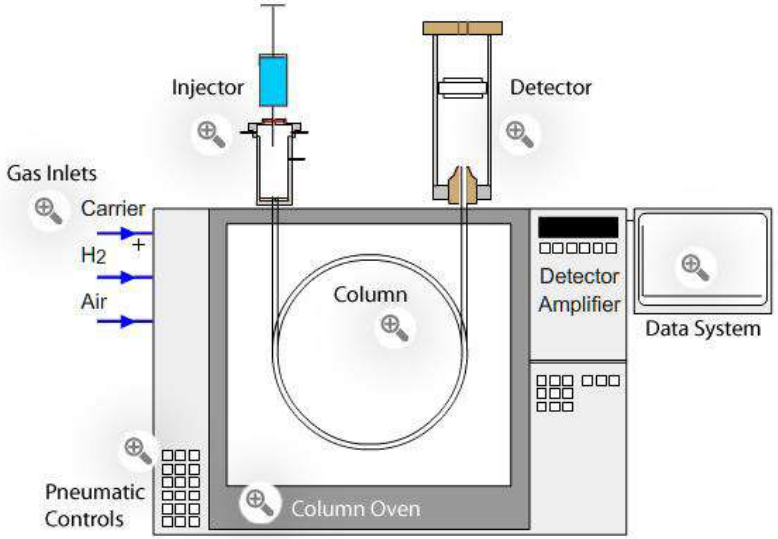
Separation techniques II.

Chromatographic methods

Gas chromatography



2023

Anita Bufa

What is gas chromatography (GC)?

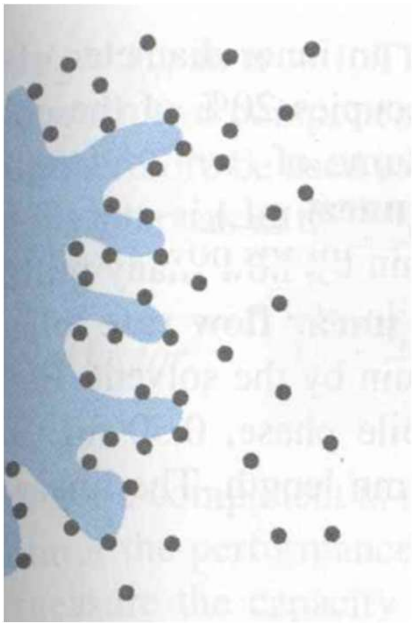
Gas chromatography is a chromatographic technique which uses a **stationary phase** and a **mobile phase**.

- **Mobile phase: gas**
- **Stationary phase: solid or liquid**

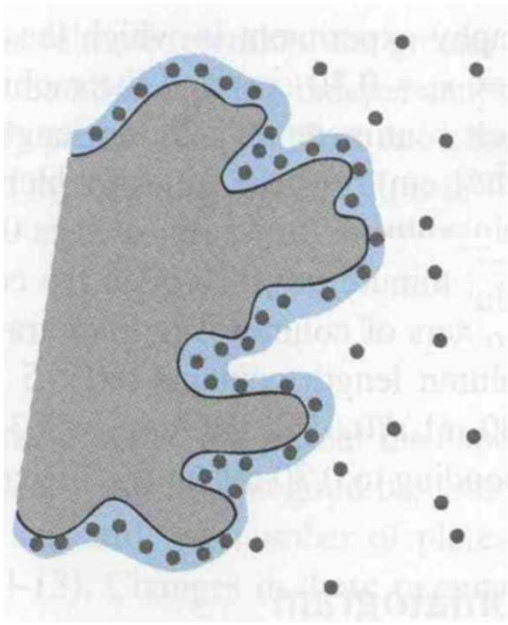
Technique of gas chromatography

Column chromatography

Mechanism of gas chromatography



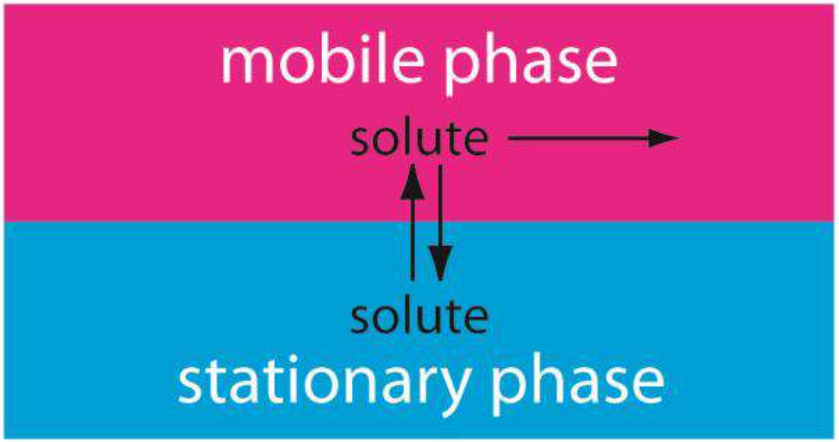
Absorption



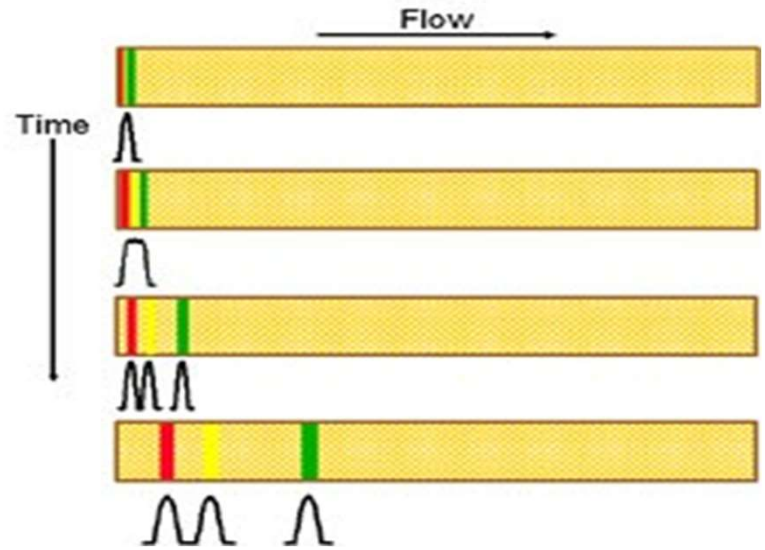
Distribution

Separation of compounds in gas chromatography

The separation is based on **partition or distribution** of the components or solutes **between two phases** (mobile and stationary phase) **in a dynamic system**.



Separation of compounds in gas chromatography



1. Injecting the sample through a **septum** into a heated **vaporizer port (liner)** - sample mixed with gas mobile phase

2. Mobile phase: through a **capillary column** separating the components based on their ability to partition between the mobile phase and the stationary phase

Separation of compounds in gas chromatography

3. Interacting with the stationary phase + reaching the **detector** in the **reverse order** of interaction strength

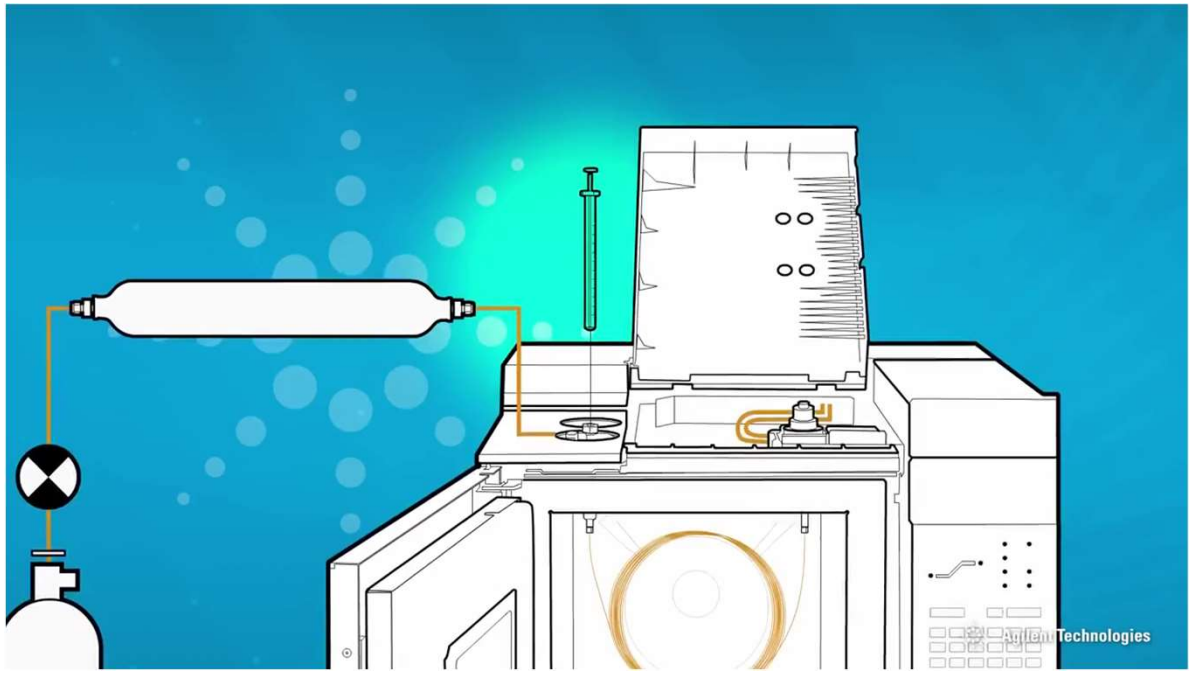
4. Detector - measuring **physical** or **chemical** properties → **qualitative** and **quantitative analysis** of the separated components

Gas chromatography

Video about the main steps of GC analysis.

Gas chromatography

Video about the basics of GC.

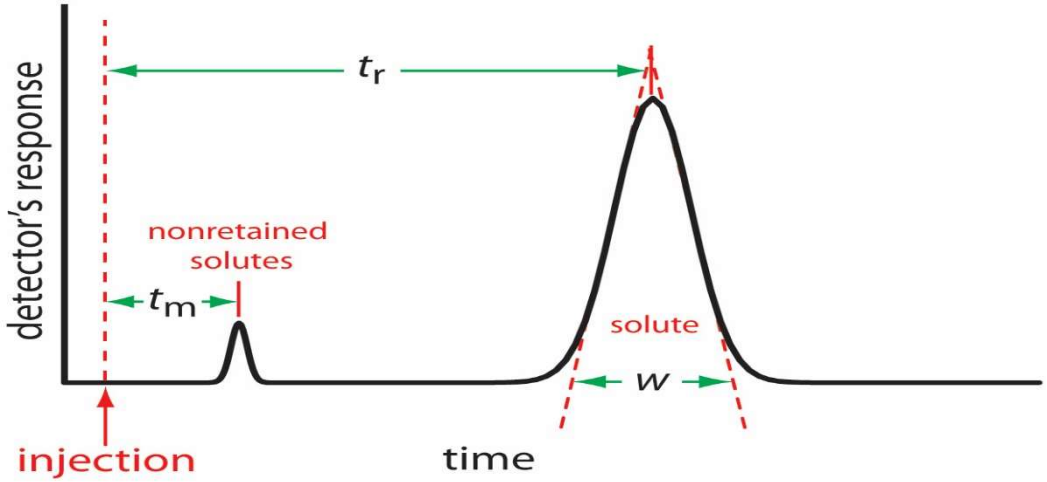


Molecular interactions:

- responsible for **absorption** and **distribution**
- **reversible**
- **weak bonds** (dispersion (68-80%), induction, H-bond)
- **polarity** is important

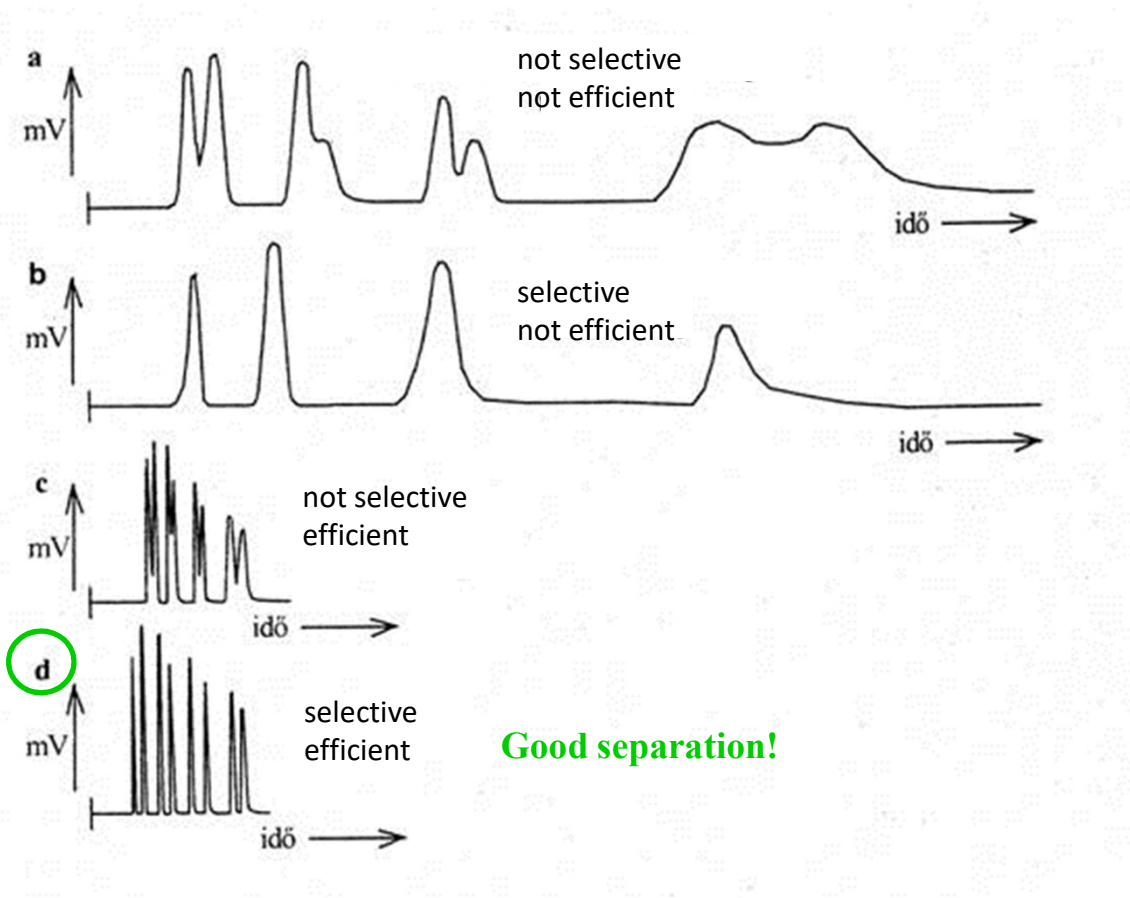
Time the components spend in the stationary phase

Chromatogram



- **Retention time**: t_r
- **Adjusted retention time**: $t_{r'}=t_s=t_r-t_m$
- **Dead time/void time**: t_m or t_0

Efficiency and selectivity



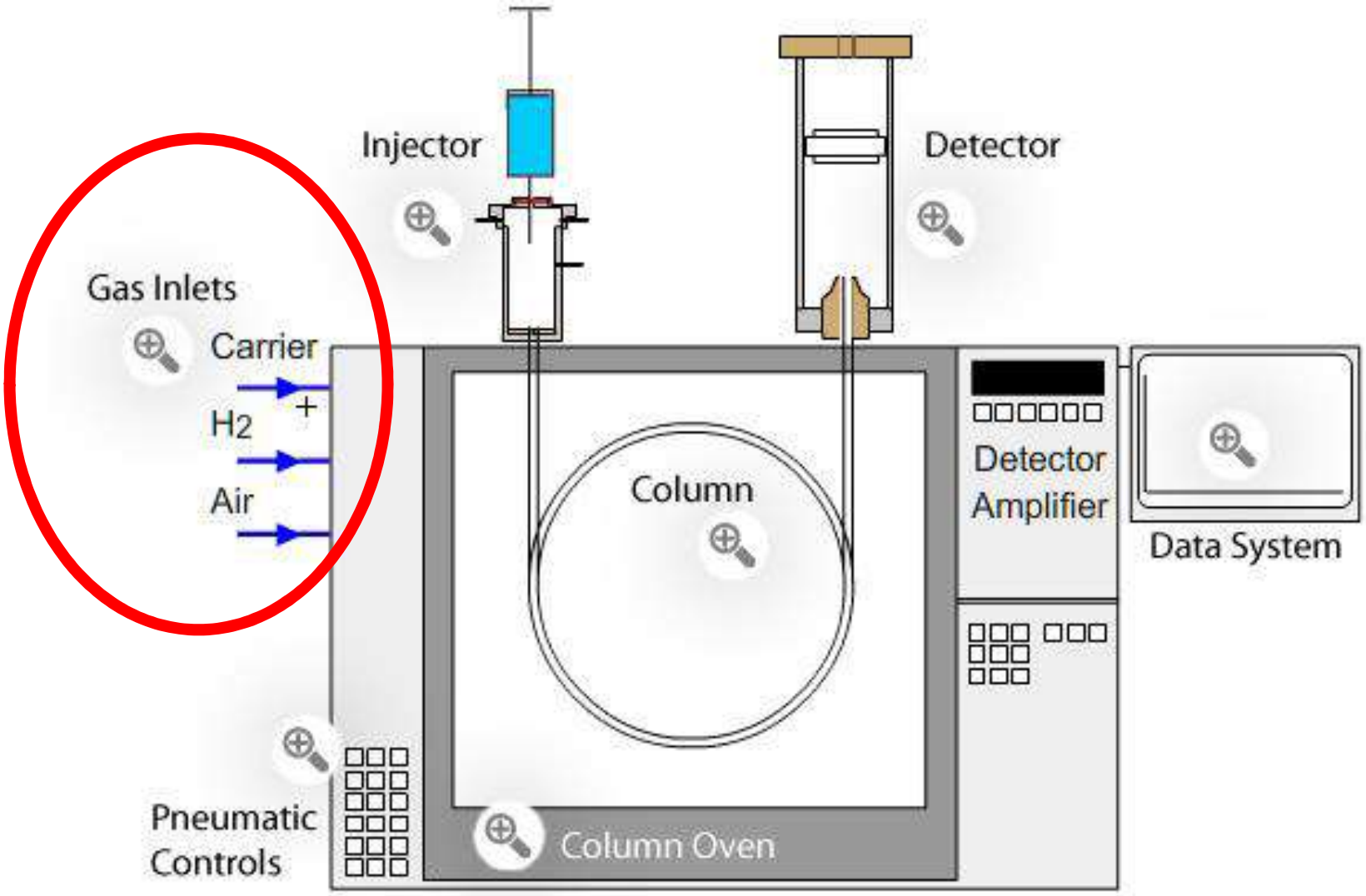
Factors affecting separation

- **length** and **internal diameter** of column
- **type** and **thickness** of stationary phase
- **type** and **flow rate** of mobile phase
- **type** of sample injection
- **volume** of sample
- **temperature**

The main parts of Gas Chromatograph

1: Mobile phase, **2:** Pressure controller, **3:** Injector, **4:** Column, **5:** Detector, **6:** Data system

1. Gas system


Injector
Detector
Gas Inlets
Carrier
+
H2
Air
Column
Detector
Amplifier
Data System
Pneumatic
Controls
Column Oven

1. Gas system: mobile phase or carrier gas and detector gases

- **Inert gaseous mobile phase**
  - H_2, He, N_2, (Ar)
  - no interaction with molecules
  - transportation of the components through the column
- **Detector gases**

- In pressurized cylinders or generators
- High purity
- Pressure regulators to control the flow rate

  Flow rate:
  - 25-150 mL/min → packed column
  - 1-5 mL/min → capillary column

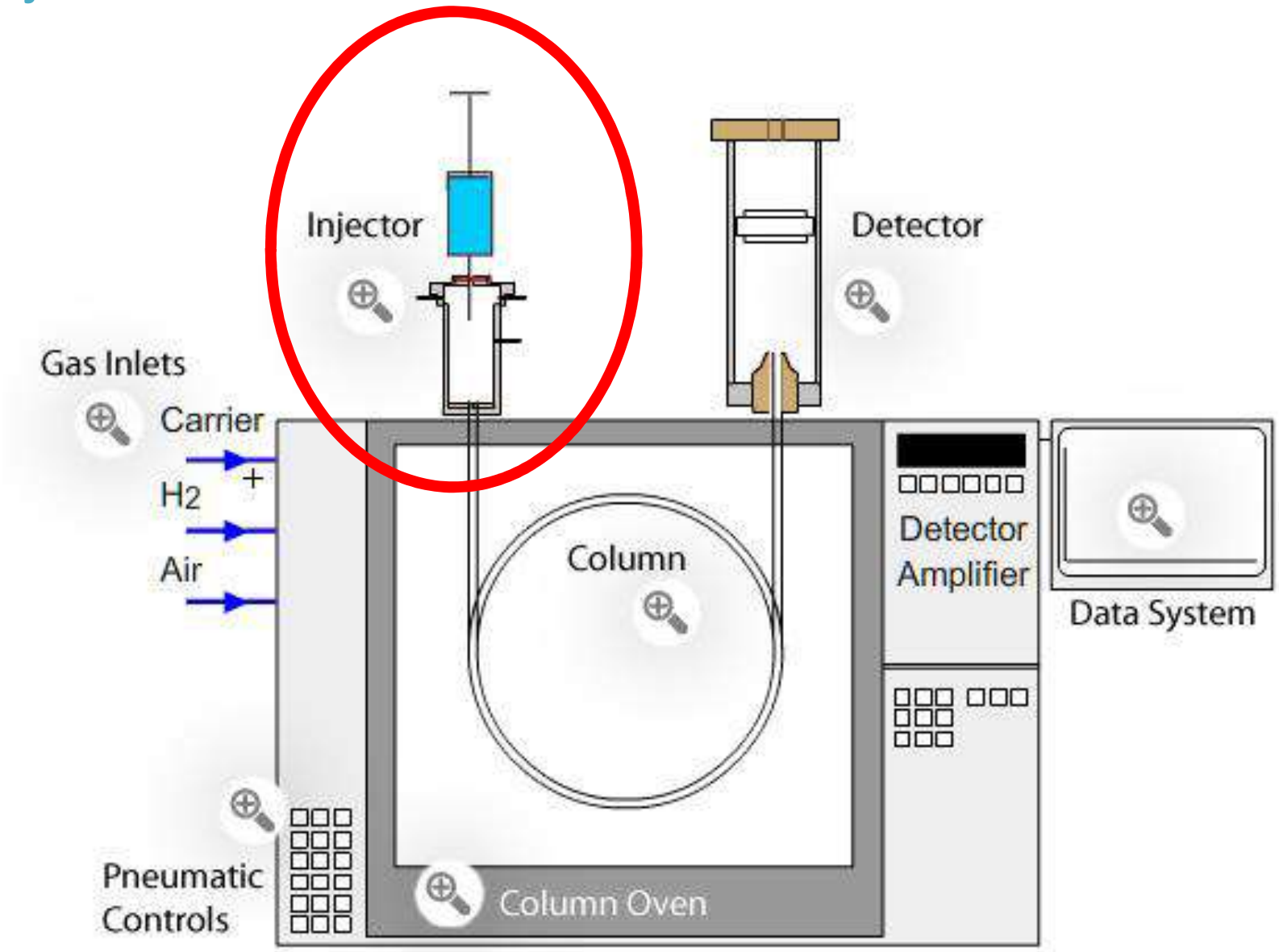
The type and flow rate of carrier gas affects the separation (retention time of components) → van Deemter equation.

2. Pressure Controller (Regulator)

1. meter: Pressure of gas cylinder (200 bar).

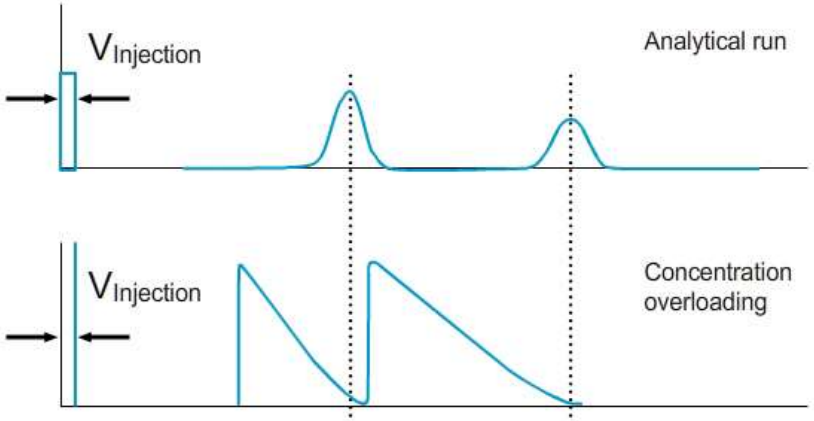
2. meter: Controlled pressure of carrier gas (5-7 bar).

3. Injector


Injector
Detector
Gas Inlets
Carrier
+
H2
Air
Column
Detector
Amplifier
Data System
Pneumatic
Controls
Column Oven

3. Injector

- it is **heated** (volatilization)
- sample injection
  - liquid (1 to 2 µL)
  - gas sample (0.2 to 5 mL)
- fast injection (plug of vapor-narrow sample band)
- small sample volume and appropriate concentration
- manual and automatic injection
- many types

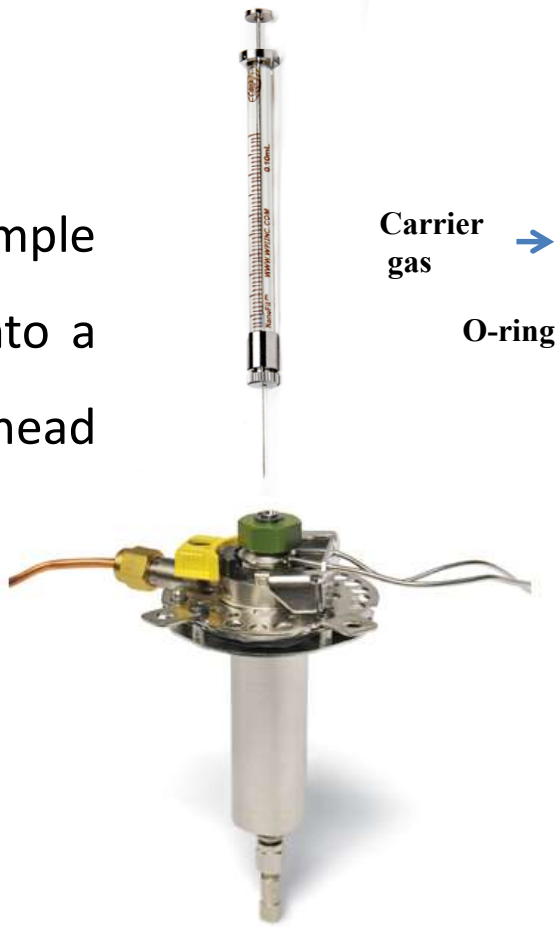


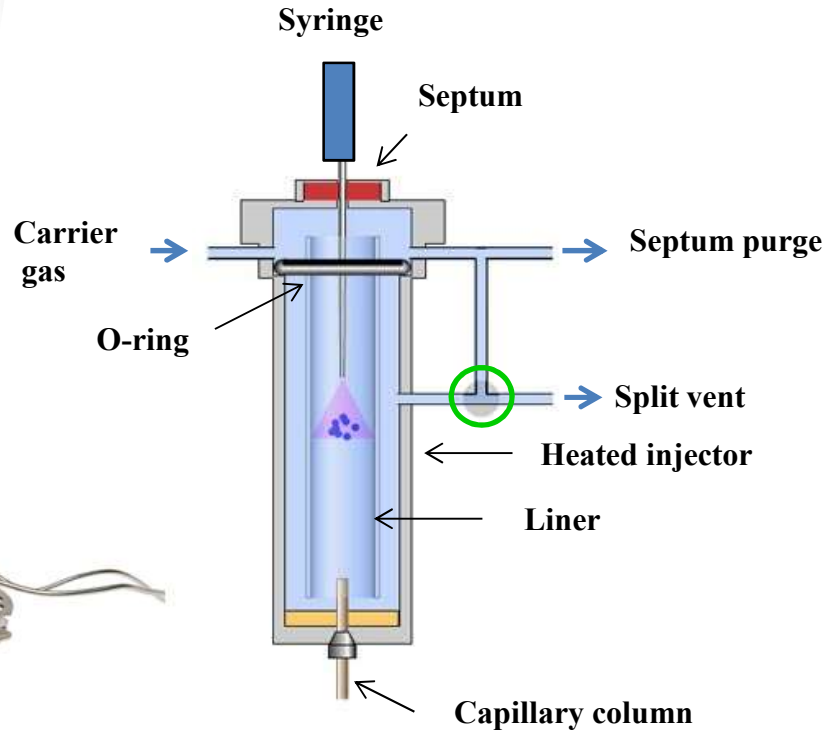
The volume of sample and concentration of the compounds affect the separation.

3. Injector

Split-splitless injector

Microsyringe to inject sample through a **rubber septum** into a flash **vaporizer port** at the head of the **column**.



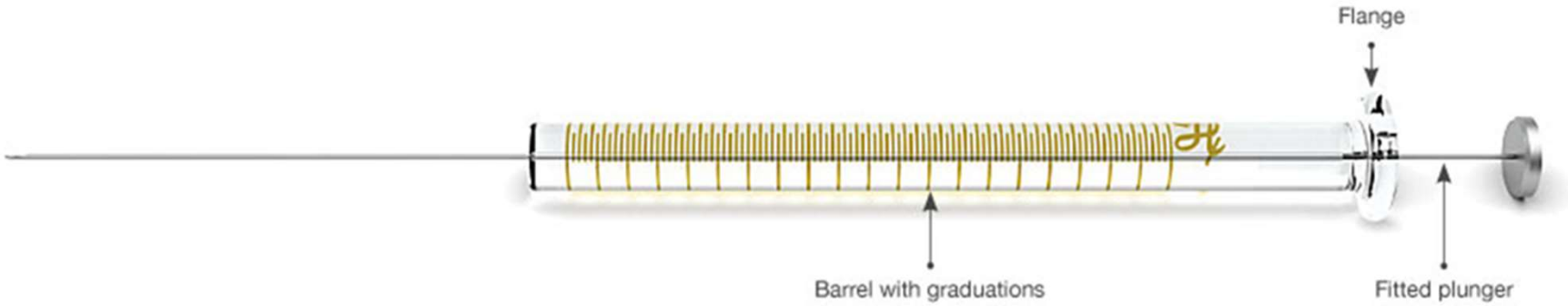


Two injection modes:

- **split mode**: the split vent is open → for high concentration sample
- **splitless mode**: the split vent is closed → for trace analysis

Injection of liquid sample

Microsyringe



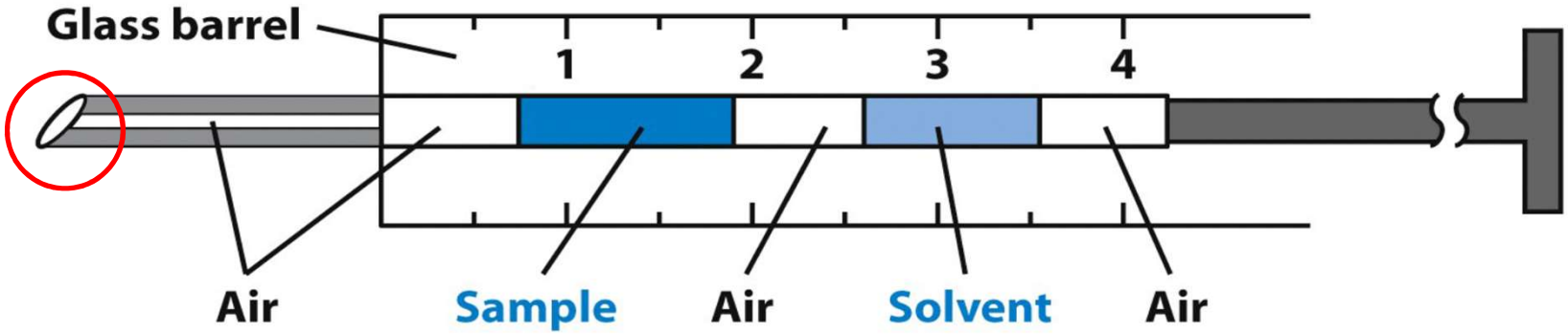
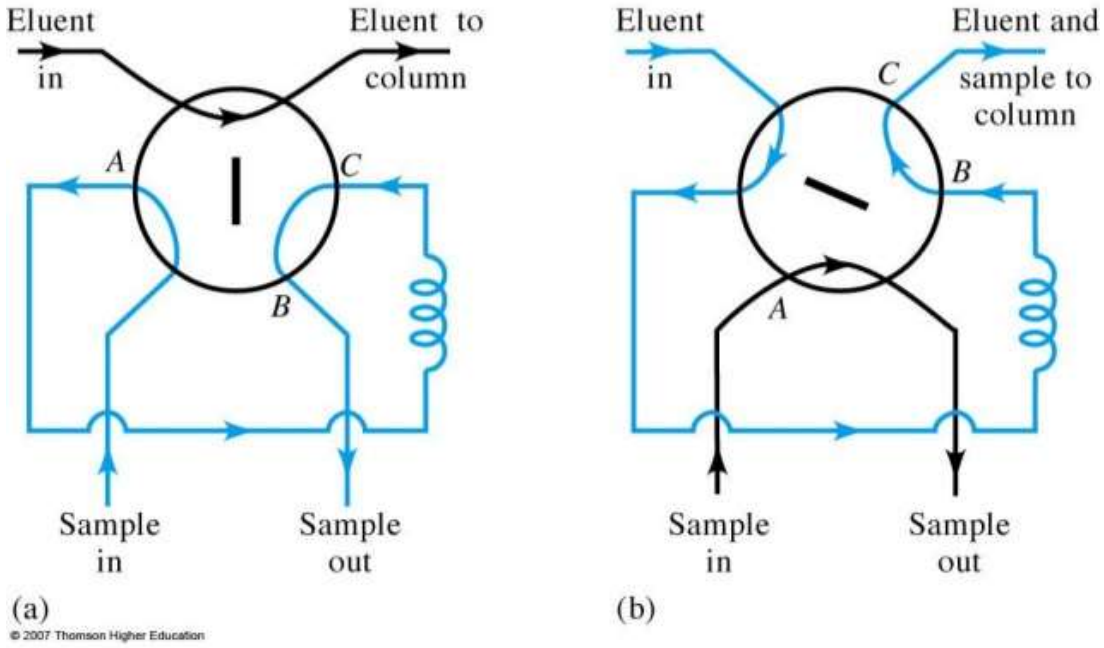


Figure 24-13
Quantitative Chemical Analysis, Seventh Edition

Injection of gas sample

Gas tight microsyringe



Loop

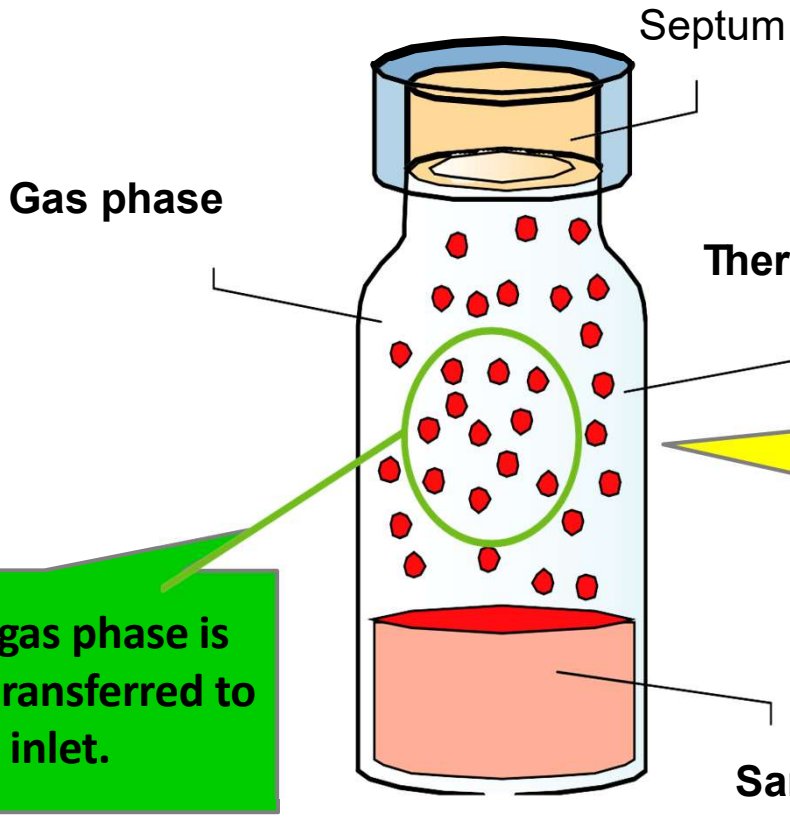
Injection of gas sample

Headspace (solvent free injection)

Video about the headspace technique.

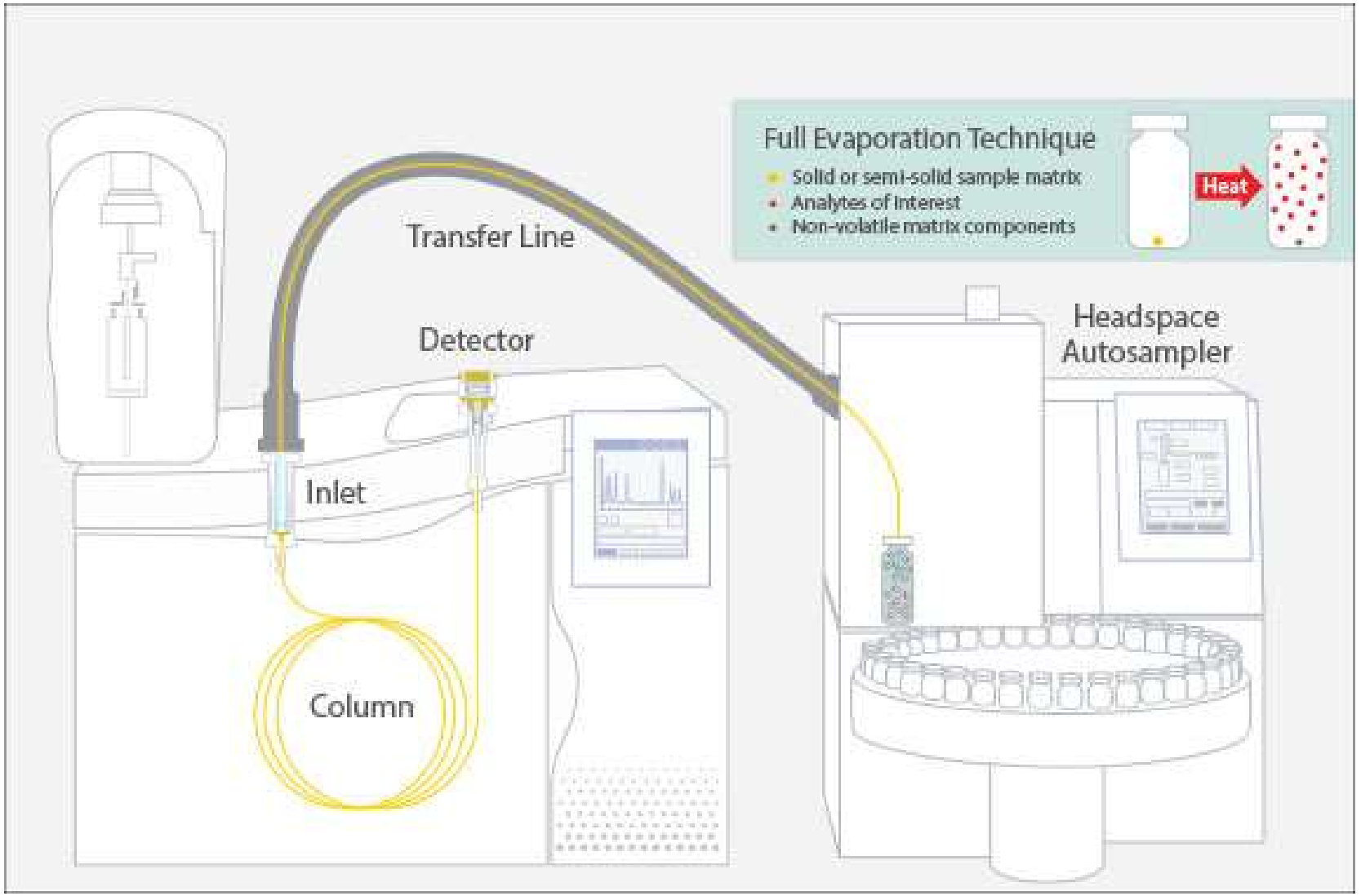
Injection of gas sample

Headspace sampling

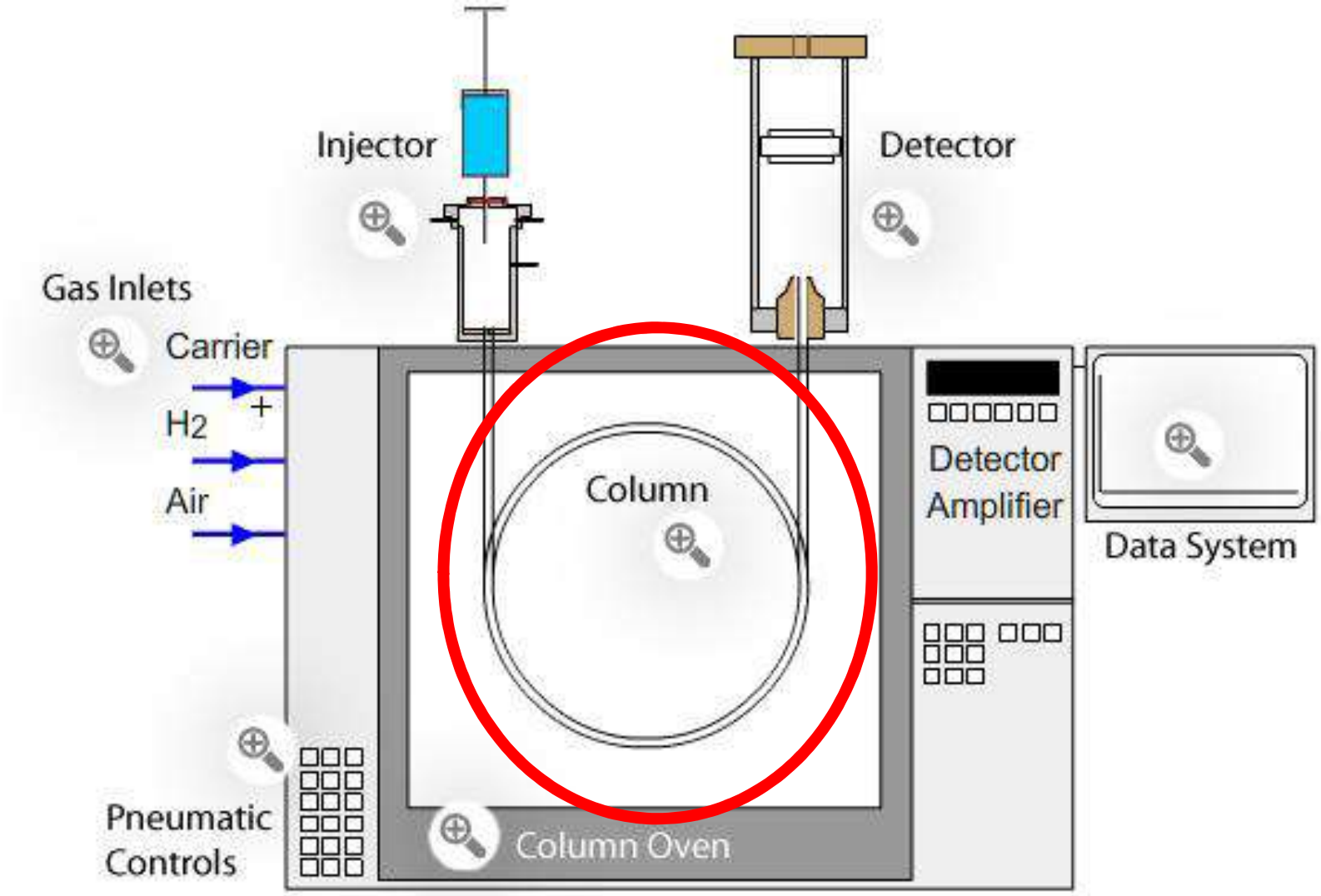


Injection of gas sample

Headspace – GC system

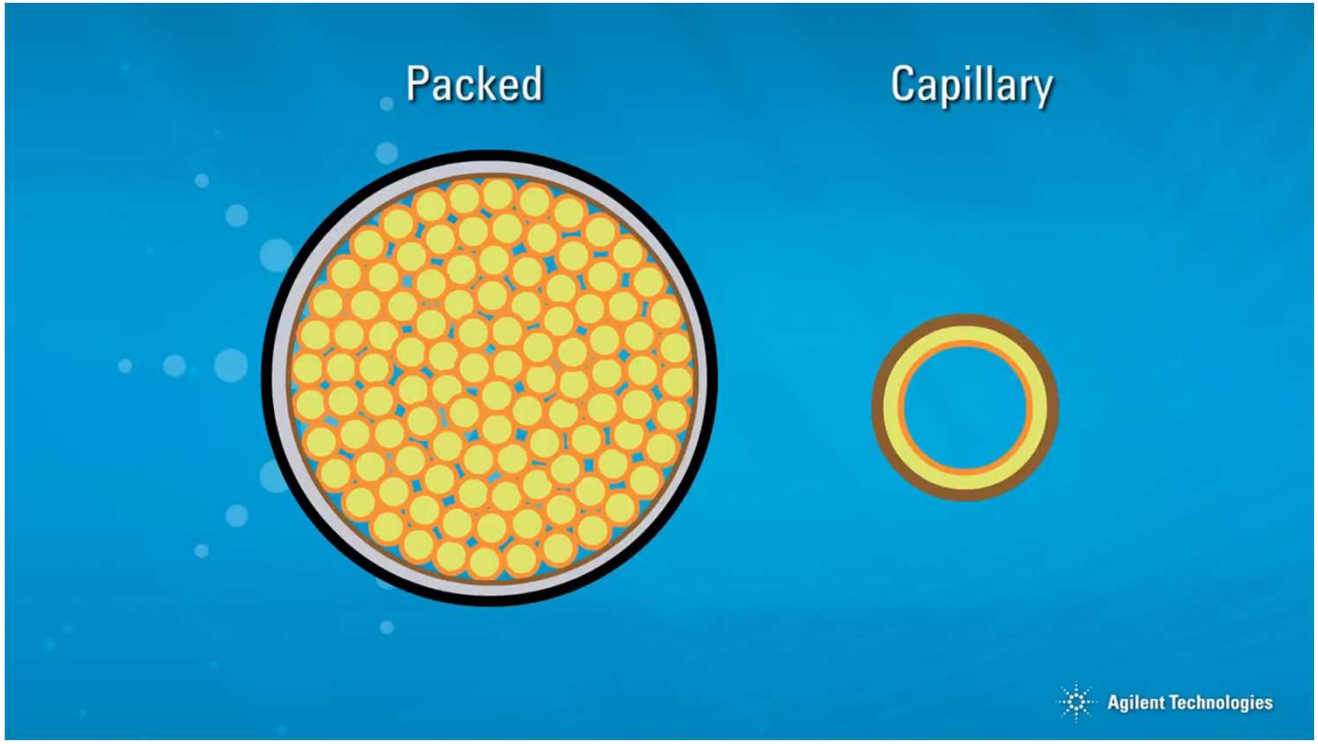


4. Column


Injector
Detector
Gas Inlets
Carrier
+
H2
Air
Column
Detector
Amplifier
Data System
Pneumatic
Controls
Column Oven

4. Column

Video about the GC columns.



4. Column

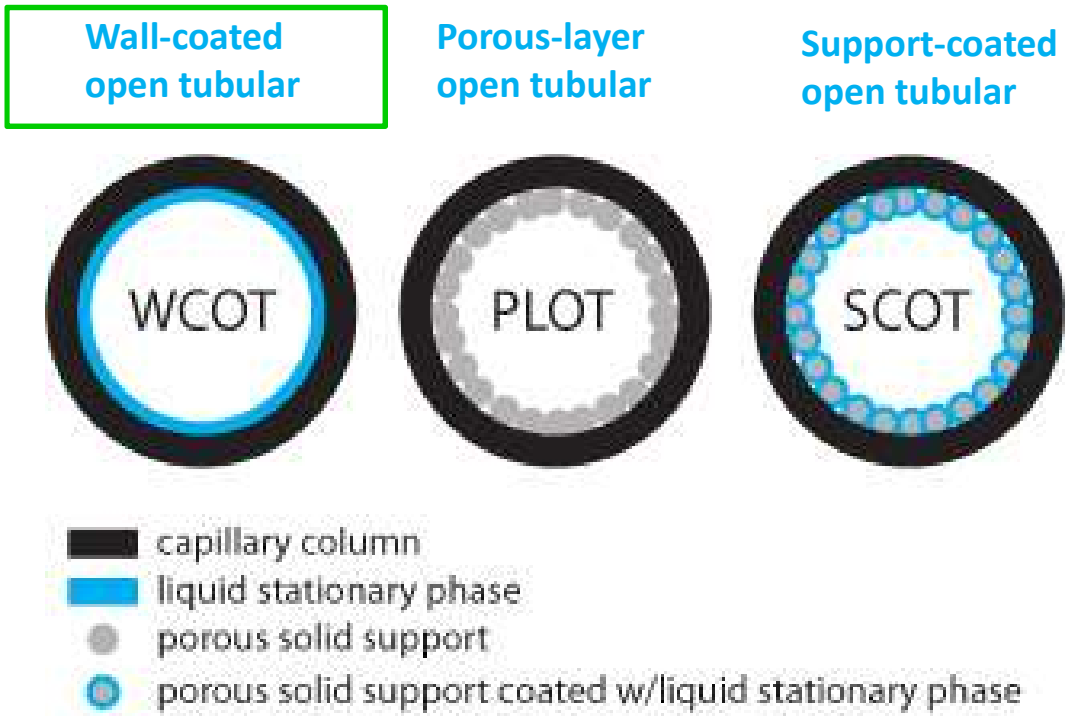
Place of separation (stationary phase)

	Packed column	Capillary column
length	1,5-10 m	10-100 m
internal diameter	2-4 mm	0,1-0,5 mm
material	stainless steel or glass	fused silica
sample amount	ml	µl
flow rate	25–150 mL/min	1–25 mL/min
N (number of theoretical plate)	up to 12 000	up to 60 000

Packed column

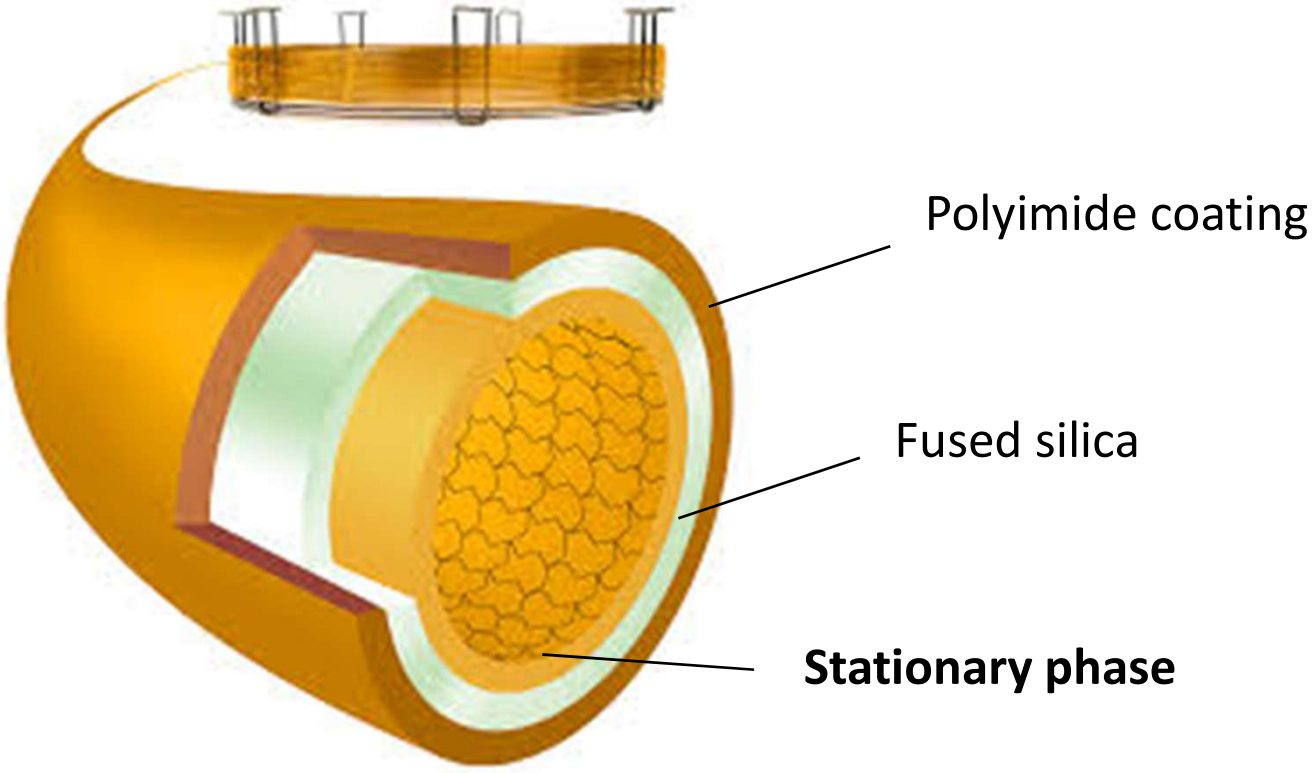
Capillary column

Capillary column



Capillary column

Wall-coated open tubular column (WCOT)



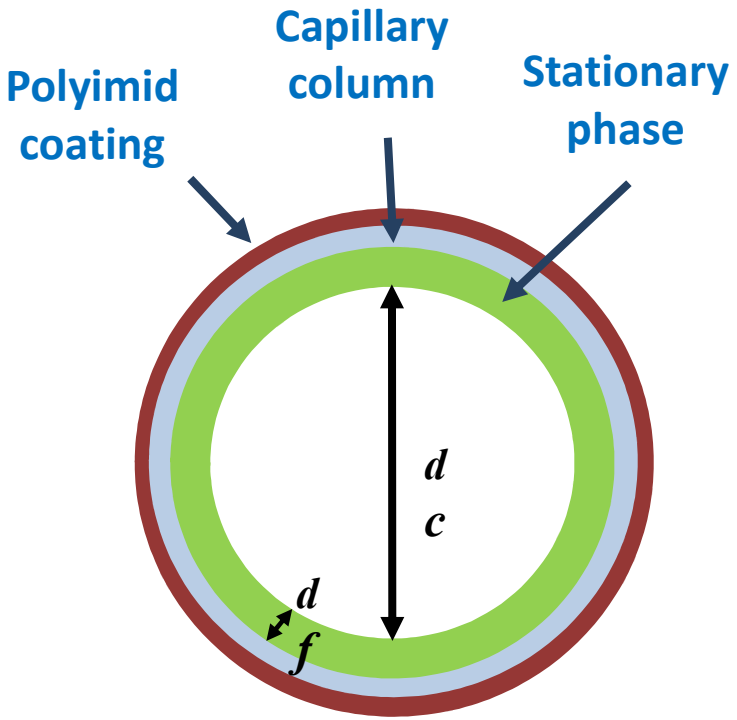
Classification of capillary columns

Based on internal diameter *(dc)* of column:

- Micro capillary (*dc* < 150 μm „micro-bore”)
- Standard capillary (150 μm < *dc* < 500 μm)
- Macro capillary (*dc* > 500 μm „wide-bore”)

Based on film thickness *(df)*:

- Thin film columns (*df* < 1 μm)
  (Small capacity, but high efficiency)
- Thick film columns (*df* > 1 μm)
  (High capacity, but small efficiency)

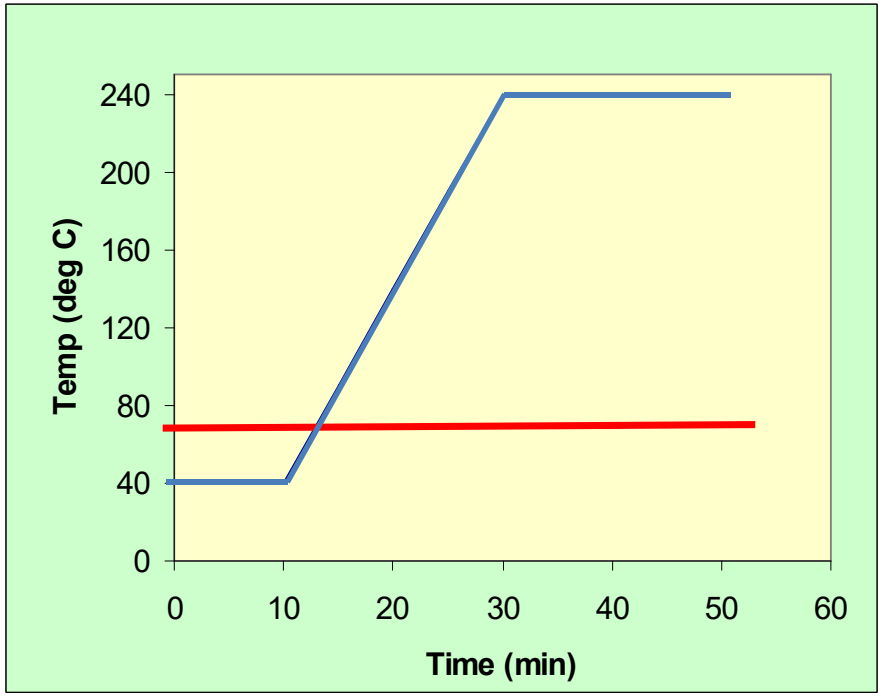


Thermostate or oven

The GC columns are in the oven.

We control the temperature of GC column via heated oven.

Oven temperature



For **isothermal** operation (**red line**), the GC is held at a steady temperature during the analysis.

In **temperature programmed** GC (**blue**) the oven temperature is increased according to a temperature program during the analysis.

Effect of oven temperature on the separation

A. Isothermal analysis

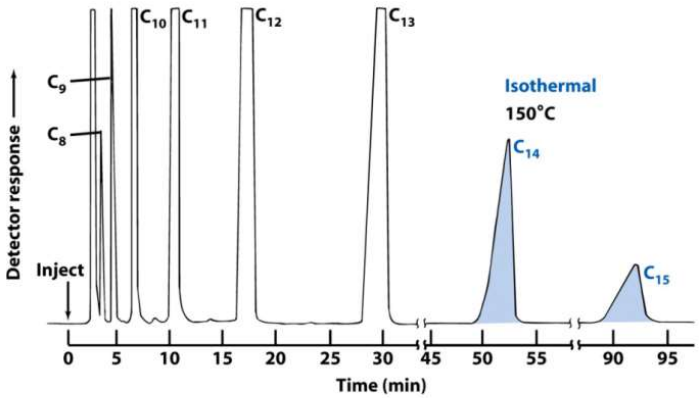


Figure 24-10a
Quantitative Chemical Analysis, Seventh Edition

One difficulty with an isothermal separation is that the separation of a low-boiling solute may lead to an unacceptably long retention time and broad peaks for a higher-boiling solute.

B. Programmed temperature

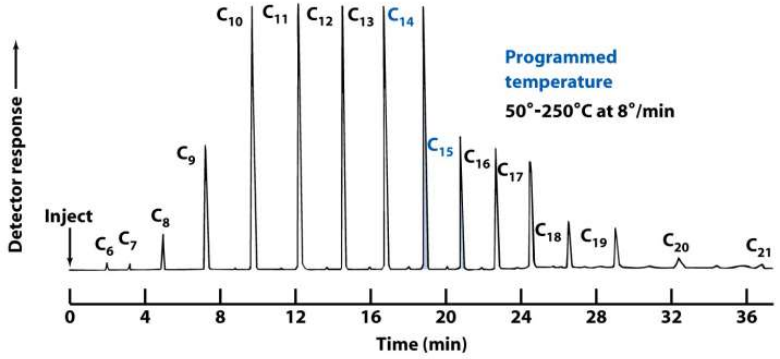


Figure 24-10b
Quantitative Chemical Analysis, Seventh Edition

Temperature programming provides a solution to this problem. At the beginning of the analysis we set the initial temperature of the column below the boiling point of the solute with the lowest boiling point. As the separation progresses, we slowly increase the temperature at either a steady rate or in a series of steps.

Solid stationary phases

Absorbents are porous and high surface area materials.

Types of absorbents:

- Organic (activated carbon, pearl polymers, Teflon)
- Inorganic (silica gel)
- Modified absorbents (graphitized activated carbon, graphitized silica gel)

Liquid stationary phases

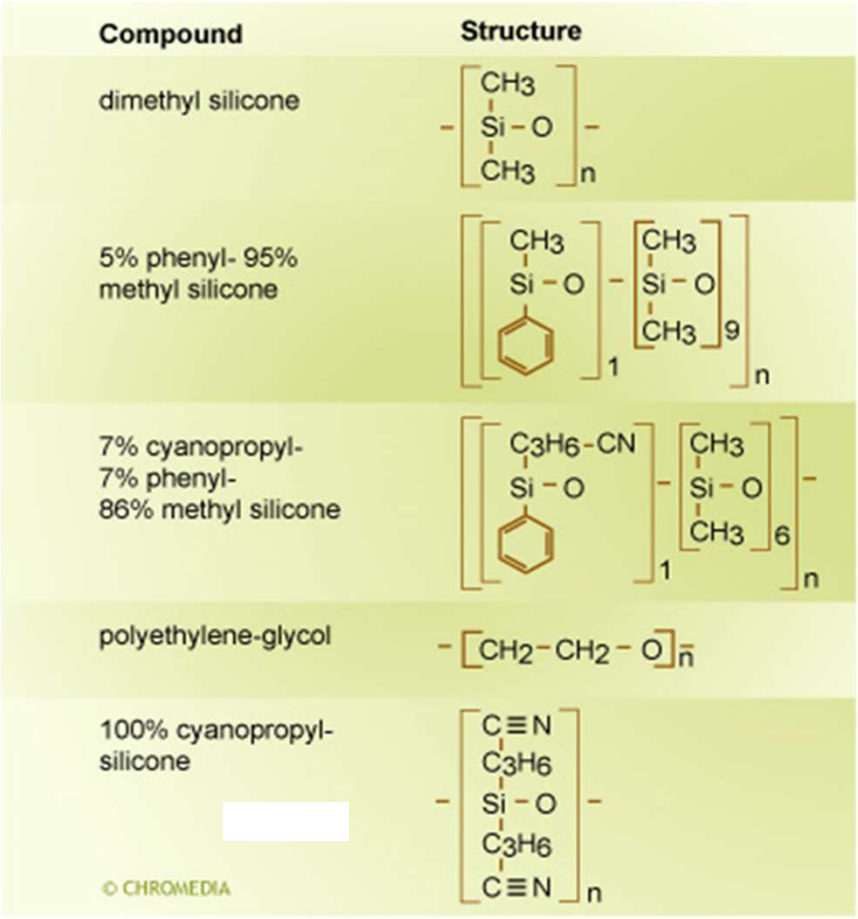
The liquid stationary phases are bonded with covalents bonds to the inner wall of the column.

Features:

- Low volatility
- Thermal stable
- Chemically inert
- Liquid at application temperature

Compounds:

- Polydimethyl-silicone
- Phenyl-methyl-silicone
- Polyethylen-glycol
- Cyanopropyl-silicone
- Nitrile
- Phthalates



Liquid stationary phases

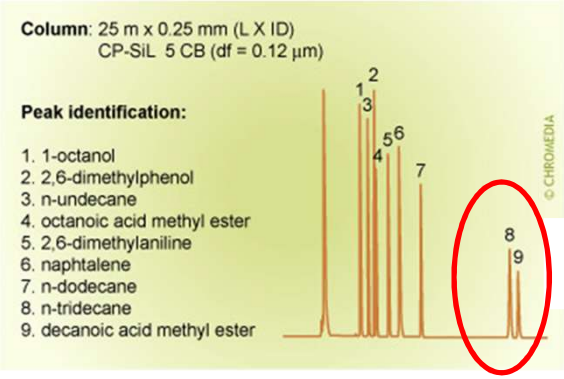
Table 12.2: Selected Examples of Stationary Phases for Gas–Liquid Chromatography

stationary phase	polarity	trade name	temperature limit (°C)	representative applications
polydimethyl siloxane	slightly polar	SE-30	300–350	alkaloids, amino acid derivatives, drugs, pesticides, phenols, steroids
phenylmethyl polysiloxane (50% phenyl, 50% methyl)	moderately polar	OV-17	375	alkaloids, drugs, pesticides, polyaromatic hydrocarbons, polychlorinated biphenyls
trifluoropropylmethyl polysiloxane (50% trifluoropropyl, 50% methyl)	moderately polar	OV-210	275	alkaloids, amino acid derivatives, drugs, halogenated compounds, ketones
cyanopropylphenylmethyl polysiloxane (50% cyanopropyl, 50% phenylmethyl)	polar	OV-225	275	nitriles, pesticides, steroids
polyethylene glycol	polar	Carbowax 20M	225	aldehydes, esters, ethers

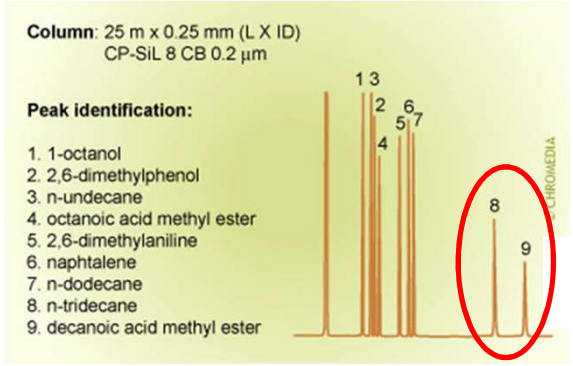
Selection of GC column

Type of stationary phase (polarity)

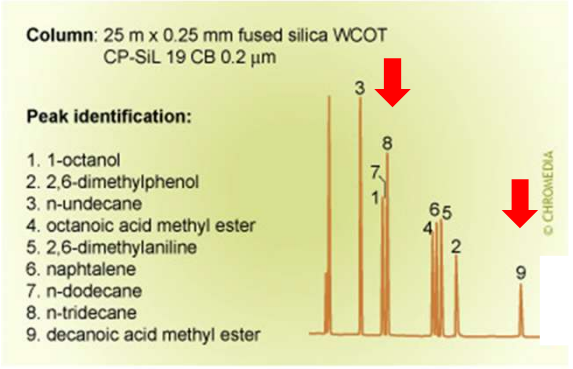
1



2



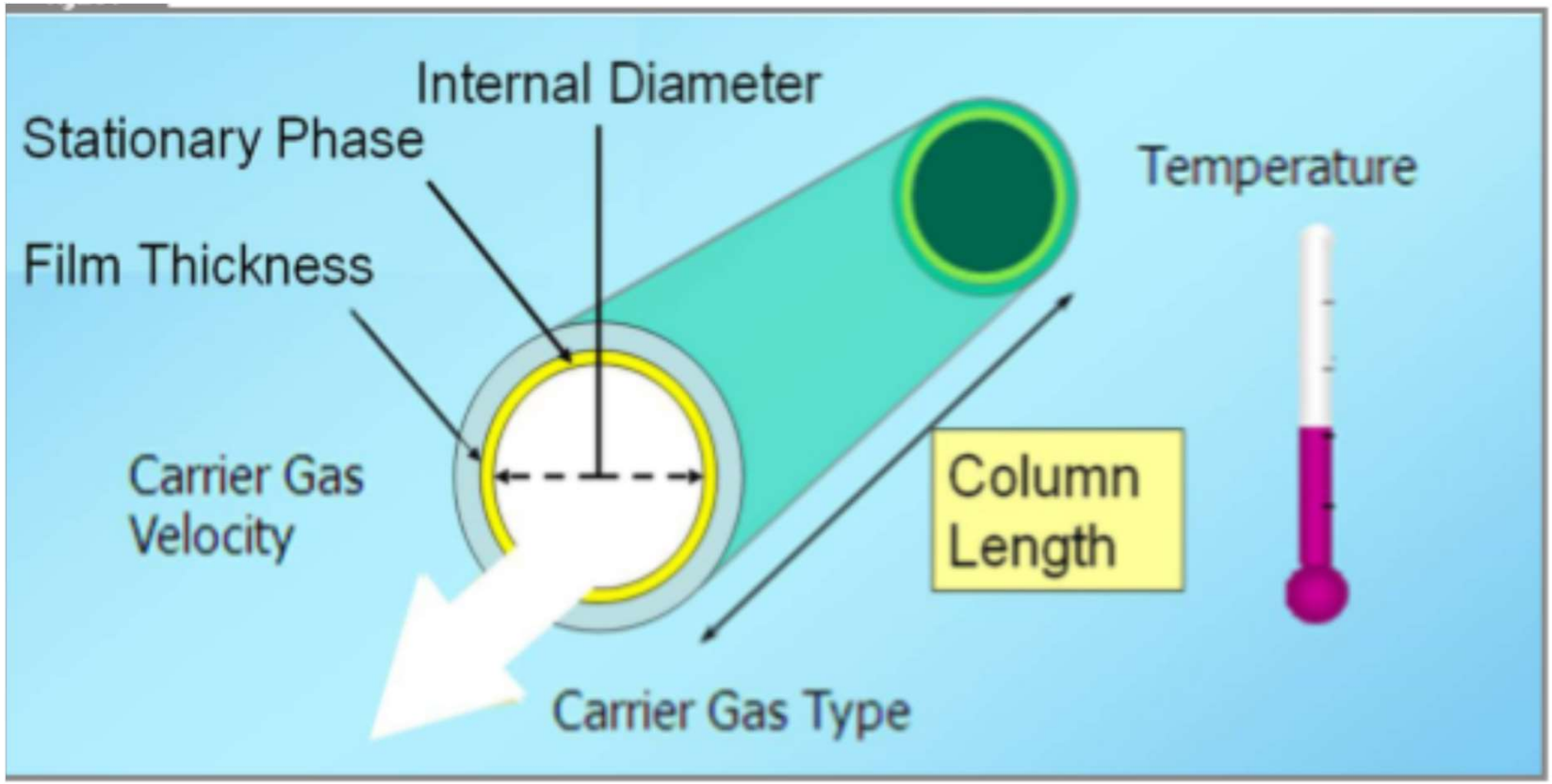
3



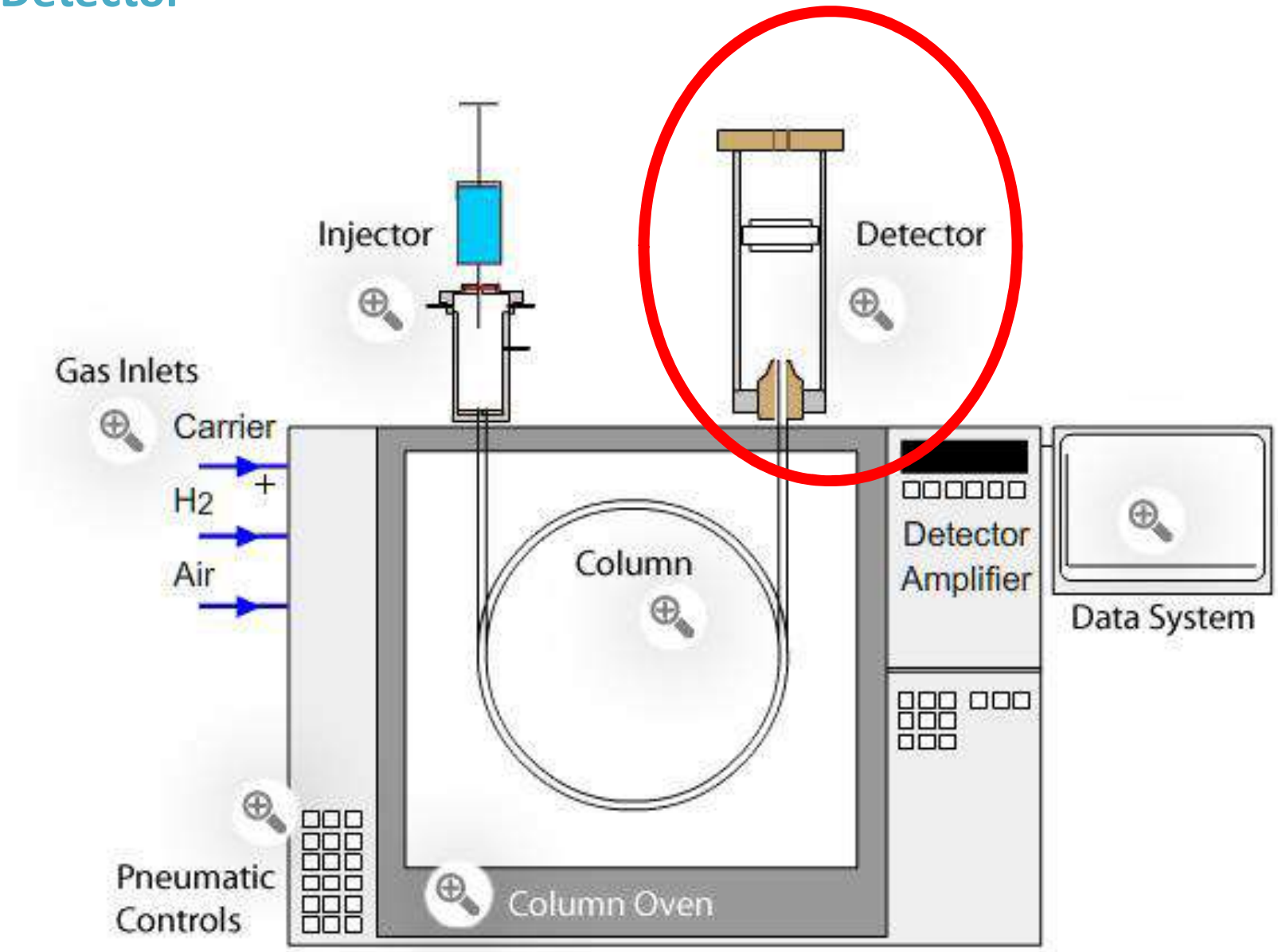
The polarity of stationary phase is increasing.

The order of the components can change depending on the stationary phase type!

Factors affecting separation



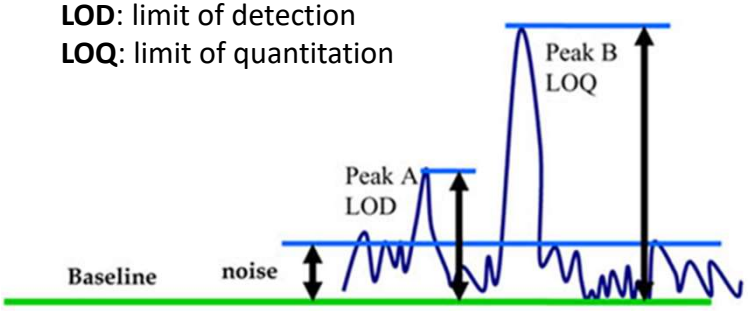
5. Detector

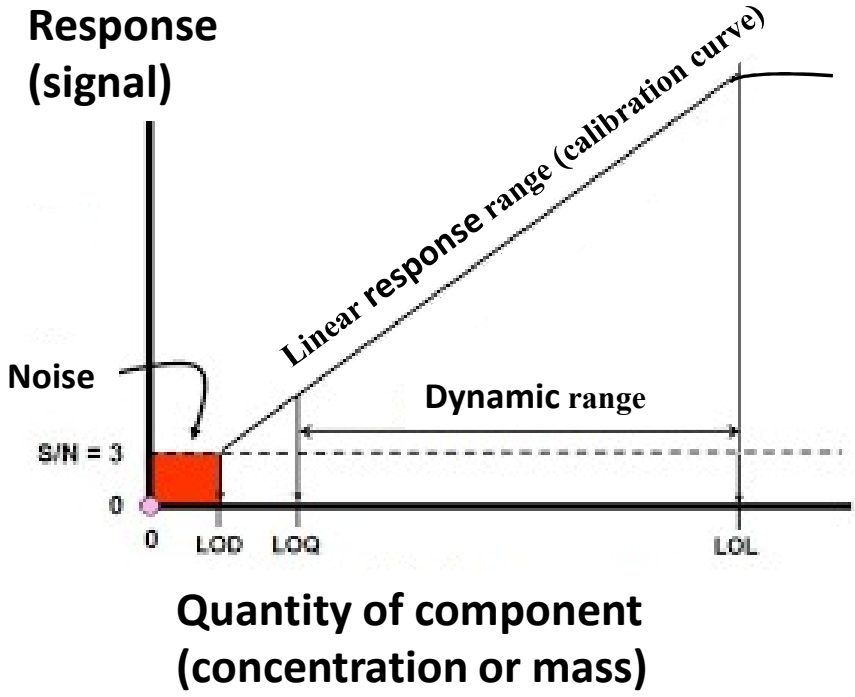

Injector
Detector
Gas Inlets
Carrier
+
H2
Air
Column
Detector
Amplifier
Data System
Pneumatic
Controls
Column Oven

GC detectors

The detector indicates the separated components and measures their physical or chemical properties. We can use the detector signal for both qualitative identification and quantitative determination of the separated components.

LOD: limit of detection
LOQ: limit of quantitation



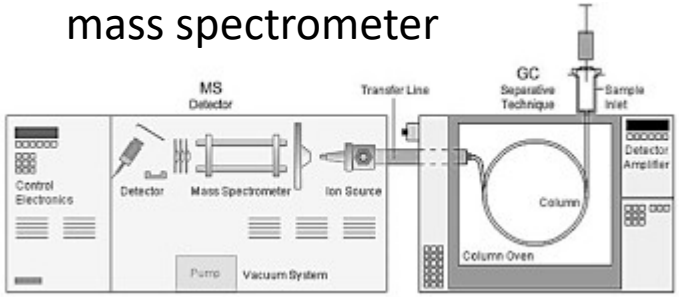


GC detectors

- Ideal detector
  - low detection limit
  - linear response over a wide range of component concentrations
  - sensitivity to all component or selectivity for a specific class of compounds

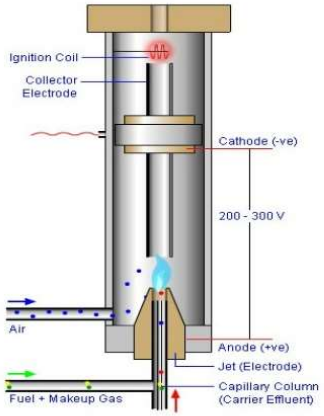
GC-MS

mass spectrometer



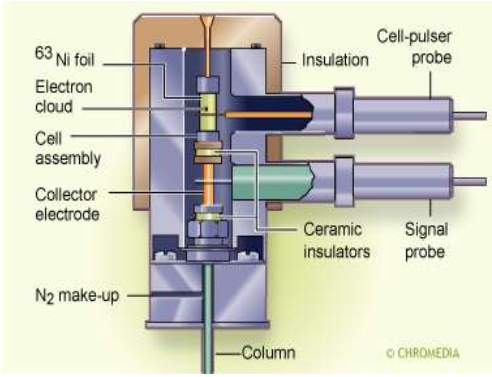
FID

flame ionization detector



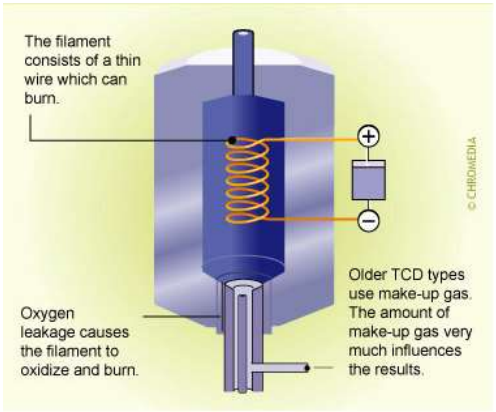
ECD

electron capture detector



TCD

thermal conductivity detector



GC detectors

Detector	Type	Support gases	Selectivity	Detectability	Dynamic range
Flame ionization (FID)	Mass flow	Hydrogen and air	Most organic compounds.	100 pg	10^7
Thermal conductivity (TCD)	Concentration	Reference	Universal	1 ng	10^7
Electron capture (ECD)	Concentration	Make-up	Halides, nitrates, nitriles, peroxides, anhydrides, organometallics	50 fg	10^5
Nitrogen-phosphorus	Mass flow	Hydrogen and air	Nitrogen, phosphorus	10 pg	10^6
Flame photometric (FPD)	Mass flow	Hydrogen and air possibly oxygen	Sulphur, phosphorus, tin, boron, arsenic, germanium, selenium, chromium	100 pg	10^3
Photo-ionization (PID)	Concentration	Make-up	Aliphatics, aromatics, ketones, esters, aldehydes, amines, heterocyclics, organosulphurs, some organometallics	2 pg	10^7
Hall electrolytic conductivity	Mass flow	Hydrogen, oxygen	Halide, nitrogen, nitrosamine, sulphur	-	-

Flame ionization detector (FID)

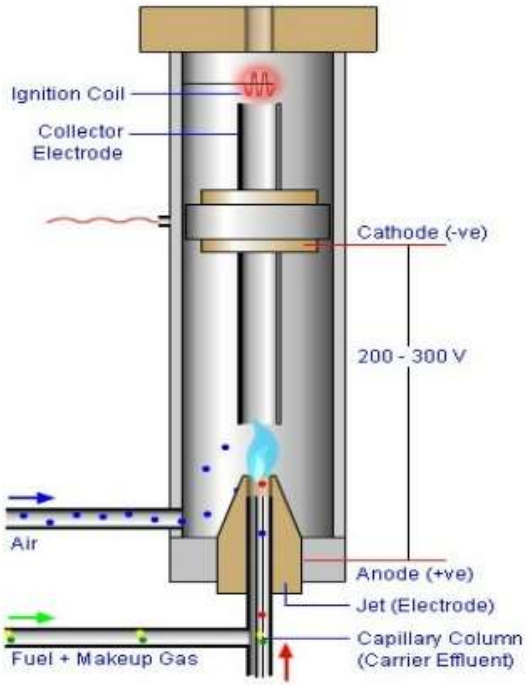
The flame ionization detector is an universal, highly sensitive detector for all organic compounds with a C-H bond.

- **The flame is mixed with hydrogen and air.**

- **The organic compounds enter the detector, they burn in the flame and, therefore, generate ions:**
  1. Pyrolysis: CnHm → n CH. + (m-n) H.
  2. Oxidation: n CH. + n O. →n CHO.
  3. Ionisation: n CHO. →n CHO+ + n e-

- **The ions move towards the collector electrode, due to the potential difference between the jet and the electrode. The resulting ionization current is amplified and fed to the data system**

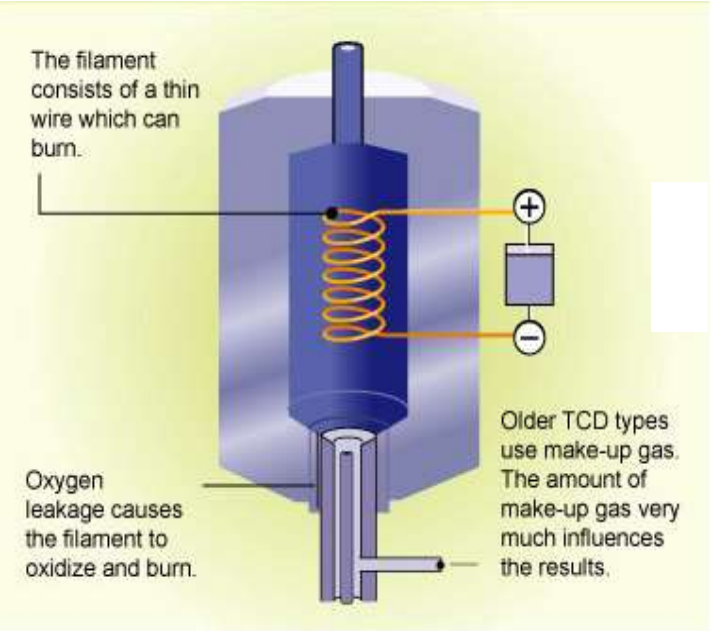
- **Large linear response range**
  (over 10^6–10^7)
- **Detection limit**
  (pg)



Thermal conductivity detector (TCD)

The thermal conductivity detector is a universal, highly sensitive detector based on the measurement of the thermal conductivity of a gas.

- **The TCD measures the difference in heat conductivity between pure carrier gas and carrier gas containing sample components.**
- **The core of the TCD is a filament, a thin wire often made of tungsten, platinum or nickel.**
- **Since the resistance of this filament is dependent on its temperature, a change in temperature will result in a change in resistance. This change can be detected electronically.**
- **He** or **H_2** is used as **carrier gas**
- **Linear response range**
  (10^4–10^5)
- **Poor detection limit**
  (ng)

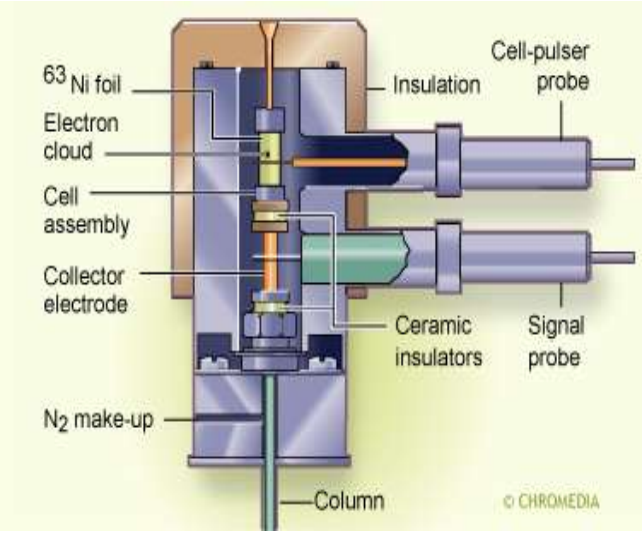


Electron capture detector (ECD)

The electron capture detector is a selective detector for electro-negative compounds, especially halogens (F, Cl, Br).

- **A (beta-ray) radio active source which ionizes the carrier gas is located in the detector. A current is produced between two electrodes in the detector supplied with a potential difference and this is monitored as a continuous background current.**

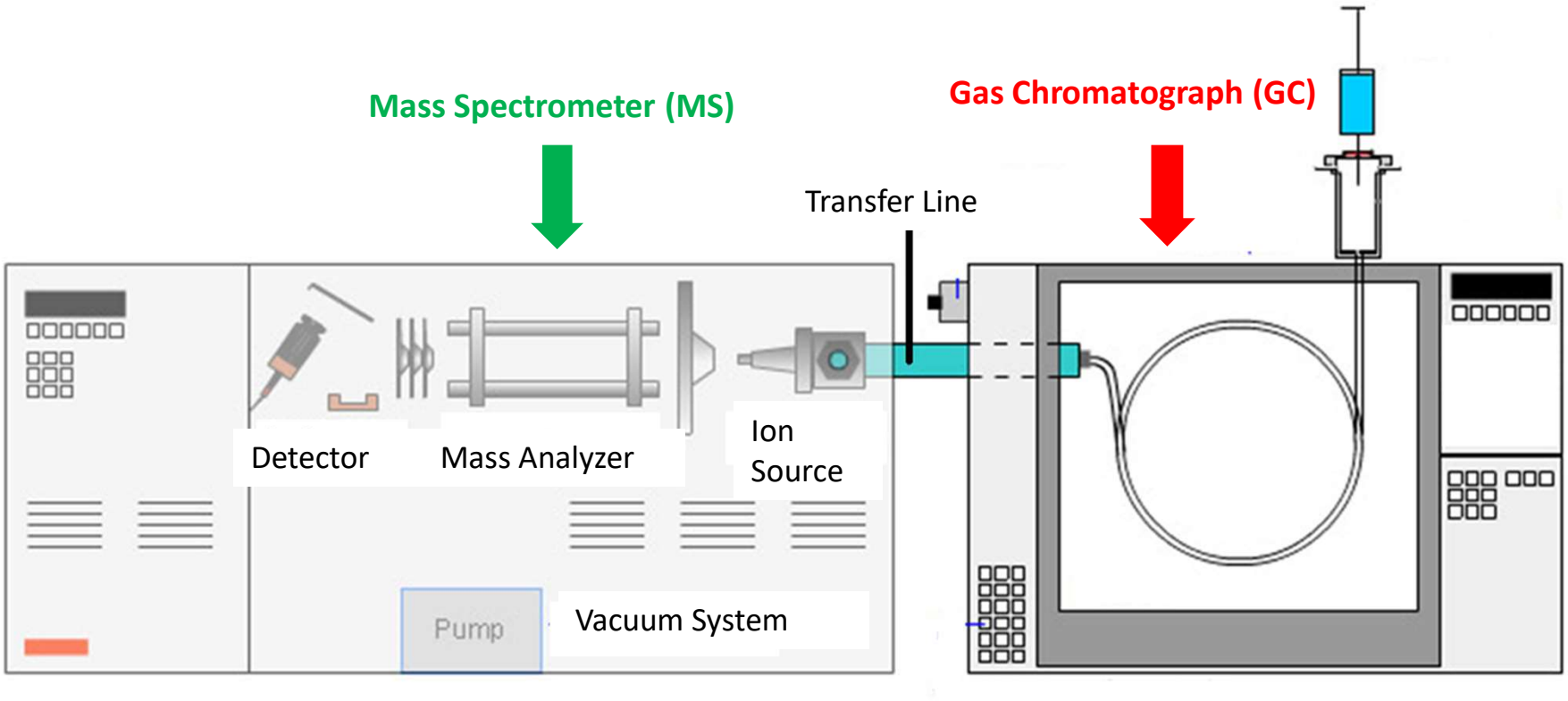
- **When there are electro-negative components present in the carrier gas, the background current is reduced, because these components capture electrons.**



- **N_2** is used as **carrier gas**
- **Linear response range**
  (10^5)
- **Detection limit** (depending on the chemical structure)
  excellent (fg)

Mass spectrometer detector (MSD)

Mass spectrometer (MS) can be used as a GC detector.

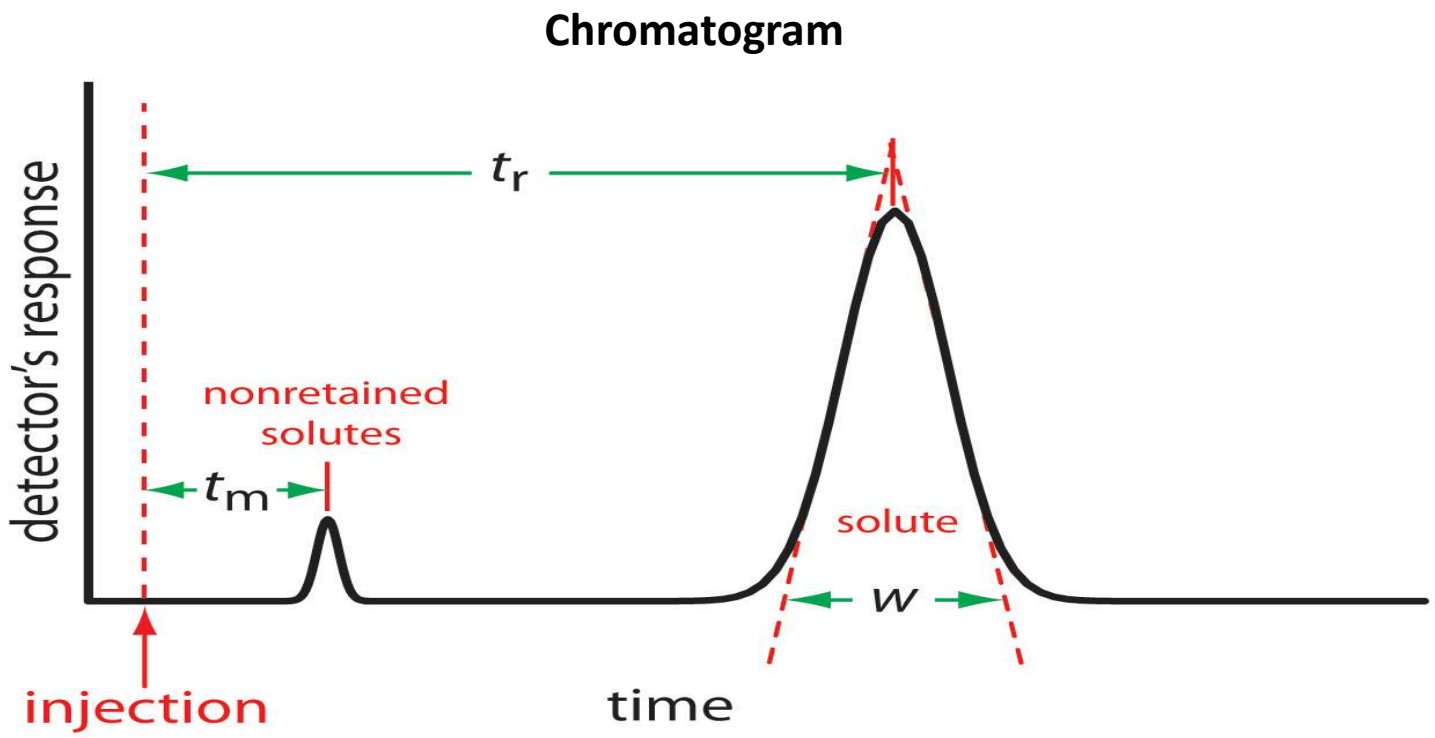


- **Molecule Structure definition**
- **Qualitative identification**
- **Quantitative analysis**

Chromatogram

The chromatogram shows the detector response as a function of elution time:

- **Retention time → qualitative analysis**
- **Peak area → quantitative analysis**



Qualitative analysis

Main methods:

- **Qualitative analysis with retention data**
  - Compare the retention time of the peak to the retention time of a known compound
  - Standard addition (Spike)
  - Retention indexes

- **Qualitative analysis with specific detectors**

- **Qualitative analysis with GC-MS**

Qualitative analysis / retention indexes

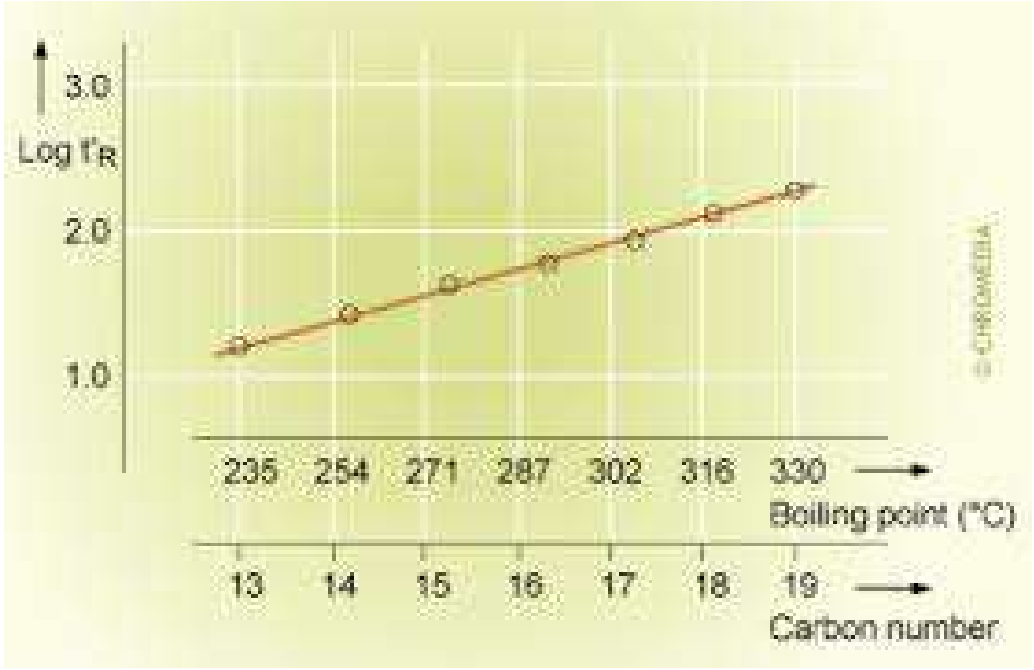
Retention indexes:

- **relative retention times are normalised to closely eluting n-alkanes**
- **system independent and long-term reproducible**
- **it can be used for the qualitative identification of components**

Kováts-index

The Kováts index (I) can be applied to organic compounds. The method interpolates peaks between bracketing n-alkanes and the adjusted retention times of normal alkanes, which increases logarithmically.

Ervin Kováts



Kováts-index

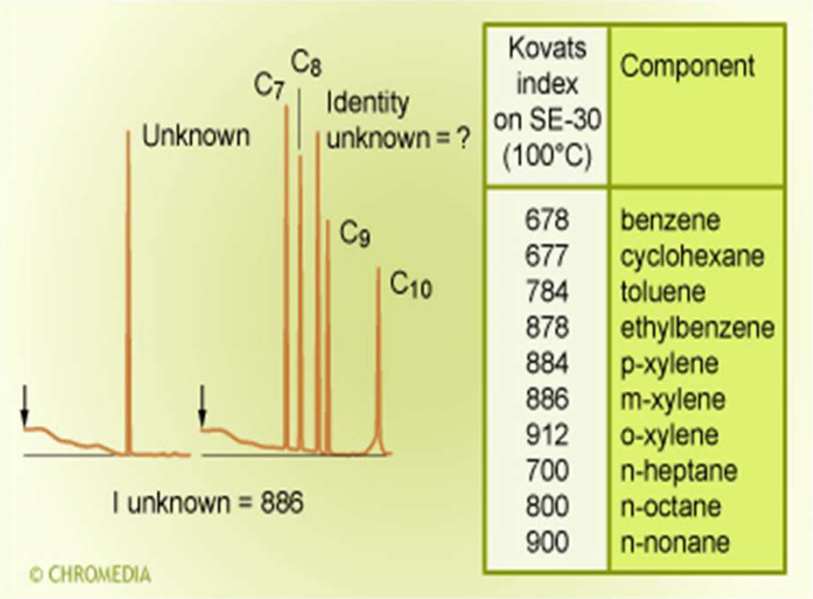
- **Kováts index of n-alkanes is 100 times their carbon number.**

  e.g. Kováts index of n-Butane is 400.

- **The Kováts index is dimensionless. For isothermal gas chromatography, the Kovats index is given by this equation:**

$$I_x = 200 * \frac{\lg t_{R'_X} - \lg t_{R'_n}}{\lg t_{R'_{n+2}} - \lg t_{R'_n}} + 100n$$

***I** is the Kováts index of the unknown*
tR'(x) is the adjusted retention time of the unknown
tR'(n) is the adjusted retention time of the smaller alkane
tR'(n+2) is the adjusted retention time of the larger alkane



Kovats index on SE-30 (100°C)	Component
678	benzene
677	cyclohexane
784	toluene
878	ethylbenzene
884	p-xylene
886	m-xylene
912	o-xylene
700	n-heptane
800	n-octane
900	n-nonane

Example (Kovats index)

Example

EXAMPLE 12.6

In a separation of a mixture of hydrocarbons, the following adjusted retention times were measured.

propane	2.23 min
isobutane	5.71 min
butane	6.67 min

What is the Kovat's retention index for each of these hydrocarbons?

SOLUTION

Kovat's retention index for a normal alkane is 100 times the number of carbons; thus

$$I_{\text{propane}} = 100 \times 3 = 300$$

$$I_{\text{butane}} = 100 \times 4 = 400$$

To find Kovat's retention index for isobutane, we use equation 12.29.

$$I_{\text{isobutane}} = 100\left[\frac{(\log t_r')_{\text{isobutane}} - (\log t_r')_{\text{propane}}}{(\log t_r')_{\text{butane}} - (\log t_r')_{\text{propane}}}\right] + I_{\text{propane}}$$

$$= 100\left[\frac{\log(5.71) - \log(2.23)}{\log(6.67) - \log(2.23)}\right] + 300 = 386$$

Retention indexes

Retention indexes

Table 24-3 Retention indexes for several compounds on common stationary phases

Phase	Retention index[a] Benzene b.p. 80°C	Butanol b.p. 117°C	2-Pentanone b.p. 102°C	1-Nitropropane b.p. 132°C	Pyridine b.p. 116°C
Poly(dimethylsiloxane)	657	648	670	708	737
(Diphenyl)$_{0.05}$(dimethyl)$_{0.95}$-polysiloxane	672	664	691	745	761
(Diphenyl)$_{0.35}$(dimethyl)$_{0.65}$-polysiloxane	754	717	777	871	879
(Cyanopropylphenyl)$_{0.14}$-(dimethyl)$_{0.86}$polysiloxane	726	773	784	880	852
(Diphenyl)$_{0.65}$(dimethyl)$_{0.35}$-polysiloxane	797	779	824	941	943
Poly(ethylene glycol)	956	1 142	987	1 217	1 185
(Biscyanopropyl)$_{0.9}$-(cyanopropylphenyl)$_{0.1}$-polysiloxane	1 061	1 232	1 174	1 409	1 331

a. For reference, boiling points (b.p.) for various alkanes are hexane, 69°C; heptane, 98°C; octane, 126°C; nonane, 151°C; decane, 174°C; undecane, 196°C. Retention indexes for the straight-chain alkanes are fixed values and do not vary with the stationary phase: hexane, 600; heptane, 700; octane, 800; nonane, 900; decane, 1 000; undecane, 1 100.

SOURCE: Restek Chromatography Products Catalog, 1993–94, Bellefonte, PA.

Table 24-3
Quantitative Chemical Analysis, Seventh Edition

Quantitative analysis

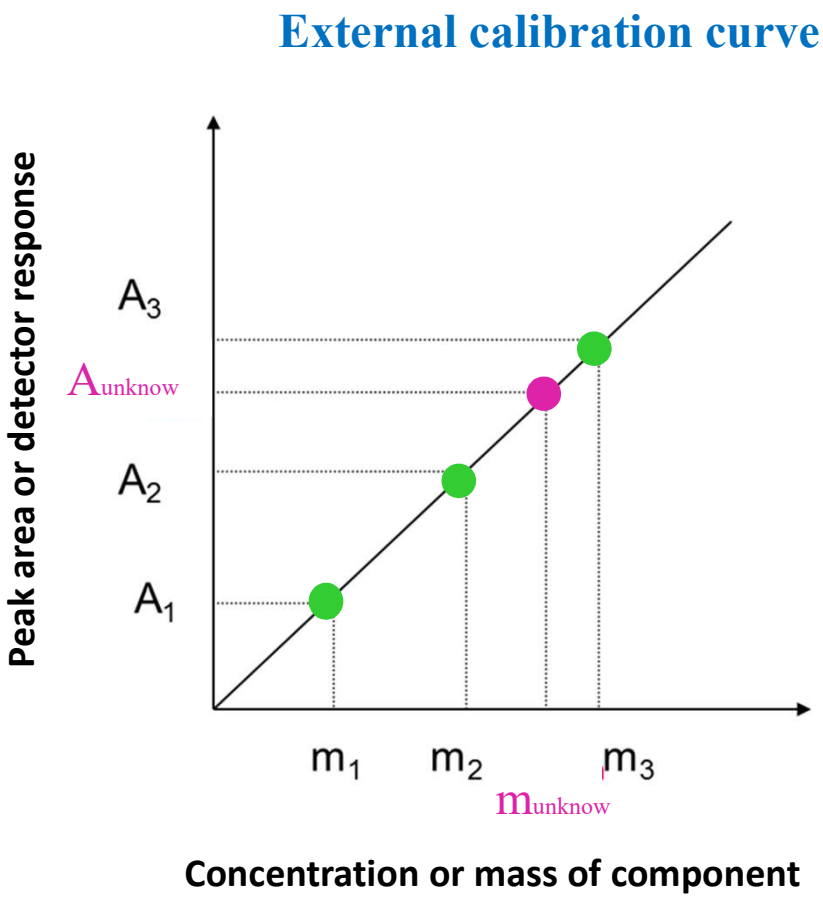
Main methods:

- **Calibration method**
- **Standard addition method**
- **Internal standard (IS) calibration method**

Calibration method

1) Measure the calibration samples of known concentration, record the chromatogram and get the peak areas (detector responses).
2) Represent the calibration curve.
3) Measure the unknown sample, record the chromatogram and get the peak areas.

We can calculate the concentration of unknown component(s) with the equation of calibration curve.



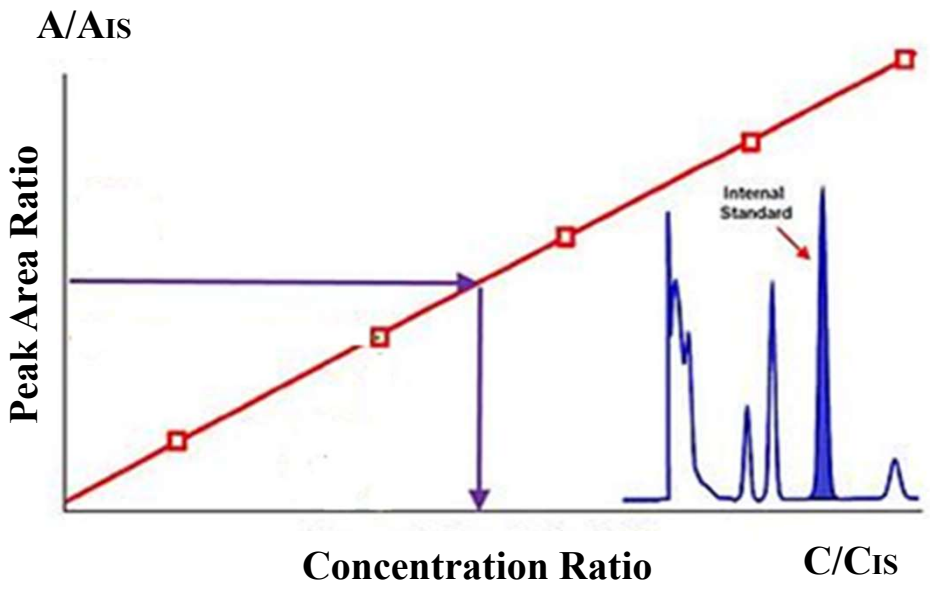
Internal standard calibration method

Internal standard (IS):

- chemically similar to analyte
- added same amount to the samples and calibration samles too
- compensation the variations of the injection volume and the system variables

We can calculate the concentration of unknown component(s) with the equation of calibration curve.

Calibration curve of internal standard method



Sample preparation before GC analysis

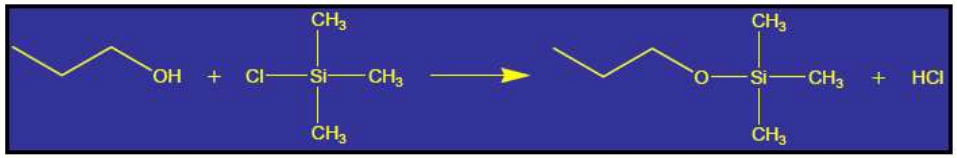
- **Cleaning**
- **Extraction**
- **Concentration**
- **Derivatization**

Derivatization

It is a chemical reaction that modifies a component so that it is easier to evaporate, separate or detect.

Derivatization increases volatility.

- A typical derivitization reactions – silylation of an alcohol:



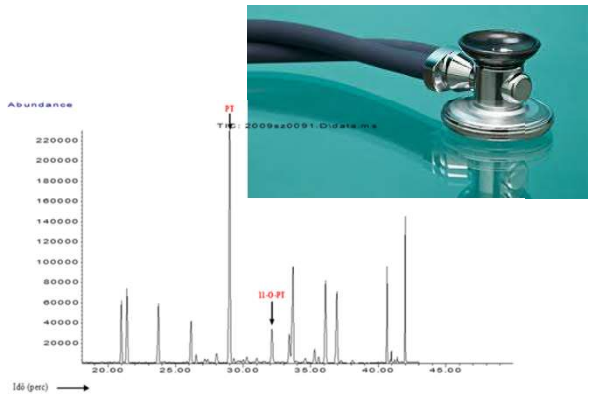
- Common derivatives that reduce polarity:

Groups	Derivative
Alcohol (–OH)	Alkyl ester, alkyl ether, silyl ether
Carboxylic acid (–COOH)	Alkyl ester, silyl ester
Amino ($-NH_2$)	Acyl derivative, silyl derivative
Imino (=NH)	Silyl derivative
Aldehyde (COH)	Dimethyl acetal
Thiol (SH)	Thioether, silylthioether

- Other derivatives contain halogens for ECD detection

S. Ahuja, "Derivatization for Gas and Liquid Chromatography", in *Ultratrace Analysis of Pharmaceuticals and Other Compounds of Interest*, Wiley, 1986.

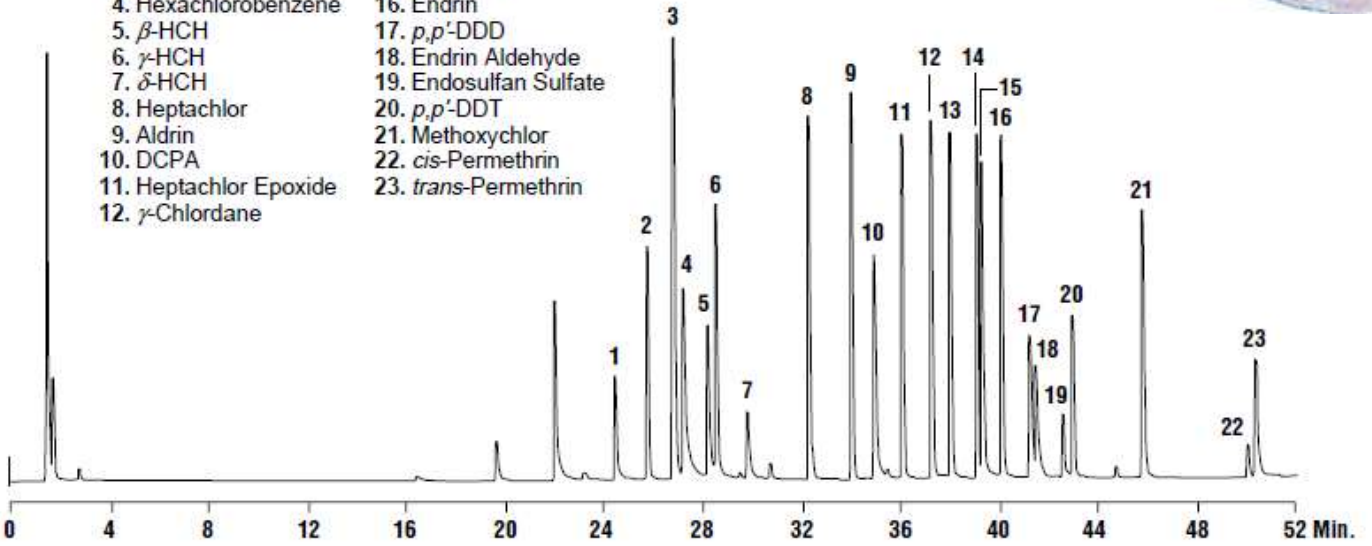
Applications of GC



Environmental applications

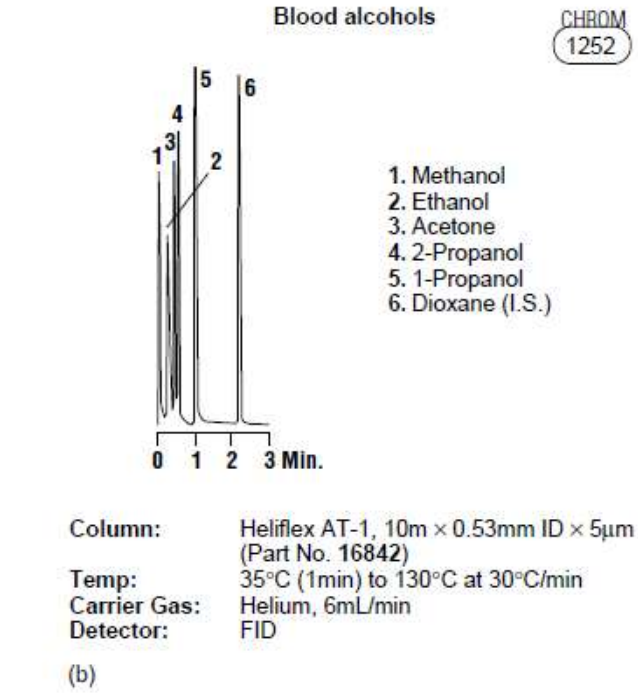
Chlorinated pesticides in water

1. Propachlor
2. Trifluralin
3. α-HCH
4. Hexachlorobenzene
5. β-HCH
6. γ-HCH
7. δ-HCH
8. Heptachlor
9. Aldrin
10. DCPA
11. Heptachlor Epoxide
12. γ-Chlordane
13. α-Chlordane
14. Dieldrin
15. *p,p'*-DDE
16. Endrin
17. *p,p'*-DDD
18. Endrin Aldehyde
19. Endosulfan Sulfate
20. *p,p'*-DDT
21. Methoxychlor
22. *cis*-Permethrin
23. *trans*-Permethrin



Column:	Econo-Cap™ EC-5, 30m × 0.32mm ID × 0.25µm (Part No. 19646)
Temp:	60°C to 300°C at 4°C/min
Carrier Gas:	Helium, 1.45mL/min
Detector:	ECD at 320°C
Injector:	250°C

Forensic science



Journal of Analytical Toxicology, Vol. 20, July/August 1996

Cocaine Contamination of United States Paper Currency

Jonathan Oyler, William D. Darwin, and **Edward J. Cone**
Addiction Research Center, National Institute on Drug Abuse, National Institutes of Health, P.O. Box 5180, Baltimore, Maryland 21224

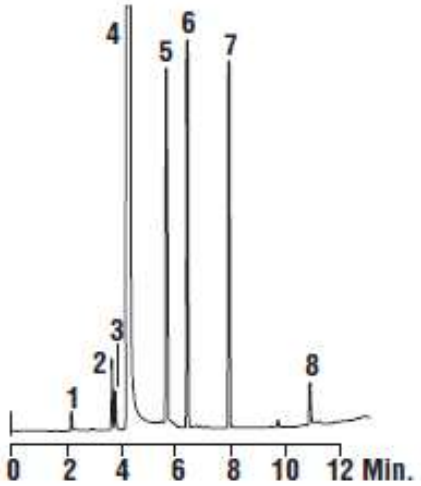
Abstract

The exchange of illicit cocaine for money by drug dealers is an everyday occurrence in cities in the United States. There is ample opportunity during the exchange, storage, and use of cocaine for paper currency to become contaminated. Because currency is exchanged frequently, it is likely that contaminated currency would be found in common use. We examined ten single dollar bills from several cities in the United States for the presence of cocaine. Individual bills were extracted with methanol (10 mL). Cocaine was purified from the methanol extract by solid-phase extraction (SPE). The SPE extract was analyzed by gas chromatography–mass spectrometry (GC–MS). Standard curves were constructed with new, uncirculated currency. Cocaine was identified qualitatively by full scan and quantitated by selected ion monitoring. Cocaine was present in 79% of the currency samples analyzed in amounts above 0.1 µg and in 54% of the currency in amounts above 1.0 µg. Contamination was widespread and was found in currency from all sites examined. Cocaine amounts were highly variable and ranged from nanogram to milligram amounts. The highest amount of cocaine detected on a single one-dollar bill was 1327 µg. These results indicated that cocaine contamination of currency is widespread throughout the United States and is likely to be primarily a result of cross-contamination from other contaminated currency and from contaminated money-counting machines.

Food Industry

Scotch whiskey

CHROM 2124



1. Acetaldehyde
2. Ethyl Acetate
3. Methanol
4. Ethanol
5. *n*-Propanol
6. Isobutanol
7. Amyl Alcohol/Isoamyl Alcohol
8. Acetic Acid

Column:	Econo-Cap EC-WAX, 30m × 0.45mm × 1.0µm (Part No. 19663)	
Temp:	50°C (2min) to 200°C at 15°C/min	
Carrier Gas:	Helium, 34cm/sec	**Split Ratio:** 20:1
Detector:	FID/250°C	**Injector:** 250°C

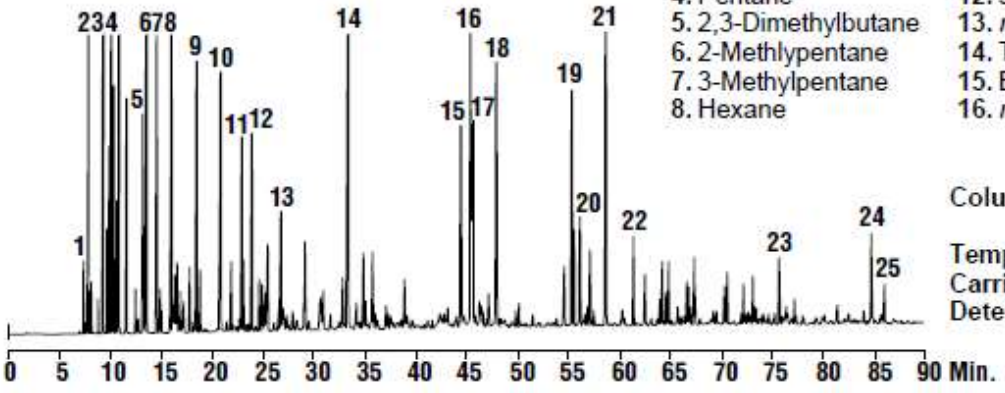
(c)

Petroleum Industry

Unleaded gasoline analysis

CHROM 1859

1. Isobutane
2. *n*-Butane
3. Isopentane
4. Pentane
5. 2,3-Dimethylbutane
6. 2-Methlypentane
7. 3-Methylpentane
8. Hexane
9. 2,4-Dimethylpentane
10. Benzene
11. 2-Methylhexane
12. 3-Methylhexane
13. *n*-Heptane
14. Toluene
15. Ethylbenzene
16. *m*-Xylene
17. *p*-Xylene
18. *o*-Xylene
19. 1-Methyl-3-Ethylbenzene
20. 1,3,5-Trimethylbenzene
21. 1,2,4-Trimethylbenzene
22. 1,2,3-Trimethylbenzene
23. Naphthalene
24. 2-Methylnaphthalene
25. 1-Methylnaphthalene



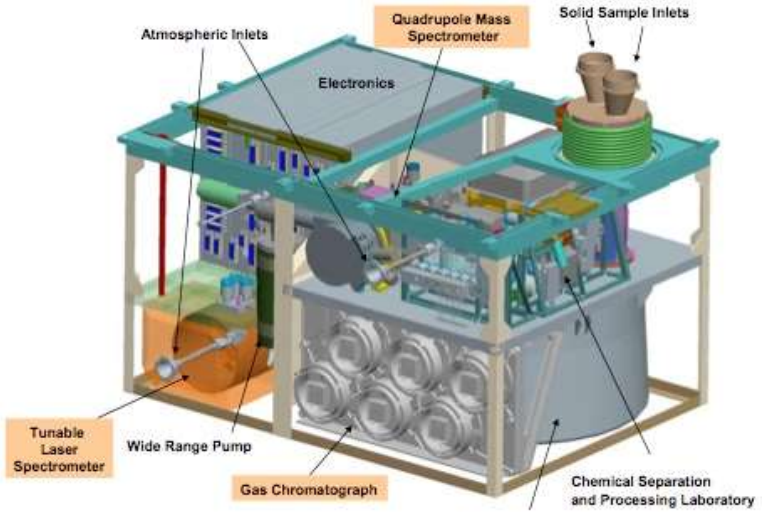
(d)

Column:	AT-Petro, 100m × 0.25mm ID × 0.50µm (Part No. 13948)
Temp:	35°C (15min) to 200°C at 2°C/min
Carrier Gas:	Helium, 0.65mL/min
Detector:	FID at 275°C

Figure 12.25—*Continued*

GC in space

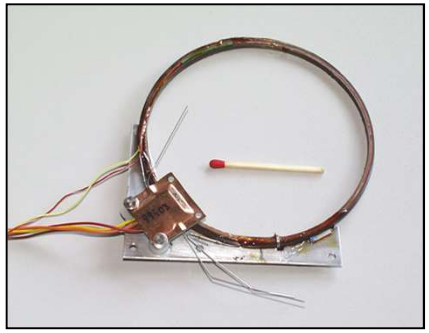
Curiosity Rover Sample Analysis at Mars (SAM)



https://mars.nasa.gov/msl/spacecraft/instruments/sam/

Titan Mission
Saturn's largest moon

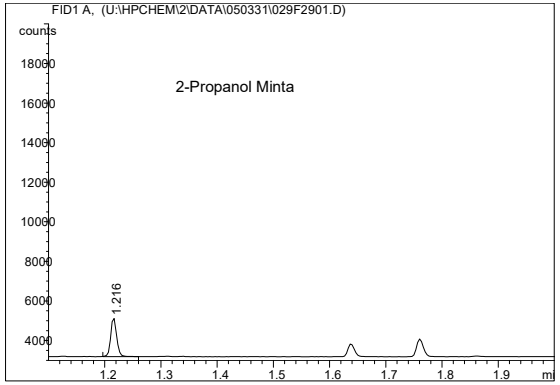
The Huygens gas chromatography–mass spectrometry team concluded that methane rain occurs on the moon, and GC–MS can distinguish between two isotopic forms of carbon — carbon-12 and carbon-13.



Pharmaceutical applications

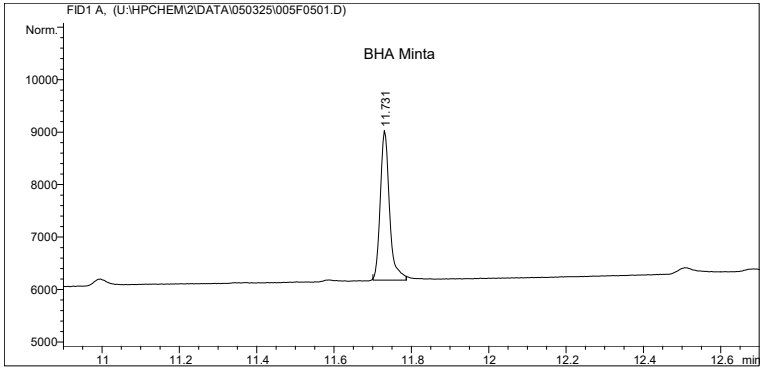
Identification and determination of trace impurities (for example residue of solvent) in medicine or drug content.

Residue of solvent



2-propanol content

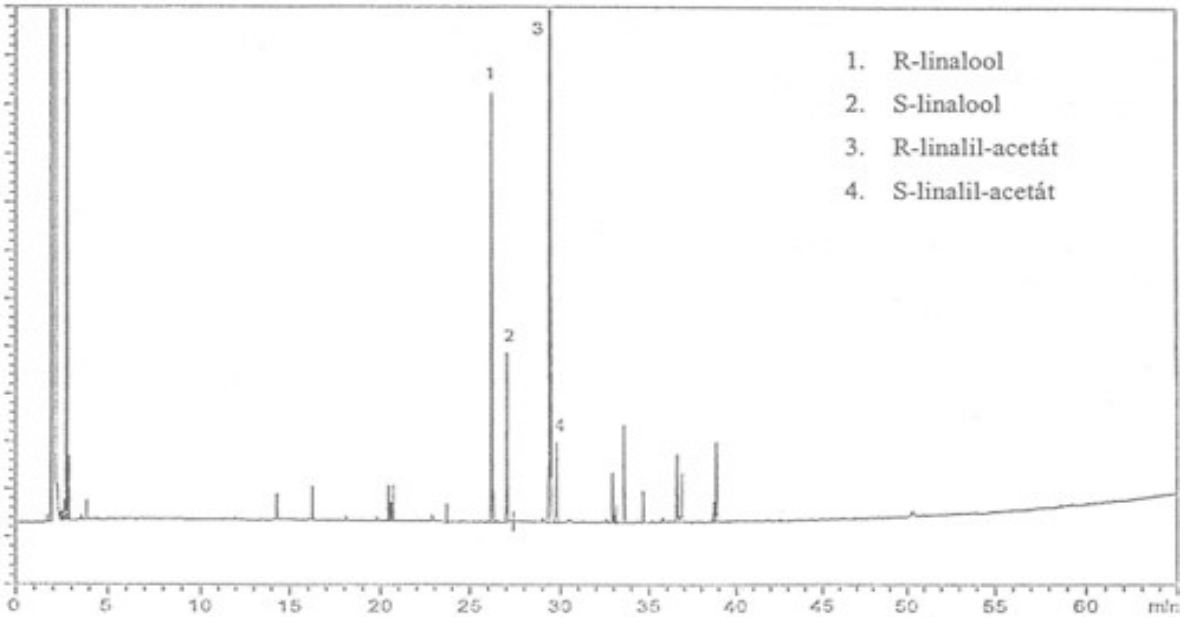
Drug content of medicine



butyl-hidroxi-anisol content

Pharmaceutical applications

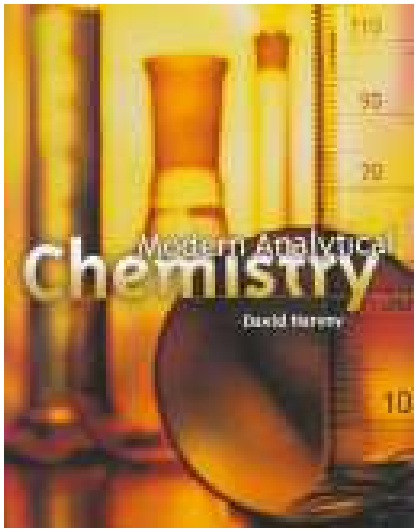
- **Analysis of bioacitve compounds of medicinal plants**
- **Analysis of essential oils**

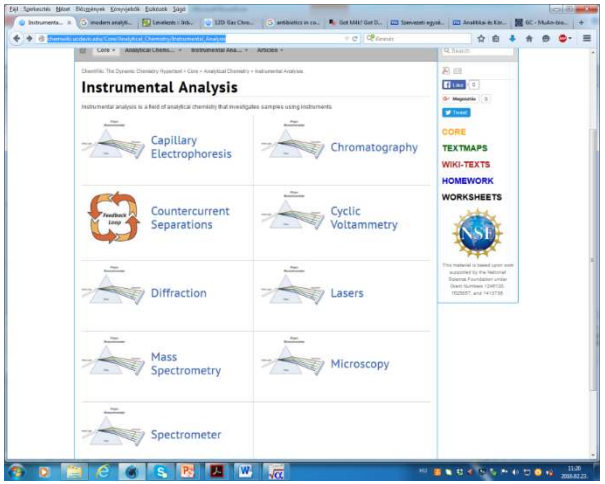


Esstential oil of Choisya ternata Aurea

Literature

Modern analytical chemistry *David Harvey*





http://chemwiki.ucdavis.edu/Core/Analytical_Chemistry/Instrumental_Analysis/

Thank you for your attention!

- See you on GC lab.
- INSTITUTE OF BIOANALYSIS
- Instructors: Anita Bufa, Viktória Poór

Agilent Technologies 6890NGC-5975 MS