
# GAS CHROMATOGRAPHY

# Different types of chromatography

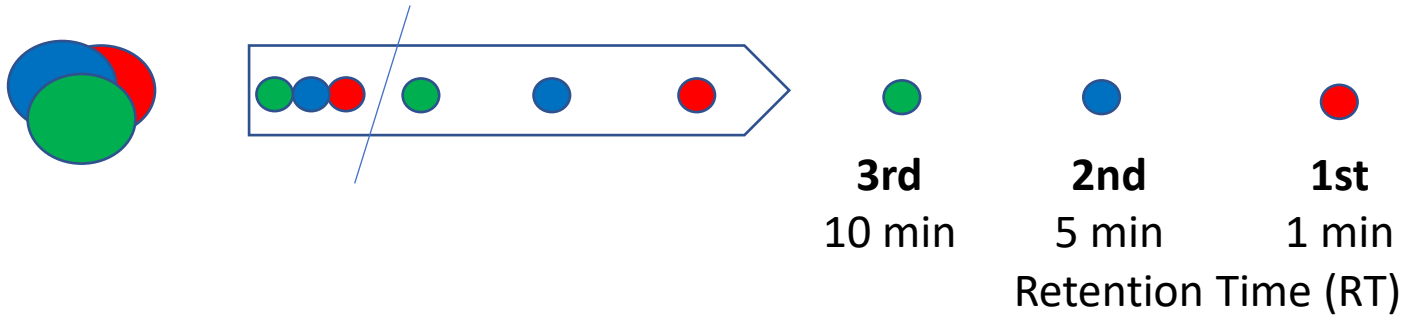
## ~~(Will be / were dealt with at some other time)~~

Chromatography technique can be explained / **classified** into three fundamental ways:

- Based on the shape of chromatographic bed.
  - ~~Planar chromatography (TLC and paper chromatpography)~~
  - ~~Column chromatography~~
- Based on the physical nature of the stationary and mobile phases.
  - **Gas chromatography (GC)**
  - ~~Liquid chromatography (LC, HPLC, UHPLC)~~
  - ~~Supercritical fluid chromatography (SFC)~~
- Based on the mechanism of the separation.
  - ~~Ion- exchange~~
  - ~~Affinity~~
  - ~~Size exclusion~~
  - ~~Hydrophobic interaction~~

# Identifying Components of a Mixture

**Chromatography** is a process by which compounds within a mixture are separated. Compounds are be separated by properties such as size, and how the compounds interact with the mobile and stationary phases of chromatography.




The sample is mixed into the **mobile phase**, usually a liquid or a gas, which is then passed over the stationary phase, usually a solid or a liquid. If a compound (compound A) within a mixture has a low affinity for the **stationary phase**, then it will not interact much with the stationary phase. However, if another compound (compound B) within a mixture has a high affinity for the stationary phase, then it will bind to the stationary phase. This results in compound A moving through the mobile phase more quickly than compound B, and thus compounds A and B can be separated from the mixture.

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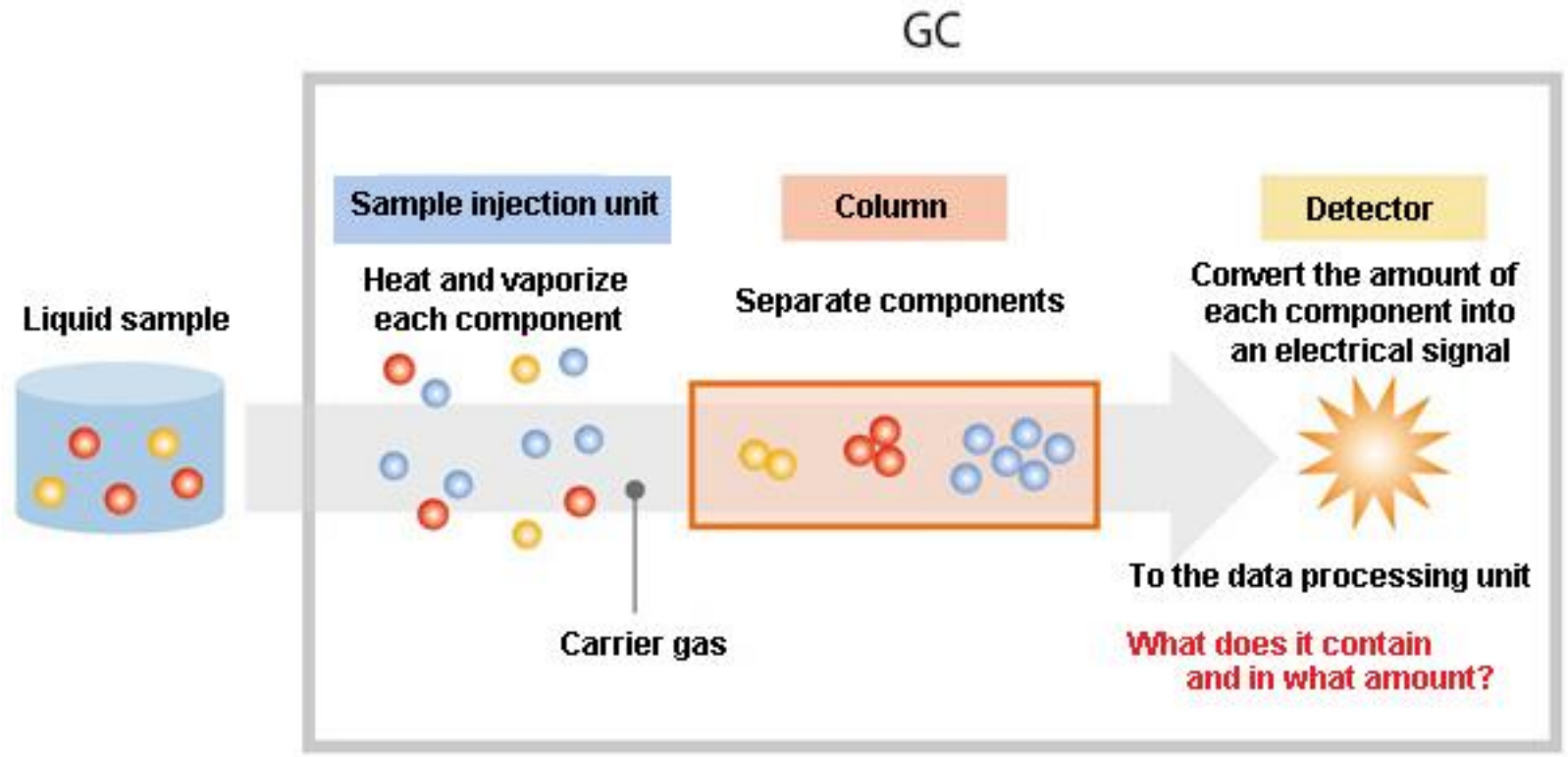
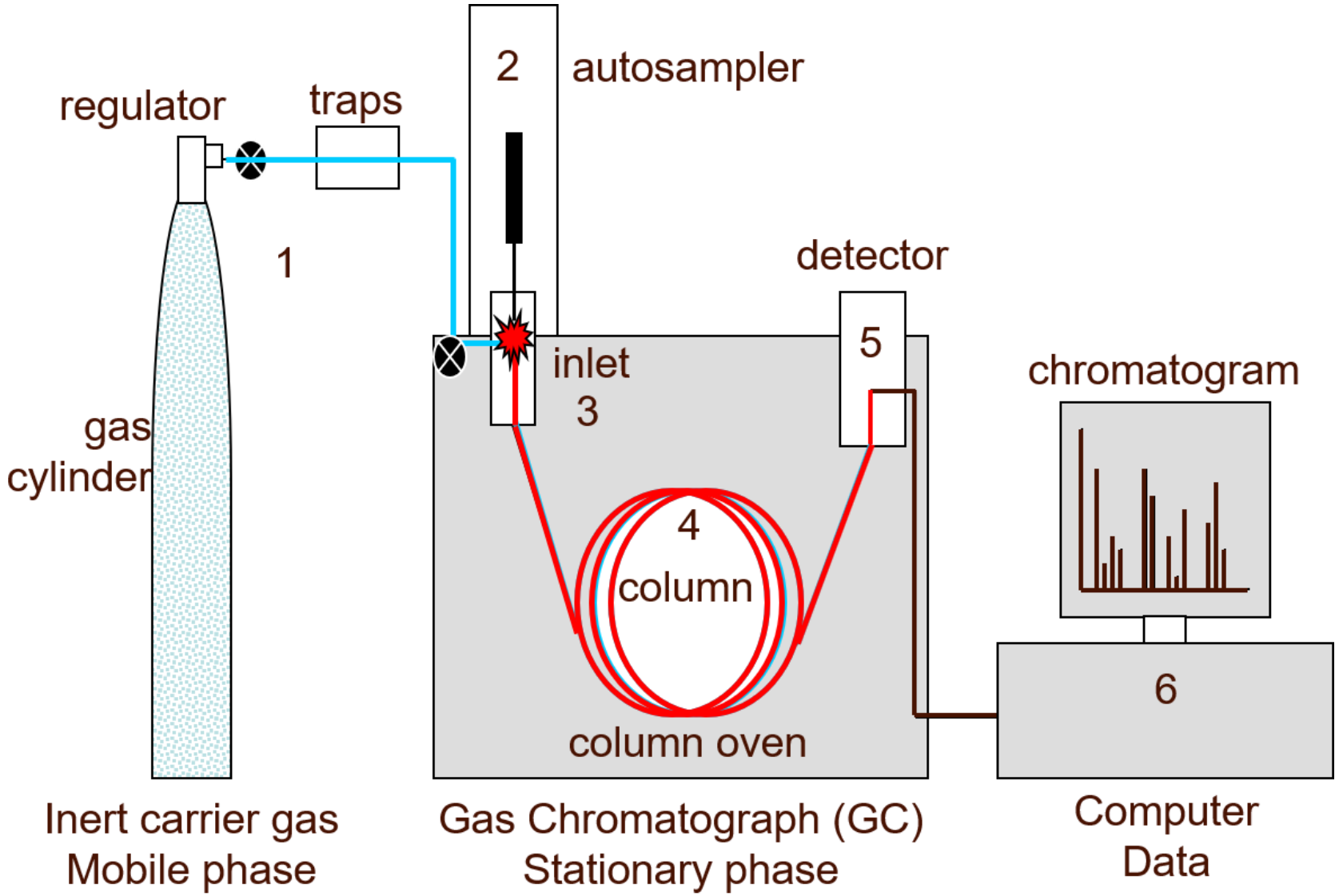
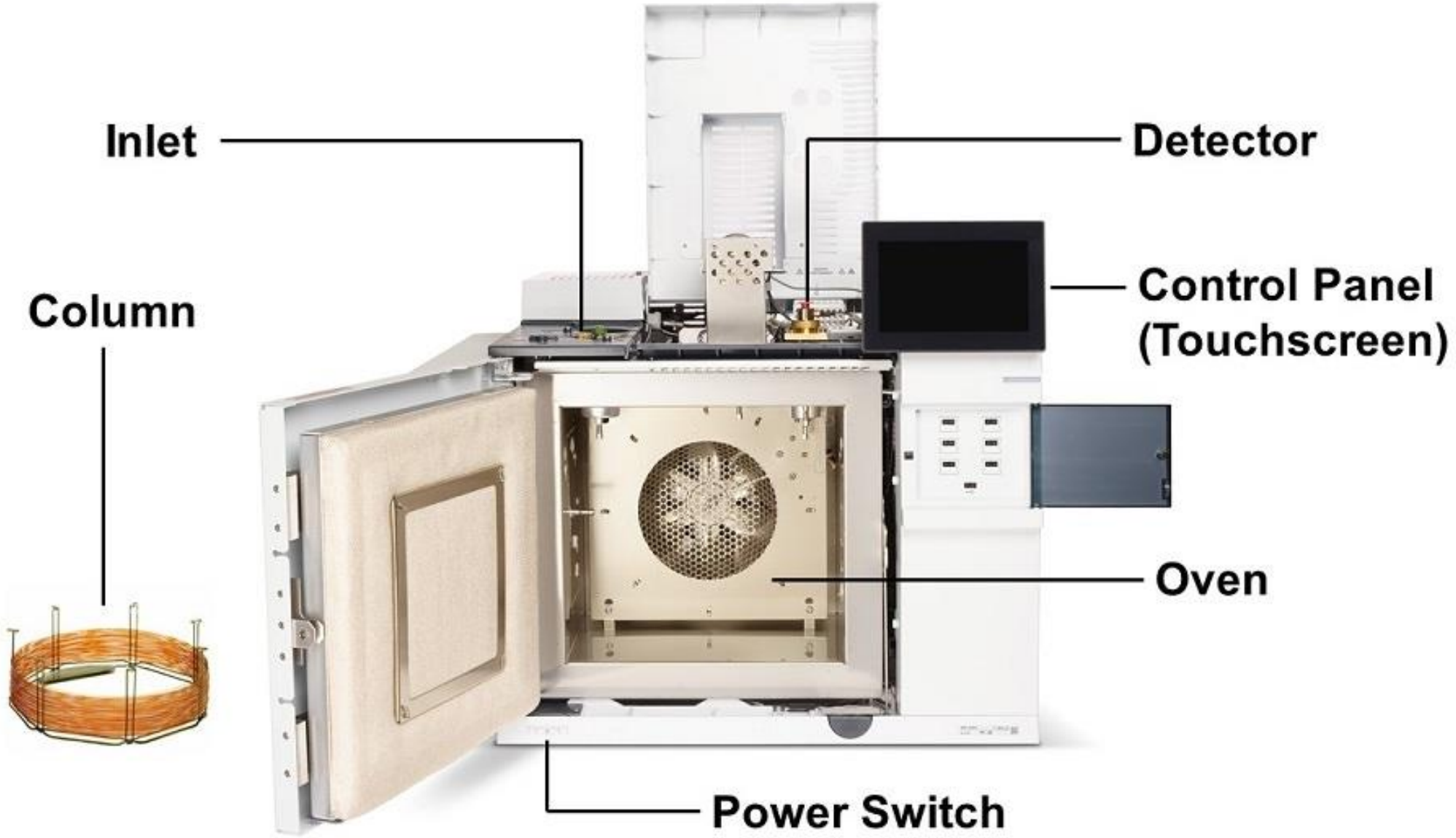



# Gas chromatography

As the name implies, **GC uses a carrier gas in the separation**, this plays the part of the mobile phase. The carrier gas transports the sample molecules through the GC system, ideally without reacting with the sample or damaging the instrument components.



# Gas chromatography: Gas chromatograph



# Gas chromatography

- The sample is **first introduced into the gas chromatograph** (GC), either with a syringe or transferred from an autosampler that may also extract the chemical components from solid or liquid sample matrices. The sample is injected into the GC inlet through a septum which enables the injection of the sample mixture without losing the mobile phase.
- Connected to the inlet is the **analytical column**, a long (10 – 150 m), narrow (0.1 – 0.53 mm internal diameter) fused silica or metal tube which contains the stationary phase coated on the inside walls.
- The analytical column is held in the column **oven which is heated** during the analysis to elute the less volatile components.
- The outlet of the column is **inserted into the detector** which responds to the chemical components eluting from the column to produce a signal.
- The signal is recorded by the **acquisition software** on a computer to produce a chromatogram.

# Gas chromatography: Sample preparation

**Liquid-liquid extraction (LLE):** This technique involves extracting the analytes of interest from the sample matrix using an organic solvent. The extracted analytes are then concentrated and injected into the GC for analysis. LLE is a relatively simple and inexpensive sample preparation technique.

**Solid-phase extraction (SPE):** This technique involves passing the sample matrix through a solid-phase cartridge, which selectively retains the analytes of interest. The retained analytes are then eluted from the cartridge and concentrated before injection into the GC.

**Solid-phase microextraction (SPME):** This technique involves extracting the analytes of interest from the sample matrix onto a coated fiber. The fiber is then desorbed into the GC inlet for analysis. This technique is fast, simple, and requires minimal sample preparation.

**Headspace analysis:** This technique involves analyzing the gas phase above a sample matrix. The sample is heated to promote the release of volatile analytes into the gas phase, which are then analyzed using GC.

# Gas chromatography: Sample preparation

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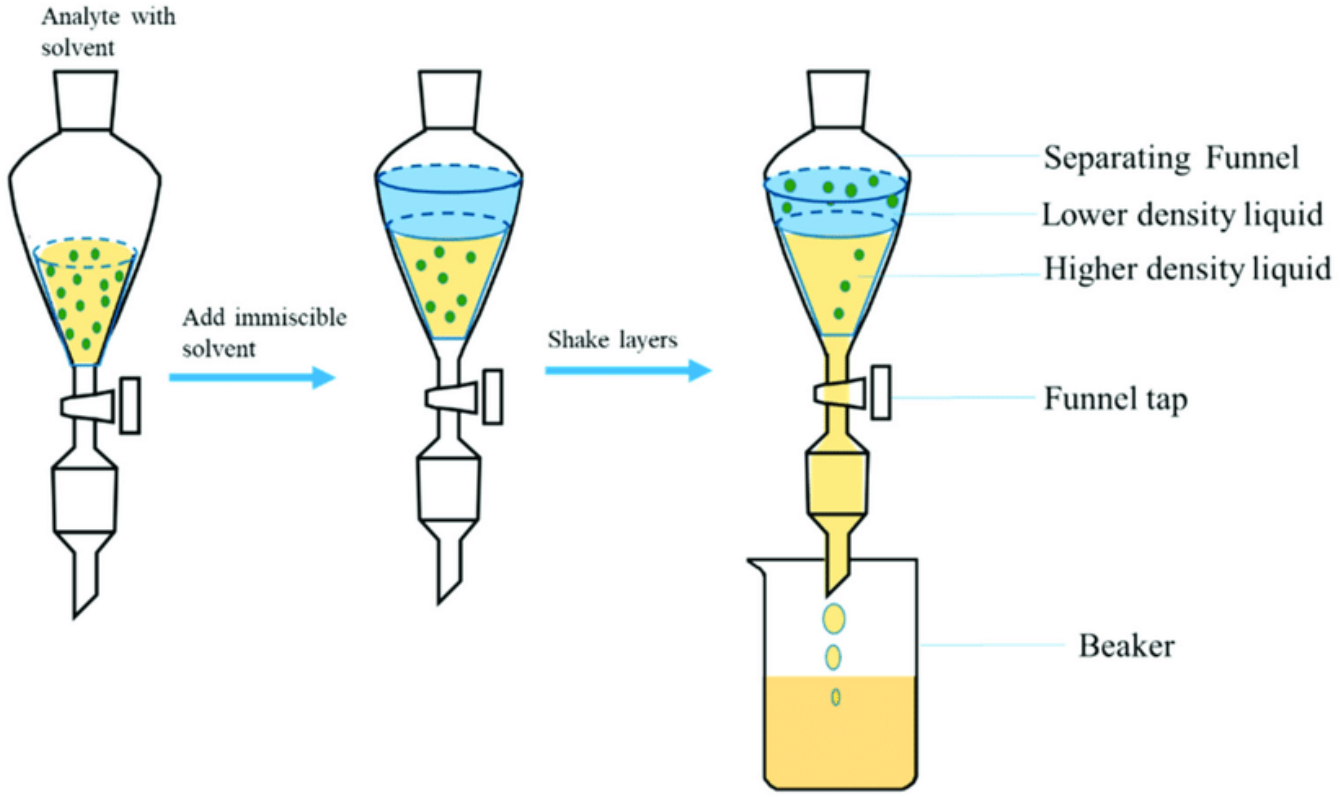
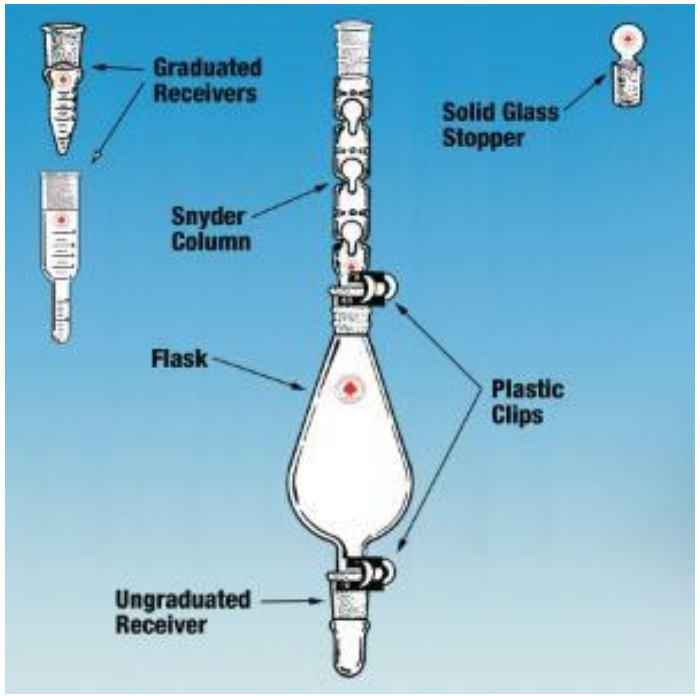
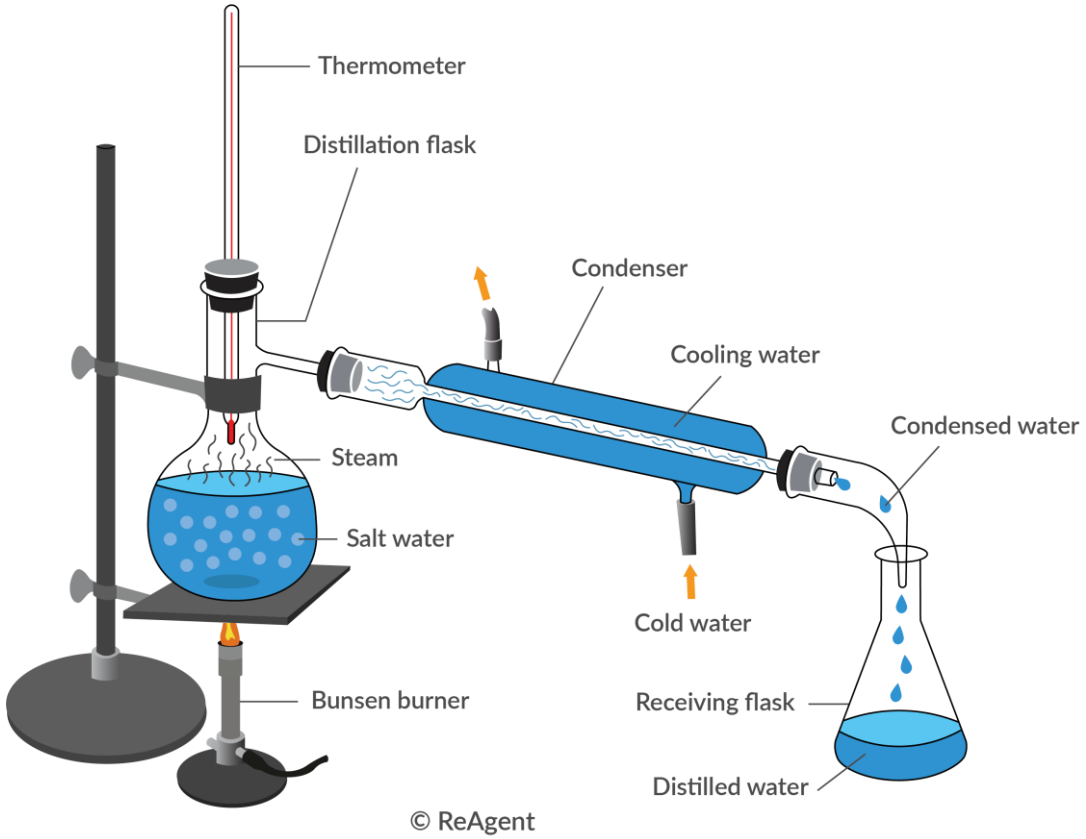


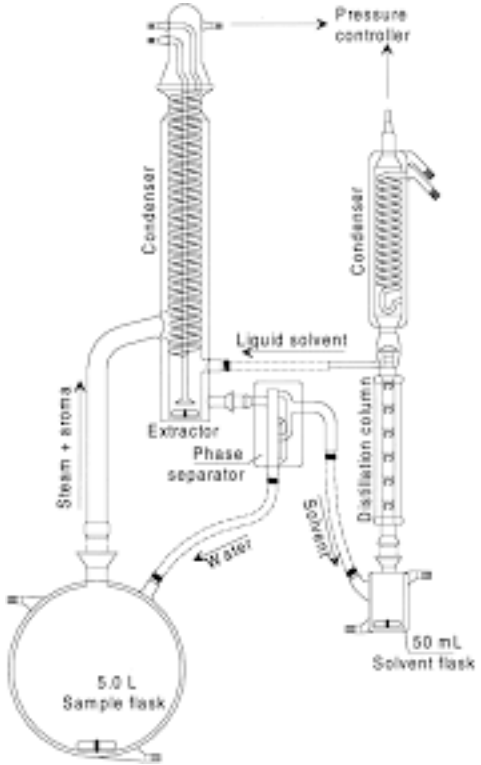
# Gas chromatography: Sample preparation

## Liquid-liquid extraction (LLE): Distillation and Simultaneous Distillation Extraction (SDE.

**Distillation:** Process involving the conversion of a liquid into vapour that is subsequently condensed back to liquid form.




**Simultaneous Distillation Extraction (SDE):** The process involves heating the sample in a flask while passing a flow of inert gas through it. The volatile compounds are vaporized and carried with the gas stream into a condenser, where they are cooled and condensed into a liquid. The condensed liquid is then collected in a separate flask, where it is concentrated by removing the solvent.



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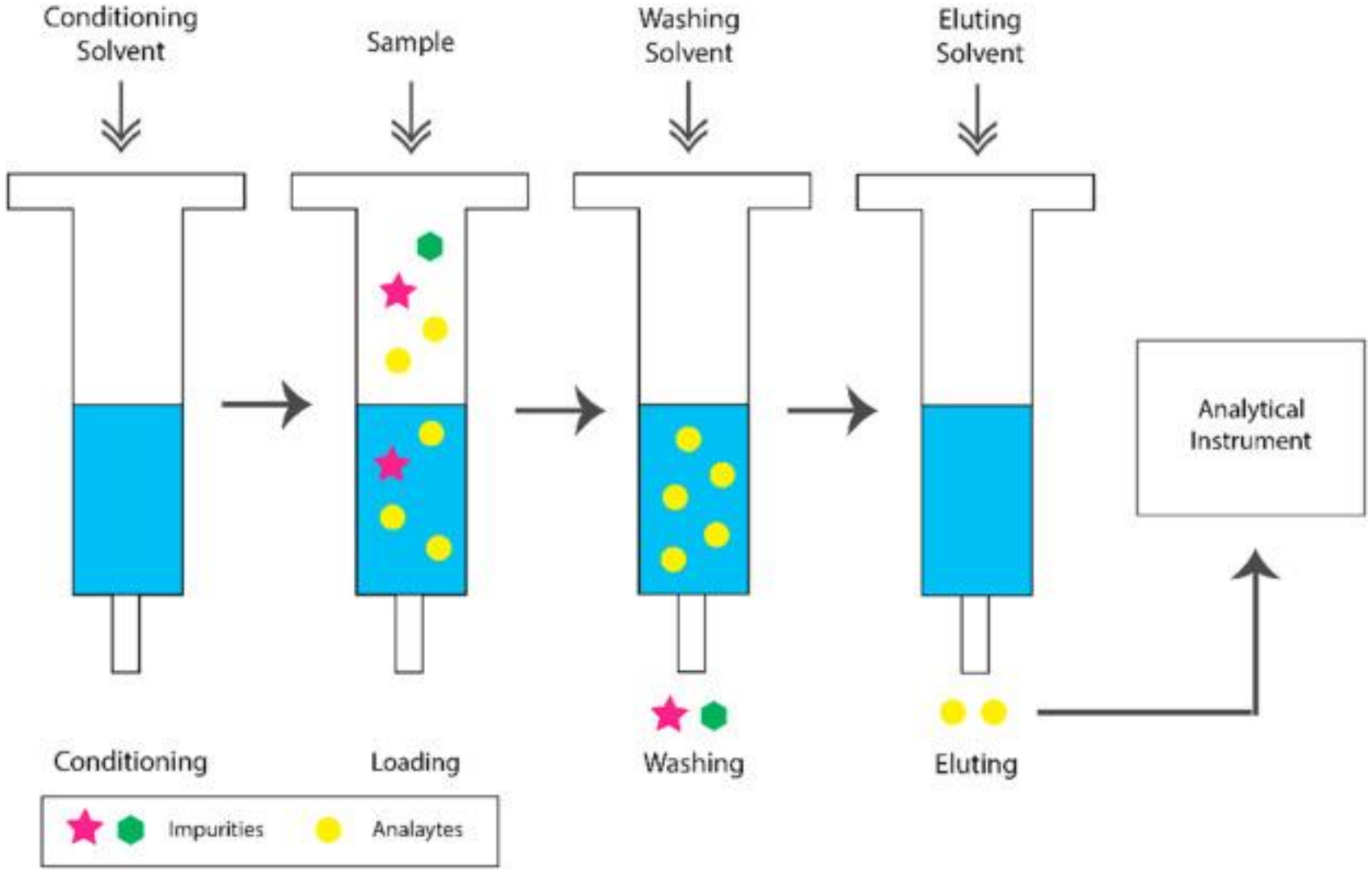

# Gas chromatography: Sample preparation

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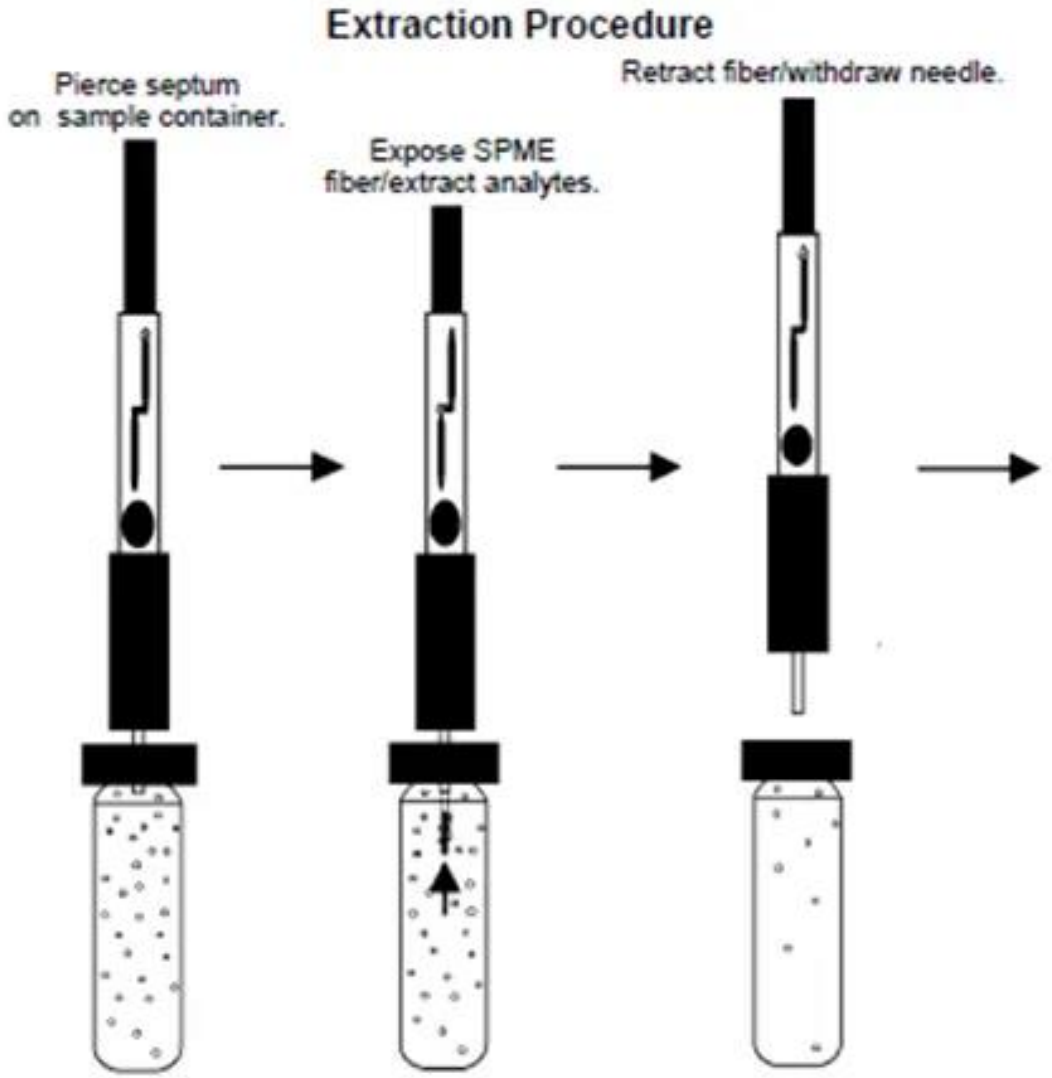
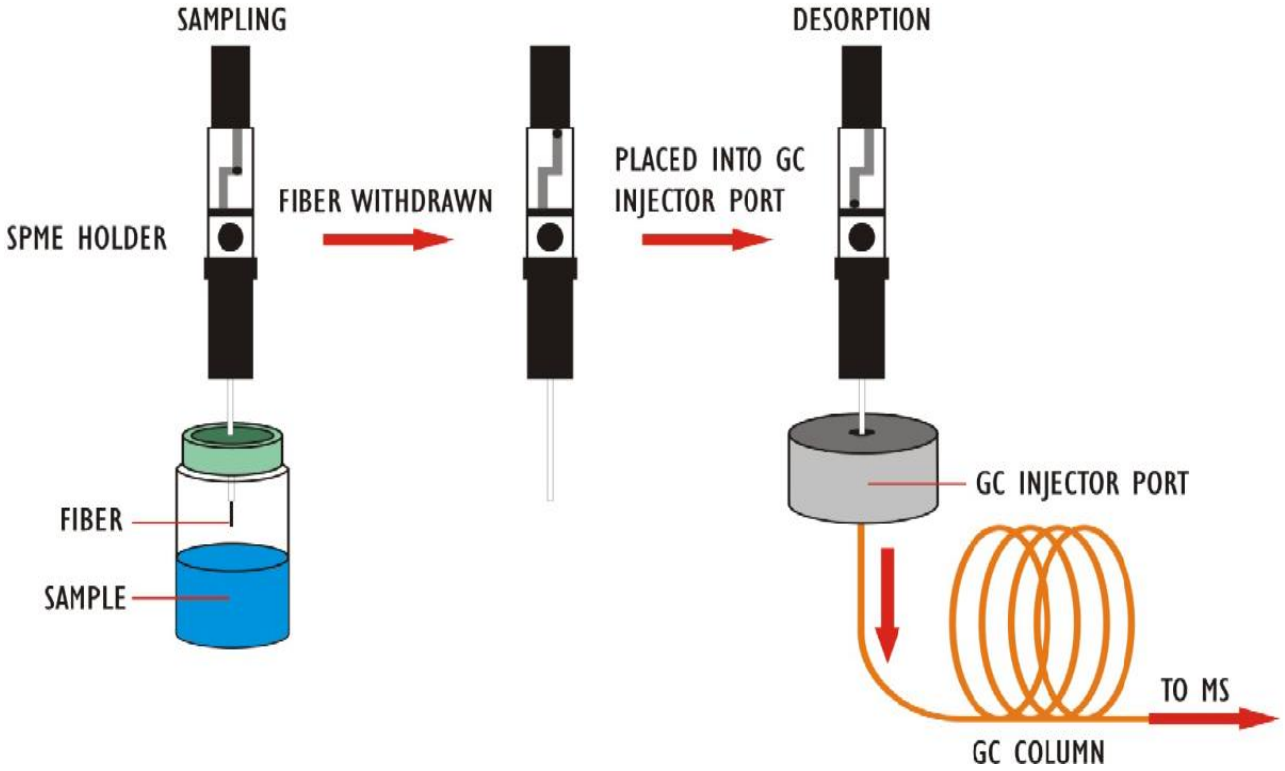
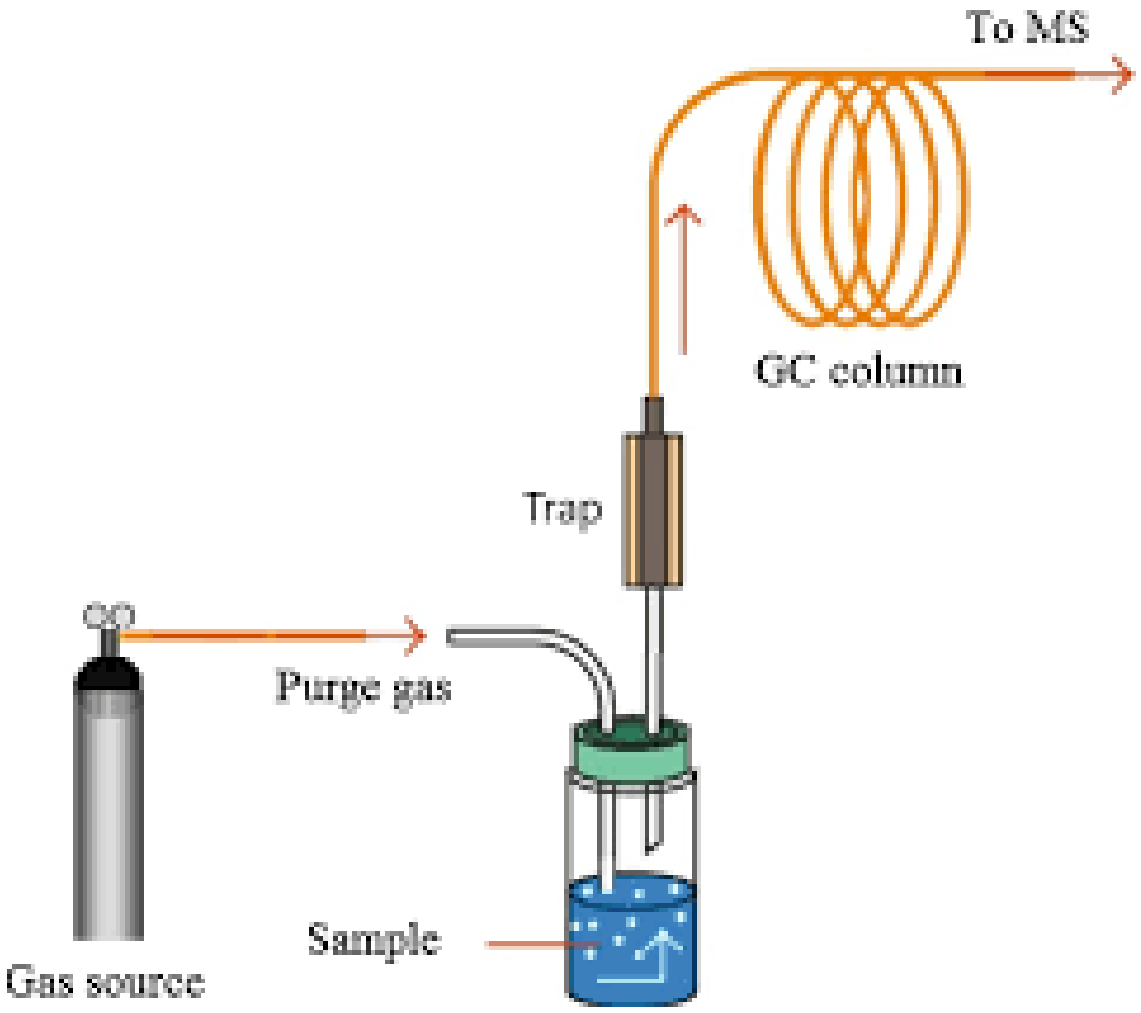


# Gas chromatography: Sample preparation

**Headspace analysis: Purge-and-Trap.** Purge and Trap (P&T) is a sample preparation technique used in Gas Chromatography (GC) to concentrate trace-level volatile organic compounds (VOCs) from a liquid or solid matrix. The technique involves three main steps: **purging, trapping, and desorption**.

During the **purging** step, an inert gas (usually helium) is bubbled through the sample matrix, which causes the VOCs to be stripped out of the matrix and carried away with the gas. The gas stream is then directed into a trap containing an adsorbent material (such as activated charcoal or a polymer resin), where the VOCs are **trapped** by adsorption.
The trap is then heated to **desorb** the VOCs, which are then transferred into the GC column for separation and detection. The desorption step can be performed either in the split or splitless mode of injection, depending on the amount of sample and the desired sensitivity.



# Derivatization

**Derivatization** in GC refers to the process of **chemically modifying a sample before analysis to enhance its detectability or to improve its chromatographic behavior**. This is typically done for samples that **lack functional groups, are non-volatile, or are thermally unstable** under GC conditions. Derivatization involves the addition of a reagent to the sample, which reacts with the analyte to form a derivative that is more amenable to GC analysis.

There are several types of derivatization reactions that can be used in GC, including **silylation, acylation, alkylation, and esterification**. The choice of derivatization reaction depends on the nature of the sample and the analytes of interest.

**Silylation** is the most common derivatization reaction used in GC. It involves the addition of a silylating reagent (such as trimethylsilyl (TMS) chloride or bis(trimethylsilyl)trifluoroacetamide (BSTFA)) to the sample, which reacts with the analytes to form volatile and stable TMS derivatives. These derivatives are more volatile and less polar than the original analytes, which improves their separation and detectability by GC.

**Acylation** involves the addition of an acylating reagent (such as acetic anhydride or trifluoroacetic anhydride) to the sample, which reacts with the analytes to form more volatile and less polar derivatives. This is particularly useful for the analysis of amino acids, sugars, and other polar compounds.

**Alkylation** involves the addition of an alkylating reagent (such as methyl iodide or propyl chloride) to the sample, which reacts with the analytes to form more volatile and less polar derivatives. This is particularly useful for the analysis of acidic compounds.

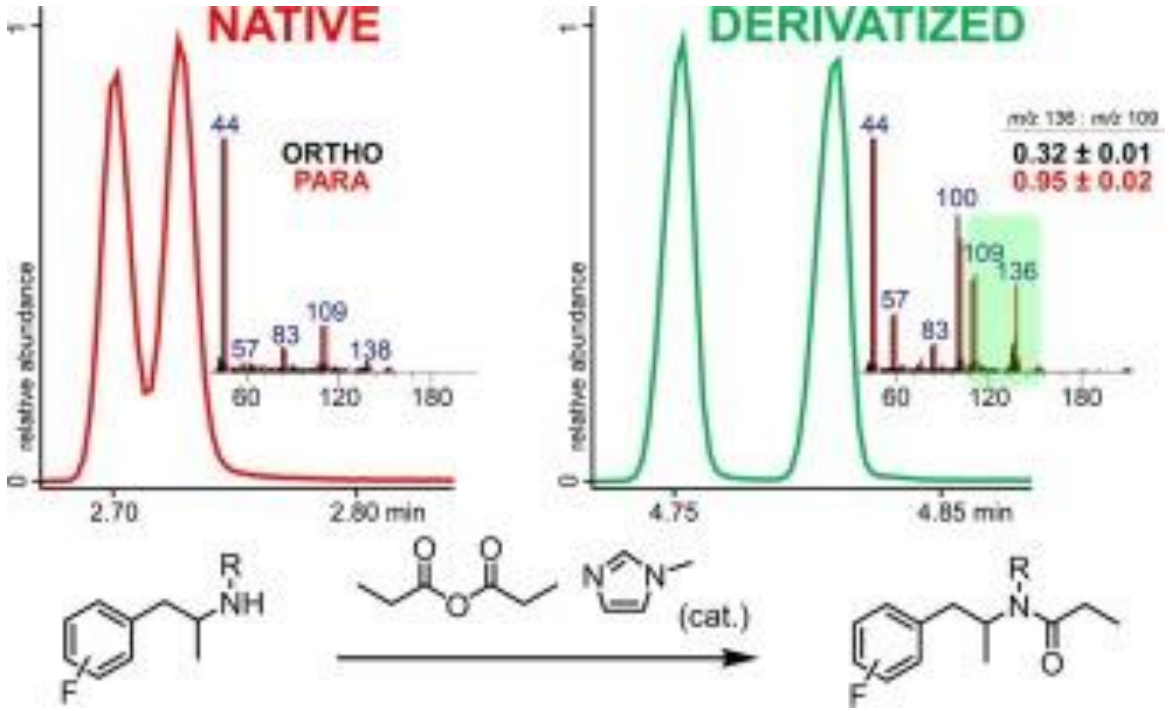
**Esterification** involves the addition of an esterifying reagent (such as diazomethane or trifluoroacetic anhydride) to the sample, which reacts with the analytes to form volatile and stable ester derivatives. This is particularly useful for the analysis of fatty acids and other carboxylic acids.

# Derivatization

**Example of samples that require derivatization:** Sugars and fatty acids

**Why?:** They are **not readily amenable to GC analysis** because they are **polar and not volatile enough** for efficient analysis by GC.

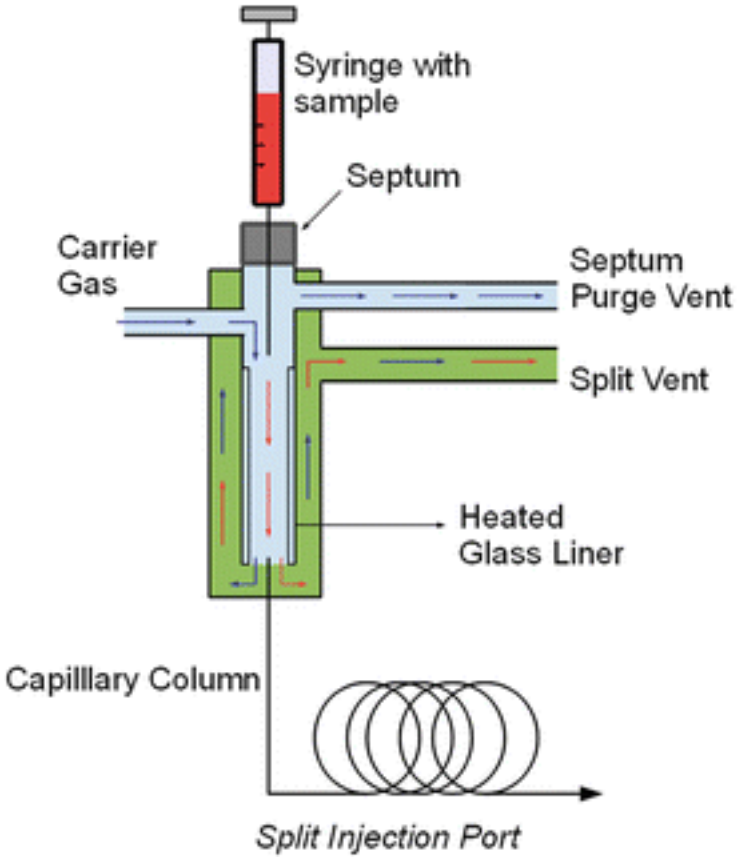
Therefore, they need to be derivatized to increase their volatility and reduce their polarity for better chromatographic separation and improved detectability. In contrast, pesticides and aroma compounds are typically not polar and are already volatile enough for efficient analysis by GC. Therefore, they do not require derivatization for GC analysis.

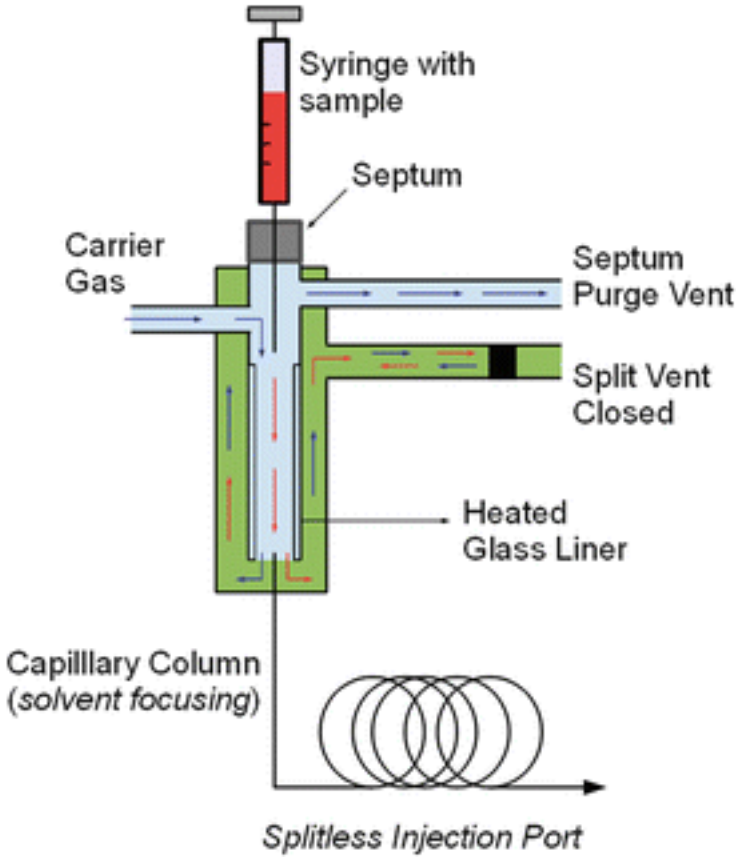


# Gas chromatography: Injection

After injection into the GC inlet, the chemical components of the sample mixture are first **vaporized**, if they aren't already in the gas phase. For low concentration samples the whole vapour cloud is transferred into the analytical column by the carrier gas in what is known as splitless mode. For high concentration samples only a portion of the sample is transferred to the analytical column in split mode, the remainder is flushed from the system through the split line to prevent overloading of the analytical column.

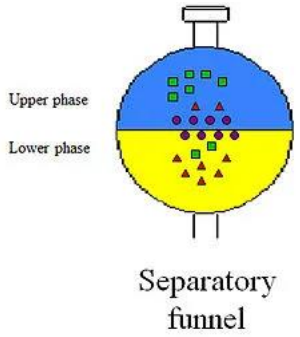
Note: The injection port should be at a higher temperature than the column for preventing further condensation. It could cause volatile or semivolatile build-up which may rise the baseline.



# Gas chromatography: Separation mechanisms

**Partition principle**: The partition principle is based on the distribution of the analyte between two phases, the stationary phase, and the mobile phase. The stationary phase is a non-volatile liquid or solid, while the mobile phase is an inert gas. The analyte partitions between the two phases, with the degree of partitioning determined by the solubility of the analyte in each phase. The more soluble the analyte in the stationary phase, the slower it moves through the column, and the longer it takes to elute.



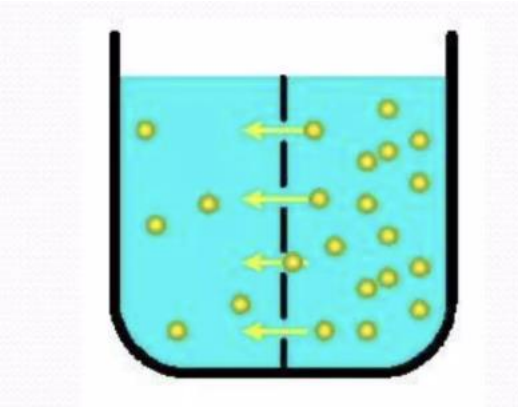

$K_D > 1$

$K_D = 1$

$K_D < 1$

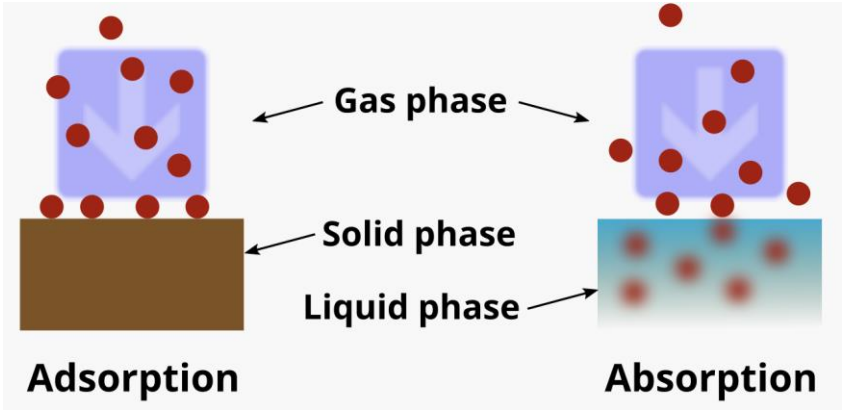
$$K_D = \frac{[A]_{upper}}{[A]_{lower}}$$

**Diffusion principle:** The diffusion principle is based on the movement of analyte molecules through the stationary phase. The rate of diffusion of a molecule is dependent on its size and shape, with smaller molecules diffusing more quickly than larger ones. As the analyte moves through the stationary phase, it interacts with the surface of the phase, and its movement is slowed down. This interaction is responsible for the separation of the components in the mixture.

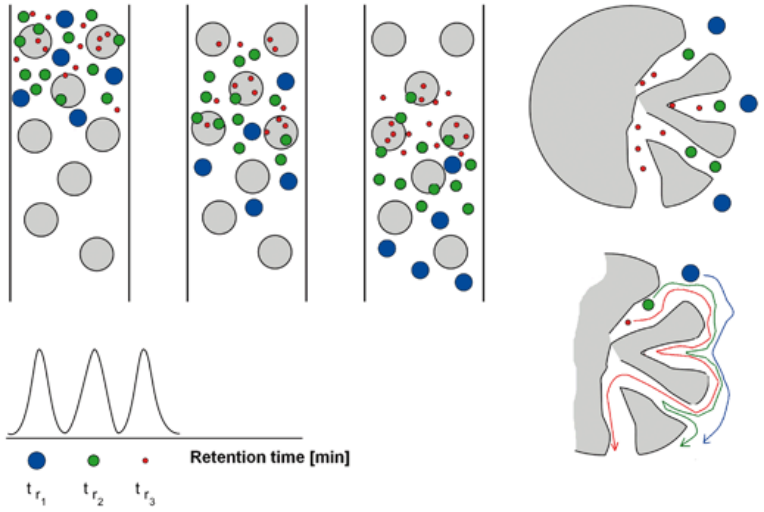


# Gas chromatography: Separation mechanisms

**Adsorption principle:** The adsorption principle is based on the interaction of analyte molecules with the surface of the stationary phase. The surface of the stationary phase has active sites that can interact with the analyte molecules through weak forces such as van der Waals forces, hydrogen bonding, or dipole-dipole interactions. The degree of interaction of the analyte with the stationary phase is dependent on the strength of the forces and the nature of the surface. The analyte molecules that interact more strongly with the stationary phase will move more slowly through the column and will take longer to elute.

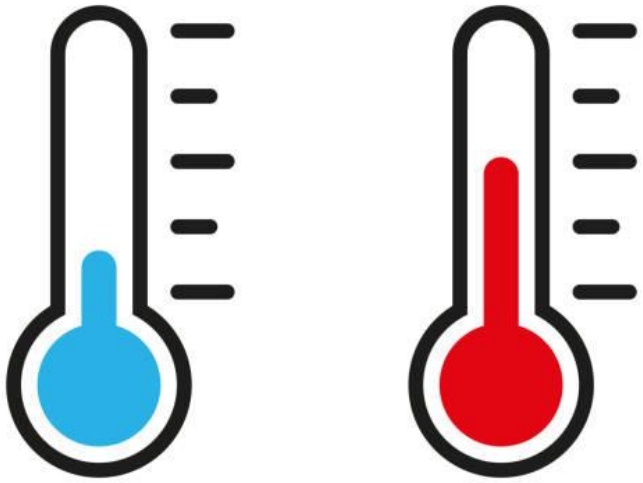



**Size exclusion principle:** The exclusion principle is based on the size exclusion of analyte molecules from the pores of the stationary phase. The stationary phase has pores of different sizes, and analyte molecules can only enter the pores that are larger than their size. Smaller molecules can enter more pores and move more slowly through the column, while larger molecules cannot enter as many pores and move more quickly. This principle is used in size exclusion chromatography.



# Gas chromatography: Separation mechanisms

**Temperature:** The volatility of the sample molecules can affect the separation efficiency and selectivity in GC. For example, high-boiling point compounds may not elute from the column under low temperature conditions, leading to poor separation efficiency. Conversely, low-boiling point compounds may elute too quickly, leading to poor separation selectivity. The temperature can be used to adjust the elution time and optimize the separation of sample components.



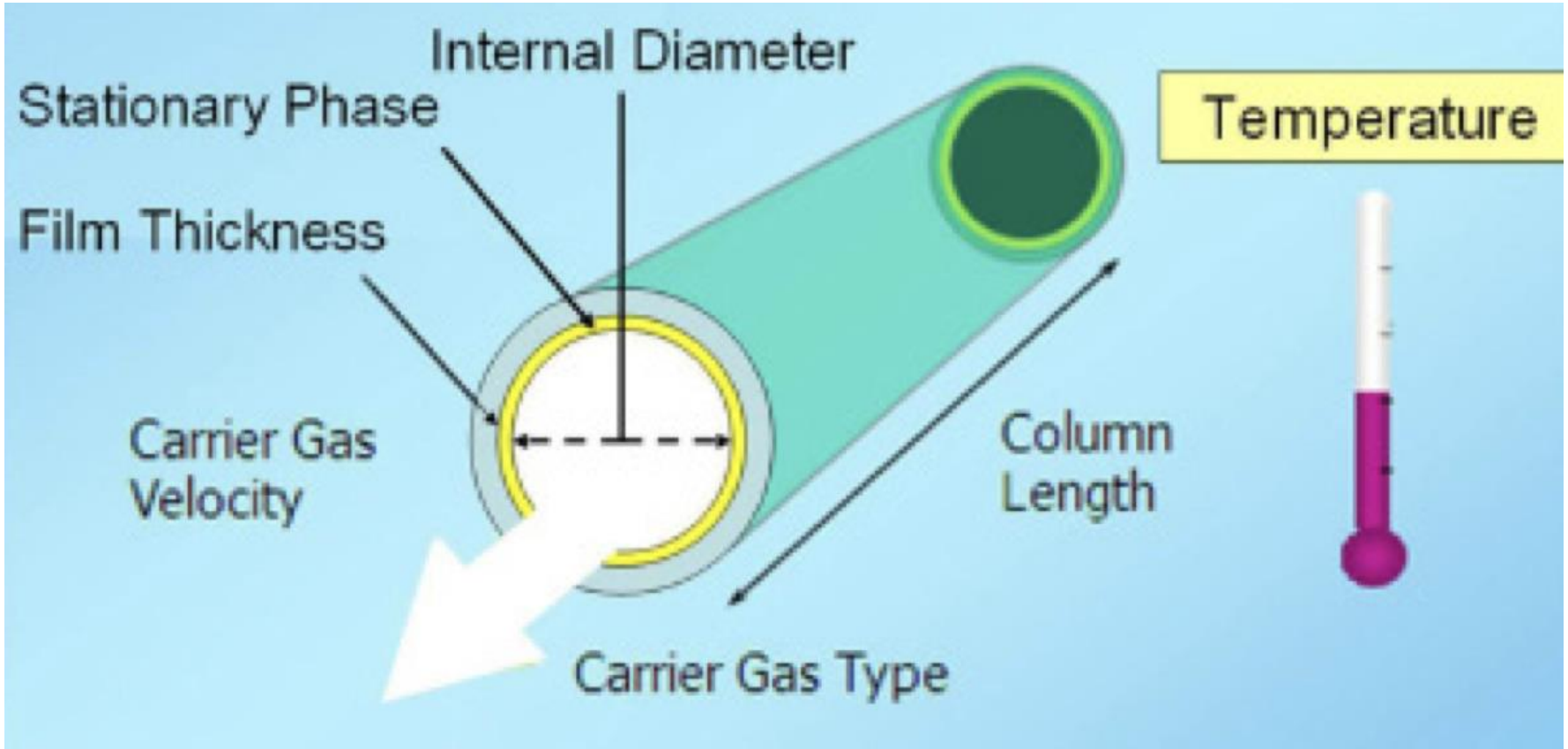
# Gas chromatography: Separation mechanisms

Diffusion principle.
Partition principle.
Adsorption principle.
Exclusion principle.

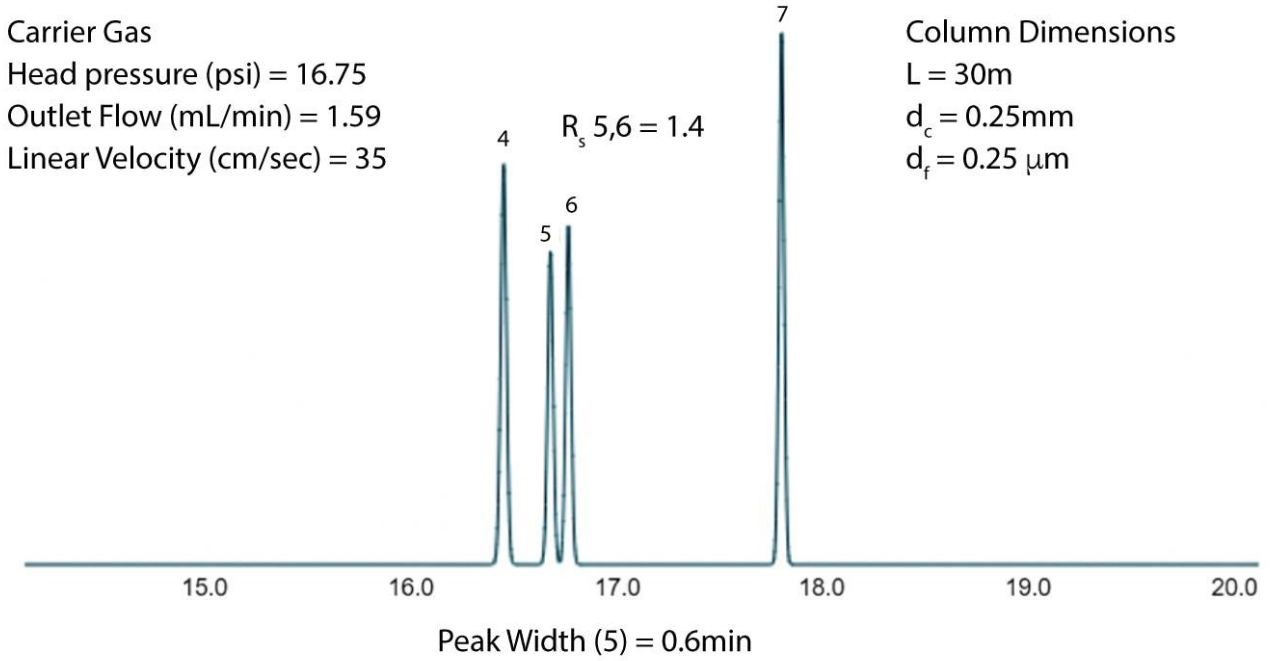
→ Mobile phase (carrier gas)

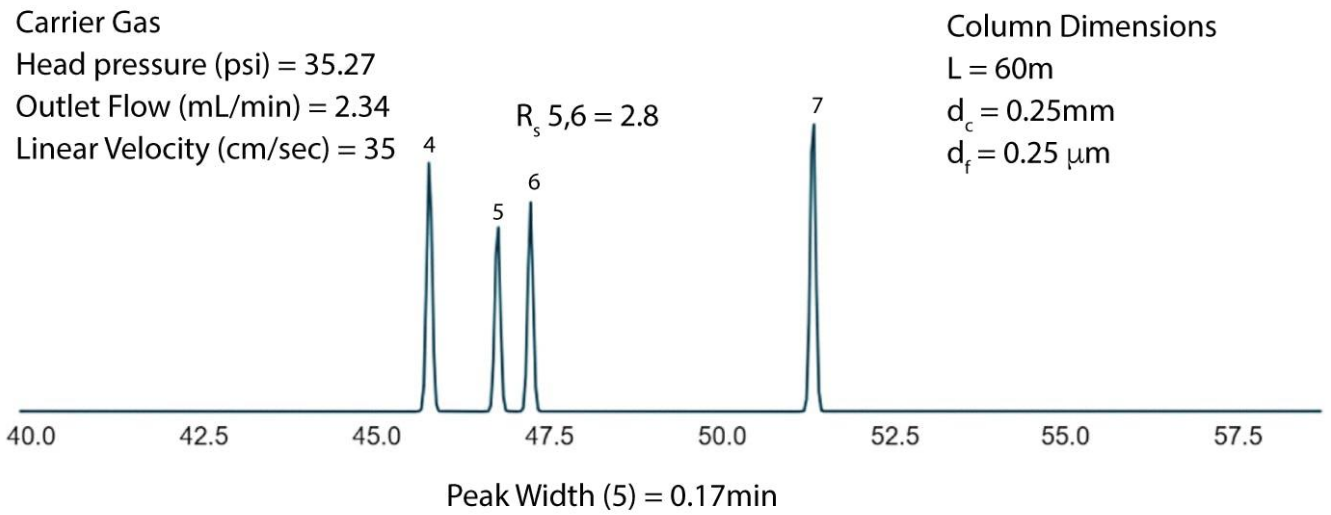
→ Stationary phase (column)

Temperature.



# Separation mechanisms



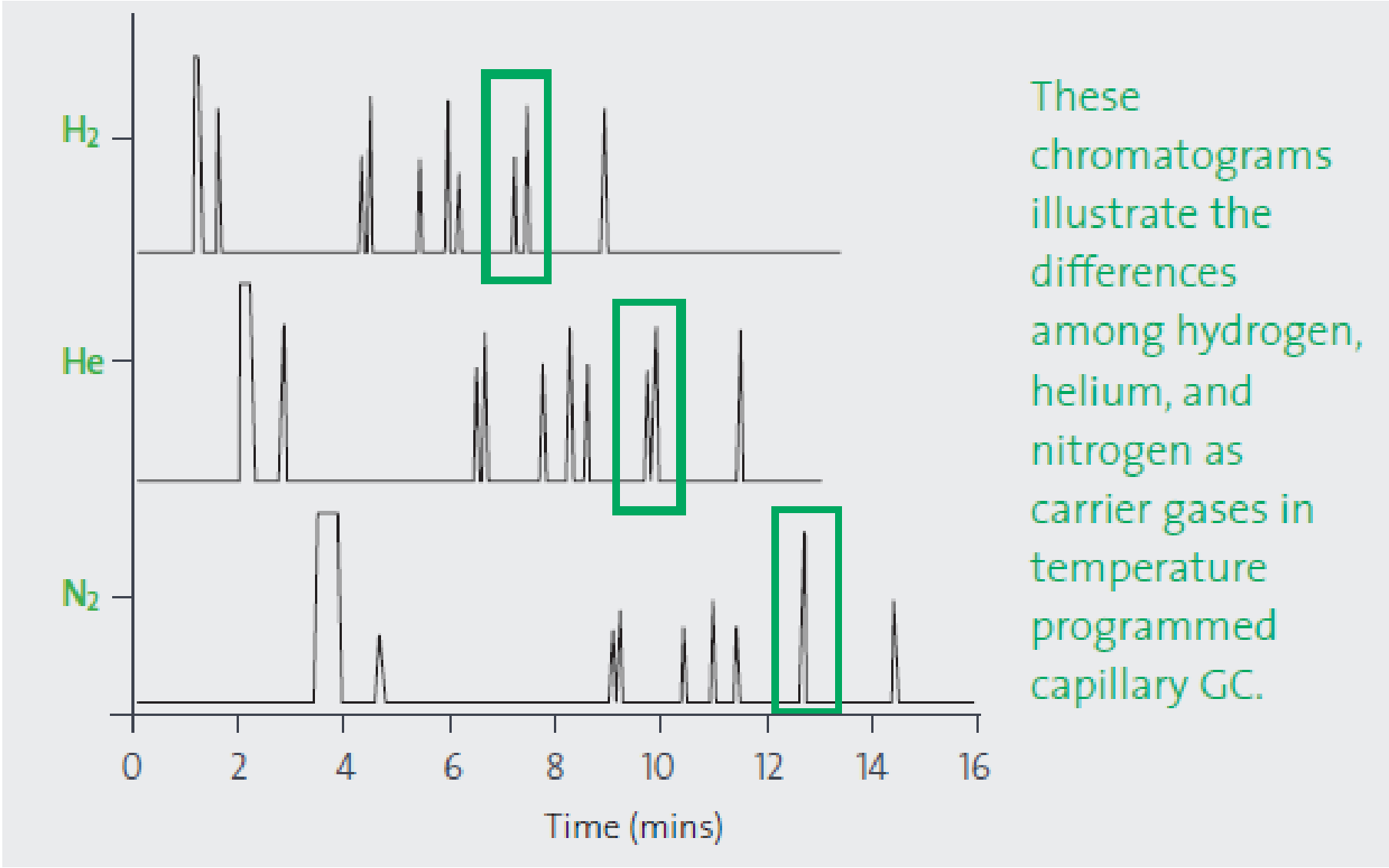



# Separation mechanisms: Carrier gas

**Type of gas**

**Pressure**

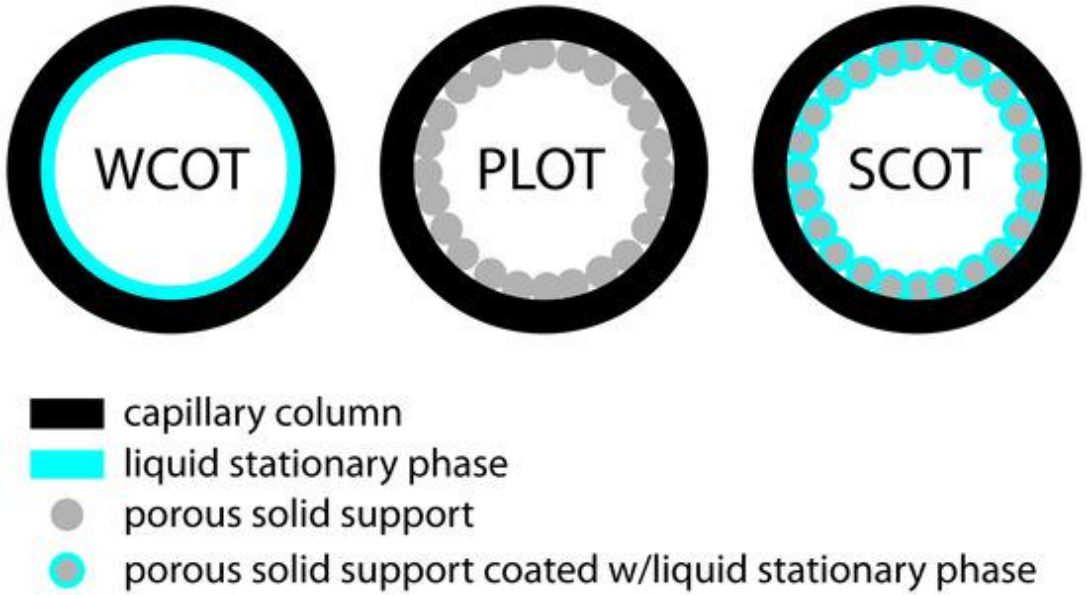
**Flow**



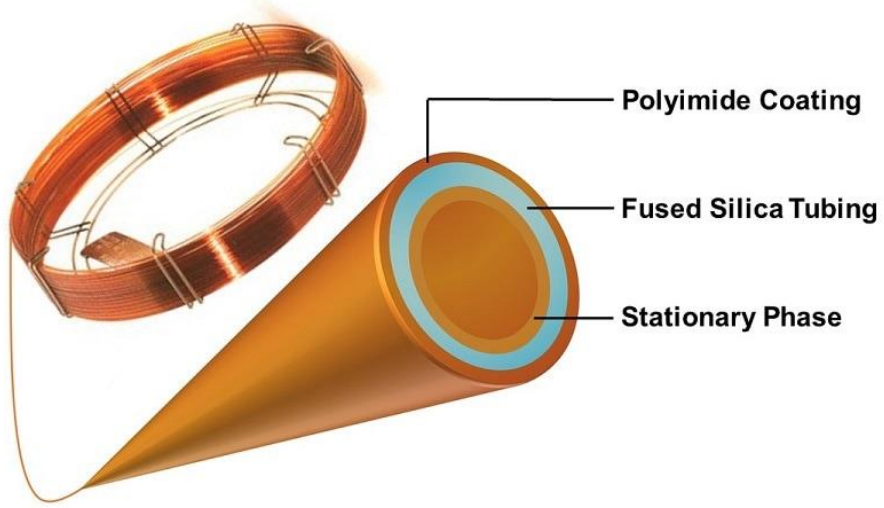

These chromatograms illustrate the differences among hydrogen, helium, and nitrogen as carrier gases in temperature programmed capillary GC.

# Separation mechanisms: Column

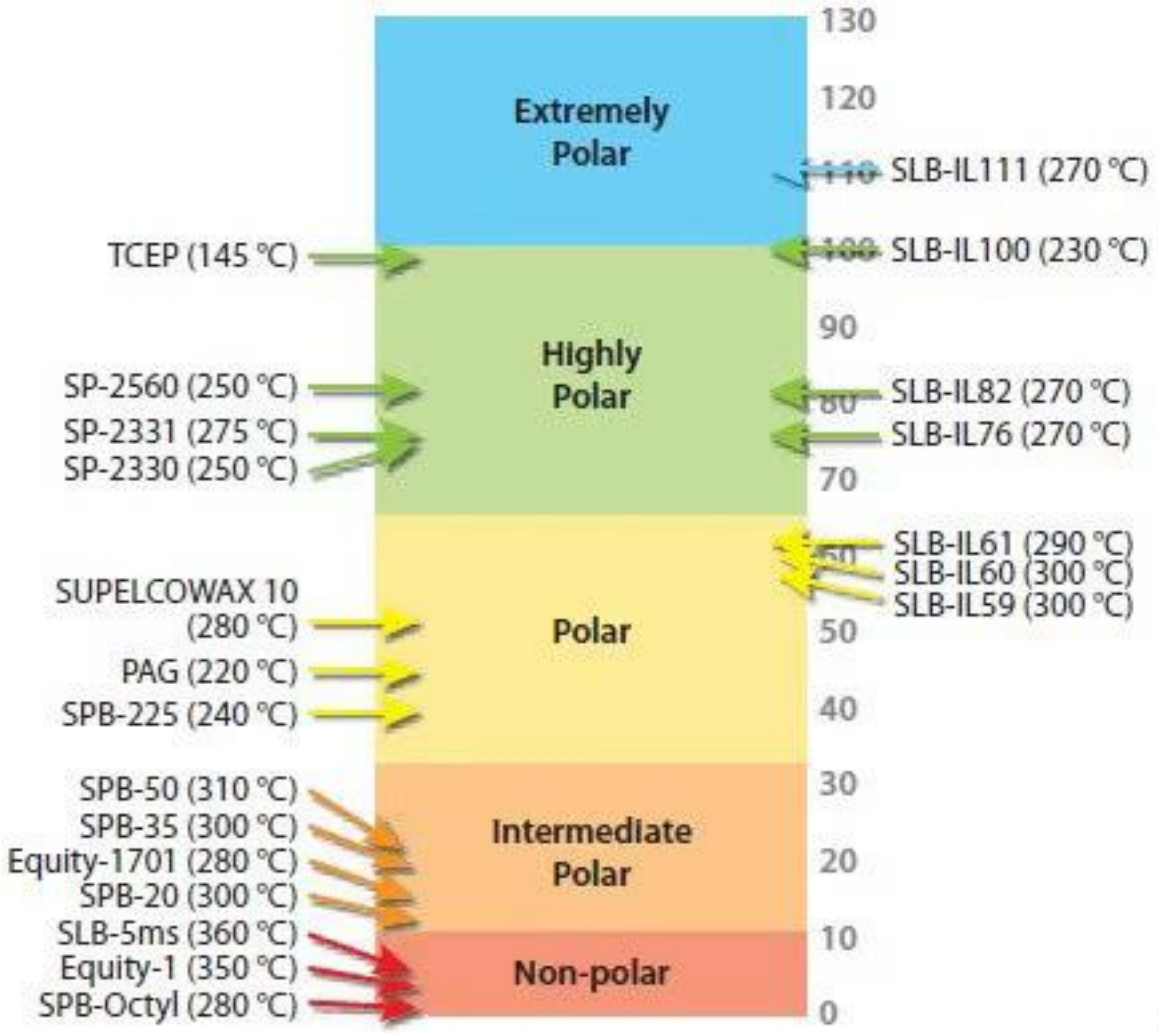
**Stationary phase**: The stationary phase is the material that coats the inner surface of the GC column and interacts with the sample components. The stationary phase can be **polar, non-polar, or intermediate and be bound in different ways.** The choice of stationary phase depends on the properties of the sample being analyzed.

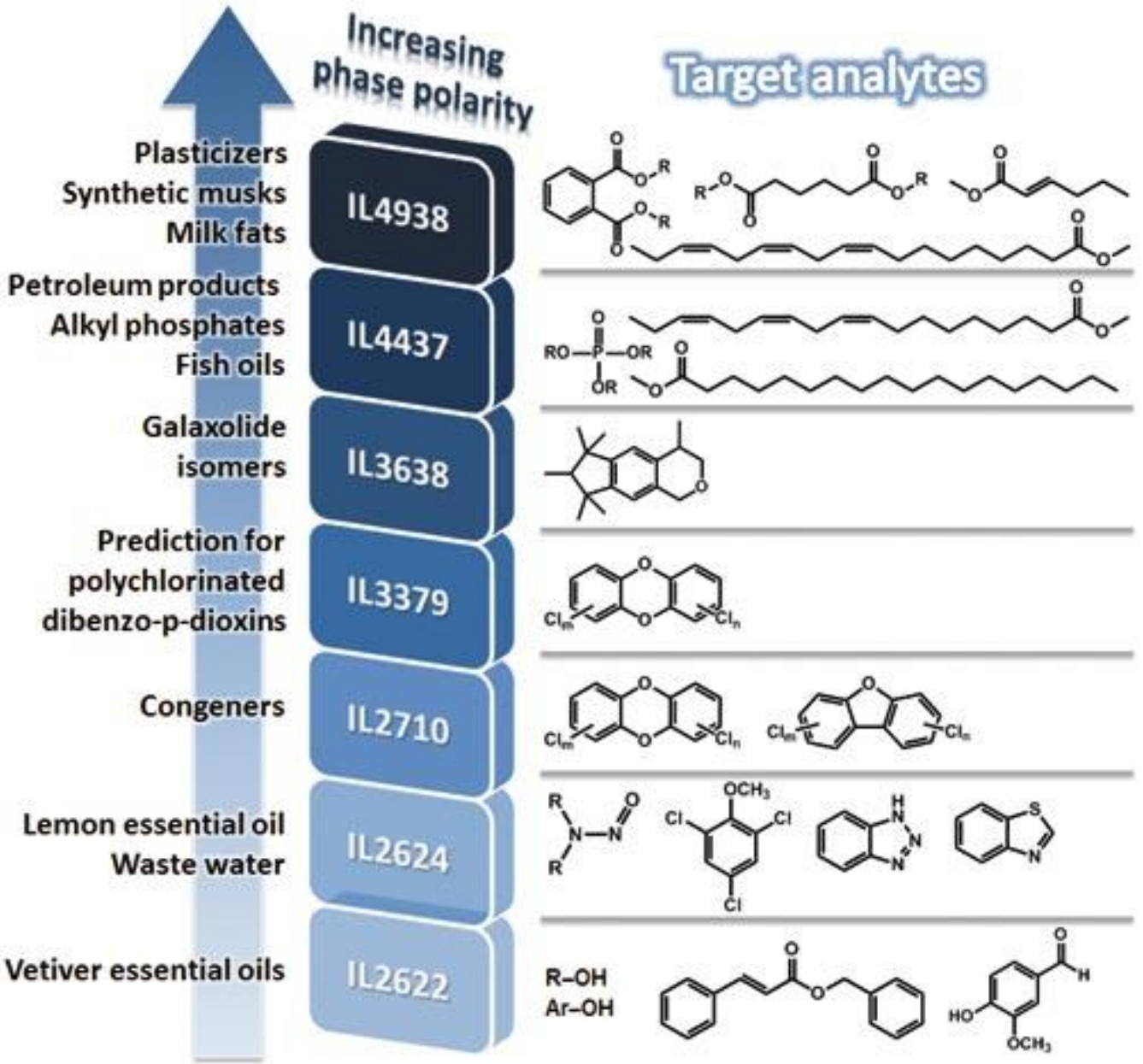



**Column dimensions:** The **length, inner diameter, and film thickness** of the column determine the separation efficiency, resolution and retention time of the sample components.



# Separation mechanisms: Column (stationary phase)



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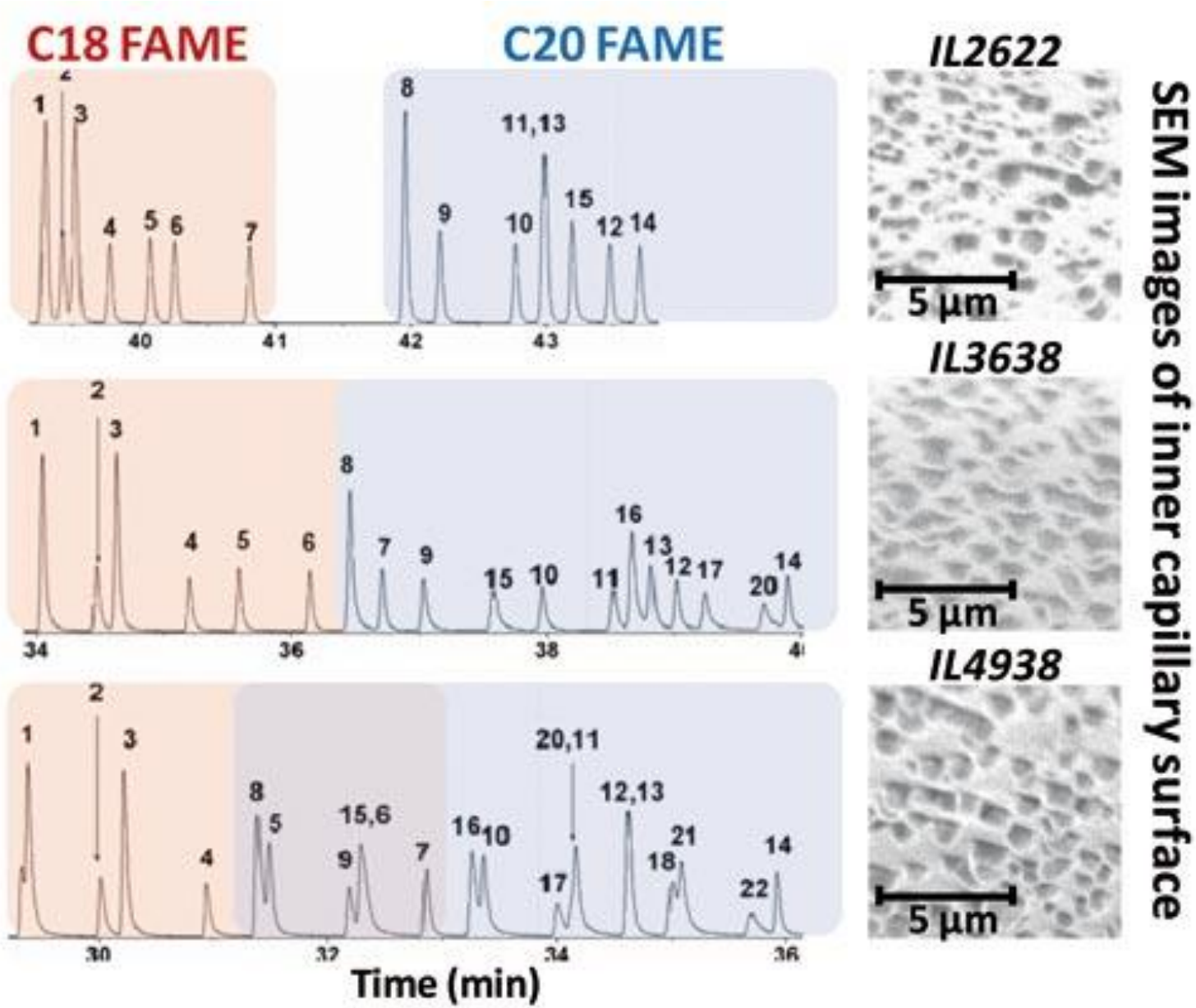
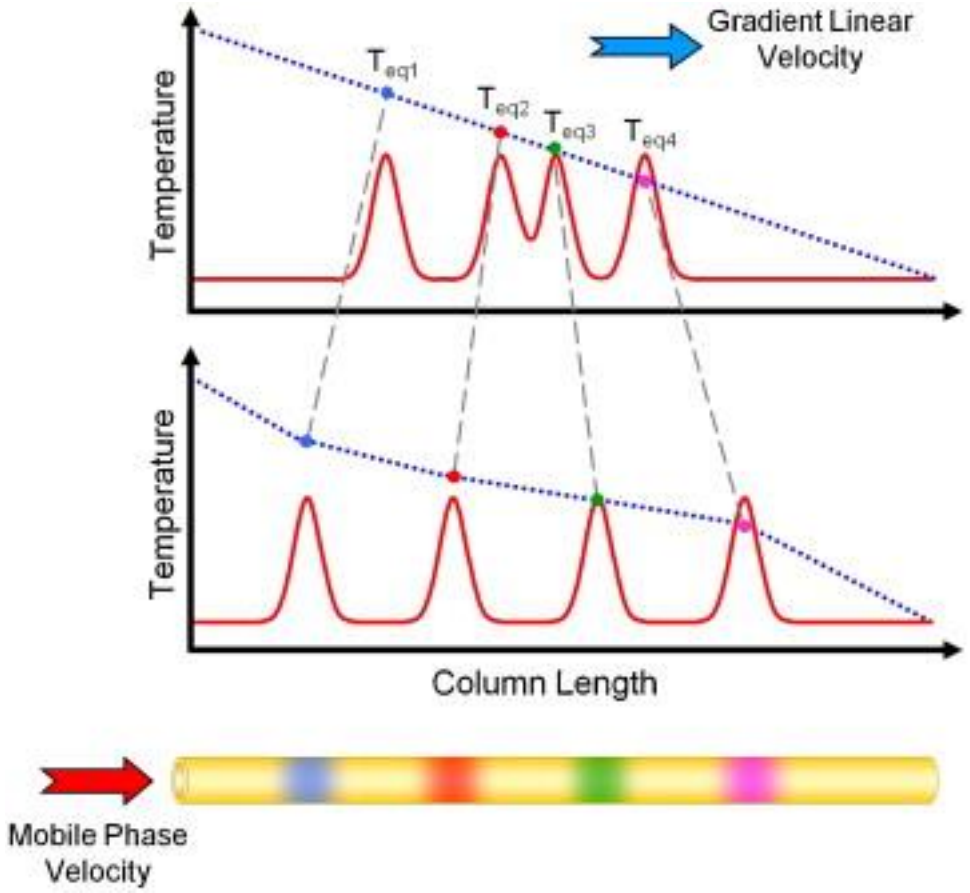
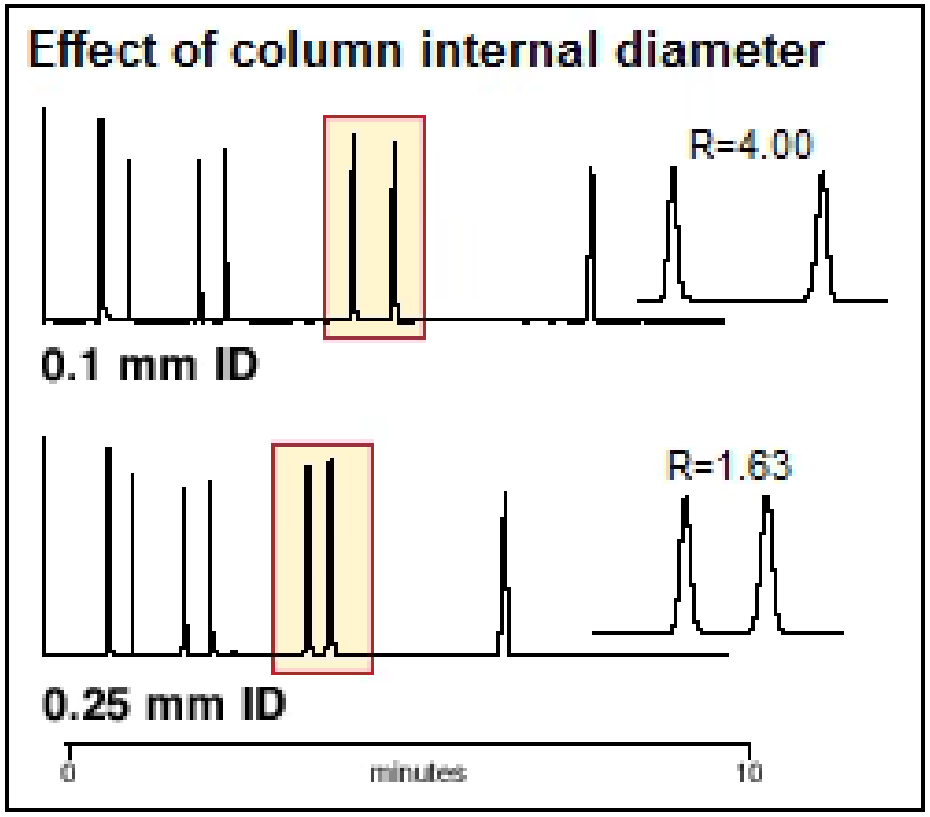


# Separation mechanisms: Column

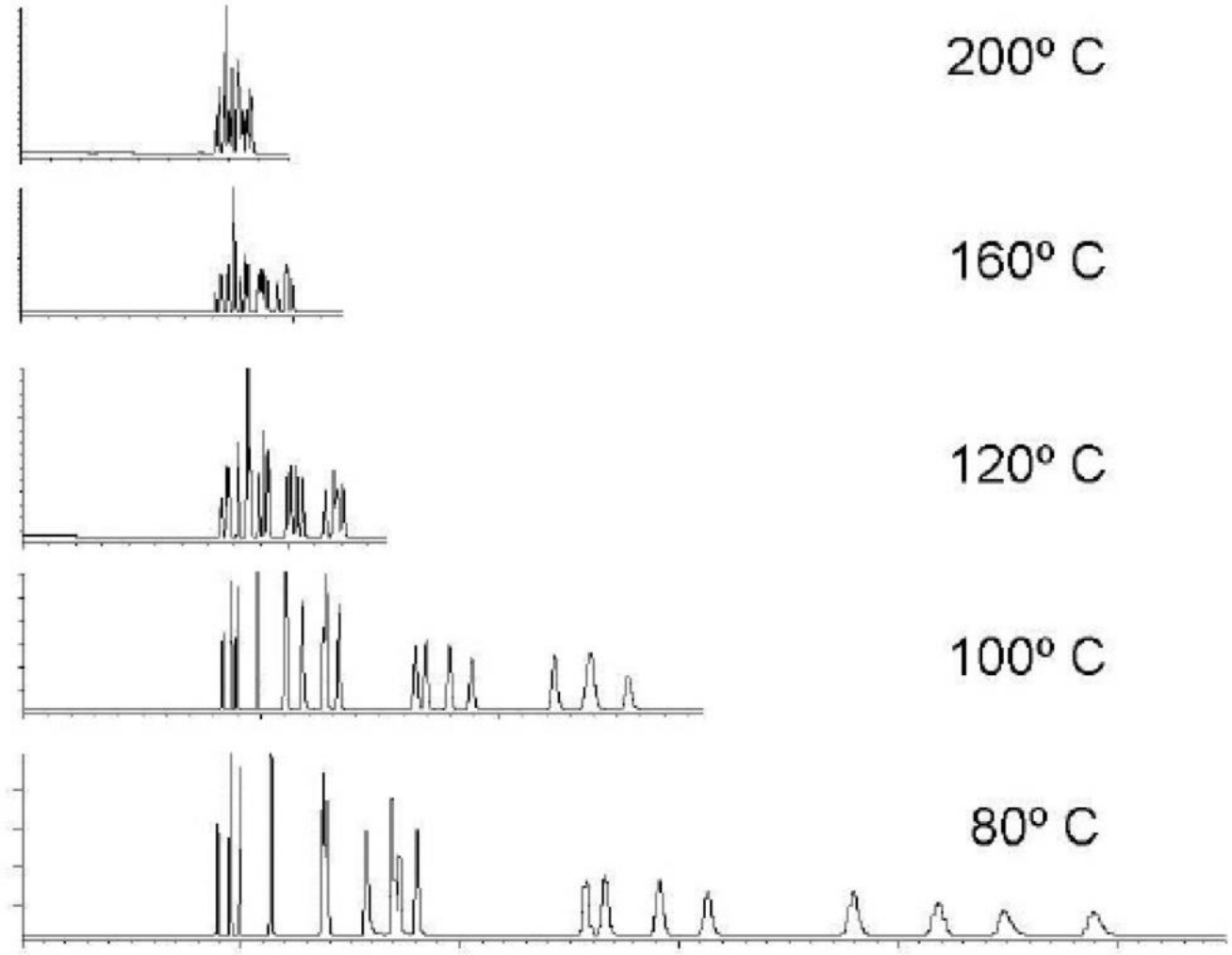
## Length



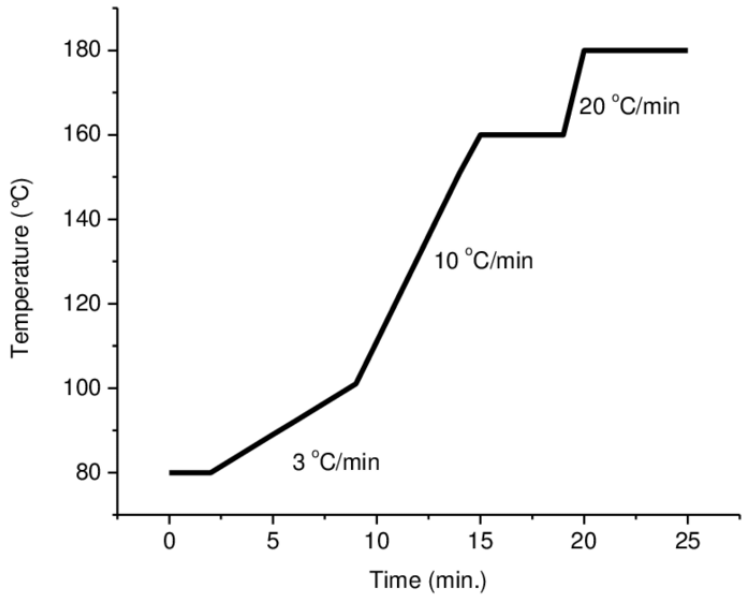

## I.D.

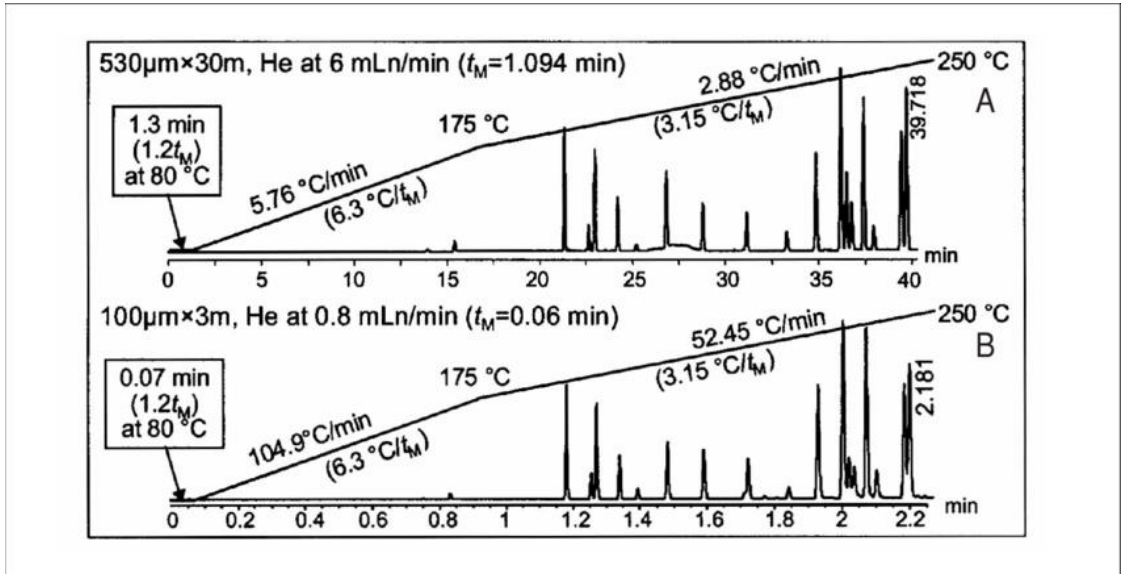


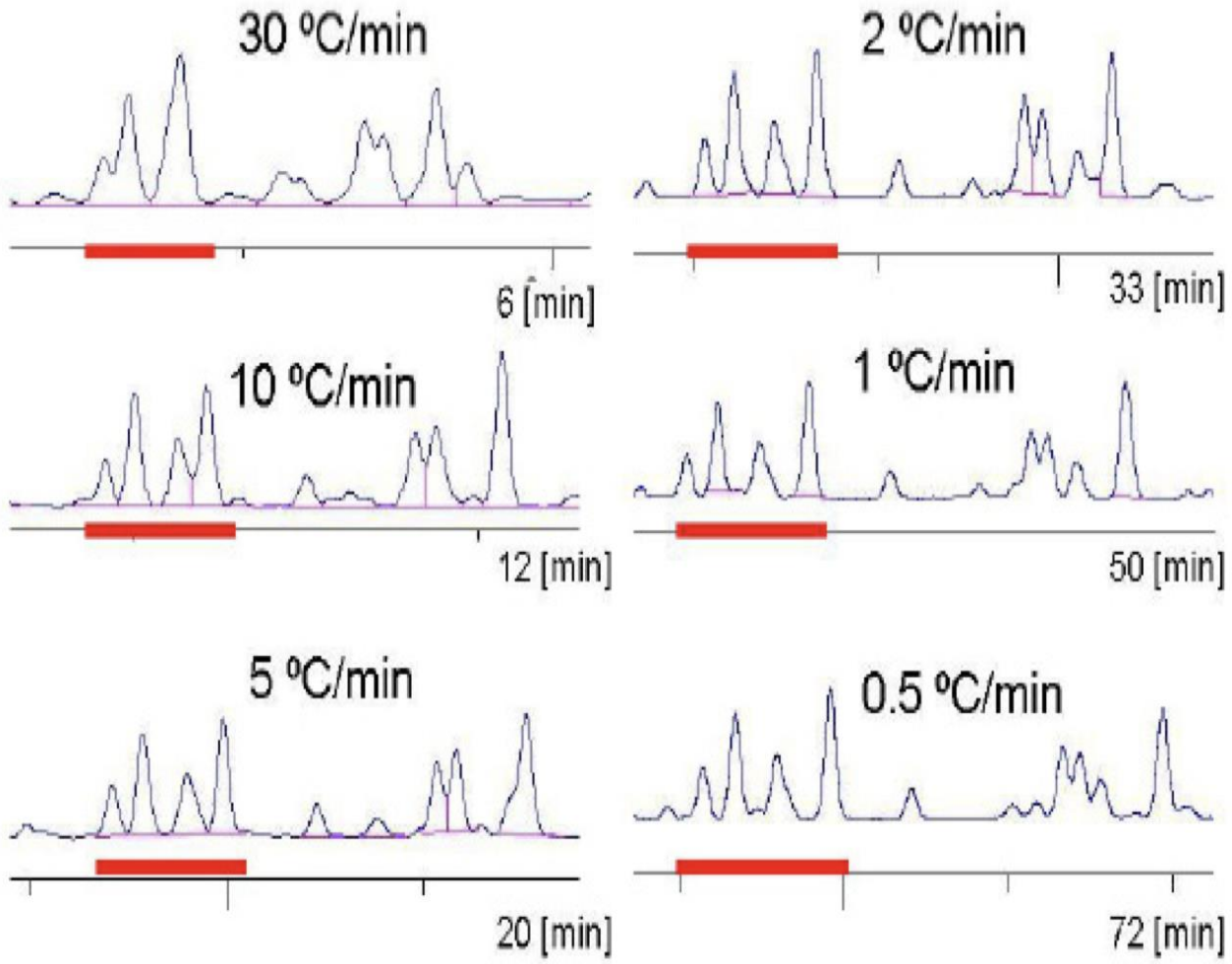
# Separation mechanisms: Temperature




# Separation mechanisms: Temperature ramp

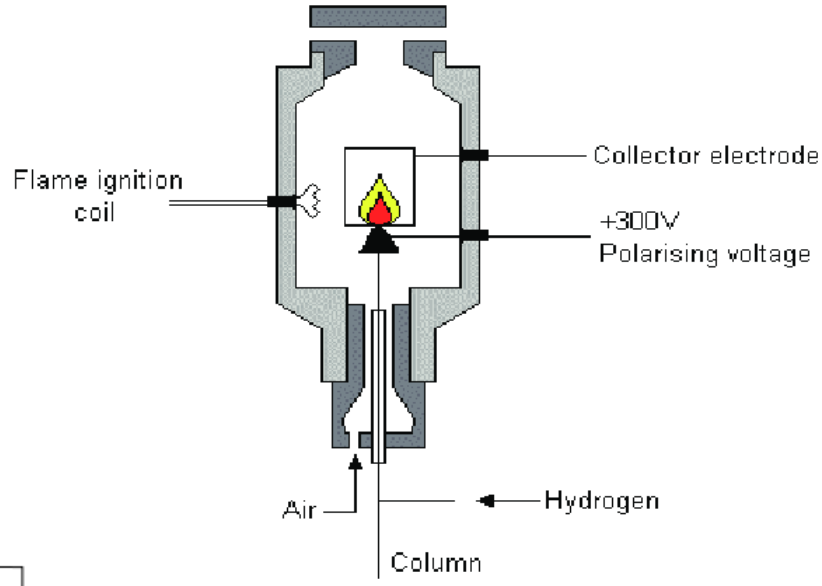


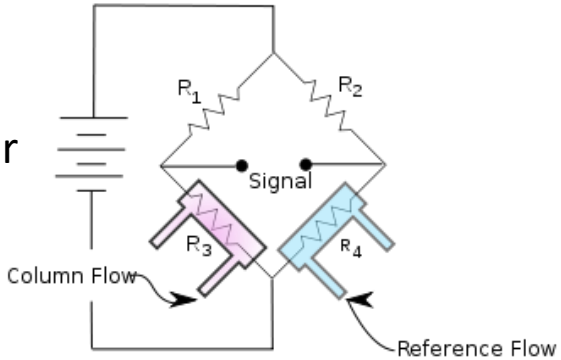



# Gas chromatography: Detectors

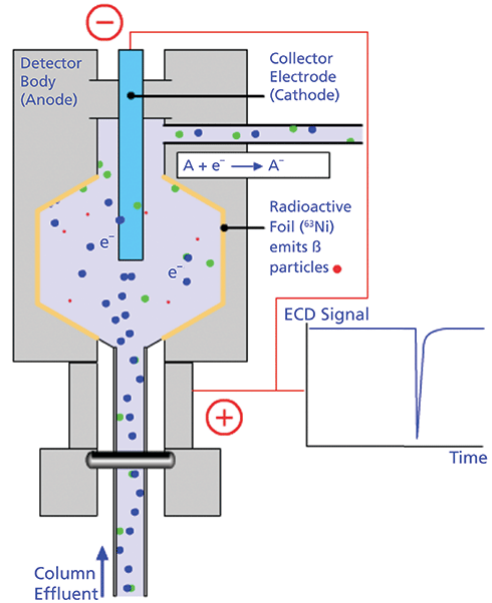
**Flame Ionization Detector (FID):** This detector is commonly used in GC because of its high sensitivity, stability, and wide linear dynamic range. It measures the amount of carbon-containing compounds that pass through the detector by ionizing them in a hydrogen-air flame. The FID consists of a hydrogen-air flame and a collector electrode. As the effluent from the GC column enters the flame, it is ionized and produces positively charged ions and electrons. The positively charged ions are attracted to the negatively charged collector electrode, producing a current that is proportional to the number of ions generated by the analytes in the sample.




**Thermal Conductivity Detector (TCD):** The TCD is a non-destructive detector that is sensitive to changes in the thermal conductivity of the gas stream. It is commonly used to detect not so volatile and non-polar compounds that cannot be detected by other detectors.




**Electron Capture Detector (ECD):** The ECD is a highly sensitive detector that is used to detect compounds that contain electronegative atoms such as halogens, nitro, or cyano groups. It works by ionizing the carrier gas with electrons emitted from a radioactive source and detecting the resulting changes in the electrical conductivity of the gas. It is used for pesticides, polychlorinated biphenyls (PCBs), chlorinated solvents, and other halogenated compounds

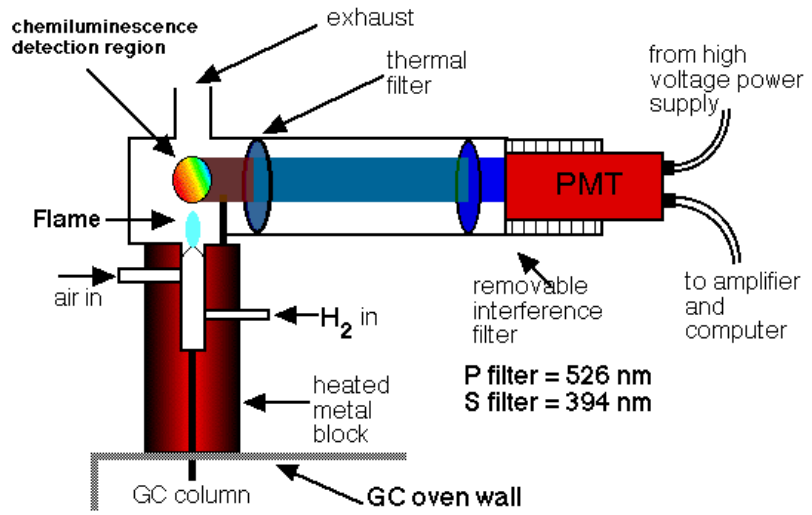


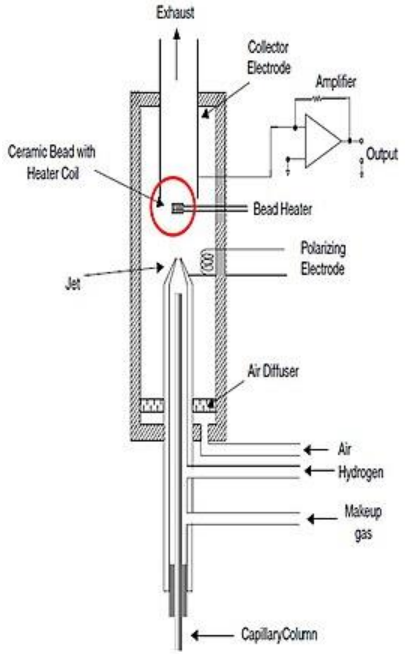
# Gas chromatography: Detectors

**Flame Photometric Detector (FPD):** The FPD is a selective detector that is used to detect compounds containing sulfur, phosphorus, or other elements that emit light when exposed to a flame. It works by ionizing the compounds in a hydrogen-air flame and detecting the resulting light emission.

**Nitrogen-Phosphorus Detector (NPD):** The NPD is a selective detector that is used to detect compounds containing nitrogen or phosphorus. It works by converting the nitrogen or phosphorus in the compound to a chemiluminescent species that can be detected by a photomultiplier tube.

**Gas chromatography-olfactometry (GC-O):** It is a sensory analysis technique that is used to evaluate the aroma and flavor of complex mixtures, such as food, beverages, and fragrances. GC-O combines gas chromatography (GC) with human sensory evaluation to identify and quantify the individual compounds responsible for the aroma and flavor of a sample

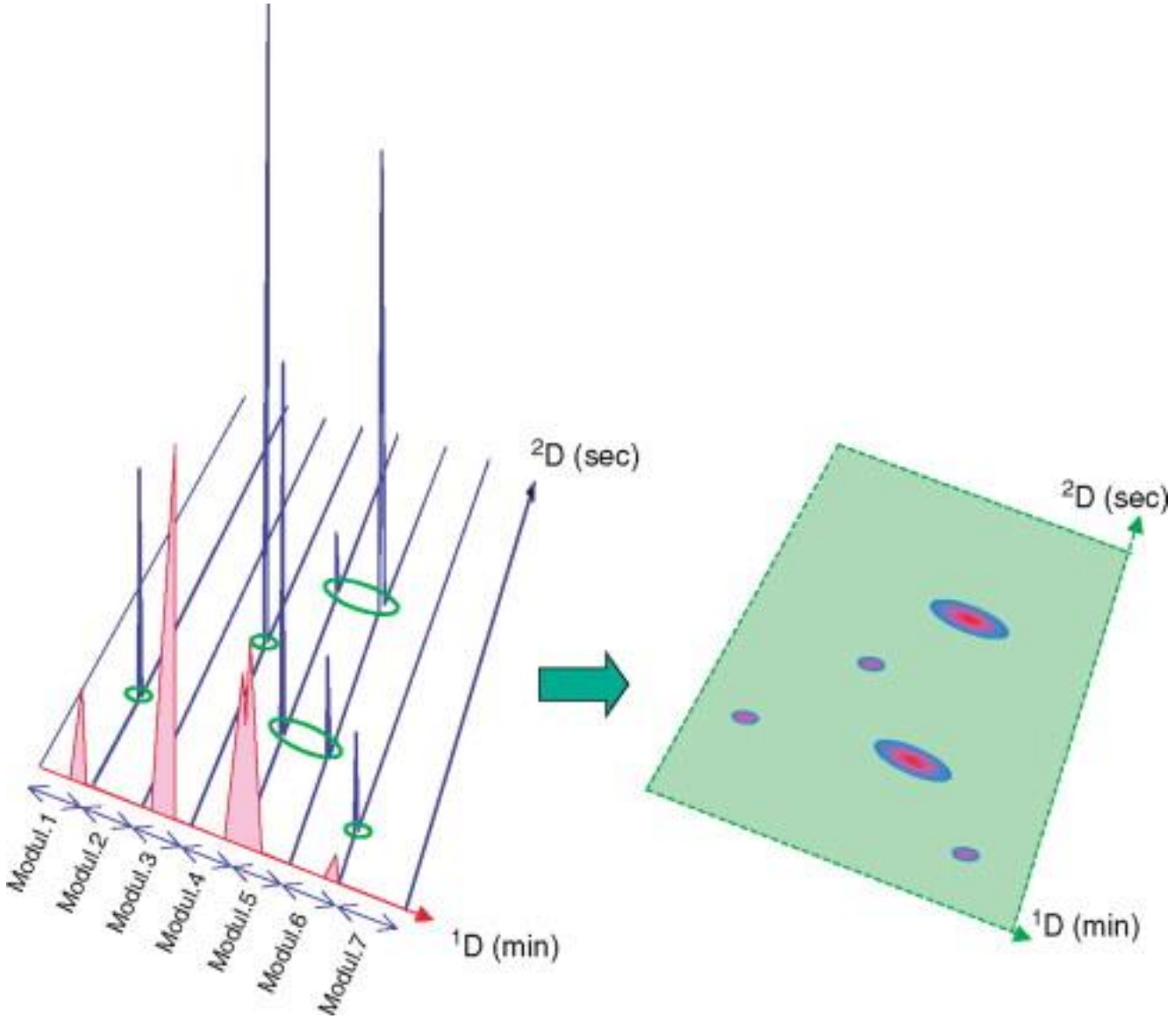


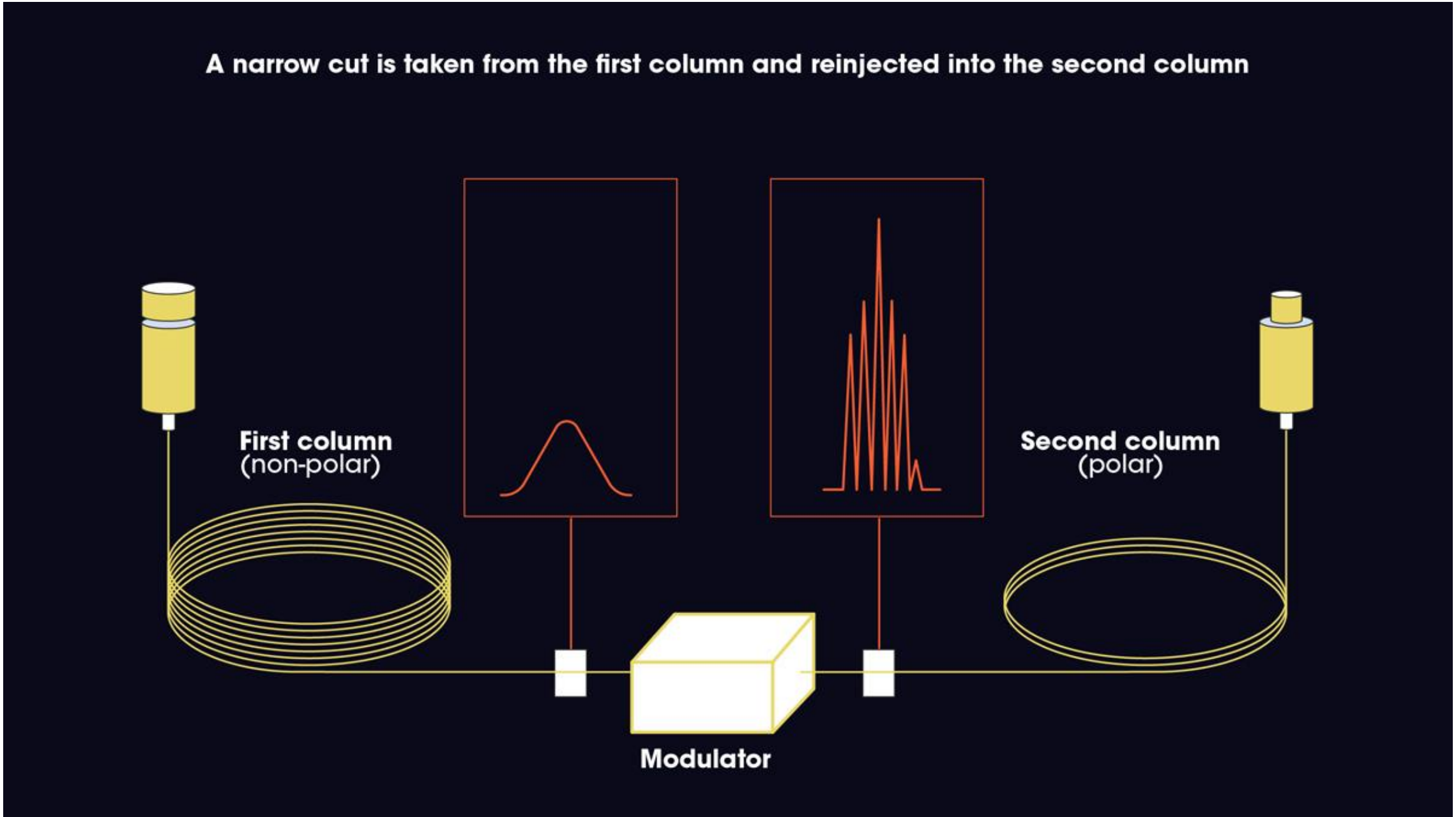

# Gas chromatography ”on steroids” 1. 2D GC

Two-dimensional gas chromatography (2D GC) is a powerful analytical technique that combines **two stages of chromatography** to provide increased resolution and separation of individual components in a sample.

In 2D GC, the sample is first separated in the first dimension based on its boiling point, using a conventional GC column. The eluted fractions from the first column are then transferred to a second column with a different stationary phase, where further separation takes place based on the chemical properties of the individual components. The elution from the second column is then detected and analyzed, providing a much more detailed chemical profile of the sample than traditional one-dimensional GC methods. 2D GC is particularly useful for complex mixtures such as natural products, essential oils, and petrochemicals, where individual components are difficult to separate using conventional GC methods



# Gas chromatography ”on steroids”  1. 2D GC



# Gas chromatography ”on steroids”  2. Hypenathed techniques

Hyphenated GC techniques refer to the **combination** of gas chromatography (GC) with other analytical techniques, such as **mass spectrometry (MS), infrared spectroscopy (IR), or flame ionization detection (FID).**

The term "hyphenated" comes from the fact that these techniques are typically **linked together** by a hyphen to indicate that they are combined. For example, GC-MS combines gas chromatography with mass spectrometry to separate and detect the individual components in a sample. Hyphenated GC techniques are widely used in various fields such as pharmaceuticals, environmental monitoring, food and flavor analysis, and forensic science. The combination of GC with other analytical techniques enhances the sensitivity, selectivity, and accuracy of the analysis

# Retention time. How long it takes a GC run?

- The following equation defines GC retention time:

$$t_R = \frac{L\,(k+1)}{\mu}$$

- There are three options to reduce $t_R$ (retention time):

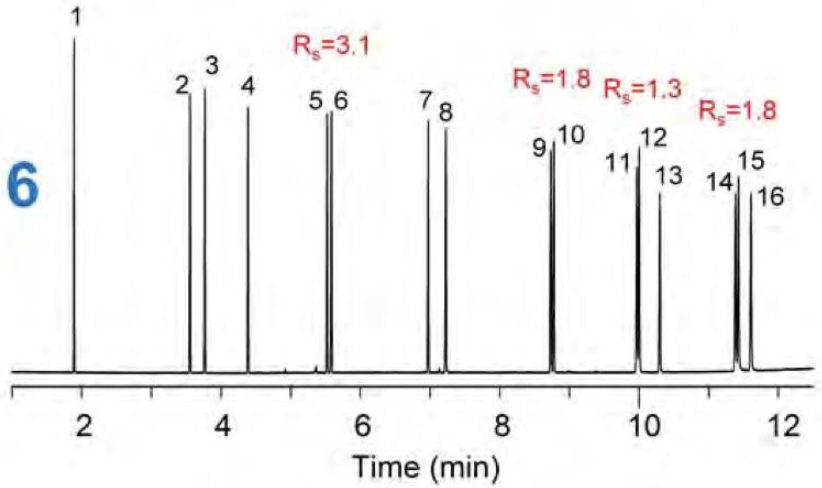
  **1**. Reduce L (column length)

  **2**. Reduce k (retention factor) by increasing temperature

  **3**. Increase μ (carrier gas linear velocity)

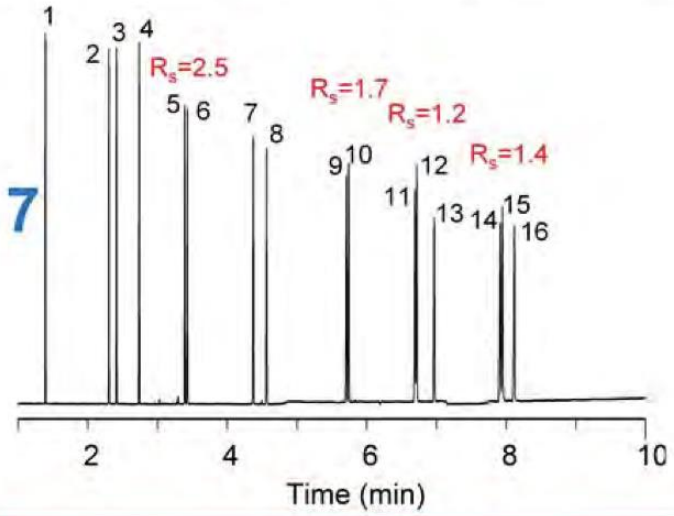
# Optimizing a GC run (1)

## Increase Ramp Rate




SLB-5ms
10 m x 0.10 mm, 0.10 µm
70 ºC (0.2 min), 20 ºC/min
to 325 ºC (3 min)
hydrogen at 60 cm/sec
0.5 µL, 100:1 split

16 PAHs




SLB-5ms
10 m x 0.10 mm, 0.10 µm
70 ºC (0.2 min), **40 ºC/min to 175 ºC, 25 ºC/min to 270 ºC**, 20 ºC/min to 325 ºC
hydrogen at 60 cm/sec
0.5 µL, 100:1 split



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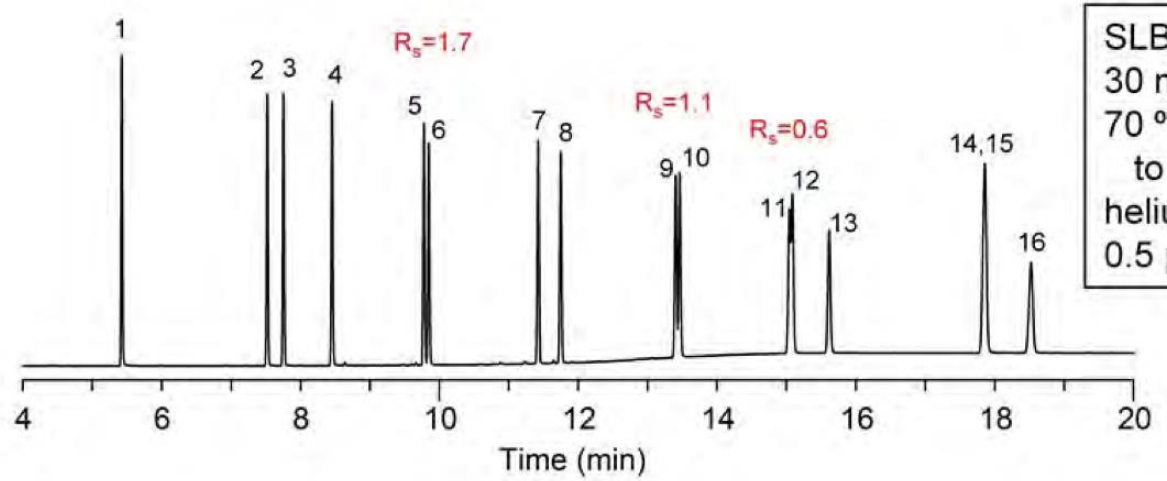
# Optimizing a GC run (2)

## Decrease Column Length

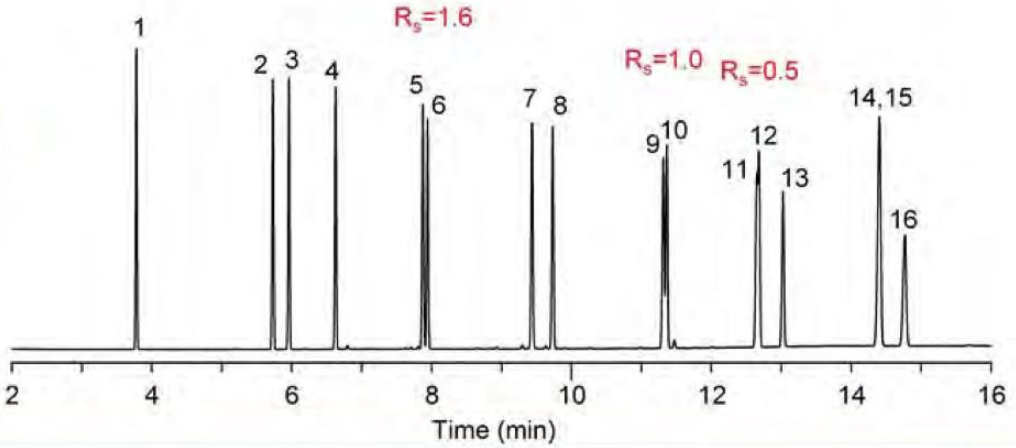
**1**




SLB-5ms
30 m x 0.25 mm, 0.25 µm
70 ºC (0.2 min), 20 ºC/min
to 325 ºC (3 min)
helium at 25 cm/sec
0.5 µL, 10:1 split

16 PAHs

**2**




SLB-5ms
**15 m** x 0.25 mm, 0.25 µm
70 ºC (0.2 min), 20 ºC/min
to 325 ºC (3 min)
helium at 25 cm/sec
0.5 µL, 10:1 split



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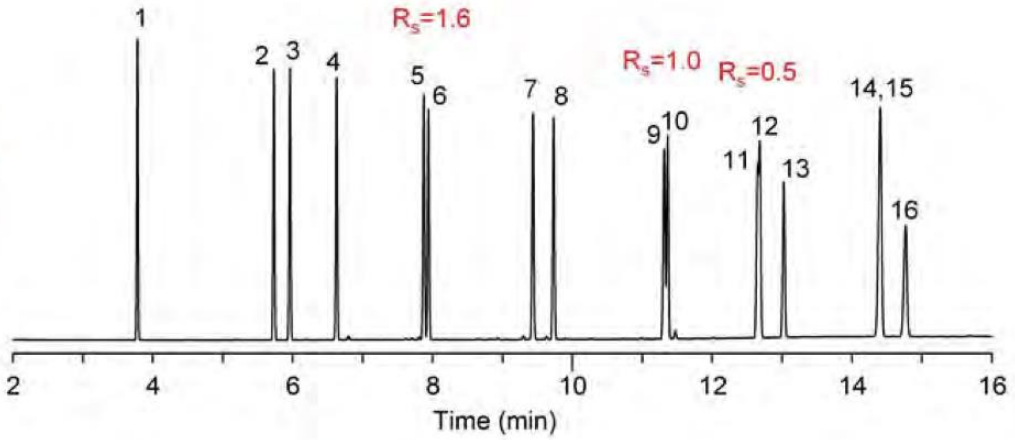
# Optimizing a GC run (3)

## Switch to Hydrogen Carrier Gas

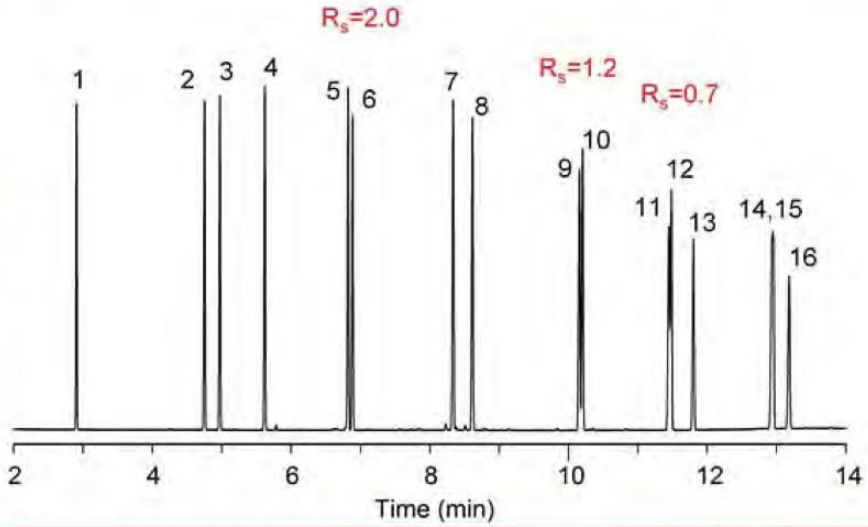
**2**




SLB-5ms
15 m x 0.25 mm, 0.25 µm
70 °C (0.2 min), 20 °C/min
to 325 °C (3 min)
helium at 25 cm/sec
0.5 µL, 10:1 split

16 PAHs

**3**




SLB-5ms
15 m x 0.25 mm, 0.25 µm
70 °C (0.2 min), 20 °C/min
to 325 °C (3 min)
**hydrogen** at **40 cm/sec**
0.5 µL, 10:1 split



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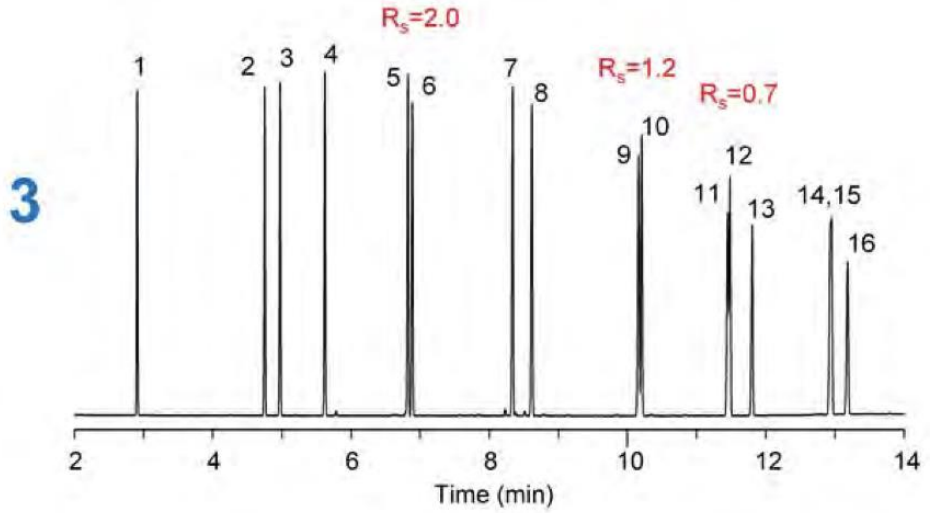
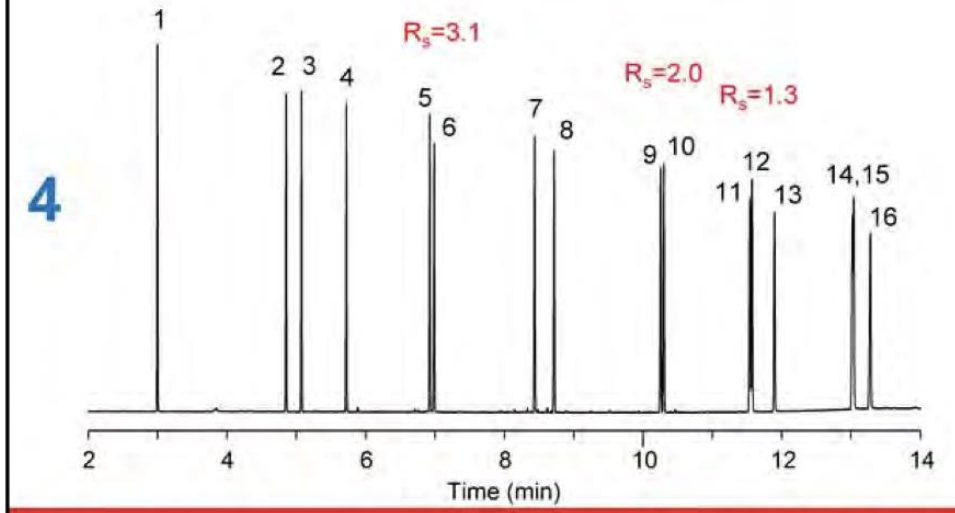
# Optimizing a GC run (4)

## Decrease Column I.D.




SLB-5ms
15 m x 0.25 mm, 0.25 µm
70 °C (0.2 min), 20 °C/min
to 325 °C (3 min)
hydrogen at 40 cm/sec
0.5 µL, 10:1 split

16 PAHs




SLB-5ms
15 m x **0.10 mm, 0.10 µm**
70 °C (0.2 min), 20 °C/min
to 325 °C (3 min)
hydrogen at 40 cm/sec
0.5 µL, **100:1** split



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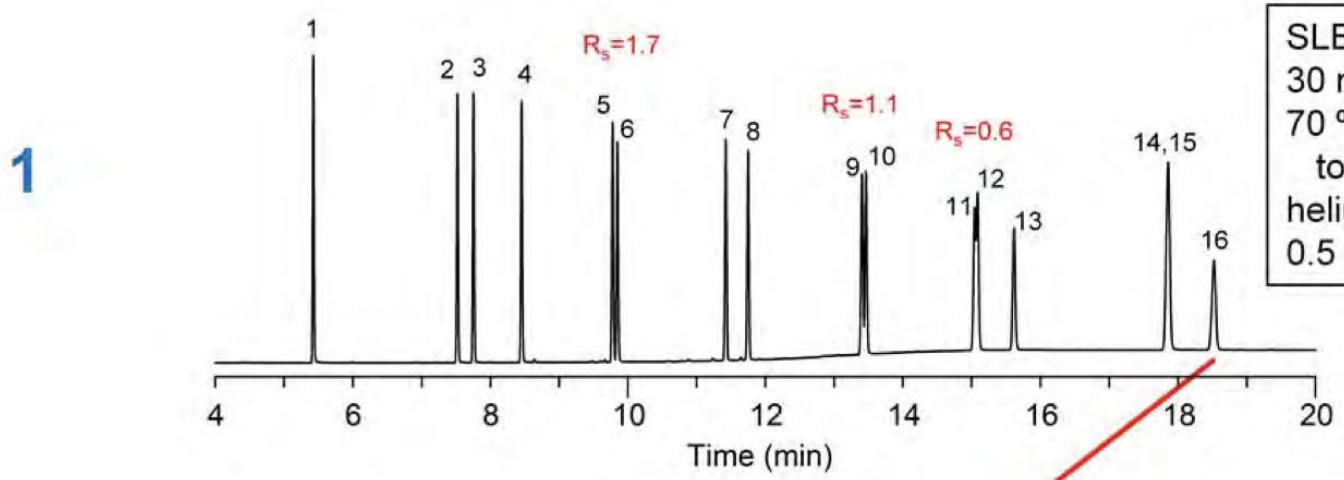
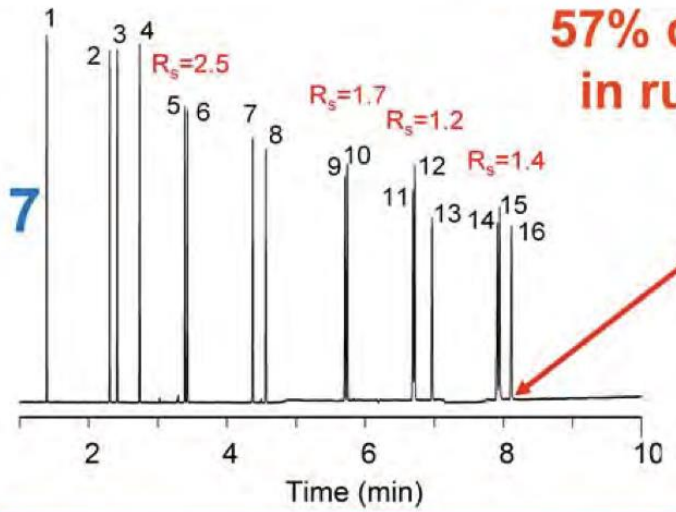
# Optimizing a GC run (5)

## Overall Results




SLB-5ms
30 m x 0.25 mm, 0.25 µm
70 ºC (0.2 min), 20 ºC/min
to 325 ºC (3 min)
helium at 25 cm/sec
0.5 µL, 10:1 split

16 PAHs




**57% decrease in run time!**

**Improved resolution!**

SLB-5ms
**10 m** x **0.10 mm, 0.10 µm**
70 ºC (0.2 min), **40 ºC/min to 175 ºC, 25 ºC/min to 270 ºC**, 20 ºC/min to 325 ºC
**hydrogen** at **60 cm/sec**
0.5 µL, **100:1** split



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