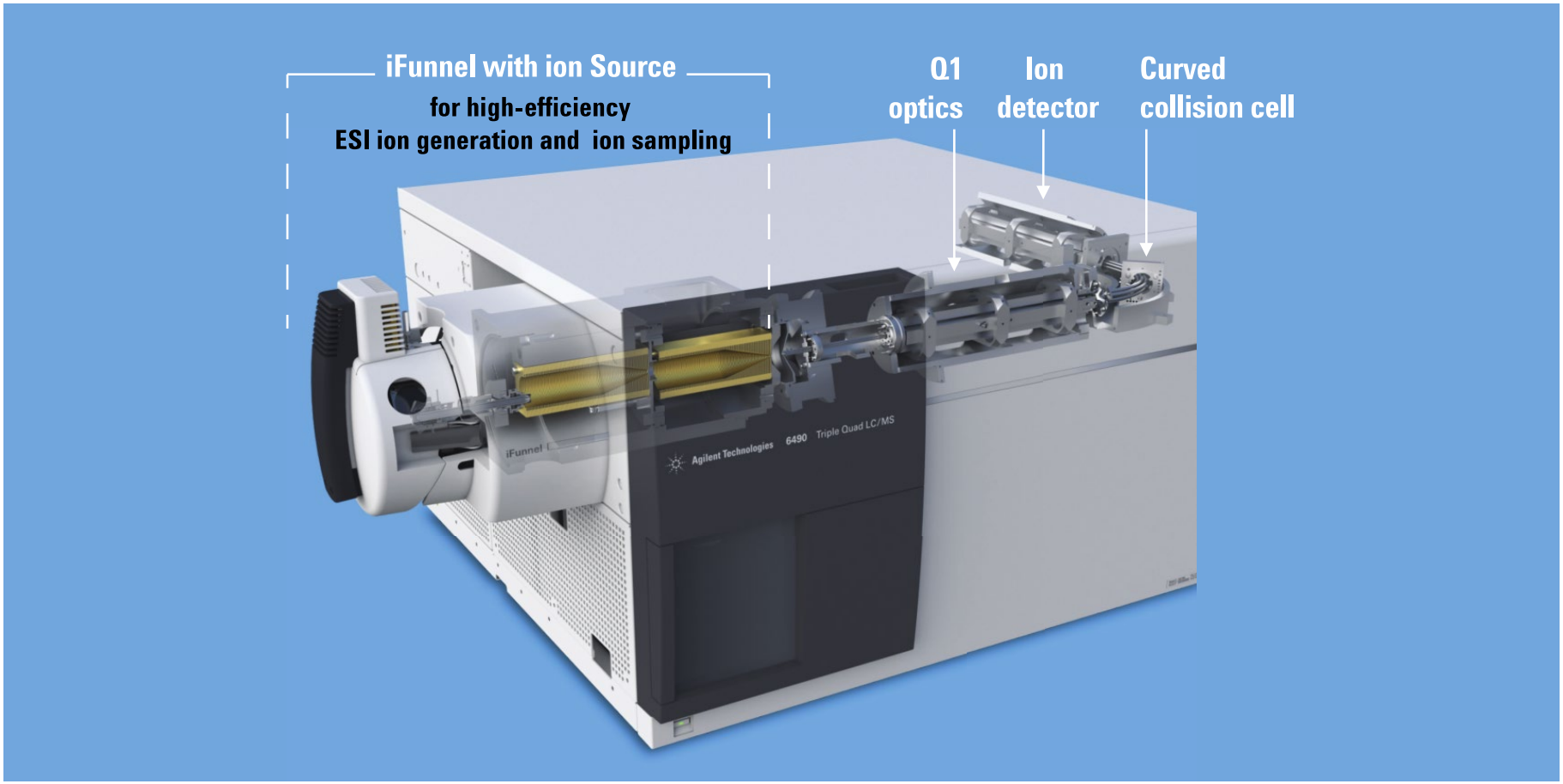
Configuration of a MS System

Analyzer – Triple Quad (QQQ)



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Configuration of a MS System

Analyzer – Triple Quad (QQQ)

API – atmospheric pressure ionization – see Single Quad

Ion transport and fragmentation

- Q1 (quadrupole) consists of four parallel hyperbolic rods through which selected ions are filtered
- Q2 is a collision cell which confines and fragments ions by collision with an inert, non-reactive gas (nitrogen or argon). The cell design has axial acceleration to keep the ions moving through the gas for high speed MS/MS analysis
- Q3 (quadrupole) filters the fragment ions allowing several modes of operation providing different information

Two main operation modes:

Product Ion Scan:

Q1: Selection of a precursor ion

Q2: Fragmentation of precursor in collision cell

Q3: All fragments are scanned resulting in a product-ion scan MS/MS spectrum

Since the fragment ions are pieces of the precursor, they represent portions of the overall structure of the precursor molecule. A **compound's fingerprint** is generated.

Selected Reaction Monitoring (SRM):

Q1: Selection of a precursor ion

Q2: Fragmentation of precursor in collision cell

Q3: Selection of a specific m/z product ion

This produces very sensitive results and is called ***single reaction monitoring (SRM)***. Running multiple SRMs for the same precursor ion is called ***multiple reaction monitoring (MRM).***

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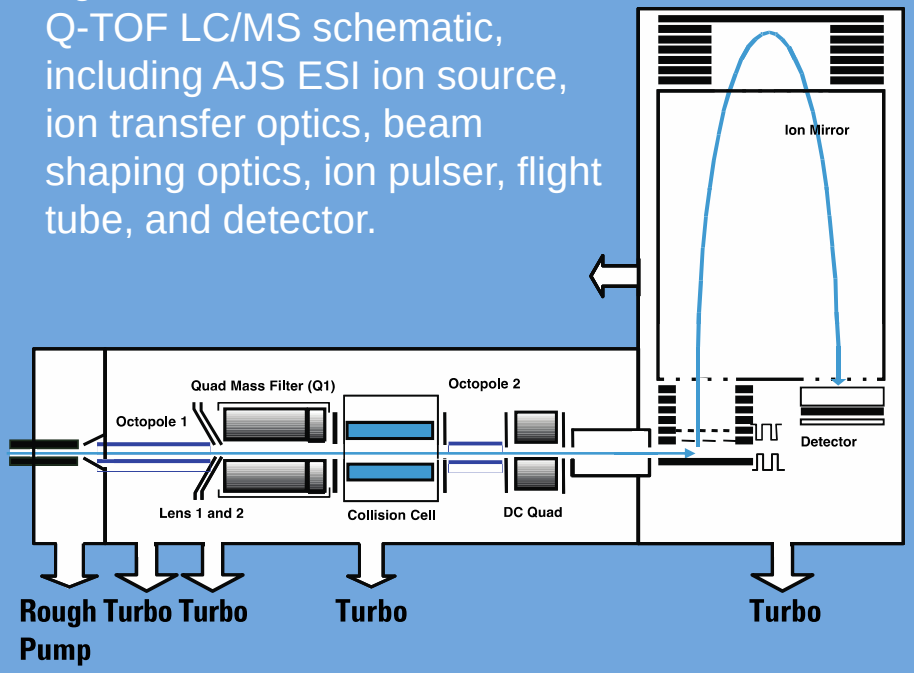
Configuration of a MS System

Analyzer – (Q)-Time-of-Flight (TOF)

Key Applications:

- Natural products screening
- Compound profiling
- Protein/peptide analysis
- Biomarker discovery
- Impurity profiling

This image shows the complete Agilent 6520 Q-TOF LC/MS schematic, including AJS ESI ion source, ion transfer optics, beam shaping optics, ion pulser, flight tube, and detector.



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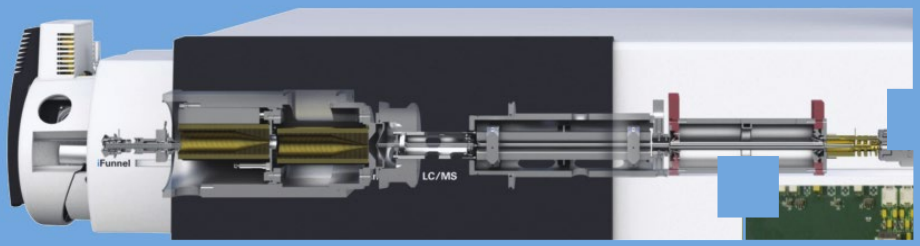
Configuration of a MS System

Analyzer – (Q)-Time-of-Flight (TOF)



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Configuration of a MS System

Analyzer – (Q)-Time-of-Flight (TOF)

API – atmospheric pressure ionization - see Single Quad

Ion transport and fragmentation (Q-TOF)

- Ions are passing through the optics and reach the quadrupole analyzer
- The quadrupole analyzer consists of four parallel hyperbolic rods which selected ions based on their *m/z* ratio pass through
- A hexapole collision cell confines and fragments ion by collision with an inert, non-reactive gas (nitrogen or argon). The cell design has axial acceleration to keep the ions moving through the gas for high speed MS/MS analysis
- Fragment ions formed in the collision cell are then sent to the TOF to record product ions as a function of precursor ions

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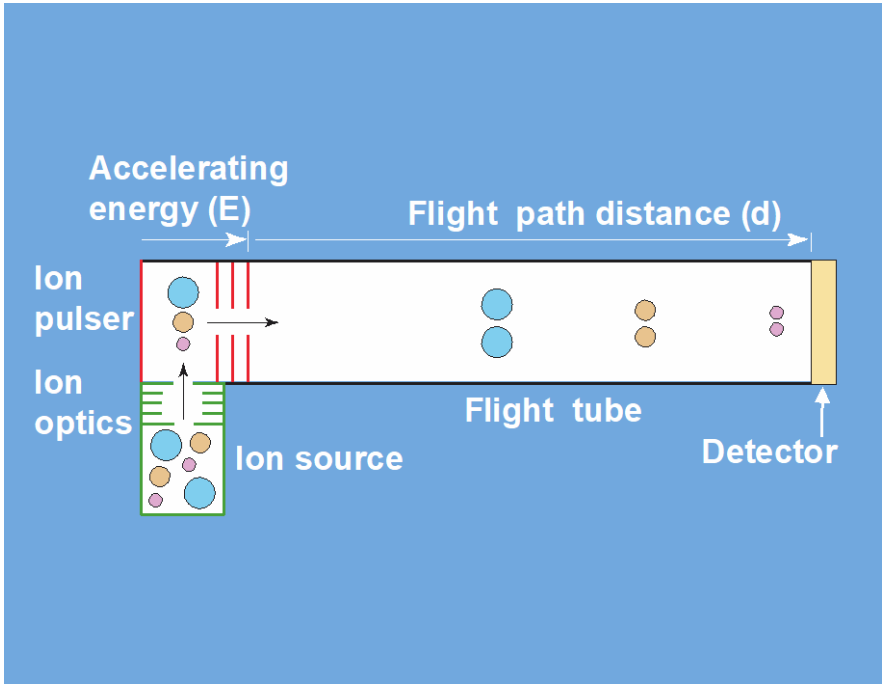
Configuration of a MS System

Analyzer – (Q)-Time-of-Flight (TOF)

Flight tube

- An ion pulser accelerates the ions which travel through the flight tube
- An ion “mirror” at the opposite end of the flight tube reflects the ions towards the detector
- An ion mirror increases the resolving power of the instrument by effectively doubling the flight distance in the same space, and by performing a refocusing operation so that ions having different initial velocities still arrive simultaneously at the detector

Because the calculation for the mass of each ion depends on its flight time, the background gas pressure must be very low. Any collision of an ion with residual gas slows the ion on its path to the detector and affects the accuracy of the mass calculation.



TOF analysis of ions of various masses, each with a single charge. For clarity and simplicity, this is shown in a linear time-of-flight mass spectrometer that does not have an ion mirror.
Source: Time-of-Flight Mass Spectrometry

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Configuration of a MS System

Analyzer – (Q)-Time-of-Flight (TOF)

Tuning and Calibration

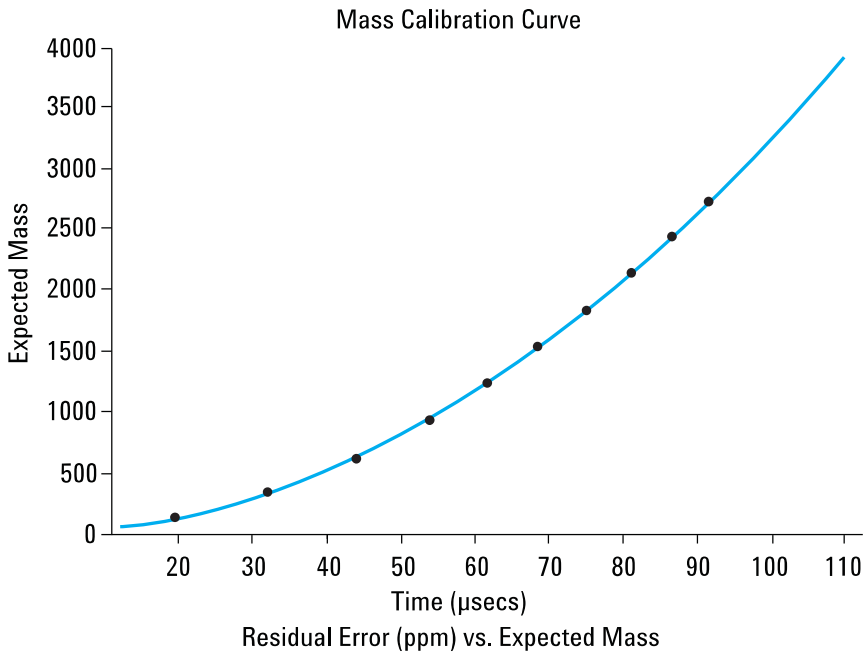
Tuning is the process of adjusting both the quadrupole (for the Q-TOF) and TOF parameters to achieve the following goals:

- Maximize signal intensity and maintain acceptable resolution, or
- Maximize resolution and maintain acceptable signal intensity

Calibration is the process of assigning accurate masses based on the known masses of standard compounds, introduced either prior to, or while running the sample.

This is very important since the calculation for the mass of each ion depends on its flight time (t):

$$m/z = [a(t - t_0)]^2$$



Example calibration curve resulting from equation (left)
Source: LCMS/TOF System Concept Guide (Fig 6)

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Configuration of a MS System

Analyzer – (Q)-Time-of-Flight (TOF)

What is the benefit of accurate mass?

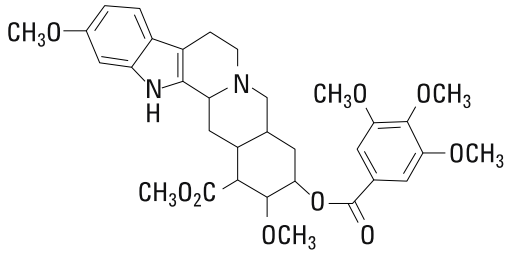
When mass accuracy is above 100 ppm as with a single quadrupole MS, compound annotation requires additional information such as retention time.

But with low ppm mass accuracy, compound annotation can be made with reasonable confidence if the user has the knowledge of the possible elements in the compound and an expectation of what is in the sample.

Confidence in identification is possible when MS/MS analysis is performed.

Example Reserpine

Reserpine $C_{33}H_{40}N_2O_9$ $[M+H]^+$: 609.28066 Da



Reserpine

Number (#) of possible elemental formulae using C, H, O & N:

Accuracy	# possible formulae	
165 ppm	209	→ SQ mass accuracy
10 ppm	13	
5 ppm	7	
3 ppm	4	
2 ppm	**2**	**→ TOF mass accuracy**

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Configuration of a MS System

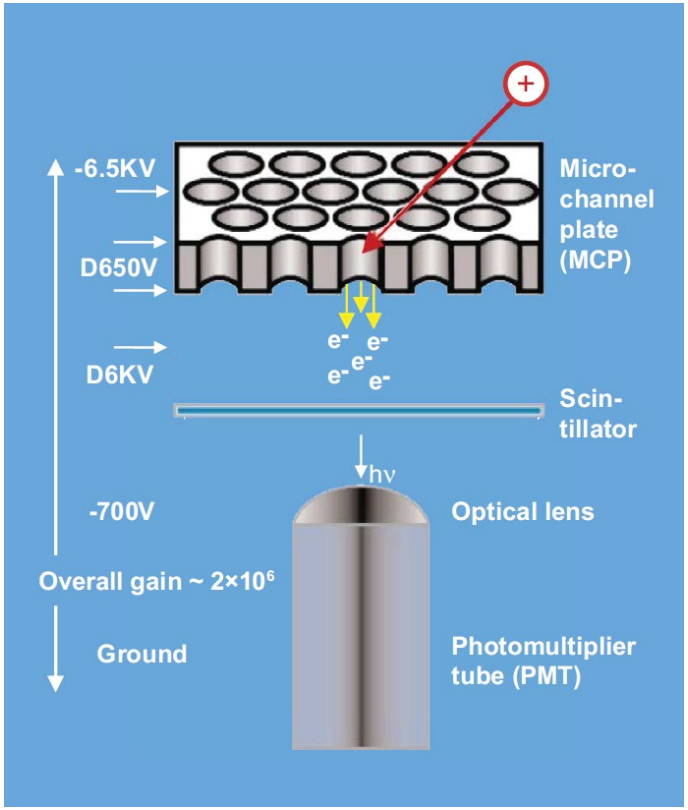
Detector – (Q)-Time-of-Flight (TOF)

Detector

The ion detector is equipped with a microchannel plate (MCP) which is a thin plate containing a set of microscopic tubes. The function of a MCP is to enhance a signal (~ 10x):

- An ion hits the front surface of the MCP which releases an electron
- An electrical signal amplification follows by collision of the freed electron with the walls of the microscopic tubes releasing more and more electrons
- An ever-increasing cascade of electrons travels to the rear of the plate

These electrons are focused onto a scintillator, which, when struck by electrons, produces a flash of light. The light from the scintillator is focused onto a photomultiplier tube (PMT). An electrical signal is produced read by the data system.



TOF detector with potentials shown for positive ion operation
Source: LC/MSD TOF System (Fig 2)

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Configuration of a MS System

Data System

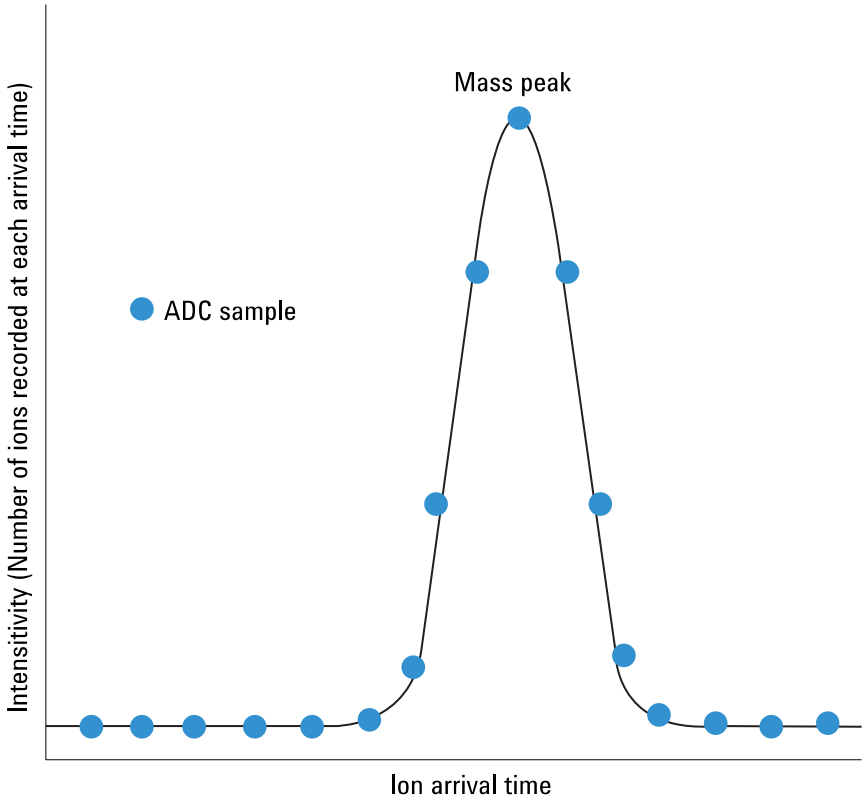
Data System

While there is an exact instant when each ion strikes the detector, it is difficult to transfer this perfectly into the digital world. Agilent's Q-TOF systems use an analog-to-digital converter (ADC).

The function of an ADC is to digitally represent the signal that comes from the ion detector. During each cycle, the detector output signal intensity is converted into a digital value.

The recorded and converted signals are displayed at the user interface as a mass spectrum.

Depending on the analysis, automated workflows and special software packages such as MS/MS libraries and compound databases are available to speed data analysis.



An ADC can record multiple ions per transient, so it accurately tracks ion signal intensity.
Source: Time-of-Flight Mass Spectrometry (Fig 4)

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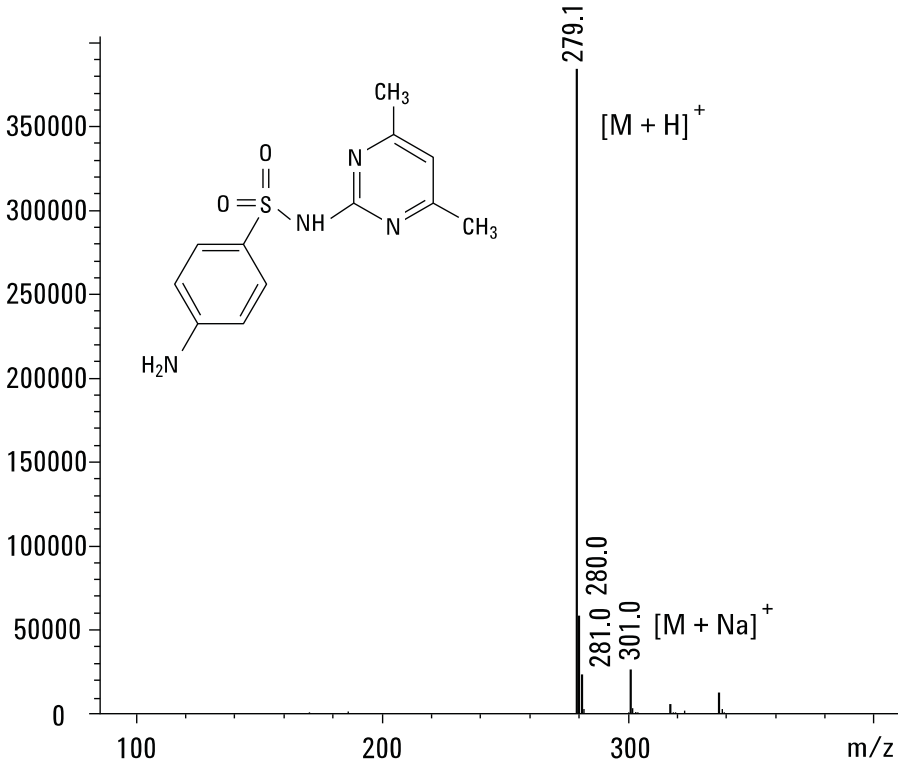
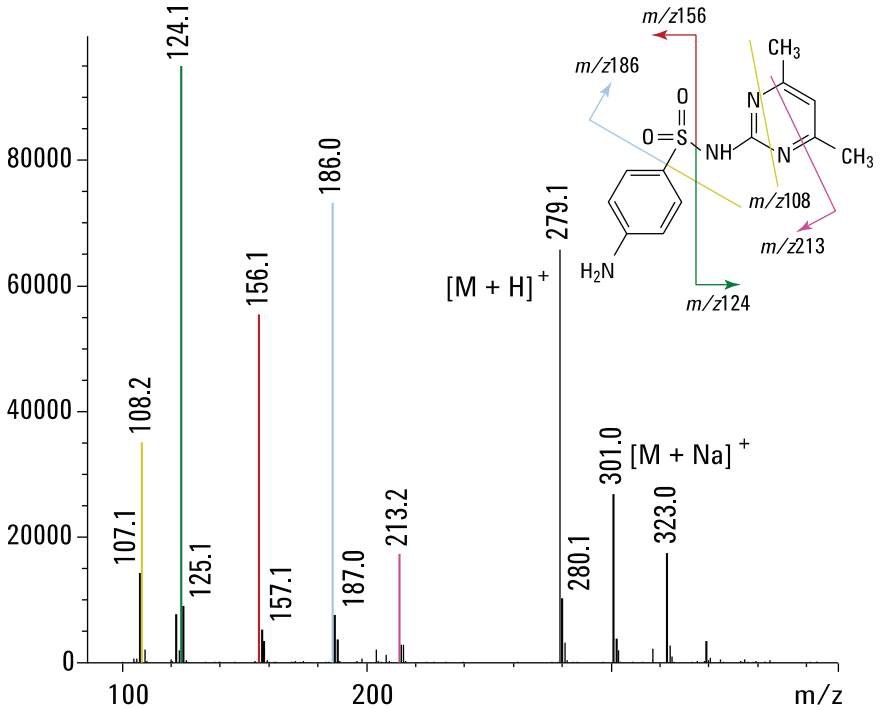
Examples

Mass spectrum (SQ) of sulfamethazine
low fragmentor



Mass spectrum (SQ) of sulfamethazine
high fragmentor (CID)



Mass spectrum of sulfamethazine with low and high fragmentor
Source: Agilent 6100 Series Quadrupole LC/MS Systems (Fig 6 & 7)

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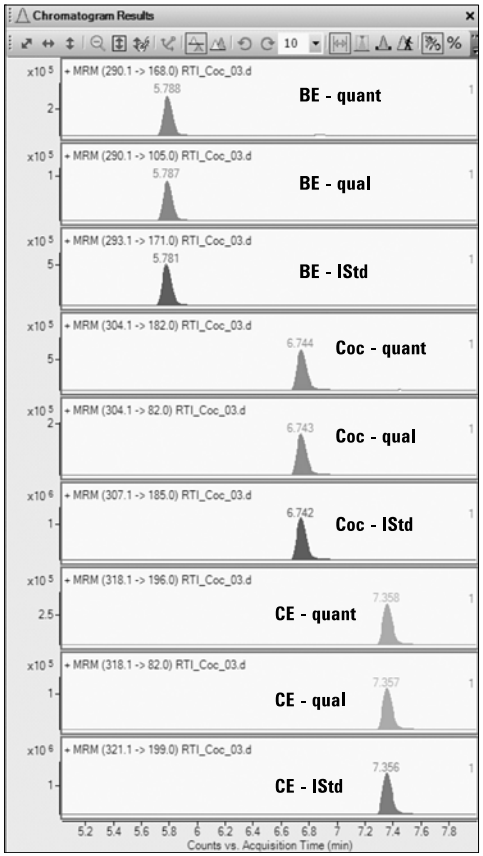
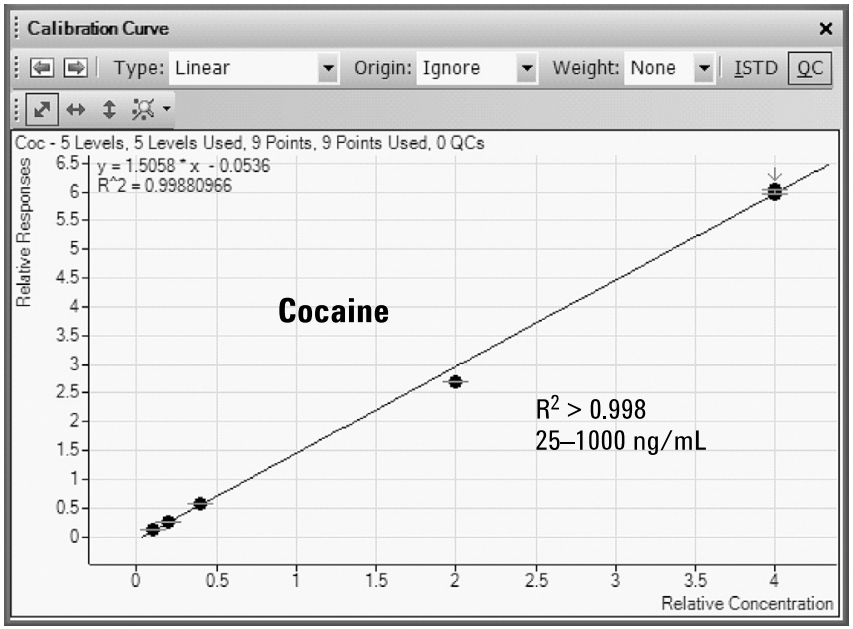
Examples

Multiple reaction monitoring chromatograms (QQQ) for a mid-level range calibrator of the cocaine metabolites.



Calibration curve of cocaine



A Comparison of Several LC/MS Techniques for Use in Toxicology
Source: Application Note 5990-3450EN For Forensic Use

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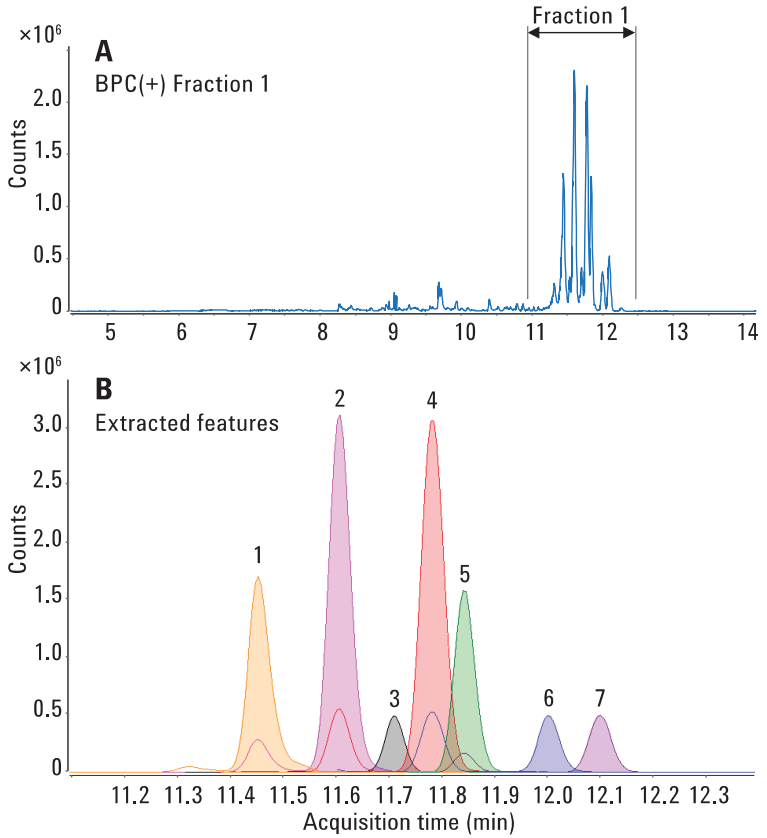
Examples

Fraction 1, positive ionization, triglycerides and cholesterol esters in plasma. Triacylglycerols (TG) and Cholesterol esters (CE) represent the most apolar lipids. Both groups are detected as ammonia adducts in the positive ESI mode.

Feature	Formula	t_R (min)	*m/z*	Identity
1	$C_{55}H_{101}O_6N$	11.45	871.7629	$TG(52:4)NH_3$
2	$C_{55}H_{103}O_6N$	11.61	873.7786	$TG(52:3)NH_3$
3	$C_{47}H_{79}NO_2$	11.71	689.6111	$CE(20:4)NH_3$
4	$C_{55}H_{105}O_6N$	11.78	875.7942	$TG(52:2)NH_3$
5	$C_{45}H_{79}NO_2$	11.84	665.6111	$CE(18:2)NH_3$
6	$C_{55}H_{107}O_6N$	12	877.8099	$TG(52:1)NH_3$
7	$C_{45}H_{81}NO_2$	12.1	667.6267	$CE(18:1)NH_3$



Unraveling the Complexity of Lipidomes by Multiple Heart-Cutting Q-TOF LC/MS with the Agilent 1290 Infinity 2D-LC Solution.

Source: Application Note 5991-5532EN

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Publication	Title	Pub. No.
Application	A Comparison of Several LC/MS Techniques for Use in Toxicology For Forensic Use	5990-3450EN
Manual	Agilent 7000 Series Triple Quad GC/MS Operation Manual	G7000-90044
Guide	Agilent 6100 Series Quadruple LC/MS System – Concept Guide	G1960-90083
Guide	Agilent G3250AA LC/MSD TOF System Concept Guide	G3300-90012
Technical Overview	Time-of-Flight Mass Spectrometry	5990-9207EN
Application	Unraveling the Complexity of Lipidomes by Multiple Heart-Cutting Q-TOF LC/MS with the Agilent 1290 Infinity 2D-LC Solution For Research Use Only. Not for use in diagnostic procedures.	5991-5532EN
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THANK YOU

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Abbreviations

Abbreviation	Definition
ADC	Analog-to-Digital
APCI	Atmospheric Pressure Chemical Ionization
APPI	Atmospheric Pressure Photo Ionization
CE	Capillary Electrophoresis
CI	Chemical Ionization
CID	Collision Induced Dissociation
Da	Dalton
DMPK	Drug Metabolism and Pharmacokinetics
EI	Electron Impact
ESI	Electrospray Ionization
GC	Gas Chromatography
GC/MS	Gas Chromatography Mass Spectrometry
HBSS	Hank's Balanced Salt Solution
ICP	Inductively Coupled Plasma
IVD	In Vitro Diagnostic
LC/MS	Liquid Chromatography Mass Spectrometry

Abbreviation	Definition
MALDI	Matrix Assisted Laser Desorption Ionization
MCP	Microchannel Plate
MMI	Multimode ionization
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry
m/z	Mass to Charge Ratio
PAH	Polycyclic Aromatic Hydrocarbon,
QQQ	Triple Quadrupole
SIM	Single Ion Monitoring
SQ	Single Quadrupole
SRM/MRM	Selected/Multiple Reaction Monitoring
(Q) - TOF	(Quadrupole) - Time-of-Flight
TEA	Triethylamine
TFA	Trifluoroacetic Acid
u	Unit

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