Fundamentals of Liquid Chromatography

The components of a Liquid Chromatograph include a pump to pressurize a liquid, which is called the Eluent, sufficiently to cause it to flow through an injector where a small volume of sample is measured and introduced, a column where separation occurs, and a detector which responds to the separated components flowing out of the column and provides a signal to the data system which displays a plot called a chromatogram with qualitative (what is it) and quantitative (how much is there) information about the components.

To be a candidate for analysis by Liquid Chromatography, the components of a sample only have to be dissolved in a common solvent and respond to a detector. Reverse Phase Chromatography is one of the more prevalent modes for separations so that is the mode we use.

In Reverse Phase Chromatography, separations are based on differences in attraction of the sample components for the stationary phase surface. The stationary phase is coated with an 18 carbon atom chain called C18…which attracts other carbon hydrogen molecules and rejects water soluble molecules. The term for this separation mode of attraction is “lipophilic” which means “fat loving”. So the more carbons/hydrogens in the molecule, the more “fat-loving” and the longer it stays on the column; less carbons/hydrogens are less attracted and go through faster…there is more to Reverse Phase separation mechanisms than that but differences in lipophilicity provide the primary mechanism to start understanding why molecules have differing attraction for C18.

The detector “sees” the components (in the present case) based upon their absorbance of UV light at 254nm. Other detectors and other wavelengths of light are used but those will be discussed separately

The central component of the Liquid Chromatograph is the column.

The column consists of a tube which must be rigid enough to contain the packing and withstand the pressure required to achieve flow. It must also be inert to the solvents which are used to affect separations.

Columns contain many types of media and are made using different technologies. The columns used with C-Vue® are called monoliths and are like a sponge with very uniform small pores. The media inside the EMD RP18e used with C-Vue® is coated with a bonded C18 surface. The columns have low back pressure but very high resolution making them ideal for C-Vue®.

The fundamental concepts of Liquid Chromatography separations are:

--all analytes must be dissolved in the eluent.

--Inside the column, there are only two places that a component may be—adsorbed to the stationary phase or desorbed in the mobile phase.
--When a component is adsorbed, it stops moving.

--When a component is desorbed, it moves at the rate of the eluent.

--The less lipophilic molecules are attracted to the C18 stationary phase proportional to their lipophilicity so they move through the column more quickly.

--The more lipophilic molecules are attracted to the C18 stationary phase proportional to their lipophilicity and move more slowly.

--Separation occurs when the adsorption (stuck to the column) and desorption (released from the column) rate for each component is different from every other component.

--The best eluent for a given separation is the one that provides just the right rates for all of the molecules of interest. Finding this special mixture is called method development. This mixture is achieved beginning with an informed guess and is improved by trial and error until an optimum mixture is found.

--The uniformity of the stationary phase coating and the uniformity of the pores through the stationary phase result in narrow and well-resolved populations of the molecules being separated. These populations are detected, in the present case, by their absorbance of ultra-violet light at 254nm and seen on the chromatograms as narrow, symmetrical peaks separated from all others. Quantitation is based upon the analytes conforming to Beer’s Law which relates concentration to absorbance.

The concept of reverse phase separations is well-explained by Professor I. Molnar’s publication in Memoriam of Professor Csaba Horvath’s work on the Solvophobic Theory. For the serious student, read the article at http://www.molnar-institut.com/HP/Literature/Solvophobic-Theory.pdf.