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METABOLOMICS: A STUDY OF METABOLOME WITHIN CELLS, BIOFLUIDS, TISSUES - A SYSTEMATIC REVIEW

[1]Krutika B. Sonone*, [2]Madhuri D. Game, [3]Pankaja D. Ingle, [4]Nandini J. Deshmukh

[1,2,3]Department of Quality Assurance, Vidyabharati College of Pharmacy, Amravati, [4]Bharati Vidyapeeth's Institute of Pharmacy, Pune

ABSTRACT

Metabolomics is the high-throughput identification and quantification of all endogenous and exogenous low-molecular-weight, tiny molecules/metabolites in a biological system. It is the study of the metabolome within cells, biofluids, tissues, or species. Precision metabolomics, single cells, epidemiologic population studies, metabolic phenotyping, metabolome-wide association studies (MWAS), and integrative omics, as well as biotechnology and bioengineering, are just a few of the uses of metabolomics in health and disease. In this review, we give a succinct summary of the current state of metabolomics, some of their emerging roles, and a number of analytical approaches for identifying, characterising, detecting, quantifying, and separating the many metabolites that are present in living things. The wider application and integration of metabolomics into systems will be accelerated by the development of analytical techniques.

KEYWORDS

Metabolites, Metabolome, Metabonome, Analysis,

INTRODUCTION

With the development of science and technology, it was suggested to apply the idea of omics from a holistic perspective because researchers discovered that simply researching a certain direction cannot explain all biological issues. A novel method for examining the pathophysiology of human disease has been made possible by the development of genomes, metabolomics, proteomics, lipidomics, and transcriptomics. The use of metabolomics as a crucial approach for researching contemporary life sciences is directly connected to the most recent advances in science and technology. In order to study small molecule metabolites of diverse metabolic route matrices and products, metabolomics analyses numerous samples, such as blood, urine, and faeces. Nuclear magnetic resonance (NMR), mass spectrometry (MS), and chromatography are technologies used in metabolomics. Clinical research, disease therapy, medication characterization, animal and plant research, agricultural research, and nutrition all benefit greatly from mass spectrometry-based metabolomics.

After genomics, proteomics, and transcriptomics, metabolomics is one of the newest "omics" disciplines. It integrates bioinformatics with high-throughput analytical methods. It deals with evaluations of metabolites, significant metabolic intermediates as well as final products, both quantitatively and qualitatively. [1]

Recent years have seen an increase in interest from both clinical and academic health circles in the innovative subject of metabolomics (also known as "metabonomics" and "metabolic profiling"), which is a part of the "omics" subclass of biological investigations. The term "metabolomics" describes the systematic identification and quantification of a biological system's small molecule metabolic products (the metabolome, which contains 40,000 metabolites in humans) at a given time.

Advanced analytical chemistry methods combined with sophisticated statistical approaches are used in the fast developing field of life science known as metabolomics to fully define the metabolome. The full set of metabolites, or small molecule compounds, present in a specific organelle, cell, organ, biofluid, or organism is referred to as the metabolome. A metabolite is any tiny molecule that can be found everywhere in any organism and has a molecular mass of less than 1,500 Da for the purposes of this review. In other terms, metabolites can be endogenous substances that are regularly created by endogenous catabolism or anabolism, such as lipids, amino acids, short peptides, nucleic acids, sugars, alcohols, or organic acids. They are referred to as "primary" metabolites. Their synthesis is encoded by the host genome, and they are necessary for several important physiological processes, including growth and development. Animals, who are auxotrophs, lack some of the genes required to synthesis some critical metabolites, therefore these substances must be obtained through diet or, in some circumstances, produced by the genes in the gut microbiota. This is because the host genome has lost a number of these genes (biotin and vitamin K). The essential amino acids (phenylalanine, histidine, isoleucine, lysine, leucine, methionine, threonine, valine, and tryptophan) and vitamins are among these important metabolites (vitamin A, 8 different B vitamins, as well as vitamins C, D, E, and K). Diseases like kwashiorkor (lack of protein or vital amino acids), scurvy (short of vitamin C), pellagra (lack of vitamin B3), or rickets can result from nutritional deficiencies in these important metabolites (lack of vitamin D). [2]

THE HISTORY OF METABOLOMICS

The youngest of the three branches of systems biology—along with genomics and proteomics—is the relatively new discipline of metabolomics. Even as recently as 2010, metabolomics was still recognised as an emerging discipline. The term "metabolome" was first used in 1998.

The history of metabolomics begins with the groundbreaking work of Horning, who employed gas chromatography for the metabolites profiling in urine in the early 1960s. The development of metabolomics research was considerably aided by the advancement of instrumental analytic tools. Several cutting-edge methods, such as NMR spectroscopy, magnetic resonance imaging (MRI), infrared spectroscopy (IR), gas chromatography-mass spectrometry (GC-MS), capillary electrophoresis MS, and HPLC-MS, have been used in metabolomics studies. Due to the technological maturity of these instruments, current metabolomics and metabolic profiling research rely nearly entirely on 1H-NMR, GC-MS, and LC-MS.[3]

Roger Williams first proposed the idea that people might have a "metabolic profile" that could be reflected in the composition of their biological fluids in the late 1940s. He did this by using paper chromatography to argue that distinctive metabolic patterns in urine and saliva were connected to conditions like schizophrenia. But it wasn't until the 1960s and 1970s that technology made it possible to analyse metabolic profiles statistically rather than qualitatively. After proving that gas chromatography-mass spectrometry (GC-MS) could be used to analyse chemicals contained in human urine and tissue samples, Horning et al. coined the phrase "metabolic profile" in 1971. Through the 1970s, the Horning group, Linus Pauling, and Arthur B. Robinson spearheaded the development of GC-MS techniques to track the metabolites found in urine.[4]

NMR spectroscopy, which was developed in the 1940s, was also developing quickly at the same time. The effectiveness of employing NMR to find metabolites in unaltered biological samples was shown by Seeley et al. in 1974. The fact that 90% of cellular ATP is complexed with magnesium in this initial work on muscle highlighted the usefulness of NMR. NMR is a key analytical method for analysing metabolism because of the advancement of greater magnetic field strengths and magic angle spinning, which has increased sensitivity. The laboratory of Jeremy K. Nicholson, first at Imperial College London and afterwards at Birkbeck College, University of London, has been substantially responsible for recent efforts to use NMR for metabolomics. Nicholson demonstrated in 1984 that 1H NMR spectroscopy might be used to detect diabetes mellitus and later led the way in the use of pattern recognition techniques on NMR spectroscopic data. [5]

In 1995, Gary Siuzdak worked with Benjamin Cravatt and Richard Lerner (the president of The Scripps Research Institute at the time) to conduct liquid chromatography mass spectrometry metabolomics research to examine the cerebral spinal fluid of sleep-deprived rats. Oleamide, a chemical of particular significance, was discovered and later demonstrated to have sleep-inducing qualities. One of the earliest metabolomics investigations that combine mass spectrometry with liquid chromatography is this one.[6]

The Siuzdak lab at The Scripps Research Institute created the first metabolomics tandem mass spectrometry database, METLIN, for describing human metabolites in 2005. Since then, METLIN has expanded to include over 450,000 metabolites and other chemical entities, with each compound having experimental tandem mass spectrometry data generated from molecular standards at various collision energies and in positive and negative ionization modes. As of July 1, 2019, METLIN appears to contain over 450,000 metabolites and other chemical agencies. The largest database of tandem mass spectrometry data is called METLIN. Additionally, Roy Goodacre, the current editor-in-chief of the specialized academic journal Metabolomics, launched it in 2005.[7]

In an effort to address the challenge of statistically identifying the most pertinent dysregulated metabolites across hundreds of LC/MS datasets, the Siuzdak lab began identifying metabolites linked to sepsis in 2005. As a result, the first algorithm was created to enable the nonlinear alignment of mass spectrometry metabolomics data. It has been developed as an online tool since 2012 under the name XCMS, where "X" stands for any chromatographic method, and as of 2019 (with METLIN) has more than 30,000 registered users.

The initial draught of the human metabolome, which consists of a database of over 2500 metabolites, 1200 medications, and 3500 food components, was finished on January 23, 2007, by the Human Metabolome Project, headed by David S. Wishart. Similar initiatives have been running for a while in a number of plant species, most notably Medicago truncatula and Arabidopsis thaliana.[8]

Metabolomics was still regarded as a "emerging field" in the middle of 2010. Further, it was highlighted that the field's ability to advance hinged in great part on the technical development of mass spectrometry instruments, which allowed for the resolution of otherwise "irresolvable technical obstacles."

Real-time metabolome profiling was first demonstrated in 2015.[9]

METABOLOME

The complete set of small-molecule compounds present in a clinical specimen is referred to as the metabolome. A cell, cellular organelle, organ, tissue, tissue sample, biofluid, or complete organism can constitute the biosensors. The small molecules that make up a given metabolome may include both exogenous chemicals and endogenous metabolites, which are substances that are not naturally produced by an organism. Examples of exogenous chemicals include drugs, environmental pollutants, food additives, toxins, and other xenobiotics, as well as amino acids, organic acids, nucleic acids, fatty acids, amines, sugars, vitamins, co-factors, pigments, and antibiotics.

In other words, there are two types of metabolomes: endogenous and exogenous. A "primary" and a "secondary" metabolome can be included in the further division of the endogenous metabolome (particularly when referring to plant or microbial metabolomes). A main metabolite plays a direct role in healthy development, growth, and reproduction. Although a secondary metabolite plays no direct role in these processes, it typically serves a vital ecological purpose. Pigments, antibiotics, and waste materials generated from partially digested xenobiotics are examples of secondary metabolites. Metabolomics is the study of the metabolome.[10]

METABOLITES

A metabolite is an intermediate or final result of metabolism in biochemistry. Small molecules typically fall within this category. Fuel, structure, signalling, stimulating and inhibiting effects on enzymes, catalytic activity on their own (often as a cofactor to an enzyme), defence, and interactions with other organisms are just a few of the many roles that metabolites play (e.g. pigments, odorants, and pheromones).

The metabolites are created by microorganisms, plants, and people.

A living thing's cells are composed of countless organic substances. Numerous other compounds are also created during metabolism. Metabolites are substances that are created during metabolism or are necessary for it. Biomolecules make up all metabolites. Biomolecules known as metabolites are frequently utilised by cells for a variety of purposes, including energy, stimulation, inhibition, defence, and others. [11]

TYPES OF METABLOLITE

The direct intervention of an extracellular synthesis occurs during typical "growth," maturation, and reproduction. A major metabolite produced on a huge scale by industrial microbiology is ethylene.

A secondary metabolite typically serves a significant ecological purpose despite not being directly involved in those processes. Antibiotics and pigments like resins and terpenes are two examples.

Actinomycin, an antibiotic derived from the fundamental metabolite tryptophan, is one example of an antibiotic that uses primary metabolites as precursors. A few types of sugar are metabolites, including glucose and fructose, which are both found in the metabolic pathways.

The following are examples of primary metabolites generated by industrial microbiology:[12]

Class	Example
Alcohol	Ethanol
Amino acids	Glutamic acid, aspartic acid
Nucleotides	5' guanylic acid
Antioxidants	Isoascorbic acid
Organic acids	Acetic acid, lactic acid
Polyols	Glycerol
Vitamins	B_2

PRIMARY METABOLITE

A type of metabolite known as a main metabolite is one that plays a key role in healthy development, growth, and reproduction. It typically serves an organism's physiological needs (i.e. an intrinsic function). Many organisms or cells normally include a main metabolite. It is sometimes known as a central metabolite, but this term has even less definitions (present in any autonomously growing cell or organism). The major metabolites lactic acid and certain amino acids are two typical examples. Keep in mind that basic metabolites don't exhibit any pharmacological effects or actions. [13]

SECONDARY METABOLITE

In contrast, a secondary metabolite frequently serves a significant ecological purpose while not being directly involved in those processes (i.e. a relational function). Secondary metabolites are organic compounds produced by any lifeform, such as bacteria, fungi, animals, or plants, but not directly involved in the normal growth, development, or reproduction of the organism. They are also known as specialised metabolites, toxins, secondary products, or natural products. Instead, they typically operate as mediators between ecological interactions, which might give the organism a selection advantage by boosting its ability to survive or reproduce. Within a phylogenetic group, specific secondary metabolites are frequently confined to a small number of species. Plant defence against herbivory and other interspecies defences frequently depend heavily on secondary metabolites. Secondary metabolites are utilised by people as pharmaceuticals, flavourings, colours, and medications. [14]

PLANT METABOLITES

In plants, metabolism refers to a series of chemical reactions that keep the plant cell alive. Primary metabolism, which produces substances known as metabolites that are directly engaged in the growth and development of the organism, on the other hand, includes all metabolic pathways that are necessary for the plant's existence.

Primary metabolites, which are vital chemical substances that fuel plant growth and development by supplying the carbon and energy required for cell division, expansion, and maintenance as well as the production of stress- and defense-related compounds, include sugars, organic acids, and amino acids.

Secondary metabolites serve as antimicrobials, attractants, or repellents depending on their function. In the plant kingdom, more than 50,000 secondary metabolites have been found. Secondary plant metabolites are the basis for the therapeutic effects of many modern medicines and herbal remedies. [15]

HUMAN METABOLITES

There are 2500 metabolites in humans. Arachidonic acid is a metabolite of prostaglandin. Both molecules are connected by a series of enzyme-catalyzed processes and share the same physical characteristics and functional groups. Tyrosine, an amino acid, produces catecholamines, while cholesterol creates steroid hormones.

A free online resource called the Human Metabolome Database contains all the details on the metabolites made by the human body (HMDB). [16]

Organic acids	Lipids	Misc	Amino acids
2 aminobutyric acid	Arachidonic acid	D-Fructose	Glycine
Alpha-Hydroyisobutyric acid	Cholesterol	D-Galactopyrane	L-Alanine
2-Methylbutanoic acid	Capric acid	D-Galactose	L-Asparagine
3-Hydroxybutyric acid	Dodecanoic acid	Glucitol	L-Aspartic acid
4-Hydroxybutyric acid	Arachidic acid	D-Glucose	L-Cystein
Aminomalonic acid	Heptadecanoic acid	Glycerol	L-cystine
Benzoic acid	Linoleic acid	D-Glucopyranose	L-Glutamic acid
Citric acid	Oleic acid	Hydroxyproline	L-Glutamine
Erythronic acid	Palmitelaidic acid	D-Maltose	L-Histidine
Fumaric acid	palmitric acid	Myro-insitol	L-Isoleucine
Gluconic acid	Stearic acid	Acetylglycine	L-Leucine
Isobutyric acid	Myristic acid	N Acetyl -L-Lysin	L-Lycine
Tartaric acid		Acetaminophen	L-Methionine
L- Lactic acid		Phosphoric acid	L-Ornithine
Malonic acid		Ribitol	L-Phenylalanine
Methylmalonic acid		Salicylic acid	L-Proline
Nicotinic acid		Urea	L-Serine

List of typical metabolites found in polar and lipid extracts of human serum.

MICROBIAL METABOLITES

Different bacteria create metabolites in different ways. These are helpful in distinguishing between various microbe species.

One of the key metabolites employed most frequently in the fermentation-anaerobic respiration process for the creation of wine and beer is alcohol. Citric acid is widely utilised in the food, pharmaceutical, and cosmetics sectors and is produced by Aspergillus niger. [17]

METABONOMICS

"The quantitative evaluation of the dynamic multiparametric metabolic response of biological systems to pathogenic stimuli or genetic alteration" is the definition of metabonomics. The word "origin" comes from the Greek roots "o" (change) and "nomos" (system of rules or regulations). This method was developed by Jeremy Nicholson at Murdoch University and has been applied in a variety of domains, including toxicology and illness diagnostics. One of the first techniques to use the scope of systems biology to study metabolism was the metabonomics approach. [18]

EXOMETABOLOMICS

The study of extracellular metabolites is known as exometabolomics, often known as "metabolic footprinting." It has applications in the development of biofuels, bioprocessing, establishing the mechanism of action of medications, and researching intercellular connections. It makes extensive use of techniques from other subfields of metabolomics. [19]

MAJOR APPROACHES IN METABOLOMICS

The study of cells through profiling all or a significant portion of their metabolites is known as metabolomics. Metabolomics was first presented as a way of functional genomics, but its applications go much beyond that; they are helpful whenever it is crucial to assess changes in metabolite levels. Applications in bacteria, plants, and animals, including humans, are given as examples. Comparing mutants, evaluating reactions to environmental stress, researching the worldwide consequences of genetic modification, comparing various growth stages, toxicology, drug discovery, nutrition, cancer, diabetes, and discovering new natural products are all uses for metabolomics. Metabolite profiling can be used as a tool in systems biology, where metabolite snapshots are utilised to examine biological dynamics using mathematical models, regardless of whether it targets certain metabolite classes or is untargeted. Targeted analysis, metabolite profiling, and metabolic fingerprinting are the three main methods applied in metabolomics studies. [20]

TARGETED ANALYSIS

The most advanced metabolomics analytical strategy is targeted analysis. It is used to precisely measure the concentration of a select group of known metabolites. Knowing the target metabolite's structure and having an analytical technique designed to accurately quantify its concentration in the sample are prerequisites for doing focused analysis. For known metabolites, targeted analysis offers very low limits of detection and is a genuinely quantitative approach. Depending on the target analyte, it can also be employed in a high-throughput mode. The primary drawback of targeted analysis for metabolomics is that it necessitates prior knowledge of the relevant chemicals and their availability in pure form. Currently, many metabolites that can be positively identified in samples utilising analytical techniques in use cannot be purified standards for many of the identified metabolites. As a result, this approach cannot currently be utilised to scan global metabolic changes or to find novel metabolic indicators. Targeted analysis will be used more frequently to study global metabolic changes in the future since it delivers fully quantitative data when additional metabolites are discovered and pure substances are accessible to create quantitative tests. [21]

METABOLITE FINGERPRINTING

All of the metabolites in the sample are not aimed at being precisely identified or quantified by metabolic fingerprinting. As opposed to this, it views a whole profile, or fingerprint, as a distinctive pattern capturing a moment in time of the metabolism in a certain cell line or tissue. The classification of fingerprints and identification of the specific profile characteristics for each pattern are done using pattern recognition software. The identification of biomarkers and diagnostics are where metabolic fingerprinting is most helpful. Spectroscopic methods such as nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy (FT-IR), Fourier transform ion cyclotron resonance mass spectroscopy (FTICR-MS), or mass spectrometry (MS), which directly acquire physical spectra without using prior separation methods like chromatography or electrophoresis, are typically used for fingerprinting.

Since the molecules in the samples are difficult to identify, metabolic fingerprinting has the drawback of not allowing direct comparison of the metabolic pathways. It has been frequently utilised to identify unique metabolic patterns of diseases via metabolic fingerprinting. Combining metabolic fingerprinting with tools for pattern recognition and discriminant analysis is very beneficial. [22]

METABOLITE PROFILING

Measuring the concentrations of a series of metabolites in a sample is known as metabolite profiling. In the biomedical sciences, metabolite profiling is a well-established process that is regularly applied to biological fluids as a type of diagnosis that helps characterise the patient's state of health. However, this is currently being expanded to considerably larger quantities. Those profiles are normally made up of a small number of metabolites (and maybe proteins). Metabolite profiling is now receiving more interest from researchers as a functional genomics extension. According to a theory, metabolite profiles of a cell's internal state could help determine a gene's function, particularly when mutations in that gene lack any discernible phenotypes.

The reasoning behind this is that although the mutation would have had an effect, the cell's regulatory mechanisms worked to offset it, leaving no obvious macroscopic observable. If one could measure the amounts of those metabolites, they would reveal the function of the mutant gene. However, those regulatory systems would have altered the quantities of metabolites in the metabolic network. The profiles then need to be as understandable as feasible because it is unknown in advance which metabolites are anticipated to be altered. The second implication is that by examining the metabolite profiles of several mutants, it may be possible to identify the underlying metabolic network.

Therefore, this use of metabolite profiling in functional genomics is comparable to transcript and protein profiling, and like these, it will be helpful to determine the full chemical make-up of the cell—the metabolome. [23]

ANALYTICAL APPROACHES

Metabolite profiling can be done using a variety of approaches. Each strategy has benefits and downsides that go along with it. Thus, to obtain a comprehensive understanding of a tissue's metabolome, a variety of analytical methodologies must be applied. NMR, gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and capillary electrophoresis-mass spectrometry are the analytical methods most frequently employed for metabolite profiling (CE–MS). A mixture of methodologies is required for the analysis of the majority of metabolites in different polarity and molecular weight ranges because there is no one analytical technology that is ideal for all of the many thousands of metabolites within a cell. [24]

METABOLITE EXTRACTION

Comprehensiveness, or representing as many cellular metabolites as feasible in the extract, is the first criteria for the extraction protocol. Enzymatic activity must be stopped for the length of the extraction procedure in order to avoid potential metabolite degradation or interconversion. High levels of acid/alkali and organic solvent are required in the extraction medium to achieve this. There is no one extraction technique that works best for all the metabolites in a cell or tissue. Usually, an extraction protocol is designed to work with a certain compound class or analytical technique. As a result of the required comprehensiveness of the extract, metabolomics presents a substantial difficulty for extraction methods. Finding the ideal balance between comprehensiveness and metabolite stability is particularly difficult since extraction circumstances that favour preservation of one metabolite class can inevitably destroy other metabolite species. [25]

Several studies have recently been conducted to evaluate the suitability of various extraction methodologies for metabolomics. In the end, the extraction methodology needs to be adjusted to the needed metabolite range and the chosen analytical method. Extraction solutions are typically spiked with one or more internal standards to determine how well metabolites were recovered from the sample and to later quantify the metabolites. [26]

NUCLEAR MAGNETIC RESONANCE (NMR)

The ability of NMR to simultaneously detect and quantify a variety of organic chemicals in the micro-molar range makes it a good choice for metabolomics studies. Since NMR is not destructive like MS, samples can be used for additional analysis. NMR sample preparation is simple and mostly automated. However, it has historically been challenging to analyse the NMR spectra of complicated mixes. Metabolic flux analysis, metabolite profiling, and fingerprinting have all been widely utilised NMR techniques. NMR's relatively poor sensitivity is a fundamental drawback for thorough metabolite profiling, making it unsuited for the investigation of many low-abundance metabolites. [27]

MASS SPECTROMETRY

MS has evolved into the method of choice in many metabolomics research because to its high sensitivity and broad range of covered compounds. Biological samples can be analysed using MS either immediately through direct-injection or after chromatographic or electrophoretic separation. The variety of metabolites that may be analysed by MS has recently been greatly increased, and compound identification accuracy has also improved, thanks to the introduction of new mass analyzers and advancements in mass accuracy. Direct-injection MS is widely used for metabolic fingerprinting and metabolite profiling because it offers a relatively quick method for analysing a large number of metabolites, especially when utilising a high-resolution mass spectrometer. However, there are limitations to direct infusion as well, mostly because of a process called co-suppression, where the signal of numerous analytes with poor ionisation efficiencies might be lost at the mass spectrometer interface. MS is frequently employed as a hyphenated technique, which means it is combined with capillary electrophoresis, liquid chromatography, or gas chromatography, in order to avoid these issues and reduce the complexity of the material (CE). In this scenario, the sample mixture is separated using chromatography or electrophoresis, and then the eluted compounds or simpler mixtures of compounds (which cannot be separated due to similar characteristics) are analysed by MS. [28,29]

GAS CHROMATOGRAPHY–MASS SPECTROMETRY (GC–MS)

Gas chromatography in combination with electron impact (EI) quadrupole or time-of-flight mass spectrometry (TOF) MS is currently the most developed technology for fast metabolite profiling (GC–MS). Over 25 years ago, the idea of automated GC-MS metabolic profiling was invented, and it later became a key metabolomics technology. Several hundred chemically varied molecules, such as organic acids, the majority of amino acids, sugars, sugar alcohols, aromatic amines, and fatty acids, can be profiled at once using this method. By using GC-MS, it is possible to directly separate and measure volatile metabolites. Others need to undergo chemical derivatization in order to be suitable for GC-MS analysis. Chemical derivatization significantly improves the GC separation of many substances, but the derivatization process itself can potentially introduce artefacts. [30]

LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY (LC–MS)

Due to its high sensitivity and a larger range of analyte polarity and molecular mass than GC-MS, LC-MS is being employed more frequently in metabolomics applications. High-performance liquid chromatography (HPLC), in particular, is a well-established technology that combines high resolution and analytical versatility. It can be customised for the examination of a particular metabolite or class of compounds or used to a wide range of compound classes. LC in conjunction with MS has a variety of applications that now exist or that can be converted for a wide range of metabolites. One advantage of LC-MS over GC-MS is that chemical derivatization of metabolites is largely unnecessary (which is required for analysis of non-volatile compounds by GC–MS).

Mass spectrometers have become more sophisticated and durable for everyday use thanks to LC-MS techniques that use soft ionisation techniques like electrospray (ESI). These techniques have developed during the past ten years. Ultra-performance liquid chromatography (UPLC) and monolithic capillary columns have recently been developed, allowing for considerably better separation of complex biological mixtures than was previously possible. The lack of transferrable mass spectral libraries for the LC-MS as a non-targeted profiling technique is a significant disadvantage. On the other hand, the structure of unknown molecules can be successfully revealed via LC-MS. Structure elucidation is greatly aided by the several sequential steps of MS that may be performed by modern ion-trap mass spectrometers. [31,32]

CAPILLARY ELECTROPHORESIS–MASS SPECTROMETRY (CE–MS)

Despite being a more recent technology than GC-MS and LC-MS, CE-MS is quickly overtaking them as one of the most important analytical methods for metabolomics. Compared to other separation methods, CE-MS offers a number of significant benefits. It offers a very high resolving power with plate numbers between 100,000 and 1,000,000, a very minimal sample volume requirement (1–20 nl on average), and a quick analysis time. Targeted and non-targeted analyses of metabolites, such as those of inorganic ions, organic acids, amino acids, nucleotides and nucleosides, vitamins, thiols, carbohydrates, and peptides, have all been carried out using CE. The capacity of the CE-MS to differentiate cations, anions, and uncharged molecules in a single analytical run is one of its key features. As a result, CE may be utilised for the simultaneous profiling of numerous different metabolite classes. It is a very appealing and promising analytical method for high-throughput non-targeted metabolomics because of this property. [33]

OTHER TECHNOLOGIES

Despite the fact that NMR and MS are frequently employed for extensive analysis, metabolomics is not restricted to these methods. Thin-layer chromatography, HPLC with UV/visible absorbance, photodiode array (PDA) or electrochemical detectors, FT-IR, "phenotype microarrays," and various other enzymatic tests are further possibilities. The metabolite coverage, quantification limits, and metabolite identification from a single biological sample can all be considerably increased by combining the use of numerous methodologies or detectors in online or parallel processing. Gamache and colleagues, for instance, enhanced the concentration range of endogenous rat urine metabolites after exposure to xenobiotic toxins by combining mass spectrometric and electrochemical array detection. [34]

RECENT ADVANCES AND APPLICATIONS

Applications for metabolomics in drug screening, clinical evaluation, pharmacological efficacy and toxicity assessment, and animal model validation are numerous.

There have been more metabolomics investigations using MS. Drug, toxin, and disease-related metabolite effects have been investigated using MS-based metabolomics techniques. Numerous disorders, including breast cancer, kidney cancer, cardiovascular disease, bladder cancer, esophageal cancer and gastric cancer, kidney disease, natural metabolic errors, toxicological effects, and nutrition have been studied using MS-based metabolomics techniques. [35]

DISEASE DIAGNOSIS

The study of each of the metabolic components' commonalities, features, and rules is eventually the goal of metabolomics research, which initially focuses on the similarities of particular components. Compared to genomes and proteomics, metabolomics has a closer relationship to physiology. The disease alters the body's pathophysiological functioning, which ultimately results in corresponding changes in metabolites. The search for illness biomarkers involves examining certain metabolites and contrasting them with typical human metabolites. A novel way of illness diagnostics will be provided by metabolomics. A diagnostic technique for the automatic diagnosis of atherosclerotic disease was developed by Johno, H. et al. using electrostatic ionisation mass spectrometry. Cholesterol sulphate and phospholipids are thought to be novel arteriosclerosis indicators. [36]

CANCER RESEARCH

Tens of millions of people still lose their lives to cancer each year, making it a serious issue that needs to be addressed in the modern era. Knowing what causes cancer and what influences it is crucial to understanding cancer. Researchers utilise metabolomics to describe the effects of multiple factors on cancer because there are numerous elements that can affect cancer, including the environment, an unhealthy diet, and hereditary factors.For instance, Yang, W. et al. discovered 18 metabolites closely associated to ovarian cancer using MS to investigate the metabolic alterations of ovarian cancer. An, Y. et al. identified seven metabolites as possible biomarkers after using LC/MS to examine the metabolic alterations of pancreatic cancer tissues. 45 Breast cancer metabolic markers were identified by Jasbi, P. et al. using LC-MS/MS, and it was discovered that the

impacted metabolic pathways are strongly associated to tumour growth, metastasis, and immune escape mechanisms.[37]

METABOLIC MECHANISM

The therapy of an illness depends critically on identifying the metabolic process at play. Researchers employ metabolomics approaches to describe the effects of numerous factors on disease mechanisms since the occurrence of diseases is influenced by a variety of factors, including environmental and hereditary factors. Breviscapine has been proven to be therapeutic in rats with amyloid 1-42–induced Alzheimer's disease, and studies have indicated that it primarily improves phospholipid metabolism through controlling serotonin levels, among other mechanisms. Zhao, G. et al. examined the hypoglycemic effect of Crassostrea gigas polysaccharides by UPLC-Q-TOF-MS to evaluate learning and memory impairment. 19 metabolites, mostly connected to amino acid, carbohydrate, and purine metabolism, were used as prospective therapeutic indicators.[38]

NUTRITION

Nutritional metabolomics, which refers to the systematic investigation of the relationship between diet and organism metabolism utilising metabolomics in various health and disease states of organisms, is one application of metabolomics in the realm of nutrition. The trace elements that the human body needs are abundant in fruits and vegetables, which might serve as a supplement. The amount of trace elements in fruit depends on a variety of circumstances. Geographical considerations and biological stress are some of them. There are numerous examples that can be used to describe the characteristics of metabolomics in nutrition.

One of them, Zhao, L. et al., examined the changes in cucumber fruit metabolites following nano-Cu stress using 1H NMR and GC-MS methods. They discovered that exposure to nanocopper altered the metabolic profile of fruit metabolites. Wang, Y. Q. et al. investigated the nutritional profiles of Chinese kale using a UHPLC-MS/MS-based metabolomics approach. Varying cabbage types have different component contents, it was discovered. [39]

FUTURE: FUTURE OBJECTIVES OF METABOLOMICS

By discovering one or a profile of prognostic biomarkers, metabolomics has the potential to be a useful tool for disease early detection. It can also be used to forecast survival and treatment response. The metabolome can be used to monitor an individual's metabolic health and flag any potential harmful consequences. It can also be used to identify any lingering disease or recurrence following therapy because it reacts swiftly to environmental stimuli, including therapeutic or surgical intervention. [40]

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