High Performance Liquid Chromatography

HOW DID WE GET HERE?

Discovery of Liquid Chromatography

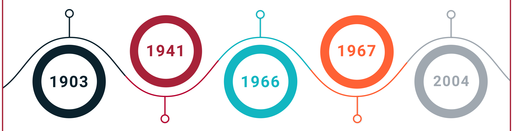
Mikhail Tsvet (Italy-1872) was a botanist at the University of Warsaw. He developed a technique to study the separation of plant pigments that he named chromatography from the Greek khrōma "color" and graphia "description of".

Introduction of High Pressure LC

After two years of research at Yale University, Csaba Horváth (Hungary-1930) introduced, at the Six International Symposium on Gas Chromatography in Rome, a new liquid chromatography system using high pressures and reducing separation time that he will later name HPLC.

Enhanced Performance with UPLC

By the beginning of the 21st century, scientists were reaching the limits of the HPLC systems. To break the separation barrier, Waters introduced Acquity UPLC which, by using even higher pressure and sub 2µm particles in the column, was able to increase both measurement speed and peak capacity.



Partition Chromatography

Archer J.P. Martin (England-1910) and Richard L.M. Synge (England-1914) developed partition chromatography during their research at the Wool Industries Research Association in Leeds. It was introduced to characterize monoamino-acids in proteins with two liquid phase chromatogram. They received the Nobel Prize in 1952 for their work.

First Commercial HPLC

Waters Corporation, created by James Waters in Framingham, MA in 1958, specialized itself in the development of liquid chromatographs. Following some prototyping work, the company introduced the first successful commercial HPLC in 1967, the ALC-100.

HOW DOES IT WORK?

Technical Background

From X-rays to electron beams there are many analytical techniques to distinguish the elements of the periodic table. For options to distinguish materials that have almost every element in common, we need chromatography. Liquid and gas chromatography are widely applicable for characterizing soft materials, pharmaceuticals, petrochemicals, adhesives and specialty chemicals.

This article will focus on liquid chromatography since our previous article already described gas chromatography. From a high-level perspective, chromatography is the science of separating the components of a system such that positive identification can occur for the target compounds. The separation takes many forms and is controlled by the columns attached to the instrument. Equally diverse are the number of detectors that exist for positive identification. Since chromatography column choice is a highly specialized skill set, this article will focus on usage and applications.

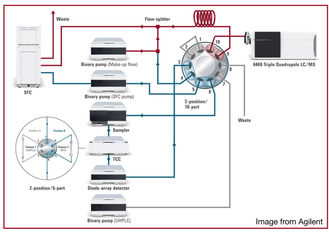


Figure 1. Ensemble of equipment that can make up a single liquid chromatography system

Liquid chromatography is about selective filtration and creating gradients to separate soluble compounds. If a compound is not soluble or is highly combustible, then gas chromatography might be more appropriate. The compound of interest or target molecule will begin its journey in some type of solvent which is known as the mobile phase. When the mobile phase is non-polar (hexanes, octanes etc.) the columns (stationary phase) will need to be polar in order to cause separation. This configuration with non-polar mobile phase is normal phase chromatography. When the mobile phase is polar (acetonitrile, water etc.) and the stationary phase is nonpolar, this is known as reverse phase chromatography.

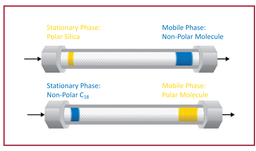


Figure 2. Modes of operation for liquid chromatography

The next steps are for highly experienced chromatographers to select the proper columns for the instrument, set up solvent gradients to maximize the effectiveness of the separation and run baseline method development to be able to accurately quantify the amount of target compound in the sample. There is a true artistry in being able to develop methods to separate compounds effectively. It takes decades of experience and storage closets full of columns and solvents.

Once the separation is completed and the target compound is isolated, the appropriate type of detector needs to be used for positive identification. Detectors can utilize UV light, visible light, photo diode arrays, refractive index, evaporative light scattering, multi angle light scattering, mass spectrometers, conductivity, fluorescence, chemiluminescence, optical rotation and electrochemistry. The selection of detector depends on the chemistry of the target molecule.

LC, HPLC or UPLC

When considering liquid chromatography there are a few categories of instruments available that are distinguished by their operating pressures. Ultra-High Performance Liquid Chromatography (UPLC) operates at 15,000 PSI while High Performance Liquid Chromatography (HPLC) HPLC operates under 6,000 PSI. The oldest systems operate under 500 PSI but those are quite rare nowadays. From a functionality point of view, any chromatography system can detect a molecule once it is separated. The reason that higher pressures are used is to speed up the separation times. A UPLC can separate a compound 2 to 3 times faster than an HPLC. If you are in the market for method development, developing a UPLC method maximizes the cost savings over the long term with faster analysis times.

Figure 3. UPLC System the leading edge of technology in chromatography

Strengths and Limitations

Liquid chromatography is an excellent tool for separating closely related compounds. The limitation is that for each target compound a method needs to be tailored so that quantification is accurate, and separation occurs. This means that there is no such thing as a quick scan with HPLC, there will always be some method development. As chromatographers know, equivalent columns from different manufacturers are not identical. How the column is packed impacts its effectiveness. This means that the chromatographer needs to stock columns from multiple manufacturers to be able to support a wide variety of analytical work.

APPLICATIONS

Liquid chromatography applications are centered on the detection of organic, biological and pharmaceutical products. The analysis can be used to monitor the stability of drug products over time and temperature. Alternatively, analysis can be done on sample to detect foreign contaminates, such as measuring water for types of organic contamination. Chromatography is often used with food and nutraceuticals as a means of monitoring product integrity. The final usage is for isolation of materials where the chromatography is used to separate materials after chemical reactions.

CUSTOMER SERVICE WORKFLOW

STEP 1 — Consult with Our Technologists

STEP 2 — Provide Your Samples

STEP 3 — Receive In-Depth Report

STEP 4 — Debrief with Our Technologists

WHY CHOOSE OUTERMOST TECHNOLOGY FOR HPLC?

1. Access to a wide range of columns and detectors for HPLC and UPLC
2. Highly experienced analysts in chemical and pharma R+D method development
3. Access to supporting techniques like IC-MS, ICP-MS and GC-MS
4. High capacity for routine sample measurement using multiple tools