CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1287901

Available online at: http://www.iajps.com *Review Article*

METABOLOMICS: CURRENT TECHNOLOGIES AND FUTURE TRENDS

Dr. A. Krishnamanjari pawar*, T .Samhitha, B. Prasanna, MD. Asif, M. Lavanya.
A.U.College of Pharmaceutical Sciences, Andhra University,
Visakhapatnam (A.P) – India.

Abstract:
The emerging field of metabolomics had profound importance in recent years as its wide applications in the field of drug discovery and drug development.
With recent advances in the field of metabolomics it can be proved as a major tool in the process of drug discovery and development.
In the agricultural/chemical industry, metabolomics may be used to develop herbicides and pesticides With increasing importance being placed on health and safety related aspects of our food, metabolomics can potentially be a valuable tool to monitor and improve the quality of what we eat; for example, in food processing and quality control, or in plant breeding for improved crop varieties and in the development of novel food stuffs.
The present review reveals the importance of metabolomics in drug discovery along with recent advances in the techniques of metabolomics along with its wide applications in various fields like drug discovery, drug toxicity profiling, food industries especially in food and allied beverages testing.
A range of analytical technologies has been employed to analyze metabolites in different organisms, tissues, or fluids. Mass spectrometry coupled to different chromatographic separation techniques, such as liquid or gas chromatography or NMR, are the major tools to analyze a large number of metabolites simultaneously.
All of these so-called 'omics approaches, including genomics, transcriptomics, proteomics and metabolomics are considered important tools to be applied and utilized to understand the biology of an organism and its response to environmental stimuli or genetic perturbation.

Keywords: *Metabolome, metabolite, metabolomics, systems biology, phenotyping.*

Corresponding author:
Dr. A. Krishnamanjari pawar,
A.U.College of Pharmaceutical Sciences,
Andhra University,
Visakhapatnam (A.P) – India.

QR code

Please cite this article in press A. Krishnamanjari pawar et al., ***Metabolomics: Current Technologies and Future Trends,*** *Indo Am. J. P. Sci, 2018; 05(06).*

INTRODUCTION:

History of Metabolomics:

The beginning of metabolomics traces back all the way to 2000-1500 B.C. when traditional Chinese doctors began using ants in order to evaluate the urine of patients to determine if the urine contained the high glucose of diabetics.

Metabolomics was in 131 A.D. when Galen created a system of pathology that combined the humoral theories of Hippocrates with the Pythagorean theory. This theory that was formed by Galen was unchallenged and remained standard until the 17th century.

Santorio Sanctorius became the man who is considered to be the founding father of metabolic studies. In 1614 he published work that he had done on "insensible perspiration" in De Statica Medicina and he determined that the total excrete (urine, feces, sweat) was less than the amount of fluid ingested. The first paper on Metabolomics, though not called metabolomics at the time, was by Robinson and Pauling in 1971. It was titled "Quantitative Analysis of Urine Vapor and Breath by Gas-Liquid Partition Chromatography" and was published in Proceedings of the National Academy of Sciences. The Human Metabolome Project led by Dr. David Wishart of the University of Alberta, Canada completed a first draft of his research on the human metabolome, which consists of 2500 metabolites, 1200 drugs and 3500 food components.

The emerging field of metabolomics had profound importance in recent years as its wide applications in the field of drug discovery and drug development. With recent advances in the field of metabolomics it can be proved as a major tool in the process of drug discovery and development. In the agricultural/chemical industry, metabolomics may be used to develop herbicides and pesticides With increasing importance being placed on health and safety related aspects of our food, metabolomics can potentially be a valuable tool to monitor and improve the quality. Drug development from the early stage of target identification, validation through clinical trials to clinical practice is a long, tortuous, and extremely costly process. Large investments were made to develop analytical approaches to analyze the different cell products, such as those from gene expression (transcripts), proteins, and metabolites. All of these so-called 'omics approaches including genomics, transcriptomics, proteomics, and metabolomics, are considered important tools to be applied and utilized to understand the biology of an organism and its response to environmental stimuli.

Definition:

Metabolite:

1. It is a Compound produced from the chemical changes of a drug in the body.
2. A by-product of the breakdown of either food or medication by the body.
3. Substance involved in metabolism.
4. Any compound detected in the body <1500 Da.

Eg of metabolites: oligonucleotides, Sugar, Nucleosides**,** organic acids, ketones, Aldehydes, xenobiotics, Amino acids.

Metabolomic:

Metabolomics is a multi-disciplinary science that includes aspects of biology, chemistry, and mathematics. It requires analytical techniques such as chromatography, molecular spectroscopy and mass spectrometry, coupled with multivariate data analysis methods. It has been suggested that metabolomics greatest potential lies in disease marker discovery and detection. For instance, numerous acquired metabolic disorders (obesity, diabetes, cachexia and hyper-cholesterolemia) are characterized by unusually high (or low) levels of certain metabolites.

Example: phenylketonuria is characterized by low levels of tyrosine.

Approaches to investigate the metabolomics:

1) Metabolic profiling: Quantitative analysis of set of metabolites in a selected biochemical pathway or a specific class of compounds. This includes target analysis, the analysis of a very limited number of metabolites, e.g.: single analytes as precursors or products of biochemical reactions.

2) Metabolic fingerprinting: Unbiased, global screening approach to classify samples based on metabolite patterns or "fingerprints" that change in response to disease, environmental or genetic perturbations with the ultimate goal to identify discriminating metabolites.

3) Metabolic foot printing: Fingerprinting analysis of extra-cellular metabolites in cell culture medium as a reflection of metabolite excretion or uptake by cells. The metabolome, the intersecting systems chemistry of life processes, is the functional outcome of the activity of the genome, functional genome, and the proteome.

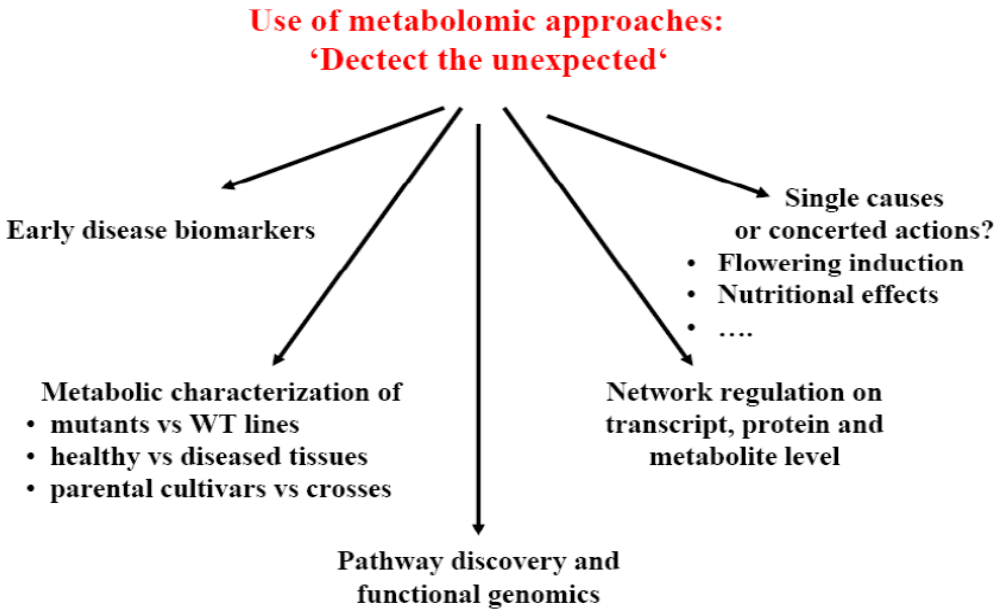

Fig. 1: Approaches for metabolomics.

ADVANTAGES: Metabolites are considered to "act as broadcasting signals from the genetic architecture and the environment", and therefore, metabolomics is considered to provide a direct "functional readout of the physiological state" of an organism, Metabolic profiling, Metabolic fingerprinting, Metabolomics are applied to identify new biomarkers and to elucidate disease mechanisms in several ocular diseases.

DISADVANTAGES: Complex profiles: Differentiating metabolomic profiles from often heterogeneous tissue samples, multiple identifying peaks (m/z values) for the same metabolite, Validation and identification of thousands of LC/MS identified metabolites with known reference standards via MS/MS.

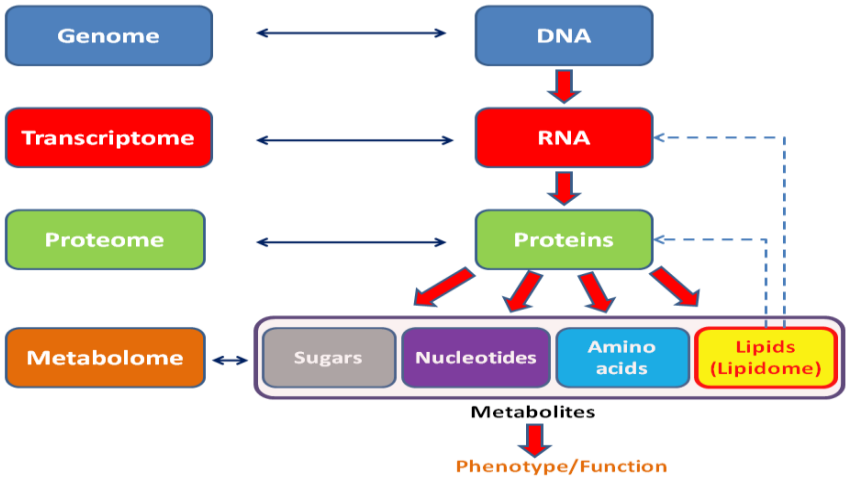

Fig. 2: Comparison of Genome, Transcriptome, Proteome and Metabolome.

METABOLOMICS TECHNIQUES:

Metabolomics is a multi-disciplinary science that includes aspects of biology, chemistry, and mathematics. It requires analytical techniques such as chromatography, molecular spectroscopy and mass spectrometry, coupled with multivariate data analysis methods. For target compound analysis and metabolic profiling, main techniques are gas chromatography (GC), high performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR). These approaches rely on chromatographic separations, often coupled with well-developed calibrations for specific analytes. The aim of metabolomics is to obtain the widest possible coverage, in terms of the type and number of compounds analyzed. This is achieved by making use of several, complementary analytical methods. In particular, the 'hyphenated' techniques of LC/MS, LC/MS/MS and LC/NMR are likely to make increased impact in the future.

An overview of each of the approaches is given below:

1) Gas chromatography (GC)
2) High performance liquid chromatography (HPLC),
3) Nuclear magnetic resonance (NMR),
4) Mass spectrometry (MS),
5) Fourier transform infrared (FT-IR) spectroscopy,
6) LC/MS,
7) LC/MS/MS
8) LC/NMR,
9) Multivariate analysis.

1) Gas Chromatography:

Developments involving gas chromatography have been responsible for the recent upsurge of interest in plant metabolomics. GC provides high-resolution compound separations, and can be used in conjunction with a flame ionization detector (GC/FID) or a mass spectrometer (GC/MS). Both detection methods are highly sensitive and universal, able to detect almost any organic compound, regardless of its class or structure. However, most of the metabolites found in plant extracts are too non volatile to be analyzed directly by GC methods. The compounds have to be converted to less polar, more volatile derivatives before they are applied to the GC column. Efficient derivatization methods are available, but relatively low sample throughput is a drawback of the GC method, particularly when there are many samples to be examined.

Advantages:

- Very high chromatographic resolving power,
- Good selection of stationary phases**,**
- wide dynamic range.

Disadvantages:

- Compounds must be sufficiently volatile (derivatized),
- Compounds must be thermally stable,
- Limited to nonpolar and slightly polar molecule.

2) High Performance Liquid Chromatography (HPLC):

HPLC, with UV detection, is probably the most common method used for targeted analysis of plant materials, and for metabolic profiling of individual classes. A derivatization step is not essential (unless needed for detection), since nonvolatile and volatile substances may be measured equally well. Selection of compounds arises initially from the type of solvent used for extraction and then from the type of column and detector. For example HPLC/UV will only detect compounds with a suitable chromophore; column selected for its ability to separate one class of compounds will not generally be useful for other types.

Advantages:

- Capable of analyze wide range of metabolites (thermally labile, polar, high molecular mass)
- Good selection of stationary phases.

Disadvantages:

- Fragmentation rules not well-established,
- No MS fragmentation libraries**,**
- Limited resolution (but UPLC is an improvement).

3) NMR:

In principle, proton (1H) NMR can detect any metabolites containing hydrogen. Signals can be assigned by comparison with that of reference compounds, or by two-dimensional NMR. 1H NMR spectra of plant extracts are inevitably crowded not only because there is a large number of contributing compounds, but also because of the low overall chemical shift dispersion.

1H spectra are also complicated by spin-spin couplings which add to signal multiplicity, although they are an important source of structural information. In 13C NMR, the chemical shift dispersion is twenty times greater and spin-spin interactions are removed by decoupling. Despite these advantages, the low sensitivity of 13C NMR prevents its routine use with complex extracts.

Advantages:

- No sample separation necessary**,**

- essentially universal detector,
- Non-destructive

Disadvantages: Low sensitivity, Results difficult to interpret, Decreased quantification, Expensive.

4) Direct Injection MS:

It is also possible to obtain metabolite 'mass profiles' without any chromatographic separation. Such profiles are obtained by injecting crude extracts into the electro spray ionization source of a high-resolution mass spectrometer.

ESI generates mainly protonated, deprotonated or adduct molecules, such as [M+H] +, [M+cation] + or [M-H]- for each species present in the mixture, with little or no fragmentation. Thus a fingerprint spectrum is obtained with a single peak for each metabolite, separated from other metabolites according to (accurate) molecular mass. Some mass analyzers are capable of ultra-high resolution and permit the mass to be determined to four or five decimal places. This allows unique formulae to be assigned to peaks with masses of a few hundred or so.

Advantage:

- The coupling of high sensitivity with high resolution provides a method of determining a rough estimate of the number of metabolites present and a valuable first indication, from the formulae, of their identities.

Disadvantage:

- Its main weakness is the inability to separate isomers of the same molecular mass.

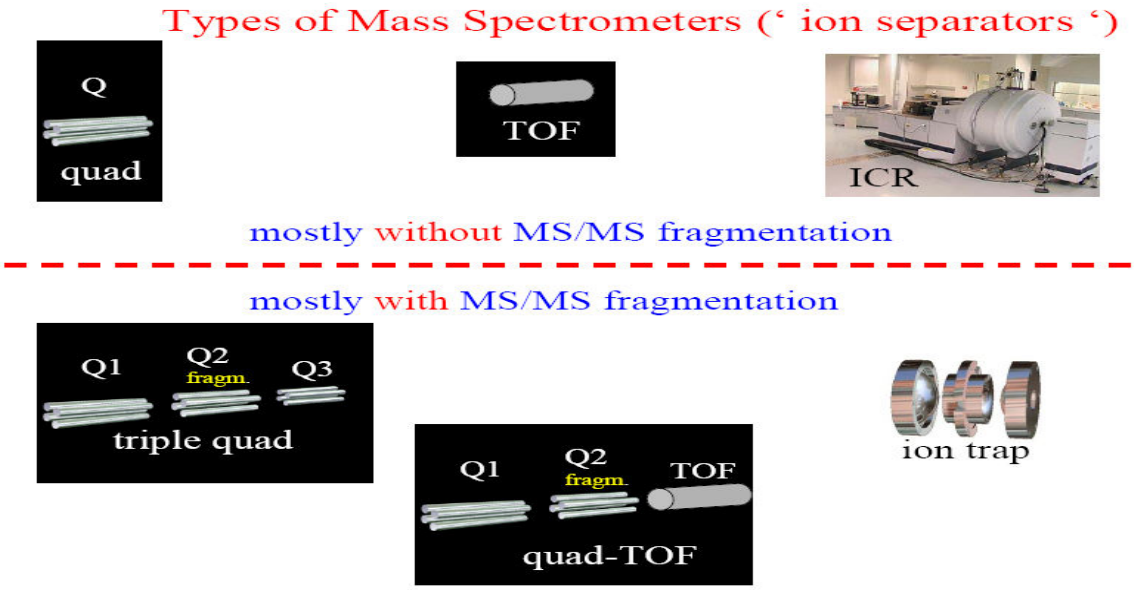

Figure: 3 Mass spectrometers.

5) LC/MS, LC/MS/MS and LC/NMR:

LC/MS, LC/MS/MS and LC/NMR are potentially powerful solutions to the problems of detector generality and structure determination. LC/MS can be used to detect compounds that are not well characterized by other methods. The electrospray ionization (ESI) technique has made polar molecules accessible to direct analysis by MS, as well as being compatible with HPLC separations.

LC/MS/MS provides additional structural information that can be a very useful aid in the identification of new or unusual metabolites or in the characterization of known metabolites in cases where ambiguity exists.

The lower sensitivity of LC/NMR means that at present it is most often used for structural characterization of unknowns, rather than for comparative analysis of numerous samples. However, NMR is a very general detection method, and can

provide unique structural information, so with improvements in sensitivity, the use of LC/NMR is likely to grow.

6) Multivariate Analysis:

Plant extracts are very complex in composition and, if many samples are examined, it is difficult to make meaningful comparisons of large numbers of spectra or chromatograms 'by eye'.

Multivariate statistical methods can be extremely useful, as they are able to compress data into a more easily managed form. This can assist in visualizing, for example, how a given sample relates to other samples - a central issue in metabolomics. Multivariate analysis is practically essential in the fingerprinting approaches, but is also helpful in techniques where individual metabolites are explicitly quantified (e.g. GC/MS). Principle component analysis (PCA) is a well-known and effective method of data compression. PCA transforms the original data (e.g. intensity values in a spectrum) into a set of

'Scores' for each sample, measured with respect to the principal component axes ('loadings'). Due to these properties, a small number of PCs can replace the many original varieties without much loss of information. Scatter plots of the scores on the first few PC loadings provide an excellent means of visualizing and summarizing the data and often reveal patterns that cannot be discerned in the original data. The scores plots may show clustering of similar samples, separation of different sample types, or the presence of outliers. Plots of the loadings themselves may be used to explore which compounds are most responsible for, say, separating samples into groups: the most important compounds (peaks) tend to correspond to high absolute loading values.

7) FTIR spectroscopy:

The attraction of FTIR spectroscopy as a fingerprinting method is the ease of sample preparation, the speed with which data can be acquired, and the high degree of reproducibility attainable. Samples that can be poured or spread to make good contact with a flat surface can be measured by the attenuated total reflectance (ATR) method, whereas powdered or dried samples are measured by diffuse reflectance.

APPLICATIONS OF METABOLOMICS:

1) In vitro NMR approach:

NMR has a long history in analytical and natural products research, and along with MS and single crystal X-ray diffraction it has become one of the main analytical tools for structure analysis.

2) Metabolomics in ADMET:

The absorption, distribution, metabolism, excretion and toxicology of drugs (ADMET), is one of the most critical areas for drug testing and drug development. It is also one of the most times consuming. While the drug discovery process is concerned almost exclusively with identifying active lead molecules, ADMET is concerned with identifying which of the leads is potentially hazardous, thereby preventing them from progressing too far down the drug development pipeline. ADMET is carried out both in preclinical and clinical trial phases of drug development. In pre-clinical studies, ADMET normally requires testing on large numbers of animals and performing detailed histological and pathological analyses. These are supplemented with clinical chemistry studies of blood, cerebrospinal fluid (CSF), urine and faeces. Metabolomics has applied to ADMET appears to meet these desired criteria. In fact, the field of metabolomics largely began with ADMET studies.

3) Toxicity assessment / toxicology:

Metabolic profiling (especially of urine or blood plasma samples) can be used to detect the physiological changes caused by toxic insult of a chemical (or mixture of chemicals). In many cases, the observed changes can be related to specific syndromes, e.g. a specific lesion in liver or kidney. This is of particular relevance to pharmaceutical companies wanting to test the toxicity of potential drug if a compound can be eliminated before it reaches clinical trials on the grounds of adverse toxicity; it saves the enormous expense of the trials.

4) Metabolomics in Organ Transplantation:

Metabolite measurements have been restricted to just a few well-known compounds (creatinine, glucose), there is a surprisingly large body of literature describing injury dependent changes for a large number of lesser-known metabolites. recently, most of these measurements have been done using 'classical' clinical chemistry methods such as GC-MS, but since 1999 a growing number of reports have described the use of true metabolomics methods (NMR, LCMS, spectral pattern analysis, etc.) to monitor organ function. In general, metabolite measurements have been performed to monitor two key aspects of organ physiology:

(i) Organ reperfusion injury and
(ii) Organ function (or dysfunction).

Most metabolite measurements associated with organ transplant analysis have been performed *ex vivo*, using biofluids such as urine, serum or bile. More recently, a few measurements have been performed *in vivo* using NMR chemical shift imaging techniques.

5) Metabolomics approaches for discovering biomarkers of drug-induced hepato toxicity and nephrotoxicity:

Hepato toxicity and nephrotoxicity are two major reasons that drugs are withdrawn from post-market, and hence it is of major concern to both the FDA and pharmaceutical companies. The number of cases of serious adverse effects (SAEs) in marketed drugs has climbed faster than the number of total drug prescriptions issued.

In some cases, preclinical animal studies fail to identify the potential toxicity of a new chemical entity (NCE) under development. The current clinical chemistry biomarkers of liver and kidney injury are inadequate in terms of sensitivity and/or specificity, prompting the need to discover new translational specific biomarkers of organ.

6) Use of metabolomics to discover metabolic patterns associated with human diseases:

Metabolomics techniques aim at detecting unexpected effects comparing stressed/unstressed or mutant/wild type

experiments. This chapter asks the question if metabolomics could also go one step further for analyzing changes in metabolic patterns that are associated with the time course of nutritional-dependent human diseases. Such diseases are typically hard to predict, and existing biological markers such as for type 2 diabetes mellitus have limited value for the assessment of individual risks. Many factors may be involved in disease progression such as genetics, nutritional habits, age, or sex, resulting in the need to study large cohorts in order to draw statistically sound conclusions. Due to this inherent biological variability, severe constraints are posed on the validation of the analytical methods used. Using diabetes as an example, the economic and scientific needs for accurate diagnostic tools are discussed with respect to the available analytical and computational approaches for cost-effective high throughput methods.

7) Metabolomics in Alcohol Research and Drug Development:

Numerous metabolomics approaches may contribute to alcohol-related research, as illustrated by studies on alcohol-related metabolic dysfunctions such as alterations in fat metabolism and thiamine deficiency. By further increasing the number and types of metabolites that can be measured in a given biological sample, metabolomic approaches may be able to help define the role of the many different metabolic pathways affected by alcohol abuse and support discovery and development of novel medications for the treatment of alcoholism and related conditions.

8) Application of Metabolomics to Cardiovascular Biomarker and Pathway Discovery:

Metabolites change rapidly in response to physiologic perturbations; they represent proximal reporters of disease phenotypes. The profiling of low molecular weight biochemicals, including lipids, sugars, nucleotides, organic acids, and amino acids, that serve as substrates and products in metabolic pathways is particularly relevant to cardiovascular diseases. In addition to serving as disease biomarkers, circulating metabolites may participate in previously unanticipated roles as regulatory signals with hormone-like functions. Cellular metabolic pathways are highly conserved among species, facilitating complementary functional studies in model organisms to provide insight into metabolic changes identified in humans. Although metabolic profiling technologies and methods of pattern recognition and data reduction remain under development, the coupling of metabolomics with other functional genomics approaches promises to extend our ability to elucidate biological pathways and discover biomarkers of human disease.

9) Application of NMR to stable isotope tracer studies, Target identification and verification by NMR profiling.

10) Application of Metabolomics to Unique Human Cardiovascular Disease Models:

Novel metabolomics techniques still suffer from signal-to noise issues, however, and applications to humans may be limited by interindividual variability. Although recent studies have evaluated the diurnal and even seasonal variation of Haemostatic and inflammatory proteins (e.g., fibrinogen, Ddimer, and C-reactive protein), systematic studies have yet to be performed for metabolites in humans. Studies to identify novel disease-related pathways are also restricted by the Inherent unpredictability of the onset of pathological states. As noted previously, human metabolomics studies are also at High risk for potential clinical confounders, such as diet or drug effects, as well as age, gender.

11) Metabolomics study on the anti-depression effect:

The emerging field of metabolomics provides a promising opportunity to generate novel approaches for addressing the therapeutic effect of drugs, molecular mechanisms, and ultimately towards exploiting new ideal antidepressants. It has been increasingly used as a versatile tool for the discovery of molecular biomarkers in many areas such as diagnosing or prognosing clinical diseases, exploring the potential mechanism of diverse diseases, and assessing therapeutic effects of drugs. Recent

metabolomics technology has successfully applied high through put analytical tools to analyze various biological samples and utilized multivariate statistics to extract meaningful biological information from the resultant complex and huge data sets. Urine has been heavily used in metabolomics studies because it is minimally invasive to the animals or human and primarily reveals an overall metabolic state of the given organism.

12) Functional genomics:

Metabolomics can be an excellent tool for determining the phenotype caused by a genetic manipulation, such as gene deletion or insertion. Sometimes this can be a sufficient goal in itself—for instance, to detect any phenotypic changes in a genetically-modified plant intended for human or animal consumption. More exciting is the prospect of predicting the function of unknown genes by comparison with the metabolic perturbations caused by deletion/insertion of known genes. Such advances are most likely to come from model organisms such as Saccharomyces cerevisiae and Arabidopsis thaliana.

CONCLUSION:

The concept of research is directed towards the development of newer chemical entities for the treatment of various life threatening diseases and disorders. The fields of drug discovery and drug development mostly rely on systems biochemistry as it offers the global biochemical networks and molecular regulations. The field of metabolomics gaining its importance in relation to the study of these global biochemical networks and their regulations which will be proved as base for future drug discovery and development.

The Future Directions of Metabolomics:

In the future metabolomics will most likely be based on finding biomarkers in order to determine when disease is present in an individual biological system. Since there is already a use of biological keys to determine disease, such as glucose in urine means diabetes, or high cholesterol being more susceptible to heart disease, so it is clear metabolomics can take advantage of biochemical pathway knowledge. Currently metabolomics is focusing on specializing on 20-100 different metabolites, and although this is just a small portion it is making strides in discovering biomarkers. There is presently a speculation that metabolomics is the key to finding universal biomarkers for diagnosing disease. This has already been begun in experimentation as in the case for biomarkers for reversible myocardial ischemia which can be found through metabolomics, rather than through genomics or proteomics. This is because there is a sign of 60% to 70% rise in citric acid cycle components when there is a restriction of cardiac flow to the heart. These metabolomics changed can be found in the plasma of the blood and that this is a good new biomarker to find signs of somebody suffering from this disease. With some diseases already being diagnosed by these metabolomics biomarkers they could easily become the future of medical detection to diseases.

REFERENCES:

1. Poonam P. patel et al, metabolomics, International Journal of Research and Development in Pharmacy and Life Sciences, 2012
2. David S Wishart, Metabolomics for Drug Discovery, Development and Monitoring, Business Briefing: Futured Discovery 2006.
3. Dr Chris W W Beecher and Reid Tripp, Metabolomics – Applications in Drug Discovery and Development, Business Briefing: Pharmatech 2004.
4. Gregory D. Lewis et al, Application of Metabolomics to Cardiovascular Biomarker and Pathway Discovery, Metabolomics in Biomarker Discovery, JACC; 2008.
5. Nicholson JK, Lindon JC. "Systems biology: Metabolomics". Nature 2008.
6. A high-throughput method for microbial metabolome analysis using gas chromatography/ mass spectrometry. O. Fiehn, and R.H. Weiss. 2007.
7. A comprehensive urinary metabolomic approach for identifying kidney cancer, Parveen, I., J.M. Moorby, 2007.
8. Richard D. Beger et al, Metabolomics approaches for discovering biomarkers of drug-induced hepatotoxicity and nephrotoxicity, Toxicology and Applied Pharmacology, 2010.
9. George G. Harrigan, Ph.D et al Metabolomics in Alcohol Research and Drug Development, Metabolomics, Alcohol Research, and Drug Development, 2008.