ADVANCE IN FOOD ANALYSIS

INTRODUCTION TO GC

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Outcome

- GC instrumentation
- Separation and columns
- Injectors
- Detectors
- Linear retention index (LRI) in GC

Gas Chromatography - Instrumentation

Gas Chromatography

A.) Introduction:

Gas Chromatography (GC) - chromatographic technique where the mobile phase is a gas.

GC is currently one of the most popular methods for separating and analyzing compounds. This is due to its high resolution, low limits of detection, speed, accuracy and reproducibility.

GC can be applied to the separation of any compound that is either naturally volatile (i.e., readily goes into the gas phase) or can be converted to a volatile derivative. This makes GC useful in the separation of a number of small organic and inorganic compounds.

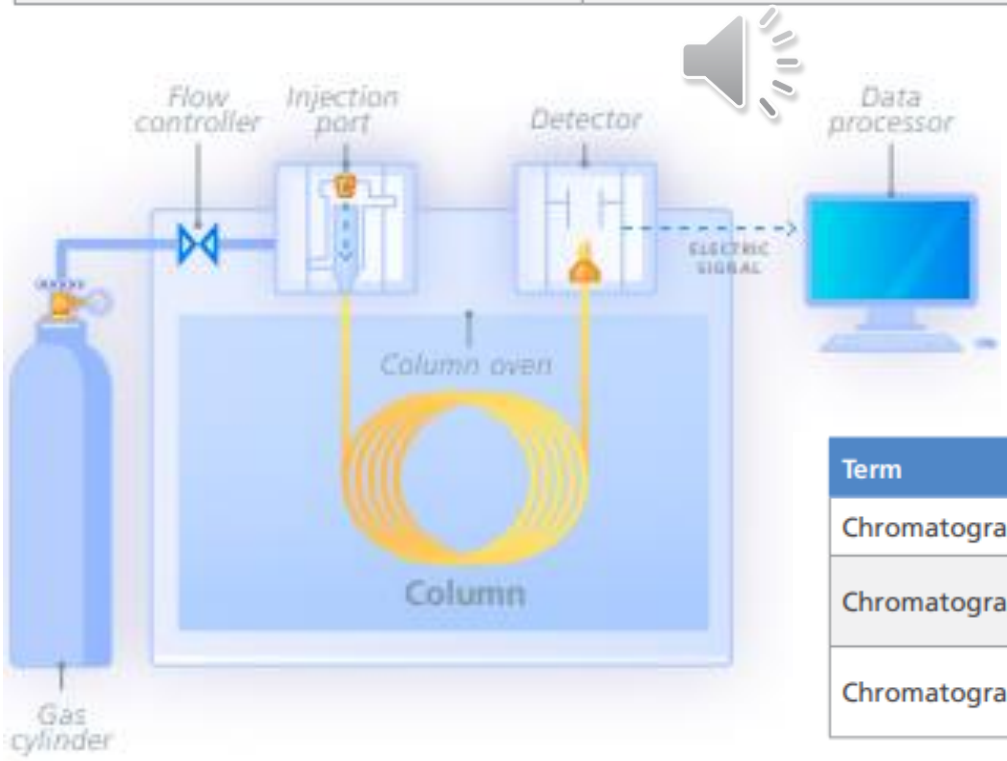
B.) Equipment:

A simple GC system consists of:

1. Gas source (with pressure and flow regulators)
2. Injector or sample application system
3. Chromatographic column (with oven for temperature control)
4. Detector & computer or recorder

Basic Gas Chromatography

Industry	Type of Analysis
Pharmaceutical	Residual solvent analysis
Food and beverages	Component analysis, food safety analysis, halal analysis of alcohol
Environmental	Air, water, soil
Petrochemicals	Simulated distillation, component analysis
Chemicals	Material, polymer, additive, gas purity analysis, gas emission in automotives
Energy and gas	Artificial photosynthesis research



Term	Definition
Chromatography	Method for Separation
Chromatograph	Instrument for Chromatography
Chromatogram	Data of Chromatography

Choice of Carrier Gas

Carrier Gas	Advantages	Disadvantages
Helium	• Safe • Relatively wide optimum linear velocity range	• Expensive
Nitrogen	• Cheap • Safe	• Optimum linear velocity range is narrow and slow • Long analysis time

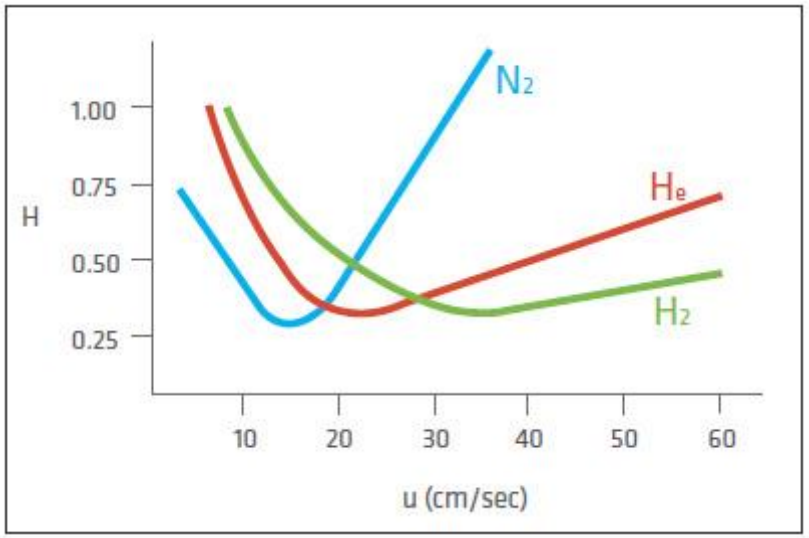
Choice of Carrier Gas

Mobile Phase/Carrier gas:

Carrier Gas or Mobile phase does not affect solute retention, but does affect:

1.) Desired efficiency for the GC System

- **low molecular weight gases (He, H_2) → larger diffusion coefficients**
- **low molecular weight gases → faster, more efficient separations**



2.) Stability of column and solutes

- **H_2 or O_2 can react with functional groups on solutes and stationary phase or with surfaces of the injector, connections and detector**

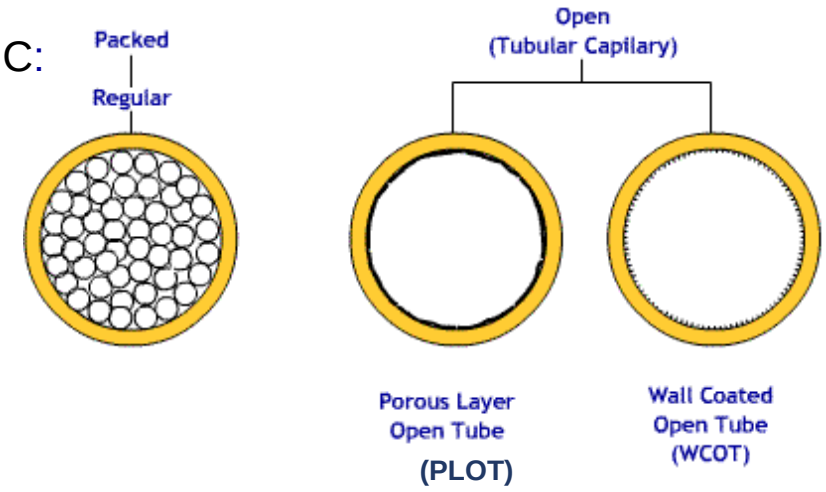
3.) Response of the detector

- **thermal conductor requires H_2 or He**
- **other detectors require specific carrier gas**

Columns

There are two main types of supports used in GC:

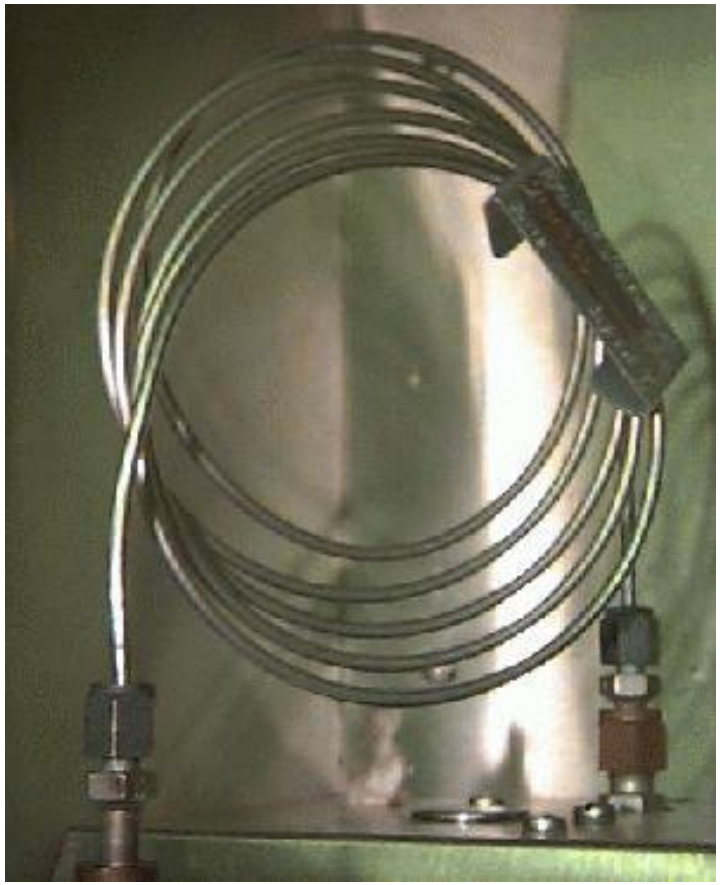
- Packed columns
  - ② large sample capacity
  - ② preparative work
- Capillary (open-tubular) columns
  - ② higher efficiency
  - ② smaller sample size
  - ② analytical applications



Recommended stationary phases for various sample types

Compound to be separated	Types of stationary phases used		
gases	alumina, silica gel, zeolites (molecular sieves) porous polymers	Gas:solid	GSC
nonpolar liquids	methylsiloxanes	Gas:liq	GLC
PCBs, petrochemical samples	phenylmethylsiloxanes, polysiloxane carboranes		
herbicides/pesticides, pharmaceuticals	phenyl polysilphenylene siloxanes		
sugars	cyanopropylphenyl methylsiloxanes		
free fatty acids, alcohols	polyethylene glycols		
alcohols , amines	phenylmethylsiloxanes (>50% phenyl)		

GC Columns



Inox - glas

« Fused silica »

Packed column versus Capillary in GC

One of the main effort of analysts has been focused on increase of separation power

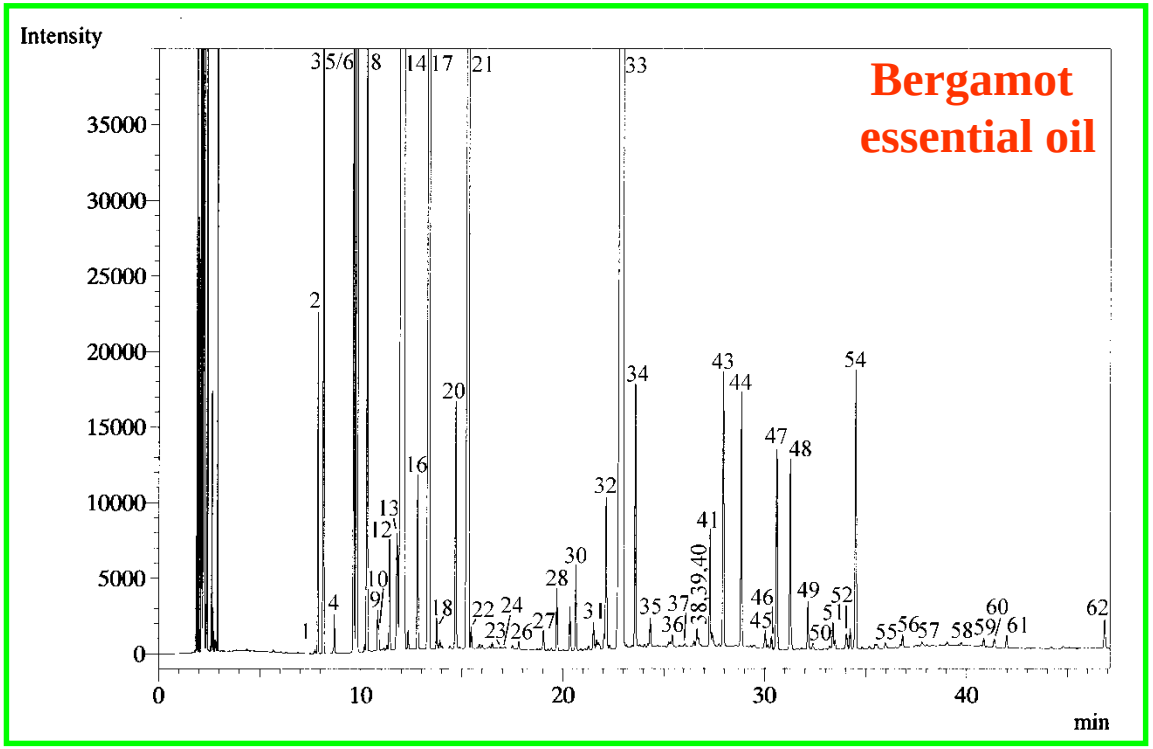
1D GC

Golay M.J.E., **1958**, Gas chromatography. New York:Accademic press

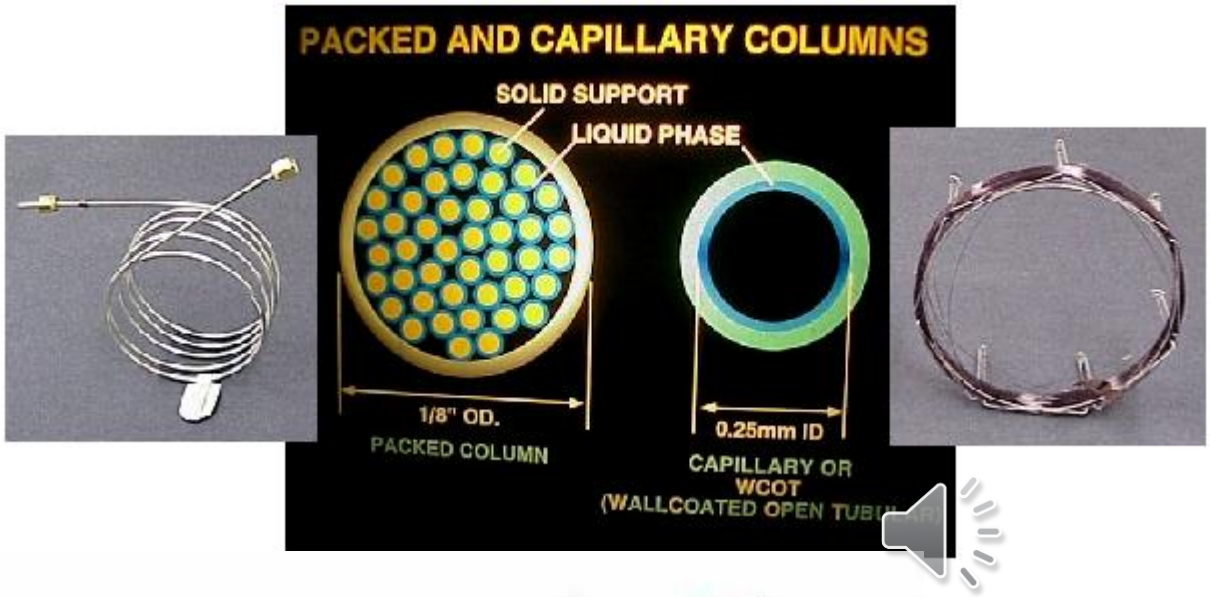
Packed column → capillary column

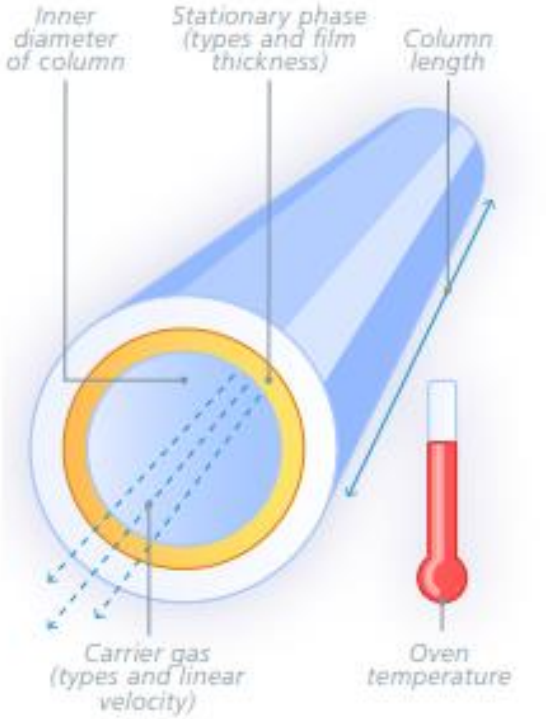
peak capacities in the 20-50 range

peak capacities in the 400-600 range

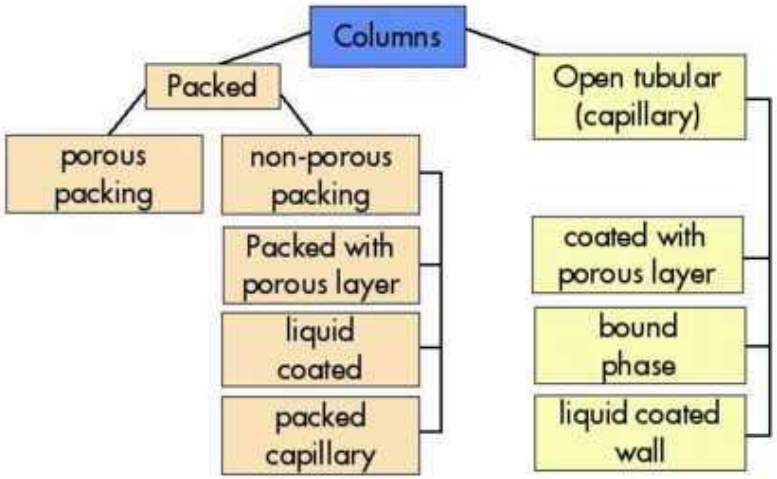


GC Columns

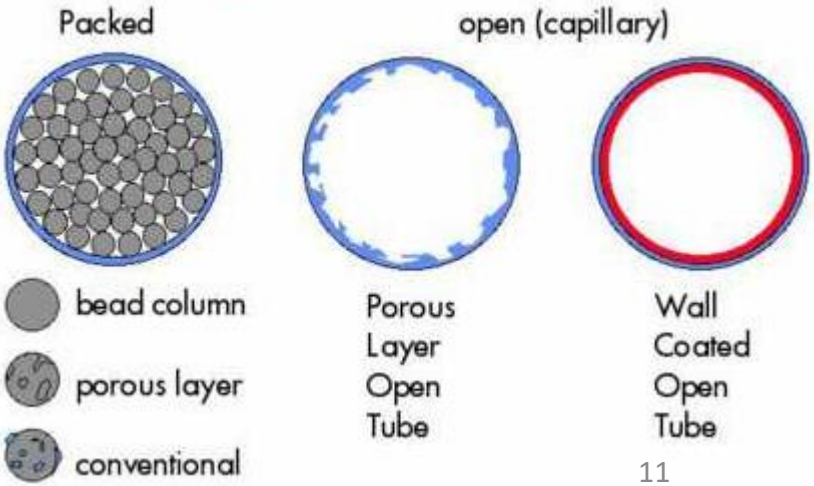




Types of columns



Types of columns



GC-COLUMNs

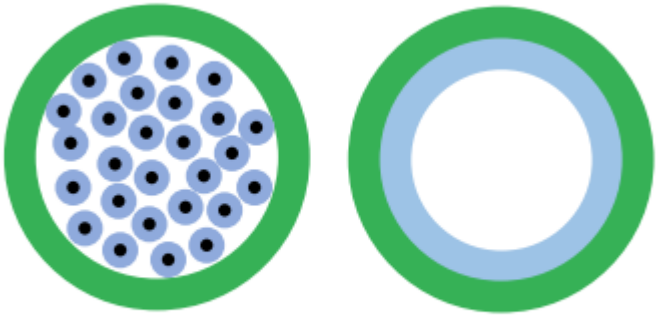
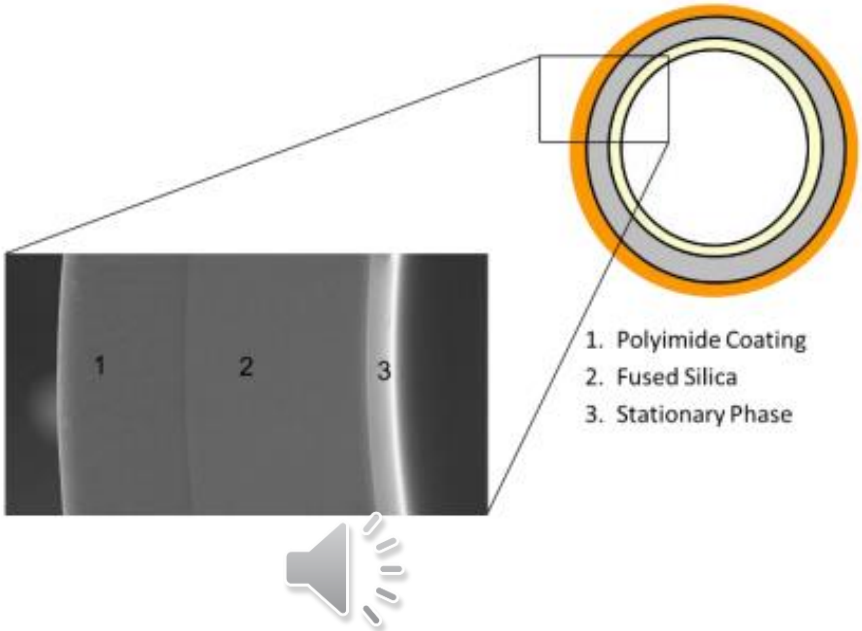


Figure 1. Packed GC columns (left) and capillary GC columns (right). Stationary phase appears in blue.

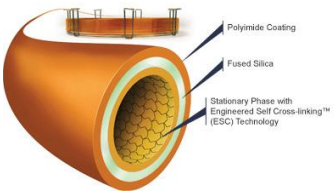


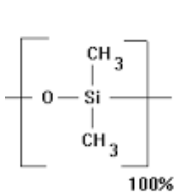
PACKED COLUMN VERSUS CAPILLARY COLUMN

PACKED COLUMN	CAPILLARY COLUMN
A column that contains a fully-packed stationary phase made up of fine particles	A column whose stationary phase is coated on the inner surface
Has a packed stationary phase	Stationary phase is coated on the inner surface
Require a large amount of the sample	Requires only a small amount of the sample
Have high pressures inside the column	Have less pressure inside the column
Short	Long
Diameter can be several millimeters	Diameter is around 1 mm
Efficiency is low	Efficiency is high
Give comparatively a poor resolution	Give a higher resolution
Less expensive	More expensive
Better for separating non polar samples since their tube is stainless steel	Better for separating polar samples since their tube is glass

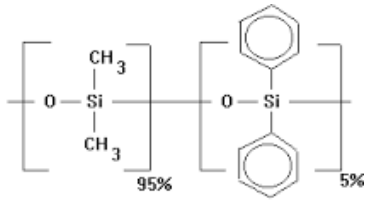
Bonded-Phase Gas chromatography

- covalently attach stationary phase to the solid support material
- bonded phases are prepared by reacting the desired phase with the surface of a silica-based support
- many bonded phases exist, but most separations can be formed with the following commonly recommended bonded-phases:
  - ② Dimethylpolysiloxane
  - ② Methyl(phenyl)polysiloxane
  - ② Polyethylene glycol (Carbowax 20M)
  - ② Trifluoropropylpolysiloxane
  - ② Cyanopropylpolysiloxane





"X"-1



"X"-5

Polydimethyl siloxane

$$HO—CH_2—CH_2—(O—CH_2—CH_2)_n—OH$$

Polyethylene glycol

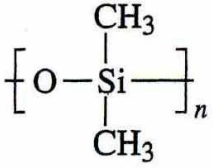
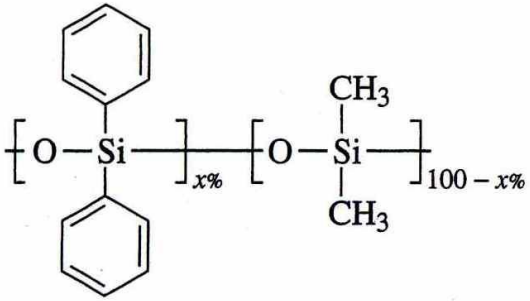
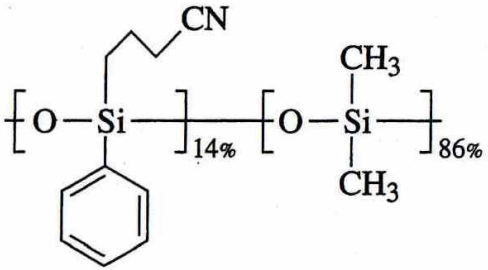
Some Common Liquid Stationary Phases for Gas-Liquid Chromatography

Stationary Phase	Common Trade Name	Maximum Temperature, °C	Common Applications
Polydimethyl siloxane	OV-1, SE-30	350	General-purpose nonpolar phase, hydrocarbons, polynuclear aromatics, steroids, PCBs
5% Phenyl-polydimethyl siloxane	OV-3, SE-52	350	Fatty acid methyl esters, alkaloids, drugs, halogenated compounds
50% Phenyl-polydimethyl siloxane	OV-17	250	Drugs, steroids, pesticides, glycols
50% Trifluoropropyl-polydimethyl siloxane	OV-210	200	Chlorinated aromatics, nitroaromatics, alkyl-substituted benzenes
Polyethylene glycol	Carbowax 20M	250	Free acids, alcohols, ethers, essential oils, glycols
50% Cyanopropyl- polydimethyl siloxane	OV-275	240	Polyunsaturated fatty acids, rosin acids, free acids, alcohols

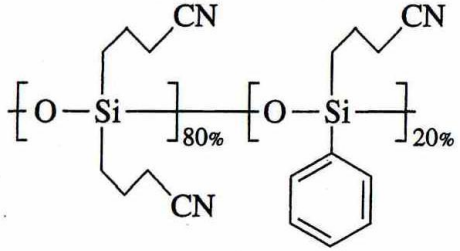
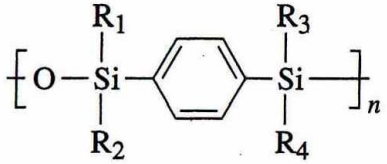
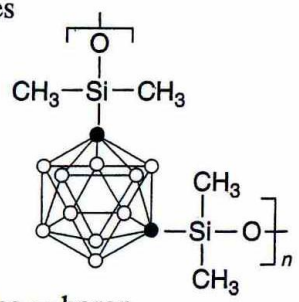
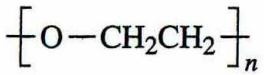
GC Columns

Stationary phase selection

Capillary Fused Silica Stationary Phases

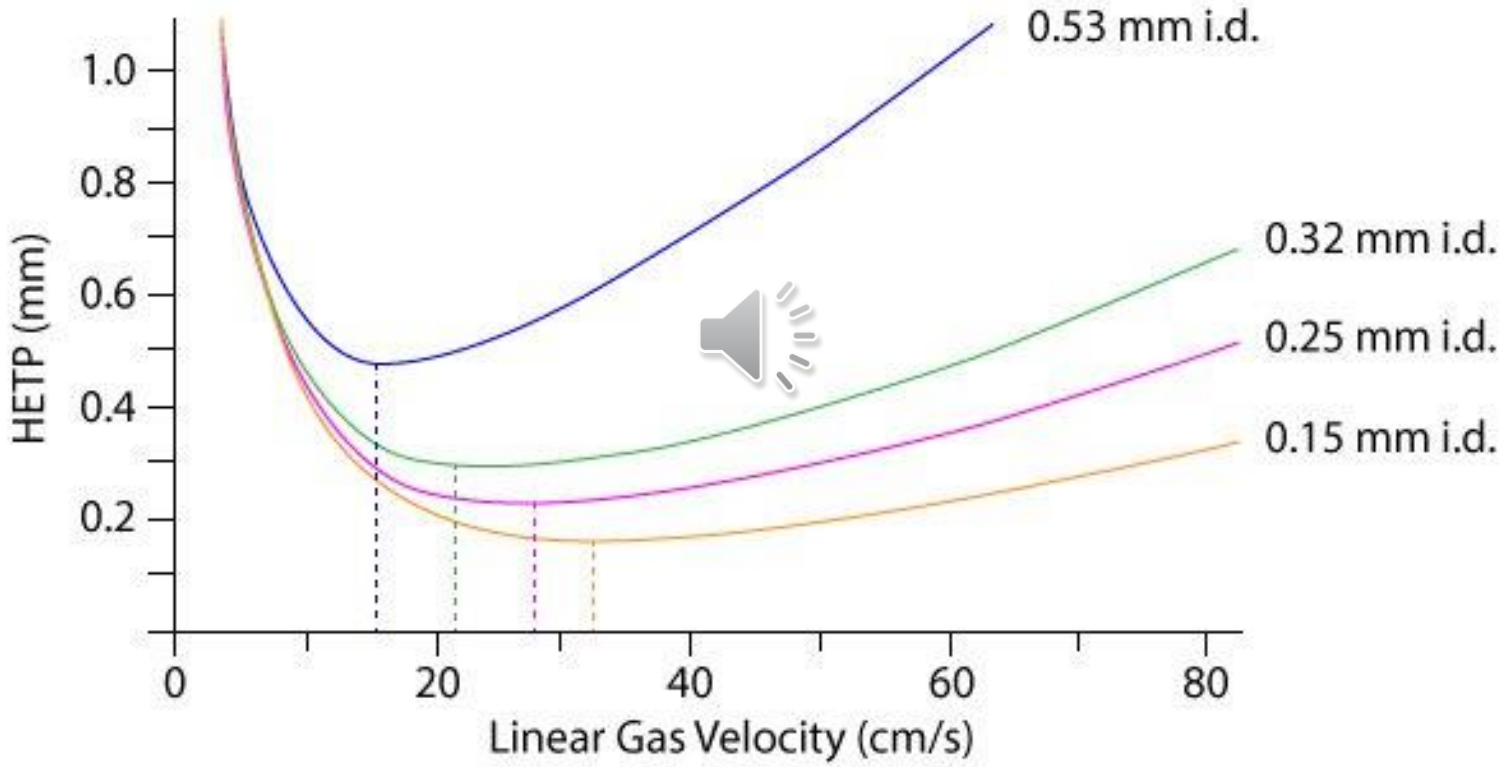
Phase	Polarity	Use	Max. Temp. (°C)
100% Dimethyl polysiloxane 	Nonpolar	Basic general-purpose phase for routine use. Hydrocarbons, polynuclear aromatics, PCBs.	320
Diphenyl, dimethyl polysiloxane 	5% Low 35%, 65% Intermediate 65%, 35% Intermediate	General-purpose, good high-temperature characteristics. Pesticides.	320 300 370
14% Cyanopropylphenyl–86% dimethylsiloxane 	Intermediate	Separation of organochlorine pesticides listed in EPA 608 and 8081 methods. Susceptible to damage by moisture and oxygen.	280

Stationary phase selection

80% Biscyanopropyl–20% cyanopropylphenyl polysiloxane 	Very polar	Free acids, polysaturated fatty acids, alcohols. Avoid polar solvents such as water and methanol.	275
Arylenes 	Vary R as above to vary polarity	High temperature, low bleed	300–350
Carboranes  open circles = boron filled circles = carbon	Vary R as above to vary polarity	High temperature, low bleed	430
Poly(ethyleneglycol) (Carbowax) 	Very polar	Alcohols, aldehydes, ketones, and separation of aromatic isomers, e.g., xylenes	250

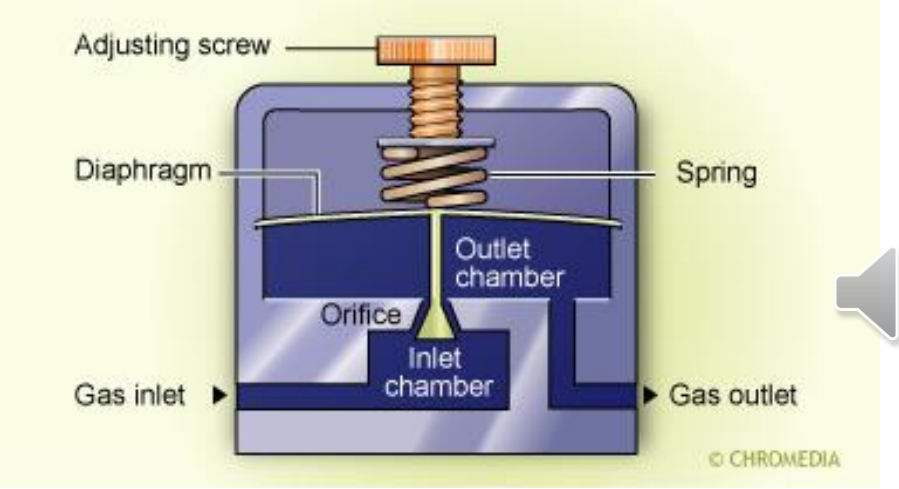
GC Columns

Practical information

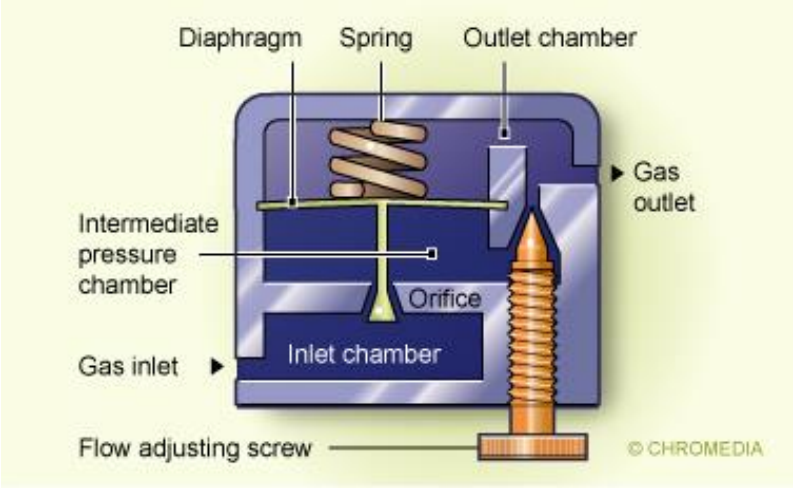


Pressure and Flow Control Modes

Pressure Regulator



Flow Controller



$$F = \frac{\pi r^4 p_c}{8\eta L}$$

P_c = column inlet pressure
L= column length

Poiseuille' law

More information are available

https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Supplemental_Modules_(Analytical_Chemistry)/Chromedia/01Gas_Chromotography_(GC)/Gas_Chromotography%3A_In_Practice/01Introduction/3Flow_control_and_pressure_regulation

Sample Injection Methods in GC

Sample Injection Methods

Inlet/Sample injector chambre

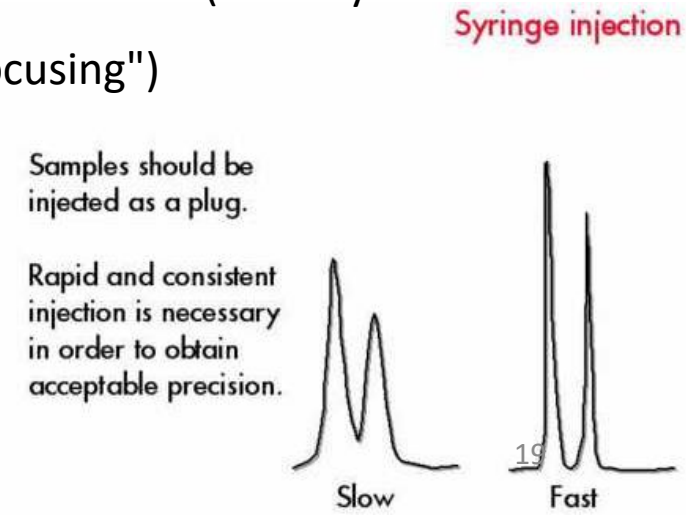
- **Ideal injection:**

1. Injection should be representative of the sample :

➔ No discrimination of the compounds (discrimination = different efficiency of injection on the studied molecules)

➔ good repeatability (analyse quantitative)

➔ Narrow injection band : rapid injection of small quantities of products (a few tens of ng / peak) and concentration at the head of the column (for very volatile molecules, sometimes need to re-concentrate them "Cryofocusing")



Sample Injection Methods

Inlet/Sample injector chambre

Objective criteria for an ideal injection

- Chemical inertia and sealing of the injector:
  - ➢ no oxidative degradation during vaporization (heated injectors)
  - ➢ no interfering signals due to sealing systems (septa and other mechanical parts)

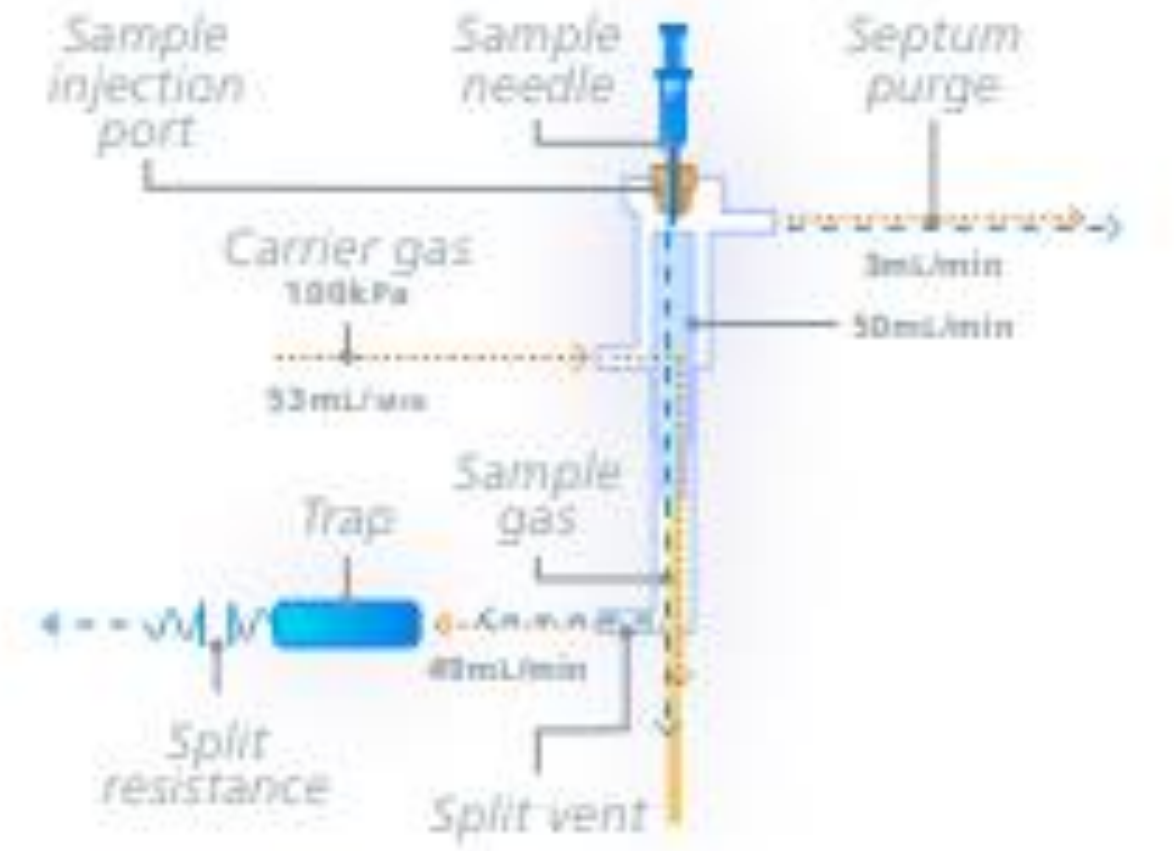
  NB: If pressure drops in the gas circuit are observed, this often indicates that the septum, worn by the multiple injections, must be changed.
- Automation possibilities
- Easy regulation of injection conditions (flow, temperature)

Sample Injection Methods

- **There are many different injection methods:**
  - split injection,
  - splitless injection
  - direct injection
  - on-column injection

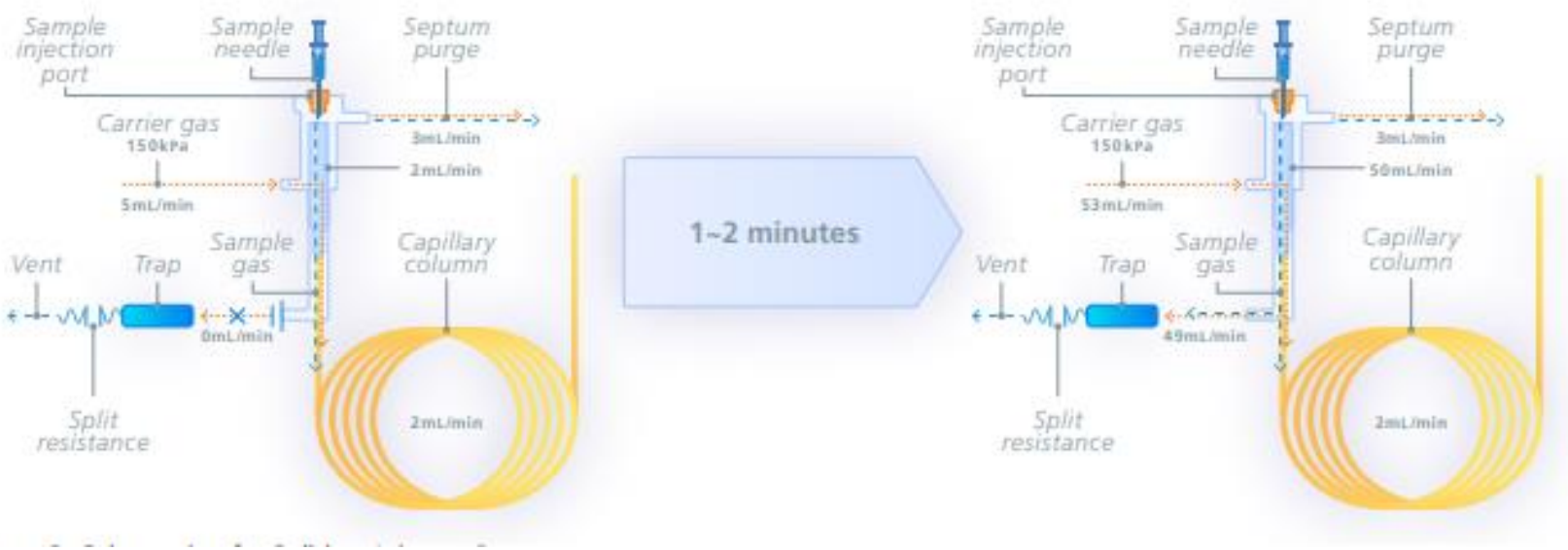
Sample Injection Methods
Split Injection

- There are many different injection methods:
  - **split injection**
  - splitless injection
  - direct injection
  - on-column injection



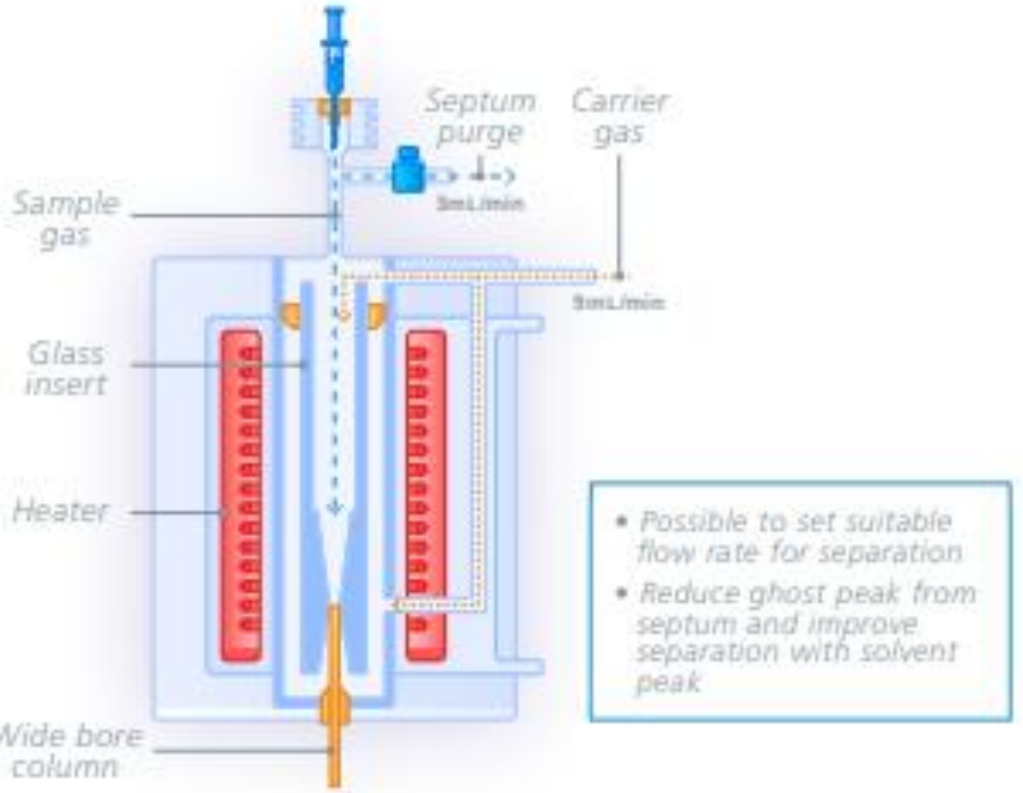
Sample Injection Methods
Splitless Injection

- There are many different injection methods:
  - split injection
  - **splitless injection**
  - direct injection
  - on-column injection



Sample Injection Methods
Direct Injection

- There are many different injection methods:
  - split injection
  - splitless injection
  - **direct injection**
  - on-column injection



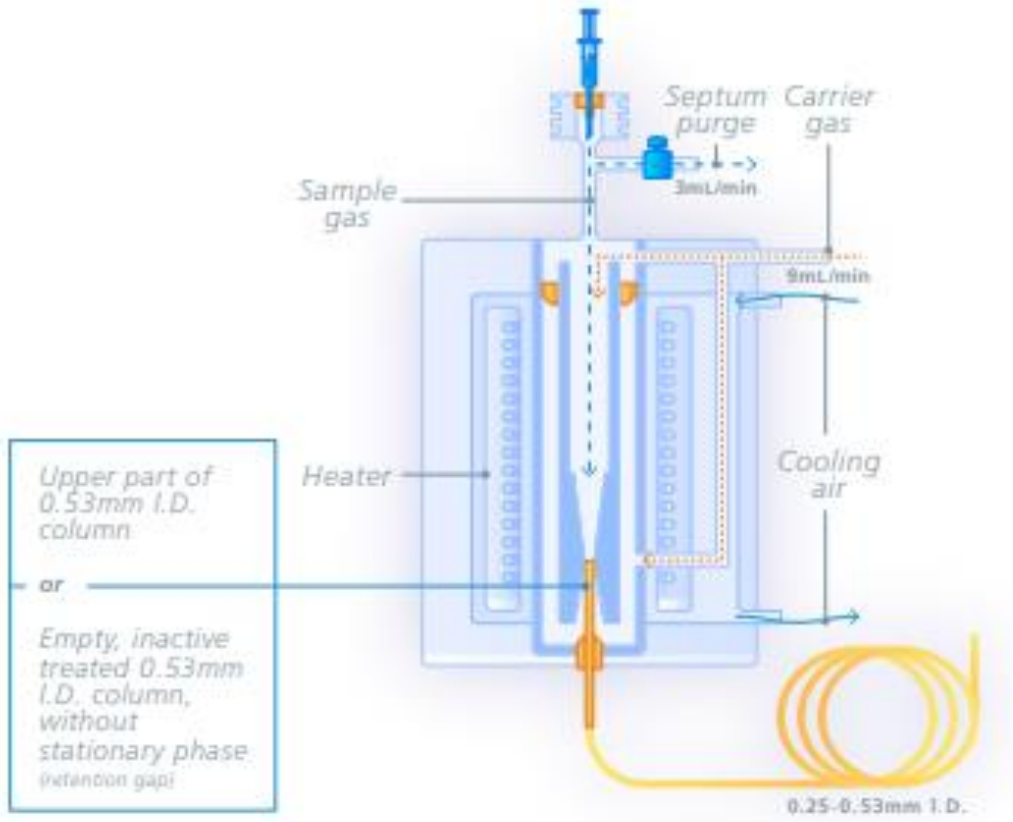
Sample Injection Methods
On-column Injection Methods

- There are many different injection methods:
  - split injection
  - splitless injection
  - direct injection
  - **on-column injection**

    a. Cold on-column caps Injection (Cold OCI)

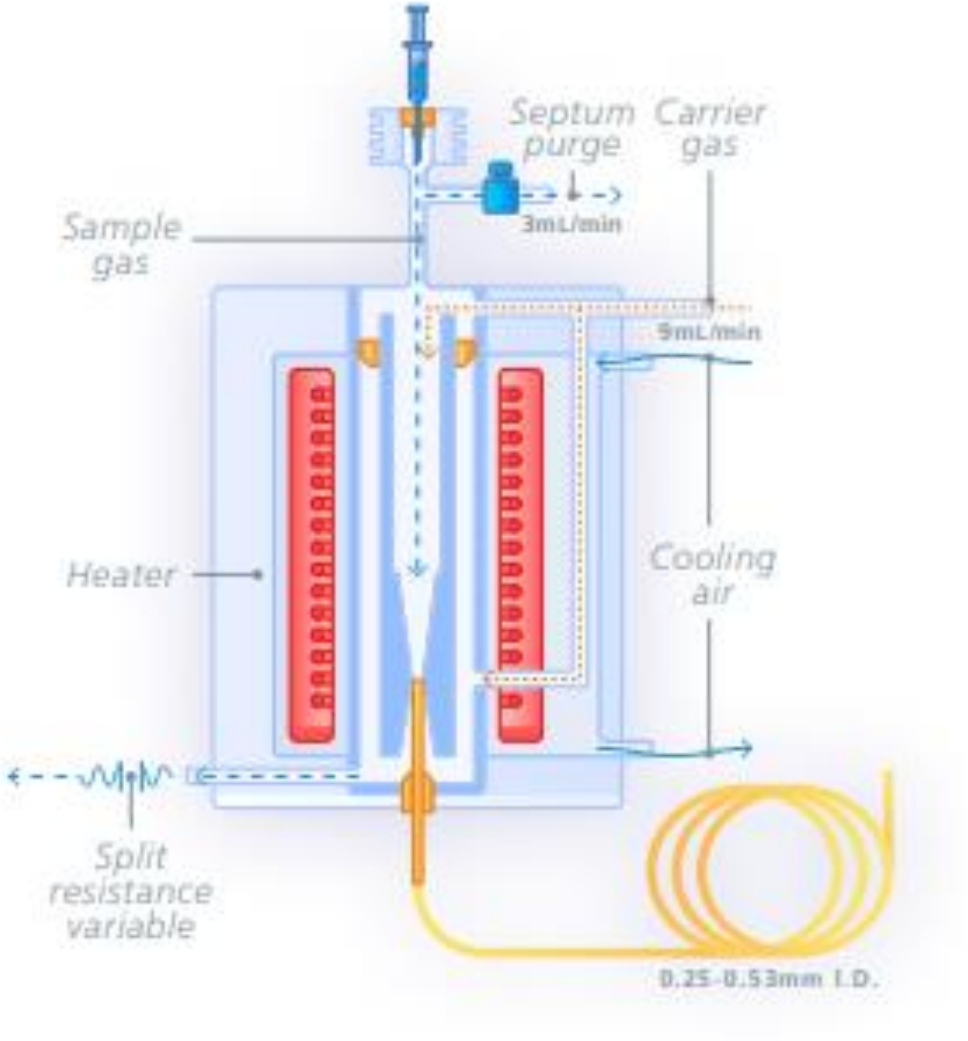
    b. PTV Injection System (Programmable Temperature Vaporizer)

Sample Injection Methods - Cold Injection Methods
Cold on-column caps Injection (Cold OCI)



- Samples with low concentrations (less than about 200 ppm per component) are also suitable.
- Risk of contamination.

Sample Injection Methods - Cold Injection Methods
PTV (Programmable Temperature Vaporizer) Injection System



GC Detectors

GC Detectors

Detector			Example of Detectable Compound	Example of Minimum Detectable Amount*
Universal Detector	Thermal Conductivity Detector	TCD	All compounds except for carrier gas	10 ppm (10 ng)
	Flame Ionization Detector	FID	Organic compounds	0.1 ppm (0.1 ng)
	Barrier Discharge Ionization Detector	BID	All compounds except for He and Ne	0.07 ppm (0.07 ng)
	Mass Spectrometer	MS	Ionized molecule	10 ppm (10 ng) in Scan mode 0.5 ppm (0.5 ng) in SIM mode 10 ppb (10 pg) in MRM mode
Selective High-sensitivity Detector	Electron Capture Detector	ECD	Organic Halogen compounds Organic mercury compounds	0.01 ppb (0.01 pg)
	Flame Photometric Detector	FPD	Sulfur compounds Organic phosphorus compounds Organic tin compounds	10 ppb (10 pg)
	Flame Thermionic Detector	FTD (NPD)	Organic phosphorus compounds Organic nitrogen compounds	0.1 ppb (0.1 pg) 1 ppb (1 pg)
	Sulfur Chemiluminescence Detector	SCD	Sulfur compounds	1 ppb (1 pg)

MS is explained in an another lesson

Features of GC Detectors.
This table serve as a rough indication, it may be different depending on the compound chemical structure and analytical condition

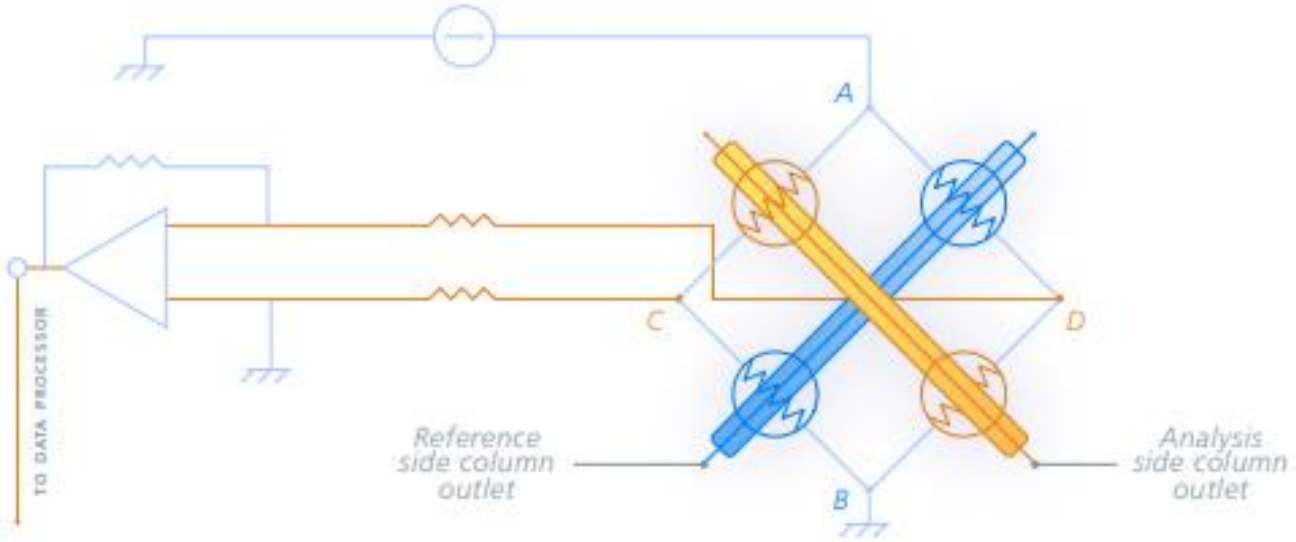
GC Detectors
Thermal Conductivity Detector (TCD)

- katherometer or hot-wire detector
  - first universal detector developed for GC

Compounds	Thermal Conductivity Constant (10^{-6} cal/s-cm-°C)
He	408
H_2	547 (Very High)
N_2	73
Ar	52
O_2	76
H_2O	60
Ethane	77
Methanol	52
Acetone	40
Chloroform	24

Process

- measures a bulk property of the mobile phase leaving the column.
- measures ability to conduct heat away from a hot-wire (i.e., thermal conductivity)
- thermal conductivity changes with presence of other components in the mobile phase



The voltage or a direct current is applied between A and B. While only carrier gas is flowing at constant flow, each filament is kept at constant temperature and shows constant voltage between C and D.

Components are eluted from an analysis side column.

→ *The temperature of filament rises up (since the thermal conductivity is smaller than that of carrier gas, resistance value changes).*

→ *Voltage between C and D changes.*

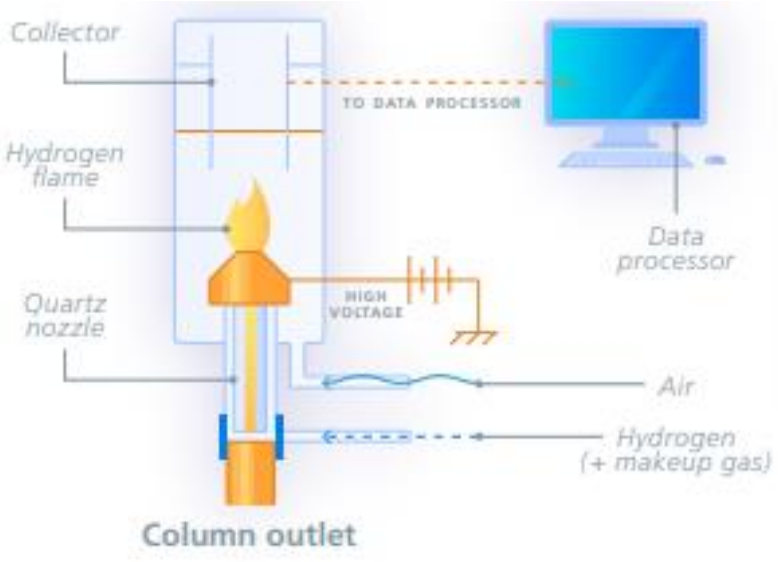
GC Detectors
Flame Ionization Detector (FID)

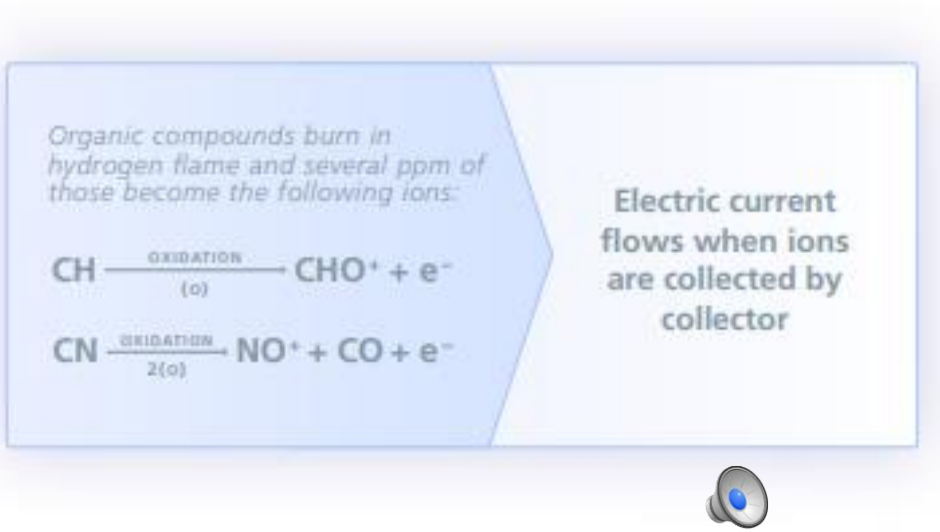
- most common type of GC detector

- “universal” detector capable of measuring the presence of almost any organic and many inorganic compound

Process

- measures the production of ions when a solute is burned in a flame in presence of H_2 and Air.
    - ions are collected at an electrode to create a current





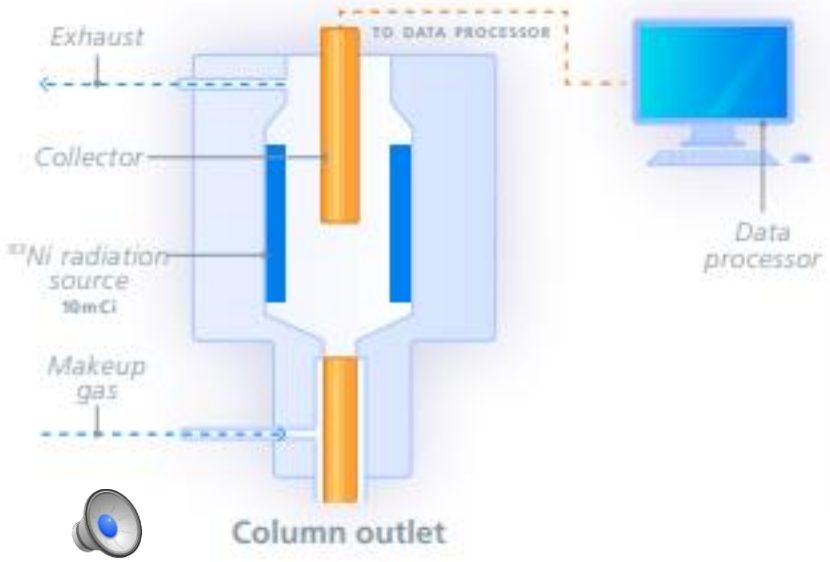
GC Detectors
Barrier Discharge Ionization Detector (BID)

- The barrier discharge ionization detector (BID– is a universal detector that offers high–sensitivity analysis by using a low–frequency dielectric barrier discharge plasma for ionization.

GC Detectors
Electron Capture Detector (ECD)

- radiation-based detector
- selective for compounds containing electronegative atoms, such as halogens
- detects also polynuclear aromatic compounds, anhydrides and conjugated carbonyl compounds
- useful for environmental testing

Relative responses	
10^0	hydrocabons
10^1	esters, ethers
10^2	alcohols, ketones, monochlorides, amines
10^3	monobromides, dichlorides
10^4	anhydrides, trichlorides
10^5 - 10^6	poly halogenated, mono and diiodo



N_2 used as carrier gas (makeup gas) is ionized by the beta ray emitted from ^{63}Ni.

$N_2 \xrightarrow{\beta \text{ ray}} N_2^+ + e^-$

Electric current flows when electrons are captured by collector (background current)

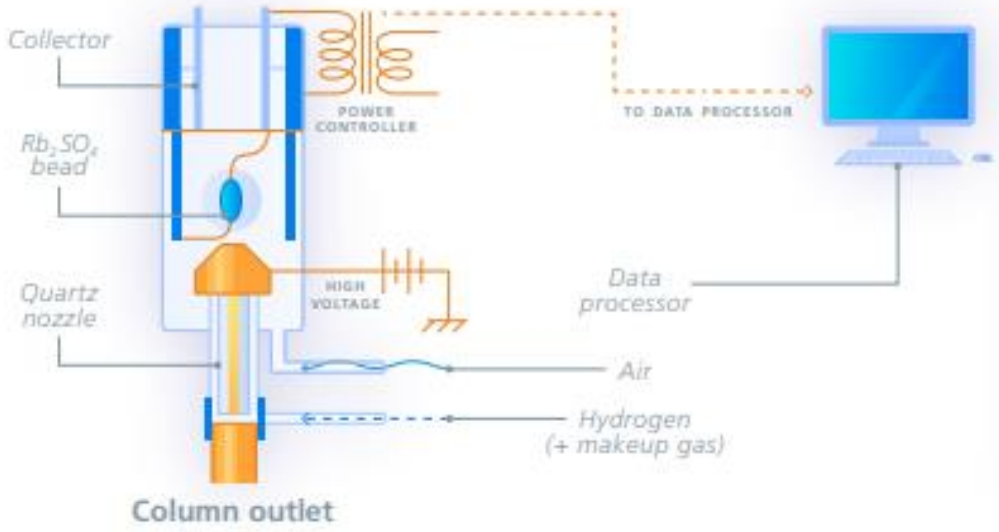
If electrophilic compounds, such as PCB, enter then

$PCB + e^- \longrightarrow PCB^-$

Since PCB is highly electrophilic, it will absorb the background electrons
— Ion current decreases
— Peak detected

GC Detectors
Flame Photometric Detector (FPD)

- The detection principle is the formation of excited sulphur (S2*) and excited hydrogen phosphorous oxide species (HPO*) in a reducing flame.



When the platinum coil to which rubidium sulfate adhered to is heated by electric current, plasma-like atmosphere is generated into the surrounding of Rb_2SO_4 bead. In this atmosphere, Rb^* (rubidium radical) is generated, and –CN and $-PO_2$ (generates by the oxidation of organic phosphorus compounds) react as follows with Rb^*, and become ions.

$$CN + Rb^* \longrightarrow CN^- + Rb^+$$
$$PO_2 + Rb^* \longrightarrow PO_2^- + Rb^+$$

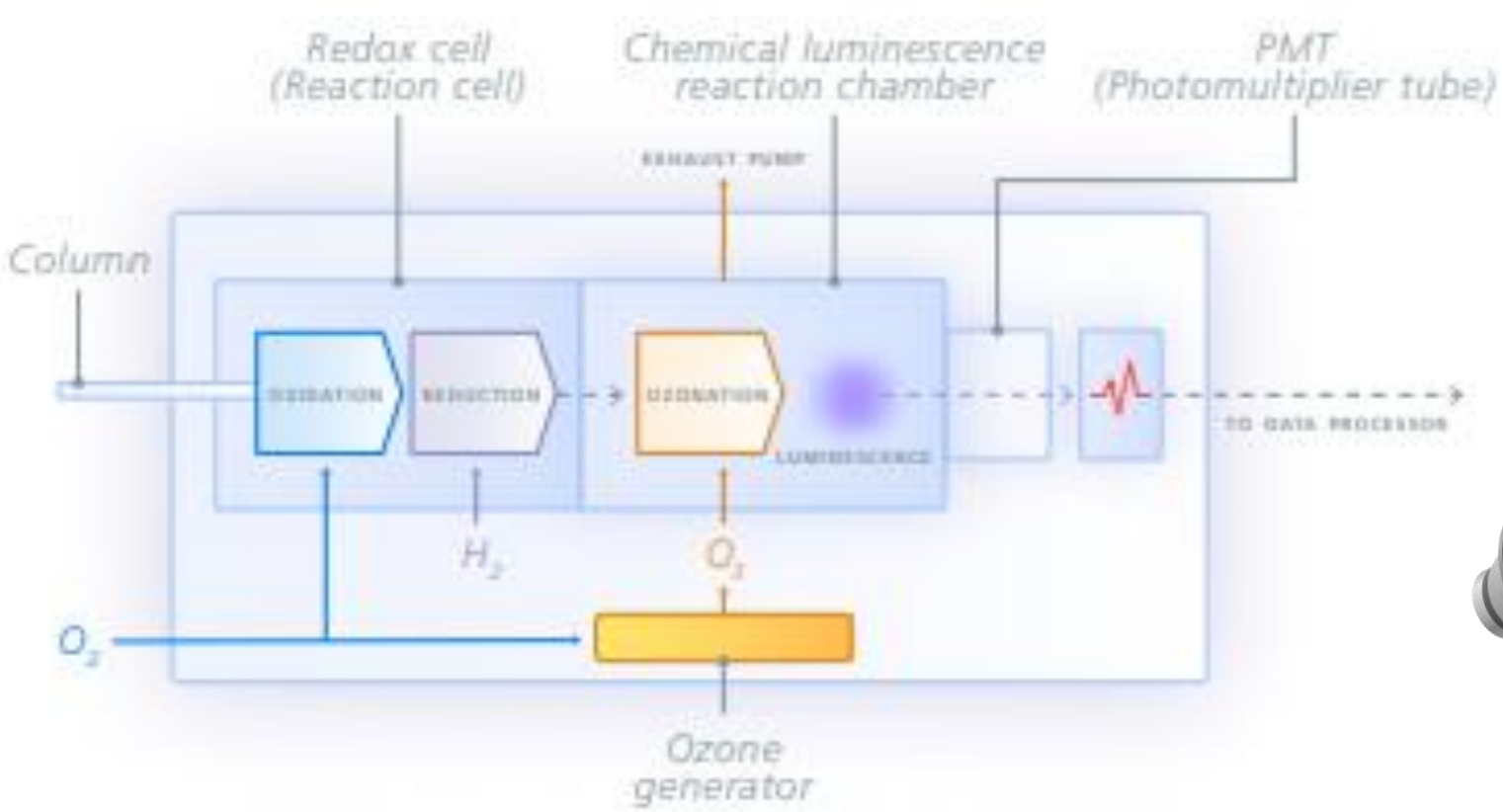
Electric current flows when ions are collected by collector

GC Detectors
Sulfur Chemiluminescence Detector (SCD)

$R\text{-}S + O \rightarrow SO + H\text{-}R + H_2O$

$SO + O_3 \rightarrow SO_2^* + O_2$

$SO_2^* \rightarrow SO_2 + h\nu\ (300\sim400nm)$

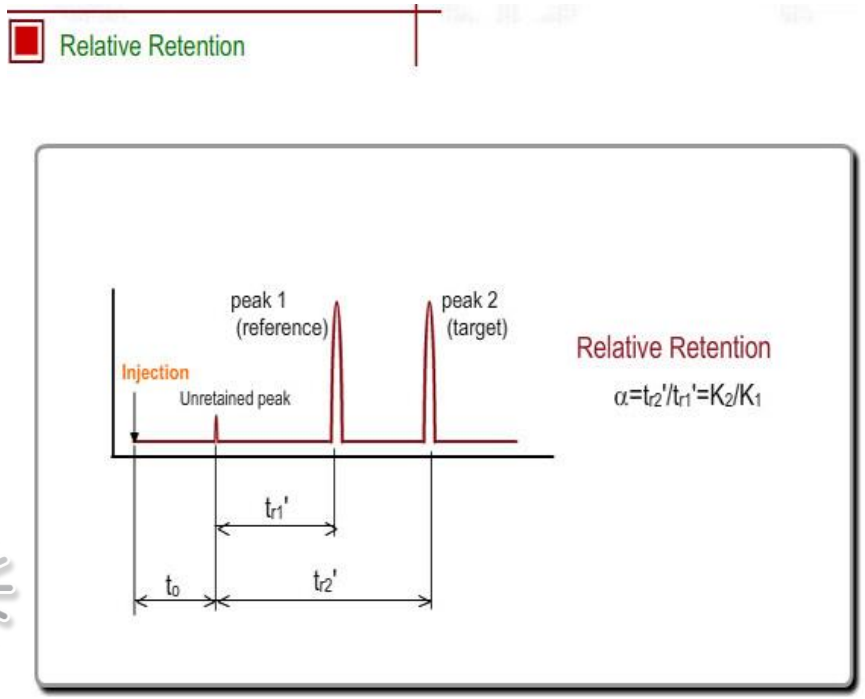


Linear retention index

Relative retention time (RRT)

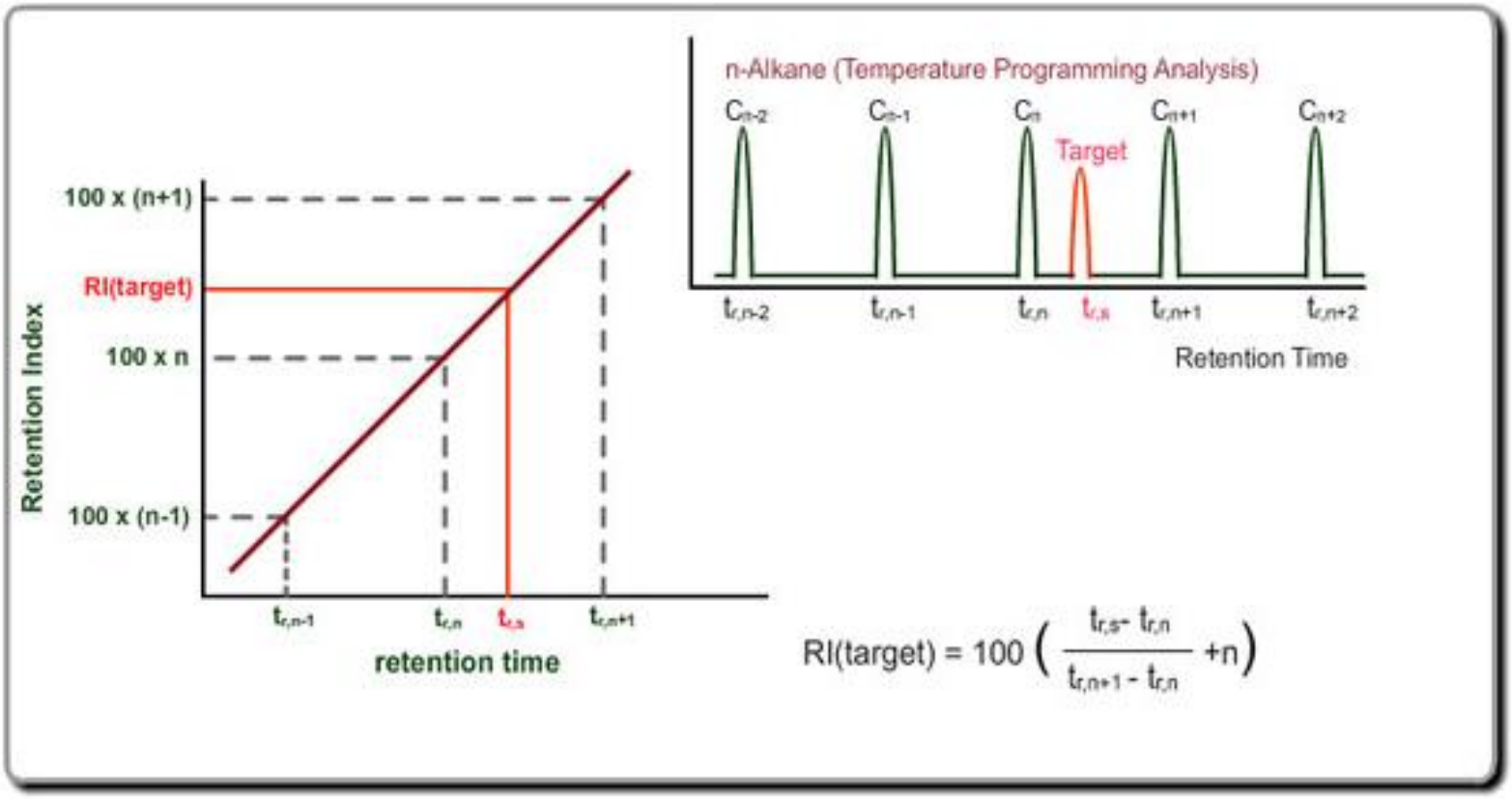
RRT (relative retention time) = t'_{rB} / t'_{rA}

- **t'_{rA}= retention time of a reference analyte intentionally added or naturally present**

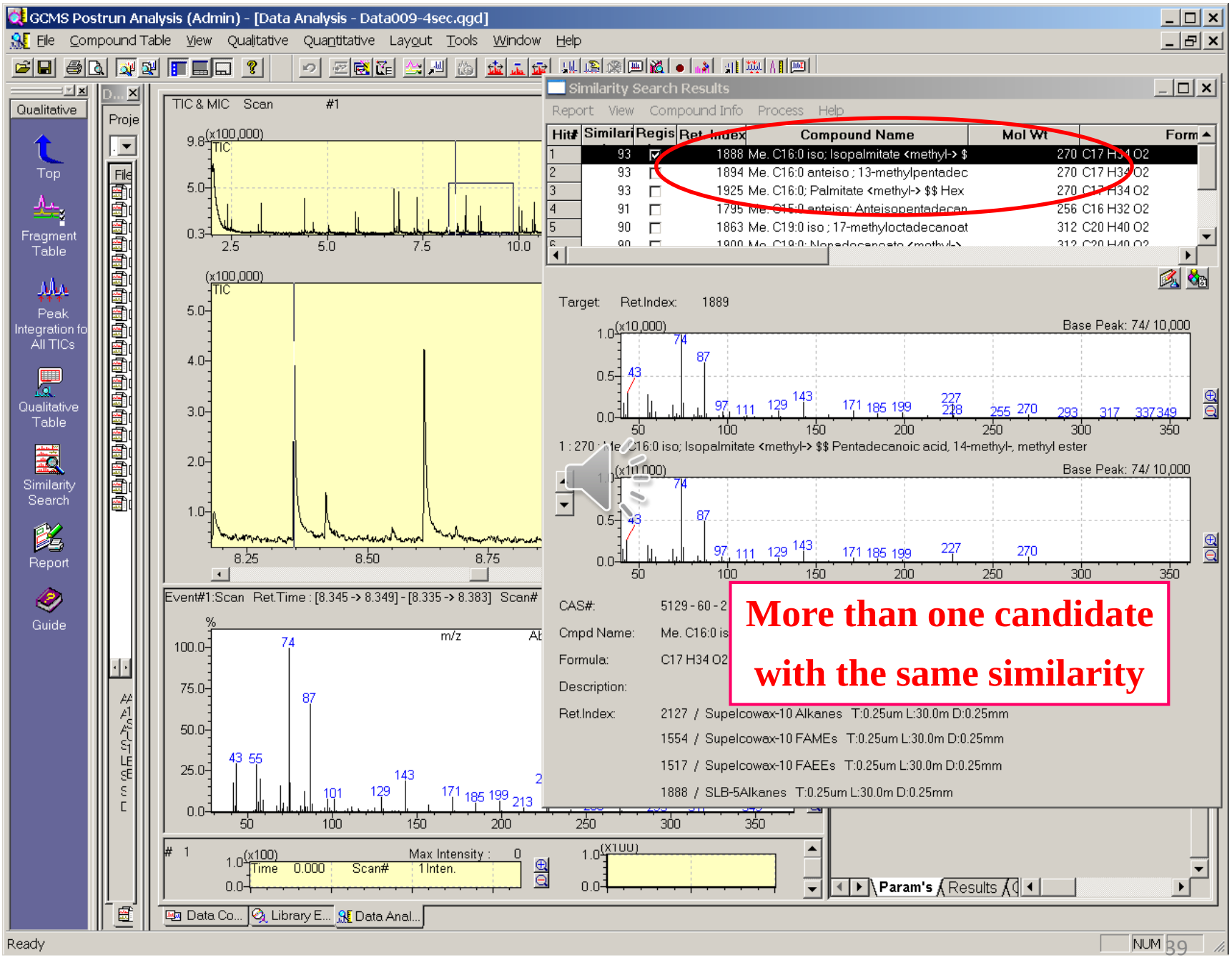


RRT depends on the stationary phase ➔ support identification of the analytes by comparison with reference using the same stationary phase

Linear retention index (Kovats retention index)

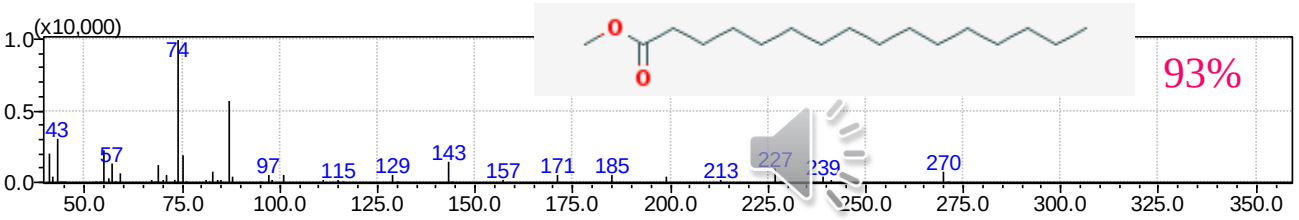


$$RI(target) = 100\left(\frac{t_{r,s} - t_{r,n}}{t_{r,n+1} - t_{r,n}} + n\right)$$


GCMS Postrun Analysis (Admin) - [Data Analysis - Data009-4sec.qgd]
File Compound Table View Qualitative Quantitative Layout Tools Window Help
Qualitative
Top
Fragment Table
Peak Integration fo All TICs
Qualitative Table
Similarity Search
Report
Guide
TIC & MIC Scan #1
(x100,000)
TIC
Similarity Search Results
Report View Compound Info Process Help
Hit# Similari Regis Ret. Index Compound Name Mol Wt Form
1 93 1888 Me. C16:0 iso; Isopalmitate <methyl-> $ 270 C17 H34 O2
2 93 1894 Me. C16:0 anteiso ; 13-methylpentadec 270 C17 H34 O2
3 93 1925 Me. C16:0; Palmitate <methyl-> $$ Hex 270 C17 H34 O2
4 91 1795 Me. C15:0 anteiso; Anteisopentadecan 256 C16 H32 O2
5 90 1863 Me. C19:0 iso ; 17-methyloctadecanoat 312 C20 H40 O2
Target: Ret.Index: 1889
Base Peak: 74/ 10,000
1 : 270 : Me. C16:0 iso; Isopalmitate <methyl-> $$ Pentadecanoic acid, 14-methyl-, methyl ester
CAS#: 5129 - 60 - 2
Cmpd Name: Me. C16:0 is
Formula: C17 H34 O2
Description:
Ret.Index: 2127 / Supelcowax-10 Alkanes T:0.25um L:30.0m D:0.25mm
1554 / Supelcowax-10 FAMEs T:0.25um L:30.0m D:0.25mm
1517 / Supelcowax-10 FAEEs T:0.25um L:30.0m D:0.25mm
1888 / SLB-5Alkanes T:0.25um L:30.0m D:0.25mm
More than one candidate with the same similarity
Event#1:Scan Ret.Time : [8.345 -> 8.349] - [8.335 -> 8.383] Scan#
Max Intensity : 0
Time 0.000 Scan# 1 Inten.
Param's Results
Data Co... Library E... Data Anal...
Ready
NUM

GC-MS and FAMEs: some disadvantages

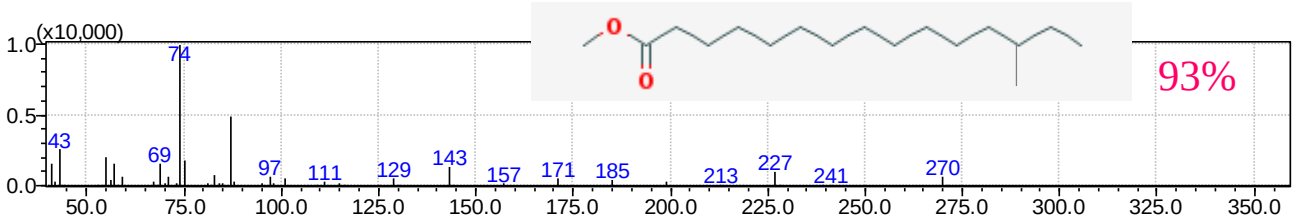
- Spectral similarity
- Low reliability of commercial libraries



93%

C16:0

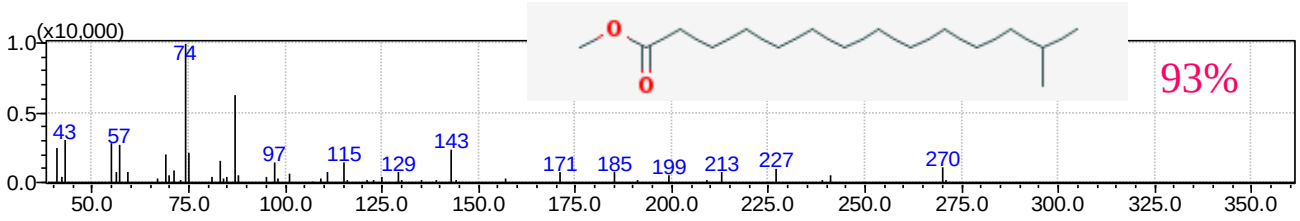
LRI: 1925



93%

iC16:0

LRI: 1888



93%

aC16:0

LRI: 1894