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A REVIEW ON HYPHENATED TECHNIQUE LIQUID CHROMATOGRAPHY- MASS SPECTROSCOPY (LC-MS)

NAME OF AUTHORS:

HIMANSHU MISHRA[1], DRASHTI PANCHOLI[1], ANAMIKA RAJPUT [1], AJAYKUMAR CHAUHAN[1], DR. SIDDHI UPADHYAY* [2], DR. UMESH M. UPADHYAY [3]

1 STUDENT, 2 PROFESSOR, 3 PRINCIPAL

CORRESPONDING AUTHOR:

Dr. Siddhi Upadhyay, Professor, Sigma Institute of Pharmacy

ABSTRACT

Liquid Chromatography-Mass Spectrometry (LC-MS) has emerged as a powerful analytical technique for the separation, identification, and quantification of a wide range of compounds in complex samples. This hyphenated approach, combining liquid chromatography with massspectrometry, has revolutionized the field of analytical chemistry by offering enhanced sensitivity, selectivity, and versatility. LC-MS has found applications in diverse scientific disciplines, including pharmaceuticals, environmental analysis, metabolomics, and proteomics. overview of the fundamental principles, components, and applications of the LC-MS technique. It explores the role of liquid chromatography in separating analytes based on their chemical properties and the subsequent mass spectrometric analysis for characterizing compounds through their mass-to-charge ratios and fragmentation patterns. Various LC-MS configurations, such as triple quadrupole, time-of-flight, and Orbitrap mass analyzers, are discussed, each offering unique capabilities for specific analytical challenges. It highlights theability of LC-MS to identify and quantify trace levels of compounds in complex matrices, making it an invaluable tool for addressing analytical questions in both research and routine analysis.

Keywords: Hyphenated Technique ,Chromatographic Analysis, Liquid Chromatography- Mass Spectrometry technique, LC-MS Applications, Method Optimisation of LC-MS, Spectrometry, LC-MS Instrumentation.

INTRODUCTION

Analytical techniques [1-6]

Definition: - Analytical methods serve as the foundation of scientific inquiry and problem-solving across diverse domains such as chemistry, biology, environmentalscience, and pharmaceuticals. These techniques are pivotal for ascertaining the makeup, configuration, and characteristics of substances, enabling scientists and experts to derive valuable insights from intricate samples.

Importance of Analytical techniques: - Analytical techniques encompass a diverse array of methods and tools that are employed to scrutinize and evaluate thecomposition, structure, characteristics, and behavior of substances and materials. These techniques hold paramount importance across numerous scientific disciplines, industries, and research domains, facilitating both qualitative and quantitative analysis. Some prevalent analytical techniques include: Spectroscopy:Spectroscopic methods revolve around the interaction of matter with electromagnetic radiation, and noteworthy examples encompass: UV-Visible Spectroscopy, IR Spectroscopy, NMR Spectroscopy, Mass Spectrometry. Chromatography: Chromatographic approaches are instrumental in segregating and identifying components within a mixture, relying on their distinctive distribution between a stationary phase and a mobile phase. Prominent instances involve: HPLC, GC, TLC.

Hyphenated Technique: -Hyphenated technique refers to the amalgamation of twoseparate analytical methods through the use of an appropriate interface. Typically, chromatographic methods are combined with spectroscopic techniques. In chromatography, this fusion streamlines the isolation of pure or near-pure fractionsof chemical constituents within a mixture, while spectroscopy furnishes precise identification by comparing the outcomes against established standards or referencelibrary spectra. Hyphenated techniques encompass various combinations, includingthe linkage of separation approaches with online spectroscopic detection methods. These combinations can encompass separation-separation, separation-identification, and identification-identification techniques, thereby extending the scope of analytical possibilities. To gain insights into molecular machinery, it is imperative to elucidate protein structures and interactions.

Liquid chromatography-mass spectrometry: - Liquid chromatography-mass spectrometry (LC-MS) is a valuable technique, particularly in shotgun proteomics. This approach facilitates the exhaustive identification of tryptic peptides originating from tissues, biofluids, and cultured cells. Additionally, it has proven instrumentalin characterizing various protein-protein interaction networks and posttranslationalmodifications. Beyond its utility in shotgun proteomics, LC-MS serves as a versatile tool for conducting structural proteomics investigations. Research on propolis has gained significant attention in Southeast Asian nations, particularly inMalaysia, due to its valuable pharmacological attributes. One notable aspect of thisresearch involves the identification of phytochemical compounds through advancedanalytical techniques such as gas chromatography-mass spectrometry (GC–MS) and quadrupole time-of-flight liquid chromatography-mass spectrometry (Q-TOF LC-MS).

Chromatography: - [52,54]

Chromatography derives its name from the Greek words “chroma,” meaning color, and“graphein,” meaning to write. In a more precise context, chromatography refers to a physical separation process used to isolate, purify, and separate compounds within a mixture based on their distinct distribution rates. These rates are determined by several factors, including: Solubility , Affinity ,Interaction with a stationary material During chromatography, the components of the mixture are distributed between two phases: the stationary phase and the mobile phase. The mobile phase moves through the system at varying speeds in a predefined direction. Every chromatographic separation technique comprises three essential components: 1.Sample2.Mobile phase3.Stationary phase The stationary phase refers to the material, which can be either solid or liquid, where the components of the mixture are separated and isolated. The nature of the stationary phase can vary. The mobile phase, on the other hand, is either a solid or liquid substance responsible for transporting the mixture, composed of a sample to be purified, isolated, and separated, across the surface of the stationary phase.

Classification: - Chromatographic methods can be categorized and summarized in three distinct ways, Classification based on the configuration of the stationary phase, such as planar andcolumn chromatography. Classification based on the physical state of both the stationary and mobile phases, including gas and liquid chromatography, Classification based on the interaction between the stationary and mobile phases, encompassing techniques like affinity chromatography, ion exchange chromatography, partition chromatography, adsorption chromatography, and size exclusion chromatography.

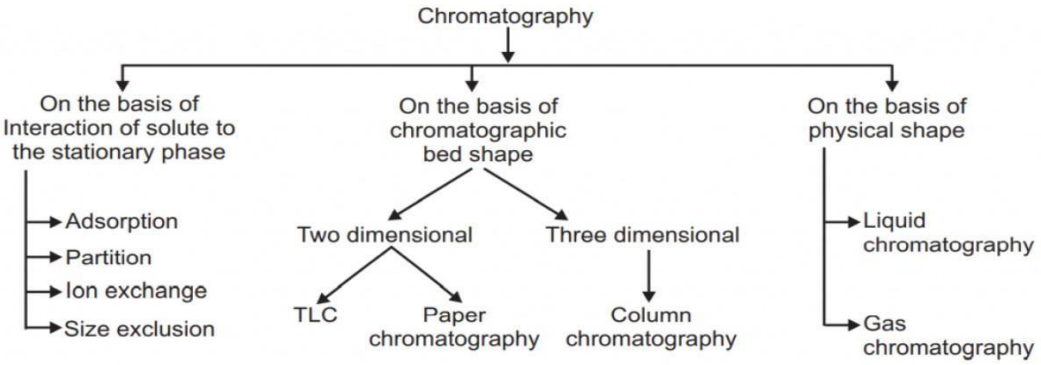

Figure 1. Classification of chromatography

Principle: - In chromatography, the molecules within a mixture become attached to the surface of the stationary phase. The mobile phase is then introduced to traverse the solid phase, carrying themixture for separation. Key factors influencing this separation process include molecular characteristics associated with adsorption (liquid-solid), partition (liquid-solid), and affinity, aswell as variations in their molecular weights. Due to these distinctions, certain components within the mixture spend more time on the stationary phase and move slowly through the chromatographic system, while others exit the system more quickly.

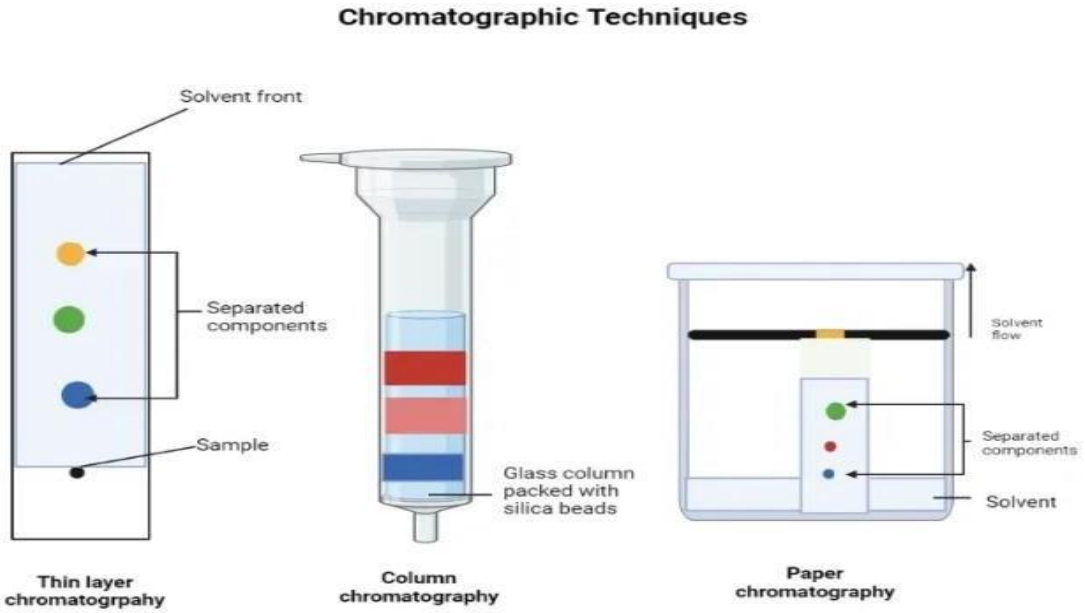

Figure 2. Chromatographic techniques (1) thin layer chromatography, (2) columnchromatography, (3) paper chromatography.

Spectroscopy: - [40,52,53,57,58]:- Spectroscopy, as a scientific field, traces its origins back to Isaac Newton's groundbreaking experiments involving the dispersion of light with a prism, initially referred to as optics. In itsinception, spectroscopy primarily focused on the examination of visible light, encompassing the study of color. It was not until the investigations conducted by James Clerk Maxwell that the scope of spectroscopy expanded to encompass the entire electromagnetic spectrum. Consequently, spectroscopy emerged as the scientific discipline dedicated to exploring the interactions between matter and electromagnetic radiation. Ferrocene is an organometallic compound that readily undergoes oxidation in atmosphere under acidic conditions. However, because of its diamagnetic nature, ferrocene does not lend itself to structural analysis by nuclear magnetic resonance (NMR) spectroscopy

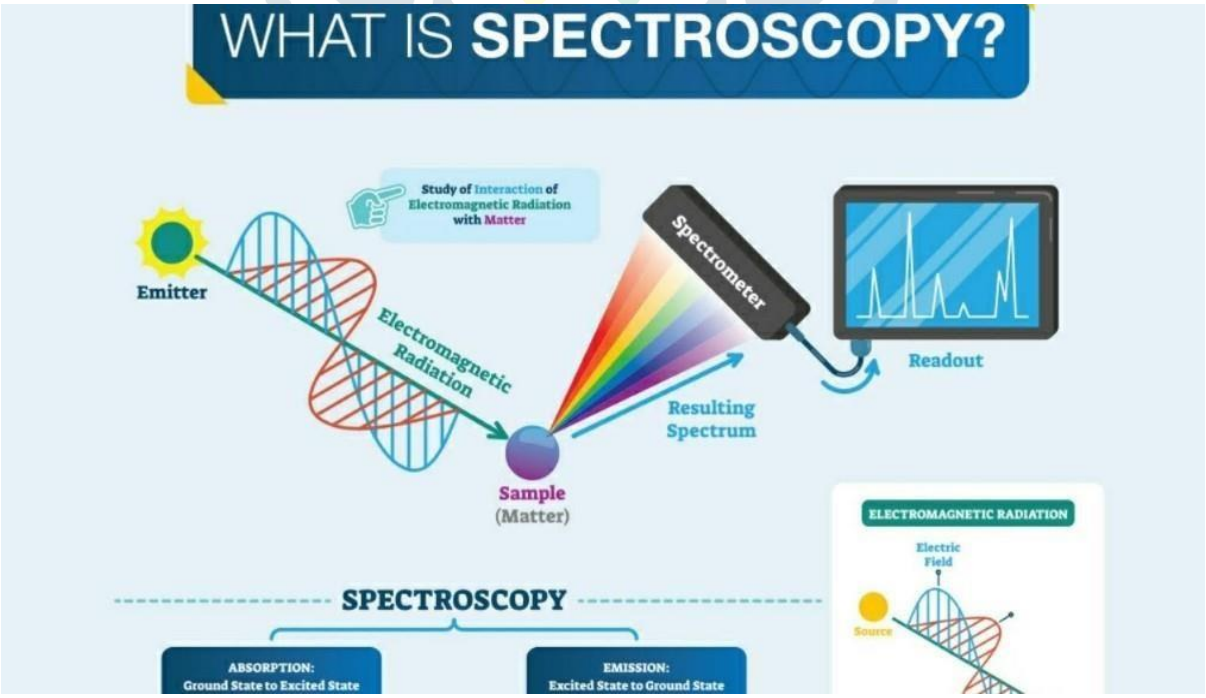

Figure 3. What is spectroscopy?

UV-spectroscopy:- Ultraviolet (UV) spectroscopy is an optical analytical technique that relies on the principles outlined in the Beer–Lambert equation, where the concentration of the absorbing species in a solution and the path length direct impact on the solution's absorbance. UV spectroscopy utilizes light within

the near-infrared, ultraviolet, and visible spectra. Consequently, it enables the quantification of the concentration of the absorbing substance in a solution for a given pathlength.

Atomic spectroscopy:- Spectroscopy primarily concerns the interaction between electromagnetic radiation and atoms,typically when the atoms are in their lowest energy state known as the ground state. The absorption of electromagnetic radiation by electrons in atoms only transpires when the photoncarries energy precisely equivalent to the discrepancy between two quantized energy levels.

Molecular Spectroscopy:- Spectroscopy pertains to the interaction between electromagnetic radiation and molecules, leading to transitions between rotational, vibrational, and electronic energy levels. Consequently, the spectra of molecules are considerably more intricate than those of atoms.

Raman spectroscopy: -The principle of Raman spectroscopy is based on scattering, a vibrational spectroscopytechnique that provides in-depth insights into various aspects of a sample, including its chemical composition, structural phases, crystallinity, and molecular interactions.

Optical Spectroscopy: -Optical spectroscopy is widely recognized as a real-time, quantitative, and minimally invasivemethod for the optical analysis of biological samples. Optical spectrometry relies on the interaction of light with biological tissue.

LIQUID CHROMATOGRAPHY

Liquid chromatography (LC) serves as a widely employed separation method within contemporary analytical laboratories. This prominence can be attributed to its diverse range ofseparation modes, including reversed-phase, size-exclusion, ion-exchange, hydrophilic-interaction, and others. Moreover, LC is highly adaptable as it accommodates the dissolution of nearly all sample types in various liquid phases, such as aqueous or organic solvents. This versatility renders LC an indispensable tool employed in both academic research and industrialapplications. However, for the analysis of exceptionally intricate, non-volatile samples found in diverse fields like biopharmaceuticals, food science, environmental analysis, and polymer research, conventional LC may not meet the analytical requirements, thus making two-dimensional (2D) LC an appealing alternative. In the historical context, the development of multi-dimensional LC methods has largely reliedon empirical, experience-based, and trial-and-error approaches, which have traditionally sufficed in academic settings. Nevertheless, in order to enhance the accessibility and appeal ofthis technique to industrial laboratories, there is a growing interest in adopting systematic andmodel-driven methodologies to streamline the method development process for multi- dimensional separations. These innovations, coupled with other fundamental advancements in2D-LC, can greatly benefit the field. In addition to the application-driven research papers discussed throughout this review, there has been a notable increase in the publication of technical advancements in multi-dimensionalseparations in recent years. Many of these advancements focus on computer-aided methods forthe development and optimization of 2D-LC techniques. Low-performance liquid chromatography and High-performance liquid chromatography Liquid chromatography (LC) methods employing support materials with particle sizes exceeding 40 micrometers in diameter often exhibit specific limitations and characteristics, including: Reduced System Efficiency and Elevated Plate Heights: These systems typically sufferfrom reduced separation efficiency and tend to have larger plate heights, which can hinder the quality of separation. Broad Peaks: The use of large, non-rigid support materials leads to broader peaks in chromatographic profiles, negatively impacting resolution and peak sharpness. Poor Limits of Detection: Owing to the broader peaks and reduced efficiency, these LCmethods often exhibit less sensitivity and less favorable limits of detection. Prolonged Separation Times: Achieving adequate separation and resolution may requirelonger elution times due to the broader peaks and reduced efficiency, making the analysis less time-efficient. Low Tolerance for Operating Pressures: Columns using large, non-rigid support materials are typically limited in their ability to withstand high operating pressures, which restricts their use in high-pressure chromatography systems. [52,54]

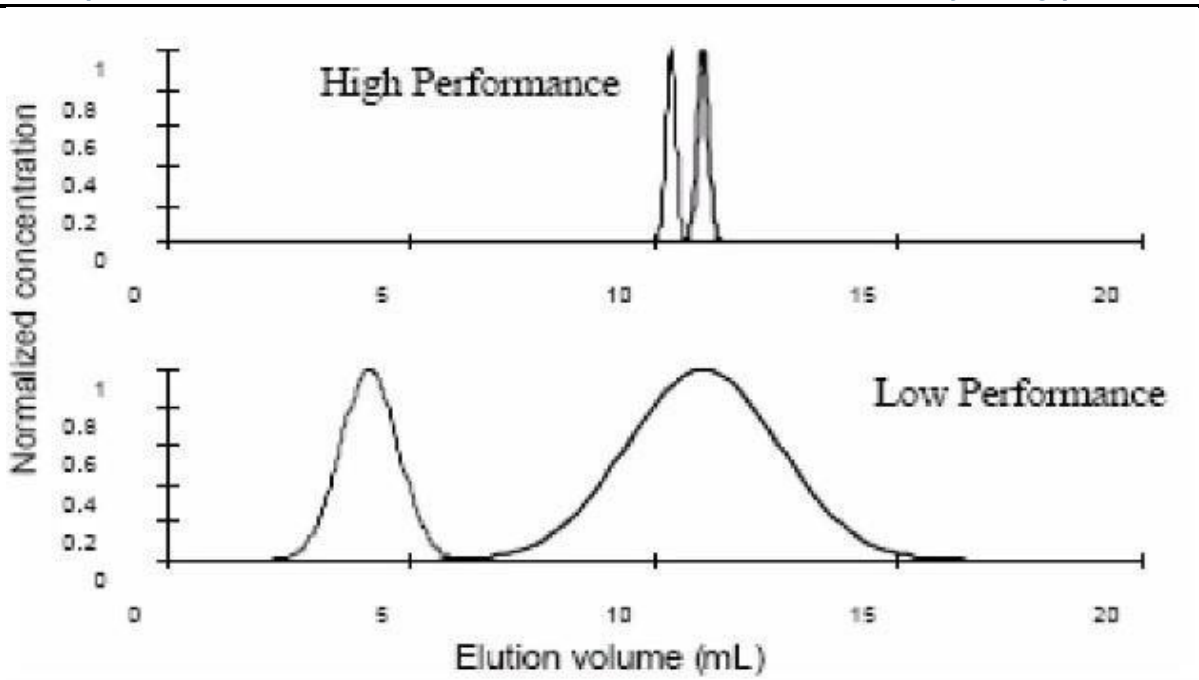

Figure 4. Normalized concentration versus elution volume (ml) of high performance andlow performance.

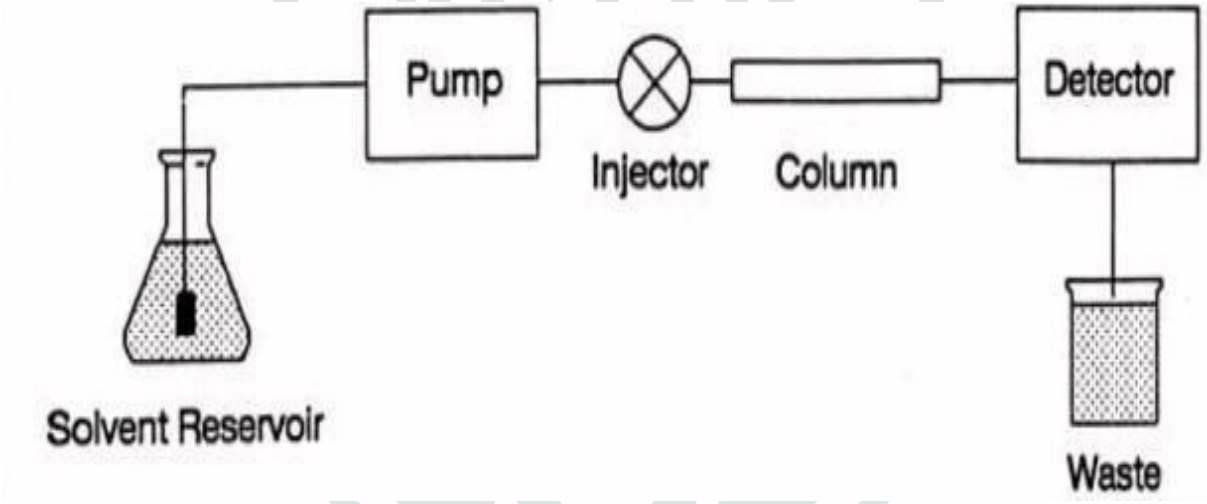

Figure 5. Liquid chromatography working,

Liquid chromatography is a widely adopted analytical technique with diverse applications,encompassing but not limited to:Environmental monitoringDrinking water analysisFood safety and quality assurance Air quality analysis, Clinical diagnostics, including neonatal screening,Pharmaceutical and biopharmaceutical analysis,Drug abuse screening.There are various system configurations for liquid chromatography, with the most efficient separations achieved using ultra-high performance liquid chromatography (UHPLC) instrumentation. This technique was initially introduced in 2004 and was initially referred to as ultra-performance liquid chromatography (UPLC). In brief, it allows for the separation of a multi-component mixture that is soluble in the liquid mobile phase. This separation occurs because the individual components partition differently between the mobile phase and the stationary phase, typically a column.Strengths and Limitations of Liquid Chromatography (LC): Strengths: Versatility: LC is widely used for a broad range of applications, making it a versatile analytical technique for separating and analyzing various compounds. Selectivity: LC can provide high selectivity when choosing the right stationary phase and mobile phase, allowing for the separation of complex mixtures. Sensitivity: LC can be highly sensitive, especially when coupled with mass spectrometry (LC-MS), enabling the detection of trace amounts of compounds.

Limitations:

Unsuitability for Volatile Compounds: LC is not ideal for the separation and analysis of volatile compounds, as it requires components with lower vapor pressures than the mobile phase. Gaschromatography is better suited for this purpose.

Limited Separation Efficiency: LC may have limitations in terms of separation efficiency for very complex mixtures or compounds with similar chemical properties, requiring additional optimization or alternative techniques.

Longer Analysis Times: LC often involves longer analysis times compared to gas chromatography, which can be a limitation for high-throughput analysis.

MASS SPECTROMETRY

Mass spectrometry is an analytical technique utilized for the identification of chemical substances. It achieves this by sorting gaseous ions in electric and magnetic fields based on their mass-to-charge ratios. The tools employed in these investigations go by the names of massspectrometers and mass spectrographs, and they function on the fundamental principle that ionsin motion can be influenced and directed by electric and magnetic fields. The two instruments, mass spectrometers and mass spectrographs, primarily diverge in their methods of detecting the sorted charged particles. In a mass spectrometer, the detection occursthrough electrical means, whereas in a mass spectrograph, detection is achieved through photographic or alternative non-electrical methods. The term "mass spectroscope" encompasses both types of devices. Nevertheless, given that electrical detectors are presently the more prevalent choice, the field is conventionally referred to as mass spectrometry. MS (Mass Spectrometry) analysis can be significantly compromised by the existence of non- volatile salts, which can lead to analyte ion suppression and instrument contamination. Mass spectrometric methods have seen growing utilization in species identification across a wide range of sample types, such as bone, meat, milk, and gelatin. In this process, target proteins are enzymatically digested into peptide fragments, most commonly employing trypsin. Numerous analytical techniques have been developed to address the requirement of detecting and quantifying small molecule nitrosamines in pharmaceutical products. The predominant approach in most of these methods involves employing mass spectrometers (MS) as the detector in conjunction with gas or liquid chromatographic separation (GC-MS or LC-MS). This combination is necessary to attain the required levels of sensitivity and selectivity. Even though GC-MS and/or LC-MS are standard technologies widely used by the pharmaceutical industry and regulatory agencies, their extensive application for quality controlpurposes, especially for trace-level nitrosamines, was not initially anticipated. Consequently, there has been a growing interest from regulatory bodies and stakeholders regarding the performance characteristics of these MS-based analytical procedures. Combining MS with LC provides insights into the hydrophilicity of the molecules, which canbe assessed through either the total ion chromatograms (TIC) or extracted ion chromatograms(EIC) of specific m/z values. Researchers in the field of mass spectrometry (MS)-based proteomics have the objective of isolating, identifying, and functionally characterizing the protein composition within a cell, tissue, or organism of their focus. Imaging mass spectrometry (IMS) is a molecular technology employed in spatially targeted research, offering molecular maps derived from tissue sections. MALDI mass spectrometry hasa well-established history of classifying bacteria and conducting various bulk analyses, especially for plate-based assays. Nevertheless, the clinical utilization of spatial data from tissue biopsies to aid in diagnoses and prognoses represents a relatively emerging prospect within the realm of molecular diagnostics. This research focuses on spatially targeted mass spectrometry techniques for clinical diagnostic purposes and tackles various aspects related tonovel imaging-based assays, encompassing analyte selection, quality control and assurance metrics, data reproducibility, data classification, and data scoring. [30,46,51,55,56,57]

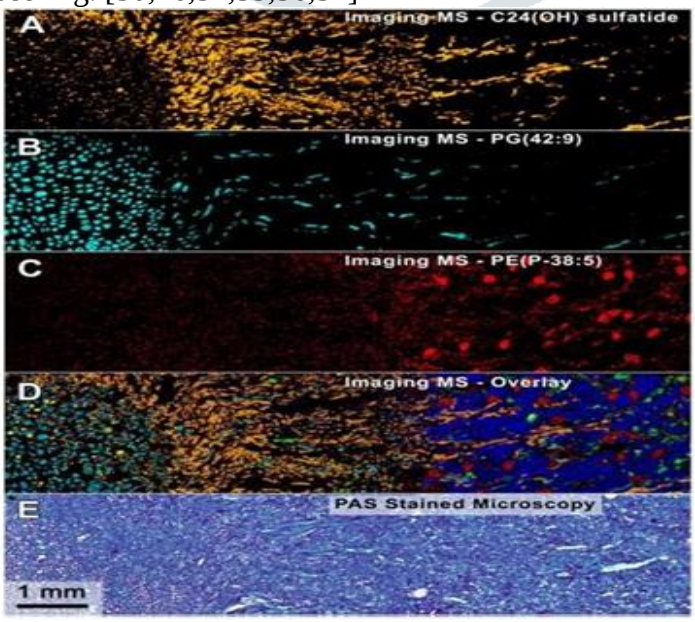

Figure 6. Maldi

Mass spectrometry is often considered the optimal detector that can be coupled with a liquid chromatograph due to its exceptional sensitivity, linear dynamic range, selectivity, and, with the use of high mass resolving power instrumentation, specificity. This technique is employed to determine the mass-to-charge ratio(m/z) of an analyte. Unlike gas chromatography-mass spectrometry (GC-MS), integrating an LC system with MS posed challenges and required several years of development. Electrospray ionization (ESI) is the prevailing ionization technique in LC-MS today, operatingat atmospheric pressure. The challenging aspect was the development of an atmospheric pressure inlet that could seamlessly connect to the high vacuum conditions needed within the mass spectrometry system. Microflow and low-flow LC-MS have emerged as valuable tools invarious applications, including biomarker detection and biopharmaceutical analysis. Mass spectrometers function by transforming analyte molecules into charged (ionized) states,enabling the subsequent analysis of these ions and any fragments generated during ionization,based on their mass-to-charge ratio (m/z). Various technologies exist for both ionization and ion analysis, leading to a wide array of mass spectrometers with various combinations of theseprocesses. In practical applications, some configurations are considerably more versatile than others. The following descriptions will primarily concentrate on the major types of ion sources and mass analyzers commonly employed in LC-MS systems found in clinical laboratories.

HYPHENATED TECHNIQUE: - [58,59]

A hyphenated technique refers to the combination or coupling of two distinct analytical methods using a suitable interface. Typically, chromatographic techniques are integrated withspectroscopic techniques. In chromatography, this integration facilitates the separation of pure or nearly pure fractions of chemical components in a mixture, while spectroscopy provides specific information for identification by comparing the results to standards or reference libraryspectra. Hyphenated techniques encompass various combinations, including the coupling of separation techniques with on-line spectroscopic detection methods. These combinations can involve separation-separation, separation-identification, and identification-identification techniques, offering a broad range of analytical possibilities. Hybrid methods have received significant attention in recent years as a primary approach for addressing complex analytical challenges. These methods involve combining separation technologies with spectroscopic techniques to enable both quantitative and qualitative analysis of unknown compounds within complex natural product extracts or fractions. The integration of two or more methods for the detection and isolation of chemicals from solutions iscommonly referred to as hyphenated separation techniques, with chromatography often beingone of the techniques employed. In the fields of chemistry and biochemistry, hyphenated methods are widely employed. When one of the method names includes a hyphen, it is commonto use a slash instead. The goal of these approaches is to obtain structural information that allows for the characterization of compounds present in a crude sample Hyphenated techniques in analytical chemistry are often categorized into two groups based onthe number of methods combined: Double Hyphenated Techniques: These combine two distinct analytical methods, often through the use of an interface or coupling device. Some examples include:

•GC-MS
•LC-NMR
•LC-IR

Triple Hyphenated Techniques: These involve the combination of three separate analytical methods, allowing for even more comprehensive analysis. Examples of triple hyphenated techniques include:

- LC-API-MS
- APCI-MS-MS
- ESI-MS-MS
- LVI-GC-MS
- LC-ESI-MS
- LC-UV-NMR-MS-ESI
- LC-MS-TSPLC-UV-NMR-MS
- LC-NMR-MS
- LC-DAD-API-MS
- LC-PDA-MS
- LC-PDA-NMR-MS
- SPE-LC-MS

These hyphenated techniques are instrumental in tackling complex analytical challenges and providing a wealth of information from diverse sources

LITERATURE REVIEW

Table 1: Literature Review Table

Sr. No.	Title	Method	Description	Ref. No.
1	An LC-MS-based workflow measures the export activity of S1P transporters	LC/MS	**Mobile phase:** **Mobile Phase A:** 20 mM ammoniumacetate, 0.1% ammonium hydroxide, and 2.5 mM methylenediphosphatein95:5water/ACN **Mobile Phase B:** Acetonitrile (ACN). **Stationary Phase:** HILIC column (Amide, 3.5 mm, 4.6 100 mm) **Flow rate:** 0.4 mL/min **Injection volume:** 5 µL	7
2	Q-TOF LC-MS compounds evaluation of propolis extract derived from Malaysian stinglessbees, Tetrigona apicalis, and their bioactivities in breast cancer cell, MCF7	Q-TOF LC-MS	**Mobile phase:** **Mobile Phase A:** 0.1 % formic acid in distilled water **Mobile Phase B:** 0.1 % formic acid in acetonitrile **Stationary Phase:** C18 column diameter of 2.1 mm, length of 150mm, particle size of 3.5 µm **Flow rate:** 0.5 mL/min **Injection volume:** 1.0 µL	8
3	Analysis of monoclonal immunoglobulins from bone marrow plasma cells using immunopurification andLC-MS	LC/MS	**Mobile phase:** **Mobile Phase A:** 100 % water +0.1 %FA **Mobile Phase B:** 90 % acetonitrile +10 % 2-propanol + 0.1 % v/v formic acid **Stationary Phase:** 1.0 × 75 mm Poroshell 300SB-C3 column with 5µm particle size **Flow rate:** 25 µL/minute **Injection volume:** 2 µL	13
4	Heterogeneous multimeric metabolite ion species observed inLC-MS based metabolomics data sets	LC/MS	**Mobile phase:** **Mobile Phase A**: 0.1% formic acid inLCMS grade water **Mobile Phase B:** ACN **Stationary Phase:** C18 column (2.1 ×150 mm, 1.8 µ m) **Flow rate:** 0.2 mL/min **Injection volume:** 5 µL	14
5	An automated online three-phase electro-extraction setup with machine-vision process monitoring hyphenated to LC-MS analysis	LC/MS analysis	**Mobile phase:** 60:40 water:acetonitrile with 0.1% aceticacid **Stationary Phase:** Phenomenex C18 LC column (50 ×2 mm, 4-µm particlesize) **Flow rate:** 0.3 mL/min	15
6	Method for detecting rare differences betweentwo LC-MS runs	LC/MS	**Mobile phase:** **Mobile Phase A:** 0.1% (v/v) formicacid	16

			plus 0.03% (v/v) trifluoracetic acid (TFA) in water **Mobile Phase B:** acetonitrile **Stationary Phase:** C18 column**Flow rate:** 0.25 mL/min **Injection volume:** 5 µ g	
7	Aquifer system and depth specific chemical patterns in fractured-rockgroundwater from the Critical Zone revealed by untargeted LC-MS- based metabolomics	LC-MS-based metabolomics	**Mobile phase:** **Mobile Phase A:** 0.1% (v/v) formicacid in water **Mobile Phase B:** 0.1% formic acid in acetonitrile **Stationary Phase:** C18 column**Flow rate:** 400 µL/ min **Injection volume:** 1 µL	17
8	The impact of different calibration matrices on the determination of insulin-like growth factor 1 by high-resolution-LC-MS in acromegalic and growth hormone deficient patients	LC/MS	**Mobile phase:** **Mobile Phase A:** 0.2 % formic acid(FA) in deionized water **Mobile Phase B:** 0.2 % FA in acetonitrile/H2O **Stationary Phase:** C18 **Flow rate:** 0.4 ml/min **Injection volume**: 10 µl	19
9	LC/MS Assessment of Glycoform Clearance ofa Biotherapeutic MAbinRabbit Ocular Tissues	LC-MS/MS	**Mobile phase:** **Mobile Phase A:** 0.1% formic acid inwater **Mobile Phase B:** 0.1% formic acid in acetonitrile **Stationary Phase:** C18 column**Flow rate:** 0.3 mL/min **Injection volume:** 10 µL	20
10	LC/MS-based lipidomicsto characterize breed- specific and tissue-specific lipid composition of chickenmeat and abdominal fat	LC/MS	**Mobile phase:** **Mobile Phase A:** acetonitrile/water (6:4, v/v) with 10 mmol/L ammoniumacetate and 0.1% formic acid **Mobile Phase B:** acetonitrile/isopropanol (1:9, v/v) with10 mmol/L ammonium acetate and 0.1% formic acid **Stationary Phase:** C30 column **Flow rate:** 350 µL/min	21
11	Application of 1 H- NMR- and LC-MS based	LC/MS	**Mobile phase:**	22
	Metabolomic analysisfor the evaluation of celery preservation methods		**Mobile Phase A:** 1 mL/L formic acidin water **Mobile Phase B:** 1 mL/L formic acidin acetonitrile **Stationary Phase:** C18 column**Flow rate:** 0.35 mL/min **Injection volume:** 5 µL	
12	Bispecific antibody characterization by a	HILIC-MS	**Mobile phase:**	23

	combination of intact and site-specific/chain-specific LC/MS techniques		**Mobile Phase A:** 50 mM ammoniumacetate in 2% acetonitrile **Mobile Phase B:** 160 mM ammoniumacetate in with 2% acetonitrile **Stationary Phase:** SCX mAb (2.1 mm × 50 mm, 3 µm) column **Flow rate:** 0.1 mL/min **Injection volume:** 1 µL (30 mg/mLsample material) was used.	
13	Authentication of chicken-derived components in collagen-containing foods using natural macromolecular marker fragments by LC-MS method	NLC-LTQ-Orbitrap analysis	**Mobile phase:** **Mobile Phase A:** 0.1% formic acid **Mobile Phase B:** 100% acetonitrileand 0.1% formic acid **Stationary Phase:** C18 column**Flow rate:** 0.3 µL/min **Injection volume:** 5 µL	25
14	LC-MS-based multi- omics analysis of brain tissue for the evaluationof the anti-ischemic stroke potential of Tribulus terrestris L. fruit extract in MCAO rats	LC and LC-MS	**Mobile phase:** **Mobile Phase A:** 0.1%aqueousformicacid **Mobile Phase B:** acetonitrile **Stationary Phase:** ACQUITY HSST3 column **Flow rate:** 0.3mL/min **Injection volume:** 1 µL	26
15	Fast and efficient digestion of adeno associated virus (AAV) capsid proteins for liquidchromatography mass spectrometry (LC-MS) based peptide mapping and post translational modification analysis (PTMs)	LC-MS	**Mobile phase:** **Mobile Phase A:** 0.1% (v/v) FA inwater **Mobile Phase B:** 0.1% (v/v) FA inacetonitrile **Stationary Phase:** C18 column **Flow rate:** 250 nL/min **Injection volume:** 1 µg	27
16	LC-MS metabolomic evidence metabolites from Oenothera rosea L´H´er. Ex Ait with antiproliferative properties on DU145 human prostate cancercell line	LC-ESI-MS/MS	**Mobile phase:** **Mobile Phase A:** 0.1% formic acid inwater **Mobile Phase B:** 0.1% formic acid in acetonitrile **Stationary Phase:** C18 column**Flow rate:** 0.4 mL/min **Injection volume:** 10 µL	28
17	Engineering an integrated system with ahigh pressure polymeric microfluidic chip coupled to liquid chromatography-mass spectrometry (LC-MS) for the analysis of abused drugs	LC/MS	**Mobile phase:** **Mobile Phase A:** water, containing0.1% formic acid **Mobile Phase B:** MeOH or ACN, containing 0.1% formic acid **Stationary Phase:** PFPP column **Flow rate:** 0.03 mL/min	29
18	Gas-permeable liquid- core waveguide coupledto LC-	LC-MS	**Mobile phase:** **Mobile Phase A:** 0.1% FA	31

	MS for studying the influence of oxygen on photodegradation processes		**Mobile Phase B:** 20 mM ammonium formate **Stationary Phase:** C18 column **Flow rate:** 120 µL/min	
19	Exploration of phytochemicals and biological functions of Kadsura coccinea pericarpium based on LC-MS and network pharmacology analysisand experimental validation	LC/MS	**Mobile phase:** **Mobile Phase A:** acetonitrile-formic acid **Mobile Phase B:** water-formic acid **Stationary Phase**: Reversed- phase Kinetex C 18 **Flow rate:** 0.3 mL/min **Injection volume:** 20 µL	33
20	In situ visual and content changes analysis of coumarins in Radix Angelicae dahuricae by LSCM combined with LC-MS technology	LC/MS	**Mobile phase:** **Mobile Phase A:** 0.1%formicacid **Mobile Phase B:** methanol **Stationary Phase:** AgilentEclipsePlus-C18 **Flow rate:** 0.3mL/min **Injection volume:** 10 µL	34
21	Mass spectrometry dataset of LC-MS lipidomics analysis of Xenopus laevis optic nerve	HPLC/MS	**Mobile phase:** **Mobile Phase A:** 50% acetonitrile, 50% water, 5mM ammonium formate,and 0.1% formic acid. **Mobile Phase B**: 88% isopropanol, 10% acetonitrile, 2% water, 5mM ammonium formate and 0.1% formicacid. **Stationary Phase:** C18 + UH- PLC Column. **Flow rate:** 260 µl/min. **Injection volume:** 5µL	35
22	Intestinal microbial 16S sequencing and LC-MS metabonomic analysis revealed differences between young and oldcats	LC/MS	**Mobile phase:** **Mobile Phase A:** water (containing0.1% formic acid) **Mobile Phase B:** acetonitrile (containing 0.1% formic acid) **Stationary Phase:** ACQUITY UPLCHSS T3 **Flow rate:** 0.35 mL/ min **Injection volume:** 2 µL	36
23	A validated workflow fordrug detection in oral fluid by non-targeted liquid chromatography-tandem mass spectrometry	LC-MS	**Mobile phase:** **Mobile Phase A**: 95% MeOH in aqueous **Mobile Phase B:** 0.5%acetic acid solution **Stationary Phase:** HALO PhenylHexyl column **Flow rate:** 15 µL/ min **Injection volume:** 5 µl	41

24	Adduct annotation in liquid chromatography/high-resolution mass spectrometry to enhance compound identification	UNIGE-LC-MS	**Mobile phase:** **Mobile Phase A:** 5 mM ammoniumformate in water, pH-3.0 bythe addition of formic acid **Mobile Phase B:** 5 mM ammonium formate in methanol. **Stationary Phase:** column HSS T3XP **Flow rate:** 300 µL/min **Injection volume:** 1.6 µL	42
25	Development of an LC-MS method for the semiquantitative determination of polyamide 6 contaminations in polyolefin recyclates	LC-MS	**Mobile phase:** **Mobile Phase A:** 5 mM ammonium formate in H2O containing 0.1% formic acid **Mobile Phase B:** acetonitrile with0.1% formic acid **Stationary Phase:** HILIC-D column **Flow rate:** 0.3 mL min−1 **Injection volume:** 2 µL	43
26	Effects of the LC mobile phase in vacuum differential mobility spectrometry-mass spectrometry for the selective analysisof antidepressant drugs in human plasma	LC-MR M/MS analysis	**Mobile phase:** **Mobile Phase A:** 5 mM acetic acid inwater **Mobile Phase B:** methanol **Stationary Phase:** C18 column **Flow rate:** 1.0 mL/min **Injection volume:** 10 µL	44
27	Simple screening procedure for 72 synthetic cannabinoids in whole blood by liquid chromatography–tandem mass spectrometry	Chromat ographic and spect rometric conditions	**Mobile phase:** **Mobile Phase A:** 0.1% formic acid in acetonitrile **Mobile Phase B:** 0.1% formic acid in water **Stationary Phase:** Kinetex C18 column **Flow rate:** 0.5 mL/min	47
28	Green adherent degradation kinetics study of Nirmatrelvir, an oral anti-COVID-19:characterization of degradation products using LC–MS with insilico toxicity profile	HPLC–DAD	**Mobile phase:** **Mobile Phase A:** 50 mM ammonium acetate **Mobile Phase B:** ACN **Stationary Phase:** Agilent Zorbax Eclipse-C18 analytical column **Flow rate:** 1 mL/ min **Injection volume:** 20-µL	48
29	Quantification of amino acids and peptides in anionic liquid based aqueous two-phase system by LC–MS analysis	LC/MS	**Mobile phase:** **Mobile Phase A:** methanol with 0.1% formic acid **Mobile Phase B:** water with 0.1% formic acid **Stationary Phase:** AAA-MS HPLCcolumn	49

			Flow rate: 0.15 mL/min **Injection volume:** 2 µL	
30	Effect of media and fermentation conditions on surfactinand iturin homologues produced by Bacillus natto NT-6: LC–MS analysis	LC/MS	**Mobile phase:** **Mobile Phase A:** ammonium acetate solution containing 0.1% (v/v) formic acid **Mobile Phase B:** ammonium acetate solution containing0.1% (v/v) acetonitrile **Stationary Phase:** Venusil XBP CNcolumn **Flow rate:** 8.0 µL/min **Injection volume:** 10 µL	50

LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY(LC-MS) TECHNIQUE.

Definition: - [9,10,12,16]

LC-MS is a powerful analytical technique utilized in chemistry and biochemistry to separate, identify, and quantify chemical compounds within a given sample. This technique integrates two distinct methodologies: liquid chromatography (LC) and mass spectrometry (MS). Liquid Chromatography (LC) is a separation technique that employs a liquid mobile phase, typically a solvent, to transport the sample through a stationary phase, often a column filled with a solid or gel material. Mass Spectrometry (MS) is a method used to determine the mass-to-charge ratio of ions in a given sample. It offers insights into the molecular weight, chemical structure, and abundance of diverse compounds. In LC-MS, the eluent, which is the liquid derived from the LC,is introduced into the mass spectrometer. Within the mass spectrometer, the separated compounds are ionized and subsequently analysed based on their mass-to-charge ratio.LC-MS finds extensiveapplication in diverse fields such as pharmaceuticals, environmental analysis, proteomics, and metabolomics. It possesses the capability to identify and quantify a wide range of compounds within complex mixtures, offering high sensitivity and specificity. Consequently, it serves as a valuable tool in both research and industry across a wide array of applications. Metabolomics, through the analysis of bodily fluids and tissues, can detect a wide range of endogenous metabolites, including organic acids, amino acids, fatty acids, sugars, and cholesterol. This approach is often complemented by advanced analytical techniques like gas chromatography-mass spectroscopy (GC-MS) and liquid chromatography-mass spectroscopy (LC-MS), along withhigh-throughput bioinformatics tools. Metabolomics has found extensive application in the research of male infertility. However, most metabolomics studies related to male infertility predominantly rely on human serum, plasma, and semen samples. A more comprehensive investigation would benefit from suitable pathological tissue models. The process of biomarker discovery aided by liquid chromatography – mass spectrometry (LC-MS) is intricate and extensive, necessitating interdisciplinary collaboration and the transfer of knowledge among clinicians, analysts, data scientists, and other stakeholders, including patients. A meticulous planning approach divides this process into five interconnected phases, where quality control (QC)measures play a crucial role in overseeing the other phases, ensuring confidence in results and reproducibility. These phases encompass clinical trial activities, including sample collection and pre-processing, sample preparation (such as extraction), LC-MS analyses, data processing, and subsequent evaluation. The utilization of LC-MS-based analysis for studying metabolites, encompassing lipids and lipid mediators, has become a pivotal element in biomarker research. Thesignificance of lipidomic and metabolomics in such investigations is underpinned by the intricatebiochemistry of endogenous compounds, which reflects highly individual variations in health and disease states. For instance, lipid biomarkers provide valuable insights into the intricate metabolicprocesses associated with dyslipidemia, cancer, and immune-mediated inflammatory disorders. Tosupport the broader integration of lipidomics and metabolomics in LC-MS-based clinical research,it is imperative to establish standardized methods, including pre-analytical sample handling, to ensure robust analysis of these metabolites, including lipids and lipid mediators.

Working of LC-MS: - [60]

Various system configurations are accessible for liquid chromatography, and the most efficient separations are conducted using ultra-high-performance liquid chromatography (UHPLC) instruments. This technology was initially introduced in 2004 and was known as ultra-performanceliquid chromatography (UPLC). In essence, a multi-component mixture that is soluble in the liquidmobile phase is separated based on the distinct partitioning of individual components between themobile phase (as depicted in Figure 7 - (1)) and the stationary phase (the column - Figure 7 - (3)).

The mobile phase, which typically consists of a solvent, serves to carry the sample through the system and is facilitated by a high-pressure pump (as illustrated in Figure 7 - (1)). Significantly, the mobile phase also plays a pivotal role in the separation process.

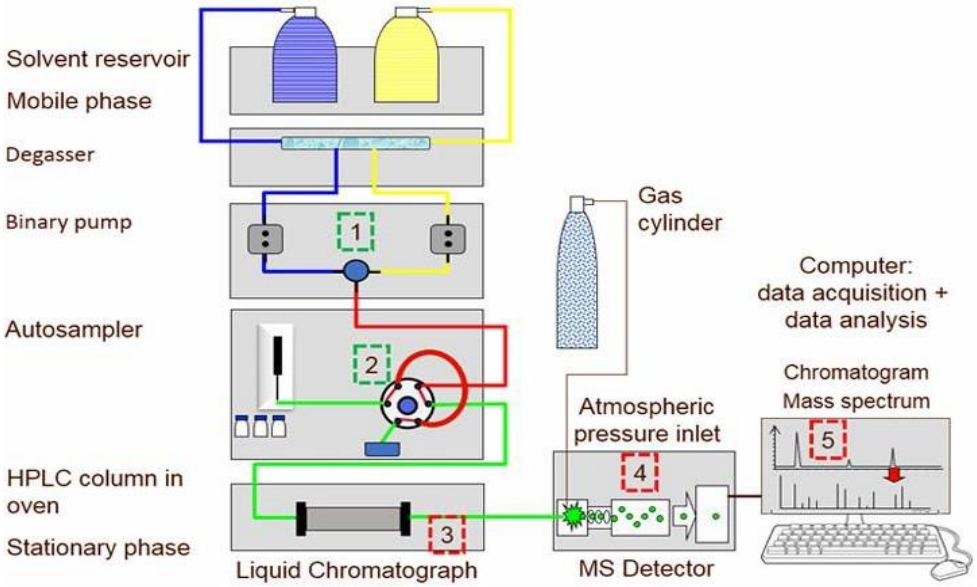

Figure 7. Presents a simplified diagram of a liquid chromatograph coupled with a massspectrometer (LC-MS).

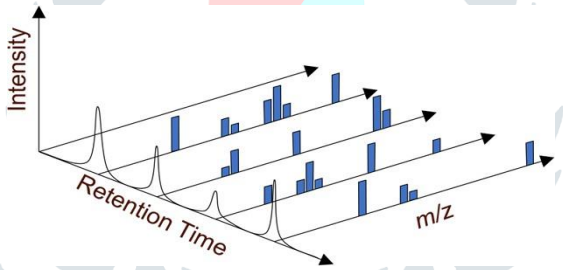

Figure 8. Illustrates a comprehensive scan of LC MS.

Instrumentation: - [15]

An automated three-phase electro-extraction (EE) method coupled with machine vision was developed and integrated with a robotic autosampler for LC-MS analysis. The system wasoptimized and evaluated using eight model compounds, namely amitriptyline, clemastine, clomipramine, haloperidol, loperamide, propranolol, oxeladin, and verapamil.This innovative approach allows for the efficient and precise extraction of target compounds, making it a valuabletool for sample preparation and analysis in LC-MS studies.

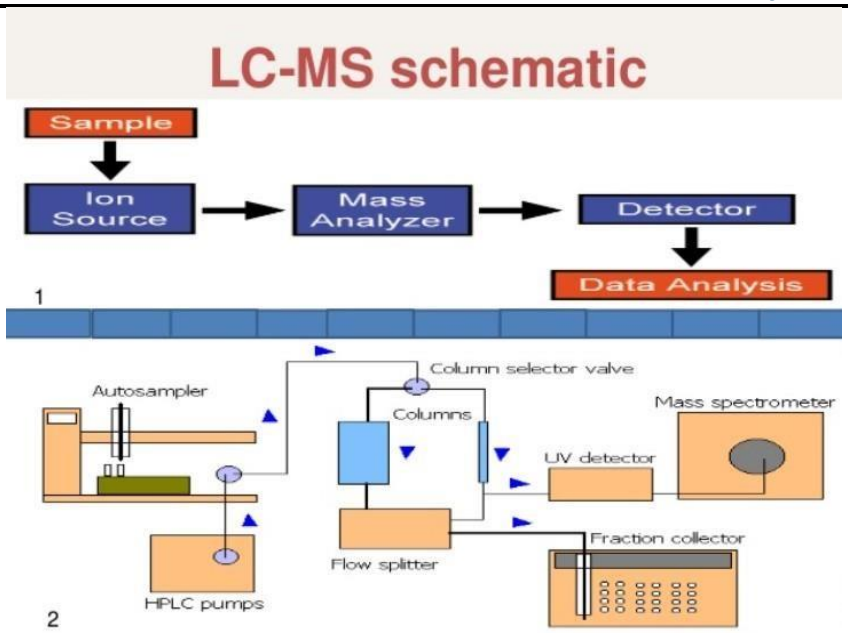

Figure 9. LC-MS schematic .

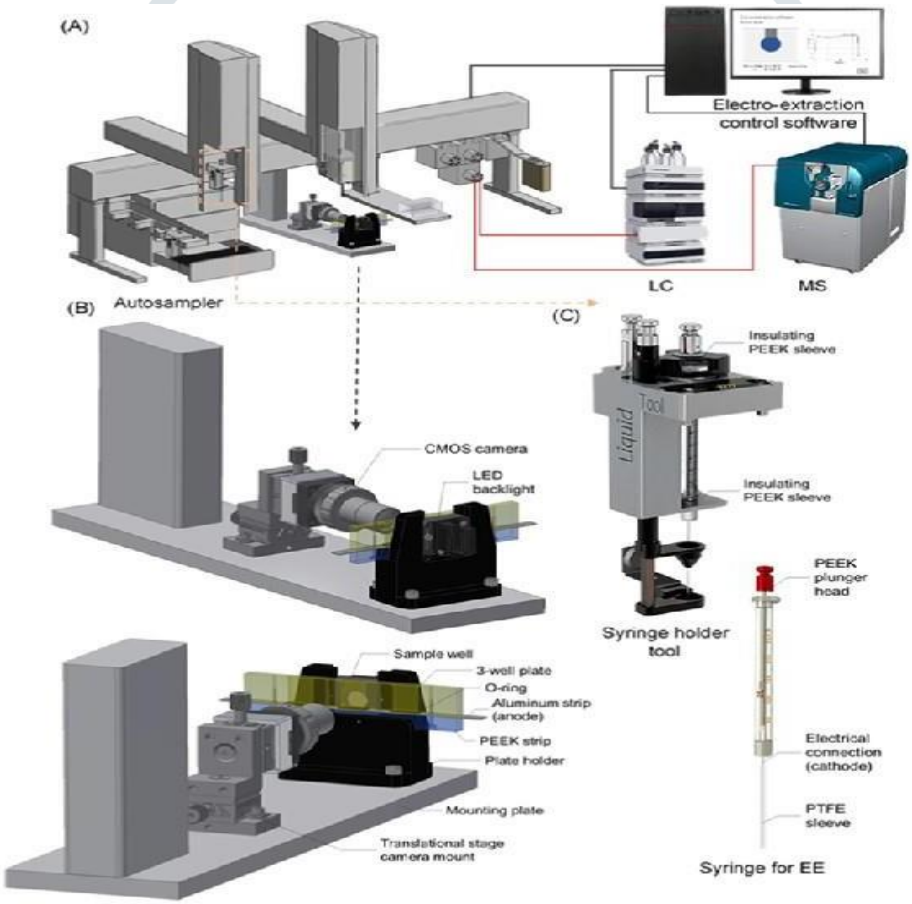

Figure 10. This figure illustrates the schematic diagram of the electro-extraction module integrated.

Metabolomics is a valuable approach for investigating male infertility by analyzing bodily fluids and tissues to detect a wide variety of endogenous metabolites, including organic acids, amino acids, fatty acids, sugars, and cholesterol. This technique is often complemented by advanced analytical methods like gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS), in combination with high-throughput bioinformatics tools. Consequently, metabolomics has found extensive applications in the field ofmale infertility research.

Method Optimisation: - [15]

Creating an LC-MS assay involves careful consideration and optimization of numerous conditionsand parameters. The specific conditions can vary widely depending on the nature of the analyte and the LC separation, making it challenging to provide a one-size-fits-all approach. Each analytetypically demands

individualized optimization. Although published methods serve as valuable starting points, the performance and ideal conditions may significantly differ between various instruments and sample matrices. Sensitivity plays a crucial role and relies heavily on the specific instrument and assay conditions in use. Mass spectrometer manufacturers are continually enhancing the sensitivity of their instruments and offer various models with different sensitivity levels. As a result, it is essential toevaluate whether the chosen instrument can deliver the required limits of detection for the intendedpurpose.

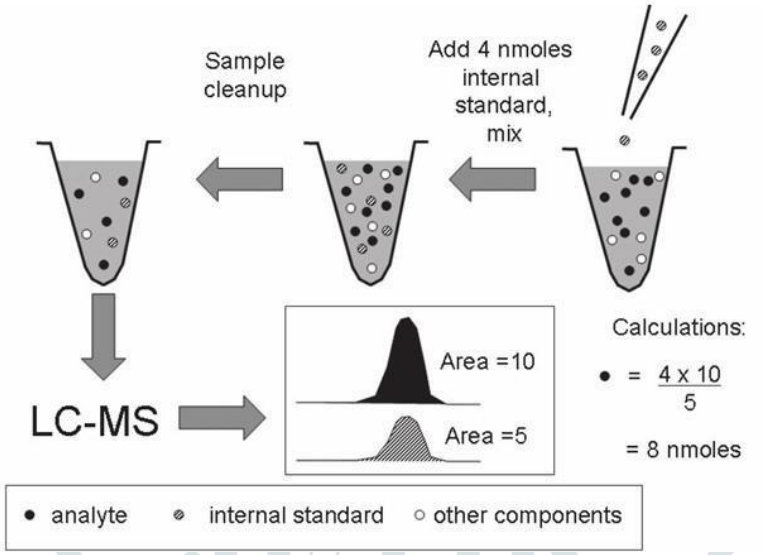

Figure 11. Method optimisation of LC-MS.

In the process known as stable isotope dilution, the assay commences with the addition of four nanomoles of an internal standard, establishing a consistent internal standard to analyte ratio, typically set at 1:2. Although there may be losses of both the analyte and the internal standard in the subsequent processing steps, the ratio between them remains unchanged. This analytical technique is often performed using liquid chromatography-mass spectrometry (LC-MS)

Uses of LC-MS Analyticle method: - [11,13,14,17,18,19,22,23,32,38]

The composition of the sample matrix, which is significantly influenced by the choice of blood collection tube used in phlebotomy, plays a crucial role in laboratory testing, as it has the potentialto impact the outcomes of various tests.designed an LC-MRM-MS assay for the molecular characterization of antithrombin in citrate plasma.The data presented in this study reveal that employing LC-MS for the analysis of monoclonal immunoglobulins, often referred to as miRAMM, provides supplementary phenotypic information at the cellular level. This approach complements other commonly used techniques like flow cytometry and histopathology. Distinguishing between covalent and non-covalent heterogeneous multimerization of molecules found in extracts from biological samples analyzed through LC-MS poses significant challenges in terms of identification and annotation. Consequently, the extent to which multimerization occursremains largely uncertain. untargeted metabolomic approach using LC-MS to establish connections between the metabolomes found in groundwater samples collected from monitoring wells situated within fractured carbonate-siliciclastic alternations along a hillslope at the Hainich Critical Zone Exploratory (CZE) in Thuringia, Germany. The use of mass spectrometry-based methods for urinary steroid profiling has previously shown its significance in aiding the differentialdiagnosis of adrenal tumors. Calibration stands as a crucial factor contributing to the variability observed in liquid chromatography-mass spectrometry (LC-MS) techniques used for insulin-like growth factor 1 (IGF-1). This variability is addressed during the validation process of the LC-MS method. The integration of nuclear magnetic resonance (NMR) and liquid chromatography-mass spectrometry (LC-MS) is well-established as a valuable approach for enhancing metabolome coverage by characterizing complementary sets of metabolites. From an analytical perspective, ensuring the proper chain association is one of the most crucial challenges to oversee during the development and production of bispecific antibodies (bsAbs). In the case of the bispecificantibody, Emici zumab, a LC/MS workflow was employed for its intact and site-specific characterization. This involved the combination of various chromatographic methods, including IEX, SEC, RPLC, and HILIC, in conjunction with high-resolution mass spectrometry. To gain a deeper comprehension of the characteristics of Host Cell Proteins (HCPs) and enhance their removal, liquid chromatography-mass spectrometry (LC-MS) has emerged as an alternative method for HCP analysis. This technique can both identify and quantify individual HCPs effectively. The existing liquid chromatography-mass spectrometry (LC-MS) methodology enables the

precise identification and quantification of 106 molecules by employing the multiple-reaction monitoring (MRM) acquisition mode.

Application of LC-MS: - [24,25,26,29,37,39,45]

1. liquid chromatography-mass spectrometry (LC-MS) is well-recognized as the industry standard for analyzing non-volatile small organic molecules. This versatile method can alsobe effectively employed in 2D-LC applications, where the simultaneous acquisition of datafrom both dimensions is essential. This can be seamlessly accomplished by incorporating a selection valve into the MHC interface, allowing for enhanced analytical capabilities in complex sample analysis.
2. In the food industry, the widespread use of collagen, a prominent biopolymer found in animals, has been leveraged to enhance the nutritional and health benefits of various foodproducts. A critical aspect of quality control in this context involves the application of liquid chromatography-mass spectrometry (LC-MS) for the precise detection of chicken- derived components within foods containing collagen. This analytical approach plays a vital role in ensuring product integrity and label transparency for consumers.
3. Liquid chromatography-mass spectrometry (LC-MS) was employed to examine the chemical composition of TT15. To identify compounds, we conducted searches in our in-house database. We applied a multi-omics approach using LC-MS to investigate differences in metabolites and proteins within rat brain tissue. Our objective was to uncoverpotential biomarkers and unravel the molecular mechanisms underlying the effects of TT15against middle cerebral artery occlusion (MCAO).
4. The application of this technology opens up new avenues for researchers and professionalsin the field of LC-MS analysis. It offers a reusable and robust solution for the separation and analysis of complex biological samples, making it a valuable tool for various applications, such as biomarker discovery, pharmaceutical research, and clinical diagnostics.
5. The potential applications of this assay are far-reaching and invaluable in the study of various target classes, especially those where conventional protein-based binding assays encounter significant challenges. By circumventing the limitations of traditional methods,our label-free LC-MS cell binding assay provides a versatile tool for researchers and pharmaceutical professionals. It empowers them to explore ligand interactions within livecells, yielding crucial insights into the pharmacological characteristics of compounds.
6. The application of LC–MS technology allowed for the identification of compounds acrossvarious categories of perfume components, including essential oils, fixatives, and dyes.

FUTURE PROSPECTS OF LC-MS

The future prospects of LC-MS (Liquid Chromatography-Mass Spectrometry) hyphenated technology are quite promising, with ongoing advancements and potential developments in variousareas:

1. Advanced Data Analysis: As data complexity increases, there's a growing emphasis on developing advanced data analysis techniques, including machine learning and artificial intelligence, to better interpret the vast amount of data generated by LC-MS experiments.
2. Single-Cell Analysis: LC-MS is being adapted for single-cell analysis, offering a deeper understanding of cellular heterogeneity and the ability to detect minute changes in cell composition.
3. Multi-Omics Integration: Integrating LC-MS data with genomics, transcriptomics, and proteomics information will provide a holistic view of biological systems, which is crucialin systems biology and understanding complex diseases.
4. Environmental Monitoring and Food Safety: LC-MS will play an increasingly significant role in monitoring environmental pollutants, food contaminants, and quality control.
5. Personalized Medicine and Clinical Diagnostics: The use of LC-MS in personalized medicine will continue to expand, enabling healthcare providers to make treatment decisions tailored to an individual's unique biochemistry and genetics.
6. Emerging Applications: LC-MS technology will continue to expand into new areas, including neuroscience, immunology, and epigenetics, as researchers discover novel applications for this powerful analytical tool.
7. Enhanced Sensitivity and Speed: A primary objective in LC-MS technology is to boost both sensitivity and speed. This will enable the detection and quantification of even lower-abundance compounds in shorter timeframes, particularly benefiting fields such as clinicaldiagnostics and environmental analysis.
8. Automation and High-Throughput Analysis: Integrating LC-MS with automation and robotics will enable high-throughput analysis, making it possible to screen a large numberof samples in a relatively short time. This is particularly important in pharmaceuticals, proteomics, and metabolomics research.
9. Integration with Other Analytical Techniques: Combining LC-MS with other analytical methods like

NMR and X-ray crystallography will provide complementary information, yielding a more comprehensive understanding of complexsamples.

10. Miniaturization and Portability: Researchers are striving to create more compact and portable LC-MS systems. This has significant implications for on-site testing and field analysis, especially in environmental monitoring and point-of-care diagnostics.

CONCLUSION

The hyphenated technique LC-MS represents a pivotal advancement in analytical chemistry, driving innovation and breakthroughs in numerous fields. Its ability to providecomprehensive information about the composition of samples has solidified its place as anindispensable tool for scientists and researchers striving to unravel the complexities of modern analytical challenges. This abstract serves as a gateway to further exploration of LC-MS and its manifold applications, making it a crucial reference for those seeking to harness the full potential of this powerful analytical technique. This technique has proveninvaluable in pharmaceutical research, enabling the identification and quantification of drug candidates and their metabolites with high sensitivity and specificity. It has empowered environmental scientists to detect trace levels of contaminants and monitor their impact on ecosystems. In proteomics and metabolomics, LC-MS is indispensable forthe characterization of biomolecules, allowing scientists to unlock the secrets of complex biological systems.

REFERENCE

1 S. and Mishra P., 2020 “A review on analytical methods of dapagliflozin: an update.”International Journal of Pharmaceutical Quality Assurance, 11. 355-360.

2 Sasipreetam D., et al. 2021 “Review on hyphenated techniques and their applications.” Journal of Cardiovascular Disease Research, 12.

3 Nagajyothi S., et al. 2017 “Hyphenated Techniques- A Comprehensive Review.” International Journal of Advance Research and Development, 2.

4 Matsui T., et al. 2023 “Assessment of inconsistencies in the solvent-accessible surfaces of proteins between crystal structures and solution structures observed by LC-MS.” Biochemical and Biophysical Research Communications 640. 97-104.

5 Muhamad W.S., et al. 2022 “Q-TOF LC-MS compounds evaluation of propolis extract derived from Malaysian stingless bees, Tetrigona apicalis, and their bioactivities in breast cancer cell, MCF7.” Saudi Journal of Biological Sciences 29. 103-403.

6 Wu X., et al. 2023 “An LC-MS-based workflow measures the export activity of S1P transporters.” Biochemical and Biophysical Research Communications 668. 118-124.

7 Karan Wadhwa and A. C. Rana. 2021 “A review on liquid chromatographic methods for the bioanalysis of atorvastatin.” Wadhwa and Rana Future Journal of Pharmaceutical Sciences .

8 Qi R., et al. 2023 “LC-MS-based untargeted metabolomics reveals the mechanism underlying prostate damage in a type 2 diabetes mouse model.” Reproductive Biology 23.

9 Rischkea S., et al. 2023 “Small molecule biomarker discovery: Proposed workflow for LC-MS- based clinical research projects.” Journal of Mass Spectrometry and Advances in the Clinical Lab 28.47–55.

10 Kruijt M., et al. 2023 “Effects of sample matrix in the measurement of antithrombin by LC-MS: A role for immunocapture.” Journal of Mass Spectrometry and Advances in the Clinical Lab 27. 61–65.

11 Sens A., et al. 2023 “Pre-analytical sample handling standardization for reliable measurement of metabolites and lipids in LC-MS-based clinical research.” Journal of Mass Spectrometry and Advances in the Clinical Lab 28. 35–46.

12 Barnidge D.R., et al. 2023 “Analysis of monoclonal immunoglobulins from bone marrow plasma cells using immunopurification and LC-MS.” Journal of Mass Spectrometry and Advances in the Clinical Lab 28. 133–141.

13 El Abiead Y., et al. 2022 “Heterogeneous multimeric metabolite ion species observed in LC-MS based metabolomics data sets.” Analytica Chimica Acta 1229. 340-352.

14 He Y., et al. 2022. “An automated online three-phase electro-extraction setup with machine- vision process monitoring hyphenated to LC-MS analysis.” Analytica Chimica Acta 1235 340-521.

15 Zhang Z., et al. 2023 “Method for detecting rare differences between two LC-MS runs.”Analytical Biochemistry 674. 115-211.

16 Sanchez-Arcos C., et al. 2022 “Aquifer system and depth specific chemical patterns in fractured- rock groundwater from the Critical Zone revealed by untargeted LC-MS-based metabolomics.” Water Research 219. 118-566.

17 Vogg N., et al. 2023 “Simplified urinary steroid profiling by LC-MS as diagnostic tool for malignancy in adrenocortical tumors.” Clinica Chimica Acta 543. 117-301.

18 Simsticha S., et al. 2023 “The impact of different calibration matrices on the determination of insulin-like growth factor 1 by high-resolution-LC-MS in acromegalic and growth hormone deficient patients.” Clinical Biochemistry 114. 95–102.

19 Dong S., et al. 2023 “LC/MS Assessment of Glycoform Clearance of a Biotherapeutic MAb in Rabbit Ocular Tissues.” Journal of Pharmaceutical Sciences 112. 2285−2291.

20 Li J., et al. 2022 “LC/MS-based lipidomics to characterize breed-specific and tissue-specific lipid composition of chicken meat and abdominal fat.” LWT - Food Science and Technology 163. 113-611.

21 Lau H., et al. 2022 “Application of 1H-NMR- and LC-MS based Metabolomic analysis for the evaluation of celery preservation methods.” LWT - Food Science and Technology 169. 113-938.

22 Duivelshofa B. L., et al. 2022 “Bispecific antibody characterization by a combination of intact and site-specific/chain-specific LC/MS techniques.” Talanta 236. 122-836.

23 Can˜o-Carrillo I., et al. 2023 “Simultaneous analysis of highly polar and multi-residue-type pesticides by heart-cutting 2D-LC-MS.” Talanta 266. 124-918.

24 Deng G., et al. 2023 “Authentication of chicken-derived components in collagen-containing foods using natural macromolecular marker fragments by LC-MS method.” Polymer Testing 120. 107-950.

25 Xu D., et al. 2022 “LC-MS-based multi-omics analysis of brain tissue for the evaluation of the anti-ischemic stroke potential of Tribulus terrestris L. fruit extract in MCAO rats.” Arabian Journal of Chemistry. 15, 104-297.

26 Guapoa F., et al. 2021 “Fast and efficient digestion of adeno associated virus (AAV) capsid proteins for liquid chromatography mass spectrometry (LC-MS) based peptide mapping and post translational modification analysis (PTMs).” Journal of Pharmaceutical and Biomedical Analysis 207. 114-427.

27 Marquez-Floresa Y.K., et al. 2023 “LC-MS metabolomic evidence metabolites from Oenothera rosea L´H´er. Ex Ait with antiproliferative properties on DU145 human prostate cancer cell line.” Biomedicine & Pharmacotherapy 165. 115-193.

28 Chen P. C., et al. 2022 “Engineering an integrated system with a high pressure polymeric microfluidic chip coupled to liquid chromatography-mass spectrometry (LC-MS) for the analysis of abused drugs.” Sensors & Actuators: B. Chemical 350. 130-888.

29 Fu¨ssl F., et al. 2021 “Native LC–MS for capturing quality attributes of biopharmaceuticals on the intact protein level.” Current Opinion in Biotechnology.

30 Groenevelda I., et al. 2023 “Gas-permeable liquid-core waveguide coupled to LC-MS for studying the influence of oxygen on photodegradation processes.” Journal of Photochemistry & Photobiology, A: Chemistry 441. 114-685.

31 Ji Q., et al. 2023 “A highly sensitive and robust LC-MS platform for host cell protein characterization in biotherapeutics.” Biologicals 82 2023. 101-675.

32 Jin Z.-L., et al. “Exploration of phytochemicals and biological functions of Kadsura coccinea pericarpium based on LC-MS and network pharmacology analysis and experimental validation.” Journal of Functional Foods 103. 105-493.

33 Gao H., et al. 2023 “In situ visual and content changes analysis of coumarins in Radix Angelicae dahuricae by LSCM combined with LC-MS technology.” Arabian Journal of Chemistry. 104-495.

34 Neag E., et al. 2023 “Mass spectrometry dataset of LC-MS lipidomics analysis of Xenopus laevis optic nerve.” Data in Brief 49. 109-313.

35 Tian T., et al. 2023 “Intestinal microbial 16S sequencing and LC-MS metabonomic analysis revealed differences between young and old cats.” Heliyon 9. 164-187.

36 Chen M., et al. 2022 “Label-free LC-MS based assay to characterize small molecule compound binding to cells.” SLAS Discovery 27. 405–412.

37 Turtoi E., et al. 2023 “Analysis of polar primary metabolites in biological samples using targeted metabolomics and LC-MS.” STAR Protocols 4 102400.

38 Jacob E. Wulff and Matthew W. Mitchell. 2018 “A Comparison of Various Normalization Methods for LC/MS Metabolomics Data.” Advances in Bioscience and Biotechnology,. 9, 339-351.

39 Yutaka Okada and Masako Tsuchida. 2019 “Detection of Oxidized Ferrocenes by LC-MS with Electrospray Ionization Using Picric Acid as the Counter Ion.” International Journal of Analytical Mass Spectrometry and Chromatography,. 7, 1-8.

40 Reinstadler V., et al. 2019 “A validated workflow for drug detection in oral fluid by non-targeted liquid chromatography-tandem mass spectrometry.” Analytical and Bioanalytical Chemistry. 411:867–876.

41 Stricker T., et al. 2021 "Adduct annotation in liquid chromatography/high-resolution mass spectrometry to enhance compound identification." Analytical and Bioanalytical Chemistry. 413:503–517.

42 Schweighuber A., et al. 2021 "Development of an LC-MS method for the semiquantitative determination of polyamide 6 contaminations in polyolefin recyclates." Analytical and Bioanalytical Chemistry. 413:1091–1098.

43 Girard M.F.C., et al. 2022 "Effects of the LC mobile phase in vacuum differential mobility spectrometry‐mass spectrometry for the selective analysis of antidepressant drugs in human plasma." Analytical and Bioanalytical Chemistry. 414:7243- 7252.

44 Dagmara Kempińska‐Kupczyk and Agata Kot‐Wasik. 2019 "The potential of LC–MS technique in direct analysis of perfume content." Monatshefte für Chemie - Chemical Monthly. 150:1617–1623.

45 Nico Symma, and Andreas Hensel. 2022 "Advanced analysis of oligomeric proanthocyanidins: latest approaches in liquid chromatography and mass-spectrometry based analysis." Phytochem Rev. 21:809–833.

46 Katarzyna Ambroziak and Piotr Adamowicz. 2018 "Simple screening procedure for 72 synthetic cannabinoids in whole blood by liquid chromatography–tandem mass spectrometry." Forensic Toxicology. 36:280–290.

47 Sara I. Aboras and Hadir M. Maher. 2023 "Green adherent degradation kinetics study of Nirmatrelvir, an oral anti-COVID-19: characterization of degradation products using LC– MS with insilico toxicity profile." Aboras and Maher BMC Chemistry.

48 Oppermann S., et al. 2018 "Quantification of amino acids and peptides in an ionic liquid based aqueous two-phase system by LC–MS analysis." AMB Expr.

49 Sun D., et al. 2019 "Effect of media and fermentation conditions on surfactin and iturin homologues produced by Bacillus natto NT-6: LC–MS analysis." AMB Expr. 9:120

50 Kumazawa Y., et al. 2018 "A novel LC–MS method using collagen marker peptides for species identification of glue applicable to samples with multiple animal origins." Herit Sci. 6:43.