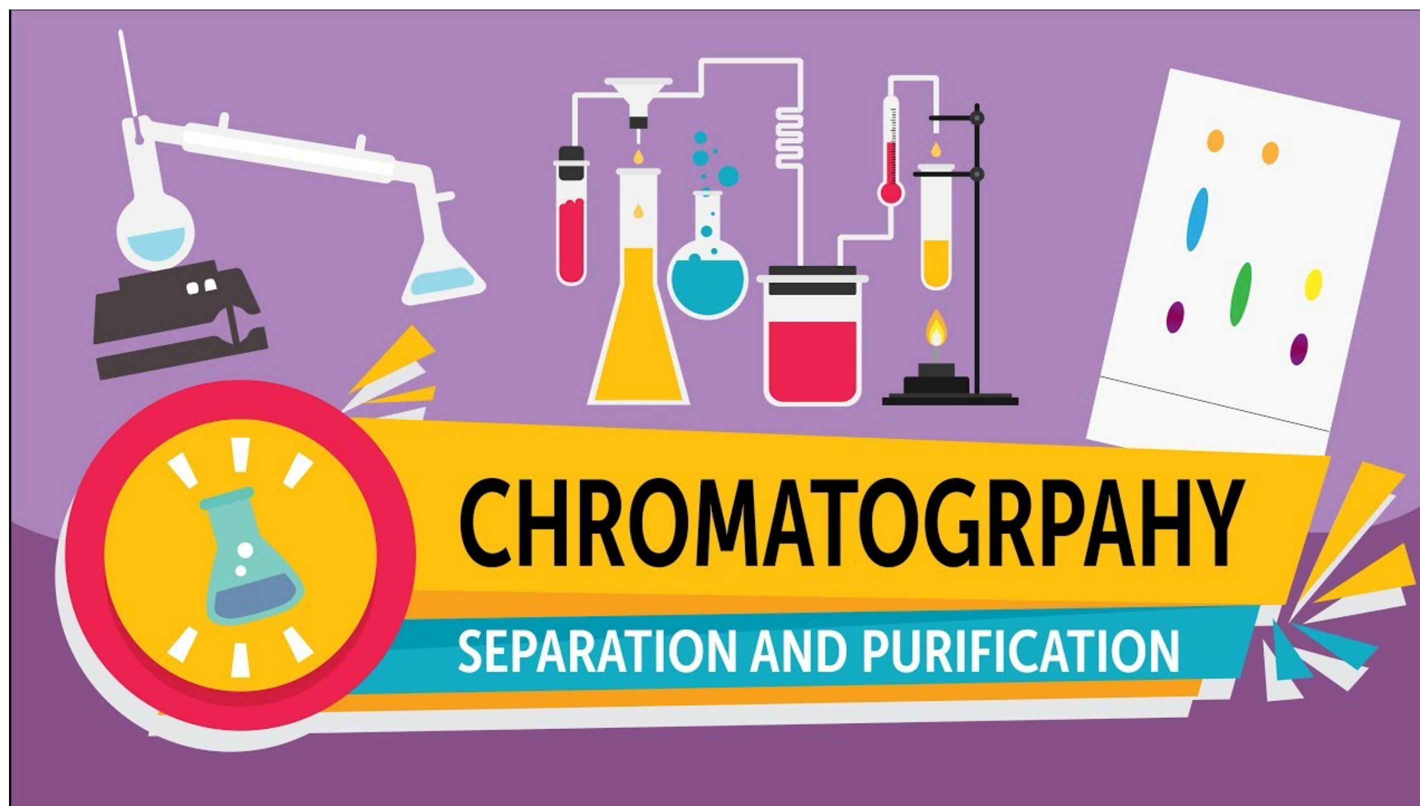
Chromatography



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Invention of Chromatography

Mikhail Tswett invented chromatography in **1901** during his research on plant pigments.

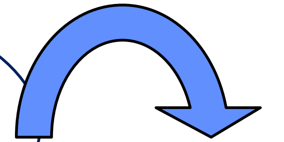
He used the technique to separate various plant pigments such as chlorophylls, xanthophylls and carotenoids.

Mikhail Tswett
Russian Botanist
(1872-1919)

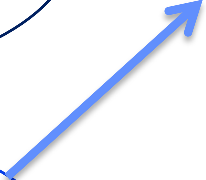
ether
chlorophyll
$CaCO_3$
Chromatography
colors

Original Chromatography Experiment

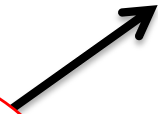
EtOH is used to flush the pigments down the column.

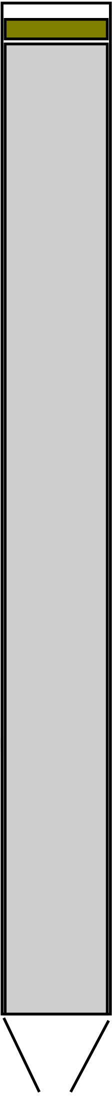


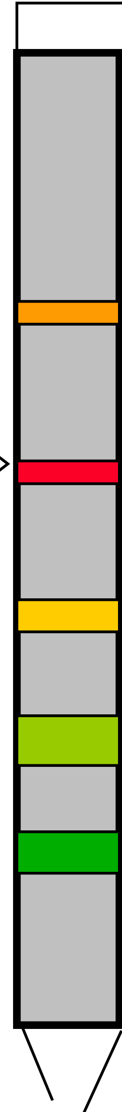
An EtOH extract of leaf pigments is applied to the top of the column.



Start: A glass column is filled with powdered Limestone ($CaCO_3$).







Separated Coloured bands of chlorophylls, xanthophylls carotenoids

Definitions

- Chromatography is a separation method. (Chromatography is not an identification method like NMR, IR, MS)

- Chromatography consist of two phases: mobile and stationary phase.

- Mobile phase is forced along the column from injection to detector as a flowing media.

- Stationary phase is anchored to the column wall or to the particles, which are packed into the column.

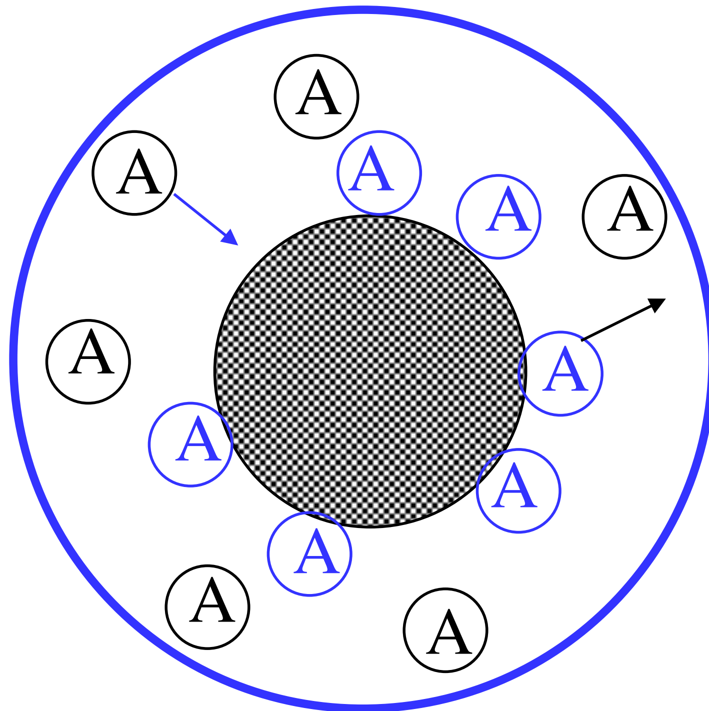
Common Types of Chromatography

Tswett's technique is based on Liquid Chromatography. There are now several common chromatographic methods.
These include:

- **Paper Chromatography**
- **Thin Layer Chromatography (TLC)**
- **Liquid Chromatography (LC)**
  - **High Pressure Liquid Chromatography (HPLC)**
  - **Ion Chromatography**
- **Gas Chromatography (GC)**

Chromatography is based on a physical equilibrium that results when a solute is transferred between the mobile and a stationary phase.

K = distribution coefficient *or* partition ratio



Cross Section of Equilibrium in a column.
"A" are adsorbed to the stationary phase.
"A" are traveling in the mobile phase.

$$K_c = \frac{c_S}{c_M}$$

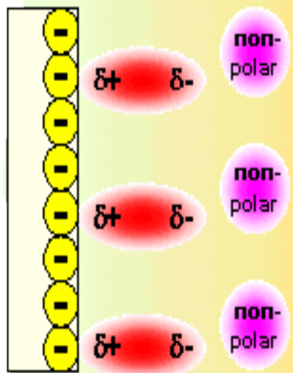
stationary
mobile

Where,

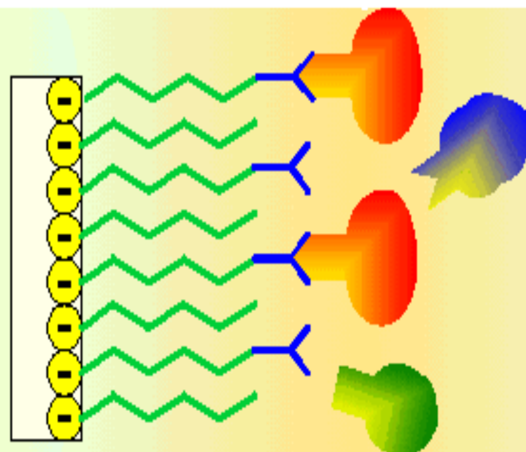
C_S is the molar concentration of the solute in the stationary phase

C_M is the molar concentration in the mobile phase.

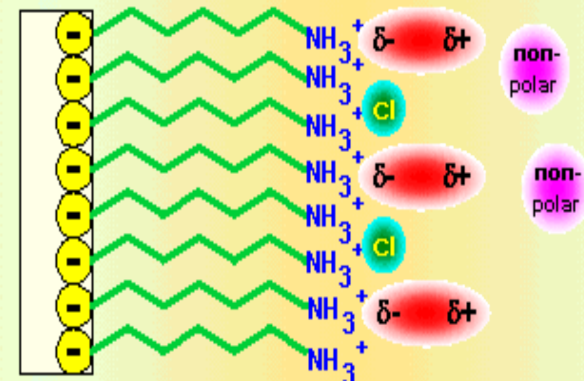
Basic Principle



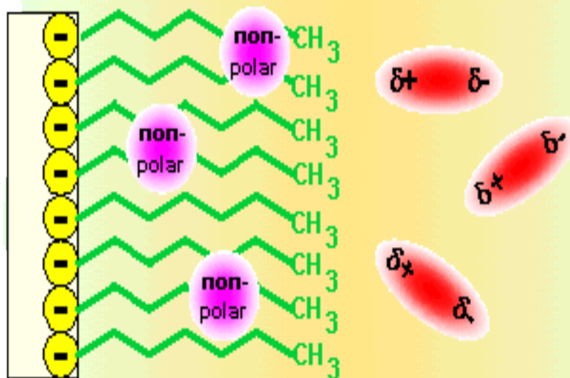
Adsorption Chromatography



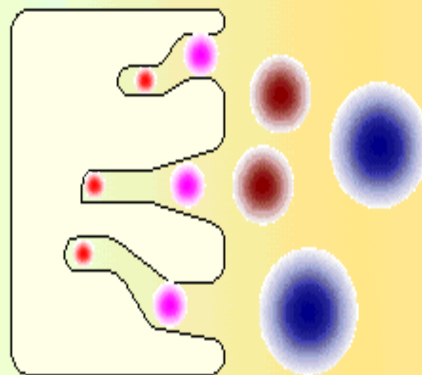
Affinity Chromatography
(covalent binding)



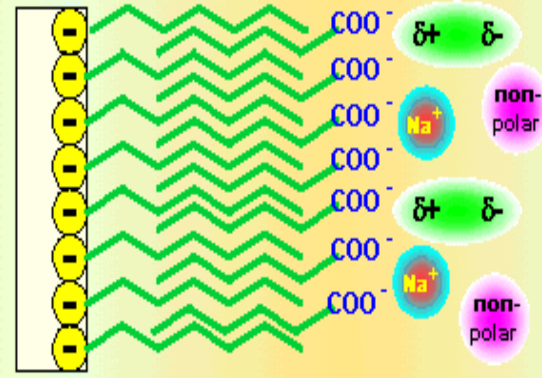
Ion-Exchange Chromatography
(cation or anion resins)



Partition Chromatography
(reversed phase)



Molecular Exclusion Chromatography
(gel filtration, gel permeation,
molecular sieve)



Ion-Pairing Chromatography
(liquid cation or anion resins)

Sample

Mobile phase

A + B

B
A

Packed
column

B
A

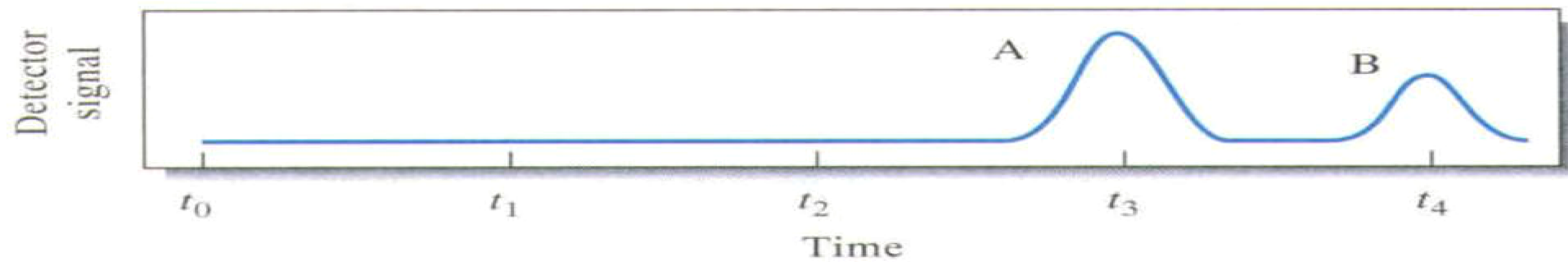
B
A

B

Detector

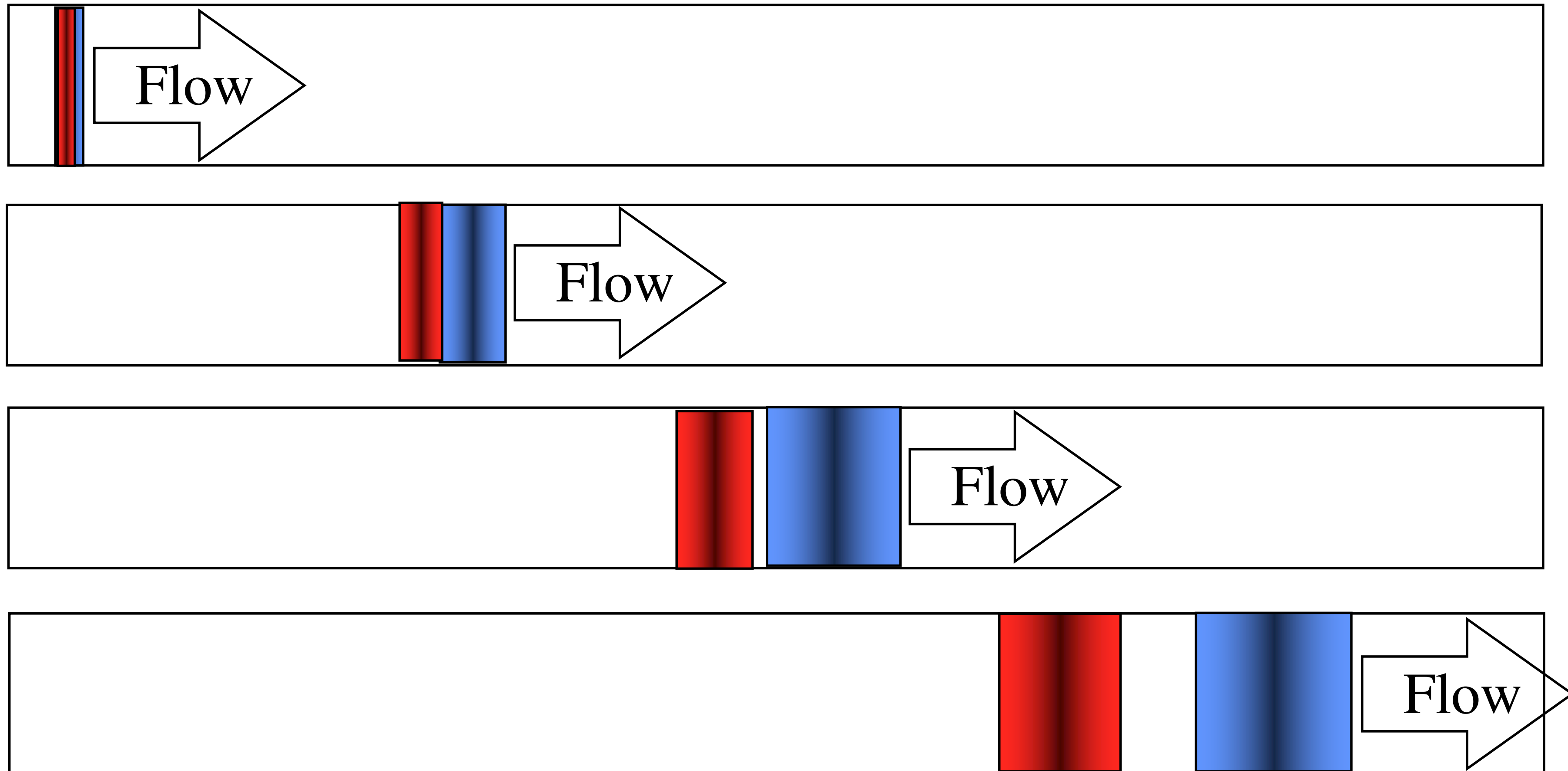
t_0 t_1 t_2 t_3 t_4

(a)



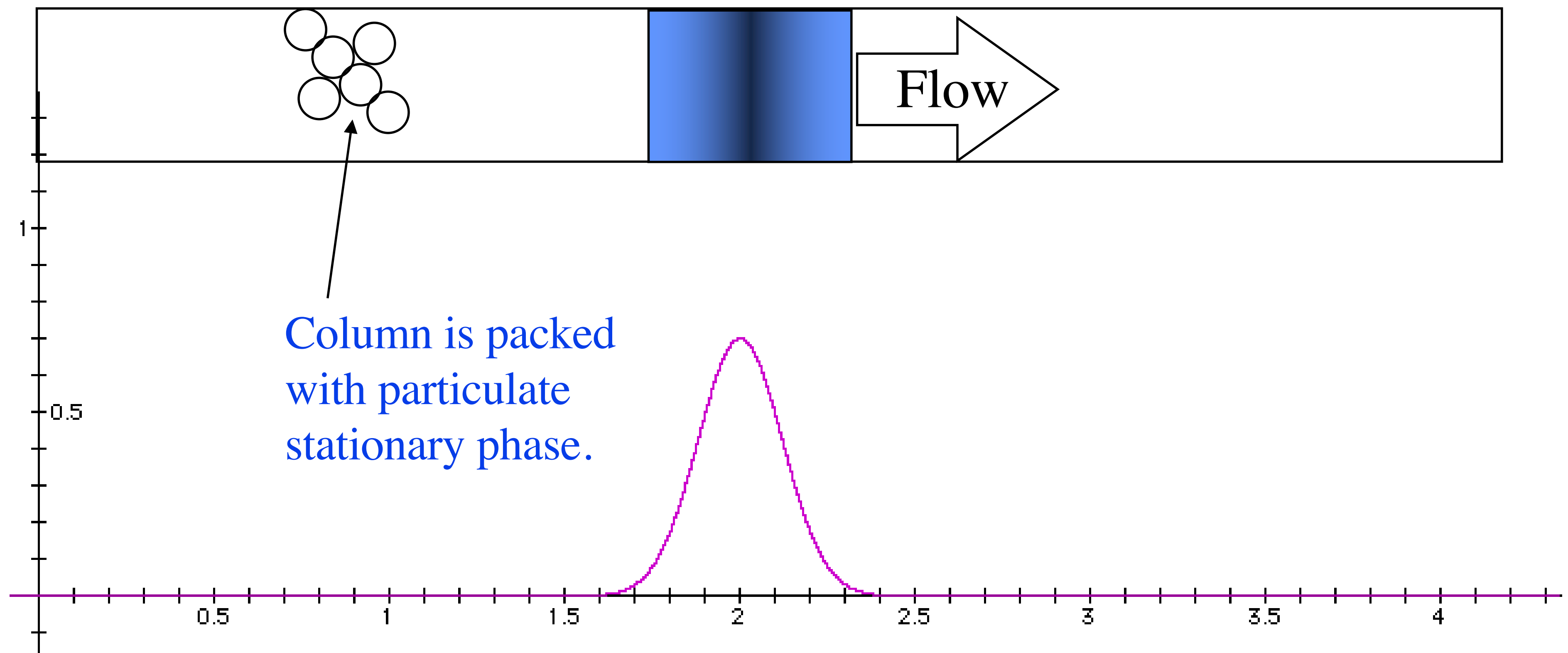
(b)

In a mixture, each component has a different distribution coefficient, and thus spends a different amount of time absorbed on the solid packing phase vs being carried along with the flowing mobile phase.



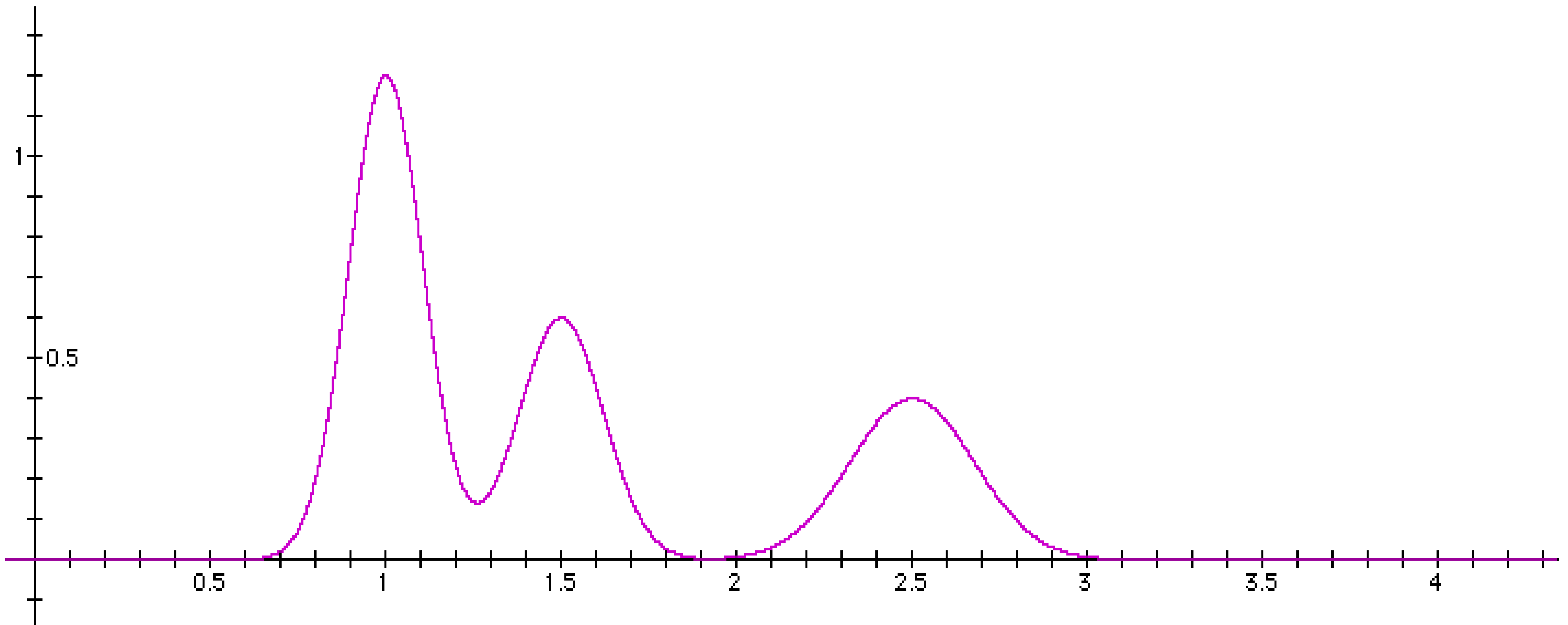
More volatile materials are carried through the column more rapidly than less volatile materials, which results in a separation.

In a chromatography column, flowing gas or liquid continuously replaces saturated mobile phase and results in movement of A through the column.



As a material travels through the column, it assumes a Gaussian concentration profile as it distributes between the stationary packing phase and the flowing mobile gas or liquid carrier phase.

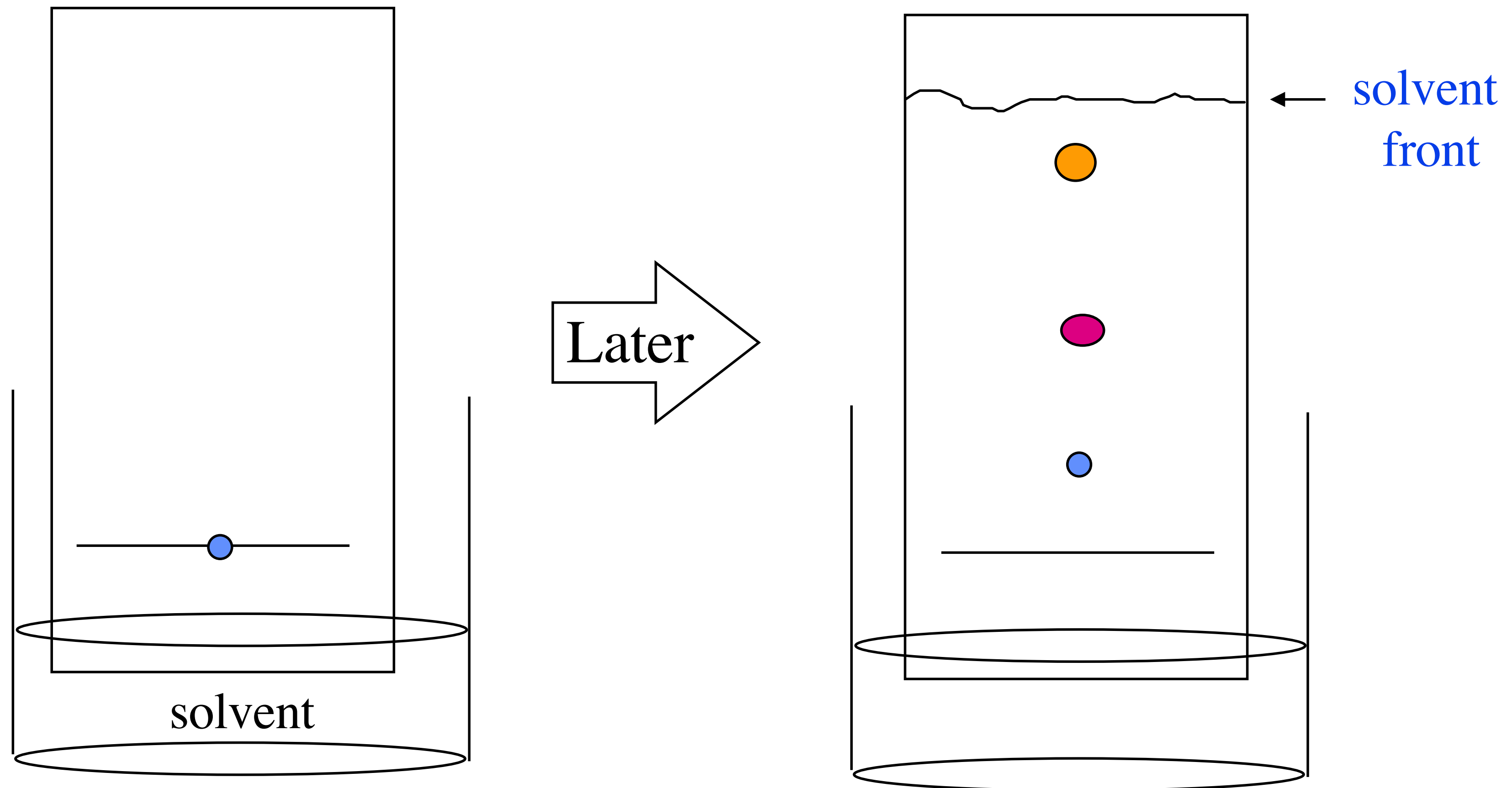
If a detector is used to determine when the components elute from the column, a series of Gaussian peaks are obtained, one for each component in the mixture that was separated by the column.



Note: The first two components were not completely separated.
Peaks in general tend to become shorter and wider with time.

Paper and Thin Layer Chromatography

The solvent moves up paper by capillary action, carrying mixture components at different rates.



GAS CHROMATOGRAPHY

Martin & Synge in 1941

Nobel prize in 1952

Mobile phase is a gas!

Stationary phase could be anything but not a gas

GC is currently one of the most popular methods for separating and analyzing compounds that is either naturally volatile (i.e., readily goes into the gas phase) or can be converted to a volatile derivative.

This is due to its…..

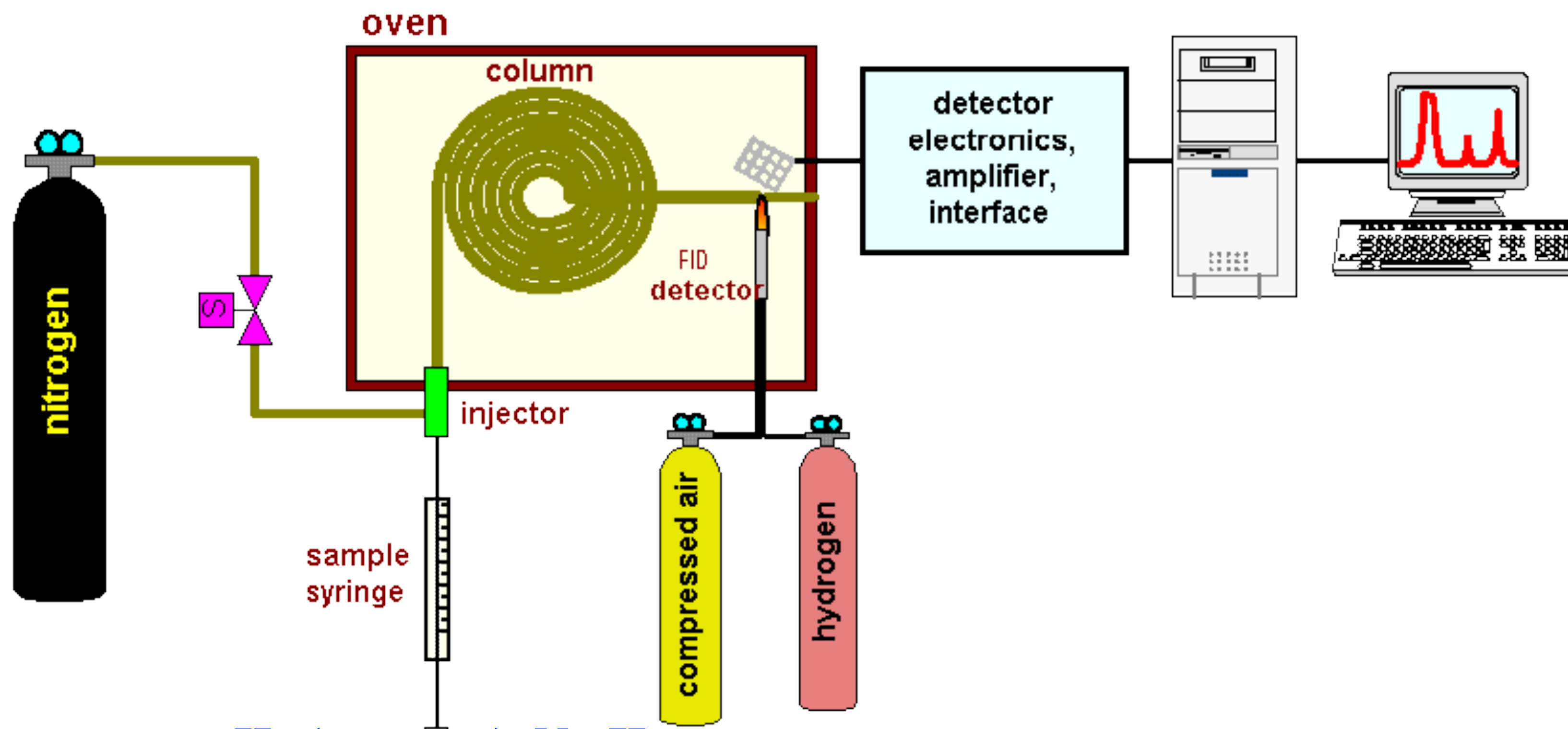
High resolution
Low limits of detection
Speed
Accuracy
Reproducibility

Separation of a number of small organic and inorganic compounds (They can be big compounds if you can make them small before separation!)

A simple GC system consists of:

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

Instrimentation



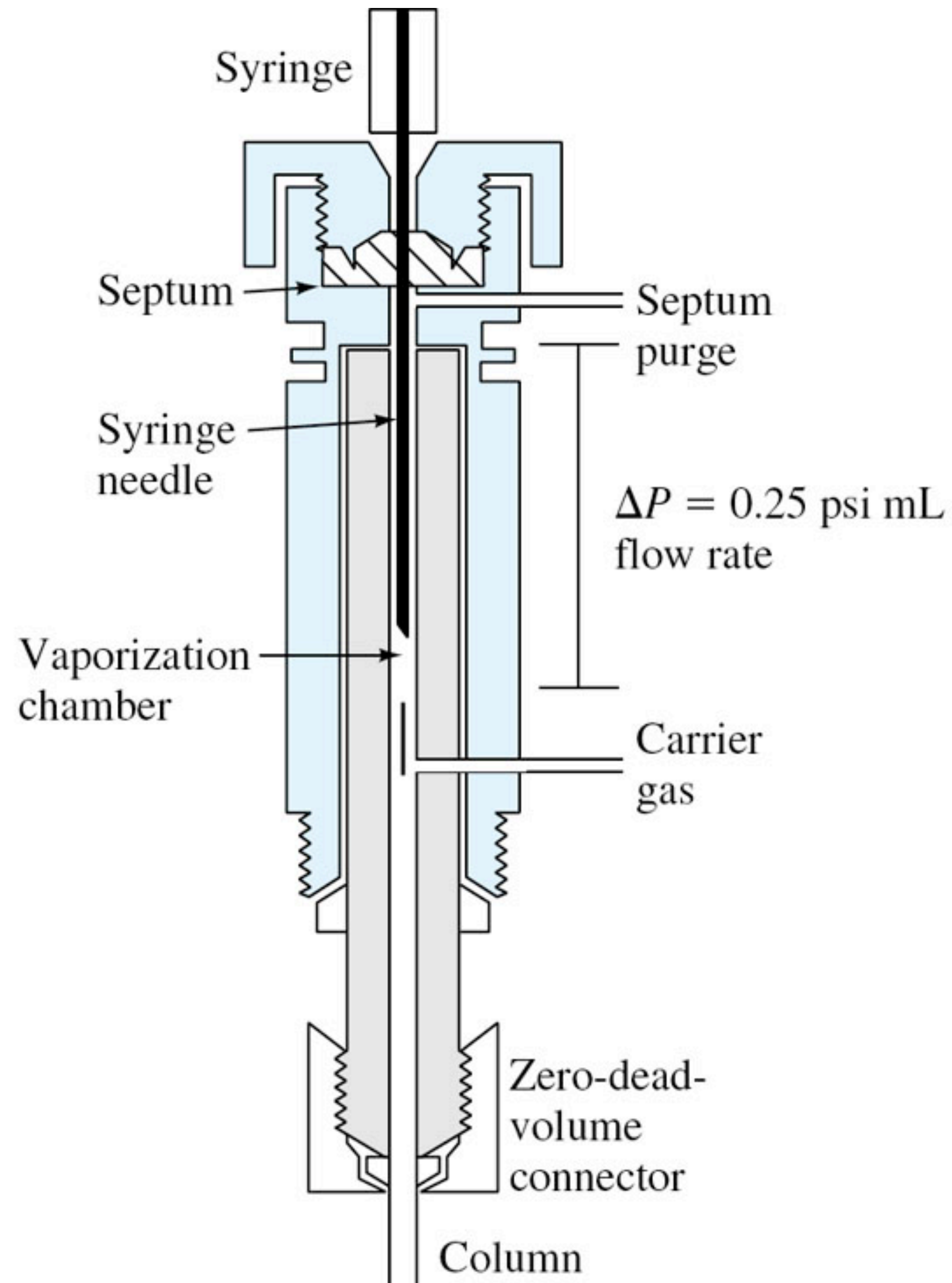
Carrier gas: He (common), N_2, H_2
P_{inlet} 10-50 psig
Flow = 25-150 mL/min packed column
Flow = 1-25 mL/min open tubular column

Column: 2-100 m coiled stainless steel/glass/Teflon/fused silica, packed vs. unpacked

Oven: 0-400 °C ~ average boiling point of sample Accurate to <1 °C

Detectors: FID, TCD, ECD, NPD, FPD, AED, PID, MSD. (SINGLE OR TANDEM)

Sample injection



Mobile Phases

GC separates solutes based on their different interactions between mobile and stationary phases.

solute's retention is determined mostly by its vapor pressure and volatility
solute's retention is controlled by its interaction with the stationary phase

Carrier gas – main purpose of the gas in GC is to move the solutes along the column, mobile phase is often referred to as carrier gas (MUST BE INERT!).

Common carrier gas: include He, Ar, H_2, N_2

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

1.) Desired efficiency for the GC System (Van Deemter!)
   - 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 conductivity detector requires H_2 or He
   - other detectors require specific carrier gas → compatibility

Stationary Phases

Stationary phase in GC is the main factor determining the selectivity and retention of solutes.

There are two types of stationary phases used in GC:

- Solid adsorbents
- Liquids coated on solid supports

1.) *Gas-solid chromatography (GSC)*

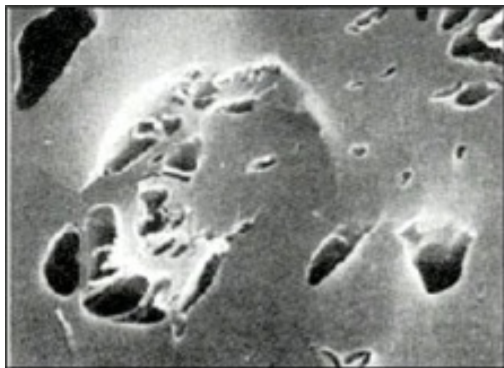
- same material is used as both the stationary phase and support material
- common adsorbents include:

alumina

molecular sieves **(crystalline aluminosilicates [zeolites] and clay)**

silica

active carbon



Magnified Pores in activated carbon

Gas-Solid Chromatography

Advantages:

- long column lifetimes

- ability to retain and separate some compounds not easily resolved by other GC methods
    - geometrical isomers
    - permanent gases

Disadvantages:

- very strong retention of low volatility or polar solutes

- catalytic changes that can occur on GSC supports

- GSC supports have a range of chemical and physical environments
    - different strength retention sites
    - non-symmetrical peaks
    - variable retention times

Gas-Liquid Chromatography

Preparing a stationary phase for GLC:

- slurry of the desired liquid phase and solvent is made with a solid support

  solid support is usually diatomaceous earth (fossilized shells of ancient aquatic algae (diatoms), silica-based material)

- solvent is evaporated off, coating the liquid stationary phase on the support

- the resulting material is then packed into the column

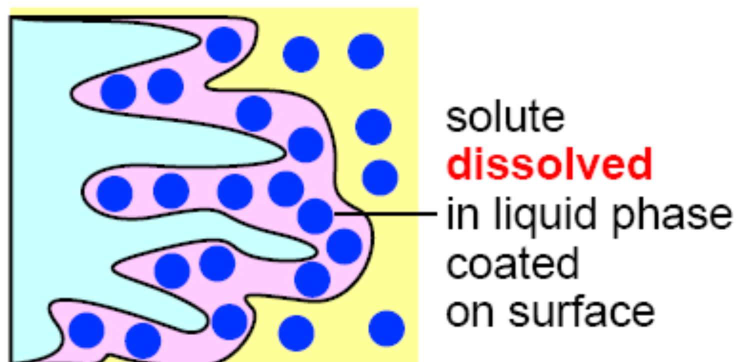
Disadvantages:

- liquid may slowly *bleed* off with time

  especially if high temperatures are used

  contribute to background

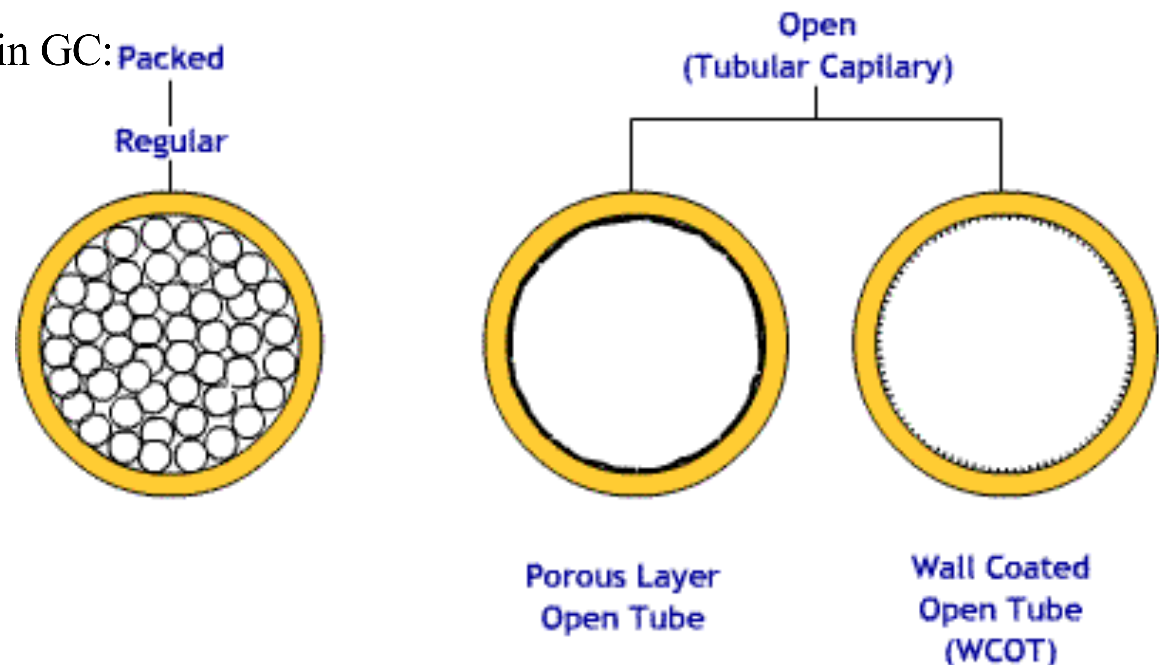
  change characteristics of the column with time



Partition Chromatography

Column support

There are two main types of supports used in GC:



Packed columns

large sample capacity
preparative work

Capillary (open-tubular) columns

higher efficiency

smaller sample size

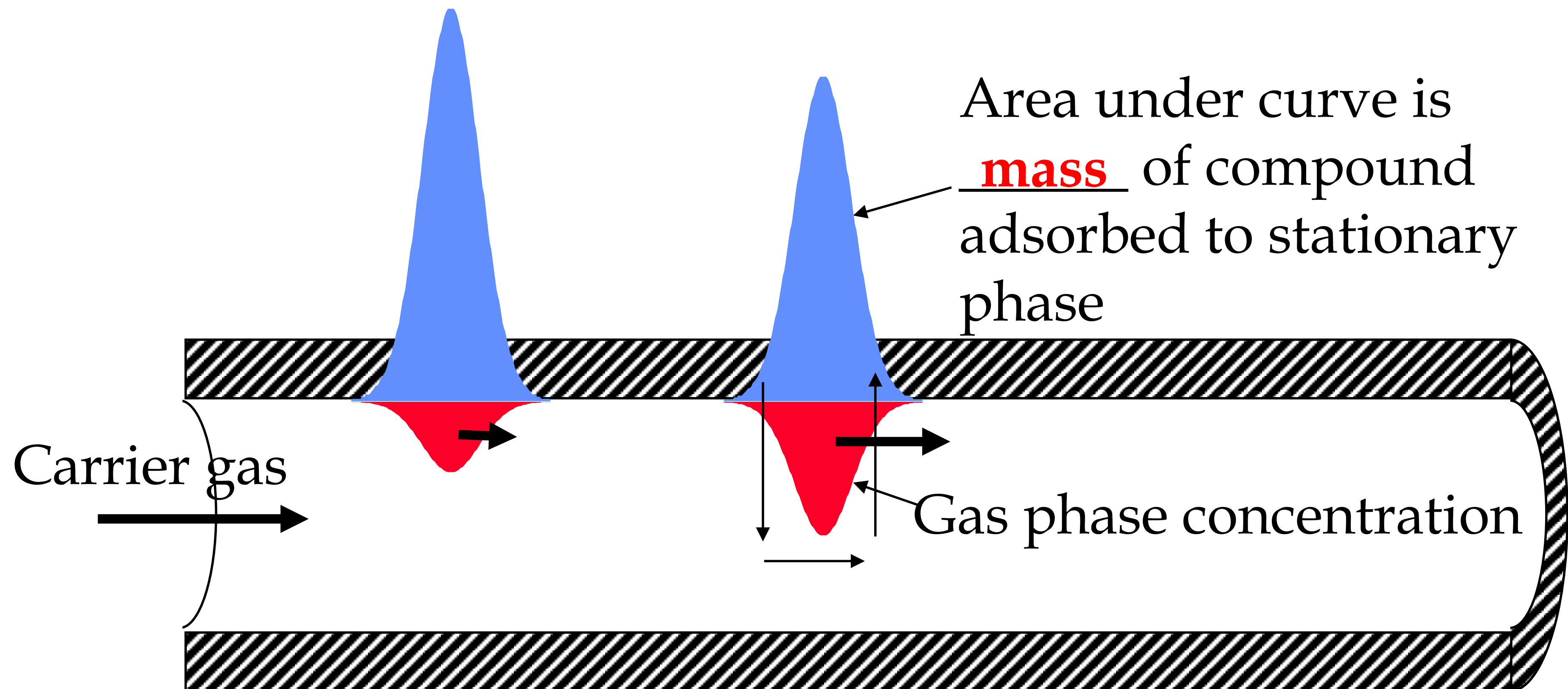
analytical applications

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
nonpolar liquids	methylsiloxanes	Gas:liq
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)	

Theory of Operation

- Velocity of a compound through the column depends upon affinity for the stationary phase



Detecting your peaks

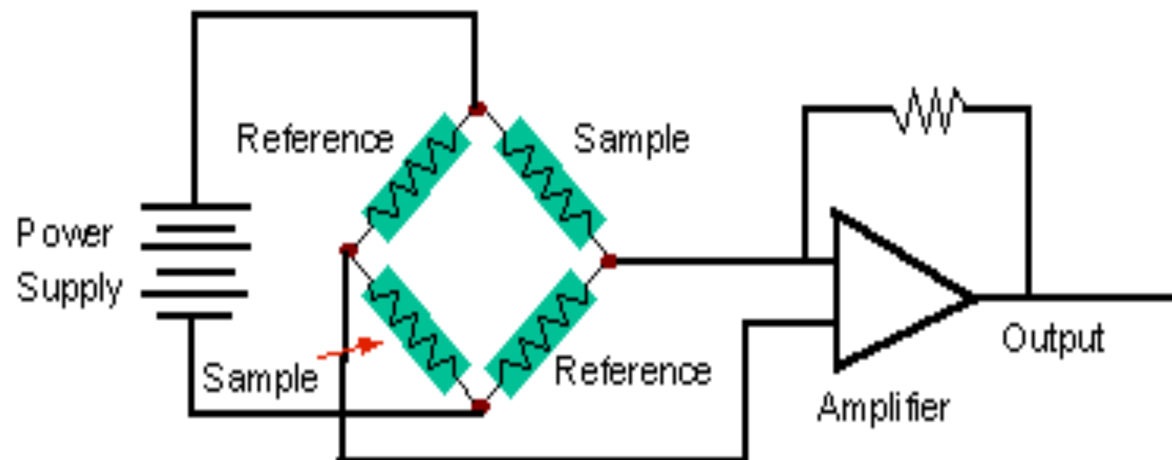
The following devices are common types of GC detectors:

1. **Thermal Conductivity Detector (TCD)**
2. **Flame Ionization Detector (FID)**
3. **Nitrogen-phosphorus Detector (NPD)**
4. **Electron Capture Detector (ECD)**
5. **Mass Spectrometers (GC-MS)**

The choice of detector will depend on the analyte and how the GC method is being used (i.e., analytical or preparative scale)

1. Thermal Conductivity Detector (TCD)

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



Advantages:

- truly universal detector
  applicable to the detection of any compound in GC

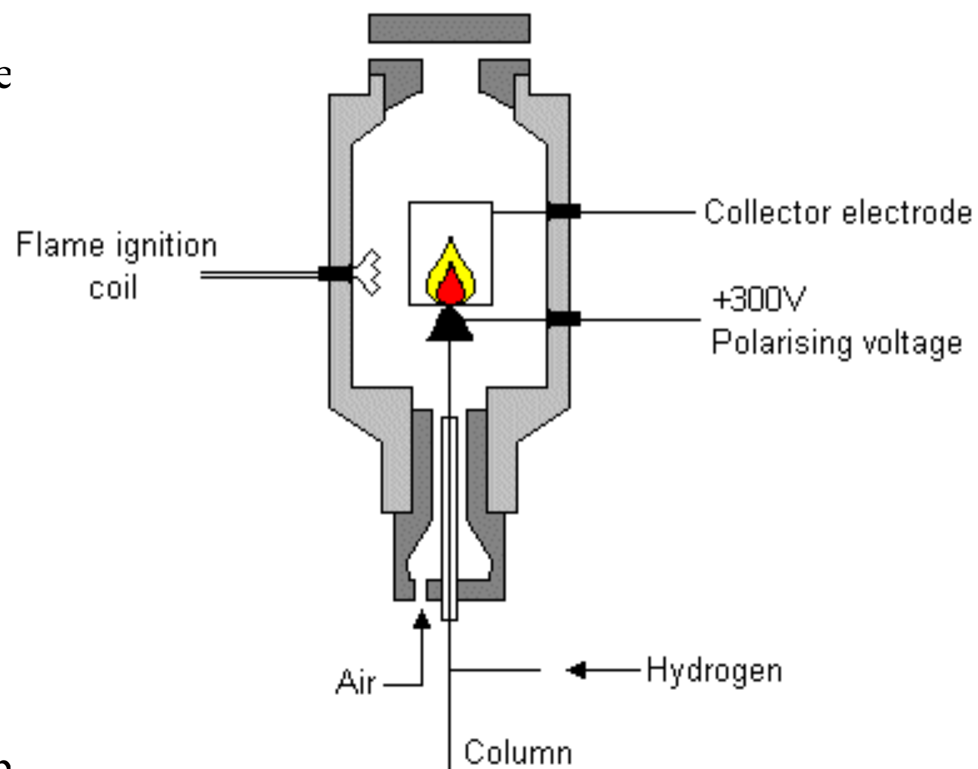
- non-destructive
  useful for detecting compounds from preparative-scale columns
  useful in combination with other types of GC detectors

Disadvantages:

- detect mobile phase impurities
- sensitive to changes in flow-rates
- limit of detection
  $\sim 10^{-7}$ M
  much higher then other GC detectors

2. Flame Ionization Detector (FID)

- most common type of GC detector
- "universal" detector capable of measuring the presence of almost any organic



Principle of operation:

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

Advantages:

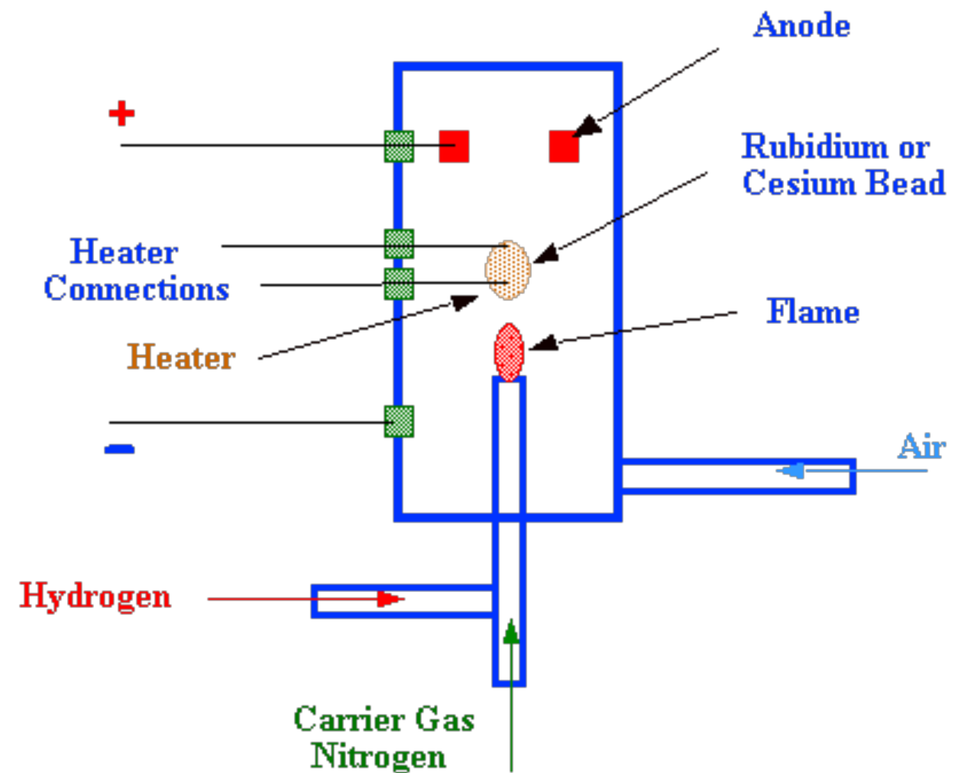
- universal detector for organics
- doesn't respond to common inorganic compounds
- mobile phase impurities not detected
- carrier gases not detected
- limit of detection: FID is 1000x better than TCD
- linear and dynamic range better than TCD

Disadvantage:

- destructive detector

3. Nitrogen-Phosphorus Detector (NPD)

- used for detecting nitrogen- or phosphorus containing compounds
- also known as alkali flame ionization detector or thermionic detector (TID)



Principle of Operation

- same basic principal as FID
- measures production of ions when a solute is burned in a flame
- ions are collected at an electrode to create a current
- contains a small amount of alkali metal vapor in the flame
- enhances the formation of ions from nitrogen- and phosphorus- containing compounds

Advantages:

- useful for environmental testing
  detection of organophosphate pesticides
- useful for drug analysis
  determination of amine-containing or basic drugs

- Like FID, does not detect common mobile phase impurities or carrier gases

- limit of detection: NPD is **500**x better than FID in detecting nitrogen- and phosphorus- containing compounds

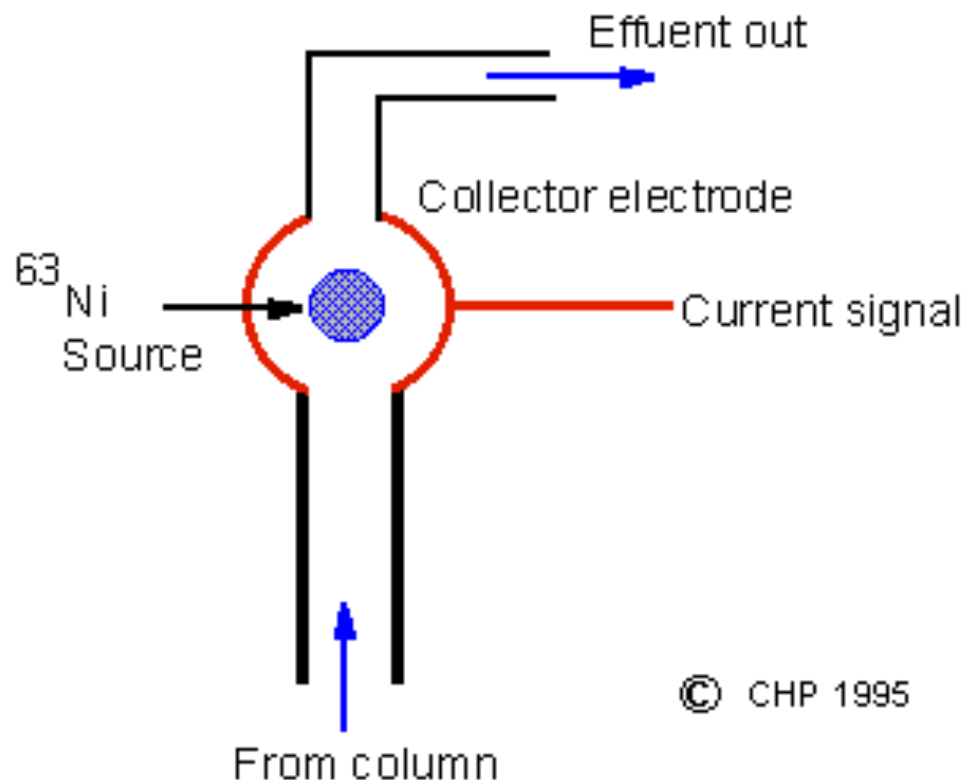
- NPD more sensitive to other heterocompounds, such as sulfur-, halogen-, and arsenic- containing molecules

Disadvantages:

- destructive detector
- NPD is less sensitive to organic compounds compared to FID

4. Electron Capture Detector (ECD)

- radioactive decay-based detector
- selective for compounds containing electronegative atoms, such as halogens



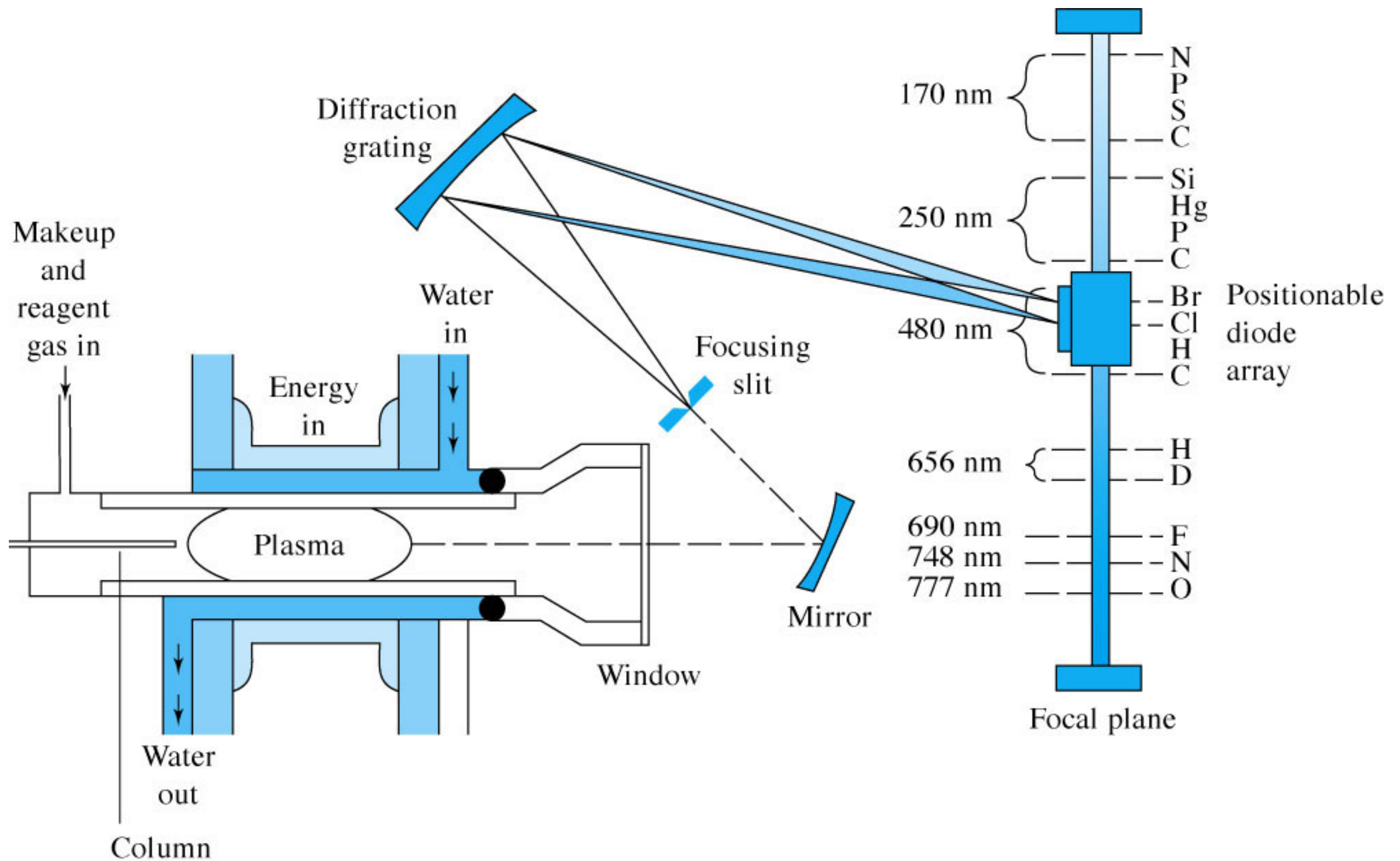
Principle of Operation

- based on the capture of electrons by electronegative atoms in a molecule
- electrons are produced by ionization of the carrier gas with a radioactive source such as ^{63}Ni (β emitter)
- in absence of solute, steady stream of these electrons is produced
- electrons go to collector electrode where they produce a current
- compounds with electronegative atoms capture electrons, reducing current

Advantages:

- useful for environmental testing
  - detection of chlorinated pesticides or herbicides
  - detection of polynuclear aromatic carcinogens
  - detection of organometallic compounds
- selective for halogen- (I, Br, Cl, F), nitro-, and sulfur-containing compounds
- detects polynuclear aromatic compounds, anhydrides and conjugated carbonyl compounds

5. Atomic emission detector (AED)



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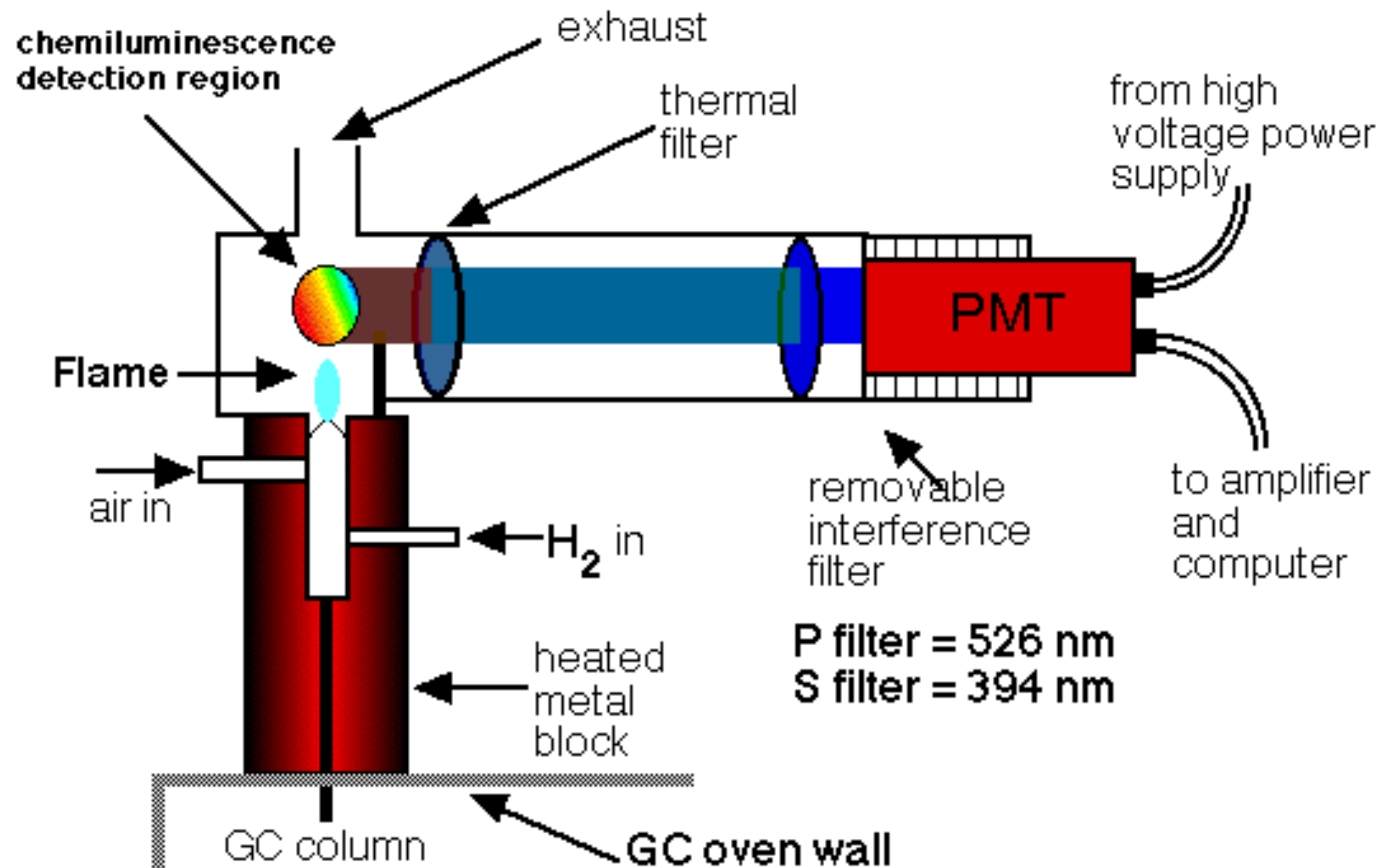
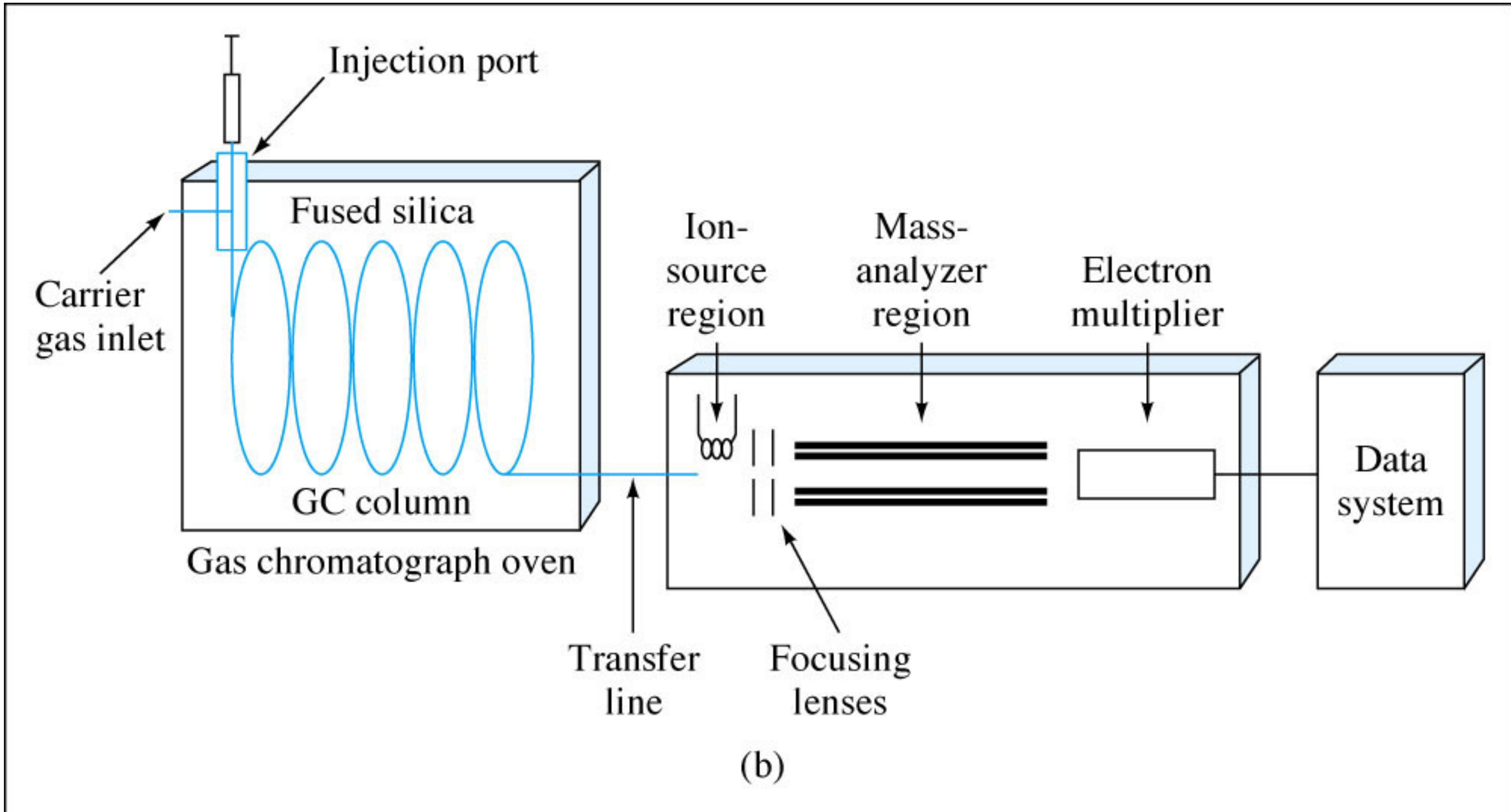
6. Flame Photometric Detector (FPD)



7. Mass Spectrometry Detector



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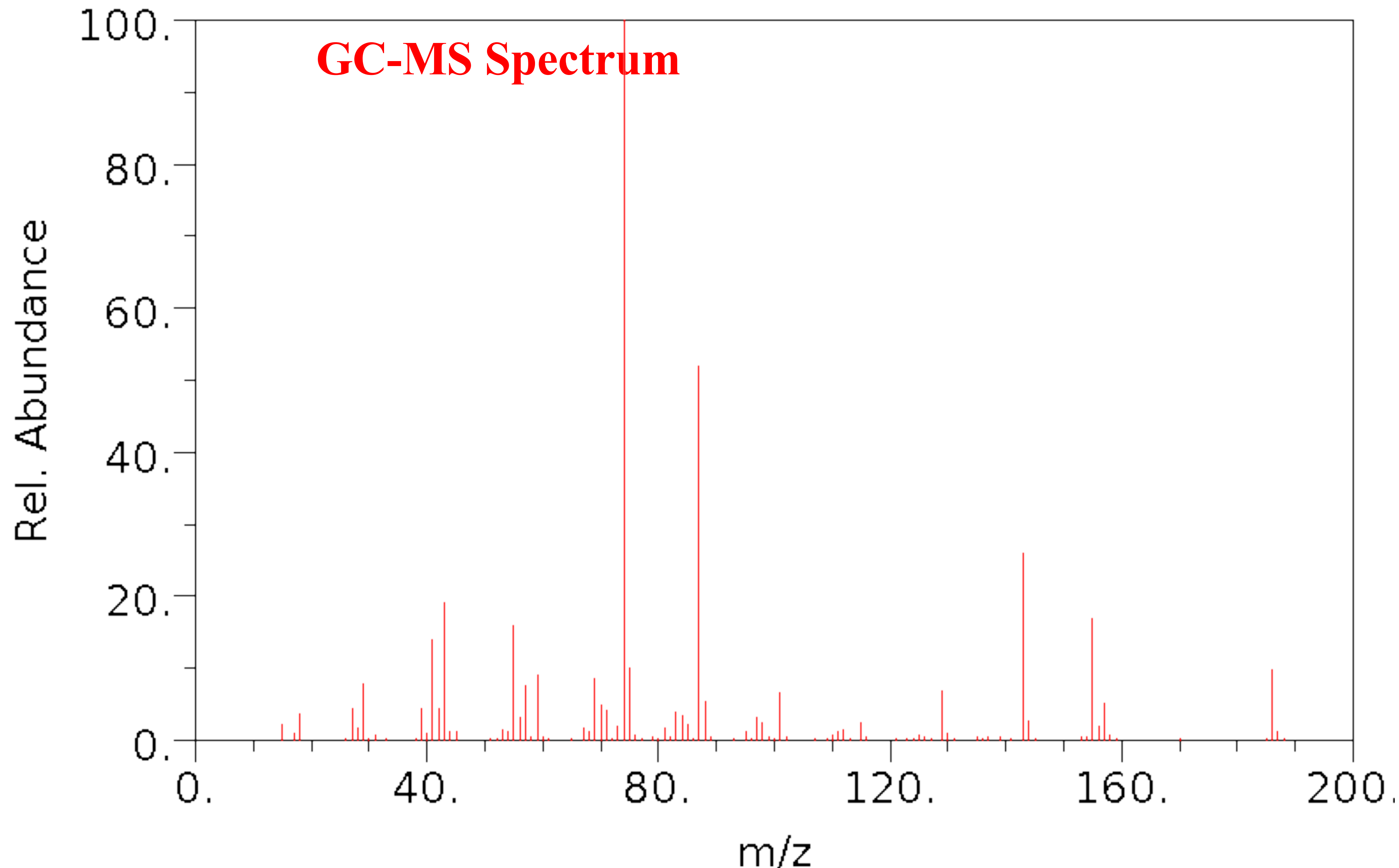
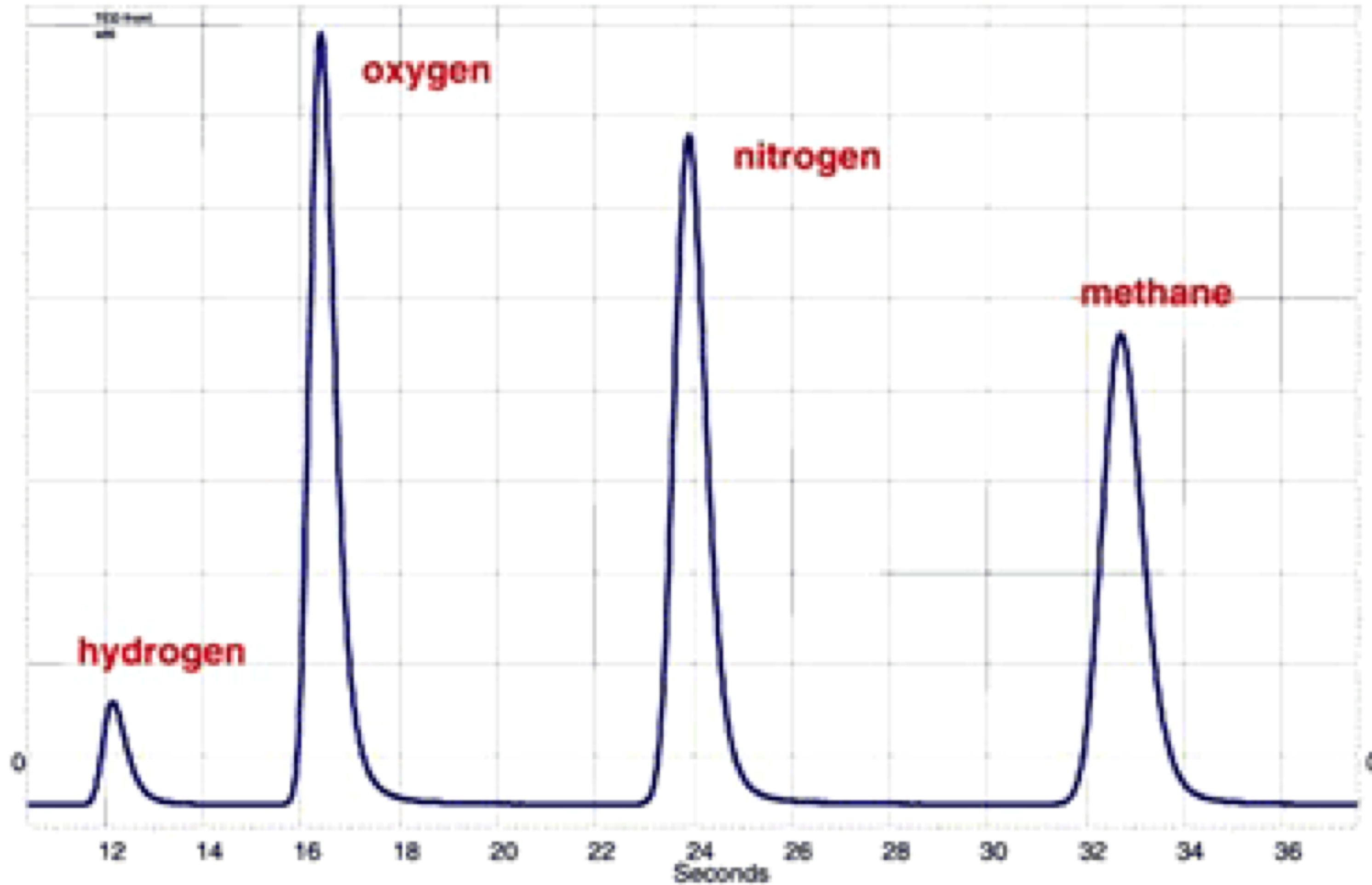
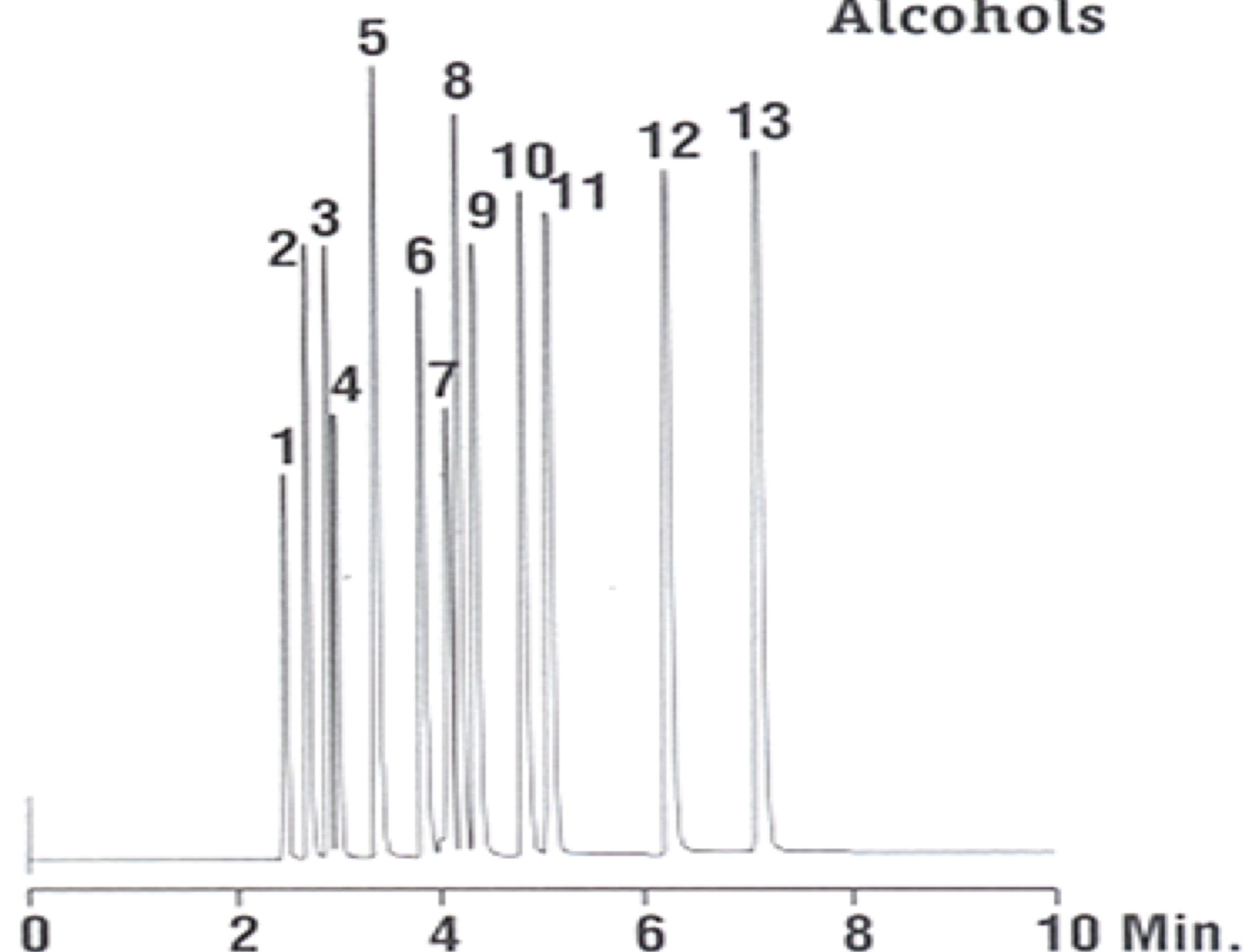
GC-MS


Decanoic acid, methyl ester
MASS SPECTRUM
GC-MS Spectrum
100.
80.
60.
40.
20.
0.
Rel. Abundance
0.
40.
80.
120.
160.
200.
m/z
NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)

Gas– Chromatograms



Decreasing order of their volatility

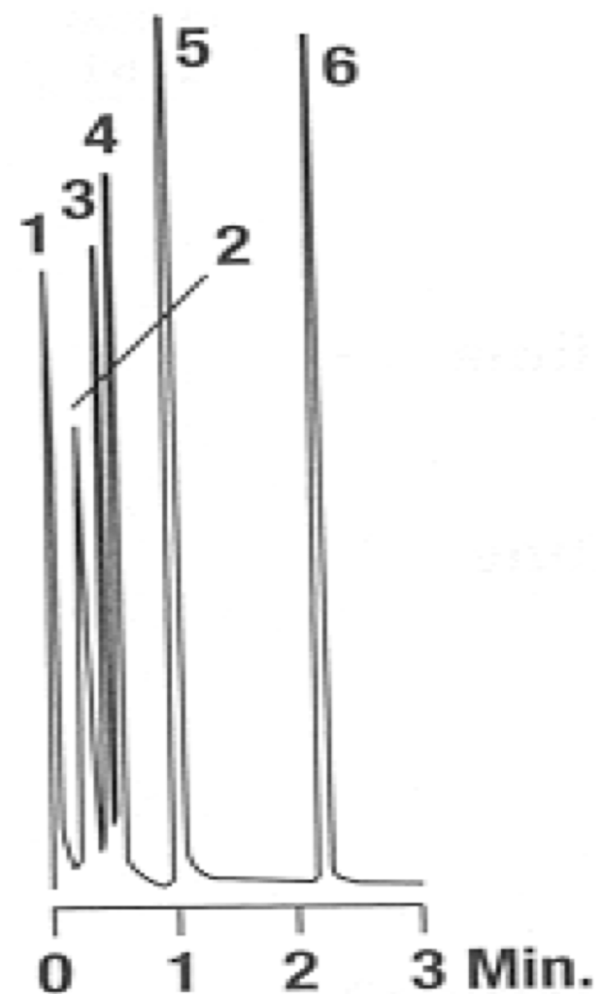


CHROM 2186

1. Methanol
2. Ethanol
3. 2-Propanol
4. Acetone
5. 1-Propanol
6. 2-Butanol
7. Ethyl Acetate
8. Isobutanol
9. tert-Amyl Alcohol
10. 1-Butanol
11. 3-Methyl-2-butanol
12. Isoamyl Alcohol
13. 1-Pentanol

Column:	Econo-Cap™ EC™-20, 30m x 0.45mm x 1.00µm (Part No. **19672**)
Temp:	55°C (3min hold) to 80°C at 10°C/min
Carrier Gas:	Helium, 22cm/sec
Detector:	FID

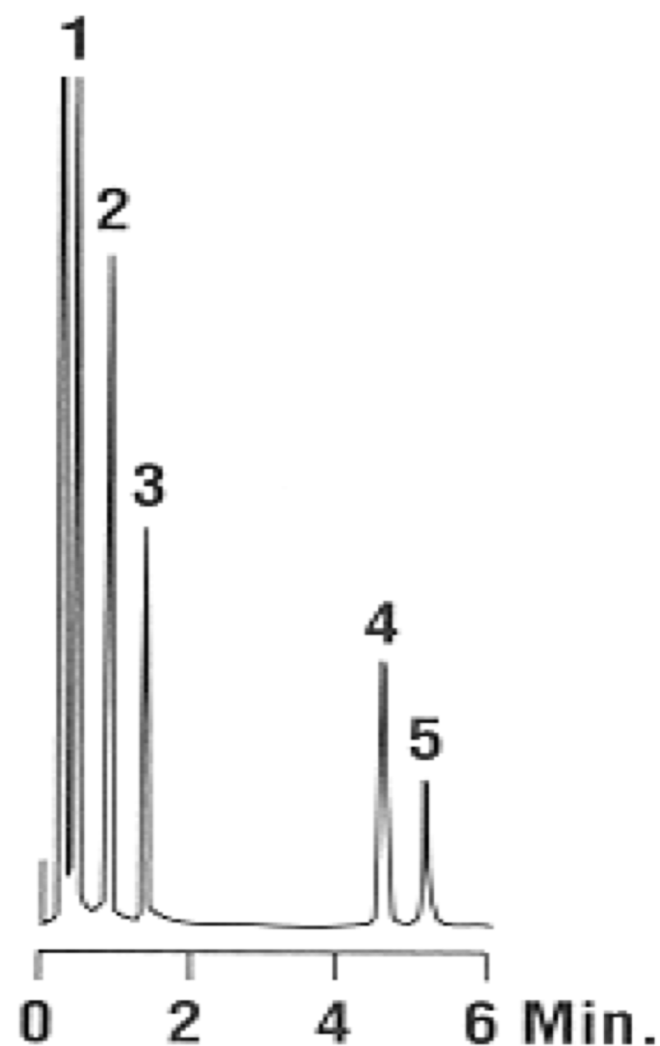
Blood Alcohols



1. Methanol
2. Ethanol
3. Acetone
4. 2-Propanol
5. 1-Propanol
6. Dioxane (I.S.)

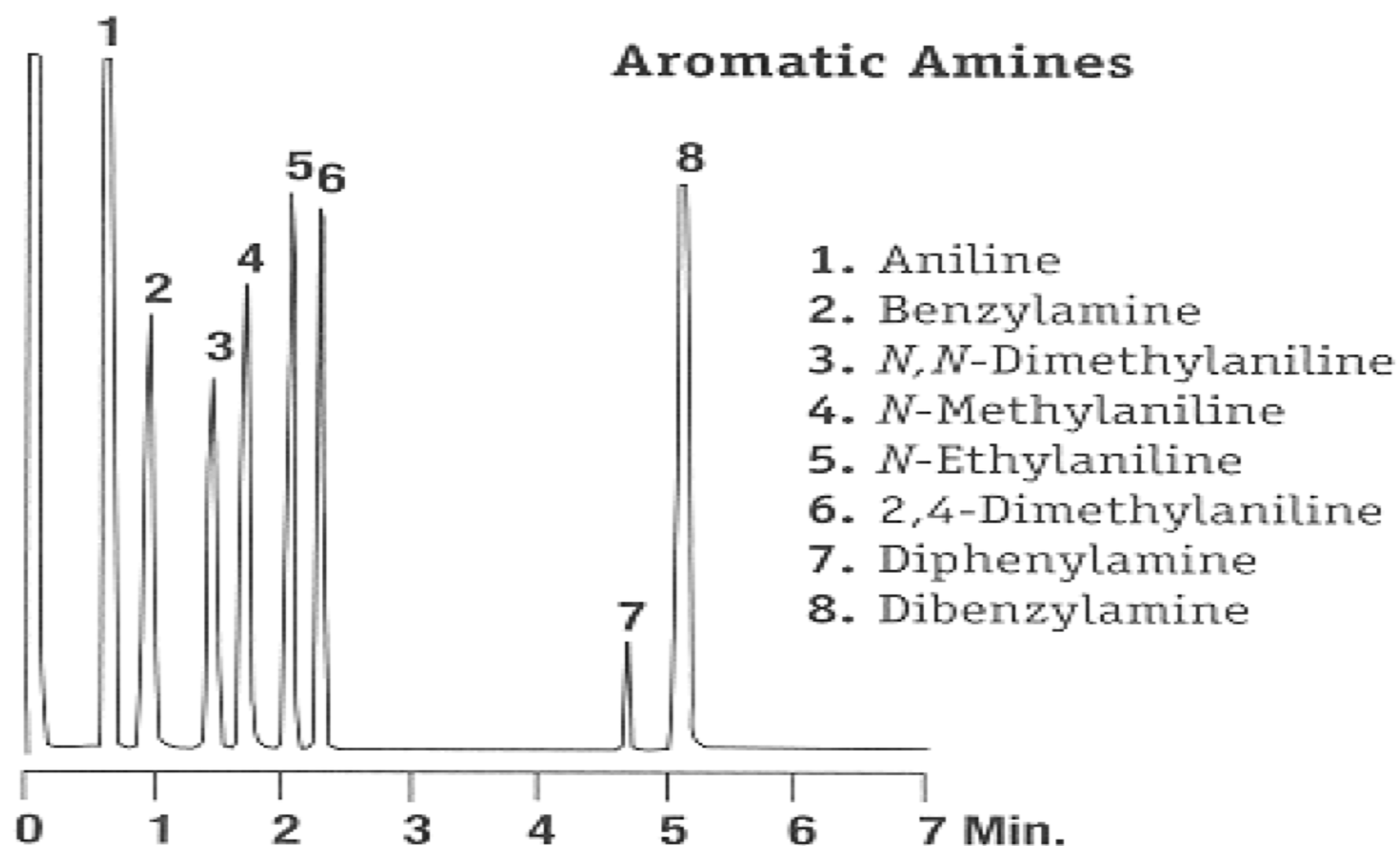
Column: Heliflex® AT™-1, 10m x 0.53mm x 5.00µm (Part No. **16842**)
Temp: 35°C (1min) to 130°C at 30°C/min
Carrier Gas: Helium, 6mL/min
Detector: FID

Ketones



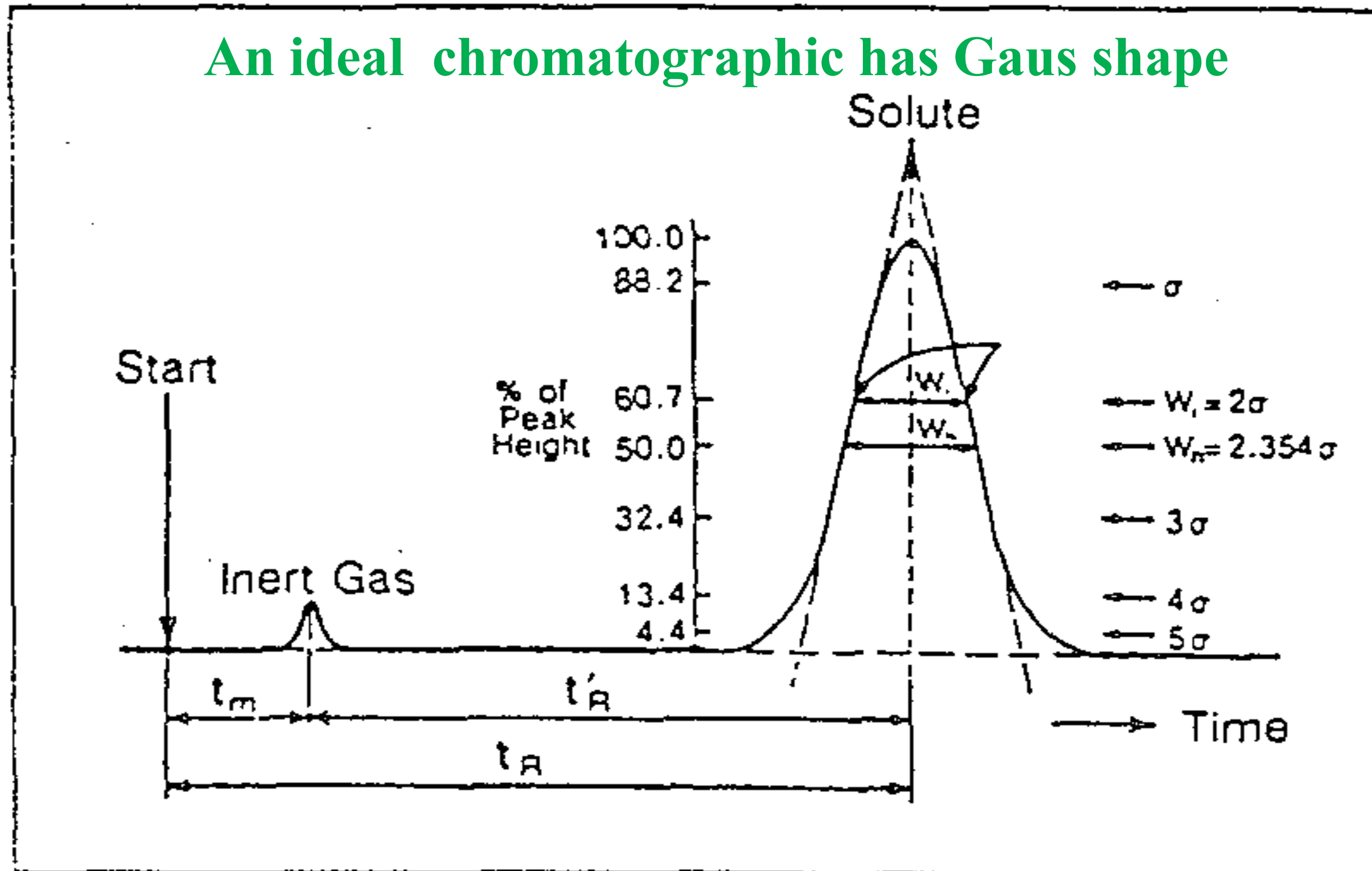
1. Acetone
2. Methyl Ethyl Ketone
3. 2-Pentanone
4. Cyclohexanone
5. 2-Heptanone

Column: Heliflex® AT™-1, 10m x 0.53mm x 1.20µm (Part No. **935110**)
Temp: 40°C
Carrier Gas: Helium, 9.1mL/min
Detector: FID



Column:	Heliflex® AT™-1, 10m x 0.53mm x 1.20µm (Part No. **935110**)
Temp:	50°C (4min) to 150°C at 10°C/min
Carrier Gas:	Nitrogen, 20mL/min
Detector:	FID

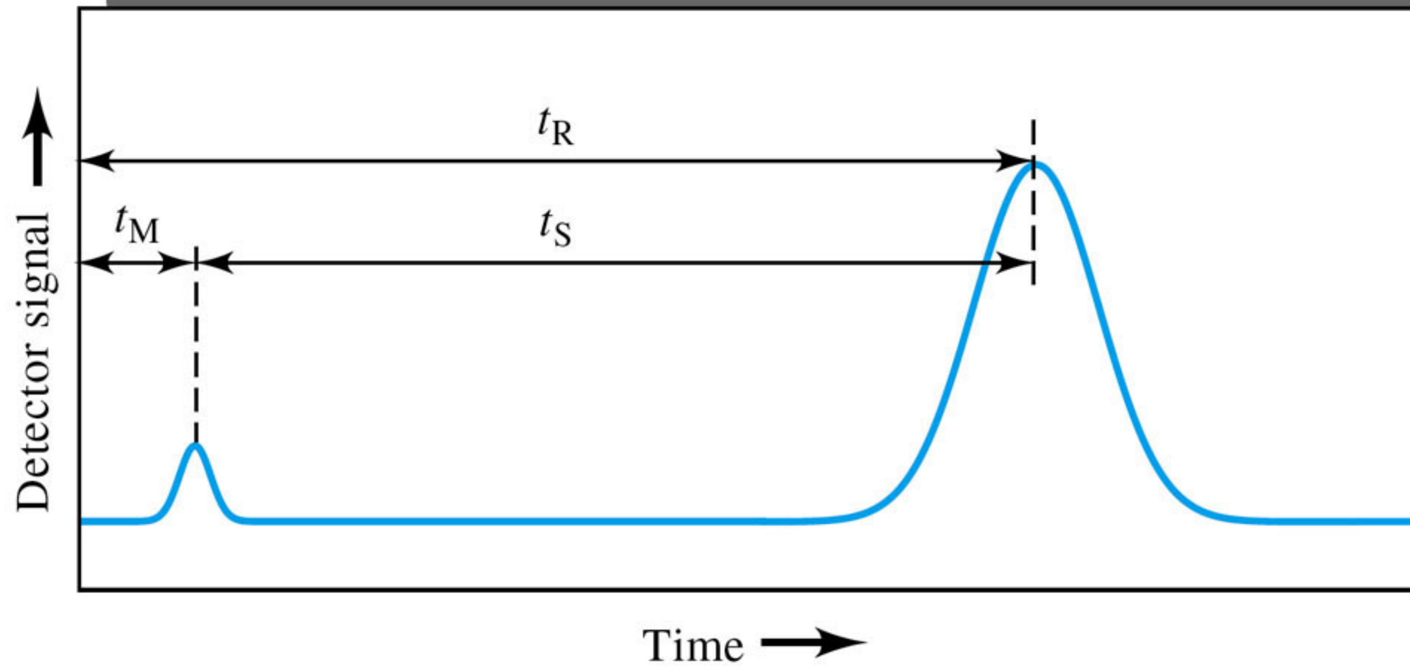
Chromatographic peak



Qualitative measure: retention time (tr)

Quantitative measure: Peak area (A)

Retention Time



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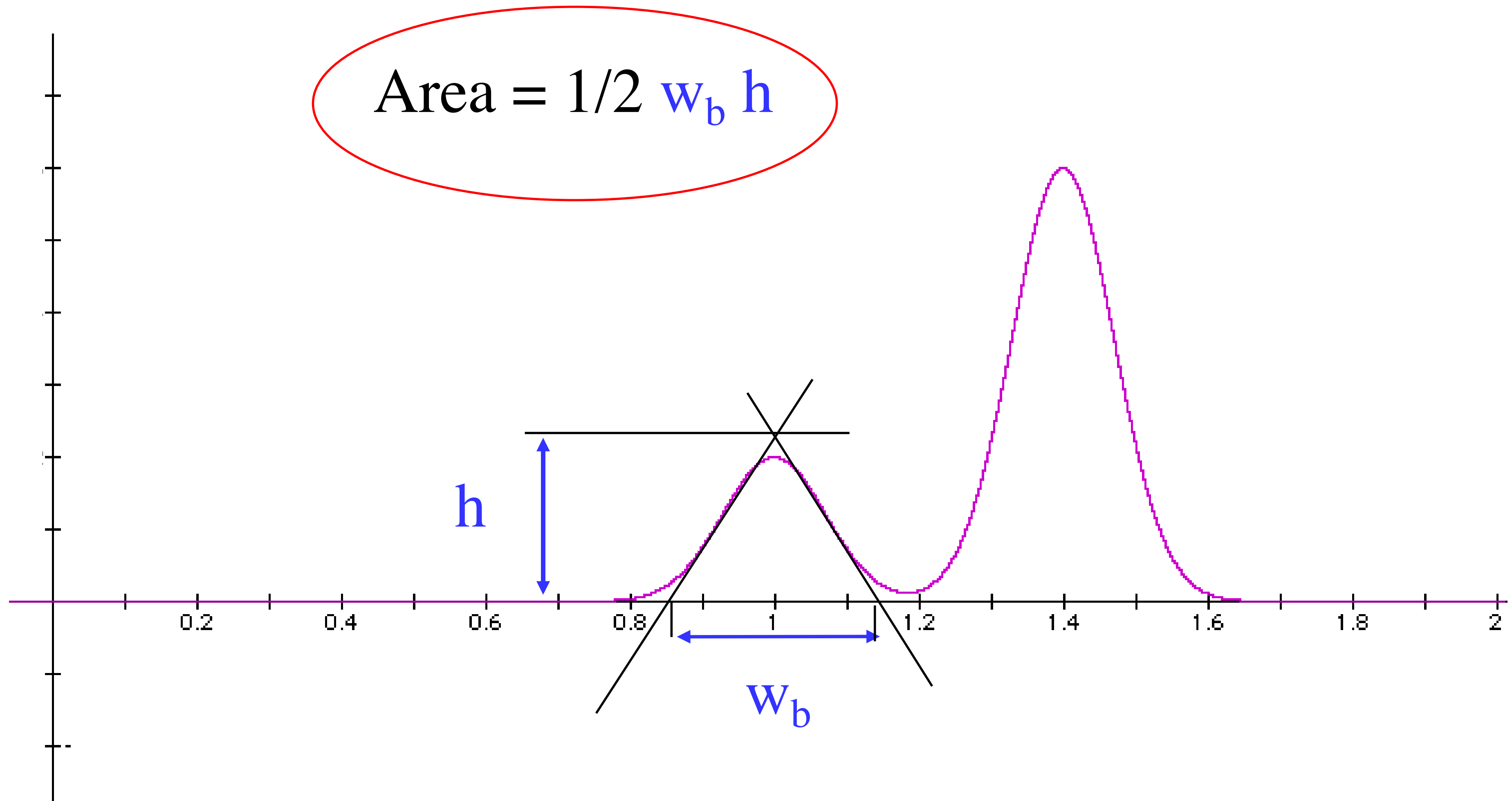
$$t_R = t_M + ts$$

t_M = retention time of mobile phase (dead time)
t_R = retention time of analyte (solute)
t_S = time spent in stationary phase (adjusted retention time)
L = length of the column

% Composition Quantity (area)

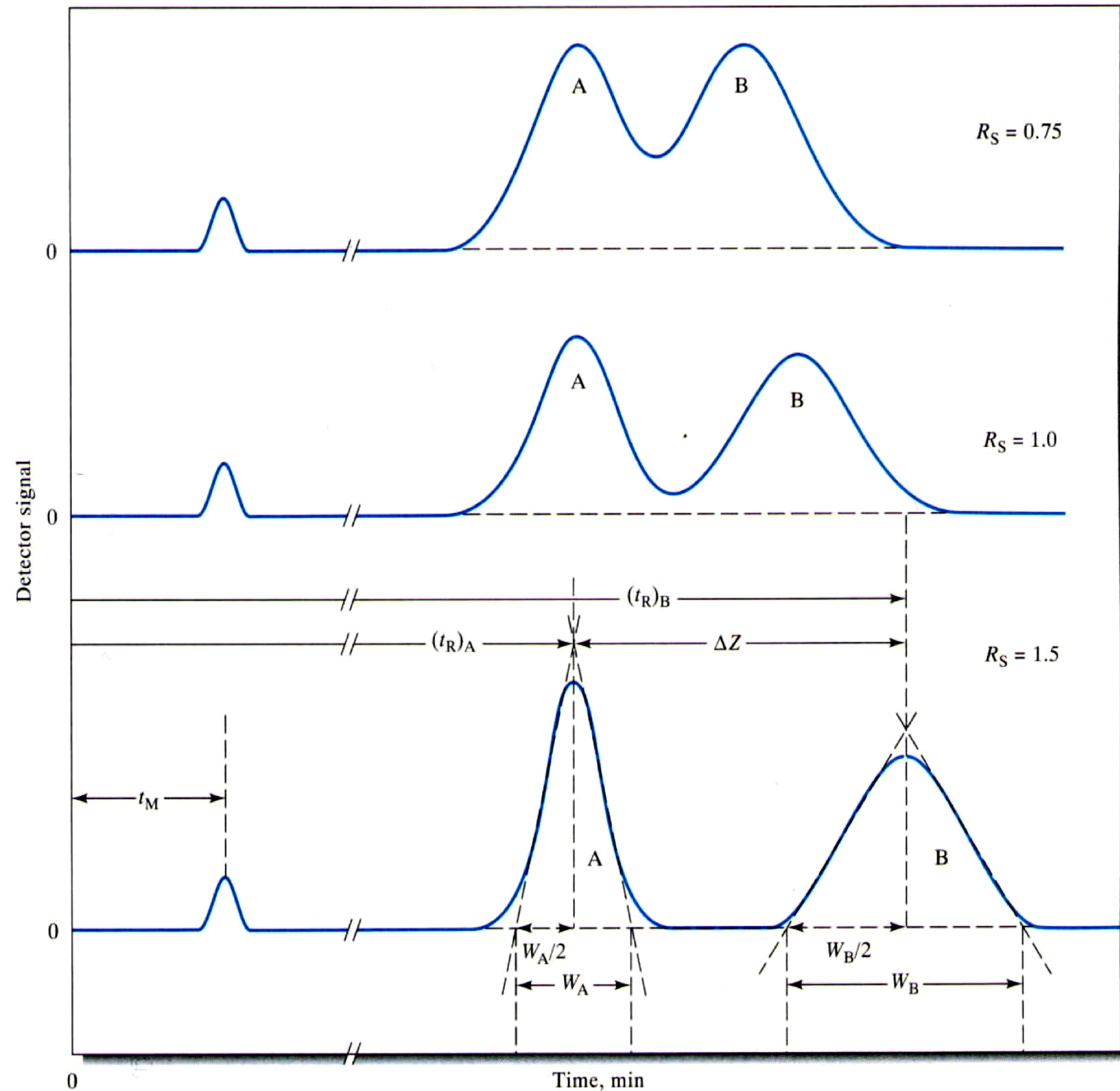


Resolution

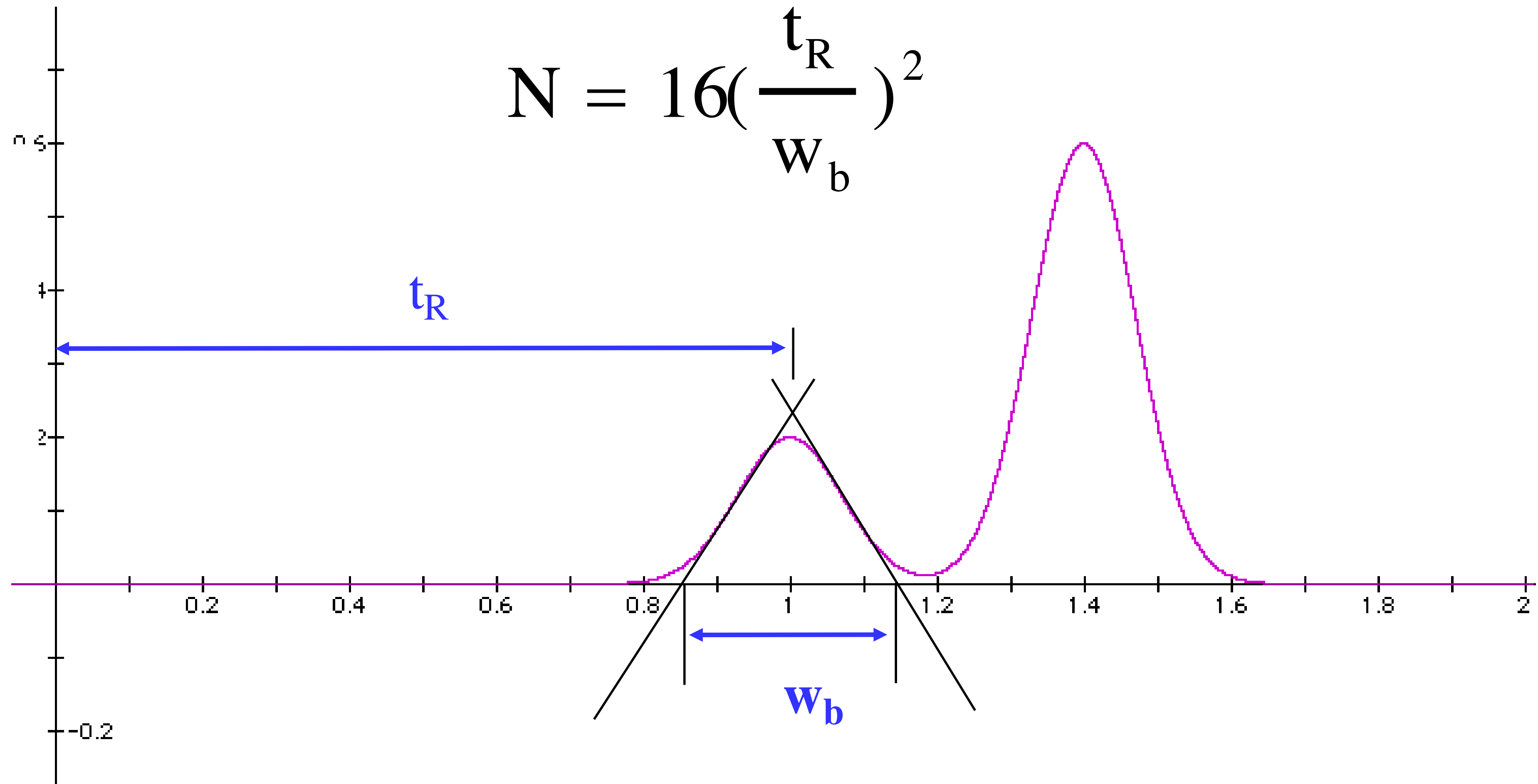
$$R_s = \frac{\Delta Z}{W_A/2 + W_B/2}$$

$$R_s = \frac{2\Delta Z}{W_A + W_B}$$

$$R_s = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$$



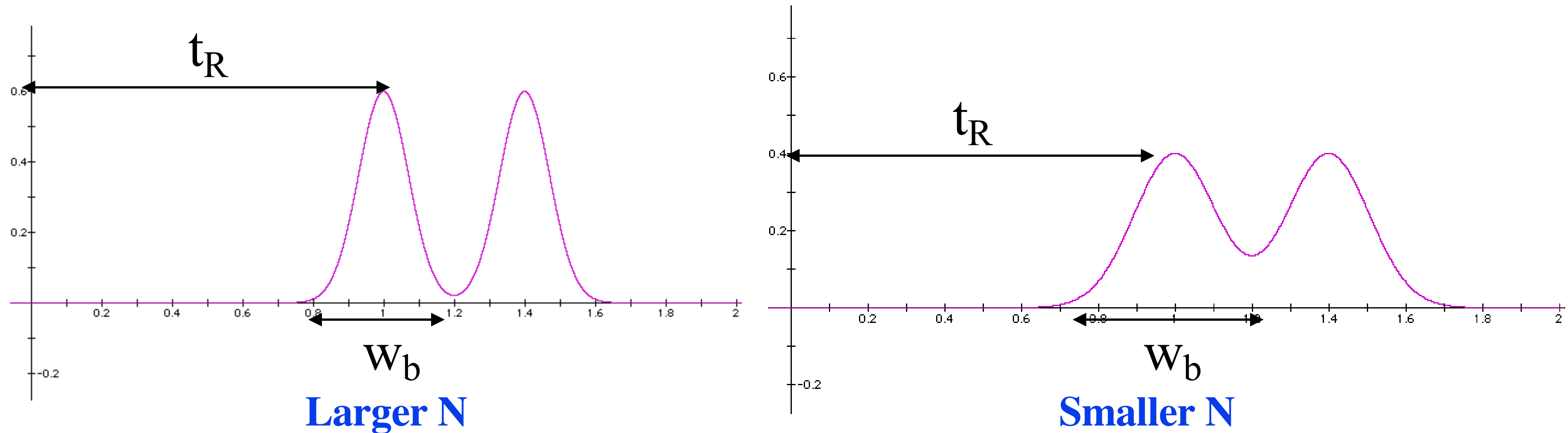
Number of theoretical plates (N)



t_R is the retention time; it is measured from the injection peak (or zero) to the intersection of the tangents.

w_b is the width of the base of the triangle; it is measured at the intersection of the tangents with the baseline.

$$N = 16\left(\frac{t_R}{w_b}\right)^2$$



When the retention time, t_R, is held constant, the column that produces peaks with narrower bases, w_b, will be more efficient – have a greater N value.

Likewise a column that produces wider peaks will be less efficient – have a smaller N value.

This is because a smaller denominator, w_b, will yield a larger overall number and a larger denominator will yield a smaller number.

TABLE 26-5 Important Derived Quantities and Relationships

Name	Calculation of Derived Quantities	Relationship to Other Quantities
Linear mobile-phase velocity	$u = L/t_M$	
Volume of mobile phase	$V_M = t_M F$	
Retention factor	$k' = (t_R - t_M)/t_M$	$k' = \dfrac{KV_S}{V_M}$
Distribution constant	$K = \dfrac{k' V_M}{V_S}$	$K = \dfrac{c_S}{c_M}$
Selectivity factor	$\alpha = \dfrac{(t_R)_\mathrm{B} - t_M}{(t_R)_\mathrm{A} - t_M}$	$\alpha = \dfrac{k'_\mathrm{B}}{k'_\mathrm{A}} = \dfrac{K_\mathrm{B}}{K_\mathrm{A}}$
Resolution	$R_s = \dfrac{2[(t_R)_\mathrm{B} - (t_R)_\mathrm{A}]}{W_\mathrm{A} + W_\mathrm{B}}$	$R_s = \dfrac{\sqrt{N}}{4}\left(\dfrac{\alpha - 1}{\alpha}\right)\left(\dfrac{k'_\mathrm{B}}{1 + k'_\mathrm{B}}\right)$
Number of plates	$N = 16\left(\dfrac{t_R}{W}\right)^2$	$N = 16R_s^2\left(\dfrac{\alpha}{\alpha - 1}\right)^2\left(\dfrac{1 + k'_\mathrm{B}}{k'_\mathrm{B}}\right)^2$
Plate height	$H = L/N$	
Retention time	$(t_R)_\mathrm{B} = \dfrac{16R_s^2 H}{u}\left(\dfrac{\alpha}{\alpha - 1}\right)^2 \dfrac{(1 + k'_\mathrm{B})^3}{(k'_\mathrm{B})^2}$	