ANALYTICAL METHODS BASED ON MASS SPECTROMETRY USED IN GENOMIC, PROTEOMIC AND METABOLOMIC STUDIES

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Abstract

This paper reviews the most important aspects of the literature on genomics, proteomics, and metabolomics studies based on analytical methods such as mass spectrometry (MS). Currently, most analyzes in the field of "omic" subjects are performed with mass spectrometers due to reproducible quantitative accuracy, and their ability to evaluate complex biofluids. This technology are faster, more sensitive, more reliable, and more automated than others.

A huge demand for a software that can process, analyse, and visualize large data sets has been generated by the ability to characterize more metabolites and allowed by recent improvements in mass spectrometry instrumentation. The application of mass spectrometry to genome annotation presents a number of challenges to standard workflows in genomics.

Key words: chromatography, metabolites, genome, bioinformatics

INTRODUCTION

Mass spectrometry (MS) is an analytical method applied in various fields of research: biology, medicine, chemistry, biochemistry or pharmacy; it is based on the principle of ionization of the analyzed substance followed by ion separation depending on the mass/ charge ratio. This analytical technique has a wide range of use and can be applied both in the case of pure substances and complex mixtures of substances [1].

Metabolomics studies the chemical processes involving metabolites, small molecule substrates, intermediates and metabolic products, while the metabolome refers to a complete set of small molecule chemicals found in a biological sample that can be represented by a cell, an organ, a tissue, a tissue extract, a biological fluid or even an entire organism. Therefore, metabolomics provides a "functional reading of the physiological state" of an organism. Another term often used in Metabolomics refers to the notion of Metabolites which are actually the intermediate products of metabolism, a term that refers especially to small molecules. Among the roles of metabolites are: structural, catalyst, signal, inhibition or stimulation for enzymes, defense and interaction with other organisms [5].

This paper presents the technical role of mass spectrometry in studies of “omic” subjects by addressing each stage of such an experiment, the workflow and methods that have been successfully implemented in various investigations.

MATERIALS AND METHODS

In order to achieve the assumed objectives of this study, there were consulted 14 scientific articles of different national and international databases. The most important aspects are presented in two different sections including: (i) the description of a metabolomics experiment based on mass spectrometry method; (ii) applications of

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The manuscript was received: 07.10.2020
Accepted for publication: 25.10.2020

mass spectrometry method in genomics, proteomics, and metabolomics studies.

RESULTS AND DISCUSSIONS

Deoxyribonucleic acid (DNA) analysis by mass spectrometry (MS) has evolved to the extent that it can be used to analyze most known types of DNA and ribonucleic acid (RNA). Implementation of MS for these forms of DNA analysis is reviewed.

(i) The description of a metabolomics experiment based on mass spectrometry method

Mass spectrometry has a big number of advantages, the best known being high sensitivity, specificity, high yield, accuracy in confirming and quantifying certain identified components, as well as the detection and identification of unknown compounds. Another important aspect is that mass spectrometry together with chromatography greatly expands the capacity of quantitative analysis of complex biological samples [13].

Mass spectrometry and metabolomics-based studies have been applied over time in various fields to identify biomarkers, for early diagnosis or to investigate changes in metabolism at different stages of the disease.

Metabolomic studies based on mass spectrometry involve several stages: (i) study design; (ii) sample collection and preparation; (iii) instrumental analysis; (iv) data processing and statistical analysis.

The science in the field of "omic" subjects reached a stage where high-throughput sequencing and MS cover all the large-scale domains: genomics, transcriptomics, proteomics, or metabolomics.

Most of researches agree that combining the results of all those techniques is the future of cell biology [12].

1. Extraction of metabolites

The conditions of the extraction process take into account both the type of solvent used and the physicochemical characteristics of the complex mixture represented by the sample, the effect of pH on it or the contact time. The efficiency of the extraction method is influenced by certain properties of metabolites, such as physicochemical diversity. The choice of an extraction solvent largely depends on the physicochemical properties of the target metabolites because due to the presence of a wide range of metabolites it is impossible to perform the extraction with a single solvent.

The combination of water and methanol has been proved over the time to help extract a wide range of metabolites, including sugars, amino acids, organic acids, acetyl coenzyme A, NADH / NADPH. For cell or microbial cultures, the most common protocol uses cold extraction solvents that involve organic solvents after removal of media or filtration.

An additional homogenization step is required for tumors and tissues. Tissues, especially hard tissues such as the heart or muscles, are frequently homogenized by grinding in the presence of liquid nitrogen. For most tissues and tumors, vortex homogenization is a sufficient technique to produce small fragments necessary for metabolite extraction [13].

2. Chromatographic separation

Liquid chromatography (LC) is a very important method in the study of complex matrices, increasing the detection limit for many analytes. Reverse phase of chromatographic separation involves non-polar stationary phase and a polar-polar phase, the last one being the most commonly used separation method. Strongly charged polar metabolites or more hydrophilic groups, such as metabolites from central carbon metabolic pathways, are not retained by the column. Thus, to increase the retention of polar metabolites, there are alternative chromatographic techniques. An example of this would be the Hydrophilic interaction liquid chromatography (HILIC) and Reverse phase chromatography with Ion Paring (IP) techniques, which are two methods intensively applied in various metabolomics studies which investigate metabolites associated with critical metabolomics pathways.

The hydrophilic interaction chromatography technique is a variant of normal phase liquid chromatography that partially overlaps with other types of

chromatographic applications, such as ion chromatography and reverse phase liquid chromatography. The HILIC method uses stationary hydrophilic phases with reverse phase eluents [4].

Paired ion reverse phase chromatography is the most common HPLC separation technique and is also applied to the separation of compounds that have hydrophobic parts and do not show a dominant polar character.

At the same time, the paired ion chromatography technique is a type of column chromatography in which the ions existing in solution can be paired or neutralized and then separated as a pair of ions on a reverse phase column. Ion pairing agents are usually ionic compounds containing a hydrocarbon chain [2].

In the field of research, the HILIC method is used with real success to monitor a large number of metabolites.

Regardless to the chosen chromatography method, metabolomics experiments using high-resolution mass spectrometry generate a fairly large amount of information in a single experiment.

Depending on the objectives of the study, the models of metabolomics experiments focus either on qualitative or quantitative information.

With the advancement of mass spectrometry instruments and data processing in various algorithms, the high-resolution mass spectrometry technique blurs the strict barriers between qualitative and quantitative analyzes.

3. Data processing and statistical analysis

The steps of processing data on unknown metabolites cannot be manually performed. For this step there is a specific Software, developed to facilitate this process and to achieve the objectives of the experiment.

There is also a software available to analyze information on ion mobility, such as Drift tube ion mobility spectrometry (DTIMS) - ion mobility spectrometry which is an analytical technique used to separate and to identify ionized molecules in the gas phase, based on their mobility in a carrier buffer gas [6].

In general, a metabolomics study contains several groups for comparisons. Thus, metabolomics analysis aims to determine the relative differences between the metabolomes of two or more groups to deduce a biological relationship. Often, difficulties arise in such analyzes due to the generation of data containing, many variables or the insufficient number of samples.

(ii) **Applications of mass spectrometry method in genomics, proteomics and metabolomics studies**

Among the various uses of mass spectrometry in genomics can be counted applications focused on the characterization of single nucleotide polymorphisms (SNPs) and short tandem repeats (STRs) which are particularly well-suited to MALDI or ESI-based spectrometry for nucleic acid analysis [10].

In a review article, published in 2010, Lee et al. reports on the practical applications of metabolomics, experiments based on mass spectrometry. Thus, the use of mass spectrometry in metabolomics analysis provides new information on the biochemical and cellular functions of living organisms and also detailed information on the composition of proteins and metabolites, as well as protein-metabolite interactions. In a single embodiment, the high sensitivity and resolution of mass spectrometry coupled with liquid chromatography (LC) or gas chromatography (GC) allows the detection and quantification of hundreds to thousands of molecules. The analysis of metabolites, based on mass spectrometry, facilitates, with a high yield, the reconstruction of metabolic networks, the discovery of biomarkers, the determination of tissue compositions and the functional annotation of both proteins and metabolites [7].

Another article published by Ren et al. (2018) reviews aspects of advanced mass spectrometry techniques and their latest applications in metabolomics, as well as the software / websites used in the analysis. Currently, mass spectrometry not only allows the analysis of information on the chemical nature of the extract obtained by methods such as LC-MS or GC-MS, but can also use

imaging methods to analyze intact tissue or cells, in order to provide location information of metabolites [11].

Dettmer et al. (2007) published a review in which presents an overview of the dynamic development of metabolomics based on mass spectrometry. The study primarily focuses on metabolic fingerprinting, a technique that analyzes all detectable metabolites in a given sample with subsequent classification. To perform this complex task, data analysis tools, metabolite libraries, and databases are required. Therefore, advances in bioinformatics applied in metabolomics are also an important part of such experiments [3].

In 2019, Liu et al. published an article on the new advances in analytical methods for the study of metabolomics based on mass spectrometry. Metabolomics study based on population cohorts is increasingly applied to identify important metabolites or critical metabolic changes related to metabolite disruption in disease states [8].

According to another article published by Luan et al. (2017), the emerging development of mass spectrometry has led to the discovery and quantification of small molecule neuroactive metabolites associated with the intestinal and brain microbiota (serotonin, bile acids, short chain fatty acids, polyunsaturated fatty acids, catecholamines). Significant progress has been made in characterizing the intermediate role of small molecule metabolites in neuronal development and neurodegenerative disorders. Also, in this article, the authors schematically present the principle of a metabolomics experiment based on mass spectrometry techniques (LC, GC, MALDI), using as samples blood, urine, and tissue, for extraction, identification, and analysis of metabolites of interest.

There is more evidence that small-molecule metabolites may play a critical role in mediating microbial effects on neurotransmission and disease development [9].

Another experiment performed by Xu et al. (2019) regarding the fecal metabolome, reveals that the analysis of intestinal microbial metabolites is essential to understand the molecular mechanisms of the interaction between the host and the intestinal microbiota. Faecal metabolomics analysis may reflect the results of ingestion, digestion, and absorption of nutrients by the intestinal microbiota and the gastrointestinal tract. Therefore, such a metabolomics experiment in faecal samples may be a way to study the outcome of host-gut microbiota interactions in clinical applications [14].

CONCLUSIONS

To date, various published studies have demonstrated the promising potential of mass spectrometry in metabolomics research.

The study of large-scale metabolomics is increasingly applied in various cases of disease and mass spectrometry is a major platform for this type of experiment, due to its unique advantages.

Numerous studies reveal that metabolomics based on mass spectrometry is becoming one of the most important techniques for precision medicine, in exploring the mechanism of the disease and in developing new therapeutic targets for neurodegenerative diseases or other diseases.

In the future, it is necessary to continuously develop new technologies (taking into account measurement sensitivity, spatial resolution, and sample consumption) for a high-efficiency analysis of metabolites and to improve the quality of the data obtained. Advances in bioinformatics applied to metabolomics also represent an important part of such experiments.

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