Chromatography

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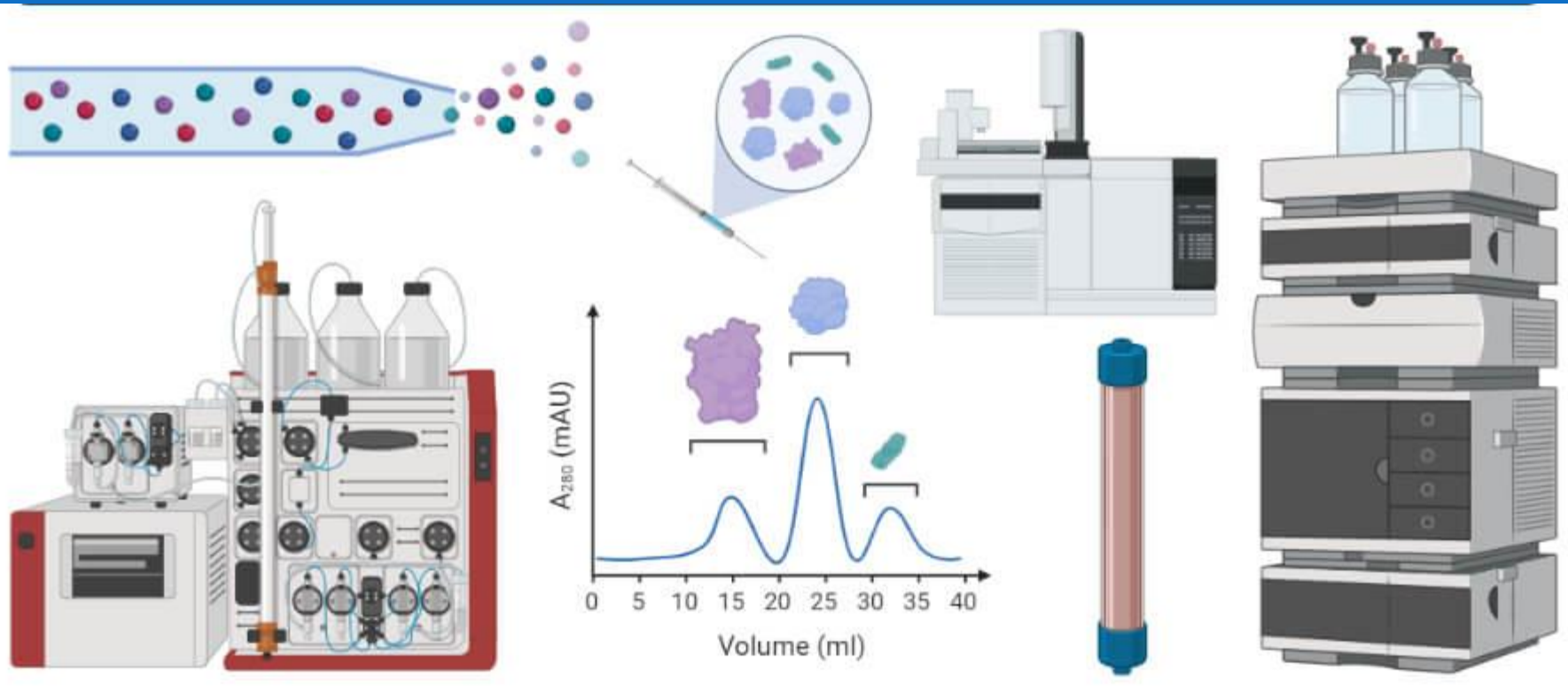
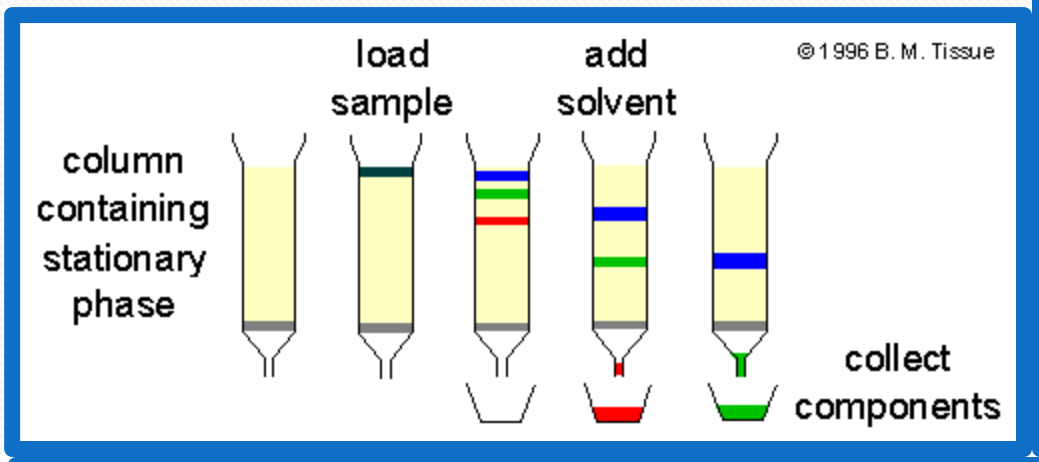
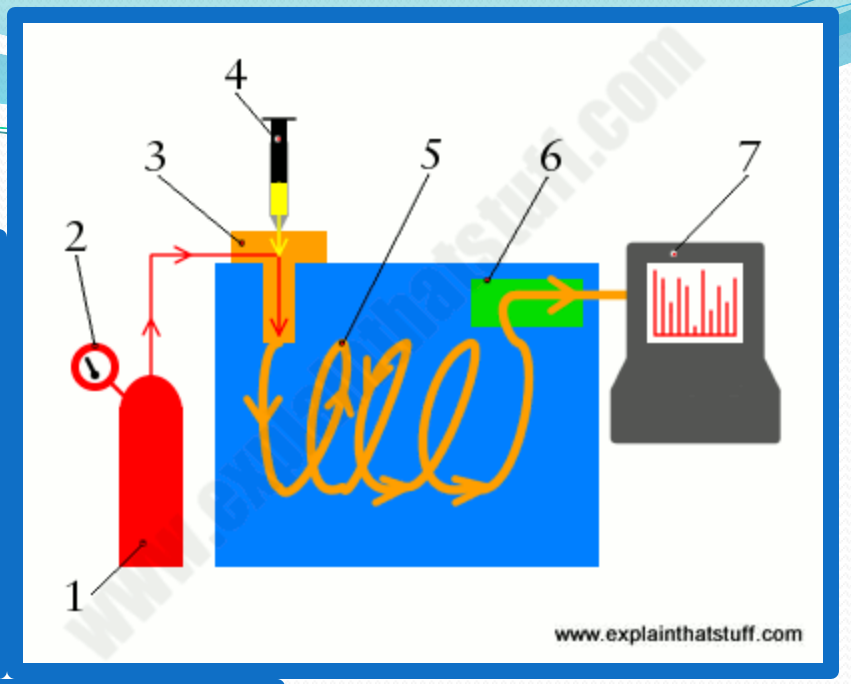


Chromatography

The word “chromatography” originated from two Greek words, chroma which means “color” and graphy which means “writing”.

Chromatography was founded in 1906 by a Russian botanist, Micheal Tswett, when he separated plant extracts on a column packed with finely divided calcium carbonate.


load sample
add solvent
column containing stationary phase
collect components
©1996 B. M. Tissue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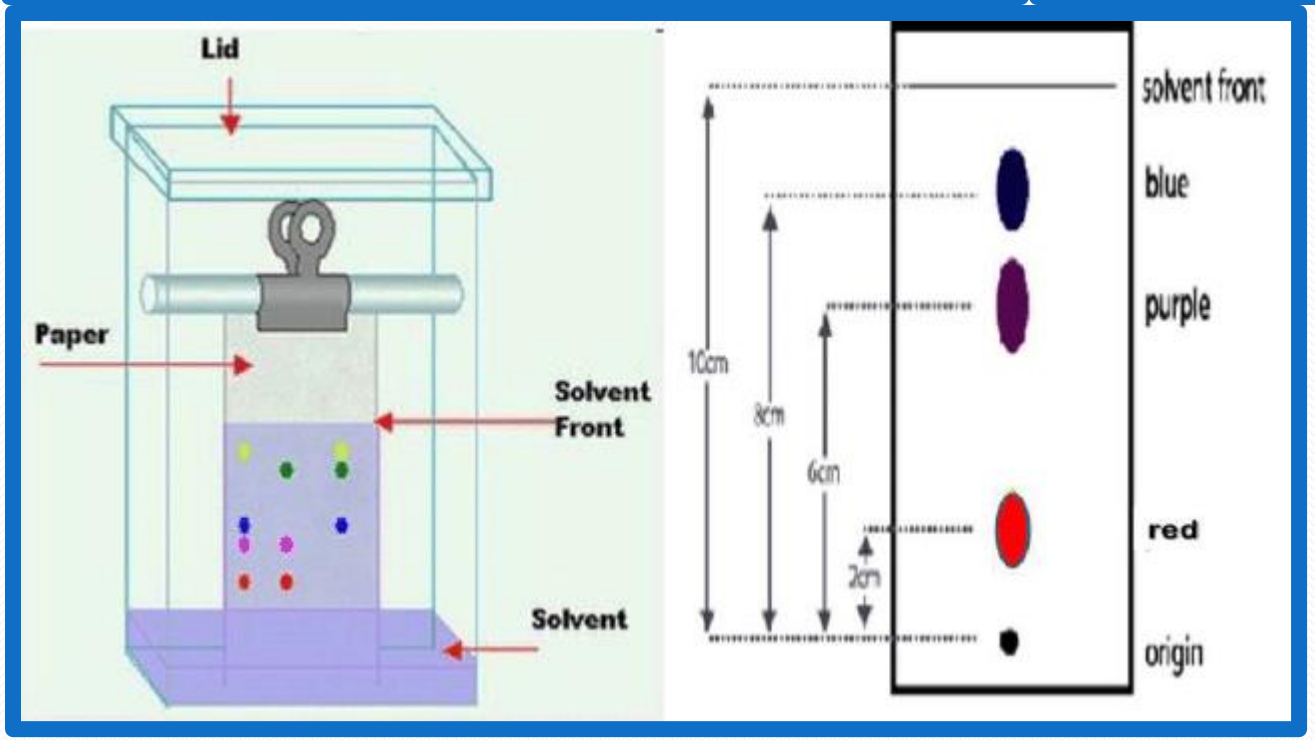

Lid
Paper
Solvent Front
Solvent

solvent front
blue
purple
red
origin
10cm
8cm
6cm
2cm

What is chromatography?

Chromatography is a powerful separation method that is usually composed of mobile phase and a stationary phase.

This method is used to separate and identify the components of complex mixtures.

In chromatography, the compounds are physically separated by distributing themselves between two phases:(a) a stationary phase(b) a mobile phase which flows continuously across the stationary phase.

Those components that are strongly retained by the stationary phase move slowly with the flow of mobile phase.

In contrast, components that are weakly held by the stationary phase travel rapidly.

As a consequence of these differences in mobility, sample components separate into discrete bands that can be analyzed qualitatively and/or quantitatively.

Purpose of Chromatography

Chromatography is a method to separate two or more compounds in a mixture based on the differences in the property of each individual substance. The properties include polarity, solubility, ionic strength, and size.

Chromatography is today one of the most useful analytical methods for separation, identification, and quantitation of chemical compounds.

Classification of Chromatographic Methods

Can be categorized based on the followings:

1. Based on physical means
   The way stationary and mobile phase are brought into contact
2. Based on the types of mobile phase
   Either gas, liquid or supercritical fluid
3. Based on the kinds of equilibria involved in the solute transfer between the phases.
4. Size of components of mixture

Interaction of analyte between stationary and mobile phases

Principles The stationary phase can be

(a) a solid packed into a column

(b) a solid coating the surface of a flat, plane material

(c) a liquid supported on a solid

(d) a liquid supported on the inside wall of an open tube.

The mobile phase can be a gas, a liquid or a supercritical fluid.

The mixture to be analysed is introduced onto the stationary phase and mobile phase carries the components through it. Each individual analyte interacts with the two phases in a different manner.

Because analytes differ in their affinity for the stationary phase vs the mobile phase, each analyte exhibits different migration and elution patterns and thus a mixture of analytes can be separated and quantified. The tracing of the output signal vs time or mobile phase volume is called a chromatogram.

The twelve types are:

(1) Column Chromatography
(2) Paper Chromatography
(3) Thin Layer Chromatography
(4) Gas Chromatography
(5) High Performance Liquid Chromatography
(6) Fast Protein Liquid Chromatography
(7) Supercritical Fluid Chromatography
(8) Affinity Chromatography
(9) Reversed Phase Chromatography
(10) Two Dimensional Chromatography
(11) Pyrolysis Gas Chromatography and
(12) Counter Current Chromatography.

There are different kinds of chromatographic techniques and these are classified according to the shape of bed, physical state of mobile phase, separation mechanisms.

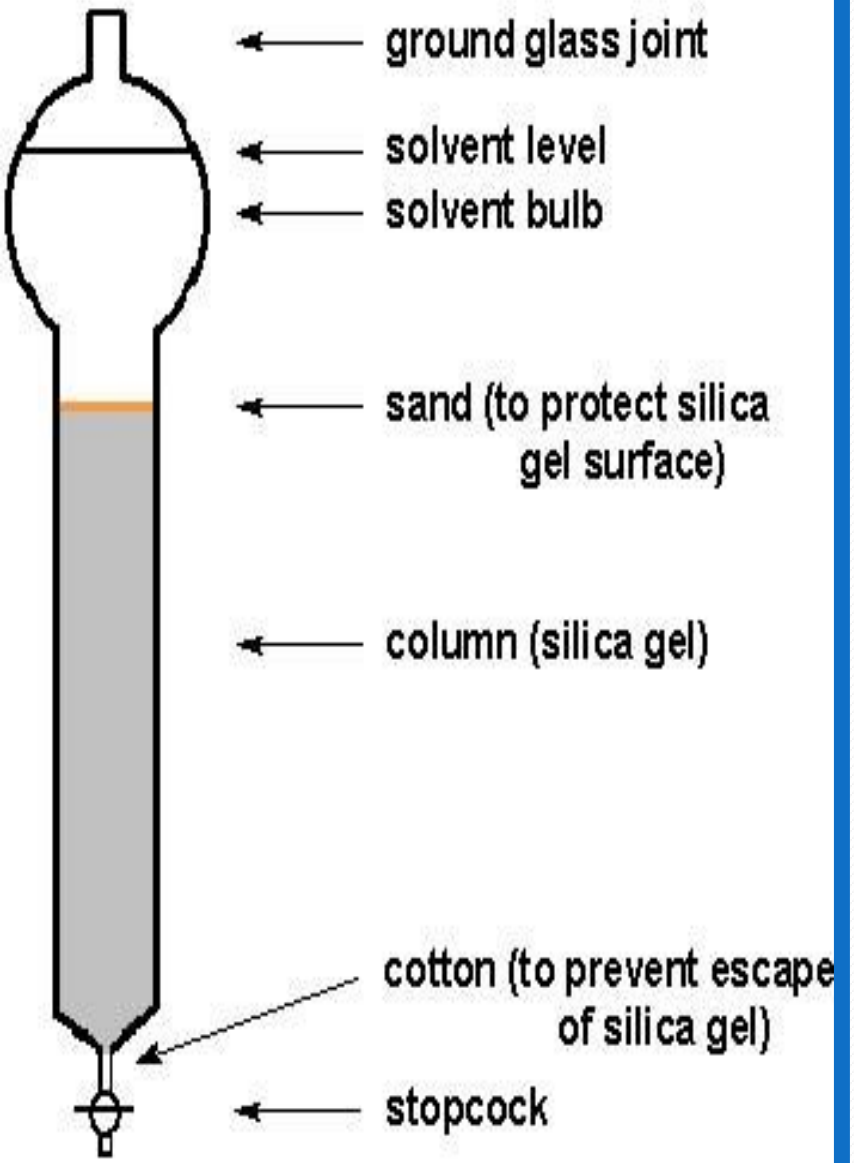
Apart from these there are certain modified forms of these chromatographic techniques involving different mechanisms and are hence categorized as modified or specialized chromatographic techniques

Column Chromatography

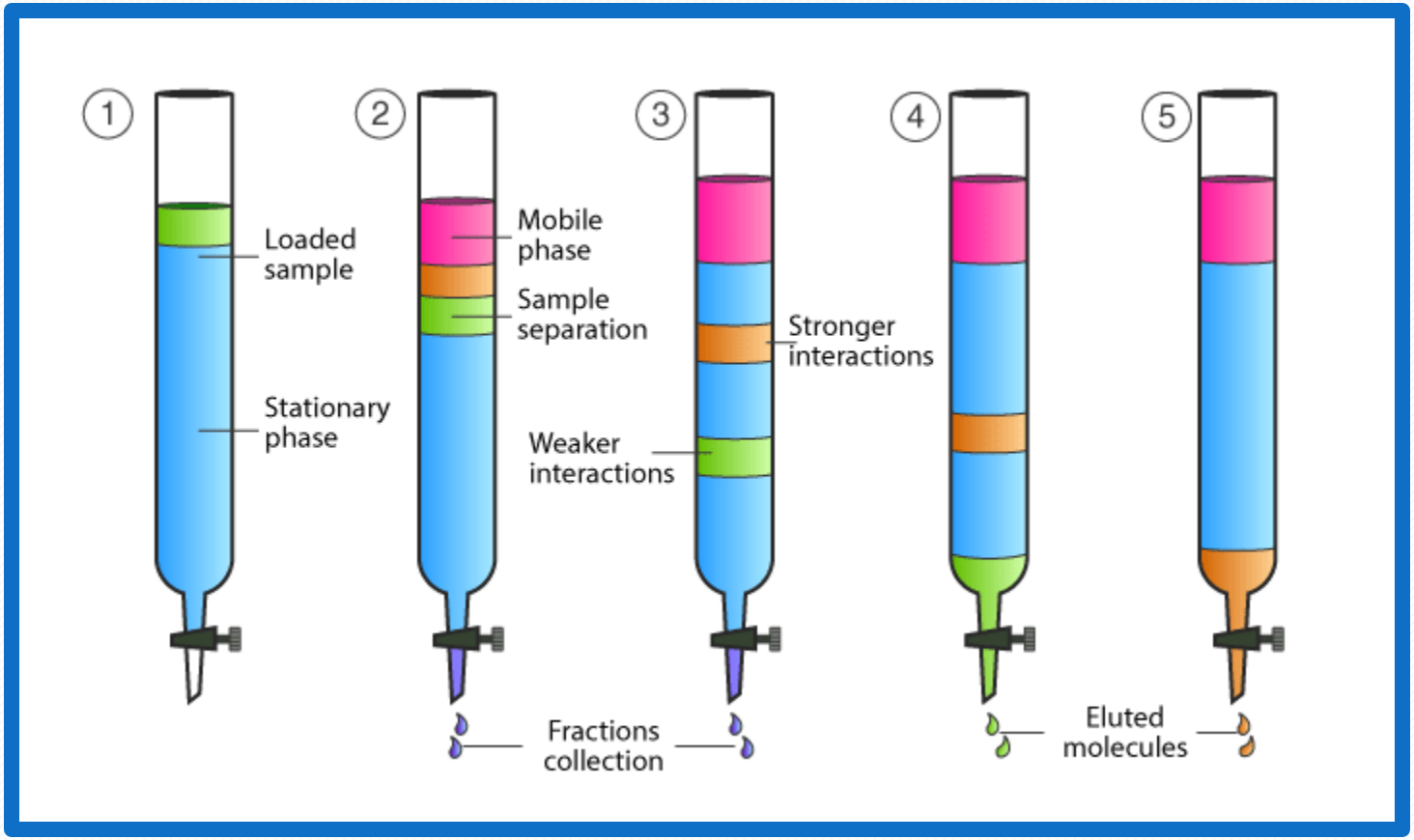
It is a technique in which the mixture of substances is introduced onto the top of a **column** packed with an adsorbent, passed through the **column** at different rates that depend on the affinity of each substance for the adsorbent and for the solvent or solvent mixture. The main **principle** involved is adsorption of the solutes of a solution through a stationary phase and separates the mixture into individual components. This is based on the affinity towards the mobile phase and stationary phase.

The main advantage of this chromatography technique is that the stationary phase is less expensive and can be easily disposed of as it undergoes recycling.

It is used to obtain pure chemical compounds from a mixture of compounds on a scale from micrograms up to kilograms using large industrial columns.



Column Chromatography



The classical preparative chromatography column is a glass tube with a diameter from 5 to 50 mm and a height of 50 cm to 1 m with a tap at the bottom.

Slurry is prepared of the eluent with the stationary phase powder and then carefully poured into the column. Care must be taken to avoid air bubbles. A solution of the organic material is pipetted on top of the stationary phase.

The individual components are retained by the stationary phase differently and separate from each other while they are running at different speeds through the column with the eluent.

At the end of the column they elute one at a time. During the entire chromatography process the eluent is collected in a series of fractions.

The composition of the eluent flow can be monitored and each fraction is analyzed for dissolved compounds, e.g.,
by analytical chromatography,
UV absorption, or
fluorescence.
Coloured compounds (or fluorescent compounds with the aid of an UV lamp) can be seen through the glass wall as moving bands.

This layer is usually topped with a small layer of sand or with cotton or glass wool to protect the shape of the organic layer from the velocity of newly added eluent.

Eluent is slowly passed through the column to advance the organic material.

Often a spherical eluent reservoir or an eluent-filled and stoppered separating funnel is put on top of the column.

The stationary phase or adsorbent in column chromatography is a solid. The most common stationary phase for column chromatography is — $C_{18}H_{37}$, followed by alumina. Cellulose powder has often been used in the past.

Also possible are ion exchange chromatography, reversed-phase chromatography (RP), affinity chromatography or expanded bed adsorption (EBA). The stationary phases are usually finely ground powders or gels and/or are micro porous for an increased surface; though in EBA a fluidized bed is used.

The mobile phase or eluent is either a pure solvent or a mixture of different solvents. It is chosen so that the retention factor value of the compound of interest is roughly around 0.75 in order to minimize the time and the amount of eluent to run the chromatography.

The eluent has also been chosen so that the different compounds can be separated effectively. The eluent is optimized in small scale pretests, often using thin layer chromatography (TLC) with the same stationary phase.

A faster flow rate of the eluent minimizes the time required to run a column and thereby minimizes diffusion, resulting in a better separation.

A simple laboratory column runs by gravity flow. The flow rate of such a column can be increased by extending the fresh eluent filled column above the top of the stationary phase or decreased by the tap controls.

Better flow rates can be achieved by using a pump or by using compressed gas (e.g., air, nitrogen, or argon) to push the solvent through the column (flash column chromatography).

Automated flash chromatography systems attempt to minimize human involvement in the purification process. Automated systems may include components normally found on HPLC systems (gradient pump, sample injection apparatus, UV detector) and a fraction collector to collect the eluent.

The software controlling an automated system will coordinate the components and help the user to find the resulting purified material within the fraction collector. The software will also store results from the process for archival or later recall purposes.

Types of Column Chromatography

Five **chromatographic** methods that use **columns** are:
Gas **chromatography** (GC),
Liquid **chromatography** (LC),
Ion exchange **chromatography** (IEC),
Size exclusion**chromatography** (SEC), and chiral **chromatography**.


The End