Miniaturized metabolomics methods for enabling the study of biomass-restricted samples

He, B.

Appendix

Summary

Nederlandse samenvatting

Curriculum vitae

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Acknowledgements

Summary

Mass spectrometry (MS)-based analytical methods are widely utilized in both academic and clinical research, providing critical insights into biological and disease mechanisms. Despite their high sensitivity and throughput, conventional MS methods face significant challenges when analyzing biomass-restricted samples. Pooling such samples to meet detection limits compromises sample heterogeneity—an essential factor in metabolomics and personalized medicine. Additionally, non-invasive sampling techniques often yield only picoliters to microliters of sample volume, creating a mismatch between available sample quantities and the capabilities of standard analytical methods.

To address these challenges, this thesis explores miniaturized analytical techniques that enhance sensitivity and performance while maintaining robustness. By optimizing micro-flow liquid chromatography-mass spectrometry (micro-LC-MS) and sheathless capillary electrophoresis-mass spectrometry (CE-MS), analytical workflows are tailored for specific metabolite classes based on their physicochemical properties. Micro-LC-MS is employed for lipid analysis, while sheathless CE-MS is applied to polar and charged metabolites such as amino acids. These methods are validated and applied to various biomass-restricted biospecimens, demonstrating their feasibility for biological and clinical research.

Chapter 1 outlines the limitations of conventional MS methods for biomass-restricted samples and introduces miniaturized MS workflows as a solution. It also provides an overview of the thesis structure and objectives.

Chapter 2 critically reviews microscale analytical techniques, emphasizing their relevance in metabolomics and small-volume biological sample analysis. It discusses recent advancements in micro-LC-MS, nano-LC-MS, and CE-MS, highlighting their sensitivity, robustness, and feasibility. While these miniaturized methods show great promise, further optimization of sampling and sample preparation is necessary to fully harness their potential. The chapter also underscores the importance of system components—such as low-flow LC and optimized MS ionization sources—designed for minimal sample amount.

Endocannabinoids play a crucial role in brain function and pathology, but their analysis in biomass-restricted samples such as human cerebrospinal fluid (CSF) remains challenging.

Chapter 3 details the development of a micro-LC-MS workflow for the selective and sensitive determination of endocannabinoids and their analogs in CSF. A modified micro-electrospray ionization (micro-ESI) spray needle (Shimadzu Mikros) was employed to enhance sensitivity and durability. The developed method enabled the analysis of 288 CSF samples, providing valuable insights into endocannabinoid profiling in clinical studies while maintaining analytical robustness.

Chapter 4 explores the application of micro-LC-MS for analyzing oxylipins, a class of bioactive lipids, in human plasma samples. Unlike endocannabinoids, oxylipins require negative ionization mode for optimal analysis, which is often affected by ionization discharge issues. These challenges were addressed using an OptiFlow ionization source, achieving superior sensitivity and robustness for oxylipin analysis in 5 µL of plasma. The developed method was validated and compared with conventional UHPLC-MS, demonstrating significant sensitivity enhancements. The workflow was applied to 40 plasma samples from a healthy aging study, showcasing its applicability in clinical research and biomarker discovery.

Miniaturized analytical methods are not limited to hydrophobic compounds analyzed via LC-MS. **Chapter 5** presents the development of a sheathless CE-MS workflow for polar and charged compounds, specifically targeting creatinine quantification in residual pediatric plasma samples. CE-MS is particularly advantageous for volume-limited samples due to its zero dead volume and high ionization efficiency. The sheathless CE-MS method demonstrated high sensitivity and reliability, enabling creatinine quantification using only 5 µL of plasma. A multi-segment injection strategy was implemented, allowing seven samples to be analyzed in a single electrophoretic run. The results correlated well with clinical measurements, validating the method's applicability in healthcare settings. Beyond creatinine, the method also identified a range of metabolites, highlighting its potential for metabolomics applications in neonatal healthcare.

The final chapter synthesizes the key findings of this thesis, emphasizing the promising future of miniaturized MS-based analytical methods for addressing complex biological and pharmaceutical questions in biomass-restricted samples. By integrating state-of-the-art microsampling devices, efficient sample processing techniques, and robust miniaturized

analytical instruments, significant biological insights can be obtained from minute sample volumes, paving the way for new advancements in clinical and metabolomics research.