Recent and current progress in LC-MS-based metabolomics techniques

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An altered metabolism often points to a disease, so it is pivotal to understand the mechanisms controlling endogenous metabolites. This need has given rise to metabolomics, the comprehensive study of the small molecule metabolites that reflect the complex network of biochemical reactions in a tissue, organ or the entire organism. However, obtaining wide coverage of the metabolome remains challenging because of the huge number of metabolites, which can span over nine concentration orders of magnitude and possess a wide variety of physicochemical properties. Acquiring the data of thousands of samples with accurate and reproducible quantifications can also prove technically difficult. Nevertheless, progress has been enormous. Metabolome analysis by mass spectrometry (MS) has witnessed exponential growth over the last decade, particularly in the clinical and pharmaceutical fields, owing to the technique's high selectivity, sensitivity, resolution, and faster data acquisition. It is becoming a powerful tool for biomarker discovery, early disease diagnosis, prognosis, identifying new therapeutic targets, investigating how new drugs work, characterizing safety and efficacy profiles, as well as stratifying patients and monitoring therapeutic response.

The advantages of LC-MS in metabolomics

In comparison to other techniques such as GC-MS, LC-MS is more tolerant to volatility, covers more metabolites, allows higher throughput and does not require the derivatization step that GC-MS usually does. Ultra-performance liquid chromatography has significantly improved resolution, peak capacity, reproducibility, and sensitivity. Reversed-phase LC (RPLC) is favored for the separation of semi-polar metabolites, such as liposoluble compounds, phenolic acids, flavonoids, alkaloids, and glycosylated species, while hydrophilic interaction LC (HILIC) is often used for polar and charged metabolites, such as sugars, amino sugars, amino acids, vitamins, carboxylic acids, and nucleotides. The ionization technique of choice for LC-MS is electrospray ionization, which must be performed in positive and negative mode for a broad coverage of the metabolome. In our lab, we have been successfully using both RPLC-MS and HILIC-MS to analyze different classes of metabolites. In addition to these two main LC-MS techniques, capillary electrophoresis MS, imaging mass spectrometry, and ion mobility MS utilizing collision cross section databases have also become more widespread.

More standards and reference materials needed

MS-based metabolomic approaches may be untargeted, targeted or a hybrid semi-targeted. The untargeted approach is widely used for unbiased and broad metabolite profiling, an example of which is comparing normal and diseased state metabolic profiles to discover biomarkers. Quantitative differences between sample groups yield a list of candidate biomarker metabolites that can help to diagnose diseases or uncover the molecular mechanisms underlying a disease. Unfortunately, there are no guidelines or clear quality assurance (QA) acceptance criteria for such untargeted approaches. Therefore, quality control (QC) and system suitability are critical for acquiring and reporting high-quality data. QC samples are important to ensure data reproducibility over time, while system suitability, which can be based on a combination of selected metabolites, is assessed to ensure that mass accuracy and chromatographic performance characteristics, including

retention time, peak shape, and peak area, conform to pre-defined acceptance criteria. Although QC samples and system suitability can ensure data quality within a particular laboratory, they do not allow standardization and harmonization across different laboratories. Hence there is a need for standard reference materials. The most widely used are SRM 1950, which is certified for more than 40 human plasma metabolites, and the urine reference material RM 8232, both developed by the National Institute of Standards and Technology (NIST). New human source reference materials for metabolomics, including frozen plasma suite, frozen urine suite, liver suite, liver extract, and liver tissue, are in the NIST pipeline. Merck has developed a variety of certified reference standards, suitable for targeted and untargeted MS-based metabolomics, that contain compounds such as oligonucleotides, amino acids metabolites, lipids, carbohydrates, bile acids, and vitamin D, catecholamines and neurotransmitters, as well as male, female, neonatal, and thyroid hormones. The Mass Spectrometry Metabolite Library of Standards (MSMLS™), containing key primary metabolites of different compound classes are another recent development. These libraries are mostly used to provide retention times and spectra for key metabolic compounds, help optimize analytical MS protocols, and qualify and quantify sensitivity and limits of detection. However, there remains a need for additional standards and reference materials.

From untargeted to targeted approach

Nearly all efforts with the untargeted approach ultimately lead to a targeted approach for the final analytical step to validate the results, during which variations of a defined number of metabolites in a specific pathway are quantified, usually by selective ion or multiple reaction monitoring mode analyses. Unlike the untargeted approach, the targeted approach has clear QA acceptance criteria and guidelines for bioanalytical validation established by the United States Food and Drug Administration and the European Medicines Agency. It provides high-quality data with great precision and accuracy, especially if an isotopically labeled internal standard, matching or analogous to the target analyte, is spiked into the samples and authentic standards early in sample preparation to normalize sample handling and analytical measurement variations. Multi-level calibration is subsequently performed to determine the concentration of the observed metabolites. Progress continues, and new methods are making headway. A recent one is parallel reaction monitoring, which has shown reliable performance in the targeted analysis of metabolites.

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