Chapter 1

Chromatography

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What is Chromatography?

- Chromatography is a physico-chemical process that belongs to fractionation methods same as distillation, crystallization or fractionated extraction.
- It is believed that the separation method in its modern form originated at the turn of the century from the work of Tswett to whom we attribute the terms chromatography and chromatogram.
- The method was used for preparation and purification purposes until the development of sensitive detectors.
- The detector signal, which is registered in continuum, leads to a chromatogram that indicates the variation of the composition of the eluting phase with time.
Chromatographic separations

- Sample is dissolved in a mobile phase (a gas, a liquid or a supercritical fluid)
- The mobile phase is forced through an immiscible stationary phase which is fixed in place in a column or on a solid surface.
- The two phases are chosen so that the components of the sample distribute themselves between the mobile and stationary phase to a varying degree.
The principle of analysis by chromatography

Figure 1.1  A *basic experiment in chromatography*. (a) The necessary ingredients (C, column; SP, stationary phase; MP, mobile phase; and S, sample); (b) introduction of the sample; (c) start of elution; (d) recovery of the products following separation.
The principle of analysis by chromatography

(a) Sample

A + B

Mobile phase

Packed column

Detector

$t_0$ $t_1$ $t_2$ $t_3$ $t_4$

(b) Detector signal

A

B

Time
The principle of analysis by chromatography

• The chromatogram, describes the passage of components.
• It is obtained from variations, as a function of time, of an electrical signal emitted by the detector. in a suitable format for the printer.
• (a). For a long time the chromatogram was obtained by a simple chart recorder or an integrator
• (b). Right, a chromatogram illustrating the separation of a mixture of at least three principal components. Note that the order of appearance of the compounds corresponds to the relative position of each constituent on the column.
The Chromatogram

• It reveals, as a function of time, a parameter the depends on the instantaneous concentration of the solute as it exits the column
• The components entering the detector will be shown as a series of peaks that would be more or less resolved from one another as they rise from the baseline obtained in the absence of analyte.
• If the detector signal varies linearly with the concentration of analyte, the same variation will occur for the area under the peak in the chromatogram.
• A constituent is characterized by its retention time, $t_R$.
• Retention time is defined by the time taken between the moment of injection into the chromatograph and the peak maximum recorded on the chromatogram.
• In an ideal case, the retention time $t_R$ is independent of the quantity injected.

• A compound not retained will elute out of the column at time $t_M$, called the **void time** or the **dead time** (sometimes designated by $t_o$).

• The separation is complete when as many peaks are seen returning to the baseline as there are components in the mixture.

• In quantitative analysis, it suffices to separate only the components that need to be measured.

• Identification by chromatography is arbitrary. For a better confirmation, another identification method has to go along with the chromatography.

• When $t_M = t_R$; there would be no separation, why?

• All components will move at the same rate through the column
Retention time

Example of a real chromatogram
The Theoretical Plate Model

- Many theories have been suggested to explain the mechanism of migration and separation of analytes in the column.
- The oldest one, called the *theoretical plate model*, corresponds to an approach now considered obsolete but which nevertheless leads to relations and definitions that are universal in their use and are still employed today.
- In this model, each analyte is considered to be moving progressively through the column in a sequence of distinct steps, although the process of chromatography is a dynamic and continuous phenomenon.
- Each step corresponds to a new equilibrium of the entire column.
- In liquid-solid chromatography, for example, the elementary process is described as a cycle of adsorption/desorption.
Column Efficiency

• As the analyte migrates through the column, it occupies an increasing area.

• This linear dispersion $\sigma_L$, measured by the variance $\sigma_L$ increases with the distance of migration.

• When this distance of migration is equal to $L$, the column length; $H$ is the same as the value for the height equivalent to one theoretical plate.

• Since $N = L/H$

• The appropriate equation for $N$ is

$$N = 5.54 \frac{t^2_R}{w^2}^{1/2}$$
Effective plate number

• If the performance of different columns has to be compared for a given compound, more realistic values are obtained by replacing the total retention times $t_R$, by the adjusted retention times $t'_R$

• $t'_R$ does not take into account the void time $t_M$ spent by the compound in the mobile phase.

• The mathematical relationships:

$$N_{\text{eff}} = 5.54 \frac{t'_R^2}{w_{1/2}^2}$$

$$N_{\text{eff}} = 16 \frac{t'_R^2}{w^2}$$
Retention factor $k$ (historically called capacity factor, $K'$)

- When a compound is injected onto a column, its total mass $m_T$ is divided in two quantities:
  - $m_M$, the mass in the mobile phase and $m_s$, the mass in the stationary phase.
- The values of these quantities are dependent on $M_T$ and $K$ but their ratio, the retention factor, is constant:
  \[
  k = \frac{m_s}{m_M} = K \frac{V_s}{V_M}
  \]
- The factor $k$, which is independent of the flow rate and length of the column, can vary with experimental conditions.
- $k$ is the most important parameter in chromatography for determining the behavior of columns.
- The value of $k$ should not be too high otherwise the time of analysis is unduly elongated.
Separation factor between two solutes

• The separation factor, $\alpha$, allows the comparison of two adjacent solutes 1 and 2 present in the same chromatogram.

\[ \alpha = \frac{t'_{R_2}}{t'_{R_1}} \]

Thus, the separation factor is given by the equation:

\[ \alpha = \frac{k_2}{k_1} \]
Resolution factor between two peaks

- To quantify the separation between two peaks, the resolution factor $R$ is used and can be obtained from the chromatogram

$$ R = 2 \frac{t_{R_2} - t_{R_1}}{w_1 + w_2} $$
• Selection guide for all of the different chromatographic techniques with liquid mobile phases.
• The choice of technique is chosen as a function of the molar mass, solubility and the polarity of the compounds to be separated.
Classification of chromatographic techniques

- Chromatographic techniques can be classified into **three categories** depending on
  - the *physical nature* of the phases,
  - the *process* used,
  - or the *physico-chemical phenomenon*, which is at the basis of the Nernst distribution coefficient $K$, also defined as:

$$K = \frac{C_S}{C_M} = \frac{\text{concentration of solute in the stationary phase}}{\text{concentration of solute in the mobile phase}}$$

- We will take here the classification based on the nature of the phase present
1. Liquid-solid chromatography

- The mobile phase is a liquid and the stationary phase is a solid.
- This category, which is widely used, can be subdivided depending on the retention phenomenon into:
  - Adsorption chromatography
  - Ion chromatography (Ion-exchange chromatography)
  - Molecular exclusion chromatography
a. Adsorption chromatography

• The separation of organic compounds on a thin layer of silica gel or alumina with solvent as a mobile phase.
• Solutes bond to the stationary phase because of physisorption or chemisorption interactions.
• The physico-chemical parameter involved is the *coefficient of adsorption*. 
Adsorption Chromatography

The stationary phase is solid. Separation is due to adsorption/desorption steps.
- Adsorbent can be packed in a column, spread on a plate, or impregnated in a porous paper.
- Both solutes and solvents will be attracted to the stationary phase.
- If the solutes have different degrees of attraction, separation would be achieved.
b. Ion chromatography (Ion-exchange chromatography)

- The mobile phase in this type of chromatograph is a buffered solution and the stationary phase consists of spherical μm diameter particles of a polymer.
- The surface of the particles is modified chemically in order to generate ionic sites.
- These phases allow the exchange of their mobile counter ion, with ions of the same charge present in the sample.
- This separation relies on the coefficient of ionic distribution.
Ion exchange chromatography

- Stationary phase has charged surface opposite that of the eluents
- Separation is based on the affinity of ions in solution for oppositely charged ions on the stationary phase
Separation by ion exchange chromatography

Conductivity detection

Mobile phase:
4mM salicylic (tris) pH 7.8

40 ppm of each component

1. Fluoride
2. Chloride
3. Nitrite
4. Bromide
5. Nitrate
6. Phosphate
7. Sulfate
c. Molecular (Size) exclusion chromatography

• The stationary phase is a material containing pores, the dimensions of which are chosen to separate the solutes present in the sample based on their molecular size.
• This can be considered as a molecular sieve allowing selective permeation.
• This technique is known as gel filtration or gel permeation, depending on the nature of the mobile phase, which is either aqueous or organic.
• The distribution coefficient in this technique is called the coefficient of diffusion.
Size exclusion chromatography

- Separation is based on molecular size. Stationary phase is a material of controlled pore size. It is also called Gel permeation chromatography.
Size exclusion chromatography

• Columns are made to match the separation of specific size ranges
• Larger species will elute first. They cannot pass through many pores so their path is shorter
• Size exclusion liquid chromatography is useful for determining size, size range and molecular weights of polymers and proteins.
Separation by size exclusion chromatography

This example shows three general classes of components.

The second has a much larger size distribution.
2. Liquid-liquid chromatography (LLC) (Partition chromatography)

A. Partition chromatography

Stationary phase is a liquid *immobilized* in the column.

It is important to distinguish between the inert support which only has a mechanical role and the stationary phase immobilized on the support.

Impregnation of a porous material with a liquid phase was used earlier but had the problem of bleeding.

In order to immobilize the stationary phase, it is preferable to fix it to a mechanical support using covalent bonds.

The stationary phase still acts as a liquid and the separation process is based on the partition of the analyte between the two phases at their interface.

The parameter involved in the separation mechanism is called the *partition coefficient*. 
Modes of separation

• **Normal phase** partition chromatography
  Polar stationary phase and nonpolar solvent
• **Reverse phase** partition chromatography
  Nonpolar stationary phase and polar solvent
• Reverse phase is now more common
3. Gas-liquid chromatography (GLC)

- The mobile phase is a gas and the stationary phase is a liquid.
- The liquid can be immobilized by impregnation or bonded to a support,
- The partition coefficient $K$ is also involved

4. Gas-solid chromatography (GSC)

- Stationary phase is a porous solid (such as graphite or silica gel) and the mobile phase is a gas.
- This type demonstrates very high performance in the analysis of gas mixtures or components that have a very low boiling point.
5. Supercritical fluid chromatography (SFC)

• The mobile phase is a fluid in its supercritical state, such as carbon dioxide at about 50 °C and at more than 150 bars (15 MPa).
• The stationary phase can be a liquid or a solid.
• This approach combines the advantages of the LLC and GLC techniques.
Qualitative analysis by Chromatographic methods

- Qualitative analysis is based on retention data
- Retention time $t_R$ is characteristic of a substance, compared to a standard.
- Reproducibility of retention depends upon several experimental conditions:
  - column length and diameter,
  - stationary and mobile phases,
  - column packing,
  - column temperature,
  - mobile phase flow rate
  - and others
Retention time, $t_R$

- $t_R$: It is the time elapsed from the point of injection to the peak maximum.
- Adjusted $t'_R$: It is the time from the maximum of unretained peak (the peak of the mobile phase or the air peak) to the peak maximum of a certain component.
- $t_M$ (hold up time): is the time required for the mobile phase to be eluted completely from the column.
IDENTIFICATION BY RETENTION TIMES

A

Same column under
Same conditions
Has been used

Unknown alcohol
components

B

Standard sample

a Methyl alcohol
b Ethyl alcohol
c n-propyl alcohol
d n-butyl alcohol
e n-amy alcohol
RELATIVE RETENTION

\[ a = \frac{t'_{R-2}}{t'_{R-1}} = \frac{K_2}{K_1} \]

- Component 1 is used as the reference; it should be present or added to the sample and compatible with the sample.
- Peak of component 1 must be close (but resolved) to the sample peak.
When component 3 is suspected, add more of this component to the sample and watch any change in its peak.
Basis for Quantitative Analysis

- The peaks in the chromatogram are the basis for quantitative analysis

**Peaks of interest should fulfill the following requirements:**
- must be undistorted
- must be well separated
- Must have a large S/N ratio
- must have a flat baseline

**Peak shape:** The ideal chromatographic peak is symmetric and narrow

- Peak integration
  The peak height or, better, the area must be determined and this is done by the computer

- Calculation
  - External standard method
  - Internal standard method
  - Internal normalization
External standards method

• Standard solutions covering the desired concentration range (preferably diluted from one stock solution) are chromatographed.

• The appropriate data (peak height or area) plotted vs. concentration to obtain a standard curve.

• An identical volume of sample is then chromatographed, and height or area of the solute of interest is compared to that obtained in the standard curve.

• Limitations: This approach requires precise analytical technique and requires that the analytical system be absolutely reproducible from injection to injection as well as from day to day.
External standard method

- This method is common to most quantitative analysis techniques.
- It allows the measurement of the concentration of one or more components that elute in a chromatogram containing, perhaps, many peaks.
- This method, employing the **absolute response factor**, $K$, is used in the following way (**Single point calibration method**).

$$C_{\text{Ref}} = K \cdot A_{\text{Ref}} \quad \text{and} \quad C_{\text{Unk}} = K \cdot A_{\text{Unk}}$$

$$C_{\text{Unk}} = C_{\text{Ref}} \frac{A_{\text{Unk}}}{A_{\text{Ref}}}$$
Multilevel External Calibration of Fatty Acids

The content % of $C_{14}$ fatty acids =

$$\frac{C_{14}}{C_{14} + \frac{C_{16}}{C_{16} + C_{18}}} \times 100$$

= the content % of $C_4$ fatty acids
Quantitative Methods
Gas Chromatography

- GC is the first instrumental chromatographic method developed commercially.
- It is relatively easy to introduce a stable flow and pressure for the mobile phase, that is the carrier gas.
- The equipment is simple. All that is really needed is cylinder of compressed air, pressure regulator and a valve.
Introduction

Principles
- Partition of molecules between mobile phase and stationary phase

Separation technique
- Gas is the mobile phase and liquid (GLC) or solid (GSC) is the stationary phase
- **GLC**: liquid is coated on an inert solid; separation is the result of variable solubilities in the liquid phase
- **GSC**: Particulate solid like molecular sieve is the stationary phase; separation is the result of variable adsorption on the solid surface
Gas Chromatography

Sample: mixture of volatile liquids (∼1µL)

Gas Chromatogram

Time (minutes)
GC Process

1. Column is selected, packed with liquid phase, and installed

2. Sample injected with microliter syringe into the injection port where it is vaporized and mixed into the carrier gas stream (helium, nitrogen, argon).

3. Sample becomes partitioned between the moving gas phase and the stationary liquid phase.

4. The time spent by different compounds of the sample in vapor phase is a function of their vapor pressure.

5. The more volatile compounds arrive at the end of the column first and pass into the detector.
Factors Affecting Separation

- Boiling Points of Components in Sample
  - Low boiling compounds have higher vapor pressures.
  - Boiling point increases with increasing molecular weight

- Flow Rate of Carrier Gas

- Choice of Liquid Phase (Solubility in the liquid stationary phase determines the retention time in the stationary phase)

- Molecular weights, functional groups, and polarities of component molecules are factors in selecting liquid phase.

- Length of Column
  - Similar compounds require longer columns than dissimilar compounds.
  - Isomeric mixtures often require quite long columns
Gas Chromatograph

Main components of a Gas Chromatograph

- 3 main components:
  1. **Injector** – introduces sample
     - Manual: syringe (glass syringe & fine needle
     - Auto-sampler: greater volume accuracy
     - Greater temp. than oven: immediate vaporization of sample
Gas Chromatograph

- gas system
- Inlet (Injector)
- column
- detector
- Readout system
gas travels through the metal tubing and is dispersed to each GC in turn
Gas Flow
Oven: Characteristics

- **Constant temp:** high enough to keep samples gaseous, but won’t break down column
- **Higher temperatures** – components move faster, sloppy separation
- **Lower temperatures** – longer retention, better resolution of separation
- **Oven columns:**

![Diagram of oven columns](image)

- Rubber septum
- Septum purge outlet
- Split outlet
- Vaporization chamber
- Column
- Glass liner
- Heated metal block
- Carrier gas inlet
2. Columns

Commonly glass, coiled to fit in oven
- Stainless steel
- Quartz or fused silica: (see below)
  - Stationary phase:
    - Liquid must be:
      - Non volatile
      - Thermally stable
      - Chemically un-reactive
    - Solid
      - Differentiated on adsorptivity

Column temperature
- must be controlled to within tenths of a degree.
- optimum column temperature is dependant upon the *boiling point* of the sample.
  - a temperature slightly above the average boiling point of the sample results in an elution time of 2 - 30 minutes.
- If a sample has a wide boiling range, then *temperature programming* can be useful.
  - temperature is increased (either continuously or in steps) as separation proceeds
3. Detectors

- Measure physical properties (preferred), not chemical properties.
- Different detectors will give different types of selectivity.
- **non-selective detector** - responds to all compounds except the carrier gas,
- **selective detector** - responds to a range of compounds with a common physical or chemical property
- **specific detector** - responds to a single chemical compound.

- Detectors can also be grouped into **concentration dependant detectors**
  - The signal is related to the concentration of solute in the detector,
  - does not usually destroy the sample
  - Dilution with make-up gases will *lower* the detectors response.
- **mass flow dependant detectors**
  - usually destroy the sample,
  - signal is related to the rate at which solute molecules enter the detector.
Various types of gas chromatographic detectors

• Flame Ionization Detector (FID),
• Thermal Conductivity Detector (TCD or hot wire detector),
• Electron Capture Detector (ECD),
• Photo Ionization Detector (PID),
• Flame Photometric Detector (FPD),
• Thermionic Detector
• VERY expensive choices: Atomic Emission Detector (AED)
  • Ozone- or Fluorine-Induced Chemiluminescence Detectors.
• All of these (except the AED) produce an *electrical signal* that varies with the amount of analyte exiting the chromatographic column.
• The AED does that & yields the emission spectrum of selected elements in the analytes as well.
- **TCD (Thermal conductivity detector)**
  - Uses a *Wheat stone bridge* – resistors compare unknown parameters to a known according to Ohm’s law

Two pairs of TCDs are used in gas chromatographs.
- One pair is placed in the column effluent to detect the separated components as they leave the column.
- Another pair is placed before the injector or in a separate reference column.
- The resistances of the two sets of pairs are then arranged in a bridge circuit.
- The heated element may be a fine platinum, gold, or tungsten wire or, alternatively, a semi conducting thermistor.
- The resistance of the wire or thermistor gives a measure of the thermal conductivity of the gas.
Thermal Conductivity Detector

Catharometer block and its connections
• The effluent from the column is mixed with hydrogen and air, and ignited.
• *Organic compounds* burning in the flame produce ions and electrons which *can* conduct electricity through the flame.
• The current resulting from the pyrolysis of any organic compounds is measured which is proportional to the carbon content of the molecule entering.
• The FID is a useful general detector for the analysis of *organic* compounds;
  – unfortunately, it destroys the sample.
Applications of GC in drug analysis

• Both quantitative and qualitative identification of the active components
• Possible contaminants, adulterants or characteristic features
• May indicate the source of the particular sample.
• Forensic analysis frequently uses GC to characterize drugs of abuse,
  • the source of manufacture of the sample
  • worldwide source of a vegetable material such as cannabis.
• Analytical procedures, chromatographic methods and retention data are published for over 600 drugs, poisons and metabolites.
  • data are extremely useful for
    • forensic work
    • hospital pathology laboratories
LC solvents depend upon the type of chromatographic mode used:

* Normal Phase
* Reversed phase
Solvent selection

• If the sample is water insoluble or nonpolar- normal phase mode is used
• If the sample is water soluble or not soluble but polar- use the reverse phase mode
• It is seldom to find a single solvent does the job. Thus mixtures of two or more solvents are used
• Two factors are considered:
  – **Solvent strength, \((\varepsilon^o)\)**
    A measure of relative solvent polarity (ability to displace a solute). It is the adsorption energy per unit area of solvent. \(\varepsilon^o\) for silica is about 0.8 of those on alumina
  – **Polarity index, \((P')\)**
    • Solvents that interact strongly with solutes are strong or polar solvents
    • Polarity of solvents has been expressed by many terms, one of which is the polarity index. Thus, the \(P'\) value measures the relative polarity of various solvents

Used for reversed phase methods
Isocratic and gradient elution

- **isocratic elution** - single solvent separation technique
- **gradient elution** - two or more solvents, varied during separation

- Gradient elution provides better resolution, higher sensitivity and shorter analysis time
Column Liquid Chromatography

- LC techniques are: Classical LC and HPLC or HS-LC (S = speed)
- Both techniques have same basic principle for separation but differ in apparatus and practice used
- HPLC gives high speed, high resolution, high sensitivity and convenient for quantitative Analysis.
HPLC

originally referred to:
High Pressure Liquid Chromatography

currently refers to:
High Precision Liquid Chromatography

– the high pressure allows using small particle size to allow proper separation at reasonable flow rates
Features of HPLC compared to Classical LC

**Particle size of the packing substance**

- Classical LC utilises large porous particles that make it difficult to speed up the flow rate by pumping due to a decrease in resolution that results from the mass transfer limitation in the deep pores. These high capacity particles are good for preparative chromatography.

- Since the mass transfer coefficient is a function of the square of $d_p$ (van deemter eq.) thus the HPLC was based on using pellicular and porous microparticles. Pellicular particles when packed into narrow columns will lead to an increase in column efficiency of 10 to 100 folds.

- Pellicular particles have dense solid cores thus they are easily packed.

- $V_s$ is significantly reduced and the sample capacity is reduced to 0.05 to 0.1 of the totally porous packing.
Column length

- Efficiency is very high due to packing thus shorter columns are used (~ 20 cm)
- For difficult separations longer (50-100 cm) are used with smallest available particles and high pressure solvent feed

Effect of sample size on column efficiency

- Efficiency increases as the sample size decreases
Columns

- LC columns could be made of **stainless steel, glass and glass lined stainless steel** that is used for extremely inert surfaces.
- In classical LC, elution takes place under gravity or low pressure by using small pumps.
- Columns can be thermostated by placing in an oven or using a water jacket.
- HPLC columns are mainly made of stainless steel packed with the microparticles.
- When same amount is injected in the HPLC column, narrower and longer peaks are obtained that leads to greater detector sensitivity.
- In HPLC solvent consumption is reduced.
- HPLC columns facilitate coupling to MS that requires flow rates <50 μL/min to avoid over pressuring the ion source in the MS.
Components

Solvent Reservoir and Degassing System
Pumps
Precolumns
Sample Injection System
Columns
Temperature Control
Detectors
Readouts
Schematic diagram of a typical high performance liquid chromatograph
Schematic of Liquid Chromatograph
Solvent Reservoir and Degassing System

- **isocratic elution** - single solvent separation technique
- **gradient elution** - 2 or more solvents, varied during separation
• **Process:**
  – Sample (mixed solutes) is injected into a liquid mobile phase
  – Enters column
  – Components interact with stationary phase
    • Solutes separate based on physical and chemical characteristics
  – Fractions leave column
  – Enter photometric OR electrochemical detector
  – Fractions are sensed and quantitated
  – Electrical signal goes to processing unit
  – Output device (printer or chart recorder) provides hard copy

• **Characteristics of HPLC**
  – Sample & carrier *can* chemically interact
  – Columns are shorter and smaller (25cm long / 2-5 mm diameter)
  – Stationary phase surface area is increased by using very small diameter packing material (5-50 um)
  – Small packing material requires high pressure (1500 – 3000 psi)
• **Injector**
  – 2-position, parallel-column loop valve
    • Sample is injected with syringe while in load position
    • Inject position – parallel loop is in series with the main column
      – Sample is flushed into the mobile phase stream
    • *higher precision than septum injections*

• **Mobile phase**
  – A liquid solvent (varies based on mechanism of separation)
  – Possible interaction with sample increases selectivity of separation process
  – Isocratic elution - single solvent used
  – Gradient elution – more than one solvent
    • Polarity is adjusted during analysis (or pH is adjusted)
    • accelerates the elution of substances which normally elute slowly (or those with widely different polarities of pKs)
    • (equivalent of temperature programming in GC)
• Pump
  – Provides pressure to move mobile phase through the column
  – Pressure depends on:
    • Length of column
    • Particle size of stationary phase
    • Viscosity of mobile phase
    • Flow-rate of mobile phase
• **Column**
  – Tubular structure made of metal
    • Internal diameter = 2-5 mm
    • Length = 25 – 50 cm
  – Filled with a highly selective packing material
    • Packing type determined by application
      – Silica particles → Adsorption chromatography
      – Silica particles coated with a chemically bonded stationary phase → Partition and Ion-exchange chromatography
      – Silica particles with a definite pore size → Exclusion chromatography
    • Particle diameter = 3,5, or 10 um

• **Detector**
  – Senses the solute fractions as they leave the column
  – 2 major categories
    • Photometric
    • electrochemical
Applications

- Identification of compounds by HPLC is a crucial part of any HPLC assay.
  - accomplished by researching the literature and by trial and error.
  - Identification of compounds can be assured by combining two or more detection methods.
- Quantification - the process of determining the unknown concentration of a compound in a known solution.
  - inject a series of known concentrations of the standard compound solution
  - chromatograph of these known concentrations
  - peaks that correlate to the concentration of the compound injected