Gas Chromatography

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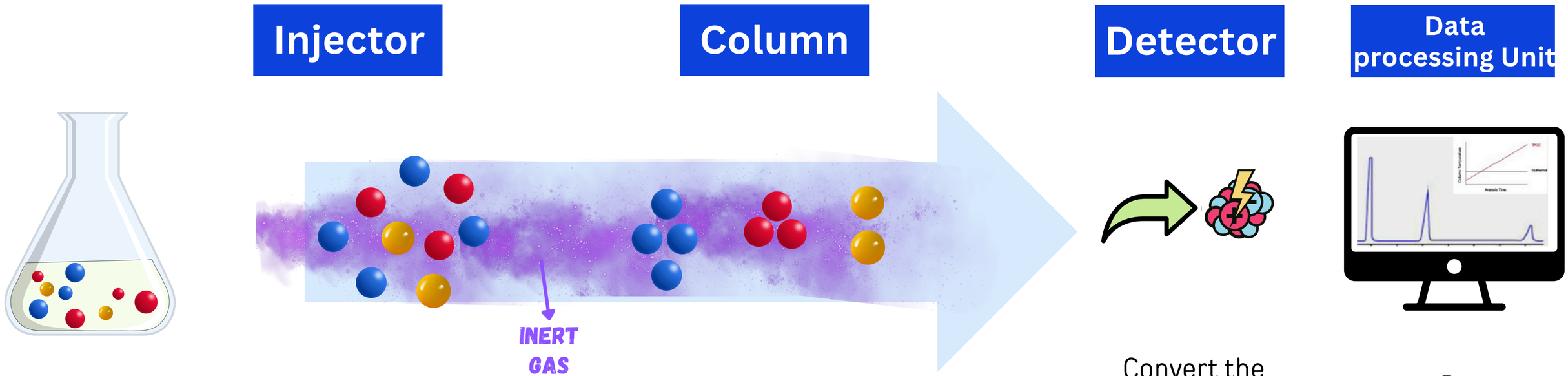
CONTENT

What is Gas chromatography?

Concept

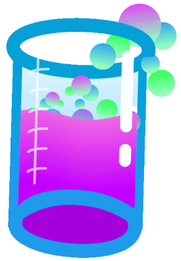
Gas chromatography (GC) is a widely used analytical technique for **separating** and **analyzing** compounds in a mixture.



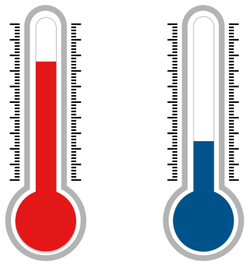
Applications

Criteria of compounds to be analysed by GC

1-Volatility



2-Thermostability



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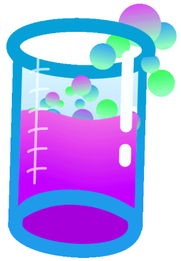

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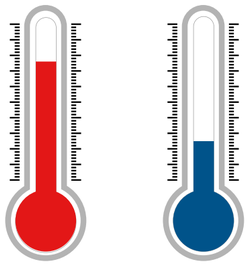
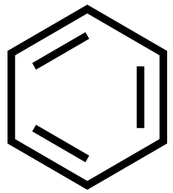


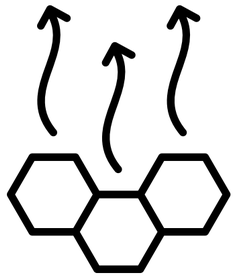
Examples

Flavors and Fragnances

Pesticides



Aromatics (ex: benzene, toluene)



Volatile organic compounds

Active pharmaceutical ingredients

Drugs and metabolites

Applications in Pharmaceutical field

Quality control and purity testing

Stability of drug formulation

Monitoring the degradation of active pharmaceutical ingredients (APIs) and the identification of degradation products

Residual solvent analysis

Separation and quantification of residual solvents present in drug formulation, such as methanol, ethyl acetate, dichloromethane, and others

https://www.drawellanalytical.com/9-key-gas-chromatography-applications-in-pharmaceuticals-analysis/

Jwaili, M. (2019). Pharmaceutical applications of gas chromatography. Open Journal of Applied Sciences, 9(9), 683-690.

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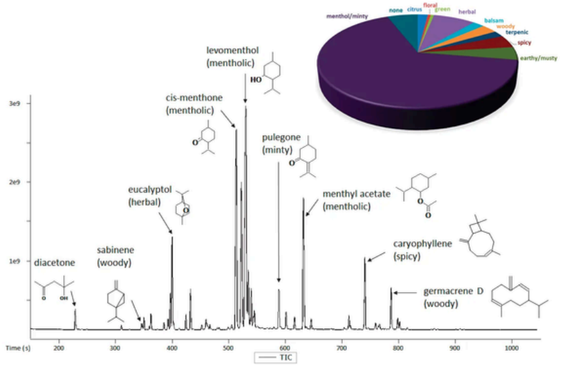
Forensic analysis

Aanalysis of drugs of abuse, such as cocaine, heroin, amphetamines, cannabinoids, and others

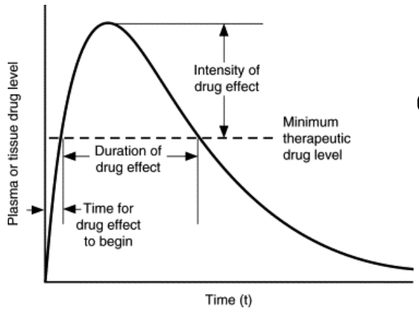
Characterization of natural products



Identification and quantification of active constituents, such as essential oils, terpenes, and other volatile compounds

https://www.azom.com/article.aspx?ArticleID=20833

Drug metabolism and pharmacokinetics



Understanding the absorption, distribution, metabolism, and elimination of drugs in the body.
Determination of drug concentrations, aiding in therapeutic drug monitoring and assessing drug metabolism

https://www.drawellanalytical.com/9-key-gas-chromatography-applications-in-pharmaceuticals-analysis/

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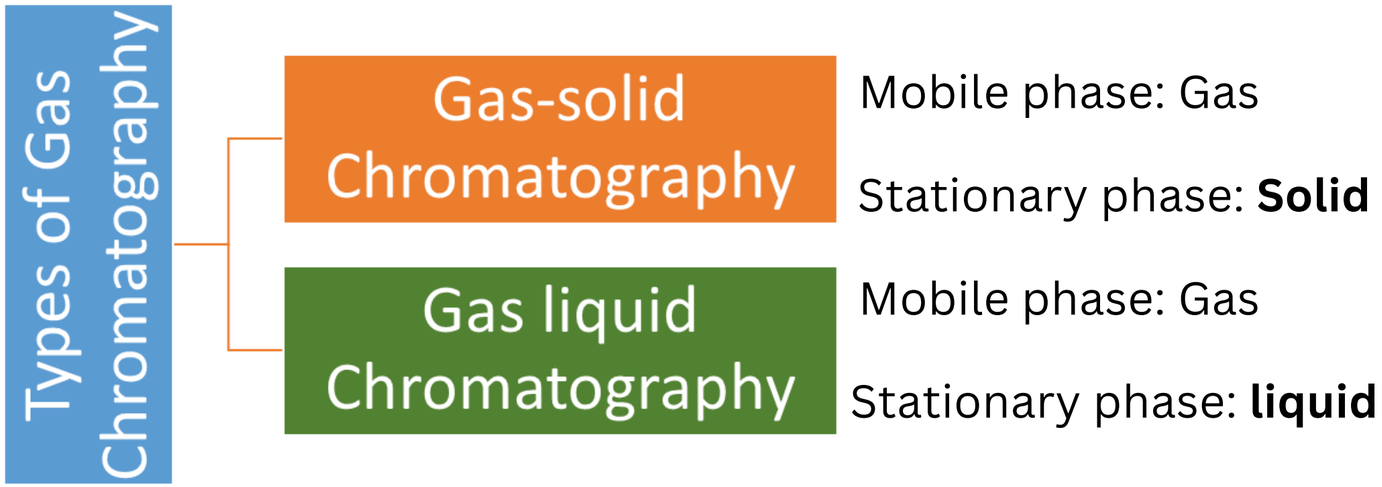
Principle of separation



Aspect	Gas-Solid Chromatography (GSC)	Gas-Liquid Chromatography (GLC)
Stationary Phase	Solid	Liquid
Separation Mechanism	Adsorption	Partitioning
Sensitivity	High	Moderate
Resolution	Moderate	High
Column Length	Shorter	Longer
Efficiency	Lower	Higher

Separation occurs because different compounds in the sample **interact differently** with the stationary phase:

- Compounds with weaker interactions move faster through the column.
- Compounds with stronger interactions are retained longer.

This leads to the components eluting (coming out of the column) at different times called **retention times**.

https://pharmasciences.in/gas-solid-chromatography-gsc-and-gas-liquid-chromatography-glc/

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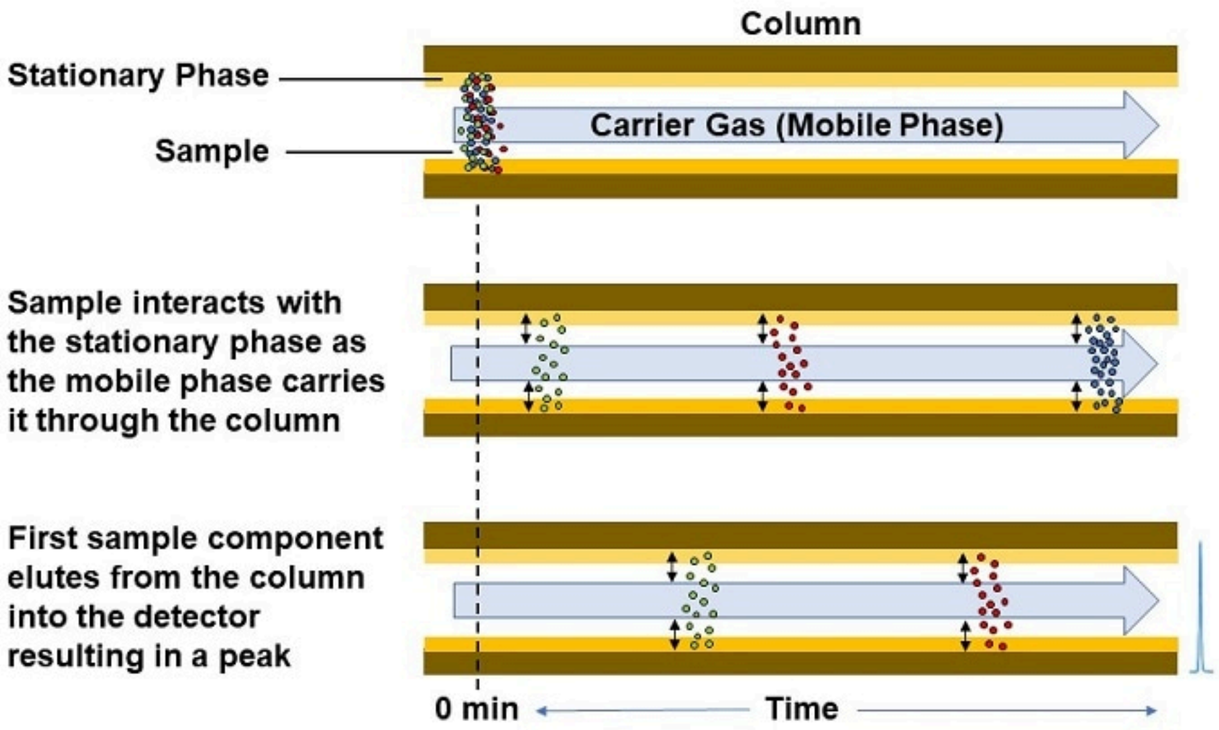

Partition as the Basis of Separation

- Compounds in the sample **partition** between the stationary phase (liquid) and the mobile phase (carrier gas)
- Partitioning depends on:
  - The compound's **solubility** in the stationary phase
  - The compound's **volatility** (tendency to remain in the gas phase)

https://www.agilent.com/en/product/gas-chromatography/what-is-gas-chromatography

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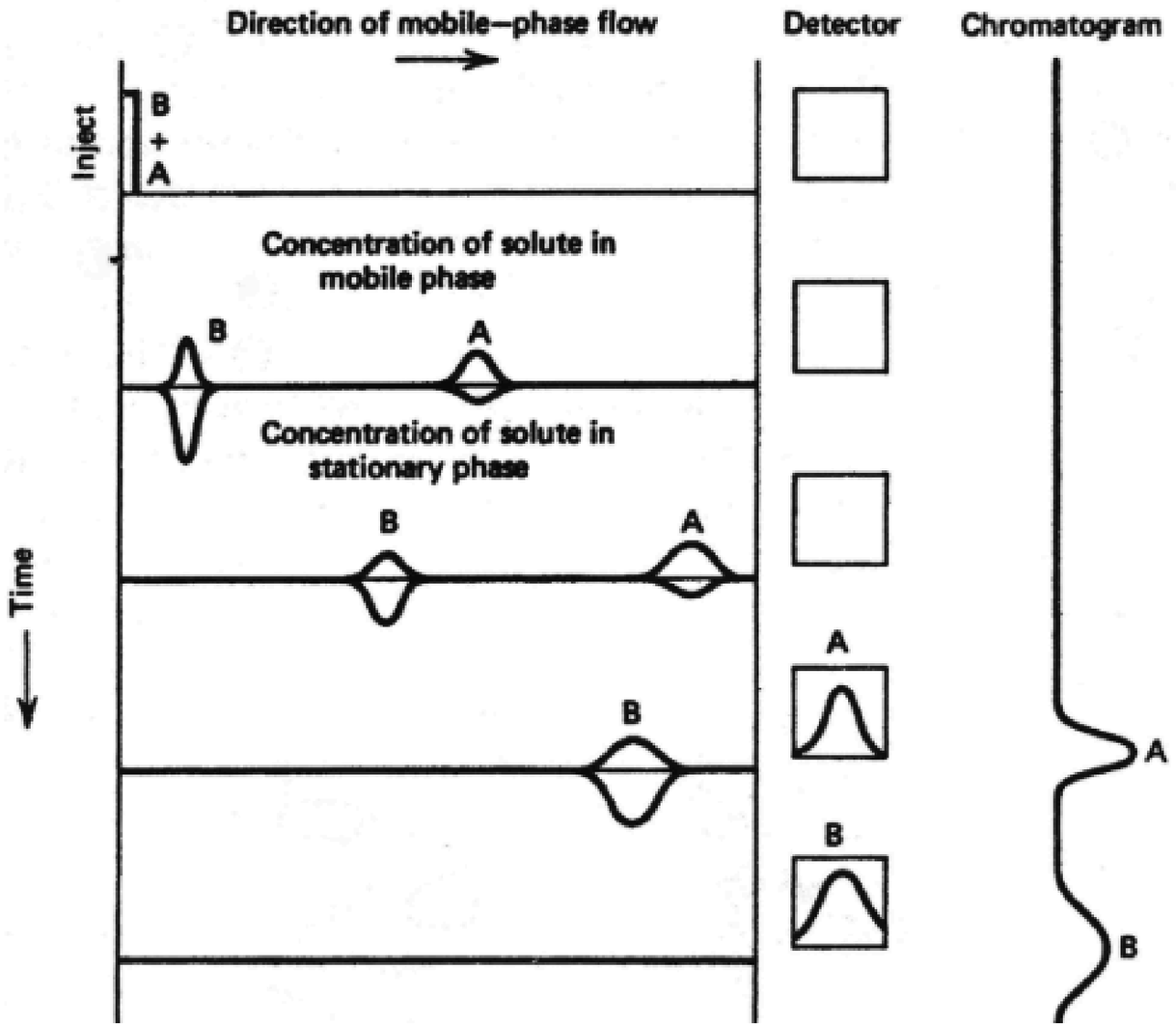


Which compound, A or B, is more retained?

Which compound, A or B, has higher solubility in the stationary phase?

Harold M. McNair, James M. Miller, Basic Gas Chromatography, John Wiley & Sons, New York,1998. Reproduced courtesy of John Wiley & Sons, Inc.

Advantages

High resolution

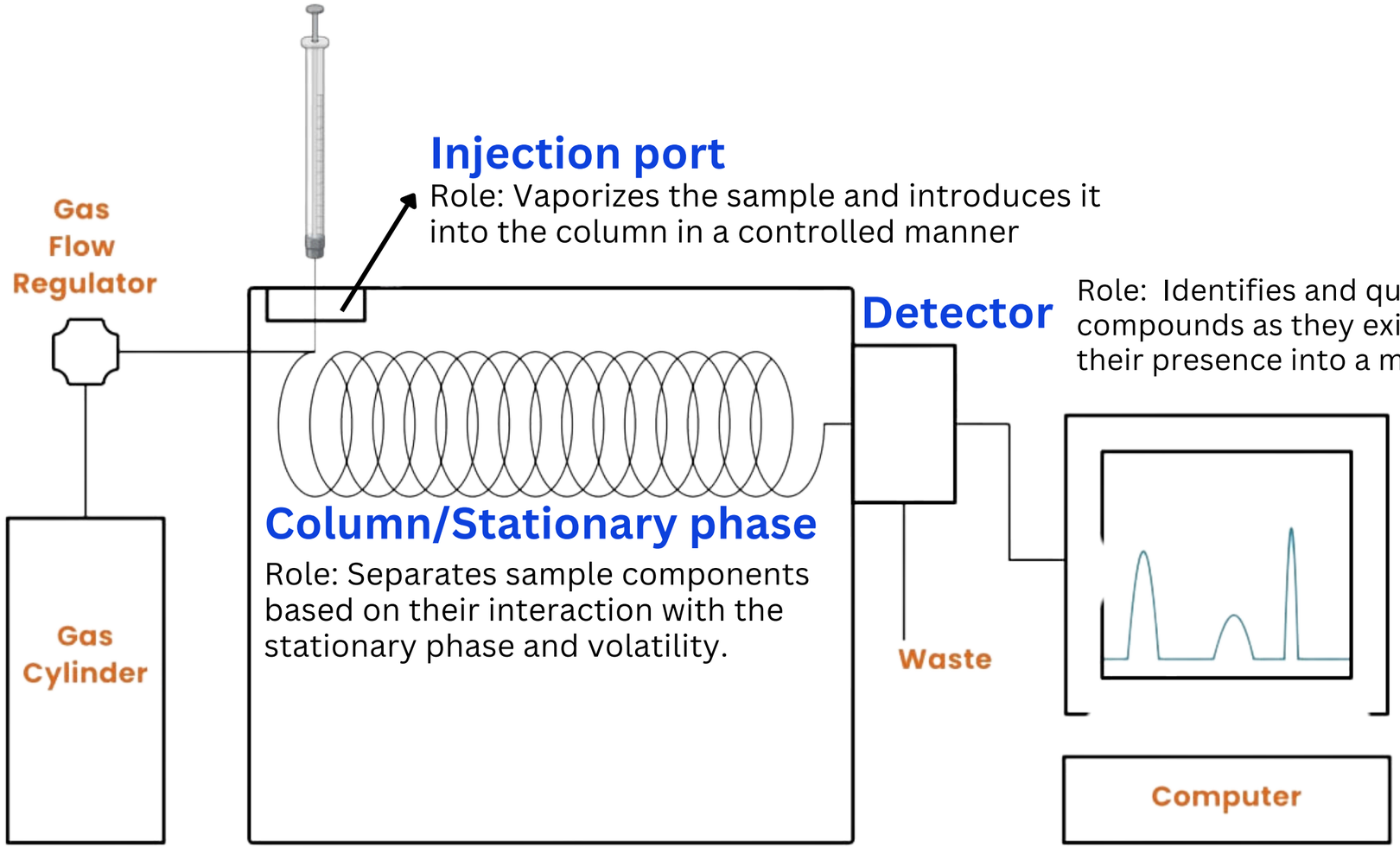
High sensitivity

High accuracy and precision

Fast analysis (seconds-minutes)

Small sample (µL-µg)

GC instrumentation



Injection port

Role: Vaporizes the sample and introduces it into the column in a controlled manner

Detector

Role: Identifies and quantifies separated compounds as they exit the column by converting their presence into a measurable signal.

Column/Stationary phase

Role: Separates sample components based on their interaction with the stationary phase and volatility.

Carrier Gas

Role: Carry the sample through the column as the mobile phase without interacting chemically.

Oven

Role: Maintains precise temperature control to optimize separation and elution of compounds.

https://bitesizebio.com/28687/carrying-gas-chromatography/

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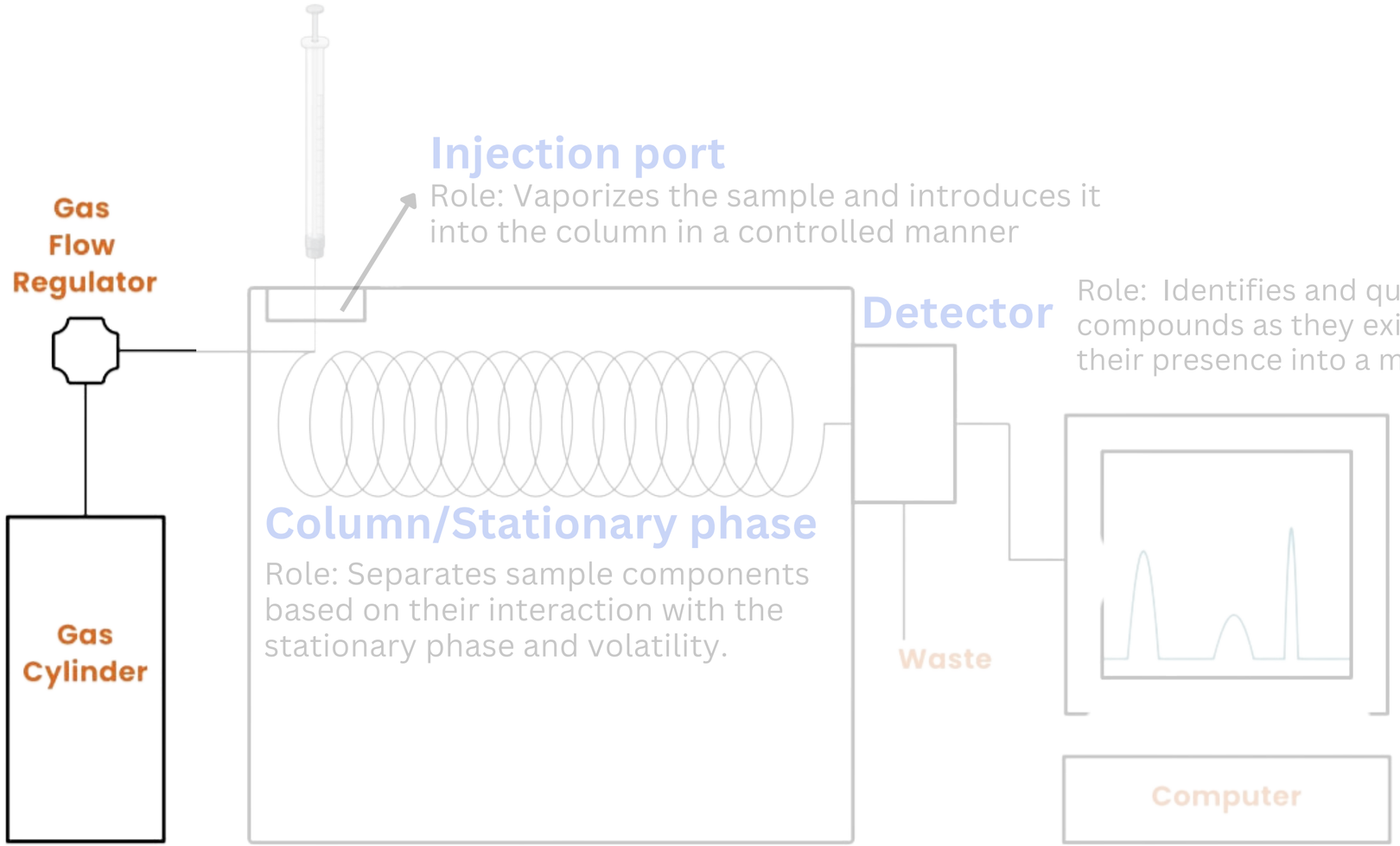

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Carrier gas

Role:

- Serves as the mobile phase to transport the vaporized sample through the GC system
- It does not interact chemically with the sample. Its primary purpose is to ensure **consistent and inert movement**.

Common carrier gases:

- **He**: most common due to inertness and optimal viscosity
- **N2**: cheaper but slower flow rate
- **H2**: fast and efficient but flammable

Key considerations:

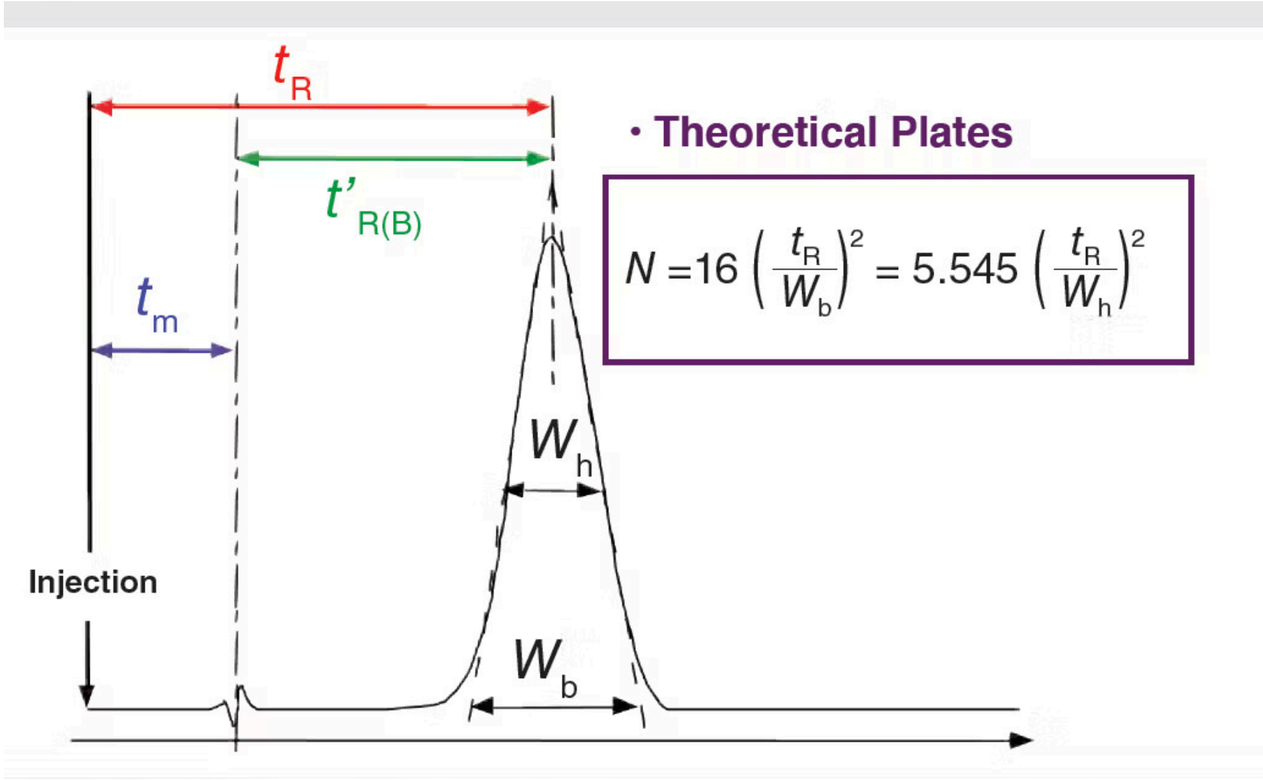
- The gas must be inert to avoid reactions with the sample or stationary phase (main difference from liquid chromatography)
- The gas must be of high purity (no oxygen or water)
- The flow rate of the carrier gas affects separation efficiency and retention times

Carrier gas: optimizing linear velocity for high column efficiency

HETP: height equivalent to a theoretical plate

HETP is the length of column required to generate one theoretical plate, with a theoretical plate being one transfer process of an analyte molecule between the mobile phase and the stationary phase

$$\text{HETP} = \frac{L}{N} = \frac{L}{16\left(t_R/t_w\right)^2}$$



Rate of band broadening

N and HETP are dependent on many parametersincluding **temperature, carrier gas flow rate, inlet and outlet pressures, column dimensions, and the choice of carrier gas.**

Shall we target conditions of hoigher or lower HETP?

https://www.chromatographyonline.com/view/is-golay-s-famous-equation-for-hetp-still-relevant-in-capillary-gc-part-1-a-common-view-of-hetp

Carrier gas: effect of flowrate

HETP: height equivalent to a theoretical plate

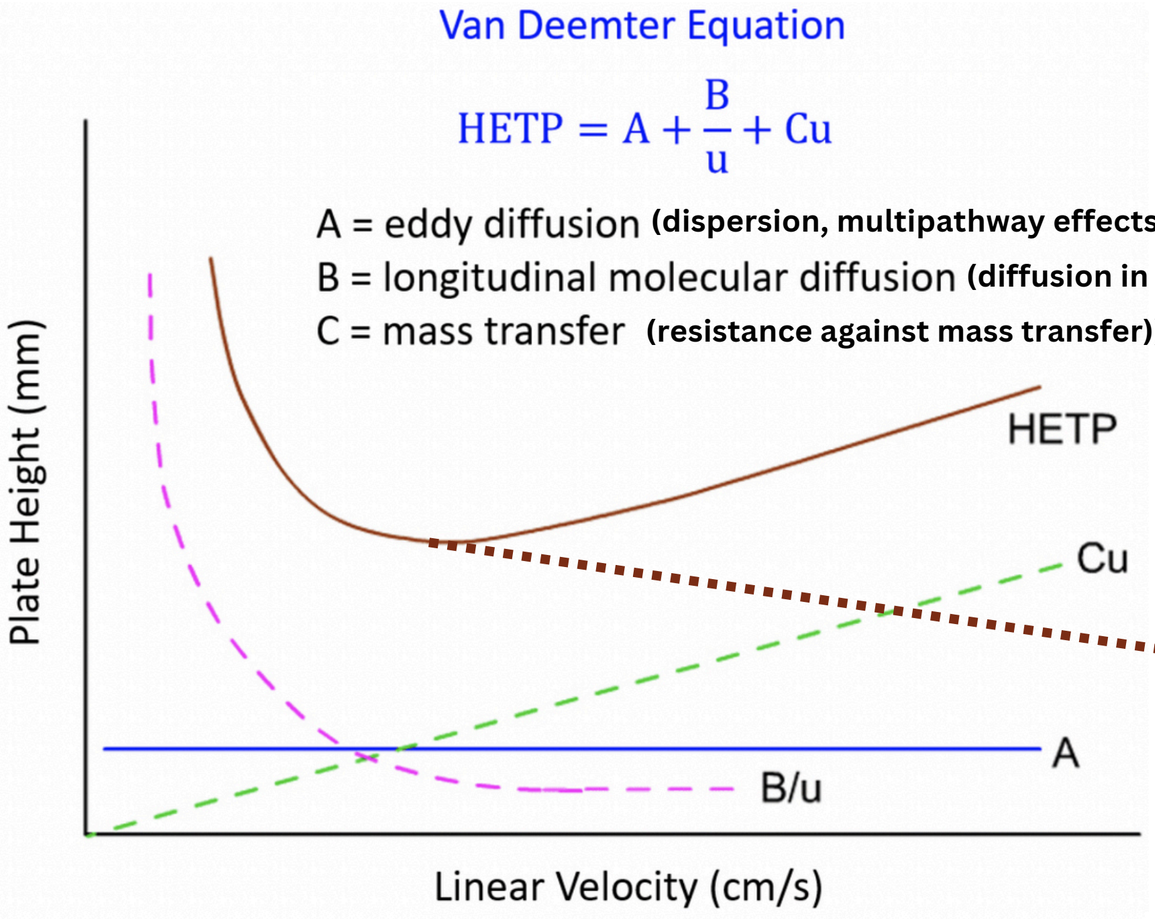
Van Deemter Equation

$$HETP = A + \frac{B}{u} + Cu$$

A = eddy diffusion **(dispersion, multipathway effects)**

B = longitudinal molecular diffusion **(diffusion in or against flow direction)**

C = mass transfer **(resistance against mass transfer))**



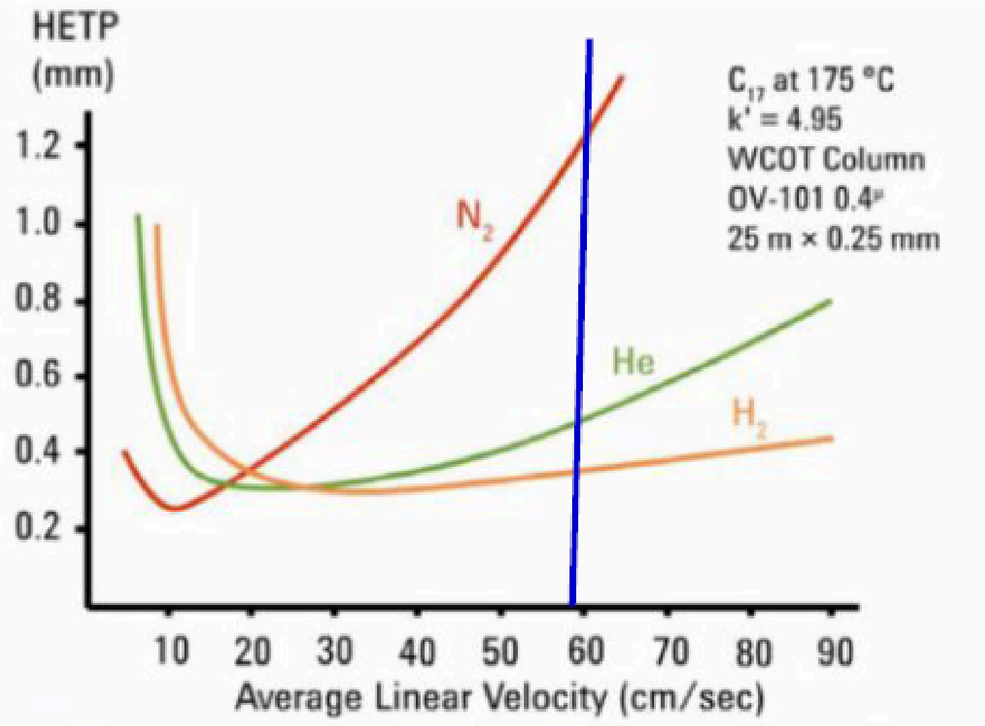
Effiency is a function of gas linear velocity or flow rate

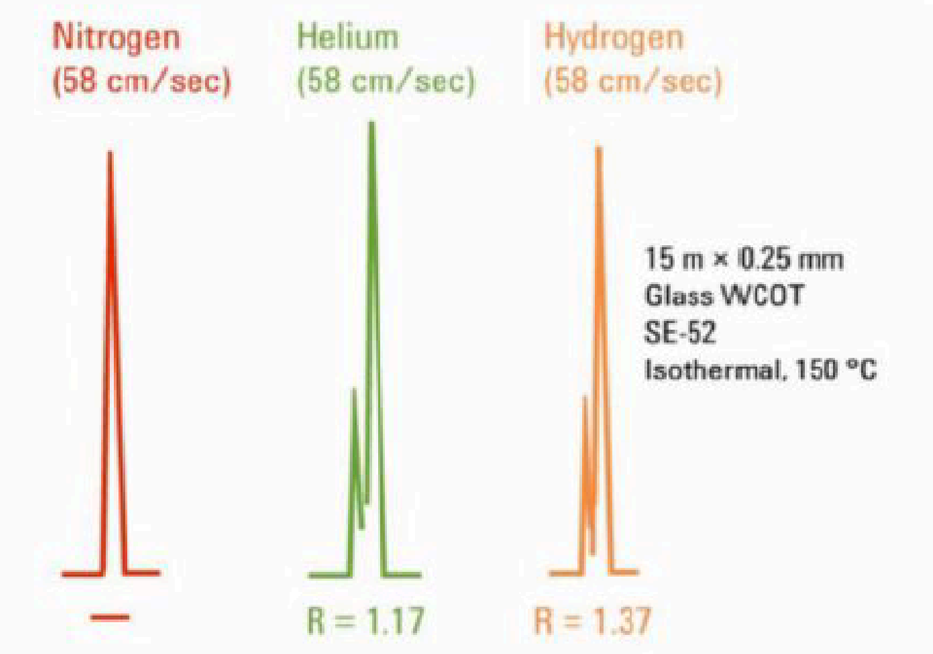
The minimum of the curve represents the smallest HEPT

Smallest HEPT=best effciency

The minimum linear velocity value is the optimum value for achieving the best efficiency

Type of carrier gas effect on column efficiency and resolution

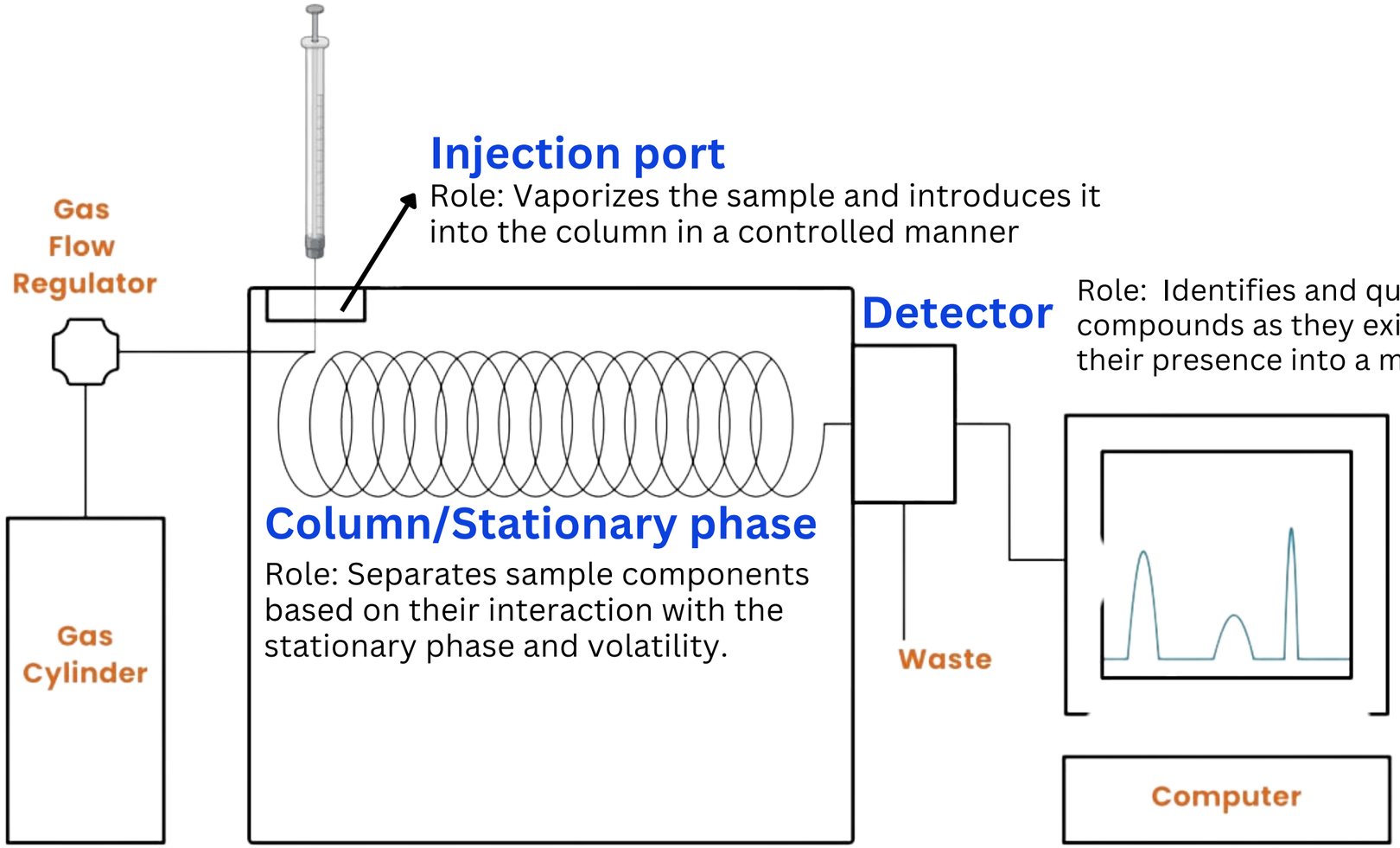




$N_{2\ HETP} > H_{e\ HETP} > H_{2\ HETP}$ ⟶ $R_{S\ H2} > R_{S\ He} > R_{S\ N2}$

Smallest HEPT= higher resolution

GC instrumentation



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https://bitesizebio.com/28687/carrying-gas-chromatography/

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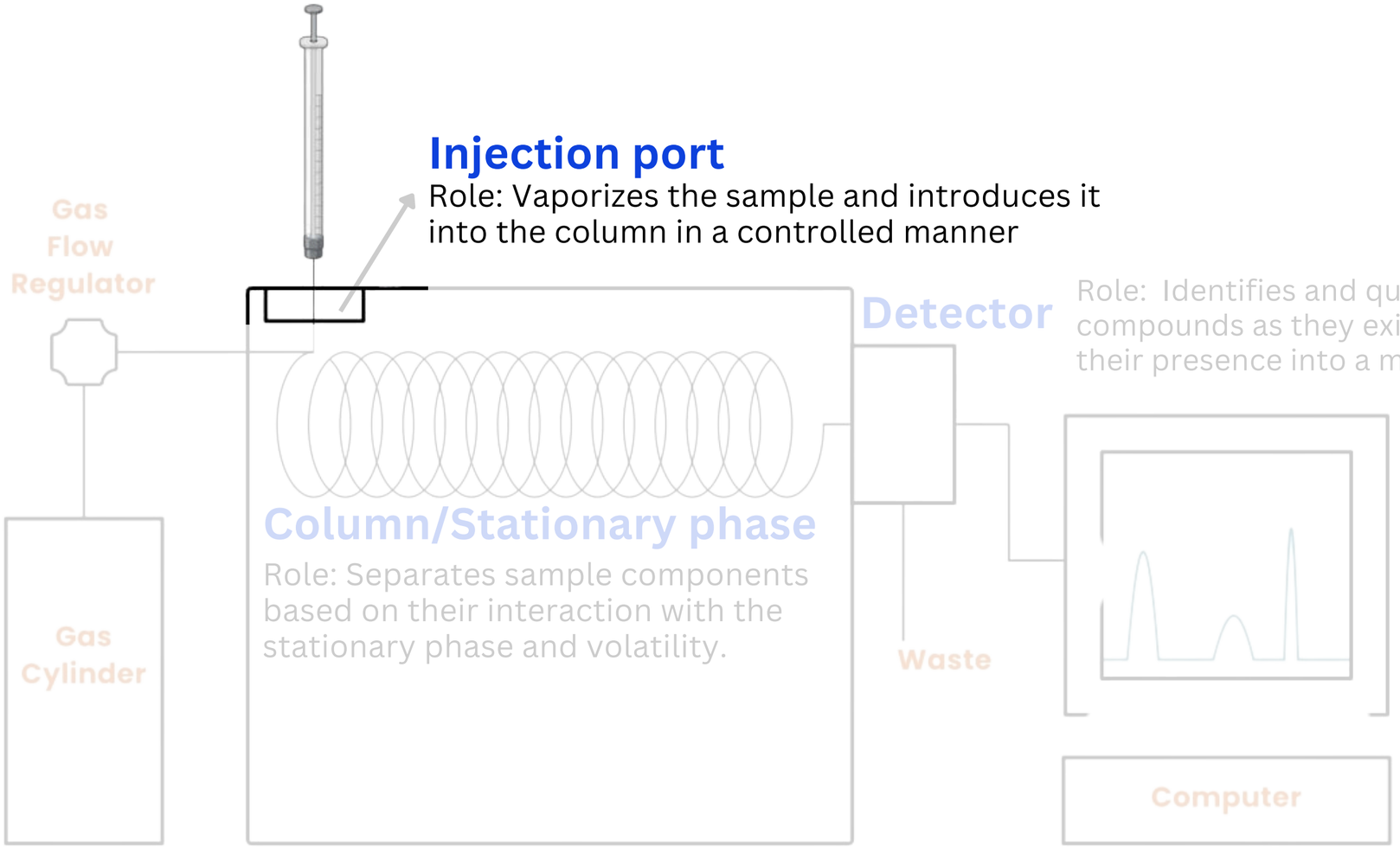

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Injector

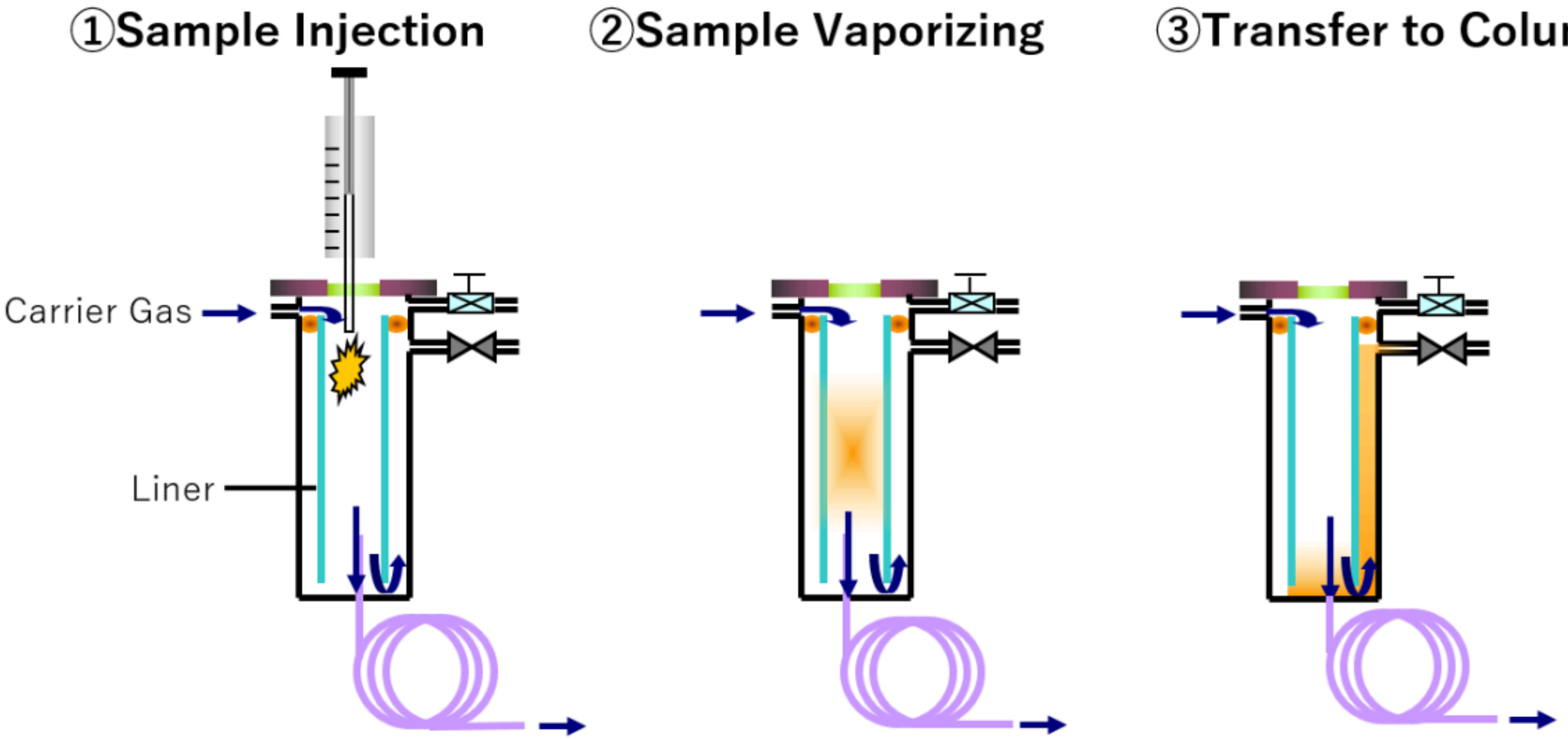
Role:

- Injects a **small** and **controlled** volume of the sample (liquid or gas) into the system.
- Vaporization: converts the liquid sample into a gas phase instanly
- Ensures the vaporized sample mixes with the carrier gas

Common types of injectors:

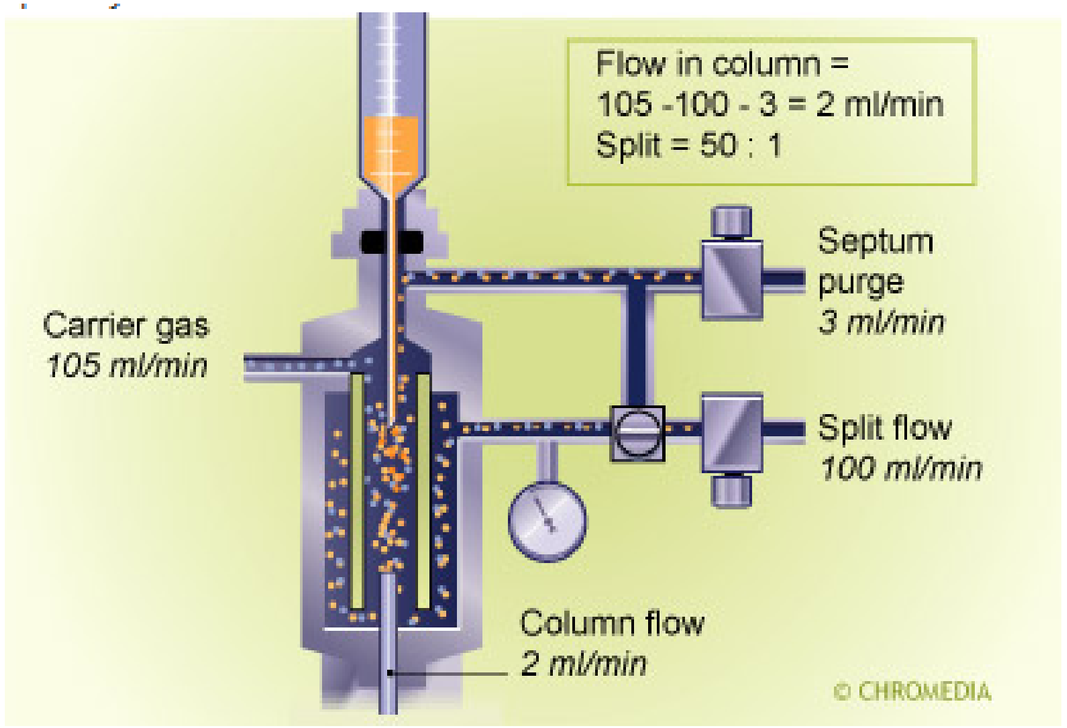
- **Split injector**: Divides the sample so only a portion enters the column
- **Splitless injector**: Directly inject the entire sample into the column
- **On-column injector**: introduces the sample directly into the column without vaporization

Split injector



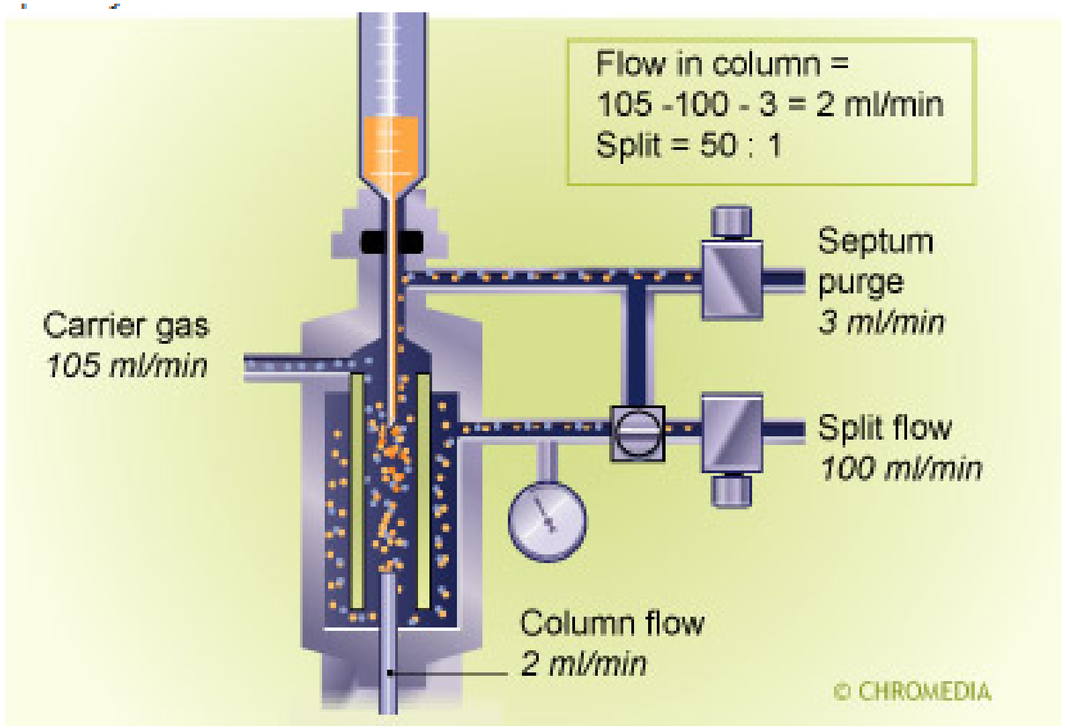
https://www.glsciences.com/technique/technique_data/gc/basics_of_gc/p3_2.html

Split injector



What is the split ratio?

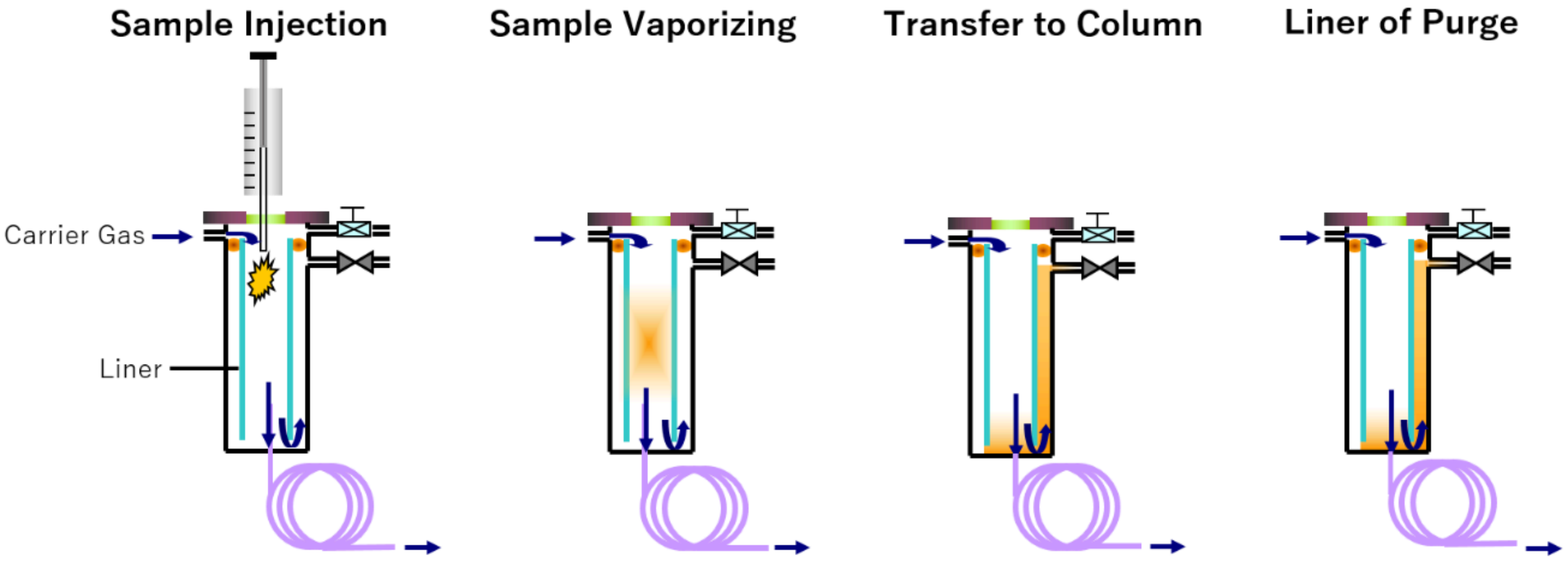
Split injector



What is the split ratio?

$$\text{Split ratio} = \frac{\text{Gas to vent}}{\text{Gas to Column}}$$

Splitless injector



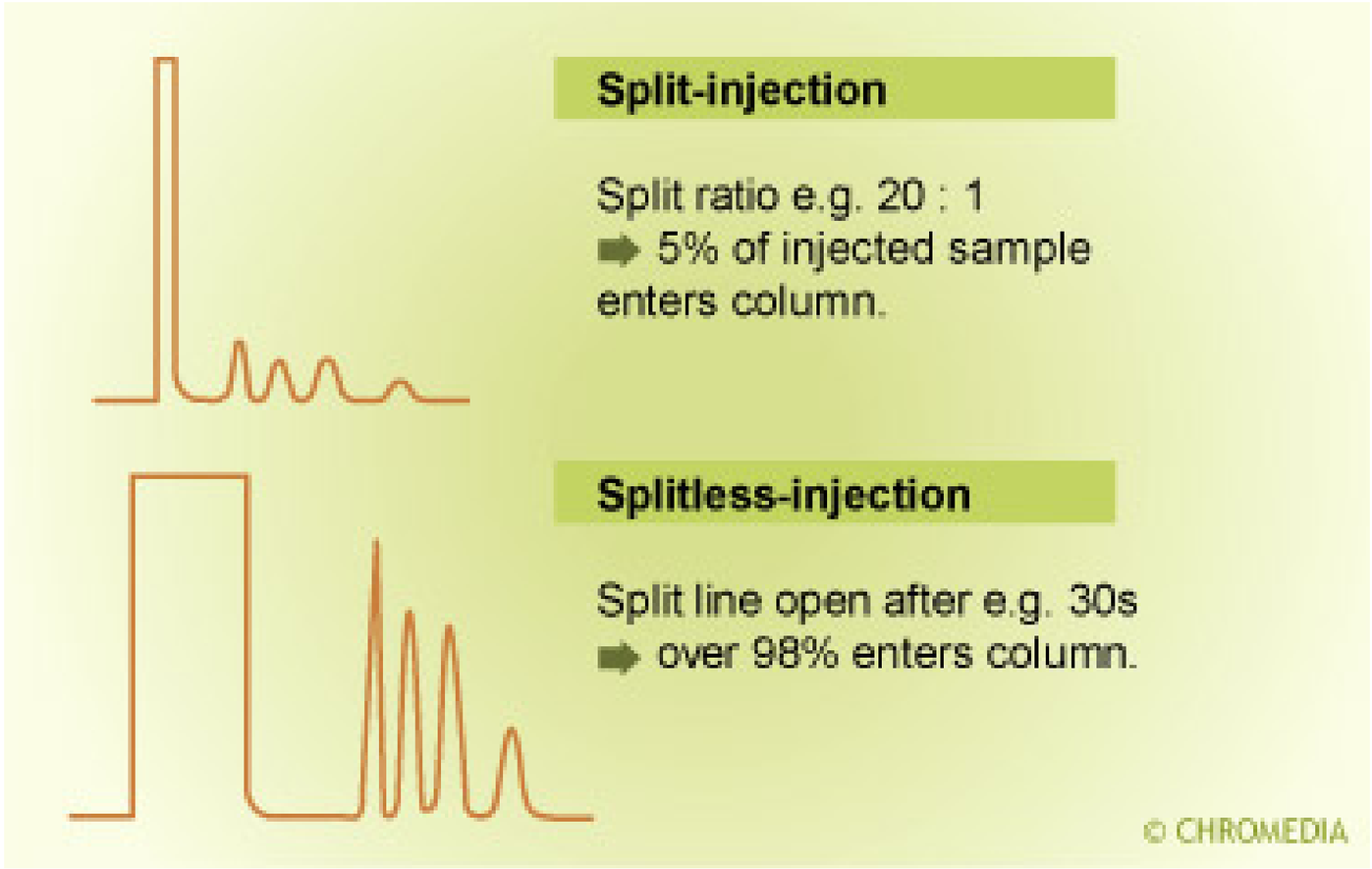
Split

vs.

Splitless

https://www.youtube.com/watch?v=TaLOF_jVRno&t=274s

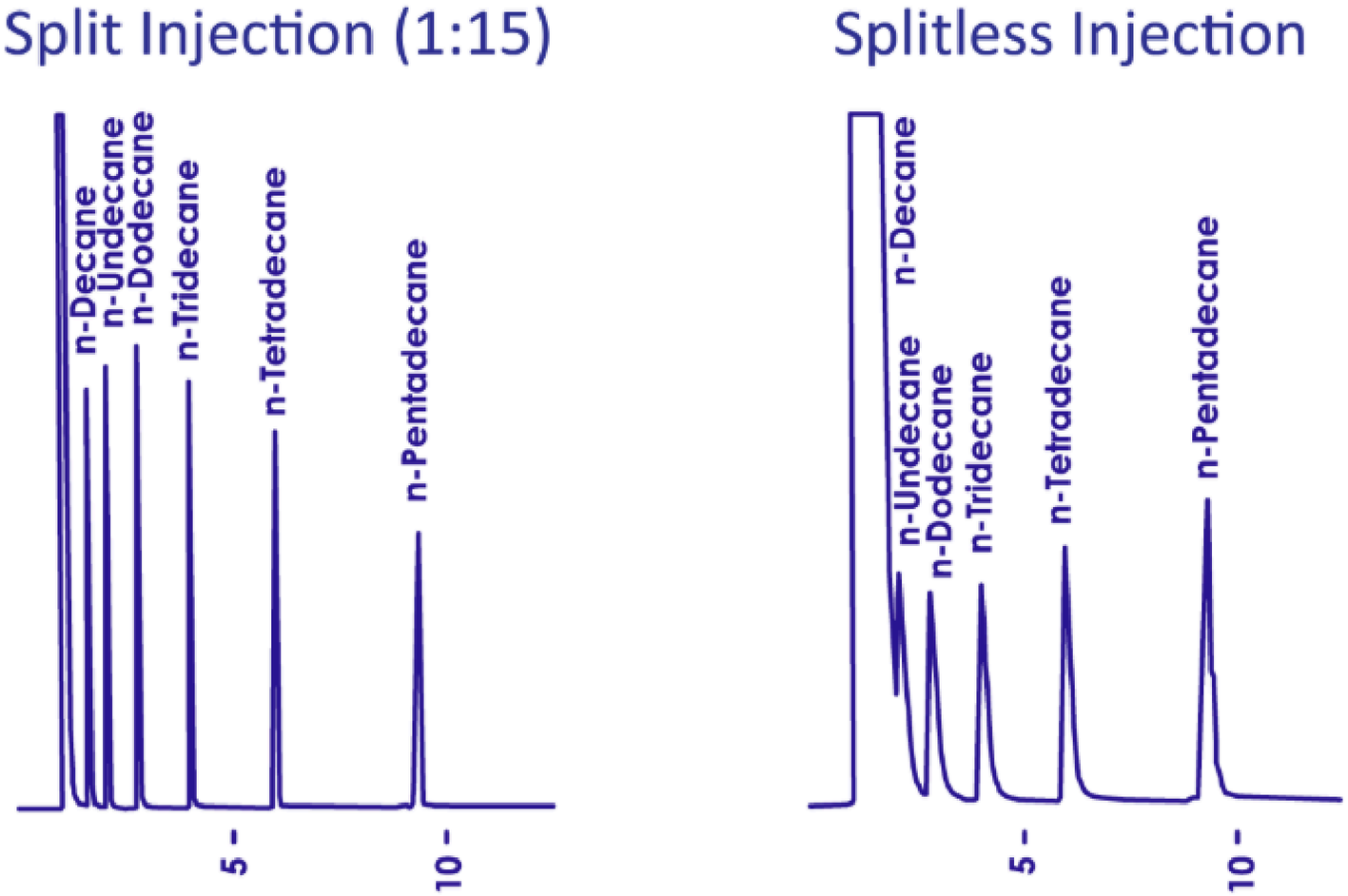
Split vs Splitless injector



For the experimental conditions, which injection method was more adapted?

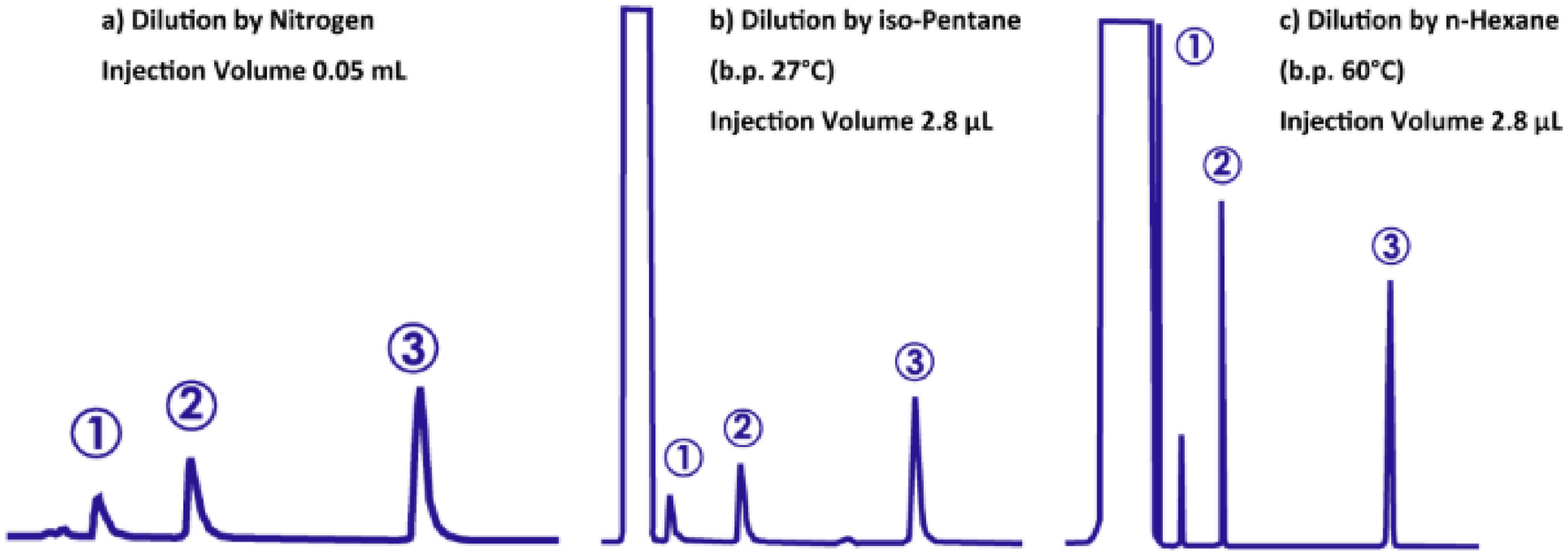
https://www.chromedia.org/chromedia?waxtrapp=obwgrDsHqnOxmOlIEcCbCoFtFeC&subNav=jmfweDsHqnOxmOlIEcCbCoFtFeCfC

Split vs Splitless injector



For the experimental conditions, which method is more sensitive? which injection method was more adapted?

https://www.glsciences.com/technique/technique_data/gc/basics_of_gc/p3_3.html



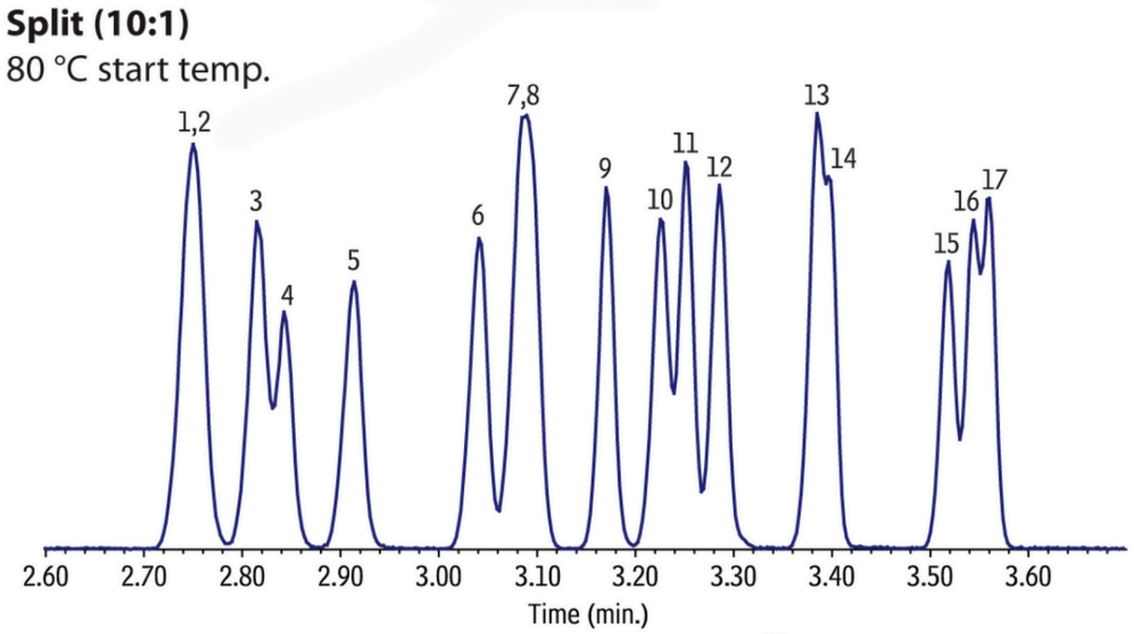
[Condition]

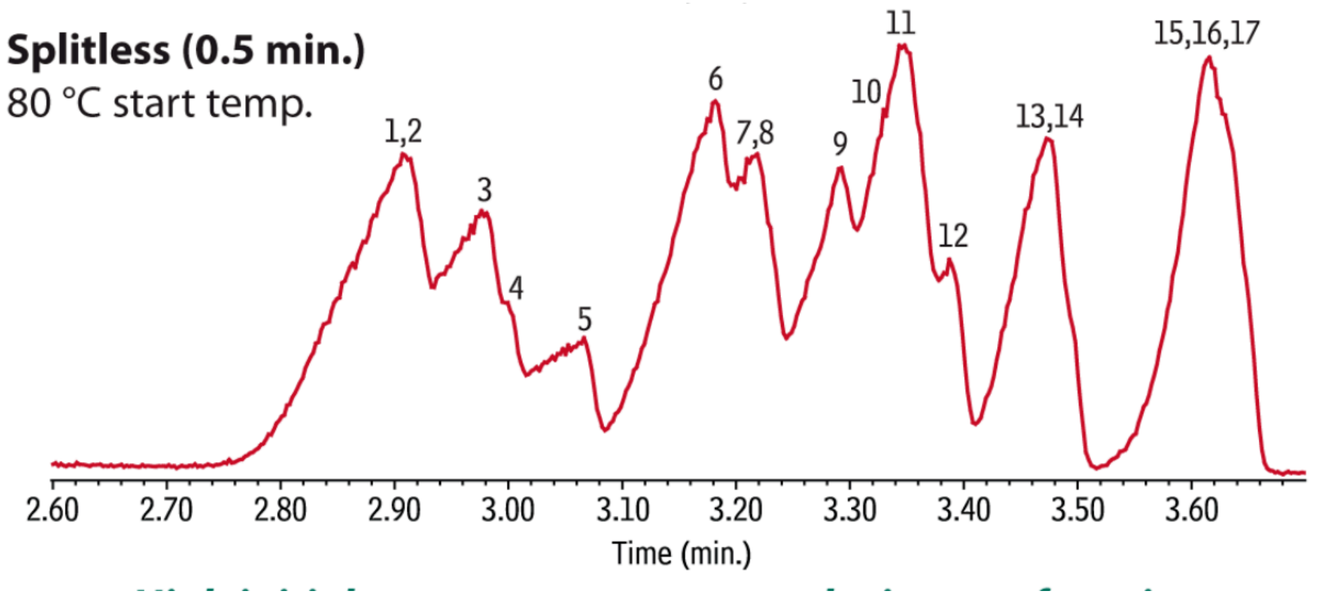
Column	: NB-1 df 0.4 μm 0.25 mm i.d. x 25m	Injection	: Splitless 1 min 200 ℃
Col. Temp.	: 40 ℃	Detection	: FID 200 ℃
Carrier Gas	: He 200 kPa		

[Sample]

① n-Heptane
② n-Octane
③ n-Nonane

Which solvent was better to dilute in (nitrogen, iso-pentane, n-hexane)?





Which injection method showed better results for the tested experimental conditions?

Split

vs.

Splitless

https://www.youtube.com/watch?v=TaLOF_jVRno&t=274s

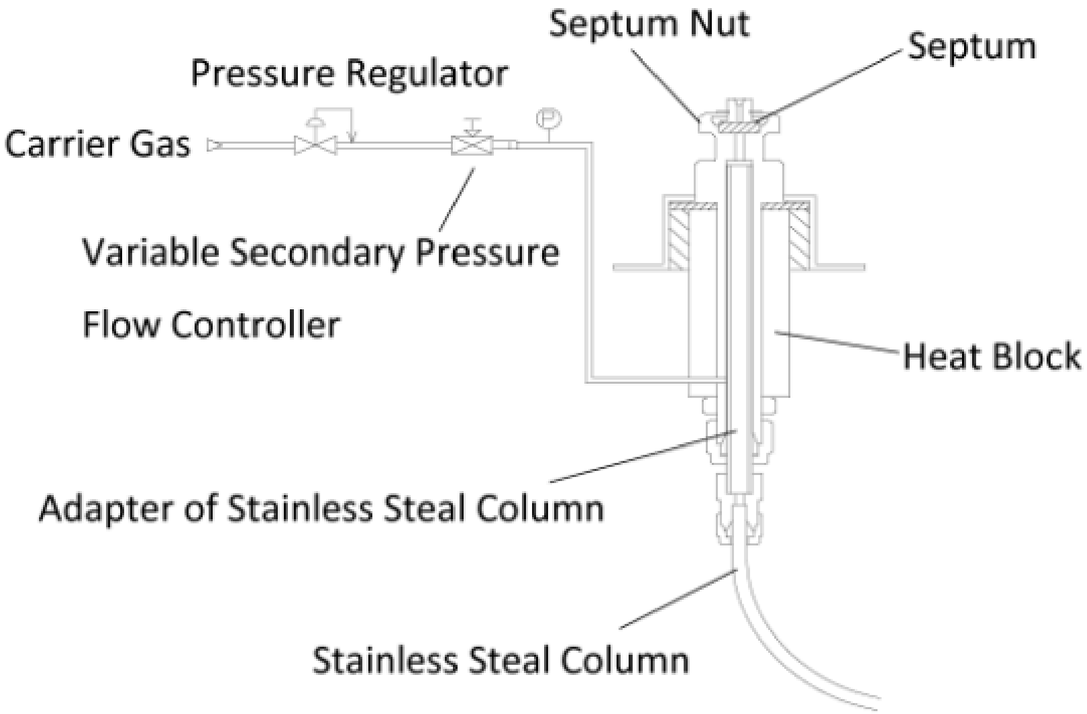
Split

- Prevents overloading of column
- Ideal for analyzing samples with high analyte concentrations (ex: solvents, environmental polluants)

Splitless

- Maximize the amount of sample introduced into the column
- High sensitivity (suitable for low concentration analytes)
- Very repeatable
- Trace-level components analysis (ex: pesticides in food)

On-column injection



- The sample is injected **directly** into the column **without vaporization** in the injector
- The tip of the microsyringe is inserted directly into the tip of the capillary column. By raising the temperature of the inlet and column, the sample is gradually vaporized directly inside the capillary column.
- A precolumn, chemically neutral (1 à 10 m) could be introduced
- **Purpose:** For **thermally sensitive** or **high-boiling compounds** that may decompose in a hot injector.
- **Advantages:** No thermal degradation of the analytes, precise introduction of the samples without losses
- **Disadvantages:** Requires careful injection to avoid overloading the column. Carefull consideration should be taken to avoid peak broadening

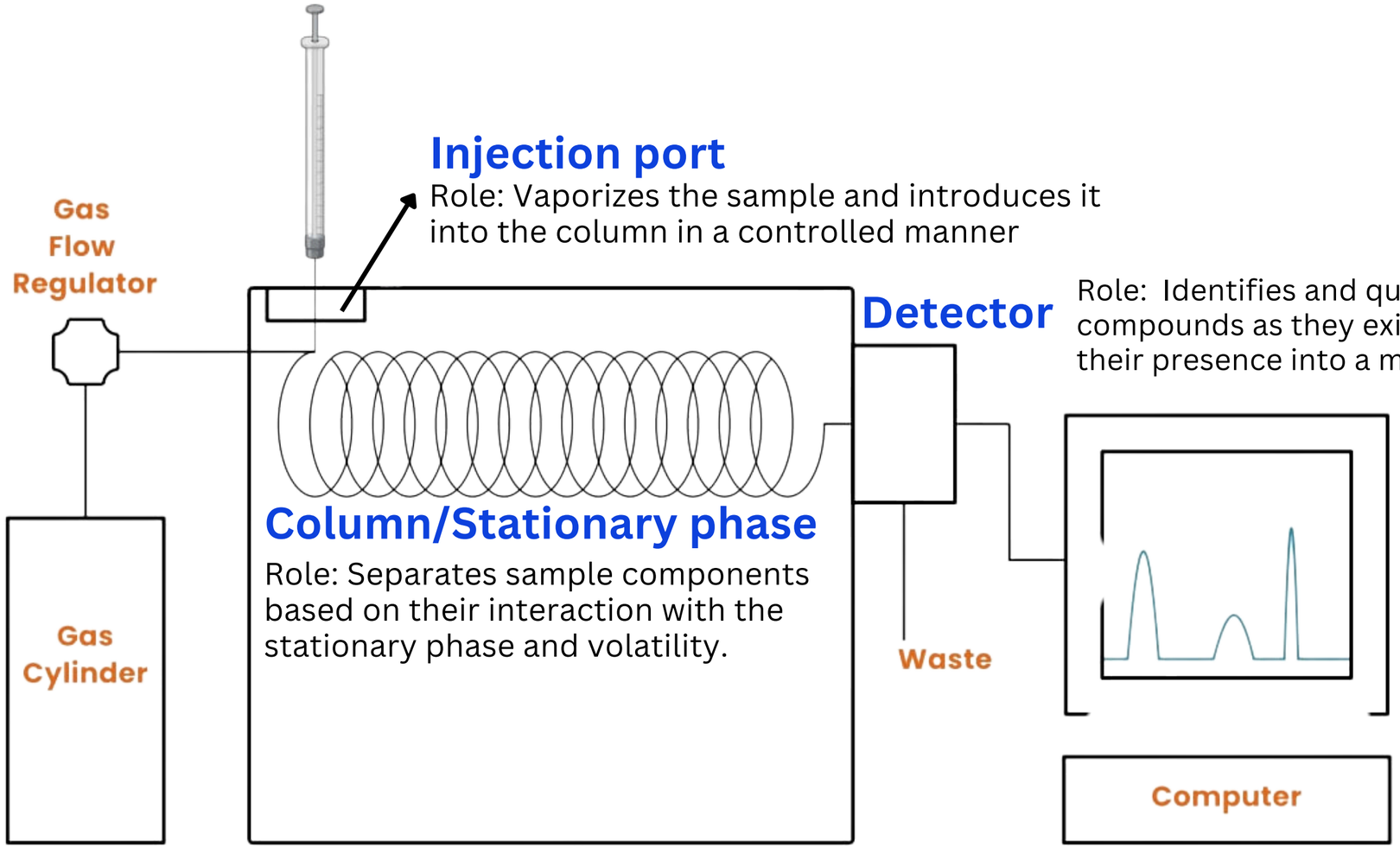
Other injection methods

- **PTV (Programmed Temperature Vaporization) Injection**
- **Headspace Injection**
- **Cold-on-column injection method**
- **....**

The injector choice in gas chromatography is **not one-size-fits-all**. It depends on the sample's characteristics (ex: thermal sensitivity, volatility), the **concentration** of analytes, and the specific analytical requirements. A well-matched injection method ensures optimal performance, accuracy, and efficiency in achieving your chromatographic goals

- **What is the sample type?**
    - Liquid or gas?
    - Volatile or non-volatile compounds?
- **What are the analytical goals?**
    - Trace-level detection or analysis of concentrated compounds?
    - High sensitivity or routine analysis?
- **What is the thermal stability of the sample?**
    - Will the analytes degrade at high injector temperatures?
    - Should a cold injection method be considered?
- **What is the expected concentration range?**
    - High concentration (split injection)?
    - Trace-level (splitless injection)?
- **Does the sample have a complex matrix?**
    - Would headspace injection help avoid matrix interferences?

GC instrumentation



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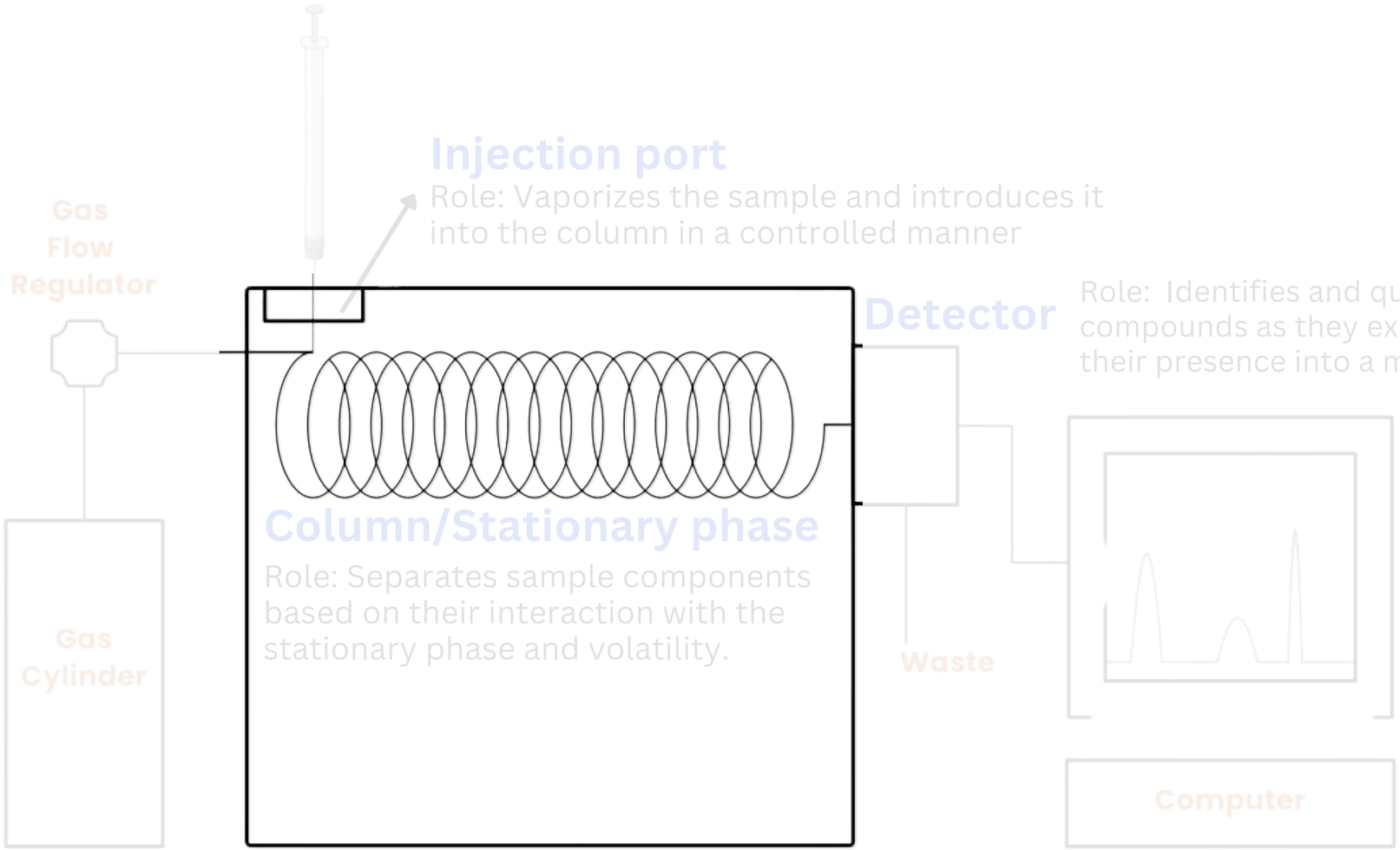

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Oven

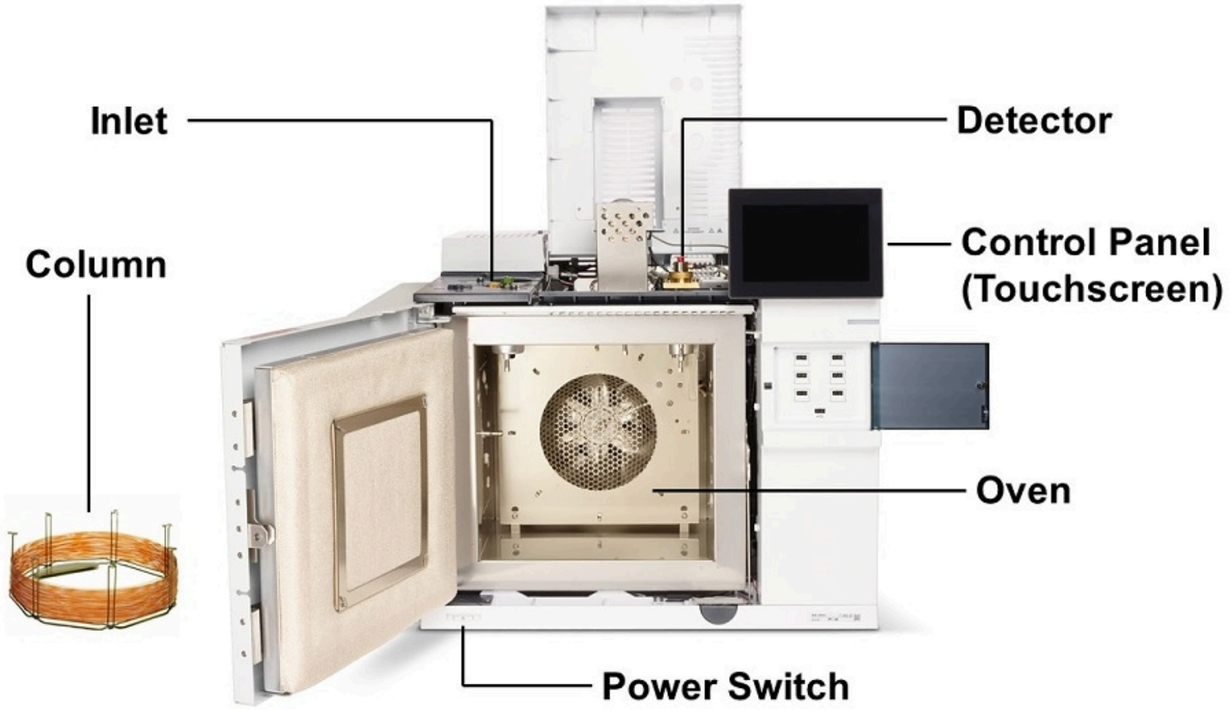
Role: Temperature control

Isothermal operation

- Constant temperature
- Used for solutes with similar retention
- Peak width increase with retention

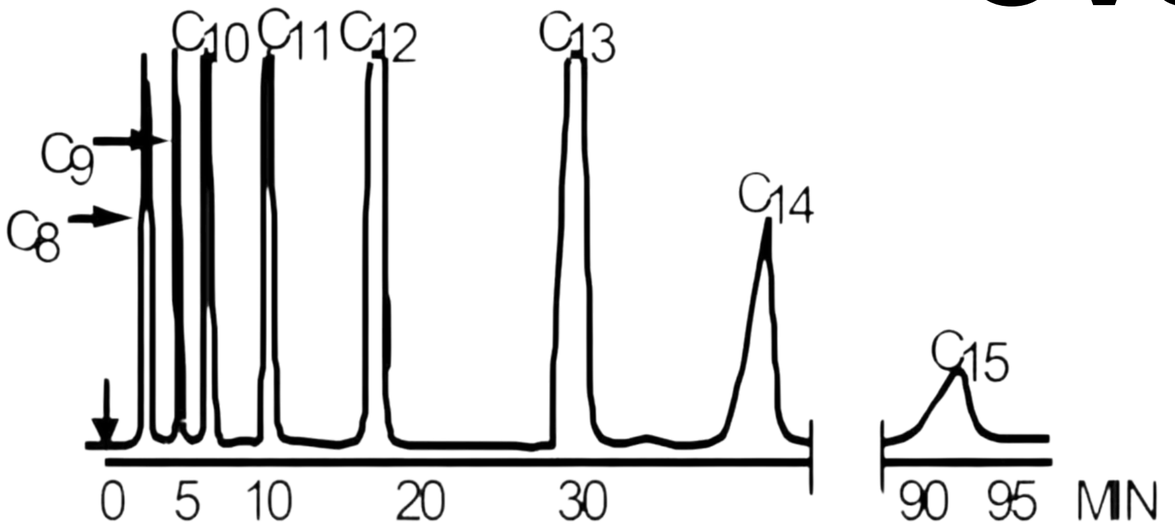
Temperature programming

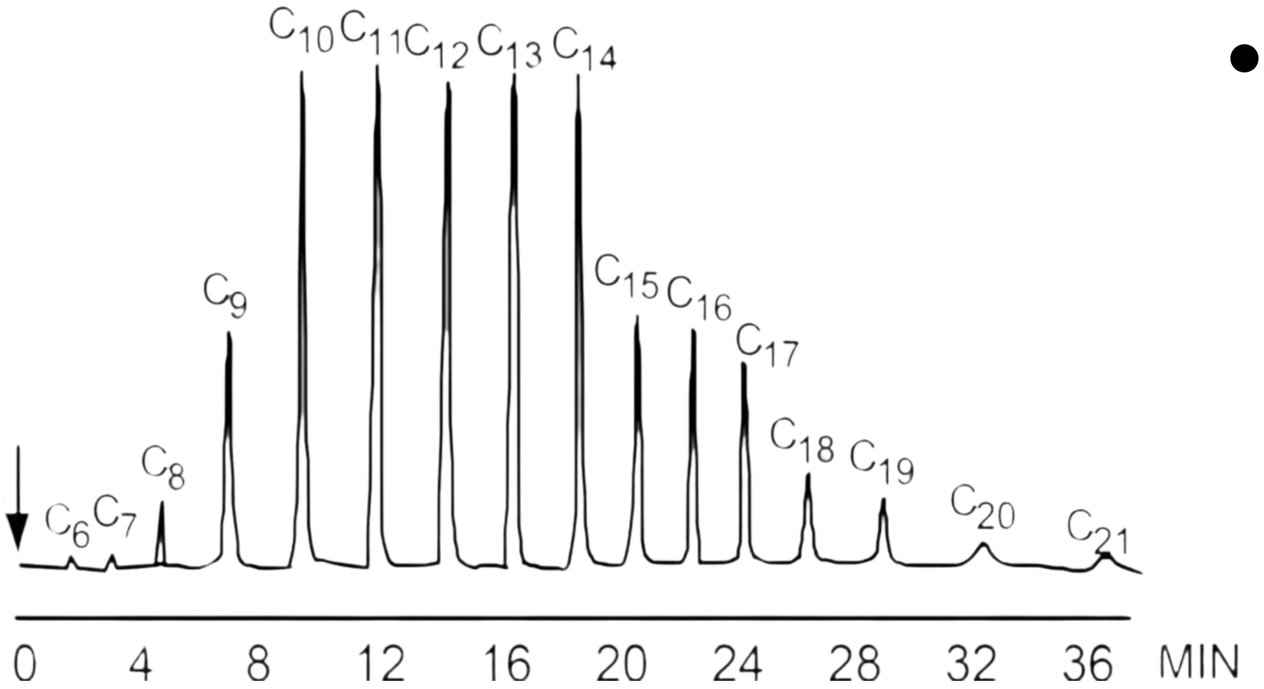
- Temperature increase continuously or in steps
- higher boiling point constituents come off after low boiling point ones with reasonable resolution and time and good peak shapes



https://www.agilent.com/en/product/gas-chromatography/what-is-gas-chromatography

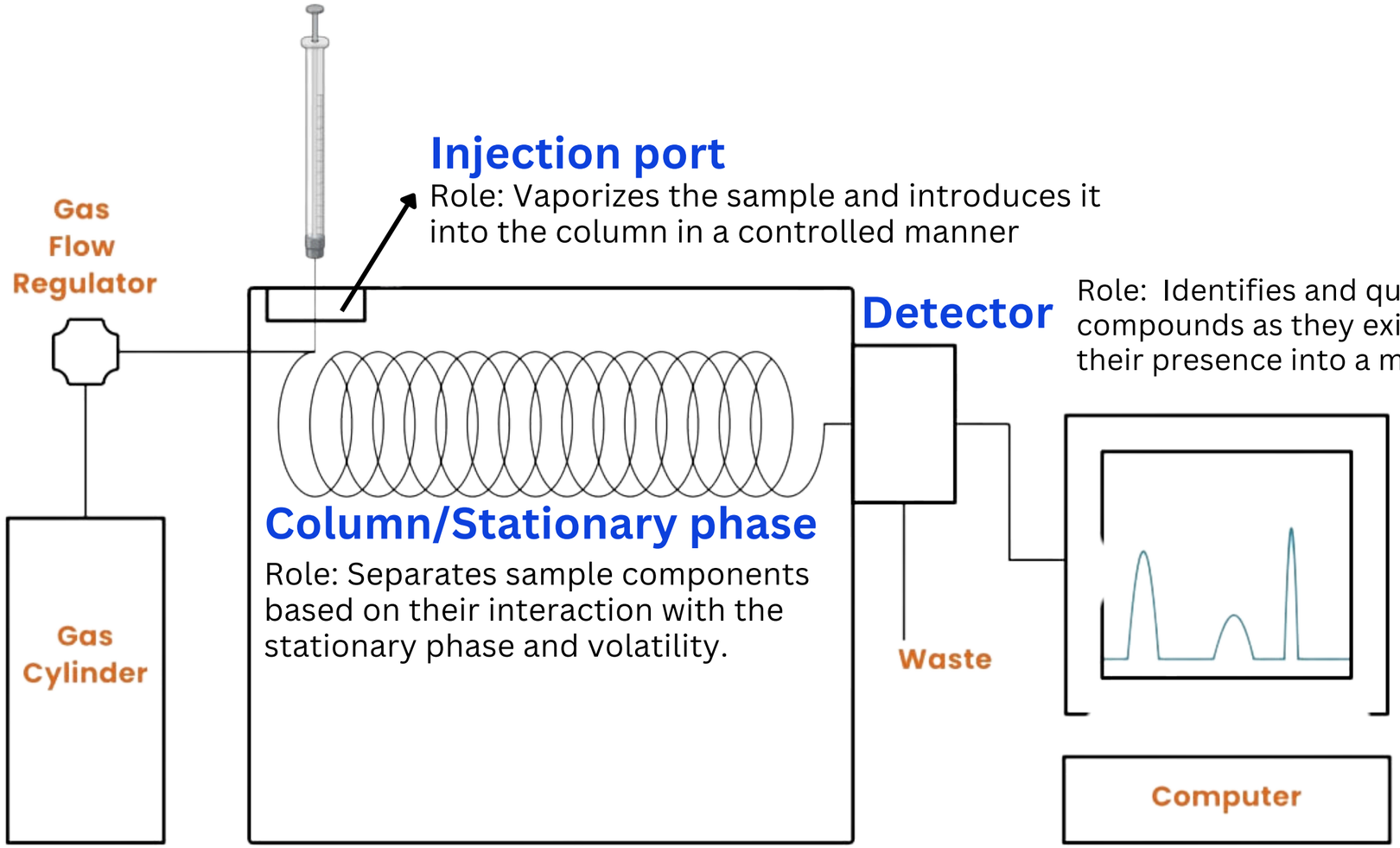
Oven





- **Which method is isothermal?**
- **What are the advantages of programmed temperature?**

GC instrumentation



Injection port

Role: Vaporizes the sample and introduces it into the column in a controlled manner

Detector

Role: Identifies and quantifies separated compounds as they exit the column by converting their presence into a measurable signal.

Column/Stationary phase

Role: Separates sample components based on their interaction with the stationary phase and volatility.

Carrier Gas

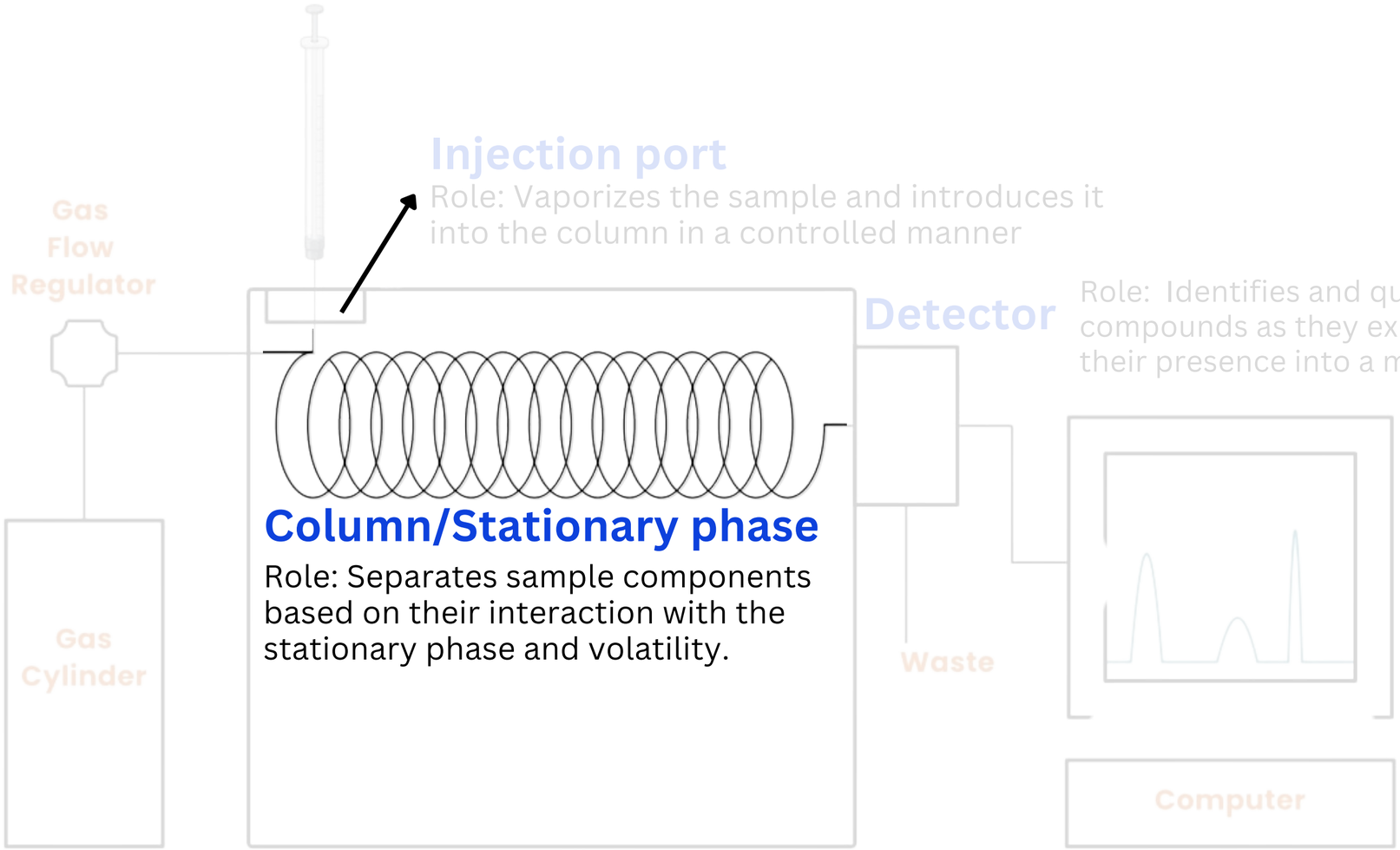
Role: Carry the sample through the column as the mobile phase without interacting chemically.

Oven

Role: Maintains precise temperature control to optimize separation and elution of compounds.

https://bitesizebio.com/28687/carrying-gas-chromatography/

GC instrumentation



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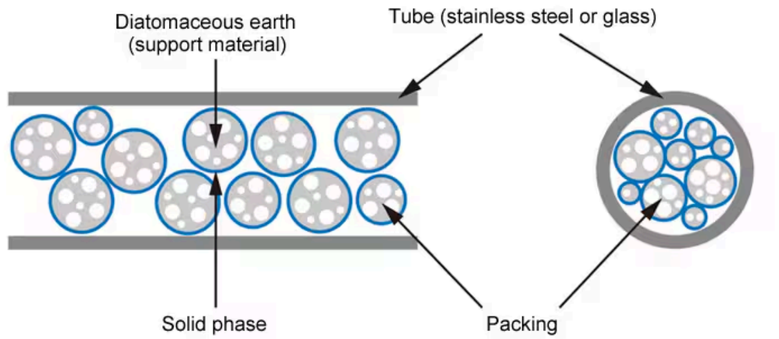
Column

Separation occurs based on differences in partitioning behavior (for gas-liquid chromatography) or adsorption (for gas-solid chromatography

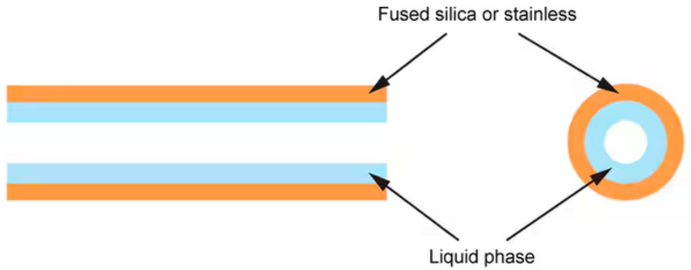
Types of columns

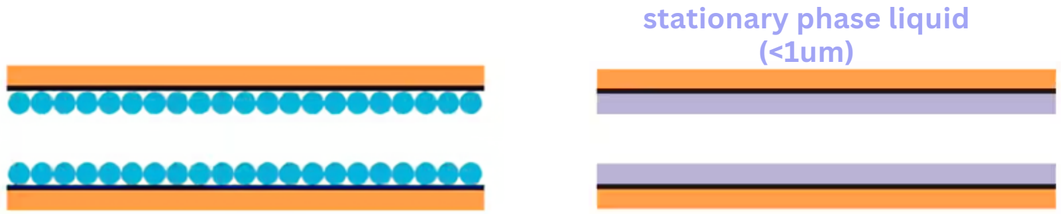
1.Packed



- **Shorter (0.5-5 m) and wider (2-4 mm) than capillary columns**
- **Used for simpler analyses or when large sample volumes are needed**

2.Open





Porous Layer Open Tubular column

Wall coated open tubular

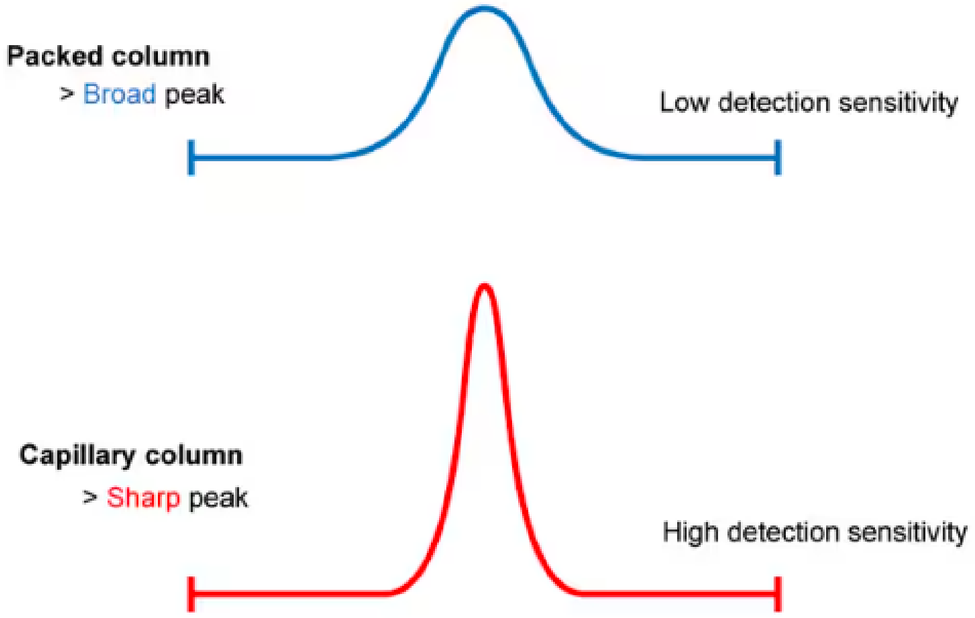
- **Narrow diameter (ex: 0.25 mm) and longer lengths (up to 60 m)**
- **Offer high resolution and better separation efficiency**

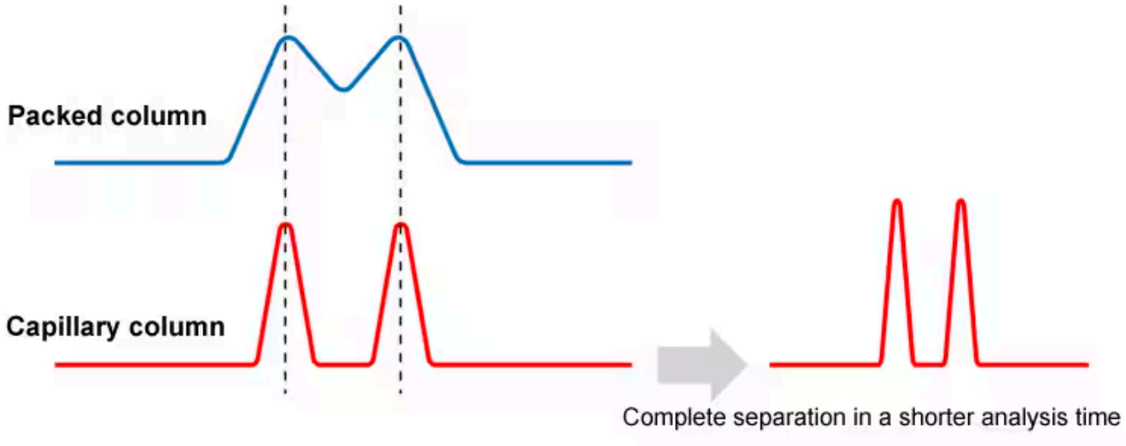
Immobilized "liquid" stationary phases are oils that have a low volatility and a high decomposition temperature. These are chemically inert so they do not react at high temperatures and are chemically attached to the support.

Stationary phases are covalently bonded groups which are bonded directly to the column.

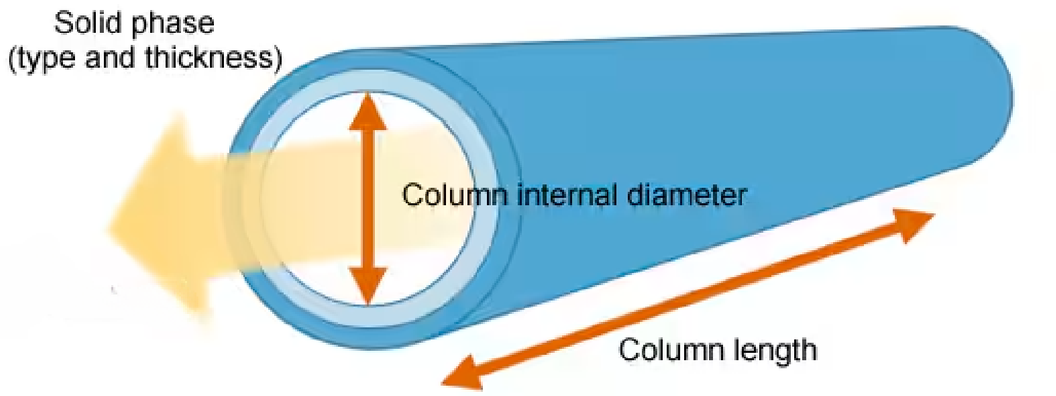
https://ueache.weebly.com/gas-chromatography.html

Column

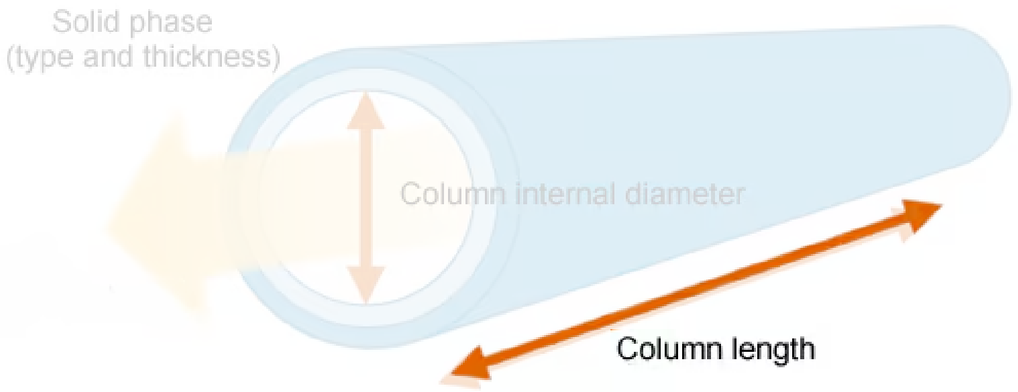

Packed column
> Broad peak
Low detection sensitivity
Capillary column
> Sharp peak
High detection sensitivity

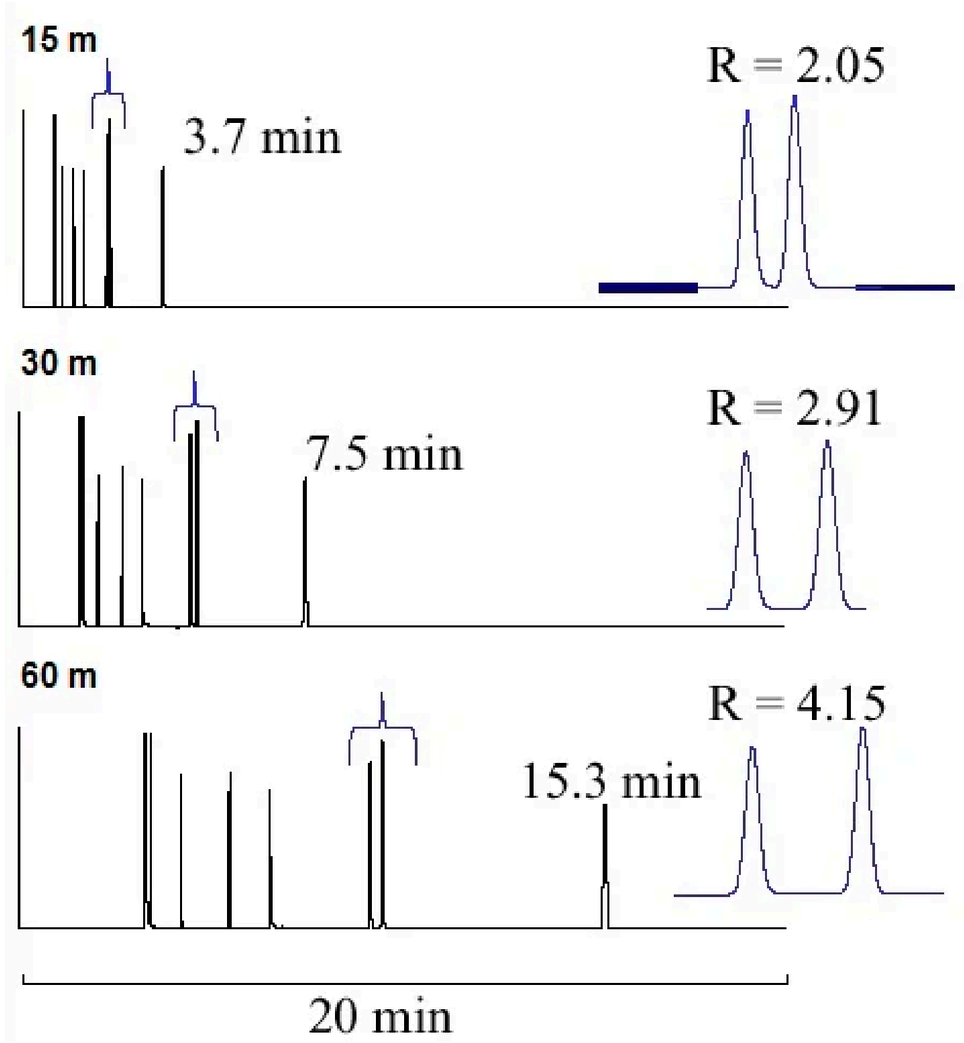

Packed column
Capillary column
Complete separation in a shorter analysis time

Column: elements affecting separation



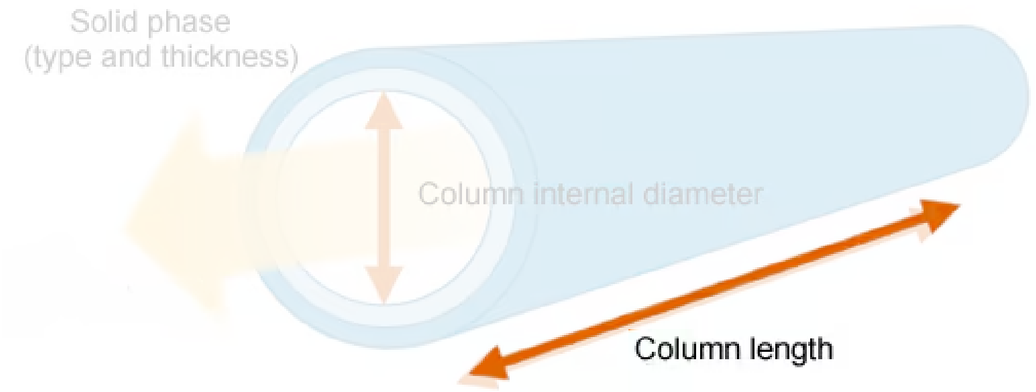
Column: elements affecting separation

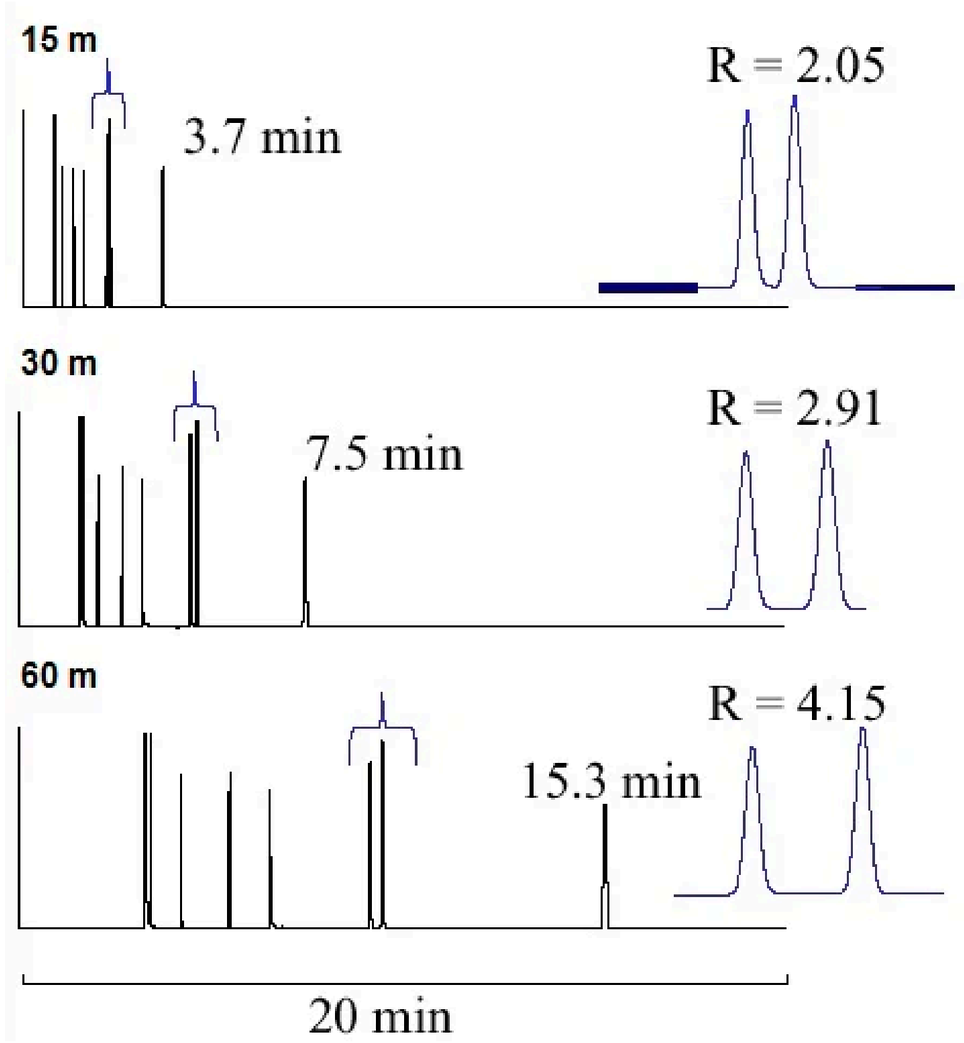




What is the effect of the column length?

Column: elements affecting separation

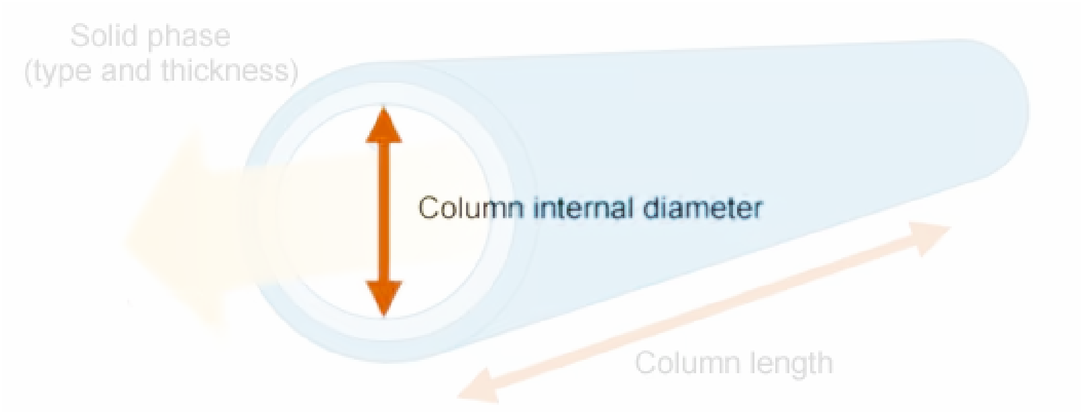




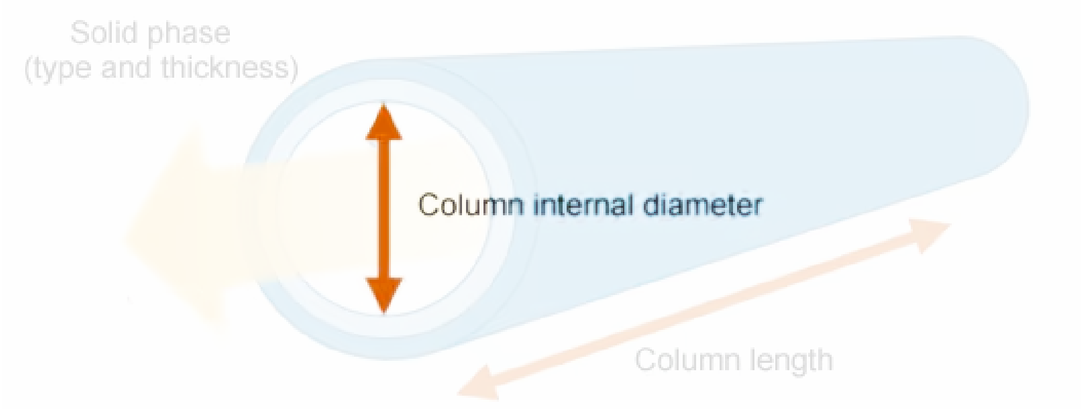
Longer columns provide higher efficiency and better separation of complex mixtures

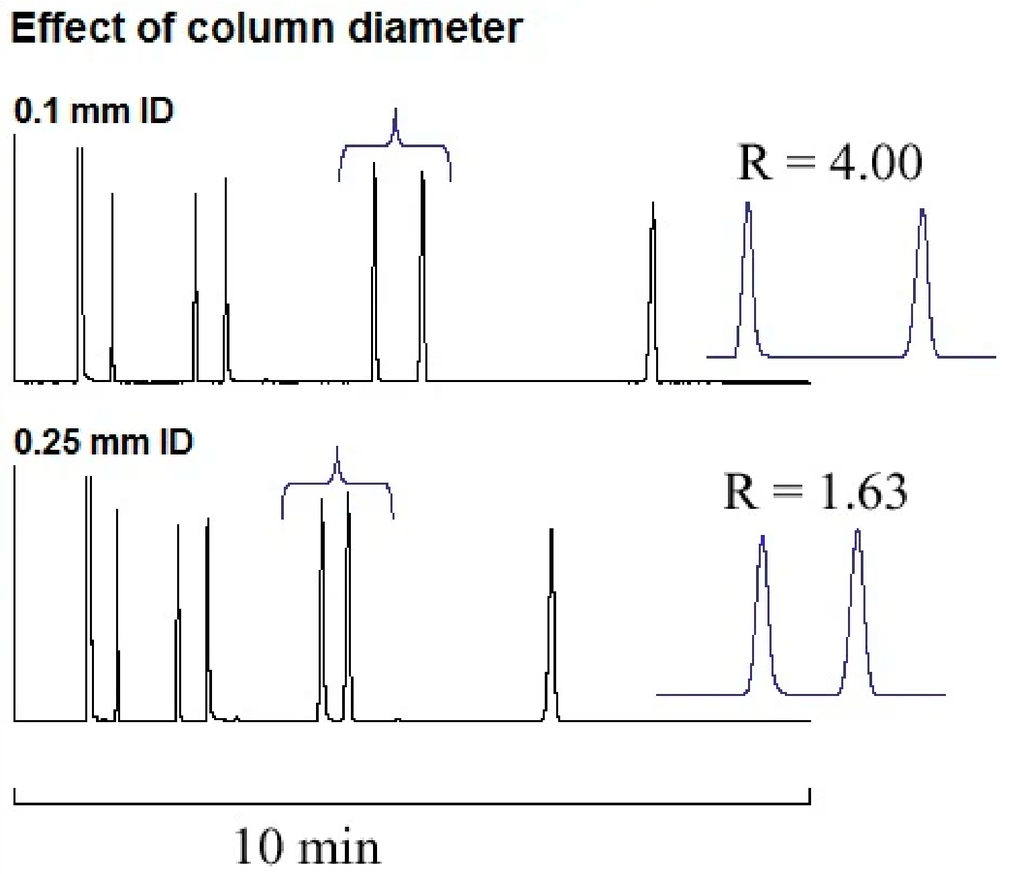
Shorter columns are faster but may compromise resolution

Column: elements affecting separation



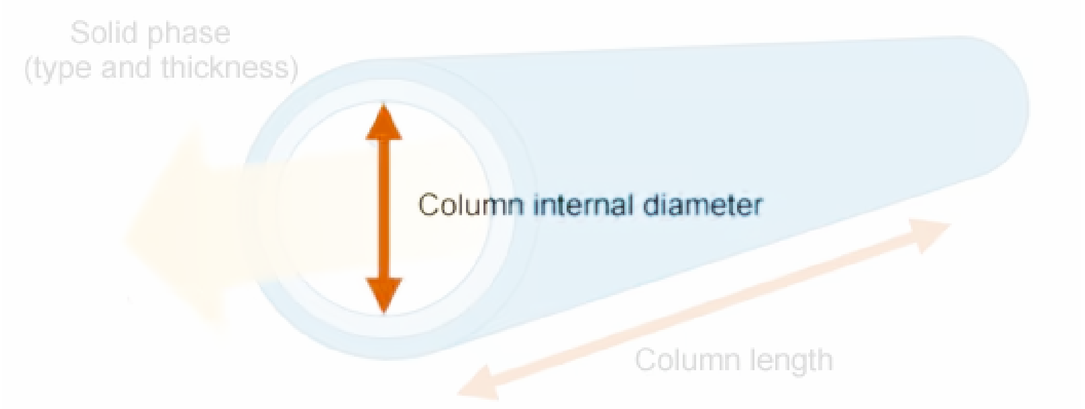
Column: elements affecting separation

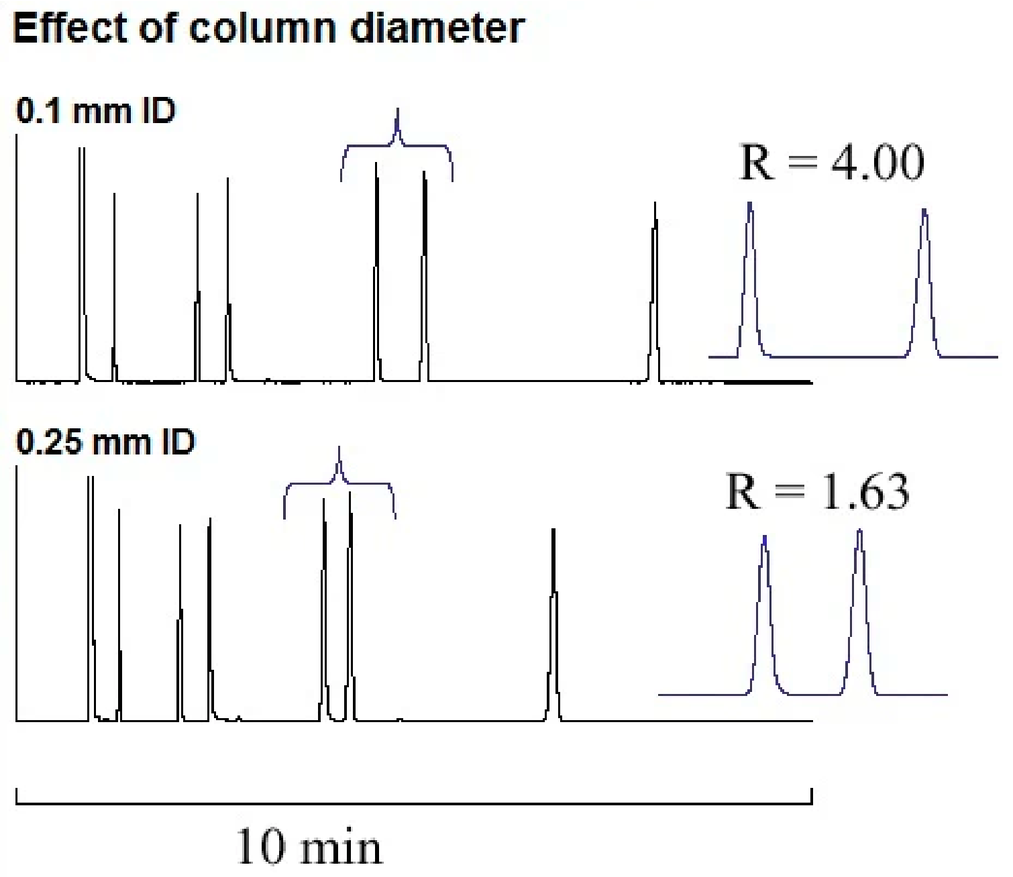




Which is better?

Column: elements affecting separation



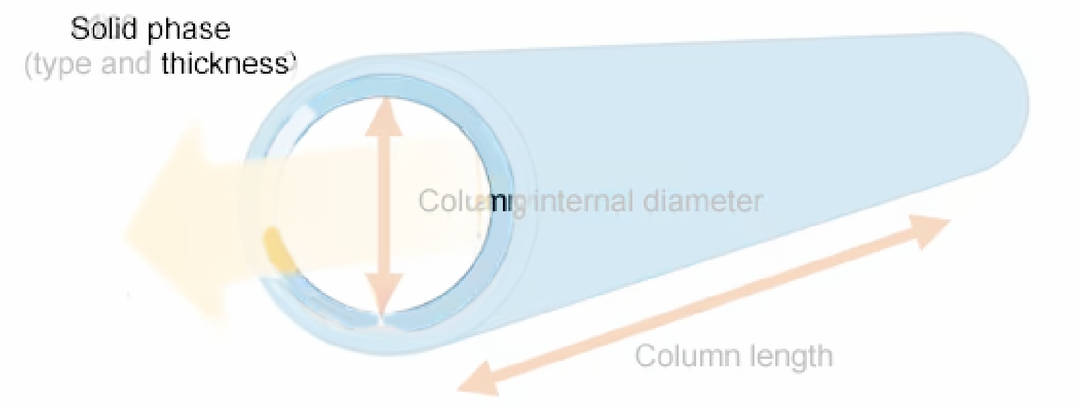


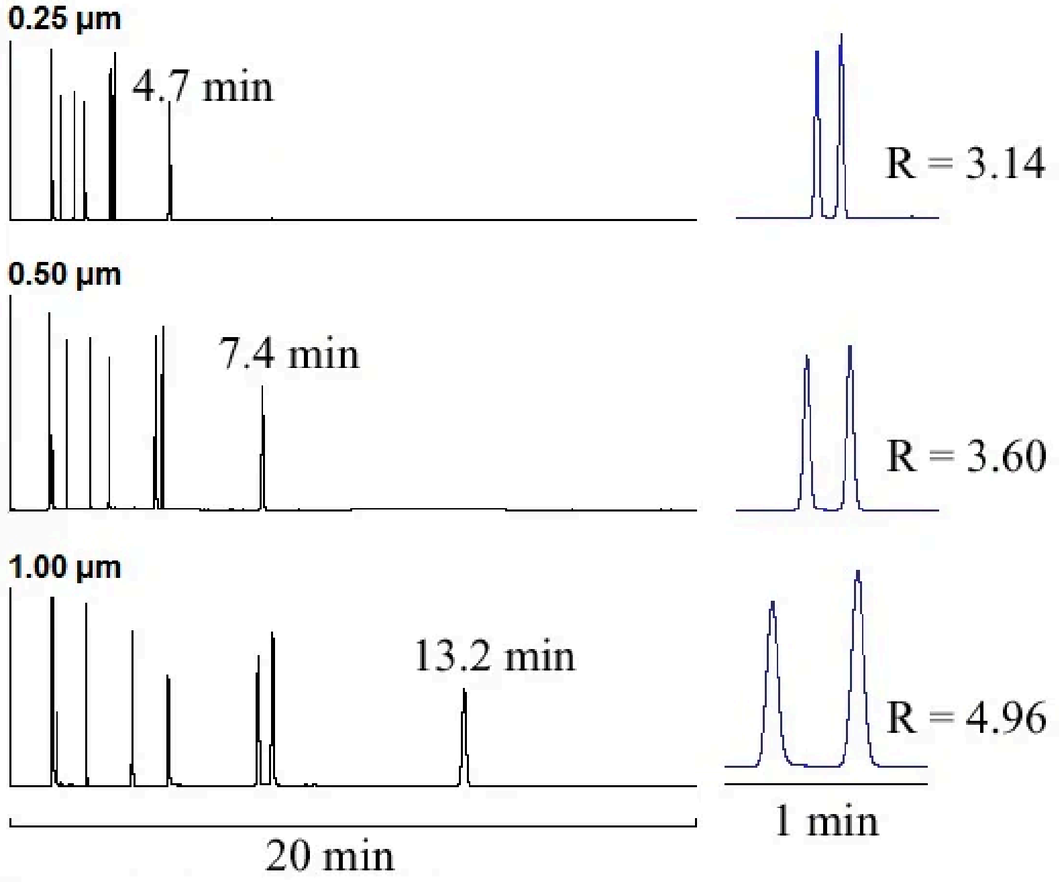
The smaller the diameter, the greater the efficiency.

Decreasing column diameter results in:

- **Faster run times for a given resolution**
- **Increased efficiency**
- **Decreased capacity**

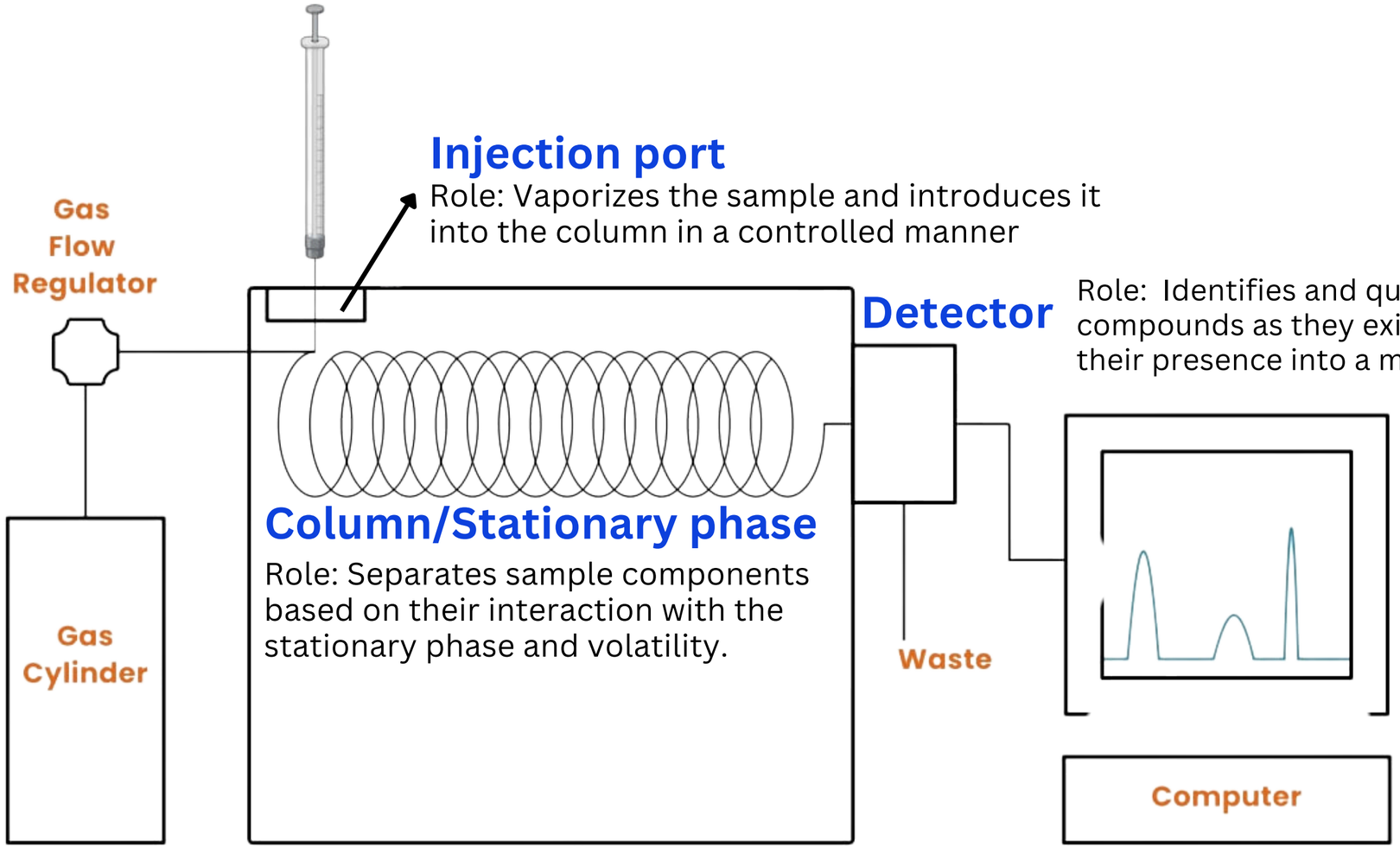
Column: elements affecting separation





- **Thicker films retain volatile compounds longer, improving separation.**
- **Thin films are better for separating high-boiling compounds.**

GC instrumentation



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Detector

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Column/Stationary phase

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Carrier Gas

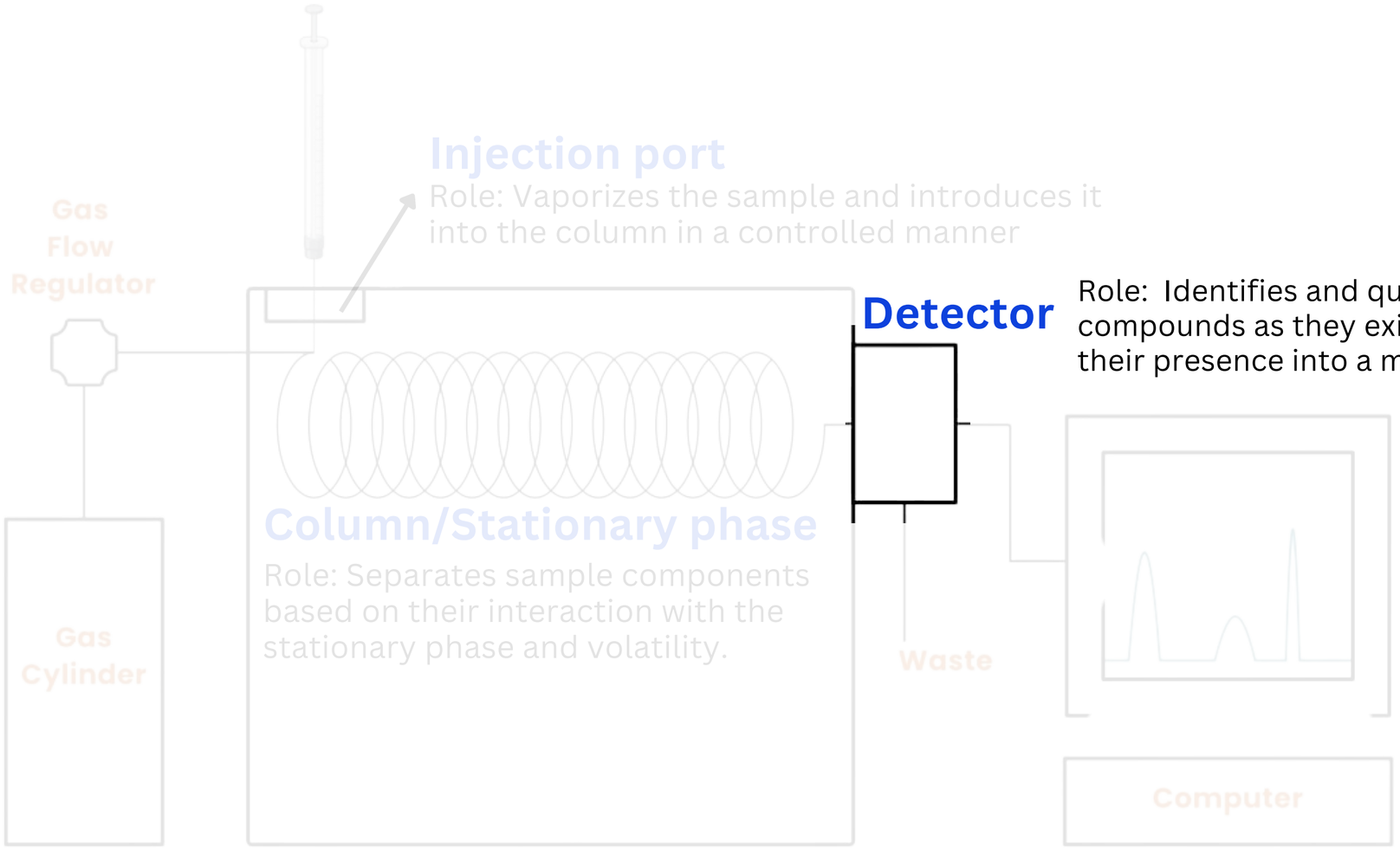
Role: Carry the sample through the column as the mobile phase without interacting chemically.

Oven

Role: Maintains precise temperature control to optimize separation and elution of compounds.

https://bitesizebio.com/28687/carrying-gas-chromatography/

GC instrumentation



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Detector

Table 7: Typical gas chromatography detectors and their detection limits.

Type of Detector	Applicable Samples	Detection Limit
Mass Spectrometer (MS)	Tunable for any sample	.25 to 100 pg
Flame Ionization (FID)	Hydrocarbons	1 pg/s
Thermal Conductivity (TCD)	Universal	500 pg/ml
Electron-Capture (ECD)	Halogenated hydrocarbons	5 fg/s
Atomic Emission (AED)	Element-selective	1 pg
Chemiluminescence (CS)	Oxidizing reagent	Dark current of PMT
Photoionization (PID)	Vapor and gaseous Compounds	.002 to .02 µg/L

https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Supplemental_Modules_(Analytical_Chemistry)/Instrumentation_and_Analysis/Chromatography/Gas_Chromatography

Detector

Detectors have variable sensitivity and selectivity

For trace elements analysis we use high sensitivity detector (MS, ECD..)
Some detectors are selective for specific elements or compound types (ex: NPD for nitrogen)

Detector choice depends on application

Universal detectors are versatile but lack selectivity
Specialized detectors provide precise results for specific analysis

Detector choice depends on Analytes

FID for hydrocarbons, ECD for halogenated compounds, MS for complex unknowns

Conclusion

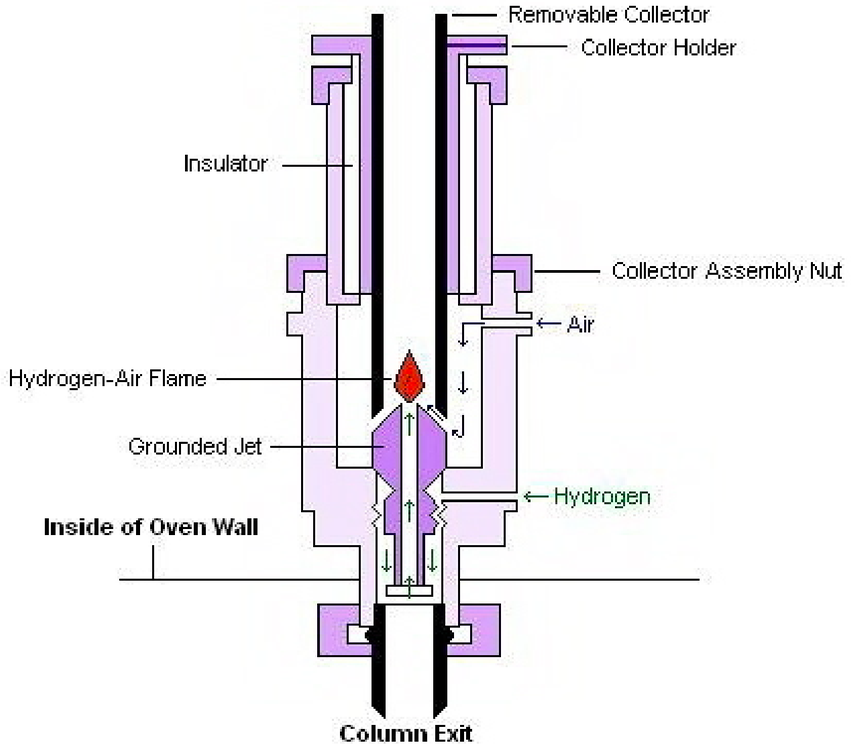
- Gas Chromatography is an important tool for separating, identifying, and quantifying volatile and semi-volatile compounds across various industries including pharmaceuticals, environment, and forensics.

- Each part of the GC system (carrier gas, injector, oven, column, detector) plays a unique role in achieving accurate and efficient analysis. Optimizing these components ensures high resolution, sensitivity, and reproducibility.

- The choice of column, injector, and detector depends on the specific properties of the sample and the goals of the analysis.

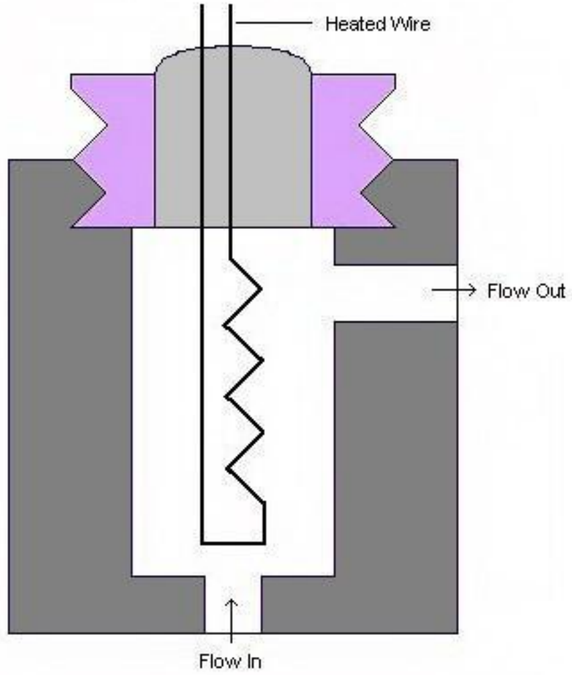
Annex

Flame ionization detector



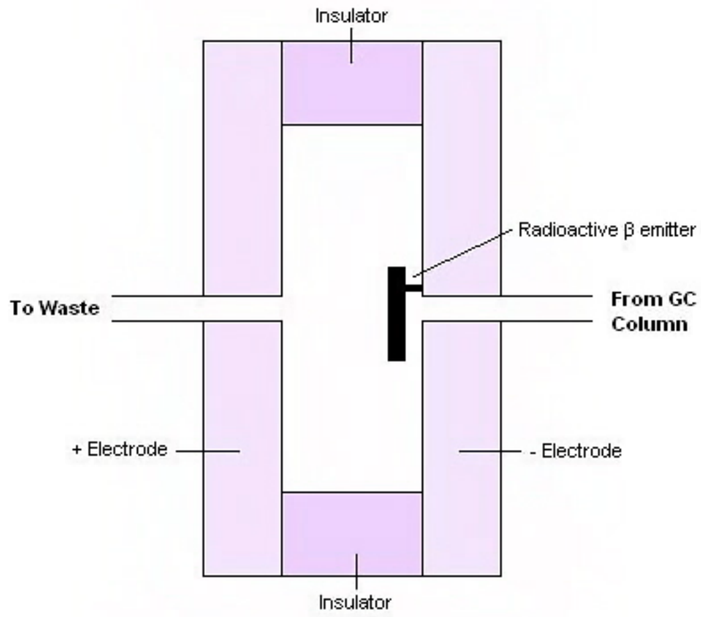
- **Detection Mechanism:** Detects organic compounds by ionizing them in a hydrogen-air flame; measures the resulting current.
- **Key Strengths:** High sensitivity for hydrocarbons; wide dynamic range; simple and reliable.
- **Limitations:** Cannot detect inorganic gases or compounds lacking carbon. Destructive technique.

Thermal conductivity detector



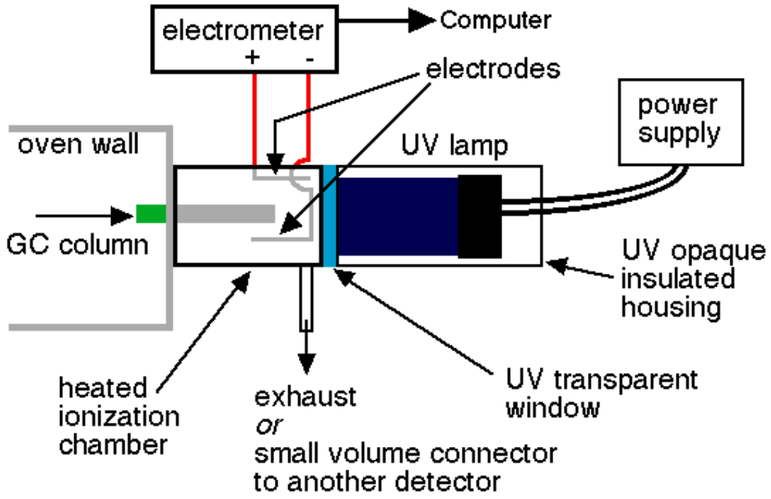
- **Detection Mechanism:** Measures changes in thermal conductivity between the carrier gas and sample gases.
- **Key Strengths:** Universal detector for both organic and inorganic compounds. Non-destructive and relatively simple
- **Limitations:** Lower sensitivity compared to other detectors (ex: FID, ECD).

Electron capture detector



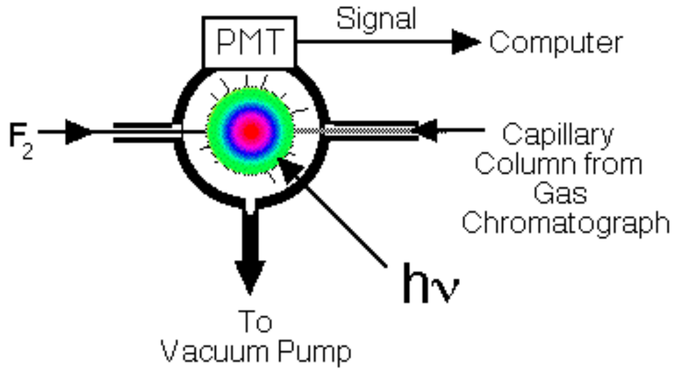
- **Detection Mechanism:** Measures the reduction of electron flow caused by electronegative compounds (ex: halogens) using a radioactive source.
- **Key Strengths:** Extremely sensitive for halogenated compounds, nitro compounds, and other electronegative analytes. Ideal for trace-level detection.
- **Limitations:** Limited to compounds capable of capturing electrons. Requires handling of a radioactive source.

Photoionization detector



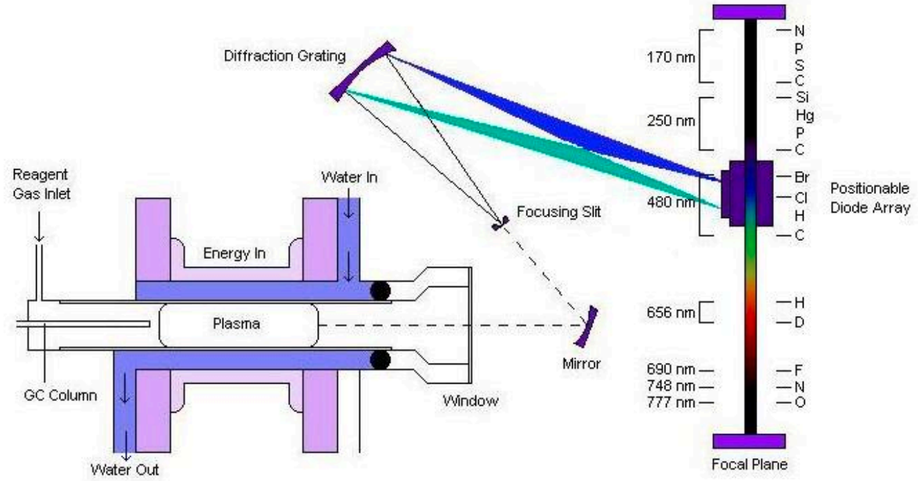
- **Detection Mechanism:** Uses UV light to ionize compounds with low ionization energy, generating an electrical signal.
- **Key Strengths:** High sensitivity for aromatic hydrocarbons and volatile organic compounds (VOCs). Non-destructive.
- **Limitations:** Ineffective for compounds with high ionization energies. Requires UV lamp maintenance.

Chemiluminescence detector



- **Detection Mechanism:** Detects light emitted from a chemical reaction, typically involving analytes like nitrogen or sulfur.
- **Key Strengths:** High sensitivity and selectivity for nitrogen- and sulfur-containing compounds. Non-destructive and robust for specific applications.
- **Limitations:** Limited to compounds that can undergo chemiluminescent reactions. Requires specific reaction conditions.

Atomic emission detector



- **Detection Mechanism:** Breaks molecules into atoms using a microwave plasma and measures the light emitted at element-specific wavelengths.
- **Key Strengths:** Element-specific detector capable of multi-element analysis. High sensitivity and versatile for many analytes.
- **Limitations:** Complex and expensive setup. Requires routine maintenance for optimal performance.

Column efficiency is dependent on:

- Flow rate/average linear velocity
- Column diameter
- Column length
- Carrier gas molecular weight

Column efficiency

$$\left(\frac{\sqrt{n}}{4}\right)$$

$$R = \left(\frac{\sqrt{n}}{4}\right)\left(\frac{k}{1+k}\right)\left(\frac{\alpha - 1}{\alpha}\right)$$

Resolution	Column efficiency	Retention	Selectivity

Selectivity

- Type of stationary phase

Selectivity

$$\left(\frac{\alpha - 1}{\alpha}\right)$$

- https://www.youtube.com/watch?v=dWsEsDikpHA
- https://www.youtube.com/watch?v=TaLOF_jVRno
- https://www.youtube.com/watch?v=iX25exzwKhI
- https://www.youtube.com/watch?v=yZf42Kk9R3I
- https://www.youtube.com/watch?v=uD-29-mV3N0