

IMPLEMENTATION OF CHROMATOGRAPHY IN MEDICINE

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Abstract

Chromatography is a separation process used to separate components in a mixture. The components of the mixture are dispersed in a liquid solution known as the mobile phase, which holds it through a structure containing another substance known as the stationary phase. Component separation requires differential partitioning between the mobile and stationary phases. The analytical goal of chromatography is to determine the qualitative and quantitative chemical makeup of a sample, and its primary purpose is to purify and extract one or more components of a sample. This paper will discuss the history and basics of what chromatography is meant and the main principles of how we can run it. besides, we will mention and focus on an application for each chromatographic type such as planar, TLC, gas, liquid. Chromatography is a scientific method used to separate and analyze substances within mixtures. It is widely applied in fields such as pharmaceuticals, chemistry, biology, medicine, and many others. Through chromatography, chemical compounds present in blood, urine, food products, and medications can be identified and studied.

Keywords

chromatographic separation technique; thin layer chromatography; High-performance liquid chromatography; elution time; applications.

Application areas of chromatography in medicine

Chromatography technique is a valuable tool for biochemists, besides it can be applied easily during studies performed in clinical laboratories For instance, paper chromatography is used to determine some types of sugar, and amino acids in bodily fluids which are associated with hereditary metabolic disorders. Gas chromatography is used in laboratories to measure steroids, barbiturates, and lipids. Chromatographic technique is also used in the separation of vitamins, and proteins.

First devised by Mikhail Tsvet in 1900, chromatography is used to separate compounds within a mixture. It involves two phases, a mobile and a stationary phase. In the mobile phase, the mixture of interest is dissolved in a fluid, which can be either a gas, solvent, or water. This is then carried through a system where a material, or the stationary phase, is affixed.

Compounds that need to be analyzed and identified are separated due to their different affinities for the affixed material in the stationary phase. This is due to different physical and chemical properties such as physical size and how strongly the compound bonds with the stationary material. These properties cause the different compounds to move at different rates, becoming affixed to the material for different periods of time. By using methods such as mass spectrometry in tandem with chromatographic techniques, a compound can be easily identified due to its known properties.

Since the invention of chromatography in the early 20th Century, there have been a plethora of techniques developed. These include planar methods such as paper chromatography and thin-layer chromatography and column-based methods such as gas chromatography and high-performance liquid chromatography. Other, more specialized, methods exist including reverse-phase liquid chromatography, chiral chromatography, and fast protein liquid chromatography.

Using RPLC to Accurately Identify the SARS-CoV-2 Spike Protein

The spike protein of the SARS-CoV-2 virus has been of particular interest as a vaccine target due to its primary role in viral pathogenesis. Whilst there is a plethora of studies that seek to categorize the spike protein's glycopeptides and glycans, intact protein analysis using RPLC (reversed-phase liquid chromatography) may offer unique analytical insights. By refining functional and structural understanding of the intact spike protein, more effective therapies can be identified and developed.

A paired chromatography-mass spectrometry method published by Waters compared difluoroacetic acid (DFA) and formic acid (FA) modified mobile phases and demonstrated that DFA enhanced method resolving power whilst maintaining mass spectrometry compatibility. A threefold gradient peak capacity increase was achieved alongside better resolution of the less abundant proteoforms.

This study demonstrates that by modifying chromatographic methods, more detailed analytical data on structural elements of the Sars-CoV-2 virus can be provided, which aids in the development of improved therapeutics and vaccines.

Remdesivir and the Use of Liquid Chromatography Coupled with Mass Spectrometry

Remdesivir is an antiviral that has shown promise in treating COVID-19. Developed initially to treat Ebola virus, it has shown efficacy in combating several RNA viruses including MERS (a precedent coronavirus.) Remdesivir has an estimated EC₅₀ at around 13.8 mg/L (decreased to 3.8 mg/L when used in conjunction with emetine) in inhibiting SARS-CoV-2 replication in Vero E6 cells. It was one of the first treatments approved by the FDA in the United States.

In a study published online in June 2020, liquid chromatography coupled with mass spectrometry was used to quantify remdesivir in the blood plasma of a COVID-19 patient and thus provide data on its efficacy as a treatment for SARS-CoV-2. This was a pharmacokinetic study. Six different drug-free plasma were also analyzed in the study.

The study was carried out and validated according to full EMA guidelines and demonstrated a successful and effective new method for the measurement of the concentration of both remdesivir and its metabolite GS-441524 in blood plasma. By using liquid chromatography in conjunction with mass spectrometry, laboratories can gain powerful analytical data for potential COVID-19 treatments in the future.

Using Chromatography to Diagnose COVID-19 in a Breath Test

Aside from the development of treatments and vaccines, there is a need for accurate and rapid detection of COVID-19 in patients. As the symptoms of the disease can present in similar ways to other respiratory conditions such as influenza, this is vital for the early diagnosis of patients and effective treatment of the condition. Breath biochemistry is a powerful diagnostic indicator for respiratory conditions.

Diagnostic tests are usually carried out in laboratories. This, however, can be costly in both time and resources. Therefore, the need to develop point-of-care tests that do not require lab support can help speed diagnosis and protect staff from potential exposure to COVID-19. One study published online in December 2020 presented a method for rapid diagnosis using gas chromatography-ion mobility spectrometry.

The results of the study showed that there is a host-response that may be due to a combination of ketosis, inflammatory response, and gastrointestinal function. The presence of volatile organic compounds detected via this method including ethanol, acetone, methanol, and heptanal, as well as an as-yet-unidentified feature, was hypothesized to provide the basis of an effective COVID-19 breath test.

Chromatography consists of two main parts:

1. Mobile phase – a moving medium (liquid or gas) that carries the components of the mixture being analyzed.

2. Stationary phase – a fixed material through which the mobile phase flows, allowing the mixture's components to separate at different speeds.

The separation process occurs because each substance in the mixture interacts differently with the stationary phase. A substance that binds more strongly to the stationary phase moves more slowly, while one that binds less strongly moves faster. As a result, the components of the mixture separate and appear at different positions.

In Conclusion

Chromatography is a powerful laboratory-based analytical method that is being employed by researchers as the scientific community strives to understand the Sars-CoV-2 virus and develop better therapies, vaccines, and diagnostic tools to aid in the fight against the Covid-19 pandemic. Research such as the studies listed in this article is providing valuable insights and data on this disease and get the world back to normal.

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