BREATH® BIOPSY

Validation of a methodology for evaluating longitudinal change of VOCs in breath

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Introduction

Exhaled breath contains thousands of Volatile Organic Compounds (VOCs), which are products of metabolic activity and biomarkers for diseases. These can be from endogenous processes in the body and exogenous sources such as environmental exposures and pharmaceuticals.

Reliable longitudinal evaluation of breath VOCs are an essential step enabling studies into:

- Understanding biological variability
- Enabling disease monitoring
- Measuring the response to therapeutic interventions
- Assessing effects of environmental exposures
- Evaluating pharmacokinetics

This study aims to examine the long term stability of the breath biopsy platform as well as its ability to detect changes over time relative to biological variability.

To evaluate this, two experiments were conducted:

- Analytical replicates: Analysis of a target set of VOCs in breath following ingestion of a peppermint capsule and the resulting "washout curve" for target over 8 hours (Figure 1) collected as 4 concurrent samples per timepoint.
- Biological variability: 9 repeats of the same experiment over a 5 weeks period in the same individual, sampled at three time points.

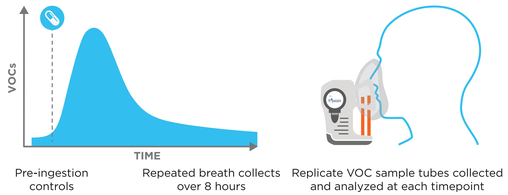

Figure 1. Design of the washout experiment to monitor changes in the breath VOC profile following ingestion of a peppermint capsule.

Methods

Breath Sampling

The study subject was a 32-year old, healthy male who standardised diet and exercise for the duration of the study. Baseline collects were performed at two time points. Thereafter the subject ingested one Obbekjaers 200mg peppermint pill. Samples were collected at 16 time points in the initial study. In the longitudinal study timepoints estimating baseline, peak and washout period were selected at -60, 45, and 180 min. Breath samples were collected using the ReCIVA Breath Sampler and analyzed using the Breath Biopsy platform in the Breath Biopsy Clinical Laboratory (Owlstone Medical Ltd, UK).

Sample Analysis and Data-processing

- Analytical replicates: Analysis of a target set of VOCs in breath following ingestion of a peppermint capsule and the resulting "washout curve" for target over 8 hours (Figure 1) collected as 4 concurrent samples per timepoint.
- Transfer onto column using split injection (Agilent Technologies)
- GC-MS analysis utilizing BENCHTOF HD mass spectrometer
- Peak area extraction using MassHunter Quant based on ion signature

Breath Collection

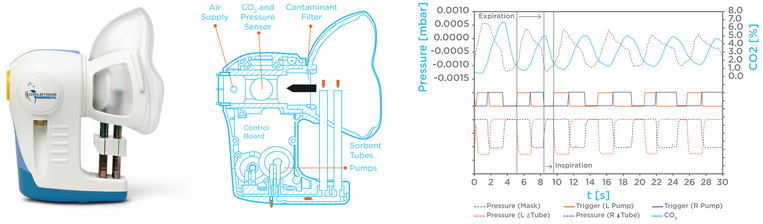

Figure 2. The ReCIVA Breath Sampler enables reliable, reproducible collection of breath VOCs and pre-concentration for enhanced sensitivity. Pressure and CO2 sensors in ReCIVA provide real-time monitoring of the patient's breathing, allowing different breath fractions to be sampled in a single collection event if desired.

Result: Washout Curve Over 8 Hours

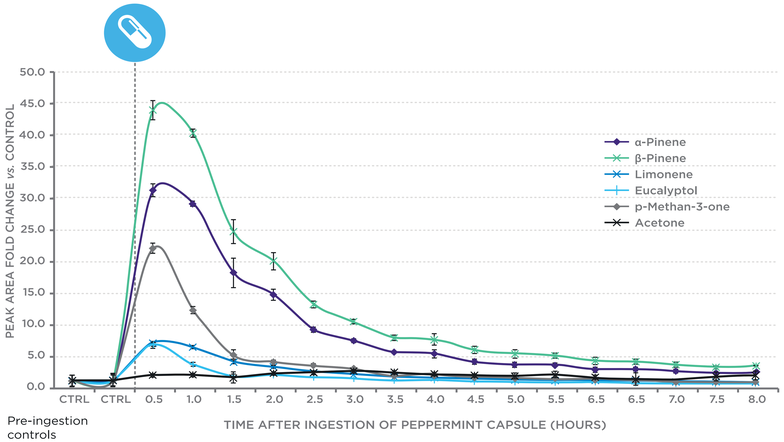

Figure 3. Washout curve following ingestion of a peppermint capsule. Breath samples from repeated collects from one individual over 8 hours (16 timepoints) were analyzed. Four replicate VOC sample tubes were collected and analyzed at each time point.

Result: Longitudinal Study Over 5 Weeks

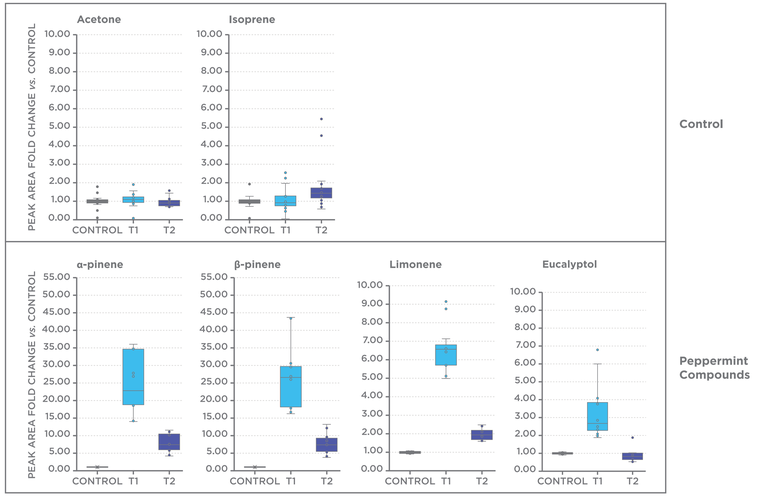

Figure 4. Longitudinal study repeating the washout experiment nine times over five weeks in a single individual. Breath samples from pre-ingestion control, and time points 1 and 2 (T1 and T2) were analyzed in each washout experiment. Two replicate VOC sample tubes were collected and analyzed at each time point.

Conclusions

- The study demonstrates that the Breath Biopsy platform can be used to study longitudinal changes of exhaled VOCs in a reliable and reproducible way.
- The evaluation of a washout curve of peppermint oil components provides a model for the study of absorption, distribution, metabolism and excretion of pharmaceuticals.

References

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