When Analytical Needs Face Clinical Reality: Comparative Evaluation of Offline Exhaled Breath Sampling Methods Using the Peppermint Experiment

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Introduction

Exhaled breath analysis holds strong promise for non-invasive monitoring, but the lack of standardization in sampling methods remains a key limitation to its clinical adoption[1]. To address this, we evaluated three widely used offline breath sampling techniques; Tedlar® bags, BioVOC-2, and the ReCIVA system, within the peppermint experiment[2], a validated perturbation model for breathomics research. The objective was to compare analytical performance as well as sampling feasibility from both technical and participant-related perspectives.

Experimental/Theoretical Methods

Seven healthy participants were included in the study. Breath sampling was performed using the three offline collection devices under the same standardized conditions following peppermint oil ingestion. Analytical performance was assessed using GC×GC-TOFMS[3], with a focus on washout curves reproducibility, signal quality, compound recovery and variability.

In parallel, feasibility was evaluated through technical criteria (e.g., ease of use, background interference, etc.) and participant-related aspects (e.g., comfort and duration). Feedback was also collected from leading clinicians and medical staff involved in clinical breath sampling to assess the potential for practical implementation of each method in clinical environments.

Results and Discussions

Tedlar® bags produced reliable and reproducible washout curves, supporting their continued use as a reference method. However, they were also characterized by a

consistent background signal, which implies a direct source of contamination, requiring particular caution during analysis. BioVOC-2 demonstrated limited sampling capacity but strong portability and simplicity, with signal intensities improving with repeated exhalations. However, variability in compound recovery and lower sensitivity for certain volatiles limit its applicability in untargeted workflows. The ReCIVA device, while technically robust and offering superior control of sample fractionation, demonstrated excellent suppression of background contamination, significantly enhancing the detection and interpretation of endogenous compounds. However, its use posed logistical challenges, including setup complexity and patient discomfort during longer sampling periods.

Despite these differences, the kinetic profiles of most key chemical families remained comparable across methods, supporting the potential for method harmonization. Variability analysis (%RSD) revealed that Tedlar® bags had the lowest overall variability, using a fully controlled workflow, followed closely by ReCIVA, while BioVOC-2 showed greater variability in repeated exhalation scenarios.

Furthermore, the study includes a detailed assessment of patient comfort and addresses practical constraints of clinical settings in relation to breath sampling. Particular attention was given to minimizing sampling time, promoting patient autonomy, and decreasing medical staff workload, while maintaining adequate control over the sampling process to ensure low analytical variability. Feedback from clinicians was also gathered to identify key requirements for advancing breathomics, with the goal of supporting future implementation and real-world integration into routine clinical practice.

Conclusions

This study provides a comparative evaluation of three commonly used offline breath sampling devices under controlled conditions. While Tedlar® bags remain a reliable reference method, their background signal requires careful consideration. BioVOC-2 offers simplicity and portability but shows higher variability and limited sensitivity. The ReCIVA device stands out for its analytical performance, especially in minimizing background contamination and improving the analysis of endogenous compounds, although its complexity may limit ease of use in routine clinical workflows.

Overall, these findings offer practical insights for optimizing breath sampling protocols and highlight the importance of balancing analytical rigor with patient comfort and operational feasibility in clinical breathomics.

References

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