**HPLC**

**’’ High Performance Liquid Chromatography’’**

Is a techniques in analytic chemistry used to separate the components in a mixture, in order to identify each component, and the quantify each component.

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But could equally well stand for ‘’ High Pressure Liquid Chromatography’’.



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Why it is used?

It is used for separating mixtures either to analyze the mixture or to separate a required products from others in the mixture. It can also be used to find a relative amounts of different components in a mixture.

It works on the same principle as a paper chromatography by showing speeded up . A liquid called a mobile phase, moves pass the solid the stationary phase, which is usually composed of irregularly or spherically shaped particles, a porous monolithic layer, or a porous membrane by a liquid( mobile phase ) at high pressure .

Paper Chromatography

the solvent moves along the paper by Capillary action



**The HPLC divided into two sub-classes based on the polarity of the mobile and the stationary phases . 1- When the polarity of Stationary phase > polarity of mobile phase called : Normal phase liquid Chromatography ( NPLC) Like ( toluene as a mobile phase and silica as a stationary phase .This type has fewer applications 2-When the opposite , polarity of Stationary phase < polarity of mobile phase called : reversed phase liquid chromatography (RPLC), this type Widely used upon (NPLC).For example : water –methanol mixture as a mobile phase and C18=octadecylsilyl as the stationary phase**

The HPLC is used for example to separated mixture of two steroid used in pharmaceutical preparation , the column is packed with a nonpolar solid, the tails of the molecules represent hydrocarbon chains C8H17, after chosen the solvent, detector wavelength and the flow rate, a single sample is run by injected about 20 microliters into the injection port , then the more polar component comes off the column first , followed by less polar. The other ingredients which is used in the formulation of the product is also appeared with a peak of a retention time 1.5 minutes.