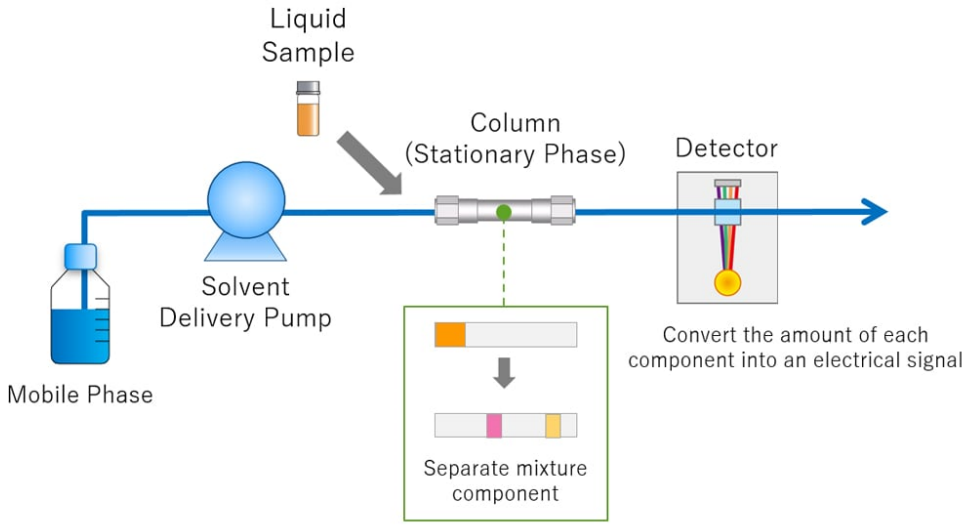
Lab 4: High Performance Liquid Chromatography

HPLC is a form of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector.



It is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. Today it is widely applied for separations and purifications in a variety of areas including pharmaceuticals, biotechnology, environmental, polymer and food industries.

HPLC is accomplished by injection of a small amount of liquid sample into a moving stream of liquid (called the mobile phase) that passes through a column packed with particles of stationary phase.

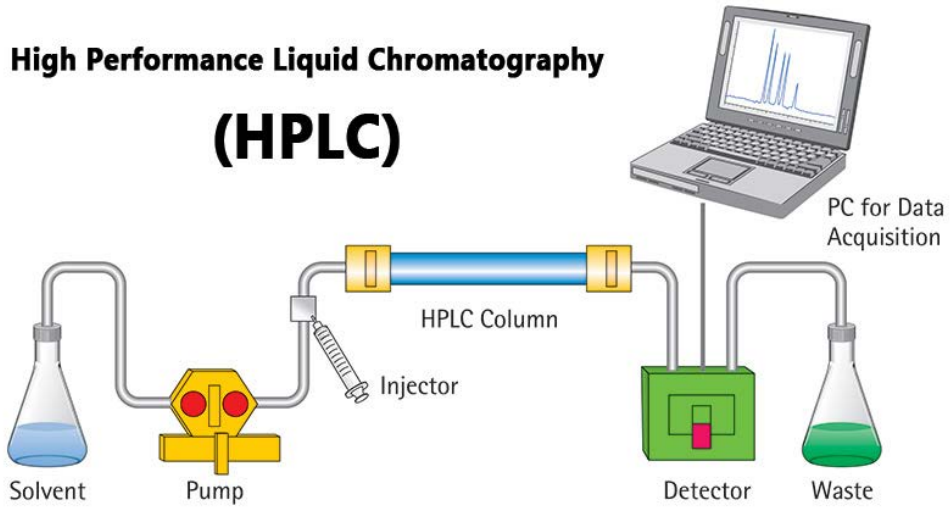
Separation of a mixture into its components depends on different degrees of retention of each component in the column. The extent to which a component is retained in the column is determined by its partitioning between the liquid mobile phase and the stationary phase.

In HPLC this partitioning is affected by the relative solute/stationary phase and solute/mobile phase interactions. Thus, unlike GC, changes in mobile phase

composition can have an enormous impact on the separation. Since the compounds have different mobilities, they exit the column at different times; i.e., they have different retention times. The retention time is the time between injection and detection.

HPLC System

1. Solvent delivery system
2. Pumps
3. Sample injection system
4. Column
5. Detectors
6. Recorders and Integrators



Solvent Delivery System:

The solvents or mobile phases used must be passed through the column at high pressure at about 1000 to 3000 psi (power per square inch pressure unit which equals to 6,894.76pascals). This is because as the particle size of stationary phase is few µ (5-10µ), the resistance to the flow of solvent is high. Hence such high pressure is recommended.

Pumps:

Two types of pumps are available:

1. Syringe Pumps
2. Reciprocating Pumps

Sample Injection System:

Several devices are available either for manual or auto injection of the sample. Different devices are:

1. Septum injectors
2. Stop flow injectors
3. Rheodyne injectors (loop valve type)

Rheodyne injector is the most popular injector. This has a fixed volume loop like 20µl or 50µl or more. Injector has 2 modes, Load position and Inject mode.

Columns:

1. Stainless steel tubing for high pressure
2.Heavy-wall glass or PEEK tubing for low P (< 600 psi).

Detectors:

Detectors used depends upon the property of the compounds to be separated. Different detectors available are:

1. U.V detectors
2. Fluorescence detectors
3. Electro chemical detectors
4. Evaporative light scattering detectors

Recorders and Integrators:

Recorders are used to record the responses obtained from detectors after amplification. They record the base line and all the peaks obtained, with respect to time. Retention time for all the peaks can be found out from such recordings, but the area of individual peaks cannot be known.

Integrators are improved version of recorders with some data processing capabilities. They can record the individual peaks with retention time. Height and width of peaks, peak area, percentage of area, etc. Integrators provide more information on peaks than recorders. Nowadays computers and printers are used for recording and processing the obtained data and for controlling several operations

General Specification

• Separation of organic, inorganic, biological compounds, polymers, and thermally liable compounds

• Qualitative and quantitative methods

Difference between Liquid and Gas Chromatography

Specifications	HPLC	GC
Sample volatility	• No volatility required • Sample must be soluble in mobile phase	• Sample must be volatile
Sample polarity	• Separates both polar and non-polar compounds • Inorganic ions	Samples are polar and non-polar
Sample preparation	Samples must be filtered	Samples should be in the same solvent as mobile phase
Sample size	Sample size based upon column	Typically 1-5µl

Limitations:

1. Qualitative analysis may be limited unless HPLC is interfaced with mass spectrometry.
2. Resolution is limited with very complex samples

Common Specific Applications

1. Quantitative/qualitative analyses of amino acids, nucleic acids, proteins in physiological samples
2. Measuring levels of active drugs, synthetic by products, degradation products in pharmaceuticals
3. Measuring levels of hazardous compounds such as pesticides and insecticides
4. Monitoring environmental samples
5. Purifying compounds from mixtures