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Review Article

A Comprehensive Review on Liquid Chromatography-Mass Spectrometry (LC-MS): A Hyphenated Technique

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A B S T R A C T

The hyphenation of liquid chromatography with mass spectrometry allows for the simultaneous analysis of compounds based on their retention times and mass-to-charge ratios, providing valuable information about the identity and quantity of analytes in a sample. One of the key advantages of LC-MS is its versatility, as it can be applied to a wide range of samples including biological fluids, environmental samples, pharmaceuticals, and food products. This makes it an essential technique in fields such as pharmaceutical analysis, environmental monitoring, metabolomics, proteomics, and forensic science. These techniques have become essential tools for researchers and scientists in various industries, including pharmaceuticals, environmental monitoring, and food safety. One of the most common hyphenated techniques is gas chromatography-mass spectrometry (GC-MS), which allows for the separation and identification of complex mixtures of compounds with high sensitivity and specificity. Another popular technique is liquid chromatography-mass spectrometry (LC-MS), which is widely used in drug discovery and metabolomics studies. These hyphenated techniques offer numerous advantages, such as increased sensitivity, improved selectivity, and faster analysis times. They have greatly enhanced our ability to detect trace levels of contaminants, identify unknown compounds, and quantify analytes accurately. In conclusion, the acknowledgment to LC-MS is crucial for advancing scientific research and improving our understanding of complex chemical systems. Its versatility and sensitivity make it an indispensable tool for modern analytical chemistry. Overall, introductions to LC-MS provide students with a solid foundation in this powerful analytical technique, allowing them to confidently apply it to their own research projects and experiments.

Keywords: Gas chromatography-mass spectrometry (GC-MS); Liquid chromatography-mass spectrometry (LC-MS);.

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INTRODUCTION

Hyphenated techniques in analytical chemistry have revolutionized the field by combining two or more separation and detection methods to provide more comprehensive and accurate results. This comprehensive review will delve into the fundamentals of hyphenated techniques, their applications, advantages, and limitations (1). One of the key benefits of hyphenated techniques is their ability to separate complex mixtures with high efficiency and sensitivity. By coupling techniques such as gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS), researchers can identify and quantify a wide range of compounds in a sample (2). However, these techniques also come with challenges such as instrument complexity, cost, and data interpretation. Understanding these limitations is crucial for maximizing the potential of hyphenated techniques in analytical chemistry (3). Hyphenated techniques in analytical chemistry have revolutionized the field by combining two or more analytical methods to provide more comprehensive and accurate results. These techniques have become essential tools for researchers and scientists in various industries, including pharmaceuticals, environmental monitoring, and food safety. One of the most common hyphenated techniques is gas chromatography-mass spectrometry (GC-MS), which allows for the separation and identification of complex mixtures of compounds with high sensitivity and specificity. Another popular technique is liquid chromatography-mass spectrometry (LC-MS), which is widely used in drug discovery and metabolomics studies. These hyphenated techniques offer numerous advantages, such as increased

sensitivity, improved selectivity, and faster analysis times. They have greatly enhanced our ability to detect trace levels of contaminants, identify unknown compounds, and quantify analytes accurately (4-6). In conclusion, hyphenated techniques in analytical chemistry play a crucial role in advancing scientific research and solving complex analytical challenges. Their versatility and effectiveness make them indispensable tools for modern analytical chemists. This review provides a comprehensive overview of hyphenated techniques, highlighting their importance in modern analytical chemistry and emphasizing the need for further research and development in this area.

Introductions to Liquid Chromatography-Mass Spectrometry (LCMS)

Liquid chromatography-mass spectrometry (LC-MS) is a powerful analytical technique that combines the separation capabilities of liquid chromatography with the detection and identification capabilities of mass spectrometry. This technique has revolutionized the field of analytical chemistry by allowing scientists to separate, identify, and quantify complex mixtures of compounds with high sensitivity and specificity. The introduction to LC-MS typically involves a brief overview of the principles behind both liquid chromatography and mass spectrometry, as well as an explanation of how these two techniques are combined in LC-MS. The importance of sample preparation, instrument calibration, and data analysis are also discussed in introductory courses on LC-MS (7). Liquid chromatography-mass spectrometry (LC-MS) has revolutionized the field of analytical chemistry by providing a powerful tool for the separation, identification, and quantification of complex mixtures of compounds. This technique combines the high resolution separation capabilities of liquid chromatography with the sensitive and selective detection capabilities of mass spectrometry. The acknowledgment to LC-MS is essential in various fields such as pharmaceuticals, environmental monitoring, food safety, and forensic analysis. LC-MS has enabled researchers to detect trace levels of contaminants in water, identify metabolites in biological samples, and quantify drugs in plasma with unparalleled accuracy and precision (8-10). In conclusion, the acknowledgment to LC-MS is crucial for advancing scientific research and improving our understanding of complex chemical systems. Its versatility and sensitivity make it an indispensable tool for modern analytical chemistry. Overall, introductions to LC-MS provide students with a solid foundation in this powerful analytical technique, allowing them to confidently apply it to their own research projects and experiments.

History and development of LC-MS

Liquid chromatography-mass spectrometry (LC-MS) is a powerful analytical technique that has revolutionized the field of chemistry and biology. The history and development of LC-MS can be traced back to the 1950s when researchers began combining liquid chromatography with mass spectrometry to improve the separation and detection of compounds (11). Liquid chromatography and mass spectrometry are two powerful analytical techniques that have revolutionized the field of chemistry and biochemistry. The origins of these techniques can be traced back to the early 20th century, when scientists began to explore ways to separate and analyze complex mixtures of compounds. Liquid chromatography, which involves the separation of components in a liquid mixture based on their different affinities for a stationary phase, was first developed in the 1950s by scientists such as Archer John Porter Martin and Richard Laurence Millington Synge. Their work laid the foundation for modern liquid chromatography techniques, which are now widely used in various fields including pharmaceuticals, environmental analysis, and food science (12). Mass spectrometry, on the other hand, traces its origins back to the early 20th century with the development of the first mass spectrometer by J.J. Thomson. This technique allows for the identification and quantification of molecules based on their mass-to-charge ratio, making it an essential tool in fields such as proteomics, metabolomics, and drug discovery (13). Over the years, LC-MS has undergone significant advancements in technology, leading to increased sensitivity, resolution, and speed. The introduction of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in the 1980s further enhanced the capabilities of LC-MS by allowing for the analysis of a wider range of compounds. Today, LC-MS is widely used in various fields such as pharmaceuticals, environmental analysis, food safety, and forensic science. Its versatility and reliability have made it an indispensable tool for researchers seeking to identify and quantify complex mixtures of compounds with high precision (14). As technology continues to evolve, we can expect further innovations in LC-MS that will push the boundaries of analytical chemistry even further.

Principles of LCMS

The principles of LC-MS involve the separation of complex mixtures into individual components using a liquid mobile phase that passes through a stationary phase. The separated compounds are then ionized and analyzed by mass spectrometry to determine their molecular weight and structure (15). LC-MS offers high sensitivity, specificity, and accuracy in identifying compounds present in a sample. It can detect trace levels of analytes even in complex matrices. Additionally, LC-MS can provide quantitative information about the concentration of compounds present in a sample.The integration of liquid chromatography (LC) and mass spectrometry (MS) techniques has revolutionized the field of analytical chemistry (16). LC-MS combines the separation capabilities of LC with the detection and identification capabilities of MS, resulting in a powerful analytical tool that is widely used in various scientific disciplines. LC separates complex mixtures into individual components based on their chemical properties, while MS identifies and quantifies these components by measuring their mass-to-charge ratios. By combining these two techniques, researchers can achieve high sensitivity, selectivity, and accuracy in their analyses (17). The integration of LC-MS has enabled scientists to analyze a wide range of compounds, from small molecules to large biomolecules such as proteins and peptides. This technique has been instrumental in drug discovery, environmental monitoring, food safety testing, and many other areas of research (18).

Overview of High Pressure Liquid Chromatography (HPLC)

Liquid chromatography is a widely used technique in analytical chemistry for separating and analyzing compounds

based on their physical and chemical properties. The principles of liquid chromatography involve the use of a stationary phase, typically a solid or liquid material, and a mobile phase, which carries the sample through the column. The separation process is based on the differential interactions between the sample components and the stationary phase. Compounds with stronger interactions will move more slowly through the column, resulting in separation based on factors such as size, polarity, and charge. Key principles of liquid chromatography include retention time, resolution, selectivity, and efficiency. These parameters are crucial for optimizing separation conditions and obtaining accurate results (19, 20). The fundamental idea of HPLC is adsorption. In high-performance liquid chromatography (HPLC), a sample is driven through a column filled with a stationary phase made up of spherically or irregularly shaped particles that have been selected or derivatized to achieve specific types of separations. The stationary phase is known as the mobile phase (21). Since ancient times, HPLC techniques have been separated into two distinct subclasses according to stationary phases and the matching polarity that the mobile phase must have. Reversed phase liquid chromatography (RP-LC) approaches use octadecylsilyl (C18) and related organic-modified particles as stationary phase with pure or pH-adjusted water-organic combinations, such as water-acetonitrile and water-methanol. Normal phase liquid chromatography (NP-LC) methods use materials like silica gel as a stationary phase with neat or mixed organic compounds. In LC-MS apparatus, RP-LC is most frequently utilised as a way to enter samples into the MS (22).

Main Components of HPLC

Pump is required Aka solvent delivery system. It maintains a constant flow of the mobile phase (solvent that runs continuously to the system such as acetonitrile, methanol, phosphate buffer, etc.) through the HPLC.

Injection Valve is required It allows for the introduction of the sample solution in the HPLC column. The sample can be injected manually or with an automated injection valve called autosamplers. Autosamplers such as syringe pumps inject the samples automatically with precision and higher accuracy as compared to manual sample injection.

Column is required It contains a specific stationary phase to separate individual compounds based on a particular physiochemical property. The majority of HPLC columns are made of stainless steel and filled with porous silica particles. Nevertheless, there is a wide range of HPLC column hardware types and packing materials available.

Detector is required It analyzes the components of the eluted mixture that is collected after being run through the column. Among the commonly used detectors are ultraviolet/visible (UV/Vis), photodiode array (PDA), fluorescence (FL), and refractive index (RI) detectors.

Types of columns used in HPLC

Liquid chromatography is a widely used technique in analytical chemistry for separating and analyzing compounds in a mixture. One of the key components of liquid chromatography is the column, which plays a crucial role in the separation process. There are several types of columns used in liquid chromatography, each with its own unique characteristics and applications. One common type of column is the packed column, where the stationary phase is packed into a tube or cylinder. Packed columns are typically used for high-pressure liquid chromatography (HPLC) and can provide high resolution separations. Another type of column is the capillary column, which has a smaller diameter than packed columns and allows for faster separations with lower sample volumes. Capillary columns are often used in gas chromatography but can also be adapted for use in liquid chromatography (23, 24). The physical properties of the target molecules (analytes) determine the most suitable HPLC column for a given separation. The molecular characteristics that impact HPLC column selection include hydrophobicity/hydrophilicity, intermolecular forces (particularly dipole-dipole), intramolecular forces (ionic), and size. HPLC column separations can often exploit multiple differences in the molecular properties of the target molecules. Generally, the structure and chemistry of the HPLC column packing (stationary phase) determines the analyte elution profile (25, 26). There are different types of chromatography columns on the basis of their composition and method of separation.

1. Normal Phase Columnsis required this type of columns has more polar stationary phase than the mobile phase. The packing material of the column should be more polar than the mobile phase and this condition is fulfilled by the silica that is polar material. But water is more polar than the silica, therefore, water is not used and methylene chloride, hexane and chloroform or a mixture of these with diethyl ether is used as mobile phase. Separation of the sample components occurs on the basis of the polarity of the sample components. Sample components having more polarity interact more with polar stationary phase resulting in separation from the less polar component that interacts with less polar mobile phase. Silica columns are widely used in the pharmaceutical analysis. The chromatography column packing in which normal phase columns are used is known as Normal Phase Chromatography (27).
2. Reverse Phase Columnsis required it has a non-polar or less polar stationary phase than the more polar mobile phase. Bonded hydrocarbons like C8 and C18 and other non-polar hydrocarbons are used as stationary phase in reverse phase columns while aqueous organic solution like water-methanol or water-acetonitrile mixture is used as mobile phase. Separation of sample components in reverse phase columns also occurs on the basis on the polarity of the sample components but it happens just opposite of the normal phase HPLC columns, therefore, this type of chromatography is known as Reverse Phase Chromatography (28).
3. Ion Exchange Columnsis required the compounds those can easily ionize are analyzed using these columns. Stationary phase in these columns remains acidic or basic having negative or positive charge while mobile phase is a polar liquid as the salt solution in water. Separation of molecules occurs on the basis of the attractive ionic force between molecules and the charged stationary phase. Due to the exchange of ions during the separation of sample components, it is known as Ion Exchange Chromatography. The compounds those can easily ionize

are analyzed using these columns. Stationary phase in these columns remains acidic or basic having negative or positive charge while mobile phase is a polar liquid as the salt solution in water. Separation of molecules occurs on the basis of the attractive ionic force between molecules and the charged stationary phase. Due to the exchange of ions during the separation of sample components, it is known as Ion Exchange Chromatography (29).

4. Size Exclusion Columnsis required Porous stationary phase in these columns allows the separation of the components according to their size. Combination of polymers like polysaccharides and silica is used as stationary phase in these columns. Small sample molecules penetrate in the pores of stationary phase while the large molecules penetrate partially into the pores. Therefore the large molecules of the sample elute first than the small molecules and this chromatography is called Size Exclusion Chromatography. These columns are generally not used in the analysis of pharmaceutical compounds (30).

Overview of Mass Spectrometry

Mass spectrometry is a powerful analytical technique used to identify and quantify molecules based on their mass-to-charge ratio. It has become an indispensable tool in various fields such as chemistry, biochemistry, environmental science, and pharmaceuticals. The process of mass spectrometry involves ionizing a sample molecule, separating the ions based on their mass-to-charge ratio, and detecting them to generate a mass spectrum. This spectrum provides valuable information about the molecular structure and composition of the sample. There are different types of mass spectrometers available, each with its own advantages and limitations. Some common techniques include gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and tandem mass spectrometry (MS/MS) (31, 32). Overall, mass spectrometry offers high sensitivity, specificity, and accuracy in analyzing complex mixtures of molecules. It continues to evolve with advancements in technology, making it an essential tool for researchers in various scientific disciplines. A sample is put onto the MS device and vaporised during a standard MS operation. One of several techniques, such as striking the sample's constituent parts with an electron beam, ionises it and causes charged particles, or ions, to form. Using electromagnetic fields, the ions are sorted in an analyzer based on their mass-to-charge ratio. The ions are found, typically using a quantitative technique. Mass spectra are generated by processing the ion signal. Three modules make up MS instruments as well. An ion source that has the ability to transfer ions from gas phase sample molecules into the gas phase (33).

Mass spectrometry (MS) is an analytical technique that separates ionized particles such as atoms, molecules, and clusters by using differences in the ratios of their charges to their respective masses (mass/charge; m/z), and can be used to determine the molecular weight of the particles. Charged molecules or molecular fragments are generated in a high-vacuum region, or immediately prior to a sample entering a high-vacuum region, using a variety of methods for ion production. The ions are generated in the gas phase so that they can then be manipulated by the application of either electric or magnetic fields to enable the determination of their molecular weights (34).

Parts of Mass Spectrometry (MS)

A typical mass spectrometer comprises three parts are an ion source, a mass analyzer, and a detector. Ion Source is required For producing gaseous ions from the sample. Analyzer is required For resolving the ions into their characteristics mass components according to their mass-to-charge ratio. Detector System is required For detecting the ions and recording the relative abundance of each of the resolved ionic species. In addition, a sample introduction system is necessary to admit the samples to be studied to the ion source while maintaining the high vacuum requirements (~10-6 to 10-8 mm of mercury) of the technique; and a computer is required to control the instrument, acquire and manipulate data, and compare spectra to reference libraries.

Methods of Ion generation

Atmospheric Pressure Ionization (API): These techniques are used to ionize thermally labile samples such as peptides, proteins and polymers directly from the condensed phase. API sources introduce the sample through a series of differentially pumped stages. This maintains the large pressure difference between the ion source and the mass spectrometer without using extremely large vacuum pumps. In addition a drying gas is used to break up the clusters that form as the solvent evaporates. Because the analyte molecules have more momentum than the solvent and air molecules, they travel through the pumping stages to the mass analyzer (35).

Electrospray ionization (ESI): Electrospray ionisation (ESI) is a powerful technique used in mass spectrometry to analyze and identify molecules based on their mass-to-charge ratio. This method involves the creation of charged droplets from a liquid sample through the application of a high voltage electric field. These droplets then undergo desolvation, resulting in the formation of gas-phase ions that can be analyzed by the mass spectrometer. ESI has revolutionized the field of analytical chemistry due to its ability to analyze complex mixtures with high sensitivity and accuracy. It is widely used in various scientific disciplines, including biochemistry, pharmaceuticals, environmental science, and forensics. Overall, ESI has proven to be an invaluable tool for researchers seeking to understand the composition and structure of molecules at a molecular level. Its versatility and reliability make it an essential technique in modern analytical chemistry (36).

Electron impact ionization (EII): Electron Impact Ionisation (EII) is a fundamental process in the field of atomic and molecular physics. It involves the collision of high-energy electrons with atoms or molecules, resulting in the ejection of one or more electrons from the target species. This process plays a crucial role in various scientific disciplines, including plasma physics, astrophysics, and analytical chemistry. EII is commonly used in mass spectrometry to generate ions for analysis. By bombarding a sample with high-energy electrons, researchers can break apart molecules and create charged fragments that can be separated and detected based on their mass-to-charge ratio. This technique allows for the identification and quantification of compounds in complex mixtures. Furthermore, EII is also important in understanding

the behavior of atoms and molecules under extreme conditions, such as those found in outer space or within plasmas. By studying the mechanisms of electron impact ionization, scientists can gain valuable insights into the fundamental processes that govern matter at a microscopic level. Electron Impact Ionisation is a powerful tool that has revolutionized our understanding of atomic and molecular interactions. Its applications are vast and continue to expand as technology advances (37, 38).

Combinations of HPLC and MS

HPLC not only separates things but also provides little extra information about how a chemical might be. In fact, it is hard in HPLC to be certain about purity of a particular peak, and if it contains only a single chemical. Adding a Mass Spectrometry to this will tell you the masses of all the chemicals present in the peak, which can be used for identifying them, and an excellent method to check for the purity. Even a simple mass spec can be used as a mass-specific detector, specific for the chemical under study. More sophisticated mass detectors such as triple quadrupole and ion-trap instruments can also be used to carry out more detailed structure-dependent analysis on what is eluting off from the HPLC system (Figure 1) (35-38).

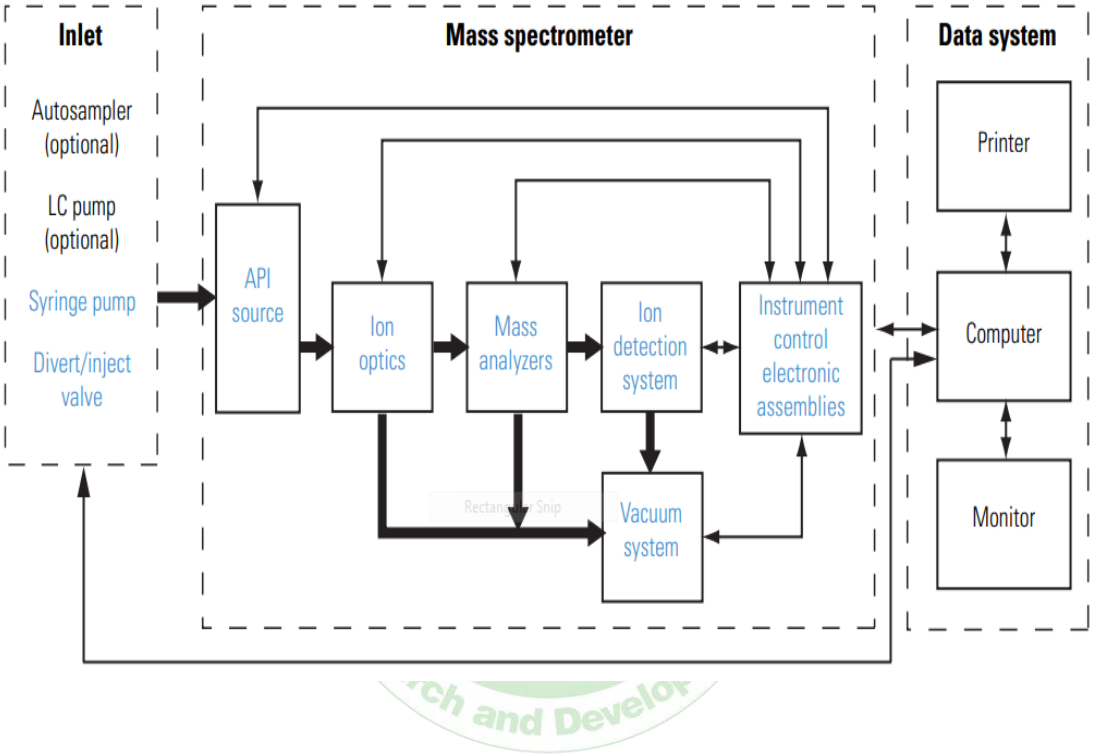

Figure 1: Workflow of Combinations of HPLC and MS

Coupling of MS to chromatographic techniques has always been desirable due to the sensitive and highly specific nature of MS compared to other chromatographic detectors. Typical LC/MS analysis begins with the liquid chromatograph (LC) separating a mixture into its chemical components. The LC pump produces a solvent stream (the mobile phase) that passes through an HPLC column (containing the stationary phase) under high pressure (39). An autosampler introduces an aliquot of sample into this solvent stream. As the solvent stream passes through the LC column, the sample separates into its chemical components. The rate at which the components of the sample elute from the column depends on their relative affinities to the mobile phase and the stationary phase. As the separated chemical components exit the LC column, they pass through a sample transfer line and enter the mass spectrometer for ionization and analysis. As the MS analyzes the ionized components and determines each mass-to-charge ratio (m/z) and relative intensity, it sends a data stream to the data system computer. In addition to supplying information about the m/z values of ionized compounds, the MS can also supply structural and quantitative information by performing MSn experiments (40).

Various Applications of LCMS

Liquid chromatography-mass spectrometry (LCMS) is a powerful analytical technique that combines the separation capabilities of liquid chromatography with the detection and identification abilities of mass spectrometry. LCMS has a wide range of applications in various fields such as pharmaceuticals, environmental analysis, food safety, forensics, and metabolomics. In the pharmaceutical industry, LCMS is used for drug discovery and development, as well as for quality control of finished products. Environmental scientists use LCMS to detect and quantify pollutants in air, water, and soil. Food safety experts rely on LCMS to identify contaminants and ensure the safety of food products. Forensic analysts use LCMS to identify drugs, toxins, and other substances in biological samples. Overall, LCMS is a versatile tool that plays a crucial role in modern analytical chemistry and has revolutionized the way scientists analyze complex mixtures in various fields (41).

Molecular Pharmacognosy:

Molecular pharmacognosy is a branch of pharmacology that focuses on the study of natural products and their potential therapeutic applications. Liquid chromatography-mass spectrometry (LCMS) has emerged as a powerful tool in this field, allowing researchers to analyze complex mixtures of natural compounds with high sensitivity and specificity. One of the key applications of LCMS in molecular pharmacognosy is the identification and quantification of bioactive compounds in medicinal plants. By separating and detecting individual components in plant extracts, researchers can determine which compounds are responsible for their pharmacological effects. LCMS also plays a crucial role in drug discovery and development by helping scientists identify lead compounds from natural sources that may have potential as new pharmaceuticals. Additionally, LCMS can be used to study the metabolism of natural products in the body, providing valuable insights into their mechanisms of action and potential side effects. In conclusion, LCMS is a versatile tool that has revolutionized the field of molecular pharmacognosy, enabling researchers to unlock the therapeutic potential of natural products for improved health outcomes (42).

Characterization and Identification of Bio active Compounds:

Liquid chromatography-mass spectrometry (LC-MS) has become an indispensable tool in the field of bioactive compound characterization and identification. This powerful analytical technique combines the separation capabilities of liquid chromatography with the sensitive detection and structural elucidation provided by mass spectrometry. LC-MS is widely used in the pharmaceutical industry for the analysis of natural products, drug metabolites, and impurities. It allows for the rapid and accurate identification of bioactive compounds in complex mixtures, such as plant extracts or biological samples. LC-MS can also be used to determine the chemical structure of unknown compounds, aiding in drug discovery and development. Furthermore, LC-MS is valuable in studying the pharmacokinetics and metabolism of bioactive compounds in vivo. By providing detailed information on compound structure, purity, and concentration, LC-MS plays a crucial role in ensuring the safety and efficacy of pharmaceutical products. In conclusion, LC-MS is a versatile tool that has revolutionized the field of bioactive compound characterization and identification, leading to advancements in drug discovery and development (43).

Quantitative Bioanalysis of various Biological Samples

Liquid chromatography-mass spectrometry (LCMS) has become an essential tool in the field of quantitative bioanalysis due to its high sensitivity, selectivity, and accuracy. This powerful technique is widely used for the analysis of various biological samples such as blood, urine, plasma, and tissues. One of the key applications of LCMS in quantitative bioanalysis is drug metabolism studies. By using LCMS to measure drug concentrations in biological samples, researchers can gain valuable insights into how drugs are metabolized in the body and how they affect different physiological processes. LCMS is also commonly used in pharmacokinetic studies to determine drug absorption, distribution, metabolism, and excretion. By accurately measuring drug concentrations over time, researchers can optimize dosing regimens and improve drug efficacy while minimizing side effects. In conclusion, LCMS plays a crucial role in quantitative bioanalysis by providing accurate and reliable data for a wide range of applications in pharmaceutical research and development (44).

Phytoconstituents / Plant Metabolomics:

Liquid chromatography-mass spectrometry (LCMS) has revolutionized the field of phytoconstituents and plant metabolomics by allowing for the identification and quantification of a wide range of compounds present in plants. Because LC-MS can analyse a wide range of metabolites, including highly polar and/or higher molecular weight molecules (oligosaccharides and lipids) and secondary metabolites (e.g., alkaloids, glycosides, phenyl propanoids, flavanoids, isoprenes, glucosinolates, terpenes, benzoids), it offers a tool for differentiating this immense plant biodiversity. In order to identify and quantify all peaks in the chromatogram as ions that are first characterised by retention time and molecular mass, LC-MS is one of the primary untargeted analytical techniques for determining global metabolite profiles. This powerful analytical technique has been instrumental in elucidating the complex chemical composition of plants, leading to a better understanding of their biological activities and potential therapeutic applications. LCMS is widely used in the analysis of phytoconstituents such as flavonoids, alkaloids, terpenoids, and phenolic compounds. By separating these compounds based on their chemical properties and then detecting them with mass spectrometry, researchers can identify specific molecules responsible for the medicinal properties of plants. Furthermore, LCMS is also used in plant metabolomics studies to investigate metabolic pathways and interactions between different metabolites. By analyzing changes in metabolite profiles under different conditions or treatments, researchers can gain insights into plant physiology and biochemistry. In conclusion, LCMS has become an indispensable tool in the study of phytoconstituents and plant metabolomics, providing valuable information for drug discovery, agriculture, and nutrition (45).

Automated Immunoassay in Therapeutic Drug Monitoring:

Therapeutic drug monitoring is a crucial aspect of patient care, ensuring that medications are administered at safe and effective levels. One of the most advanced technologies used in this process is Liquid Chromatography-Mass Spectrometry (LCMS). LCMS offers high sensitivity and specificity, allowing for accurate measurement of drug concentrations in biological samples. In recent years, LCMS has been increasingly utilized in automated immunoassays for therapeutic drug monitoring. This application allows for rapid and precise analysis of multiple drugs in a single sample, streamlining the monitoring process and improving patient outcomes. By combining the specificity of immunoassays with the sensitivity of LCMS, healthcare professionals can obtain reliable results with minimal sample volume. Overall, the integration of LCMS into automated immunoassays for therapeutic drug monitoring represents a significant advancement in clinical practice. It offers a more efficient and accurate method for measuring drug levels, ultimately

leading to better patient care and treatment outcomes (46, 47).

Two Dimensional (2-D) Hyphenated Technology

One major application of 2-D LC-MS is in proteomics, where it can be used to separate and identify thousands of proteins in a single sample. This has revolutionized the field of biological research by allowing scientists to study protein expression patterns and interactions on a large scale. Another important application is in metabolomics, where 2-D LC-MS can be used to analyze the small molecules present in biological samples. This has led to advancements in understanding metabolic pathways and identifying biomarkers for various diseases. Overall, the applications of 2-D LC-MS in hyphenated technology are vast and continue to expand as researchers discover new ways to utilize this powerful analytical tool (48).

With its application in a multitude of analytical and bioanalytical techniques for the analysis of proteins, amino acids, nucleic acids, carbohydrates, lipids, peptides, etc., as well as in the primary classification in the fields of genomics, lipidomics, metabolomics, proteomics, etc., LCMS has developed into a potent two-dimensional (2D) hyphenated technology. The initial preference for LCMS may have stemmed from the need for more potent analytical and bioanalytical techniques that could precisely and specifically separate the target analytes from high complexity mixtures (49).

The combination of this hybrid class of HPLC and MS to perform both routine qualitative discovery and quantitative directed analysis of complex mixtures is conceivably one of the most significant combinations in developments and separations, where mass spectrometry plays a major role in the field of science by detecting various analytical & bio analytical techniques in the past decade. It gives an increased level of robustness and accuracy out of their LC systems and improved detection abilities when coupled with a MS system (50).

Pharmacology and toxicology

Liquid chromatography-mass spectrometry (LC-MS) has become an indispensable tool in pharmacology and toxicology due to its high sensitivity, selectivity, and versatility. In pharmacology, LC-MS is used for drug discovery, development, and monitoring of drug levels in biological samples. It allows for the identification and quantification of drugs and their metabolites with high precision and accuracy. In toxicology, LC-MS is used to detect and quantify toxic substances in biological samples such as blood or urine. It plays a crucial role in forensic toxicology by identifying drugs or toxins present in post-mortem samples. Furthermore, LC-MS can also be used to study the metabolism of drugs and toxins in the body, providing valuable insights into their mechanisms of action and potential toxicity. Overall, the applications of LC-MS in pharmacology and toxicology are vast and continue to expand as technology advances. Its ability to provide detailed information on drug metabolism, toxicity, and efficacy makes it an invaluable tool for researchers and clinicians alike (51, 52).

Omics Study

Metabolomics

Metabolomics is a rapidly growing field in the realm of biological sciences that focuses on the comprehensive analysis of small molecules, known as metabolites, within cells, tissues, and organisms. By studying the metabolic profile of an organism, metabolomics provides valuable insights into its physiological state and biochemical processes. This holistic approach allows researchers to better understand how genetic and environmental factors influence metabolism and ultimately impact health and disease. Metabolomics has a wide range of applications in various fields such as medicine, agriculture, nutrition, and environmental science. In medicine, metabolomics is used for biomarker discovery, personalized medicine, drug development, and understanding disease mechanisms. In agriculture, it helps improve crop yield and quality by optimizing nutrient uptake and stress responses. In nutrition science, it aids in determining the effects of diet on metabolism and overall health (52).

Metabolomics aims at identification and quantitation of small molecules involved in metabolic reactions. LC-MS has enjoyed a growing popularity as the platform for metabolomic studies due to its high throughput, soft ionization, and good coverage of metabolites. The success of LC-MSbased metabolomic study often depends on multiple experimental, analytical, and computational steps. This review presents a workflow of a typical LC-MS-based metabolomic analysis for identification and quantitation of metabolites indicative of biological/environmental perturbations (53, 54). Challenges and current solutions in each step of the workflow are reviewed.The thorough examination of every metabolite in a biological system is known as metabolomics. Because it offers a quantitative evaluation of low molecular weight analytes (<1800Da), which characterise the metabolic state of a biological system, it enhances transcriptomics and proteomics. Nicholson also created the word "metabonomics" to refer to research on how metabolic activities alter in response to pathophysiological triggers or genetic alterations. But in reality, these phrases are very similar and frequently used interchangeably, particularly in the context of human disease research. Numerous fields of study have used metabolic analyses, including integrative systems biology, functional genomics, biomarker identification, and studies of biological and environmental stress (55).

Proteomics

Proteomics is a rapidly growing field in the realm of biological sciences that focuses on the study of proteins and their functions within living organisms. By analyzing the entire set of proteins present in a cell, tissue, or organism, researchers are able to gain valuable insights into various biological processes and pathways. One of the key goals of proteomics is to identify and characterize all the proteins in a given sample, as well as to understand how these proteins interact with one another (56). This information can help scientists better understand complex diseases such as cancer, Alzheimer's, and diabetes, leading to improved diagnostic tools and potential therapeutic targets. The last ten years have seen a remarkable advancement in peptide LC-MS

instrumentation, to the point where protein sample preparation—including extraction and digestion—is no longer a major point of failure in proteomic workflows or the overall success of proteome research. The degree of sample cleanliness in regard to non-protein impurities has a significant impact on the rate of protein identification. A small disc of membrane-embedded separation material is held in place by a single pipette tip known as the "StageTip," which combines protein extraction, digestion, and fractionation processes. This innovation stems from the current trend of streamlining sample preparation procedures and handling small amounts of biological material (57). Extrapolating these protocols to plant material is challenging given protein scarcity and the abundance of interfering compounds in plant cells, but it is an exciting challenge because the benefits for research of SM will outweigh development efforts (58).

Lipidomics

Lipidomics is a rapidly growing field of study that focuses on the comprehensive analysis of lipids in biological systems. Lipids are essential molecules that play crucial roles in various cellular processes, including energy storage, signaling, and membrane structure. By studying the composition and function of lipids, researchers can gain valuable insights into the underlying mechanisms of diseases such as cancer, diabetes, and cardiovascular disorders (59). The advancement of lipidomics has been made possible by technological innovations in mass spectrometry and chromatography, allowing for the identification and quantification of thousands of lipid species in complex biological samples. This detailed analysis has led to the discovery of novel lipid biomarkers for disease diagnosis and prognosis. Overall, lipidomics holds great promise for advancing our understanding of lipid biology and its implications for human health. As research in this field continues to expand, we can expect to uncover new therapeutic targets and strategies for treating a wide range of diseases (60).

CONCLUSIONS

Liquid chromatography-mass spectrometry (LC-MS) has become an indispensable tool in analytical chemistry due to its ability to separate and identify complex mixtures of compounds with high sensitivity and specificity. The hyphenation of liquid chromatography with mass spectrometry allows for the simultaneous analysis of compounds based on their retention times and mass-to-charge ratios, providing valuable information about the identity and quantity of analytes in a sample. One of the key advantages of LC-MS is its versatility, as it can be applied to a wide range of samples including biological fluids, environmental samples, pharmaceuticals, and food products. This makes it an essential technique in fields such as pharmaceutical analysis, environmental monitoring, metabolomics, proteomics, and forensic science. The future prospects for the LC-MS as a hyphenated technique are promising and exciting. As technology continues to advance, so too does the potential for this powerful analytical tool. The combination of liquid chromatography (LC) and mass spectrometry (MS) allows for the separation and identification of complex mixtures with high sensitivity and specificity. One of the key advantages of LC-MS is its ability to analyze a wide range of compounds, from small molecules to large biomolecules, making it a versatile tool in various fields such as pharmaceuticals, environmental science, and metabolomics. Additionally, advancements in instrumentation and software have improved the speed and accuracy of data analysis, further enhancing the capabilities of this technique. As researchers continue to push the boundaries of LC-MS technology, we can expect to see even greater advancements in sensitivity, resolution, and throughput. The future looks bright for LC-MS as it continues to be at the forefront of cutting-edge analytical techniques. In conclusion, LC-MS is a powerful hyphenated technique that plays a crucial role in modern analytical chemistry by providing accurate and reliable data for the identification and quantification of compounds in complex samples. Its importance cannot be overstated in advancing scientific research and improving our understanding of chemical processes.

Conflict of Interest

None

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