Optimization and Comparison of Methods for Sampling Volatile Organic Compounds in Breath by Solid Phase Microextraction and Gas Chromatography-Mass Spectrometry

Eray Schulz[1,2], Mark Woollam[2], Paul Grocki[2], and Mangilal Agarwal[1,2,3]

[1]IUPUI Department of Chemistry and Chemical Biology, [2]Integrated Nanosystems Development Institute, [3]IUPUI Department of Mechanical and Energy Engineering

Abstract

Volatile organic compounds (VOCs) can be detected in exhaled breath as potential biomarkers for medical conditions. The gold standard for analysis is gas chromatography-mass spectrometry (GC-MS) and can be coupled with various sampling methods. One method is Tedlar bag sampling, which allows for reduced sampling volumes, facile collection of many samples in the field, and requires no additional devices that require disinfection between uses. Our work aims to compare different methods for preconcentrating VOCs using solid phase microextraction (SPME). A previously optimized SPME fiber coating was used for extracting VOCs from breath collected using two methods. One method directly extracted VOCs from the Tedlar bag (Tedlar-SPME) and the other cryothermally transferred VOCs from a Tedlar bag to a vial (Cryotransfer). Various extraction periods (5-, 10-, and 15- minute) were tested for Tedlar-SPME to develop a time-efficient method with high sensitivity. The Cryotransfer method was previously optimized and allows for both long-term storage at -80℃ as well as SPME GC-MS analysis. Cryotransfer displayed the greatest sensitivity, having the highest signal for most VOCs detected in exhaled breath samples. However, smaller VOCs including acetone and isoprene were detected with the highest sensitivity using Tedlar-SPME. Overall, both methods are capable of detecting a wide variety of VOCs in breath including previously identified biomarkers for different diseases. The Cryotransfer method may be optimal when collecting a large number of samples using Tedlar bags, as it allows for long-term storage of VOCs at -80℃, while Tedlar-SPME may be more efficient when targeting relatively smaller VOCs.

Background

Volatile organic compound (VOC) biomarkers are expressed in exhaled breath and detected by canines through their sense of smell (Figure 1). This has inspired efforts to use gas chromatography-mass spectrometry (GC-MS) to identify biomarkers of disease[1,2]. Commonly used breath sampling techniques involve the use of thermal desorption units, though these are expensive and require additional hardware to operate. Therefore, developing new sampling methods using solid phase microextraction (SPME) that are rapid and easy to use, all while maintaining a high level of sensitivity, is of interest to advance VOC analysis through breath biopsy.

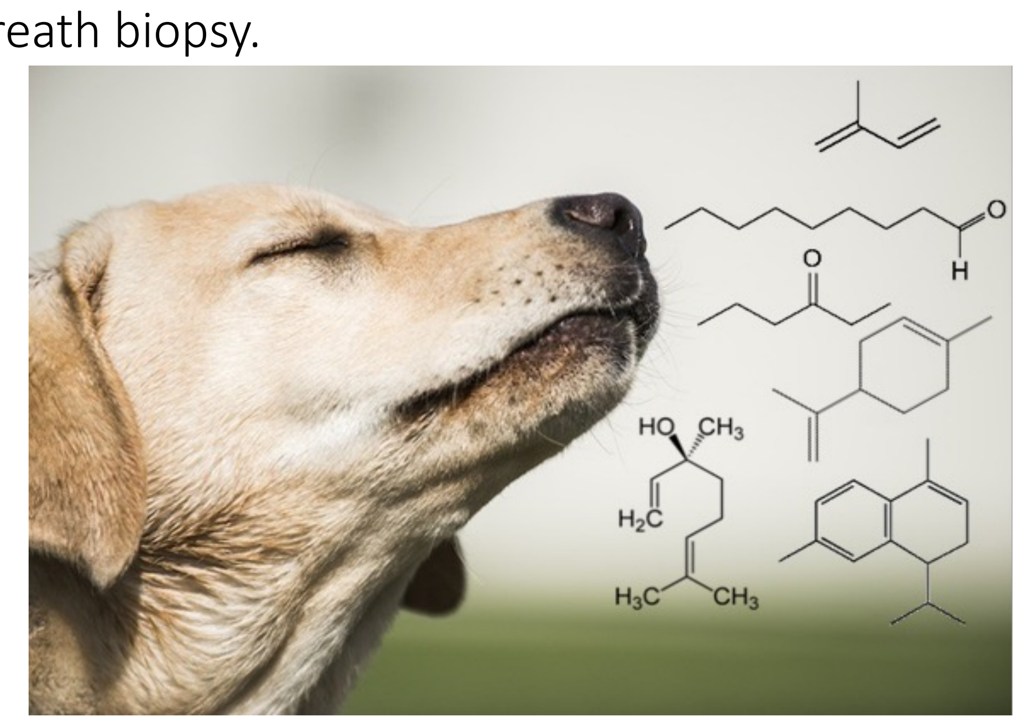

Figure 1. A canine sniffing VOCs that may serve as possible biomarkers for disease states.

Project Goals

Our work aims to develop, optimize, and compare two different breath collection methods that are capable of sampling a wide variety of endogenously produced VOCs from one volunteer. These include directly extracting VOCs from a Tedlar sampling bag (Tedlar-SPME), and the cryothermal transfer of exhaled breath to headspace vials (Cryotransfer).

Materials and Methods

Comparing SPME Fibers and Arrows

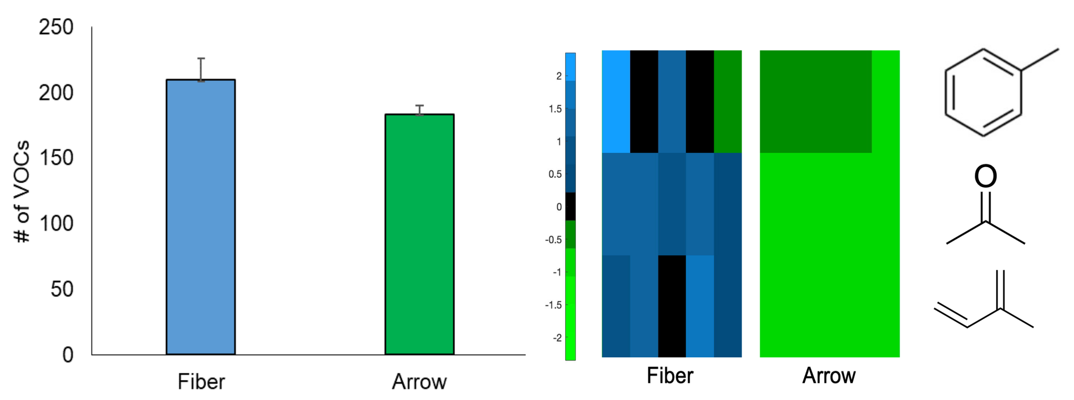

Figure 2. The performance of a SPME fiber relative to an arrow for Tedlar bag incubation. The SPME fiber detected more VOCs than the arrow, mainly due to its ability to adsorb low molecular weight VOCs.

Tedlar-SPME

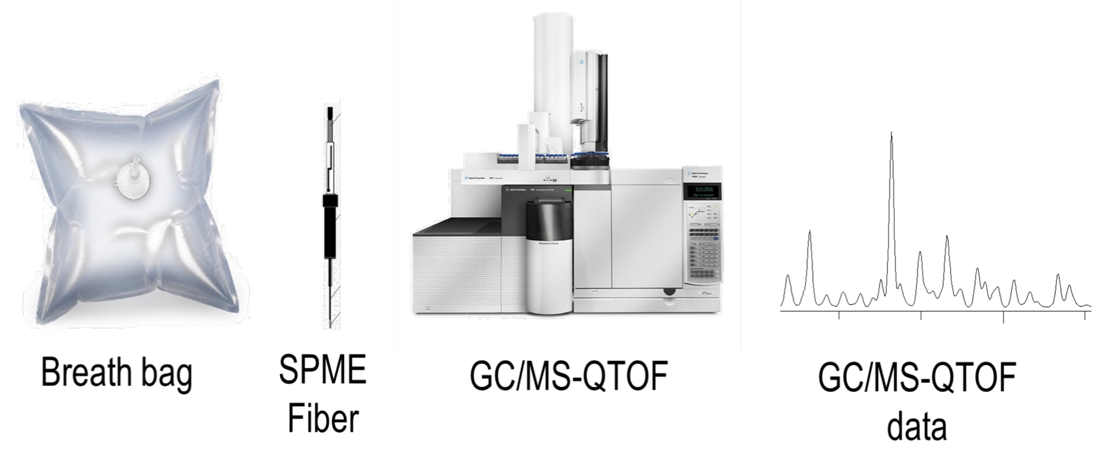

Figure 3. The materials used during the Tedlar-SPME process. Once a volunteer fills a bag, a SPME fiber is inserted through the septum and exposed to breath VOCs. Once the desired extraction period is reached, the SPME fiber is injected into the GC-MS for analysis.

Cryotransfer

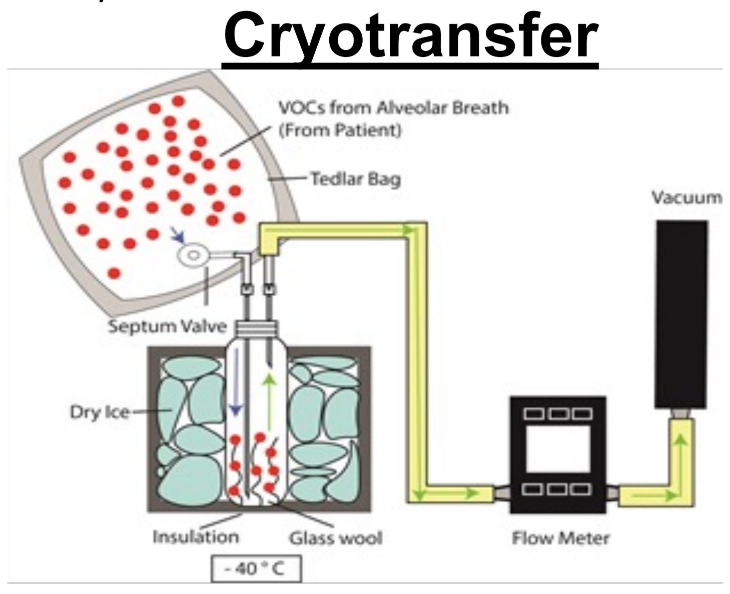

Figure 4. A schematic of the Cryotransfer process. Once vacuum is established, the bag valve is opened, and the sample flows into the vial. Glass wool is used to adsorb VOCs. Once complete, the vial can be stored at -80℃ or be incubated with a SPME arrow/fiber for 45 minutes at 60℃.

Results

Tedlar-SPME Extraction Optimization

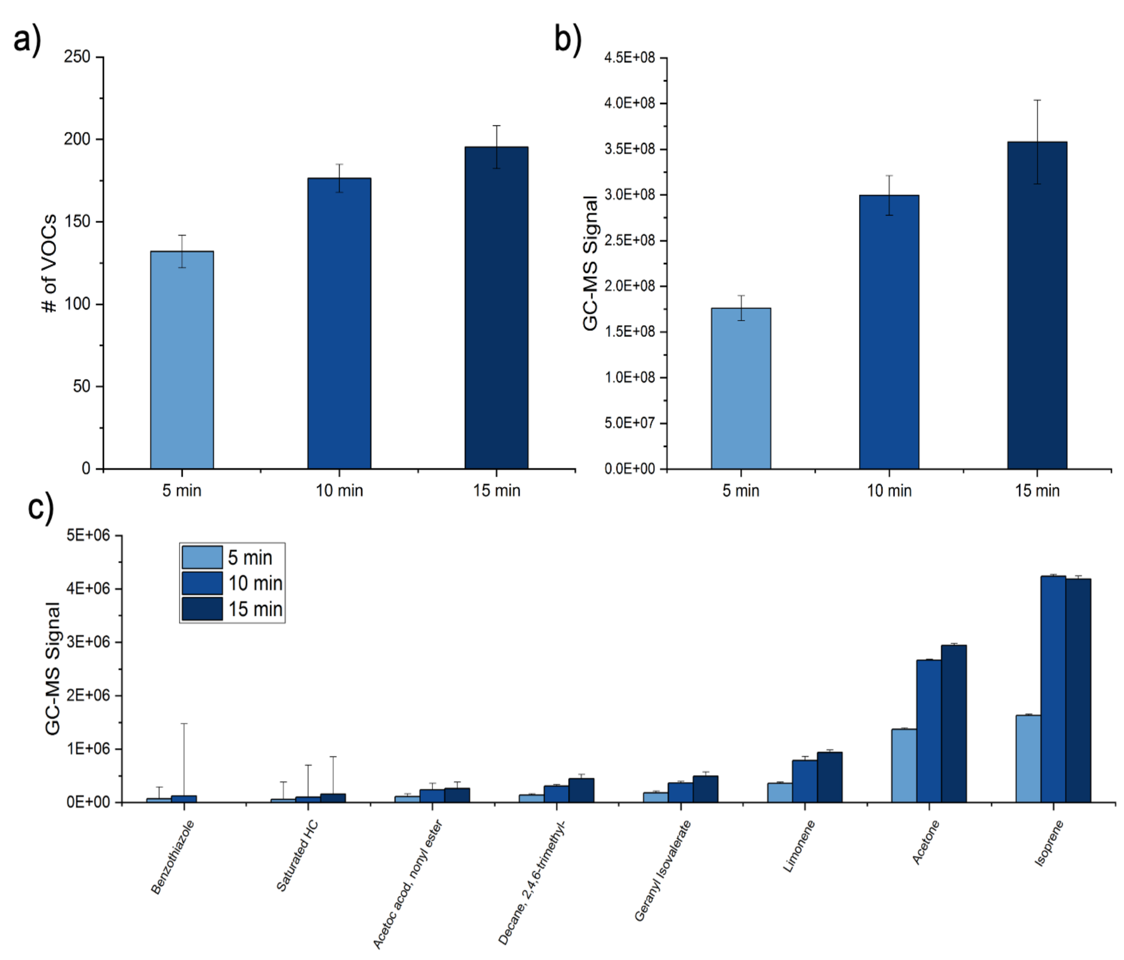

Figure 5. Different SPME fiber extraction times were tested (5-, 10-, 15-minute) by inserting the fiber into the valve of a Tedlar bag filled with exhaled breath. A stepwise increase was observed in VOC sensitivity; therefore the 15-minute extraction time was selected, as this allowed for a relatively quick sampling process to be conducted while having maximum sampling sensitivity.

Results (cont.)

Breath Fractionation in a Tedlar Bag

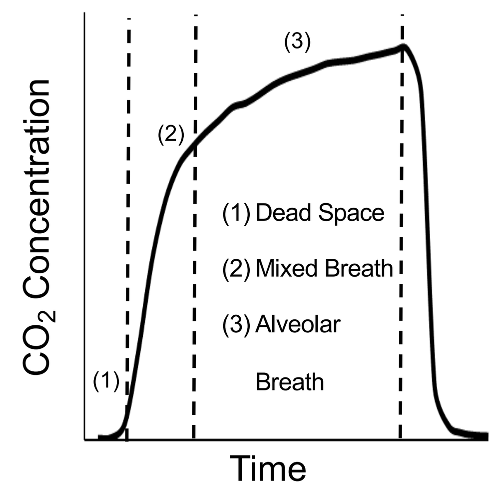

Figure 6. A clinical capnograph was used to observe alveolar breath when exploring breath fractionation, as shown in phase 3 of the graph.

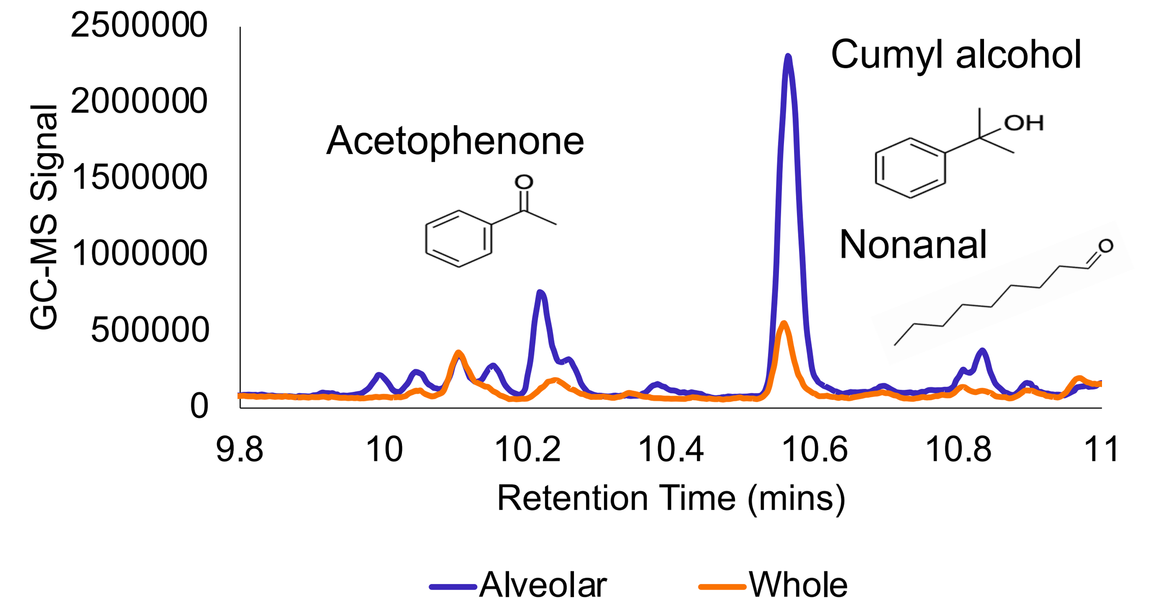

Figure 7. Alveolar breath displayed increased signals of acetophenone, cumyl alcohol, and nonanal, relative to whole breath.

Method Comparison

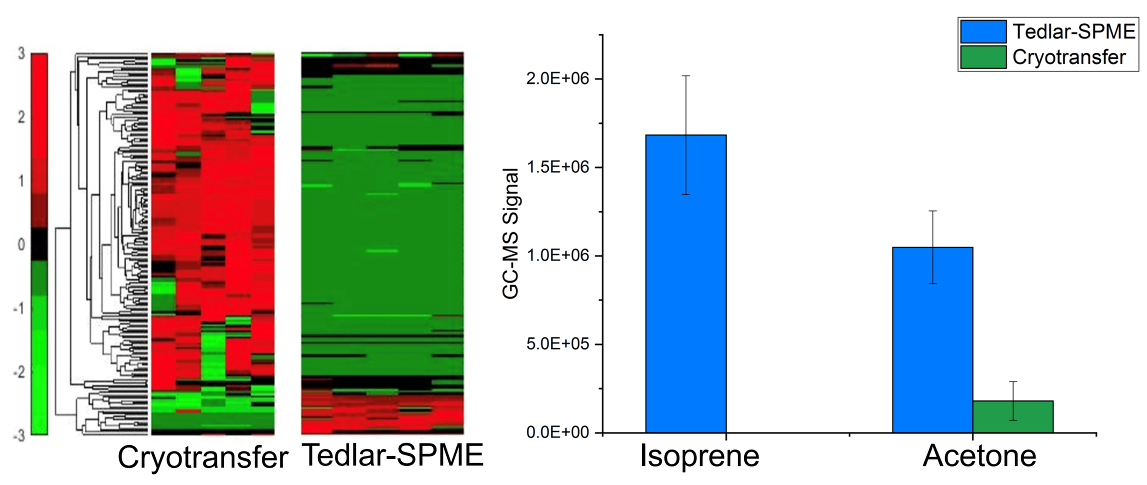

Figure 8. The heatmap illustrates Cryotransfer's increased sensitivity to detecting VOCs in breath relative to Tedlar-SPME. Tedlar-SPME demonstrated an increased sensitivity for low molecular weight VOCs such as isoprene and acetone, outperforming Cryotransfer.

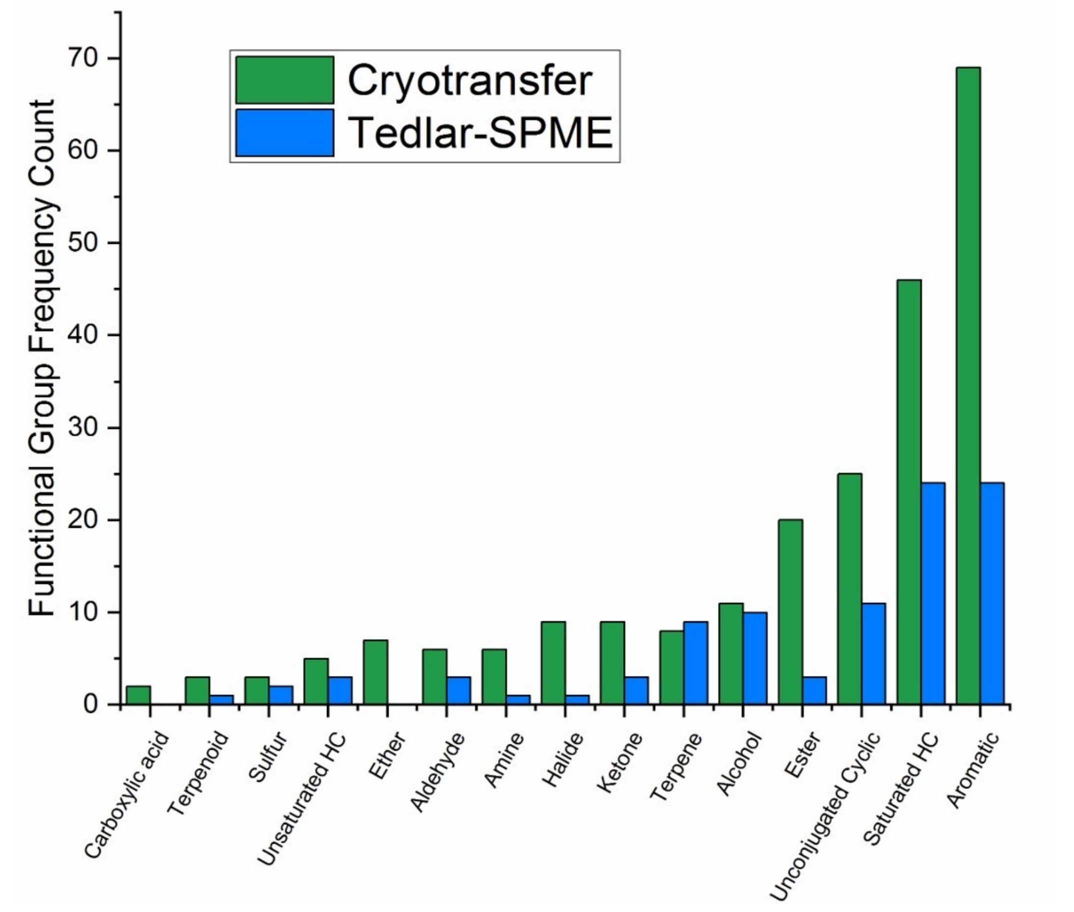

Figure 9. Functional group frequencies demonstrate the enhanced ability of Cryotransfer to detect VOCs expressed in breath, comprised of aromatics, hydrocarbons, and esters. These VOCs were also detected using Tedlar-SPME, but in lower abundance.

Conclusion

The Cryotransfer method provides the highest level of sensitivity for detecting VOCs in breath. Certain low molecular weight VOCs such as isoprene and acetone are more easily detected using Tedlar-SPME. Tedlar-SPME provides a rapid sampling method which may be beneficial for low molecular weight VOC sampling, while cryotransferring breath provides long-term storage of samples. These results may one day help to advance the field of VOC biomarker discovery through exhaled breath analysis.

Acknowledgements

The author would like to thank Mariana Maciel for her input on this study. We would also like to thank the National Science Foundation (grant #1502310) and Agilent Technologies.

References

1. A. Siegel et al. 2017 J Breath Res 11 0260
2. M. Woollam et al. 2019 Sci Rep 9: 2526