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THE ROLE OF CHROMATOGRAPHY IN BIOMEDICAL RESEARCH

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ABSTRACT

This article examines the significance of the chromatographic method in medical research, its main types, and areas of application. Specifically, the role of chromatographic methods in pharmaceuticals, clinical diagnostics, and forensic medicine is analyzed. The importance of modern chromatographic technologies in increasing accuracy and efficiency is also highlighted. The article discusses the molecular-level identification capabilities of methods such as HPLC, GC, and LC, as well as the principles of "Green Chromatography".

Keywords: Chromatography, HPLC, GC, LC, TLC, Medical research, diagnostics, pharmaceuticals, forensics.

INTRODUCTION

Chromatography is one of the most important and widely used methods of modern analytical chemistry. It is of particular importance in the medical field as an effective technology for the separation, identification and purification of various substances. Chromatographic methods are widely used in the development of drugs, biological sample analysis and disease diagnosis. At the same time, this method is also an important analytical tool in the fields of forensic medicine and criminalistics.

High-performance liquid chromatography (HPLC).

High-performance liquid chromatography (HPLC) remains a key analytical method in various scientific fields due to its high flexibility, accuracy and efficiency in separating, classifying and measuring compounds in complex mixtures. Current research is aimed at improving HPLC approaches, expanding its scope and comparing its performance with other analytical instruments. This review summarizes the main results of recent research, discusses the principles, applications and comparative advantages of HPLC, and the current research landscape [1].

HPLC is based on the principle of partitioning the analytes between a mobile phase and a stationary phase placed in a cylindrical tube called a "column". The sample is passed through the mobile phase and the components are separated according to their properties. This leads to different rates of movement; accordingly, the components are separated according to their elution from the column and detected using a suitable detector (e.g. UV, fluorescence, electrochemical or mass spectrometry). Depending on the type of analyte, important separation methods are selected, such as reversed-phase (the most common), normal phase, ion exchange, size-exclusion and affinity chromatography.

Recent studies have highlighted the effectiveness of HPLC in separating complex mixtures, especially in pharmaceutical analysis. In contrast, gas chromatography (GC) is limited to volatile compounds, while capillary electrophoresis (CE) has advantages in terms of speed and resolution for ionic species [1-5]

High-performance liquid chromatography (HPLC) advances

The field of HPLC is constantly evolving, and recent advances have focused on:

Ultra-high-performance liquid chromatography (UHPLC): This is a significant development that uses columns with particles smaller than 2 µm or core-shell particles. UHPLC systems operate at extremely high pressures (1000 bar or more), which dramatically reduces analysis time, increases resolution, and increases sensitivity compared to conventional HPLC [1-2].

Column technology: Improvements in stationary phase (column chemistry and design), including monolithic columns and porous core-shell particles, have increased separation efficiency and selectivity. Also noteworthy are hybrid particles with improved pH stability.

Improvement of detectors: The coupling of HPLC with mass spectrometry (LC-MS and LC-MS/MS) has become an indispensable tool for structural analysis and high sensitivity in trace element determination. Developments in other detectors such as diode array detectors (DAD) and evaporative light scattering detectors (ELSD) have also expanded the capabilities of HPLC [1-4].

Green analytical chemistry: This includes reducing solvent consumption by a factor of 5-10 through techniques such as UHPLC, replacing toxic reagents such as methanol with safer alternatives (ethanol, pure water), and using alternative mechanisms such as temperature-sensitive liquid chromatography (TRLC) that can use pure water as the mobile phase.

Automation and method development: Automated methods based on the principles of Quality by Design (QbD) are accelerating and making HPLC separation optimization more efficient.

Nano LC: The development of nano-LC systems allows for the analysis of very small amounts of samples with high sensitivity, which is typically suitable for metabolomics and proteomics [2]

High-performance liquid chromatography (HPLC) in medicine.

HPLC is widely used in pharmaceutical analysis, food safety, and environmental monitoring. Studies have proven the role of HPLC in the detection of contaminants in food products and its indispensable role in the analysis of complex matrices.

Pharmaceutical analysis: This is the main application area, where HPLC is used for drug discovery, development (e.g., compound profiling, stability testing of drug substances), quality control of raw materials and finished products, and pharmacokinetic studies[1-3].

Environmental monitoring: HPLC is essential for the detection and quantification of contaminants such as pesticides, herbicides, heavy metals, and volatile organic compounds (VOCs) in water, soil, and air samples.

Food safety and quality analysis: Widely used for the analysis of vitamins, lipids, additives, and the control of contaminants such as mycotoxins, pesticide residues, and preservatives.

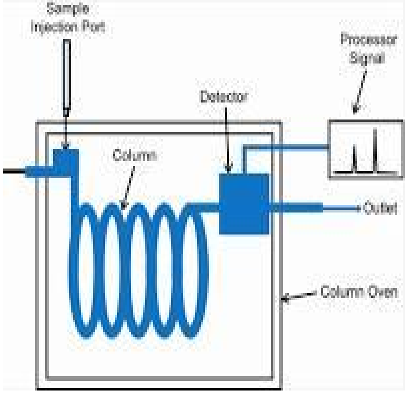
Biochemical and clinical research: Used for protein and nucleic acid analysis, metabolomics, and therapeutic drug monitoring. Its high resolution is essential for the detection of brain neurotransmitters, the separation of fatty acids, the assessment of oxidative stress (e.g., urinary 8-hydroxyguanosine, a marker of DNA damage), and the analysis of Coenzyme Q10 and vitamins.

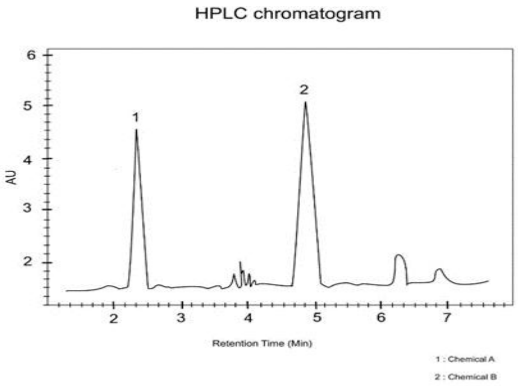
Recent studies confirm the continued position of HPLC, while indicating a desire for more powerful and efficient iterations such as UHPLC. The focus on the combination of HPLC and mass spectrometry (MS) is revolutionizing analytical capabilities, especially for the analysis of complex samples and the identification of drug metabolites. The drive for "green" HPLC and automation also reflects global trends in improving laboratory productivity and ensuring sustainability.

Gas chromatography (GC).

GC is used to analyze volatile compounds that evaporate without decomposition. Substances are separated according to their different distribution between the gas and liquid phases.

Gas chromatography (GC) separates volatile chemicals by boiling point and stationary phase interactions, which is useful in drug testing and environmental monitoring, but is limited to thermally stable molecules. The separation and analysis of volatile compounds in a gas is a common application of gas chromatography (GC). The material is vaporized and then passed through a capillary column using helium or nitrogen. The components are separated by their boiling points and their interactions with the stationary phase in the column. To perform gas chromatography, a vaporized sample is introduced into a hot injector. The components are separated based on their partitioning properties as the sample is carried along the column by a carrier gas. The detector then quantifies the component retention times to produce a chromatogram. GC is used in drug screening, forensic toxicology, air pollution monitoring, pesticide residue detection, and petrochemical and perfume quality control. When used with FID or MS detectors, GC's high resolution, short analysis times, and high sensitivity are its main advantages. GC can only be used with volatile and thermally stable chemicals, the instruments are expensive, and skilled operators are required. [5].





Liquid chromatography (LC)

Liquid chromatography is an analytical technique that separates mixtures into their components, based on the distribution of substances between a stationary phase (sorbent) and a liquid phase that moves along it (mobile phase). LC is widely used for the analysis of complex mixtures and the purification of substances in important fields such as food safety, environmental monitoring, healthcare, and pharmaceuticals.

Thin-layer chromatography (tlc).

TLC is characterized by its simplicity. It uses a solid layer (such as silica gel) on a glass or aluminum plate as the stationary phase. The mobile phase (solvent) rises up under the influence of capillary forces. Separation occurs based on the affinity of the substances for the silica gel. The key parameter in TLC is the retardation coefficient.

Thin-layer chromatography is a fast and inexpensive option for rapid qualitative analysis. The capillary effect is the basic concept. To start the process, a small layer of stationary phase material, such as aluminum oxide or silica, is coated on the plate. The sample components are then passed along the plate in a solvent system to separate them according to their polarity. TLC technology uses a small sample that is applied to the bottom of the plate and then placed in a chromatographic chamber filled with solvent. The components are separated according to the heights they reach as the solvent flows across the plate. The separated components can be observed using special staining reagents or ultraviolet light. They are widely used in rapid qualitative pharmaceutical analysis, screening of plant extracts for bioactive compounds, forensic and drug identification, and food mixture identification. Although the TLC method is convenient for rapid qualitative analysis (screening) of substances, it is desirable to use high-resolution methods such as HPLC or LC-MS for measuring their exact concentration (quantitative analysis) [5].

TLC is widely used as a rapid screening method in pharmaceutical, forensic, and food analysis

Analytical capabilities of chromatography in forensics and forensic medicine

Chromatographic methods in forensic examination allow not only the separation of substances, but also their identification (confirmation) with very high accuracy. The following technical advantages of chromatography are crucial in this area:

Detection Sensitivity: Hybrid methods such as LC-MS/MS and GC-MS allow the detection of trace amounts (nanogram and picogram levels) of toxic substances in biological samples (blood, urine, hair). This is especially important in cases where very small amounts of the substance remain in the body after a long time after the crime.

Selectivity and Overcoming Matrix Effects: Although biological samples are composed of complex components such as proteins and lipids, the chromatographic column separates the desired analyte (analyte) from this "noisy" background with high selectivity.

Molecular Identification: Chromatography coupled with mass spectrometry (MS) provides a unique "molecular fingerprint" (mass spectrum) of each chemical compound. This serves as the main evidence to prove the nature of the substance in court without leaving any room for error.

Volatile Substance Analysis: Gas chromatography (GC) can be used to accurately assess the level of intoxication or cause of death of a person by detecting ethanol, carbon monoxide, or other volatile toxic compounds in the blood

1. Detection of Heroin and Drugs – In 2005, GC–MS was used in Britain to detect low concentrations of heroin metabolites in blood and urine samples. This was crucial in proving the criminal’s connection to the sale and use of drugs. 2. Detection of forgeries in judicial documents – In 2012, in the USA, the composition of the ink on a document was analyzed using TLC and it was found to be different from the original document. This was the main evidence in revealing financial fraud and document forgery by a criminal.

3. Chemical analysis in determining the cause of death – In 2017, forensic toxicologists investigating the cause of death in Germany used the HPLC–MS/MS method to identify a toxic compound that had entered the blood before death. This result was decisive evidence in determining the nature of death and bringing the criminal to justice.

Future development directions.

Modern research in the field of chromatography is mainly aimed at increasing the efficiency of methods, improving their accuracy, and ensuring environmental sustainability. In recent years, significant progress has been made in the direction of miniaturization and automation, which allows for rapid analysis in real time. In particular, the development of microfluidic and portable chromatographic systems is creating great conveniences in clinical diagnostics, environmental monitoring and forensic practice.

The introduction of artificial intelligence and machine learning technologies allows for automatic analysis of chromatographic data, peak detection and increased reproducibility of results. At the same time, hybrid and multidimensional chromatographic methods, such as combinations of GC-MS and LC-MS, allow for more in-depth and accurate analysis of complex mixtures [2].

Conclusion

The analytical data obtained show that chromatography is an integral and high-tech part of modern medicine, pharmacy and forensics. Methods such as HPLC, GC and LC-MS, considered during the study, provide not only high accuracy in the analysis of biological samples, but also the possibility of identifying substances at the molecular level.

In particular, the use of chromatographic methods in forensic toxicology allows for the detection of extremely small amounts of toxic substances (at the nanogram level) and the provision of irrefutable evidence in criminal cases. At the same time, the TLC method retains its cost-effectiveness in rapid screening of the quality of drugs.

The future of chromatography is associated with further miniaturization of analytical processes, automatic data processing using artificial intelligence, and ensuring environmental safety based on the principles of "Green Chromatography". These approaches, along with increasing the speed of laboratory analysis, serve to minimize the negative impact on the environment.

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