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A Review on Role of LC-MS in Metabolomics

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Abstract- Metabolomics, a replacement and rapidly growing area directed to the great analysis of metabolites of biological objects. Metabolites are characterized by various physical and chemical properties, traditionally studied by methods of analytical chemistry focused on certain groups of chemical substances. Metabolomics aims at identification and quantitation of small molecules involved in metabolic reactions. LC-MS has enjoyed a growing popularity because the platform for metabolomic studies thanks to its high throughput, soft ionization, and good coverage of metabolites. Because of significant improvements in LC-MS technology, metabolomics is increasingly used as a tool to discriminate the responses of organisms to varied stimuli or drugs. This review presents a workflow of a typical LC-MS-based metabolomic analysis for identification and quantitation of metabolites indicative of biological/environmental perturbations.

Key words: Metabolomics, Metabolites, LC-MS, Analytical Chemistry

I. INTRODUCTION

A modern biology study is characterized by employment of varied technological platforms describing properties of a biological object at genome, transcriptome, proteome and metabolome levels. Combined use of those platforms gives opportunity for systemic studies of varied processes in living systems; these studies include sequential "flow" of data from genes to phenotype of particular biological object. Thus, metabolomics represents the logical end point because a metabolite profile is that the most informative characteristics of the phenotype [1] .

During the past few years, technologies for global molecular profiling have revolutionised our understanding of biology with improved sequencing technologies resulting in major advances in genomic and transcriptomic studies and more selective and sensitive mass spectrometers driving rapid expansion of proteomic and metabolomic studies. Within parasitology, this resulted, as an example , within the relatively rapid whole genome sequencing of an entire range of pathogens (Plasmodium , Leishmania , Trypanosoma, Schistosoma , …) and therefore the application of metabolic fingerprints for the identification of biomarkers in parasitic infection. While genomics and transcriptomics study the start line of the molecular cascade leading towards a selected phenotype, metabolomics can study the last word expression of the genotype and is thus the profiling technology that works closest to the eventual phenotype. additionally , genome and proteome studies often struggle with functional annotation of identified sequences, while the metabolome consists of relatively few low relative molecular mass molecules – called metabolites –, many of which are key actors of cellular processes which are universal across organisms, like energy metabolism. Metabolomics is of particular interest for the study of our favorite model organisms, the unicellular trypanosomatid parasites, including pathogens like Trypanosome and Leishmania, as their organic phenomenon is regulated almost exclusively at the post-transcriptional level, in order that genome and transcriptome studies may need limitations, especially when studying the rapid effects of drug treatment or the mechanisms of drug resistance [2].

Metabolomics is idealized because the large-scale, qualitative, and quantitative study of all metabolites during a given biological system. Unlike transcripts and proteins, the molecular identity of metabolites can't be deduced from genomic information. Thus, the identification and quantification of metabolites must believe sophisticated instrumentation like MS, NMR spectroscopy, and laser-induced fluorescence detection. Each of those technologies has its own unique advantages and drawbacks [3].Optimal selection of a specific technology depends on the goals of the study and is typically a compromise among sensitivity, selectivity, and speed. Liquid chromatography coupled to mass spectrometry (LC–MS) may be a widespread metabolomics method due to its sensitivity, and its measurement strategies are broadly

classified into targeted and non targeted approaches. Reliable disease biomarkers are essential for establishing a complicated healthcare system where preventive measures, early diagnosis, and treatment are provided supported personalized risk assessments[4].Metabolomics may be a promising approach within the look for disease biomarkers because the metabolite concentrations of body fluids are considered as quantitative traits which will describe and define phenotypic characteristics of every individual, which are generated through interactions between genes and environmental influences. Metabolite profiling by nuclear resonance (NMR) has been described as a particular and reproducible method for biomarker discovery. Recently, metabolomics technologies supported mass spectrometry (MS) have made remarkable advances. The MS-based analysis of targeted metabolomics (T-Met) of primary and secondary metabolites has significantly contributed to biomarker discovery and therefore the elucidation of disease pathophysiology's; thus, this method has become widely employed as a flexible analytical technology. The methods for metabolite profiling have matured from those developed in small-scale studies with tens to many human subjects, toward developing more comprehensive analyses to realize understandings of disease states from large-scale studies with thousands of participants, like population-based prospective cohort studies[5].

General considerations

One of the most goals of metabolomics is that the differentiation between conditions supported the distribution of metabolites. Thus, results from metabolomics studies are commonly considered as quantitative. Nevertheless, many aspects of the analytical procedure can critically affect the precision and accuracy of LC–MS/MS methods. Their effect within the quantitative results is analyte dependent and may vary from individual to individual and even from sample to sample. Therefore, the influence of those factors on metabolomics studies shouldn't be underestimated. This impact substantially differs counting on the metabolomics approach selected. The driven force of untargeted metabolomics is that each one detectable metabolites are included as potential biomarkers. Therefore, this strategy aims at the detection of virtually all biomarkers. the large number of urinary products makes the control of the quantitative results an impossible task. On the opposite hand, targeted metabolomics implies the quantification of a limited number of metabolites. Targeted metabolomics studies are often conducted by using fully validated methods and, therefore the influence of every aspect of the entire procedure might be theoretically determined. In summary, the influence of things altering the quantitative data is usually under considered in metabolomic work flows, mainly when untargeted metabolomics is performed[6].

II. PREANALYSIS

Sample collection:

The first step is collection of samples, the utilization of standardized collectors and/or stabilizers is a smaller amount common in urine sampling. Thus, although sterile collectors made from non adsorbing material are normally utilized in metabolomic studies, some light-sensitive analytes are often altered by the utilization of common transparent tubes. the utilization of preservatives could also be helpful for the conservation of particular analytes. for instance , the addition of HCl to the sample has been found to be suitable for the preservation of some urinary metabolites like acid . Unfortunately, a universal preservative for the entire preservation of urine metabolome doesn't exist[7].thanks to these considerations, a number of the results obtained by untargeted metabolomics might, to some extent, be questionable. On the opposite hand, the effect of sample collection on quantitative methods should be compulsory checked before its application to targeted metabolomics studies[8].

Sampling time:

Although urinary metabolome of a specific individual are often seen as static, it's suffering from several factors both external, like diet, and internal, like circadian rhythms. Thus, sampling time may need a critical impact on metabolomics results. The detection of adrenal hormones may be a typical example for circadian variation. For these compounds, urinary concentrations varying up to 10 fold are often obtained counting on the sampling time. the gathering of all samples from a study at an equivalent time can minimize this effect, but differences on circadian rhythms counting on life pattern, for instance , night-shift versus day-shift workers, can't be discarded. The gathering of 24-h urine samples is then preferred to neutralize circadian fluctuations. Besides, the utilization of 24-h urine samples also facilitates the normalization of the results. However, this selection made the sample collection more difficult. Metabolomic studies getting to determine specific biomarkers for a characteristic diet have revealed how important it's on the metabolomic outcome. Various families like lipids, amino acids or acylcarnitines are reported to be altered by dietary habits. Therefore, these considerations should be taken under consideration both when interpreting untargeted metabolomic results and through method validation in targeted approach[9].

Stability & storage:

Besides the sampling, the time passed between collection and analysis may additionally be critical for the evaluation of the urinary metabolome. Ideally, urine samples should be analyzed within 2h after collection. From a metabolomics point of view, this ideal situation is difficult to be put into practice since collection of an adequate number of cases and controls usually requires an outsized period of your time . Immediate freezing of the sample is suggested so as to avoid undesired microbial degradation. However, urine metabolome shows an adequate short stability at 4 ◦C and it remains mostly unaltered during the first 24-h. As a summary, a short-term storage at 4 ◦C previous to the long-term storage at -80 ◦C seems to be an adequate protocol for metabolomic studies. In targeted metabolomics, the short- and long-term stability of the chosen metabolites should be tested during method validation. One among the most problems regarding stability of urine is that the occurrence of microbial contamination. Microorganisms present within the sample can alter the concentration of a several metabolites[10].Thus, undesired secondary reactions like deconjugation, oxidation, demethylation or hormone deterioration are reported to occur in urine. Microbial degradation are often minimized by the addition of some preservatives.

Sample treatment

Filtration:

In metabolomics studies, the preservation of the whole metabolome is one among the key steps .For this reason, un selective sample treatments are often preferred. Although the direct injection of the urine sample into the LC–MS/MS system is feasible , a previous filtration to get rid of materials in suspension are often employed. This filtration is usually performed on filters of cellulose with 0.2–0.45 μm pore size. the utilization of special membranes with cutoff of 3–30 k Da has also been reported so as to hide hydrophilic metabolites. Additionally, filtration also prevents bacterial growth during storage[11].

Hydrolysis of conjugates:

Intrinsic complexity of urine may be a double-edged sword in metabolomics. On one hand, the massive number of metabolites and their relatively high concentrations make urine ideal for metabolomics. On the opposite hand, the massive variability of metabolites and their frequent indirect implications on the tested status hamper the direct usefulness of the results obtained. phase II clinical trial metabolism may be a key example of the newest . An outsized number of endogenous compounds undergo phase II clinical trial metabolism consisting on the conjugation with polar groups. Thanks to this conjugation, many endogenous components aren't exclusively excreted unconjugated but also as glucuronides or sulfates[12].Conjugation with other compounds like N-acetyl cysteine or the amino acids, taurine and glycine, are common for species like volatile organic compounds or bile acids, respectively. The relative abundance of the various conjugates may depend upon several factors like polymorphisms on specific enzymes or the diet. Therefore, the implication of a metabolite within the studied status might remain hidden by the between-group variability of the conjugation reactions. One possibility to attenuate this effect is to perform a hydrolysis step previous to the LC–MS analysis. In untargeted metabolomic studies one among the goals during sample preparation is to stay the sample as intact as possible. Thus, so as to preserve all unknown metabolites in their original form, hydrolysis isn't common. Contrarily, this practice is frequent for targeted approaches designed to research metabolic pathways during which phase II clinical trial plays a crucial role[13].

Both enzymatic and chemical hydrolysis are often used. So as to pick the foremost appropriate one, the analyst should take into consideration factors just like the hydrolysis efficiency, the potential generation of artifacts and therefore the production of additional interferences altering the LC–MS/MS response. As an example, enzymatic hydrolysis is preferred for steroid analysis, whereas chemical hydrolysis is taken into account simpler for the urinary detection of catecholamines[14].

Derivatization:

One of the well-known advantages of LC–MS/MS procedures is that the absence of a derivatization step as an analytical prerequisite. However, derivatization can provide substantial benefits to metabolomics strategies. The foremost evident is that the possibility to enhance the quantification limits of targeted metabolites. Besides, specific derivatization reactions can improve key steps of the procedure like the clean-up the extraction and therefore the chromatography. Derivatization is additionally a convenient resource so as to bypass the shortage of ionization in ESI of some chemical features like carbonyl or hydroxyl groups16. Thus, although the direct quantification of some metabolites of the tricarboxylic acid cycle has been reported, several aspects like the chromatography, the sensitivity of the tactic and therefore the potential separation of isomers clearly improve after derivatization. Remarkably, derivatization also provides new insights to untargeted metabolomics. the foremost important one is that the development of stable-isotope labeling studies. As an example, the utilization of dansylation allowed for labeling untargeted metabolites containing primary amine, secondary amine or phenolic hydroxyl group. This strategy improves both sensitivity and LC behavior of selected submetabolome. Additionally,

the derivatization with a labeled dansyl chloride improved also the quantitative parameters obtained in untargeted metabolomics approaches. Several derivatization reagents are developed to use this strategy to different branches of the metabolome. Thus, 5-diethylamino-naphthalene-1-sulfonyl chloride has been described as alternative to dansyl derivatization. Additionally,p-di methyl aminophen acyl bromide and omega-bromoacetonyl quinolinium bromide are described for the determination of carboxylic acids and thiols, respectively.

III. INSTRUMENTAL ANALYSIS

LC considerations:

Contrarily to other chromatographic systems, LC features a broad portfolio of stationary phases, column dimensions and mobile-phase additives which may be wont to found optimal chromatographic conditions. Among them, the utilization of C18 stationary phases is that the default choice for untargeted metabolomics approaches. However, these columns are unable to properly resolve polar compounds. For this reason, the utilization of hydrophilic interaction LC (HILC) is becoming a well-liked complement in untargeted approaches. These columns are capable of retaining polar/ionic metabolites which, combined with the results on C18 columns, leave the determination of the most important number of metabolites in one sample. Despite the great coverage obtained by the mixture of both chromatographic methods, specific chromatographic conditions are still required for the right determination of some metabolites. As an example, proper reversed-phase chromatography of urinary bis-sulfates requires an outsized amount of salts within the mobile phase. In summary, targeted approaches leave the choice of optimal chromatographic conditions for the chosen metabolites whereas the utilization of general conditions in untargeted studies might hamper the right determination of some parts of the urinary metabolomics[15].

Ionization:

Although LC–MS/MS is usually considered as a universal technique, it's its main bottleneck within the ionization process. Indeed, only those molecules which are ionized within the interface are often detectable by LC–MS/MS. Several air pressure interfaces are developed to hide the ionization of the widest range of chemical structures. Among them, ESI is far and away the foremost popular one for metabolomic investigations. Nevertheless, some studies used other ionization sources like air pressure chemical ionization and air pressure photo ionization. generally , these interfaces are considered complementary to ESI as some analytes are efficiently ionized only by one among them. Therefore, the choice of the interface in untargeted methods will limit the a part of the metabolome detected. a mixture of the three interfaces has been reported because the most comprehensive strategy for the detection of biomarkers in untargeted metabolomics. almost like other analytical characteristics, the pre selection of the metabolites in targeted methods also will guide the choice of the foremost adequate interface. Thus, ESI is preferred within the detection of phase II clinical trial metabolites whereas the utilization of field-free air pressure photoionization has been reported to extend the sensitivity for a few steroid[16].

Analyzers:

From a metabolomics point of view, LC–MS analyzers are often divided between low- and high-resolution analyzers. High-resolution analyzers are preferred for un targeted approaches thanks to the structural information of the potential biomarkers provided by the formula calculated supported the accurate mass measurements. Hence, most of the untargeted approaches use either TOF or orbitrap analyzers. On the opposite hand, structural information is a smaller amount critical for targeted approaches since comparison of retention times and ions abundances with reference allow unequivocal identification. Thus, sensitivity became the foremost important parameter for targeted studies. For this reason, SRM approaches using triple quadrupole analyzers are preferred for targeted methods.

Data Interpretation:

After data collection by LC-MS, the first spectra must be subject to noise signal filtering, alignment, feature detection, and normalization to get the data which will be further processed. Different vendors provide different built-in software for processing . As for the spectra produced by LC-MS, the height area integration results are often extracted as data , followed by integrating all data for various samples during a uniform format, which may be a very crucial step in metabolomics study. the utilization of high-throughput detection analytical tool can obtain vast amount of knowledge . However, if not be reasonably processed, these interfering data are going to be detrimental to the study work. Pattern recognition and multi-dimensional statistical methods are often wont to extract useful information from the vast amount of knowledge. These methods can conduct data dimensionality reduction, making it easier for visualization and classification. Currently, two commonly used algorithms in data analysis are the pattern finding-based unsupervised method and supervised method. additionally , at each stage of knowledge

process and analysis, it must be also careful for the standard control of knowledge and therefore the validity check of model [17].

Unsupervised Method:

Unsupervised method is employed to explore completely unknown characteristics of the info. The first data information is classified in accordance with the characteristics of the sample, and therefore the target data with similar characteristics are classified within the homological class and are visually expressed by appropriate visualization technologies. Common methods utilized in this field include cluster analysis and principle components analysis (PCA).

Supervised Method:

If there's some prior information and assumptions related with the info , the supervised method is more suitable and simpler than unsupervised method. Supervised method establishes class information on the idea of existing knowledge and makes use of the established class information to spot , classify, and predict the unknown data19. Because there are train samples available for learning within the model establishment, this method is known as as supervised learning. Common methods utilized in the field include linear discrimination analysis and partial least square discriminant analysis (PLS-DA) [18].

New Trends in LC-MS Development

There are still many challenges in current LC- and LC-MS-based metabolomics study, a neighborhood of which lies within the instrument. For instance, the resolution of LC isn't enough high, it's difficult to hide all chemicals during a run, especially for top complexity sample. Thanks to the bias of various analytical methods to chemicals, some specific compounds often can't be detected even in an improper method; another challenge comes from informatics, the structural elucidation of unknown compound may be a bottleneck for the rapid development of metabolomics and another challenge is to explore of high-efficient data analysis methods. Bino et al. (2004) proposed that currently, there are two restriction factors limiting the analytical platform in metabolomics research: The first one is the way to improve the separation capacity of LC; the other is the way to improve the identification ability to compounds, then the developmental trend in LC-MS is expanded around these two points[19].

IV. CONCLUSION

The present review, we've described the foremost relevant issues to be taken under consideration when performing targeted metabolomics analyses of human urine by LC–MS/MS. Regarding the near future, LC–MS/MS will remain the favored analytical technique to guage the metabolome in any biological specimen, and especially in urine. Alone, or together with other sorts of instrumentation it'll play a pivotal role during this field. As a consequence, it's anticipated that targeted methods by LC–MS/MS are going to be increasingly used for the study of the metabolomics. The derived results will have incidence in many fields from personalized medicine, to clinical diagnosis, to doping control analyses. More importantly, they could help researchers by providing new hints on the biological interactions, which successively might cause the invention of latest biological mechanisms.

CONFLICT OF INTETEREST

The authors declare that there are no Conflicts of Interests

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REFERENCES

1. Rinschen MM, Ivanisevic J, Giera M, Siuzdak G, Identification of bioactive metabolites using activity metabolomics, Nat. Rev. Mol. Cell Biol, 2019; 20: 353–367.
2. Cajka T, Fiehn O, Toward merging untargeted and targeted methods in mass spectrometry-based metabolomics and lipidomics, Anal. Chem, 2016; 88: 524–545.
3. Nikolskiy I, Mahieu NG, Chen YJ, Tautenhahn R, Patti GJ, An untargeted metabolomic workflow to improve structural characterization of metabolites, Anal. Chem, 2013; 85: 7713–7719.
4. Wang R, Yin Y, Zhu ZJ, Advancing untargeted metabolomics using data-independent acquisition mass spectrometry technology, Anal. Bioanal. Chem,2019; 411: 4349–4357.
5. Cai Y, Guo Y, Chen F, Targeted metabolite extraction of multiplexed MS/MS spectra generated by data-independent acquisition, Anal. Chem, 2016; 88: 8757–8764.

6. Naz S, Gallart-Ayala H, Reinke SN, Mathon C, Development of a liquid chromatography-high resolution mass spectrometry metabolomics method with high specificity for metabolite identification using all ion fragmentation acquisition, Anal. Chem, 2017; 89: 7933–7942.
7. Beckonert O, Keun HC, Ebbels TM et al, Metabolic profiling, metabolomic and metabolomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts, Nat. Protoc. 2007;2(11): 2692–2703
8. Khamis MM, Adamko DJ, El-Aneed A, Mass spectrometric based approaches in urine metabolomics and biomarker discovery, Mass Spectrom,Rev, 2017;36(2): 115–134.
9. Deng P, Zhan Y, Chen X, Zhong D, Derivatization methods for quantitative bioanalysis by LC–MS/MS, Bio analysis. 2011; 4(1): 49–69.
10. Al Kadhi O, Melchini A, Mithen R, Saha S, Development of a LC–MS/MS method for the simultaneous detection of tricarboxylic acid cycle intermediates in a range of biological matrices, J. Anal. Methods Chem, 2017; 539-545.
11. Tang DQ, Zou L, Yin XX, Ong CN, HILIC-MS for metabolomics: an attractive and complementary approach to RPLC-MS. Mass Spectrom, Rev, 2016; 35(5): 574–600.
12. Simon Cubbon, Carla Antonio, Julie Wilson, Jane Thomas-Oates. Metabolomic applications of HILIC-LC-MS, Mass Spectrom Rev, 2010; 29(5):671-84.
13. Evans CR, Jorgenson JW, Multidimensional LC-LC and LC-CE for high-resolution separations of biological molecules, Anal Bioanal Chem, 2004; 378: 1952–61.
14. Domingo-Almenara X, Montenegro-Burke JR, Benton HP, A computational solution for streamlining metabolomics analysis,Anal. Chem, 2018; 90: 480–489.
15. Chaleckis R, Naz, S, Meister I, LC-MS-Based metabolomics of bio fluids using all-ion fragmentation (AIF) acquisition. In Clinical Metabolomics: Methods and Protocols, Giera, M., Ed.; Humana Press: New York, NY, USA, 2018; 1730: 45–58.
16. Pluskal T, Nakamura T, Villar-Briones A, Yanagida, Metabolic profiling of the fission yeast S. pombe: Quantification of compounds under different temperatures and genetic perturbation, Mol. BioSyst, 2010; 6: 182–198
17. Bogusz M, Aderjan R, Corrected retention indices in HPLC: Their use for the identification of acidic and neutral drugs, J. Anal. Toxicol, 1988;12: 67–72.
18. Bevilacqua, Marta, Marini, FedericoLocal classification: Locally weighted-partial least squares-discriminant analysis (LW-PLS-DA) science.gov, 2014; 08-01.
19. Guodong Wang LC-MS in plant metabolomics In book: Plant Metabolomics Edition: 1 Springer Netherlands,45-61.