High-Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) was developed in the late 1960s and early 1970s. Today it is widely applied for separations and purifications in a variety of areas including pharmaceuticals, biotechnology, environmental, polymer and food industries.

HPLC has over the past decade become the method of choice for the analysis of a wide variety of compounds. Its main advantage over GC is that the analytes do not have to be volatile, so macromolecules are suitable for HPLC analysis. 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 your separation. Since the compounds have different mobilities, they exit the column at different times; i.e., they have different retention times, t_R. The retention time is the time between injection and detection. There are numerous detectors which can be used in liquid chromatography. It is a device that senses the presence of components different from the liquid mobile phase and converts that information to an electrical signal. For qualitative identification one must rely on matching retention times of known compounds with the retention times of components in the unknown mixture. It is important to remember that any changes in operating conditions will affect the retention time which will affect the accuracy of identification. Thus HPLC is most often used when one is performing a target compound analysis, where one has a good idea of the compounds present in a mixture so reference standards can be used for determining retention times. For a sample of largely unknown composition qualitative identification can be determined by liquid chromatography-mass spectrometry. A mass spectrum of any or all peaks in the chromatogram is compared with spectra contained in spectral libraries on the system's computer. It should be noted that LC-MS systems are very complex, expensive instruments which are not commonly found in an academic teaching environment.

Quantitative analysis is often accomplished with HPLC. An automatic injector providing reproducible injection volumes is extremely beneficial, and are standard on modern commercial systems.

HPLCs are really rather simple. The apparatus consists of a mobile phase reservoir which is just a clean solvent jug, a solvent delivery system consisting of a pump for delivering precise, reproducible and constant amount of mobile phase, a sample inlet, the column, a detector with associated electronics, and some kind of interface to the outside world such as a computer. The pump which is used to deliver the mobile phase solvent at a uniform rate often operates at pressures ranging from 500 - 5000 p.s.i. These high pressures are needed because the stationary phase column packing consists of very small,,

tightly packed particles. It takes a lot of pressure to push the mobile phase through this stationary phase at a reasonable flow rate. Why the small particles? The mobile phase mass transfer term in the van Deemter equation (discussed in Chapter 26 of Skoog/Holler/Nieman, 5th Edition, pp. 681-687) is dependent on both the square of the particle diameter of the stationary phase as well as the column diameter. Thus there is always a good reason to go to smaller stationary phase particles and smaller columns.

Good separation of a given pair of compounds by HPLC depends on the choice of column (which has
already been done for you) and on the efficiency of the overall system. The relative position of the various components in
the sample on the chromatogram is affected by a solute-solvent type of interaction with the column substrate competing with a solute-solvent interaciton with the mobile phase.. Column efficiency is concerned with the broadening of an initially compact band of solutes as it passes through the column. The broadening is a result of column design and of column operating conditions. For samples with a broad range of retention times, it is often desirable to employ solvent programming, whereby the mobile phase composition is varied continuously or in steps as the separation proceeds. This is the answer, in liquid chromatography, to what is called The General Elution Problem. This is treated quite nicely in your Skoog/Holler/Nieman
text in Chapter 26 for chromatographic separations in general, pp. 689-693. Basically, the analysis of mixtures of widely varying composition frequently leads to a very wide spread in retention times. The longer the retention time, the broader the peak, so for those components which take a long time to elute, detector sensitivity is diminished and analysis times can be very long.. With solvent programming, successively eluted substances experience stronger solute-mobile phase interactions and so emerge from a column more rapidly than they would under conditions in which the solvent was not varied.. So long as one does not experience peak overlap (i.e. resolution remains tolerable), solvent programming gives a superior separation.

HPLC is just one type of liquid chromatography, meaning the mobile phase is a liquid. In this lab you will use what is called reversed phase HPLC. Reversed phase HPLC is the most common type of HPLC. What reversed phase means is that the mobile phase is relatively polar, and the stationary phase is relatively non-polar. Thus non-polar compounds will be more retained (i.e. have longer retention times) than a polar compound. In normal phase HPLC, the mobile phase is relatively non-polar and the stationary phase is relatively polar. Other more general types of HPLC include partition, adsorption, ion-exchange, size-exclusion, and thin-layer chromatography.

General

- Separation of organic, inorganic, biological compounds, polymers, and thermally labile compounds
- Qualitative and quantitative methods

Common Specific Applications

- Quantitative/qualitiative analyses of amino acids, nucleic acids, proteins in physiological samples
- Measuring levels of active drugs, synthetic byproducts, degradation products in pharmaceuticals
- Measuring levels of hazardous compounds such as pesticides and insecticides
- Monitoring environmental samples
- Purifying compounds from mixtures

Limitations

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

Complementary or Related Techniques

- Gas chromatography provides analyses volatile analytes with superior resolution
- Supercritical fluid chromatography provides analyses of volatile, non-volatile and thermally labile compounds
- Capillary electrophoresis provides superior analyses in many biological/pharmaceutical applications
- Ion Chromatography provides analyses of ionic compounds, as does capillary electrophoresis

References used to devise this web page:

1. "Handbook of Instrumental Techniques for Analytical Chemistry" Frank Settle, Editor: "High Performance Liquid Chromatography", Phyllis Brown, Kathryn DeAntonois, Prentice Hall, 1997, pp. 147-164.

2. "Principles of Instrumental Analysis", 5th Edition. Skoog, Holler, Nieman, Saunders College Publishing, 1998, pp. 673-697, 725-766.

Additional References

"Introduction to Modern Liquid Chromatography" by L.R. Snyder and J.J. Kirkland, 2nd Edition. John Wiley & Sons, 1979.

LC-GC Magazine. Advanstar Communications. www.chromatographyonline.com

HPLC Links

A general treatment of chromatography including an overview of plate and rate theory.
http://ull.chemistry.uakron.edu/analytical/Chromatography/

Tutorials on molecular spectroscopy and chromatography.
http://www.shu.ac.uk/schools/sci/chem/tutorials/