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MASS SPECTROMETRY-BASED WORKFLOW OPTIMIZATION FOR COMBINED METABOLOMICS AND LIPIDOMICS ANALYSIS FROM BLOOD MICROSAMPLES

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ABSTRACT

Blood microsampling has emerged as a promising alternative to conventional venipuncture for metabolomics studies, granting advantages such as minimal invasiveness, ease of collection, suitability for multiple sampling, and optimal use in longitudinal designs[1]. This study aimed to optimize a liquid chromatography-mass spectrometry-based workflow enabling both untargeted metabolomics and lipidomics analyses from a single dried blood spot (DBS). In parallel, extraction optimization was performed for targeted metabolomics on DBS (Whatman) using TMIC MEGA kits. For the untargeted protocol optimization three commercially available microsampling devices—Capitainer and Whatman (whole blood) and Telimmune (plasma)—were evaluated. Among five extraction solutions tested, pure methanol provided the best compromise for simultaneous extraction of polar metabolites and lipids. Based on these results, a two-step consecutive extraction protocol was developed, using methanol followed by water to enhance the recovery of more polar metabolite classes and improve metabolome coverage. Short-term stability of polar metabolites and lipids was also evaluated at room temperature (RT) for up to five days. Capitainer showed the best results, preserving the stability of all evaluated classes of compounds for up to five days at RT. Regarding targeted protocol optimization, modifications to the original extraction protocol for panels A and B increased metabolite coverage in DBS with the highest improvement observed for Panel B. Overall, this work suggests that methanol extraction enables integrated metabolomics and lipidomics analysis from a single spot, and that a two-step approach can further enhance polar metabolite coverage. Targeted workflow optimization also improved metabolite coverage in DBS, emphasizing the importance of tailoring device selection and extraction protocols to study aims, matrices and analytical scope.

Reference

1. Bossi, E. *et al.* (2025) 'Pre-analytic assessment of dried blood and dried plasma spots: integration in mass spectrometry–based metabolomics and lipidomics workflow', Analytical and Bioanalytical Chemistry, 417(9), pp.1791-1805. doi:10.1007/s00216-025-05760-z.