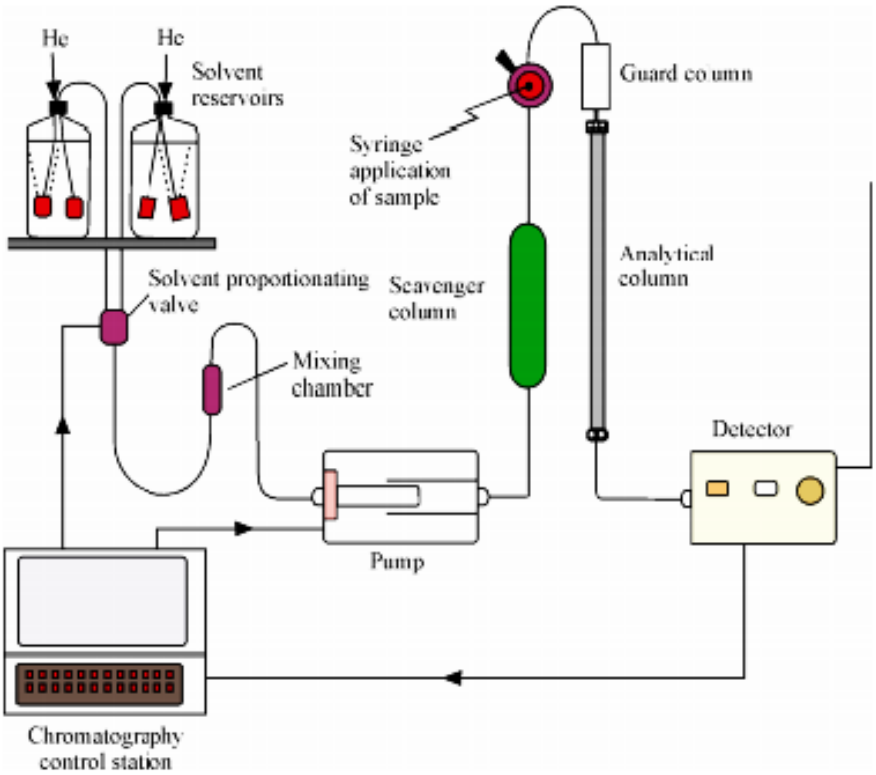
COLUMN CHROMATOGRAPHY

The column is packed with the stationary phase in a finely divided state, commonly used materials being alumina, silica gel and magnesium oxide. These are dry materials and therefore the material is an example of adsorption chromatography. The mobile phase is chosen on its selectivity of the system (e.g. solubility, surface tension and viscosity). The mobile phase is allowed to trickle slowly through the column. A small sample of the mixture to be separated is dissolved in a small quantity of the eluent and this is added at the top of the column. As further eluent is added, the mixture components are able to descend the column, their flow rate depending upon the strength of their adsorption on the stationary phase. Thus, components which are weakly adsorbed soon break away from those slow-moving components which are strongly adsorbed. This technique lends itself to relatively large scale applications, particularly if apparatus of large dimensions are employed. It is not usually used as a form of qualitative analysis but is especially suited to instances when quantities of mixtures are being analysed. The process can be very slow and very long columns are needed, in many cases, to achieve distinct separation.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY



The basic principle of separation by high performance liquid chromatography is similar to column chromatography though it differs with regard to the size of the column and the sample. It is often used for quick estimation of additives, contaminants and natural components of foodstuffs, polymer analysis and detection of drugs in blood. In HPLC, microgram amounts of sample is allowed to pass through a column containing stationary solid inert phase coated with non-volatile liquid phase by means of pressurized flow of a liquid mobile phase where components migrate at different rates due to different relative affinities. High purity solvents without any dissolved gases should be used because any impurity may affect the retention time and hence separation of the constituents. The eluent system consists of reservoirs from which one or more solvents can be selected. The size of the particles of solid used in the columns is often 10-20 times smaller than in column chromatography in order to maximise surface area. The very small size of the solid particles allows for more frequent adsorption and desorption of the components giving better separation of similar compounds. However, the small particle size creates a considerable resistance to the flow of the mobile phase and thus the liquid solvent is pumped through under high pressure up to 14000kPa.

As a result, HPLC is a faster and more sensitive technique than normal column chromatography. Retention time is influenced by mobile liquid flow rate, column temperature, column length, nature of the stationary phase and nature of the mobile phase. The sample is swept into the column in the mobile phase so the sample is often dissolved in the solvent used as the mobile phase for that determination before being injected into the column. The technique is used for both quantitative and qualitative analysis. The time taken between injection and detection for a component to pass through the column (the retention time) is characteristic of a component for the conditions of the experiment. HPLC can be used for quantitative by measuring the areas under the peaks in the chromatogram provided calibration has been performed. The advantages of HPLC include: it is a very fast separation technique, precise qualitative and quantitative analysis, technique applicable to small samples, allows for reproducible analysis, very versatile, non-destructive method etc. The disadvantages include: cannot be used for volatile compounds like hydrocarbons, high purity solvents are required, it is expensive etc.