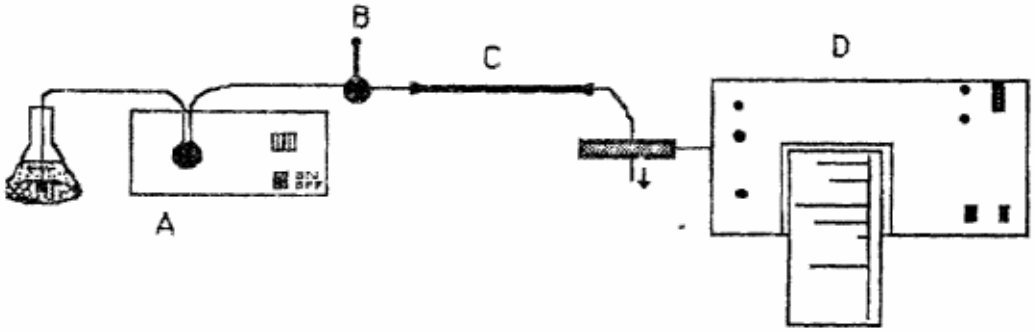
An Introduction to High Performance Liquid Chromatography

High Performance Liquid Chromatography, or HPLC, is the most common analytical separation tool and is used in many aspects of drug manufacture and research. HPLC is used for:

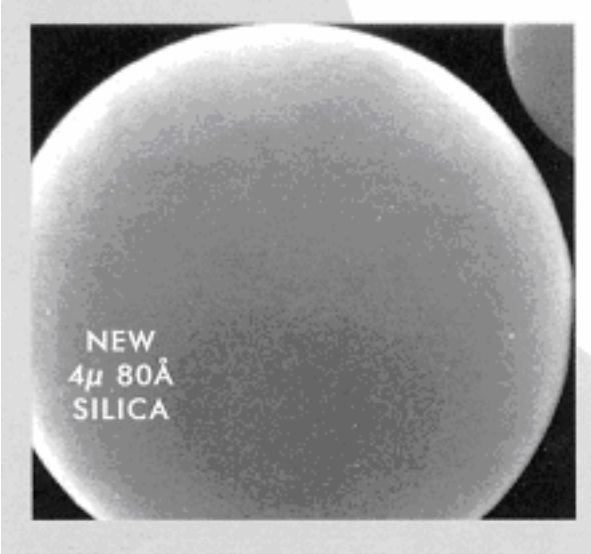
1. Qualitative and quantitative analysis of unknown mixtures – determining what is there, and how much.
2. Separation of mixtures for later analysis –preparative HPLC.

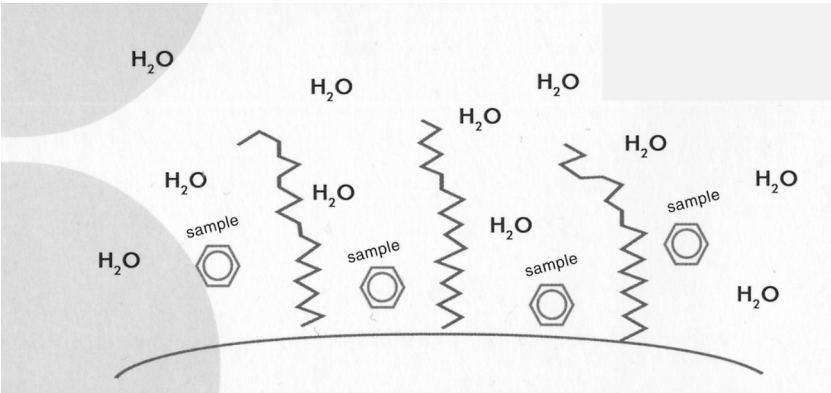
In chromatography a small volume of a mixture of chemicals is passed through a column using a solvent and different molecules exit the column at different times – this is called separation. The separation of a compound involves its physical interaction with a stationary phase and a mobile phase. In chromatography a tube is filled with stationary phase (typically surface-modified silica particles or silica "gel") and a mobile phase (solvent) is passed through the system. In HPLC the stationary phase is extremely small. A standard particle size for column chromatography is 60 microns, while that for HPLC is typically 5 microns, or the size of a speck of dust! Solvent flow through such dense material requires a high pressure, so in HPLC the stationary phase is packed in a stainless steel tube, and solvent is pumped through the system under high pressure, up to several thousand pounds per square inch (psi). This pressure results in a flow rate of several ml's per minute. Why is the stationary phase in HPLC is so tiny? A small, high-surface-area stationary phase maximizes the interaction between the substance to be separated and the stationary phase, which results in better separation.

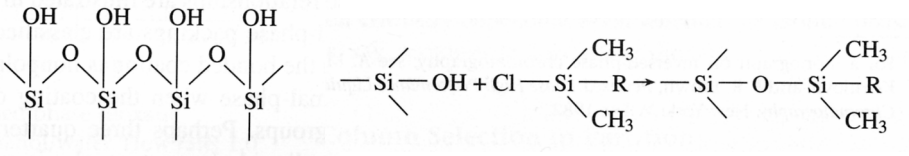
The primary parts of an HPLC are a solvent PUMP (A), an injector (B), the column (C), and a detector/recorder (D): Each of these components are connected in a series to each other by steel tubing. The pump controls the flow of solvent through the system. Upon leaving the pump, solvent enters the injector, then passes through the column, and finally through the optical unit of a detector. The injector allows a convenient and controlled introduction of sample directly onto the column, while the detector indicates when a particular analyte (also called a band) elutes (or leaves the column). If desired, the separated components can be individually collected for later analysis.
The two important things to know about each analyte appearing as a peak in the chromatogram are 1 the retention time and 2 the peak area. An example of a chromatogram of diet Pepsi (using much different conditions than we will use) is below:



The stationary phase in HPLC comprises high surface area particles of silica that have a ‘greasy’ coating (just one molecule thick!) on the surface.







A chromatographic separation works in the following way:

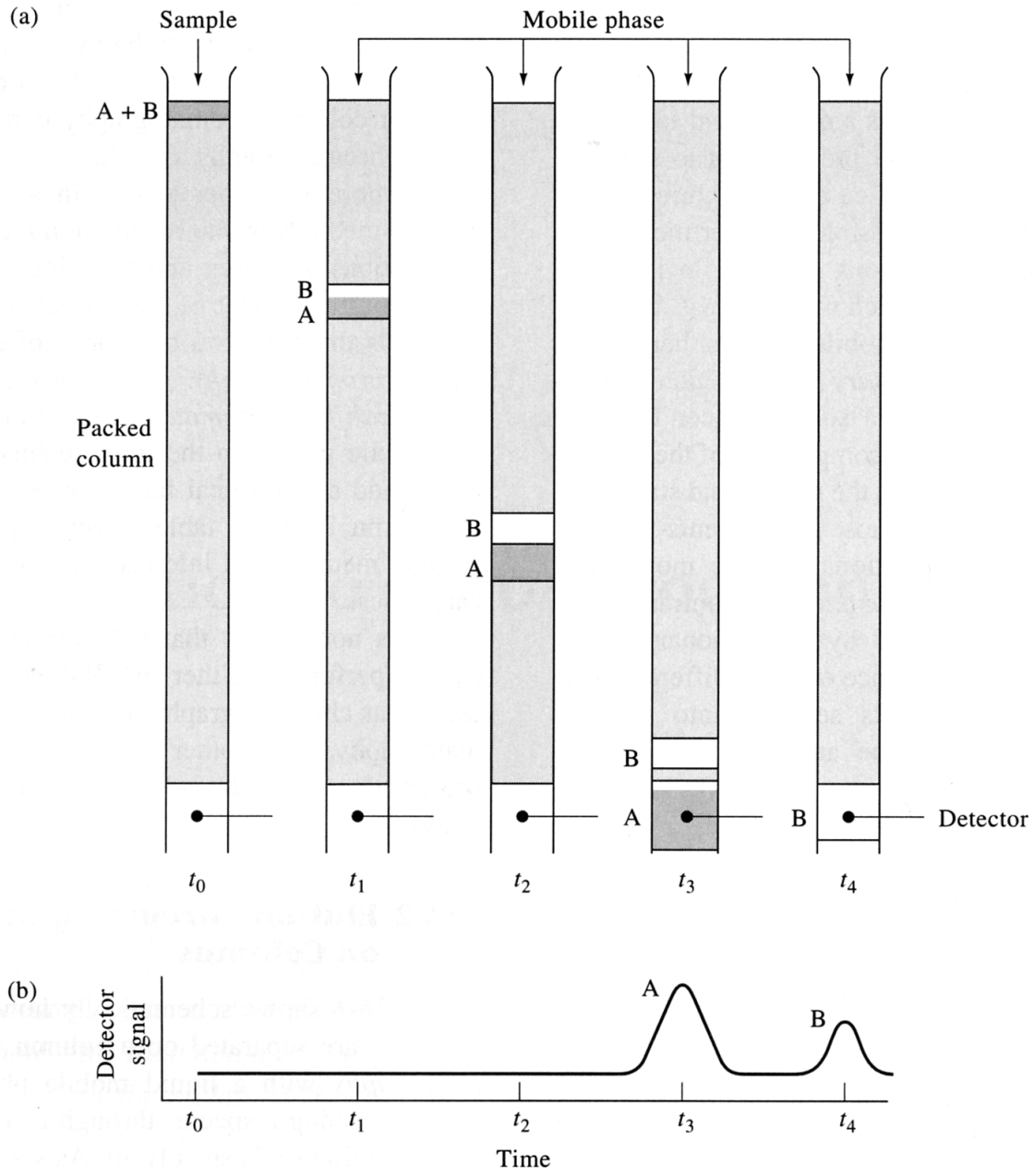


Figure 26-1 (a) Diagram showing the separation of a mixture of components A and B by column elution chromatography. (b) The output of the signal detector at the various stages of elution shown in (a).

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The most important pieces of information that you will need for each peak are the ***retention time*** (this tells you what the analyte is) and the ***peak area*** (this tells you how much of it is there). The retention time should be the same for the standard and the sample – so you can separate your analyte it from the other components of the sample.

An example: