TERRAMOR TECHNOLOGIES
PRECISION LAB TESTING

WHAT IS CHROMATOGRAPHY ?

High-Performance Liquid Chromatography (HPLC)

HPLC is an acronym for High Performance Liquid Chromatography. It is a powerful technique used in separating, quantifying, and identifying different components in a mixture. HPLC is the biggest and most essential chromatography method used in laboratories worldwide. Many research fields and industrial sectors also depend on this technology.

HPLC is based on a simple fact that compounds can behave differently in water, and it uses their polarity to separate and purify compounds. Polarity defines the physical properties of a compound, such as their melting point, boiling point, and their solubility in water. For example, oil does not mix in water, which is why it is known as a non-polar liquid. While Ethanol mixes very well with water and is known as a polar liquid. Other non-polar items are Fats, Petrol, Oil and Gasoline because they do not mix with water.

Applications of High-Performance Liquid Chromatography :

HPLC is used as an analytical tool within the medical and biological fields. It can be used for the analysis of samples to know the presence or absence of compounds. It can also assist in the identification of unknown compounds. In chemistry, it is used to monitor reactions and also to ascertain the purity of a product. Furthermore, it can be used to purify compounds for further use.

A rapidly evolving use of Chromatography is in the testing of Cannabis. As a result of the booming cannabis market in the U.S., states have mandated cannabis testing to ensure consumers are informed about the products they purchase. HPLC is used to ensure that THC levels are below 0.3% in Hemp and to determine the values of the other Components such as CBD, CBG, CBN etc. THC is associated with the psychoactive effect in cannabis, while CBD is the compound in Cannabis that has a pain-relieving effect. The products can be better trusted and safe for consumers when tested. There are a lot of cannabis-based products in the market today, and some of them are

High-Performance Liquid Chromatography (HPLC) :

High-performance liquid chromatography is used to identify, quantify, and isolate the components of liquid mixtures. A

reservoir is used to pump a solvent that mixes with a liquid sample. The Mixture (Solvent and Liquid sample) passes through a High Performance Liquid Chromatography Column into a Diode Array Detector (DAD), and then an electronic output would be generated as a Chromatograph. A vessel would be used to collect the waste outside of the machine. In this process, the HPLC Column is used as the separation component of the system. It contains silica , and other packing material with various pore sizes.

Mobile phase :

In HPLC the liquid sample that goes through the pores is known as the mobile phase. The larger molecules would become trapped by smaller pores as the mobile phase goes through the stationary phase. This would allow the smaller molecules to elute or exit the column faster. This process is known as the Separation and Isolation process.

In this process, smaller molecules would have faster retention time, while larger molecules would have a slower retention time. This retention time is the period it takes molecules to travel down to the Diode Array Detector. The molecule retention times are measured against known standards, which enables the molecules in the sample to be easily identified. The internal pressures on the mobile phase can get up to 400 atmospheres and the longer the molecules spend in the column, the wider the peaks on the chromatograph

Types of HPLC :

There are three major types of HPLC, which are reverse-phase HPLC, normal phase HPLC and Ultra-HPLC (UHPLC).

Reverse-phase HPLC :

In this phase, the stationary phase is modified by hydrocarbon chains, which cause the column to become non-polar. The reverse-phase HPLC uses a polar solvent, and the polar molecules in the sample develop strong attractions with the solvent, which makes them elute significantly faster. Reverse-phase HPLC uses resins with small hydrophobic groups that are attached. Organic modifiers like Propanol or Acetonitrile are added to the elution buffer instead of using salt ingredients to elute hydrophobic species. This would decrease the concentration of water in the mobile phase and also weakens the hydrophobic attraction of the groups.

Normal Phase HPLC:

Normal Phase High Performance Liquid Chromatography is the most common method used around the world. It makes use of non-polar solvents as the silica particles and mobile phase. In this phase, polar compounds stick to the polar silicon much longer in the stationary phase when compared with non-polar compounds. This means non-polar compounds would elute much faster in normal High Performance Liquid Chromatography. The pore sizes are between 2.5 and 3.5 microns.

Ultra-HPLC :

This type of HPLC uses less solvent, runs faster, and generally dissolves better. Their column and the stationary phase particles are smaller in size. The pores are sub-2 microns, which ensures a better resolution and separation molecules. It also has a shorter column that allows for rapid detection. Ultra-HPLC cannot be used with unfiltered or dry samples because it has smaller pores which can become clogged. A clogged sample can consume a lot of time and can be expensive to repair.

Most of the High-performance Liquid Chromatography machines use a UV-detector to measure the UV-light that is provided by the molecules in the sample. While some of the machines are coupled with a mass spectrometer that identifies the molecule by the m/z ratio. There is no need to know what the retention time is in Ultra HPLC machines.

Components of High-Performance Liquid Chromatography :

The major components are HPLC column, The HPLC Sample, Injection of Sample, The Mobile Phase and The HPLC Run

HPLC Mobile Phase :

The mobile phase is a mixture of an organic solvent and water. The organic solvent is usually methanol or acetonitrile. This phase is called the mobile phase because of its mobility. It

moves through the column and systematically flushes out compounds from the column along a concentration gradient.

The concentration gradient ensures that the percentage of non-polar solvents increases while the percentage of water in the mobile phase decreases over time. This makes the mobile phase more non-polar.

HPLC column :

The column is the central or workhorse of the High-performance Liquid Chromatography machine. It has resin in the column, which is also called the stationary phase and is made of silica. The column is very compact and composed of a variety of other substances. The silica functions by using long carbon chains that are non-polar. A longer chain would result in a more non-polar compound, and most times 18-carbon or C18 Columns chains are used.

The HPLC Sample :

There are various sample types, and they vary based on the type of compound in question. High-performance liquid Chromatography can be used to analyze various biological specimens and samples such as saliva, toxins, fungi, bacteria, blood, urine, and of course, cannabis.

The HPLC Run :

There are quite a number of models that can be used to perform HPLC run, but the most popular and commonly use is the reversed-phase HPLC. The machine would separate compounds beginning with the most polar and end with the non-polar compounds.

In the Run mode, the machine separates compounds beginning with the most polar and ending with the non-polar compounds. A high-powered pump moves the sample and the mobile phase through the column For all modes, and a run takes about 8-65 minutes.

Gas Chromatography :

Gas Chromatography is used to separate and analyze volatile compounds, unlike High-Pressure Liquid Chromatography that is used to separate non-volatile compounds. It is also used to measure and separate various types of gasses. Using GC requires a lot of expertise because they are very sensitive but can be automated to be used in the analysis of small samples. GC is a very common technology used for the analysis and separation of chemical compounds that can be vaporized. For example, GC can be used for separating the different components of a mixture or testing the purity of a particular substance. Gas Chromatography can also be used to prepare compounds from various mixtures. We use GC for analyzing Terpenes as well as Residual Solvents in cannabis.

They have become an indispensable analytical technology for organic analysis today.

Mobile Phase in Gas Chromatograph :

The mobile phase in GC is a gas carrier, and usually, helium or nitrogen is used. Nitrogen is mostly used and makes up about 90% of the machine. Gas Chromatography also has a stationary phase, which is a microscopic layer of polymer or liquid. The stationary phase rests on inert solid support inside a metallic or glass tubing known as the column. The machine used in carrying out a gas chromatography is known as a gas chromatograph.

In GC, the process of separating compounds in a mixture is done by the gas mobile phase and the liquid stationary phase. The channel or column that the gasses are transported is located in an oven, and the temperature of the gas is controlled. Furthermore, the concentration of the phase in the gas is a function of the vapor pressure of the gas. GC is very similar to HPLC, and it provides a fast way of determining alkaloids in mixtures or compounds.