# Instrumentation of HPLC

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# INTRODUCTION

- HPLC- High Performance Liquid Chromatography

Or

High pressure liquid chromatography

Definition:

- It is a chromatographic technique used to separate components of mixture for the purpose to identify, quantify or purify the individual components of the mixture.
- This is widely used in field of biochemistry and analytical chemistry.

## First, What is Liquid Chromatography?

Liquid chromatography is a separation technique that involves:

The placement (injection) of a small volume of liquid sample into a tube packed with porous particles (stationary phase) • where individual components of the sample are transported along the packed tube (column) by a liquid moved by gravity.

The modern form of liquid chromatography is now referred to as "flash chromatography" • The components of the sample are separated from one another by the column packing that involves various chemical and/or physical interactions between their molecules and the packing particles.

The separated components are collected at the exit of this column and identified by an external measurement technique, such as a spectrophotometer that measures the intensity of the color, or by another device that can measure their amount

# What is HPLC?

HPLC is an abbreviation for High Performance Liquid Chromatography (It has also been referred to as High Pressure LC)

HPLC has been around for about 35 years and is the largest separations technique used • The history of HPLC: • Beginning of the 60's: start of HPLC as High Pressure Liquid Chromatography • End of the 70's improvements of column material and instrumentation – High Performance Liquid Chromatography •

# What is HPLC?

## HPLC is a separation technique that involves:

The injection of a small volume of liquid sample into a tube packed with tiny particles (3 to 5 micron (µm) in diameter called the stationary phase)

where individual components of the sample are moved down the packed tube (column) with a liquid (mobile phase) forced through the column by high pressure delivered by a pump.

In principle, LC and HPLC work the same way except the speed, efficiency, sensitivity and ease of operation of HPLC is vastly superior. These components are separated from one another by the column packing that involves various chemical and/or physical interactions between their molecules and the packing particles. These separated components are detected at the exit of this tube (column) by a flow-through device (detector) that measures their amount. An output from this detector is called a "liquid chromatogram".

# Types of HPLC techniques

- Based on mode of chromatography
- Based on principle of separation
- Based on elution technique
- Based on scale of operation
- Based on type of analysis

# Based on modes of chromatography

## Normal phase mode:

- The **stationary phase is polar** in nature & the **mobile phase is non-polar.**
- This is not advantageous in pharmaceutical application since most of the drug molecules are polar in nature and takes longer time to be eluted and detected.

## Reverse phase:

- The **stationary phase is non-polar** & the **mobile phase is polar** in nature.
- Since most of the drugs and pharmaceuticals are polar in nature, they are not retained for a longer time and eluted faster.

# Based on principle of separation

- **Adsorption chromatography:** Separation of components takes place because of the difference in affinity of compounds towards stationary phase.
- **Ion exchange chromatography:** An ion is used to separate a mixture of similar charged ions.
- **Size exclusion or gel permeation chromatography:** A mixture of components with different molecular sizes are separated by using gels which acts as sieve.

# Based on elution technique

- **Isocratic separation:** In this technique, the same mobile phase combination is used throughout the process of separation. The same polarity or elution strength is maintained throughout the process.
- **Gradient separation:** In this technique, a mobile phase combination of lower polarity or elution strength is used followed by gradually increasing the polarity or elution strength.

# Based on the scale of operation

- **Analytical HPLC:** Where only analysis of the samples are done. Recovery of the samples is not done
- **Preparative HPLC:** Where the individual fractions of pure compound can be collected using fraction collector. The collector samples are reused.

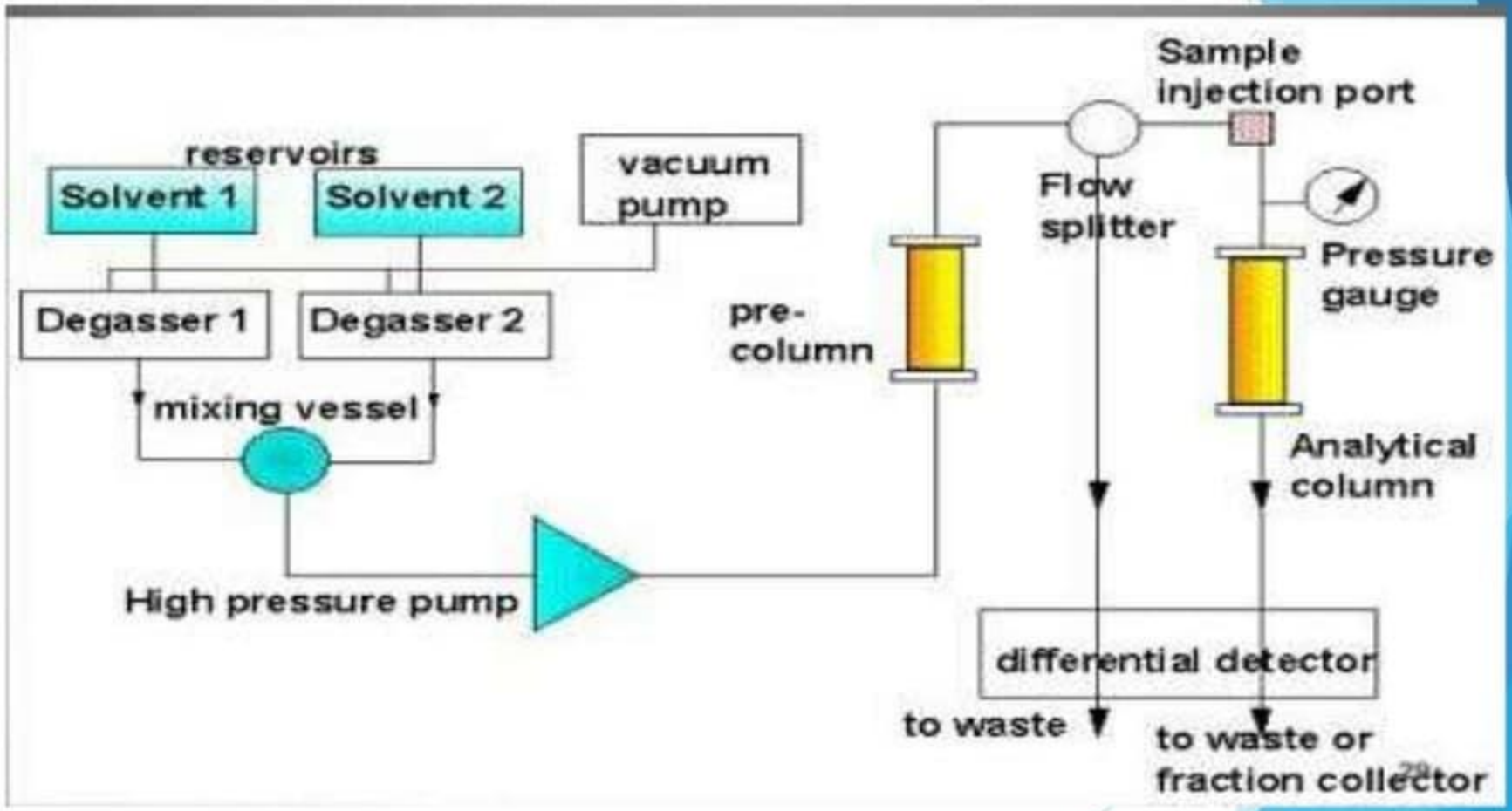
# Based on the type of analysis

- **Qualitative analysis:** Which is used to identify the compound, detect the impurities, to find the number of components, etc
- **Quantitative analysis:** Which is done to determine the quantity of the individual or several components in a mixture. This can be done by comparing peak area of the standard and sample

# INSTRUMENTATION

HPLC instrument consists of following components:

- Pump
- Mixing unit
- Solvent degassing
- Injector
- Column
- Detectors
- Application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Solvent 1
Solvent 2
vacuum pump
Degasser 1
Degasser 2
mixing vessel
High pressure pump
Sample injection port
Flow splitter
Pressure gauge
pre-column
Analytical column
differential detector
to waste
to waste or fraction collector