VOLUME - 10, ISSUE - 08, AUGUST- 2021 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Pharmaceutical

LC-MS TECHNIQUE & ITS METABOLOMIC BASED APPROACHES IN SCIENCE AND TECHNOLOGY

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ABSTRACT The metabolome is the complete set of metabolites found during a biological cell, tissue, organ or organism, representing the end product of cellular processes. Metabolomics is rising platform that enables one to research global endogenous metabolites with low molecular weight in biological systems. On the other hand, an acceptance of various analytical technique is predicated on procedures with successfully carried out for completely different types of metabolites on which Liquid chromatography (LC) in combination with tandem mass spectrometry (MS/MS) has enjoyed a growing quality. LC-MS is the best platform for metabolomic studies because of its glorious sensitivity, resolution, high throughput, soft ionization, and good coverage of metabolites. Also, an ability that can accurately and precisely discriminate target analytes from high complexity mixtures in a sensitive and selective way. In this review article we have discussed an omics and its different types, mainly focused on metabolomics and various application of metabolomics using LC-MS analytical technique. Here we collected all information regarding applications of metabolomics known by LC-MS. Highlight necessary pit falls in the process of metabolomics using LC-MS techniques and address multiple advantages of implementing LC-MS technique over another analytical techniques for metabolomics during a varied approaches.

KEYWORDS : Omics, Metabolomics, Targeted and untargeted metabolomics, Liquid chromatography-mass spectroscopy (LC-MS).

INTRODUCTION:

The "omics" sciences talk over with a bunch of analytical methodologies that aim to attain the collective characterization and quantification of pools of biological molecules, like genes, transcripts, proteins and metabolites, that translate into the structure, function and dynamics of cells, tissues or organisms [1]. Genomics is the part of biological science which deals with the study of whole genomes of organisms specializing within the structure, function, evolution, mapping, and written communication of genomes. A genome is the complete set of DNA, together with the sum all of its genes [2]. Transcriptomic are the techniques accustomed study of an organism's transcriptome, of all of its RNA transcripts [3]. Proteomics is the unit which involved the large-scale study of proteins. The proteome macromolecule is that the whole set of proteins that is created or modified by an organism or system. Proteomics science has enabled the identification of ever-increasing numbers of proteins molecule [4]. Metabolomics is that the scientific study of chemical processes involving metabolites, the tiny molecule substrates, intermediates and products of metabolism. The metabolome represents the entire set of metabolites in an exceedingly biological cell, tissue, organ or organism, that unit is the end product of cellular processes [5].

Recently, translational studies with comprehensive omics analyses are performing for clinical application [6-8]. The favourite technology for global metabolic profiling (metabolomics) are called hyphenated MS platforms, like gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) or capillary electrophoresis-mass spectrometry (CE-MS) [9] and detection of possible omics on the basis of their mass-to-charge ratio (m/z). Every of those techniques has been applied to various kinds of metabolites, and each technique has tailored advantages for specific types of metabolites [10]. Specifically, the MS-based analysis of global metabolomics (G-Met), which quantifies whole sets of metabolites in biological specimens without targeting specific molecules, has proven useful in the search for novel biomarkers [11-14]. In this review we discuss the metabolomics and the various approaches of LC-MS based metabolomics has advantageous in detection and investigation of metabolic profile. Improvements in instrument sensitivity and resolution, as well as ever-growing databases for the structural identification of metabolites, mean that larger metabolome coverage will currently be achieved [15].

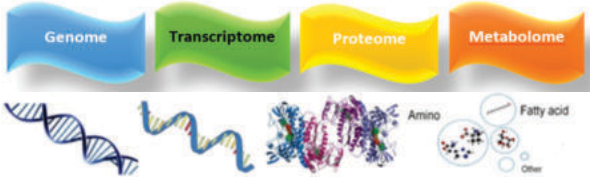

Fig. No.1. The omics cascade

There are different kind of omics which are represented as below-

Table No.1. Types of Omics

OMICS	
Genomics	Cognitive genomics; Comparative genomics; Functional genomics; Metagenomics; Neurogenomics; Personal genomics
Epigenomics	Epigenomics; Nucleomics
Proteomics	Immunoproteomics; Nutriproteomics; Proteogenomics; Structural genomics
Bioinformatics	Glycomics; Lipidomics; Foodomics; Transcriptomics; Culture; Ionimics; Phenomics
Metabolism	Metabolomics; Metabonomics
Nutrition, pharmacology, toxicology	Nutritional genomics; Nutrigenetics; Nutrigenomics; Pharmacogenomics; Pharmacomicrobiomics; Toxicogenomics
Miscellaneous	Mitointeractome; Psychogenomics; Stem cell genomics; Connectomics; Microbiomics; Cellomics; Tomomics; Ethomics; Videomics (or vide-omics); Multiomics:

Metabolomics (or metabonomics) is the analytical technique

which facilitate within the study of the metabolic profile of tiny molecules in an exceedingly biological organism including body fluids (urine, blood, cerebrospinal fluid, saliva, among others), tissues (heart, liver, kidney, brain, among others) and exhaled breath. Metabolomic analytical technologies can determine molecules with a comparatively low molecular weight (<1500 Da), including carbohydrates, lipids, amino acids, biogenic amines, organic acids vitamins, polyphenols, intermediates of the many biochemical pathways. They can influence by genetic and epigenetic effects, environmental and dietary exposures as well as lifestyle [16]. The metabolome, in turn, is defined because the complete set of small molecular metabolites [17] present in an exceedingly cell, tissue, organ or organism and represents all of the end product of cellular processes [18-19].

Metabolomics provides a purposeful view of an associate degree organism, as determined by the sum of its genes, RNA, proteins, and environmental factors including, as an example, nutrition, medications and treatments. Metabolomics aims to assess metabolic changes in an exceedingly comprehensive and global manner so as to infer biological functions and supply the detailed careful biochemical responses of cellular systems [20]. Metabolomics approaches have applied to clinical, pharmaceutical and toxicological applications[21] also has the potential to deliver diagnostic biomarkers for the detection and prognosis of diseases[22] and the prediction of the efficacy and safety of pharmaceutical interventions and recently has also involved in the study of the food and nutrition domains through Food omics approaches[23].

Metabolomics analysis technique is often categorized as two complementary methods: "untargeted-discovery-global" and "targeted-validation-tandem" supported on the target of the study. So as to systematically determine and quantify metabolites from a biological sample and achieve comprehensive characterization of biomarker targets, the analysis considers both endometabolome and exometabolome. Untargeted discovery metabolomics incorporates a hypothesis-generating manner[24] and permits for full scanning of the metabolome, pattern identification, and "metabolic fingerprinting" for the global classification of phenotypes with interacting pathway interactions[25].The targeted metabolomics approach focuses on identifying and quantifying selected metabolites (or metabolite classes), like substrates of an enzyme, direct product of protein, a particular category of compound or members of a selected pathway. In the targeted approach, the chemical properties of the investigated compounds are known, and sample preparation can be tailored to overcome matrix effects and interference from attendant compounds. Targeted metabolomics is hypothesis testing and generally performed for validation of an untargeted analysis. Untargeted approaches are especially useful for finding novel mechanisms or biomarkers, whereas targeted approaches are great tools for follow-up pathway analyses because of a higher degree of sensitivity and easy identification of compounds [26]. Untargeted and targeted approaches should be performed consecutively in order to achieve an accurate identification and absolute quantitation of the metabolites [27].

Table No.2. Types of Metabolomics

Untargeted Metabolomics	Targeted Metabolomics
• Discovery • Hypothesis generating • Global metabolomics profiling-comprehensive scanning • Metabolomics fingerprinting • Metabolomics foot printing • Classification/forming metabotypes • Qualitative identification • Relative quantification • >1000s metabolites measured • No chemical commercial standard required	• Validation • Hypothesis driven • Absolute quantification of specific features • Validation of identified feature (Requires commercially available chemical standard for validation) • Metabonomics • ~20 metabolites measured

The development of a valid holistic LC– MS method is one of the most critical steps in a metabolomics project, because not only the detection of as many metabolites as possible is required, but also high instrumental stability and repeatability throughout analysis, so as to facilitate chromatographic alignment, the correct definition of metabolic space and marker discovery with as few false negatives/positives as possible[28]. Most MS applications in metabolomics use a separation method before mass detection, typically LC, gas chromatography (GC) or capillary electrophoresis (CE). GC-MS and LC-MS are widely used techniques and can detect a large form of compounds. However, the configuration of MS instruments for those two methods is distinct because of the ionization procedures used; GC-MS instruments make use of the hard-ionization method, electron-impact (EI) ionization, whereas LC-MS principally uses soft-ionization sources (e.g., atmospheric pressure ionization (API) (e.g., electrospray ionization (ESI)) and atmospheric pressure chemical ionization (APCI). LC is probably the foremost versatile separation method, as it compatible with most standard solvents, avoid difficult derivatization steps[29], additionally it permits separation of compounds of a large range of polarity with little effort in sample preparation (compared to GC-MS). Using reverse-phase columns, semi-polar compounds (phenolic acids, flavonoids, glycosylated steroids, alkaloids and other glycosylated species) can be separated, and, using hydrophilic columns, polar compounds can be measured (sugars, amino sugars, amino acids, vitamins, carboxylic acids and nucleotides) [30].

Mass Spectroscopy is a developing technology in metabolomics applications, there are numerous configurations of mass spectrometers used for LC-MS applications, in terms of ion acceleration and mass detection, ion-production interfaces and ion fragmentation capabilities. Moreover, over the years, there have been constant changes in the hardware and the software of mass spectrometers to meet the demands for robustness, practicality, applicability and efficiency of the analyses. The performance of soft-ionization mass spectrometers, employed in LC-MS applications, can be delineated (and compared) by means of several intrinsic parameters: mass resolving power (or resolution); mass accuracy; linear dynamic range; and, sensitivity [31]. Enhancement of those parameters enables more effective identification of the MM of the analyte injected into the MS instrument [32].

Metabolite identification in LC-MS is principally supported matching the detected mass with available mass databases and derivative signal annotations [33]. LC-MS-based methods, metabolomics give an unprecedented level of both qualitative and quantitative characterization of the metabolome via biofluid and tissue analysis[34].Therefore the liquid chromatography-mass spectrometry (LC-MS)-based metabolomics has proven to be advantageous in investigating the metabolic profiles compare there to of alternative varied techniques like rapid and high-throughput techniques, such as nuclear magnetic resonance (NMR), capillary electrophoresis-mass spectrometry (CE-MS) and gas chromatography-mass spectrometry (GC-MS) have been used in metabolomic studies.

Researchers are presently developing their methods based on the experience obtained within the targeted analysis field or

untargeted analysis field. Most efforts are directed at improving metabolite detection and identification, mainly taking advantage of recent developments in mass spectrometry technology [35] and data analysis, to look through massive datasets trying to unravel biochemical phenomena and identify trends in complex data. Metabolomics datasets are massive (especially those generated by LC–MS) and can be an attractive asset for chemometricians, programmers, and statisticians [36]. This is expected to grow further with the increased accessibility of data (and metadata) open repositories [37].

Table No.3. Advantages and disadvantages of the commonly used spectrometry based analytical techniques in metabolomics

Technique	Time duration	Advantages	Disadvantages
High-resolution NMR spectroscopy	~10 min	• Simultaneous detection of many different compounds like carbohydrate, lipid without any initial sample pre-treatment • Technique non-destructive • Good libraries of spectra Easy to process	• Poor Sensitivity, • Co-resonances for 1D-NMR spectroscopy 2D-NMR spectroscopy is time consuming • In vivo NMR, very poor sensitivity (hyperpolarization or higher field strengths could improve it)
Direct-infusion MS	3–4 min	• Used in both aqueous and lipophilic metabolites in various studies. • Minimal carry-over, as no chromatography involved • Good reproducibility • Simple to optimize	• Ion suppression substantial problem, • Identification can require chromatography, e.g. for isobaric species, • Metabolite identification is a major challenge
GC–MS	20–30 min for fatty acids 30–45 min for aqueous metabolites	• Chromatography is robust and reproducible • Only positively charged ions are measured • Metabolite identification is aided by adoption of common ionization parameters in electron impact and wide availability of the • Can be quantitative	• Metabolites need derivatization • Not all metabolites are suitable for derivatization • Many polar compounds are not detectable
LC–MS	~15–30 min	• Chromatography reduces the effect of ion suppression and can separate isobaric species • Both positively and negatively charged ions are measured • Suitable for measuring intact lipids, dipeptides, tripeptides, and other macromolecules, • Better sensitivity • High resolution power • Derivatization not necessary • Suitable for polar, semi polar and non-polar metabolites • Offers a wide range of stationary phases with different functionalities (RP, hydrophilic interaction chromatography, ion-exchange) • Allows analysis of thermally labile analysts • Mass accuracy • Reduces the complexity of the sample • Increasing evaporation and nebulization efficiency	• Chromatography can drift during a sample run, which makes data processing difficult • Suffer more from matrix effects
Triple quadrupole (targeted)MS	15 min per chromatography run ~ 60 min for more comprehensive screens	• Highly sensitive • Highly quantitative • Targeted Results readily transferable because concentrations can be measured	• Targeted, so discovery of novel biomarkers unlikely • Time consuming to set up quantitative assays

LC-MS-BASED METABOLOMICS APPLICATIONS FOR IDENTIFYING NOVEL METABOLITES:

- **Determination and Identification of a Specific Marker Compound for Discriminating Shrub Chaste Tree Fruit from Agnus Castus Fruit Based on LC/MS Metabolic Analysis**

Yahagi, T., et al., (2016) reported that Shrub Chaste Tree Fruit (SCTF) and Agnus Castus Fruit (ACF); it is used as both an over-the-counter drug and as an ingredient in health foods for treating premenstrual syndrome (PMS). To ensure the efficacy and safety of both SCTF and ACF products, it is important to precisely authenticate their botanical origins and to clearly distinguish between SCTF and ACF. Therefore, in there study, they analyzed the extracts of commercially available SCTF and ACF products by LC/MS and applied the data to multivariate analysis to find marker compounds with which to discriminate SCTF from ACF. In this they used liquid chromatography coupled with tandem mass spectrometry

(LC-MS/MS) is often applied to plant metabolomics, because it can detect broad metabolites with high sensitivity and provide useful information for identification [38].

- **Potential urinary biomarkers of esophageal squamous cell carcinoma for diagnosis and staging**

Xu, J., et al., (2016) reported in their study of metabolomics using liquid chromatography-mass spectrometry (LC-MS) combined with multivariate data analysis (MVDA) to discriminate global urine profiles in urine samples from esophageal squamous cell carcinoma (ESCC) patients and healthy controls (NC). The results suggest that the combination of LC-MS analysis and MVDA may have potential applications for ESCC diagnosis and staging. They observed that currently, the most widely employed methods for metabolomics studies are NMR, MS coupled with various chromatographic techniques but particularly LC-MS offers several advantages, including high sensitivity and a wide dynamic range. Therefore, they used this approach to facilitates the screening and early detection of EC then they conducted LC-MS/MS experiments to identify the potential biomarkers with large contributions to the discrimination. A total of 83 potential diagnostic biomarkers for ESCC were screened out, and 19 potential biomarkers were identified [39].

- **LC-MS-Based Metabolic Fingerprinting of Aqueous Humor (AH)**

Pietrowska, K., et al., (2017) reported application of metabolomics has a huge potential for studying human AH may improve knowledge about the molecular mechanisms of eye diseases. (e.g., cataract, glaucoma, pseudo exfoliation syndrome, or age-related macular degeneration). They developed a method for extraction and analysis of AH metabolites by LC-QTOF-MS which allows for detection of the highest number of metabolites by a single analytical platform. This separation step reduces the complexity of the biological sample and allows the analysis of different classes of metabolites at different time points. As per their observation with the among analytical method for AH fingerprinting, the LC-MS allowed for the highest metabolome coverage or detection of more than one thousand metabolic features with good repeatability as well as suitable to study the potential role of amino acids, polar and nonpolar compound, lipids, oxidative stress, or microbial metabolites in development of ocular diseases[40].

- **Renal cell carcinoma histologic subtypes**

Jing, L., et al., (2019) in their study, they performed the first untargeted metabolomics profiling analysis using recent technological progress with respect to the speed and resolution of LC-MS metabolomics analyses on all three main RCC histologic subtypes. These results provide differences between oncogenesis mechanisms for different RCC subtypes. Compared to commonly used analyses (histologic, immune histologic and genetic), metabolomics analyses with LC-MS allows the characterization of different zones within a single tumour (necrosis, carcinoma or sarcomatoid), and therefore provide additional information on tumour behaviour. They detected significant differences in metabolic profiles (amino acid and fatty acid metabolism) among RCC subtypes, also the second aim of their study was to elucidate differences between RCC subtypes at a molecular level which should be helpful for the discovery and development of new specific therapeutic treatments strategies. Moreover, it presents the advantages of being fast, cheap and robust [41].

- **LC-MS metabolomics comparisons of cancer cell and macrophage responses to methotrexate and polymer-encapsulated methotrexate**

Al-Natour, M. A., et al., (2019) reported here in his study, a cell-based global metabolic profiling approach was applied to study the effects of MTX in both free drug form and when encapsulated in -poly(lactide-co-glycolide) (PLGA) nanoparticles on a cancer cell line, A549, and also on human-like THP-1 macrophages. For that purpose, they chose global LC-MS based metabolite profiling. Metabolomics methods potentially allow all the end products of every cellular process to be measured, and any alterations in metabolite levels might act as signals which can describe the effects of certain stimuli on cells very comprehensively. A total of 400 and 800 different metabolites were identified in THP-1 and A549 cells, these included amino acids, lipids, carbohydrates, nucleotides, cofactors and energy metabolism metabolites. That's why they believe the presented work shows that metabolomics is a valuable analytical technique that can be used to understand mechanisms of action of drugs and formulations in various clinically important cell lines [42].

- **Simultaneous determination of pesticides, mycotoxins, and metabolites as well as other contaminants in cereals by LC-LC-MS/MS**

Kresse, M., et al., (2019) They developed 2D LC-MS/MS method for the simultaneous determination of 350 pesticides, 16 mycotoxins as well as the growth regulators Chlormequat and Mepiquat. This method is applicable to cereals and products thereof. They used LC-MS/MS-technology, because of development of mass spectrometry technology has increased both the selectivity and the sensitivity of the LC-MS/MS-technology. This has enabled a reduction in clean-up steps and, furthermore, the simultaneous determination of more mycotoxins within one analytical run [43–45]. The method was so robust and accurate that nearly 90% of the pesticides and all the tested mycotoxins, growth regulators and tropane alkaloids fulfilled the validation criteria of the SANTE guideline document. For the verification, eight proficiency tests were passed successfully: three for the pesticide analysis, three for the mycotoxin analysis, and two for the analysis of the tropane alkaloids. In addition, other contaminants, the six most important ergot alkaloids (e.g. ergotamine/ergotamine) and two modified mycotoxins (deoxynivalenol-3-glucoside and zearalenone-sulfate, also known as masked mycotoxins) were detected during the routine analysis of rye and corn samples [46].

- **Effects of a Sudden Drop in Salinity on Scapharca subcrenata Antioxidant Defenses and Metabolism Determined Using LC-MS Non-targeted Metabolomics**

Zhang, M., et al., (2020) in their study effects of a sudden drop in salinity on the antioxidant defense system and related gene expression of the ark shell Scapharca subcrenata were examined. The sudden drop in seawater salinity after a rainstorm was simulated, and differentially expressed nine metabolic markers were identified by LC-MS non-targeted metabolomics. As per their survey, they observed that LC-MS technique is advantageous and suitable for the analysis of metabolites. So during their study, they used the LC-MS technique or observed good for the analysis of metabolites with poor volatilization or poor thermal stability, high flux, resolution, and sensitivity on the other hand evaluate the variation of metabolites and key metabolic pathways in the gills of S. subcrenata under different salinities that simulated the process of a sudden drop in sea water salinity after a rainstorm. Results of this study provide an understanding of the physiological adaptation process of marine invertebrates in response to low salinity also illustrate that how these nine metabolites can be used as metabolic markers for the response of S. subcrenata to a sudden drop in salinity [47].

- **Isolation of bioactive natural products**

Demarque, D. P., et al., (2020) reported metabolomics is a powerful tool in the analysis and identification of metabolites responsible for biological properties. In this study, they established a mass spectrometry-based metabolomics

strategy to discover compounds with larvicidal activity against Aedes aegypti within the Arbo Control Brazil Project. The Metabo Analyst and GNPS platforms, they used LC-MS and LC-MS/MS data, respectively, were chosen to identify compounds that differentiate active and inactive samples. The capacity of metabolomics to predict metabolite differences between active and inactive samples using LC-MS and LC-MS/MS data. In this study they used, mass spectrometry (MS) and nuclear magnetic resonance (NMR) are the most common analytical techniques in metabolomics analysis. The advantages of MS compared with NMR are: the possibility of coupling a chromatographic method with MS analysis provided important information regarding the retention time of active compounds, sensitivity, small sample volume and the possibility of coupling with a chromatographic technique [48].

- **Development and validation of an LC-MS/MS method for the bioanalysis of psilocybin's main metabolites, psilocin and 4-hydroxyindole-3-acetic acid, in human plasma**

Burton, L., et al., (2021) reported in their study, Psilocin is an indole alkaloid which is the active metabolite of psilocybin, a serotonergic psychedelic substance. The pharmacokinetic properties of psilocin are only partially characterized. Therefore, they developed and validated a rapid LC-MS/MS method to quantify psilocin and its metabolite 4-hydroxyindole-3-acetic acid (4-HIAA) in human plasma. They state that LC-MS/MS method was convenient and reliable for measuring psilocin and 4-HIAA in plasma and will facilitate the clinical development of psilocybin. Compared to other bioanalytical methods that measure psilocybin in human plasma, the developed method was at least 8-times more sensitive, uses small amounts of sample, and includes a short run time, also able to determine the pharmacokinetic properties of psilocin, 4-HIAA and psilocin glucuronide in humans. Furthermore, for drug screening analysis, they state that this method can also be adjusted to determine the metabolites of psilocybin in non-invasive biological matrices such as urine. Overall, the current bioanalytical method will be an important tool to further progress the development of psilocybin as a therapeutic agent [49].

- **LC-MS/MS Based Metabolomics Reveal Candidate Biomarkers and Metabolic Changes in Different Buffalo Species**

Shi, W., et al., (2021) in their study state that Buffalo milk contains higher protein, fat, lactose, and total solid contents that is unsaturated fatty acids (UFAs) which are important for human health. The composition of milk seems to vary among different buffalo species. In their present study, diverse significantly different metabolites were identified among the Mediterranean, Murrah, and crossbred buffalo, and the different metabolites were mainly enriched in fat synthesis related pathways which affected the end fat content in the milk. For these specific metabolites can be used as candidate biomarkers in the identification of milk quality and molecular breeding of high milk fat buffalo. They observed that compared with other chromatographic methods coupled with MS, such as LC-MS was a more sensitive technology for global metabolic profiling of complex biological samples. It can detect most of the metabolites, especially the low abundance compounds [50], which thus make it suitable for biomarker identification. Results showed that milk fatty acid in Mediterranean buffalo was significantly higher than Murrah buffalo and crossbred buffalo [51].

CONCLUSION:

LC-MS is a truly advantageous analytical technique over alternative modern analytical instrumentation for the analysis of metabolomics. It has broad range of applications that helps to educational research, clinical analysis, food characterization, quality control. This review describes about omics, types of omics, metabolomics, LC-MS, various advantages and disadvantages of spectroscopic techniques with ten applicable approaches using LC-MS-based metabolomic techniques to resolve practical problems. Its concise, efficient, machine-controlled system provides quick, reproducible and effective results that serve a key role in advancement of Science and Technology. This versatile analytical technique for metabolomics may well be explored for higher prospects in future.

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