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

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**Stellingen**

**Behorende bij het proefschrift**

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1. The issue of volume mismatch between collected samples and sample analysis workflow can be addressed by employing a miniaturized analytical workflow, which allows high detection sensitivity with a minimal sample volume. (Chapter 5)
2. Sensitivity loss is likely to happen when there is limited current and the charge on each sample droplet is lower than the number of ionizable molecules. With micro or nano flow rates, smaller droplets with higher surface-to-volume ratio will not only increase the detection sensitivity, but also broaden the coverage of metabolites. (Chapter 3&4)
3. System robustness can be compromised during the development of miniaturized techniques. To mitigate this issue, it is crucial to focus on two aspects: ensuring efficient sample preparation and developing robust analytical systems. (Chapter 3&4)
4. Future developments should put more emphasis on miniaturized sampling and sample preparation methods in order to further increase the sensitivity and strengthen the robustness of miniaturized analytical methods. (Chapter 2)
5. The advantage of reducing the column diameter is not a gain in sensitivity but the ability to obtain similar detection sensitivity as with conventional LC systems with a considerable reduced sample volume. (Hilhorst et al., Bioanalysis, 2014) This emphasizes that lower flow rates minimize in-source dilution and thereby preserve sensitivity. Sheathless CE follows the same principle of reducing dilution effects, but further improves detection efficiency through direct electrospray coupling.
6. Along with the compelling advantages provided by miniaturized sampling and separation, detection by ESI–MS must also fit the paradigm of miniaturization. (Needham et al., Bioanalysis, 2015) This highlights the importance of the efficient ionization provided by nano-ESI-MS. By using finer tip emitters and electrostatic-driven droplet formation, miniaturized ESI-MS generates smaller droplets and a more stable spray, leading to higher ionization efficiency.
7. Micro-LC falls far behind nano-LC regarding sensitivity increase, but the gain comes at almost no cost: Micro-LC can be installed on the same instruments as microbore LC and thus offers equal robustness, method adaptability and ease of use. (Fitz et al., Frontiers in Molecular Biosciences, 2022) While sensitivity is important, successful bioanalytical applications require optimization of the entire workflow—including robustness, method flexibility, efficiency, and system integration. Micro-flow provides a practical balance of these factors, making it a strong candidate for future bioanalysis.

8. The way forward for bioanalytical MS is micro flow LC–MS, yet bioanalysts today may not even recognize the look of the fully ‘integrated’ instrument 20 years from now. (Needham et al., Bioanalysis, 2017) Eight years later, early signs of this integration are emerging. Platforms like EVOSEP and chip-based LC-MS systems demonstrate efforts toward standardization and automation of miniaturized workflows. These developments suggest a shift toward compact, robust, and high-throughput solutions in future bioanalysis.
9. It is our choices, Harry, that show what we truly are, far more than our abilities. (Albus Dumbledore)
10. Some of us think holding on makes us strong, but sometimes it is letting go. (Hermann Hesse)

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