# Developing and Optimizing a Robust HRAM GC-MS Pipeline for On-breath Global Biomarker Analysis

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## Aims

Address historical challenges currently limiting progress towards widespread use of clinical breath tests for disease detection, diagnosis and treatment.

Develop a process for reliable analysis of volatile organic compounds from breath samples using HRAM GC-MS by:

- Maximizing the number of detectable on-breath compounds
- Minimizing process variability to increase sensitivity to biological signals

## 1. Background and Objectives

There is a critical need for better ways to detect, monitor and treat diseases. Early detection and precision medicine have emerged as areas that have great potential to save lives and reduce costs by improving how we diagnose and treat illnesses. The most notable advantage of breath testing is that collection can be completely non-invasive, which makes it pain-free, easy to use and well tolerated by patients [1].

Volatile organic compounds (VOCs) on breath have attracted growing interest as a promising biomarker source that may be relevant for a wide range of clinical applications. Over 1,000 different VOCs have been detected in human breath and these have both endogenous and exogenous origins (Figure 1).

VOCs can be carried in the blood and exchange readily into air in the lungs. As such, biomarkers relevant to illness anywhere in the body could be detectable on breath and there is a rapidly growing body of early-stage published work to support this.

Accurate biomarker identification is critical for developing clinically viable breath tests. The lack of progress beyond early stages is largely due the diversity of methods used and variation in how results are reported. Making progress depends on finding an approach to breath analysis that produces consistent, reliable and reproducible results.

Breath Biopsy OMNI provides an end-to-end pipeline for robust collection and global analysis of VOCs on breath.

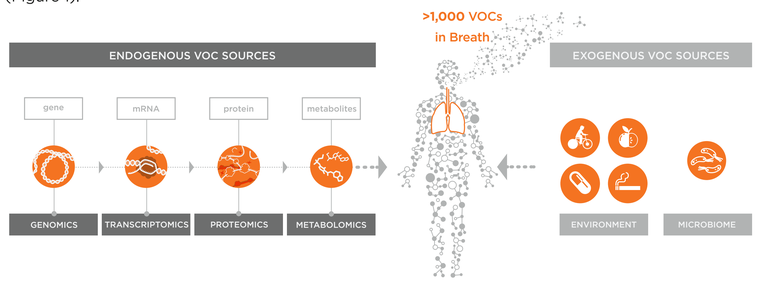
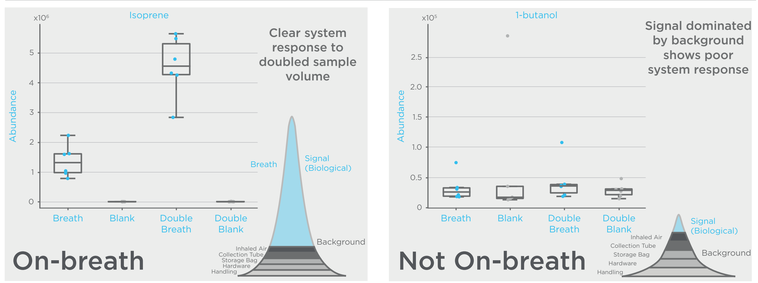



*Figure 1. Volatile organic compounds (VOCs) from endogenous and exogenous sources can be detected on breath. VOCs produced by metabolic processes all over the body are carried to the lungs by the blood.*

## On-breath VOCs

On-breath compounds are those with an individual area under the peak in breath samples that is more than three standard deviations above background.




## 2. Method


*Figure 2. Breath Biopsy OMNI is a complete, end-to-end solution for global breath VOC analysis. In addition to an award-winning collection system and HRAM GC-MS analysis capabilities we include expert support for study design and management, statistical analysis and biological interpretation. Plus, there's several reporting options for you to choose from.*

Human breath samples were collected from subjects during regular tidal breathing and VOCs captured into a set of four thermal desorption tubes using the Breath Biopsy Collection Station. VOCs were selectively captured from the alveolar breath fraction and stored prior to analysis. Blank samples were also collected to allow evaluation of Breath VOCs relative to ambient background.

VOCs were released for analysis using thermal desorption for injection onto a thick film gas chromatography column with an oven profile ranging from 30 to 280°C. Samples were analyzed using Thermo Scientific GC-Orbitrap mass spectrometers in electron ionization (EI), full scan mode from m/z 30-450. A feature extraction workflow using Thermo Scientific Chromeleon and Compound Discoverer software was applied to enable investigation of sample composition.

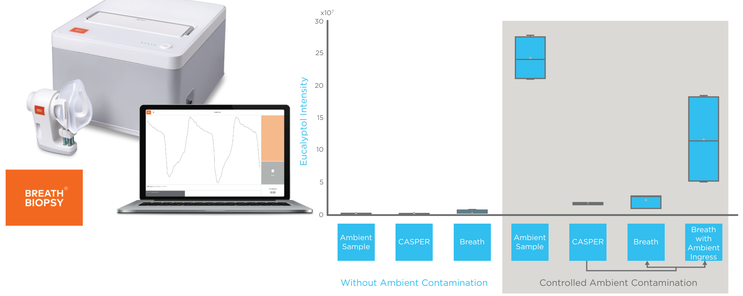



*Figure 3. Breath Biopsy Collection Station (left) including CASPER® Portable Air Supply (top). CASPER eliminates >90% of ambient VOCs dramatically reducing background signal in samples.*

## 4. Conclusions

Breath analysis offers significant potential to improve early detection of disease and advance precision medicine. Progress has been limited by a lack of suitable methods for reliable, reproducible breath analysis.

Starting with the gold standard method HRAM TD-GC-MS, we have worked towards a robust workflow for breath collection and analysis based on incremental method improvements and thorough quality control.

Our method draws distinction between VOCs in breath and on-breath VOCs that are significantly increased in exhaled breath over background and are therefore more likely to have clinical relevance.

On-breath compounds have been distinguished using BoB studies. Identifying on-breath compounds could, by excluding irrelevant signals, dramatically improve the potential to identify meaningful biomarkers.

We believe that misidentification of compounds may also contribute to limited success in validating prospective biomarkers. We have sought to change this by developing a Breath Biopsy Library of VOCs that offers higher confidence in identity assignments for breath research.

We are now investigating targeted methods for breath analysis based on the same analytical methods and aim build a comprehensive human volatilome database to provide insights into the VOCs typically present on-breath in the human population.

Find out more about Breath Biopsy OMNI

## 3. Results

### Linearity & Dynamic Range

We are able to detect compounds over a large dynamic range (0.5-200 ng) with an excellent coefficient of 0.9988 due to an automated gain controller (AGC).

The AGC enables calibration of overlapping signals. For example, achieving coefficients >0.99 for compound ranging from 1.5 ng/µl to 150 ng/µl and for a co-eluting compound between 0.3 ng/µl and 10 ng/µl.

In practice, this enables identification of sub nanogram compounds whilst quantitating compounds at approximately 500 ng/µl in the same analytical retention time.

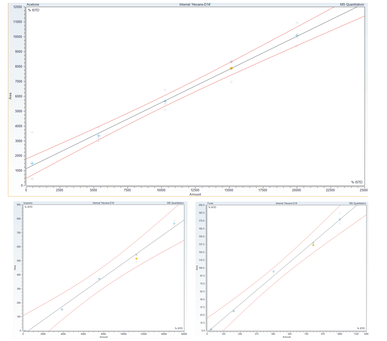


*Figure 4. Example calibration curves. Acetone ranging from 0.5 to 200 ng (top). Isoprene 1.5 to 150 ng (lower left) co-elution with furan calibration curve 0.3 to 10 ng (lower right).*

### Variable Electron Voltage (VeV)

Electron ionisation is typically operated at 70 eV and is a hard ionisation technique, resulting in a small proportion of molecular ions. Reducing eV may increase molecular ion recovery which may improve compound identification.

We compared the effect of 70 eV and 35 eV on sensitivity and signal-to-noise ratio. 1-octene and p-menthone are shown as examples. Lower eV can improve molecular ion recovery but presents issues when identifying compounds by comparing to standard spectra in e.g. the NIST library, which may limit the quality of tentative assignments.

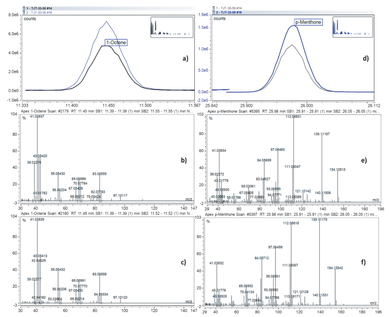



*Figure 5. HRAM mass spectal results for 1-octene showing (a-c) and p-menthone (d-f) demonstrating fragmentation at 70 eV (b & e) and 35 eV (c & f). 1-octene shows little difference, p-menthone shows a notable change illustrated by the increase of m/z 139.111 relative to m/z 112.088.*

### Measuring Progress with Breath or Blank

To facilitate method development, we used breath or blank (BoB) studies, which compare breath samples to representative blanks in order to offer a standardized approach for breath research that can be used in conjunction with any breath collection and analysis workflow.

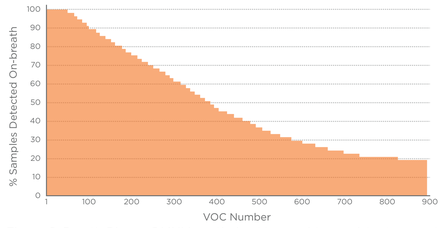



*Figure 6. Breath Biopsy OMNI is optimized to provide consistent, reproducible results for breath analysis. Arranging VOCs by frequency of detection on breath provides an overall representation of ability to detect VOCs on breath. In this study a median of 517 on-breath VOCs were detected per sample.*

Comparing breath vs. blanks allows the discrimination of on-breath VOCs from other VOCs that are likely to be contaminants ensuring that only relevant VOCs are included for detailed analysis.

In one instance, BoB analysis was applied on 57 breath samples, obtained from four different volunteers, with an equal number of representative blanks (Figure 3). The method detected a median of 1,454 VOCs per sample of which a median of 517 were shown to be on breath.

### References

1. Holden KA, Ibrahim W, Salman D, et al. Use of the ReCIVA device in breath sampling of patients with acute breathlessness: a feasibility study. ERJ Open Res. 2020 Oct;6(4).