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Modern developments and emerging directions in hydrophilic interaction chromatography-mass spectrometry for metabolomics and proteomics

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ABSTRACT

Proteomics and metabolomics have drawn more interest during the past ten years than any other -omics methods. Both methods have matured to the point where they are relevant in many clinical applications, including as the discovery of biomarkers, enhanced illness diagnosis, staging, and prognosis, as well as a greater understanding of many physiological processes. Due to its simplicity and reproducibility, reversed-phase liquid chromatography mass spectrometry is regarded analytically as the gold standard in proteomics and metabolomics. The complexity of the proteome cannot be resolved by RPLC-MS alone, because highly polar metabolites are often poorly preserved. Due to its orthogonal separation process, hydrophilic interaction chromatography in this context constitutes an appealing supplementary technique. This review provides a summary of the research literature regarding the use of HILIC-MS for proteomics and metabolomics. In contrast to proteomics, which discusses the analysis of complex samples and protein post-translational modifications therein using bottom-up, middle-up/down proteomics, and intact protein analysis, metabolomics focuses on the analysis of bioactive lipids, amino acids, organic acids, and nucleotides/nucleosides. The technical aspects of HILIC-MS utilisation in proteomics and metabolomics are covered in the review, with special focus paid to the stationary phases, mobile phase parameters, injection volume, and column temperature. Additionally shown and discussed are recent trends and advancements in the use of HILIC-MS in proteomics and metabolomics, emphasising the benefits the method can offer in addition to or as a complement to RPLC-MS as well as the current drawbacks and potential remedies.

Keywords: Electrostatic repulsion hydrophilic; interaction chromatography; Hydrophilic interaction chromatography; HILIC-MS; Metabolomics; Proteomics

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INTRODUCTION

Proteomics and metabolomics have attracted the clinical community's most recent attention among all -omics methods [1]. The term proteomics refers to the thorough examination of the proteome, or the entire set of proteins that an organism or biological system produces or modifies [2]. The proteome of an organism is not constant, unlike the genome, and evolves dynamically over time [3]. Because of this, biological markers of altered physiological conditions, such as the development of diseases, are often studied, including variations in protein expression levels, chemical modifications, and conformational changes [4]. The tremendous complexity of the proteome and broad dynamic range provide significant difficulties in large-scale proteomics research [5]. In a complex organism, there are thousands of proteins [6]. Additionally, these distribute due to the diversity of PTMs, which could have given rise to hundreds of thousands of distinct protein species [7]. Additionally, it is predicted that the dynamic range of expression levels for various proteome components will lead to variations in concentration in the order the most typical method for identifying and measuring proteins is liquid chromatography coupled to mass spectrometry [8]. The most popular method is bottom-up proteomics, which identifies proteins from peptides produced by the hydrolysis of particular enzymes [9]. Other methods involve the use of enzymes that hydrolyze uncommon amino acids or distinctive subunit sequences [10]. The outcome of middle-down or middle-up proteomics is the analysis of bigger peptides or subunits. Last but not least, proteins the broad examination of metabolites, intermediaries, and end products of cellular metabolism in biological samples is known as metabolomics. Lipids, amino acids, organic acids, vitamins, hormones, nucleosides/nucleotides, minerals, and xenobiotics like pharmaceuticals, food additives, and pollutants are all included in the metabolome. It is extremely complex and exhibits a wide variety of compounds with distinct physicochemical characteristics, the presence of many isoforms, and wide dynamic ranges between minute amounts of compounds and species that are quite abundant. There are two types of metabolomics approaches: targeted and untargeted. Targeted metabolomics involves the highly accurate and repeatable quantification of known biomarkers from one or more metabolic classes as opposed to untargeted metabolomics, which involves a thorough and unbiased analysis of all metabolites present in a biological sample

in search of potential biomarkers. A variety of analytical Proton nuclear magnetic resonance spectroscopy or capillary electrophoresis, gas chromatography, and liquid chromatography with mass spectrometry are some of the methods that have been investigated for proteomics and metabolomics investigations.

DISCUSSION

The most used method among these continues to be LC-MS employing atmospheric ionisation interfaces, particularly electrospray ionisation. In comparison to other methods, LC-MS provides a wider dynamic range and greater sensitivity. Proteomics and metabolomics often use LC-MS techniques that have high throughput, high resolving power, and/or high selectivity. Similar to proteomics, metabolomics has received more attention in recent years for its potential to enhance illness diagnosis, staging, and prognosis, identify biomarkers and therapeutic targets, and shed light on physiological processes. Switched phases Because of how simple it is to use, LC-MS has long been regarded as the gold standard in proteomics and metabolomics. Reproducibility of usage and retention period. However, a wide range of highly polar and ionisable metabolites are not adequately retained by RPLC despite numerous attempts to boost the retention of polar molecules. Furthermore, RPLC-MS alone cannot resolve the great complexity and dynamic range of the material analysed in proteomics. Therefore, a complementary LC separation before RPLC-MS is frequently considered when a thorough analysis is required in order to either enrich for particular populations or to reduce the complexity of the proteome by fractionating the sample components into several sub-samples.

CONCLUSION

In metabolomics and proteomics, hydrophilic interaction liquid chromatography is an effective substitute for or addition to RPLC-MS, assisting in overcoming some of the aforementioned difficulties. Alpert coined the phrase "HILIC" for the first time in 1990. HILIC due to its high peak capacity and selectivity for separation in LC-MS analysis is currently the second most popular chromatographic mode employed. The polarity of the stationary phase, the composition of the mobile phase, and the type of substances being examined make HILIC a hybrid technique between RPLC, normal phase LC, and ion exchange chromatography. The partition of analytes between a stationary phase that is hydrophilic and a mobile phase that is mostly hydrophobic is what causes the retention in HILIC. HILIC frequently employs silica, amino propyl, amide, diol, and zwitterionic columns as stationary phases. A biological system is made up of a large number of molecules with a wide range of physical and chemical properties that exist throughout a wide dynamic range in biological samples. Metabolomics is the systematic study of all the metabolites that are present in a biological system. To attain greater metabolite coverage, a variety of analytical approaches are required. Since the discovery and development of electrospray ionisation and matrix-assisted laser desorption ionisation techniques, the use of mass spectrometry in metabolomics has grown tremendously. Significant advances have also occurred in separation-based MS techniques. Liquid chromatography-mass spectrometry, capillary electrophoresis-mass spectrometry, and ion mobility-mass spectrometry, as well as separation-free MS techniques direct infusion-mass spectrometry, matrix-assisted laser desorption ionization-mass spectrometry, mass spectrometry imaging, and direct analysis in real time mass spectrometry in the past decades. This analysis offers a succinct summary of current sophisticated MS techniques and their most recent uses in metabolomics. Additionally assessed are the MS result analysis applications and websites. After genomes, transcriptomics, and proteomics, the growing omics discipline of metabolomics has emerged. It is an essential component of systems biology. In metabolomics, biological fluids, human tissues, and cells are the typical analytical items. Metabolites are the byproducts of intricate cellular regulatory networks1, and through feedback loops, they can also affect or even change regulation.2 Physiological, pathological, and metabolic status can be immediately understood through the comprehensive study of metabolites, which can then be integrated with chemical and informatics technologies. Additionally, endogenous small-molecule metabolic alterations can be identified, and related biomarkers can be acquired.

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