Evaluating high-capacity sorptive extraction (HiSorb™) for VOCs as biomarkers related to respiratory and liver diseases in culture media

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Introduction

Analysis of volatile organic compounds (VOCs) in breath has the potential for rapid, non-invasive disease diagnosis for early detection to improve patient outcomes. Sorbent-packed tubes are typically used to collect breath VOCs in the biomarker discovery phase, with thermal desorption (TD) coupled with gas chromatography–mass spectrometry (GC–MS) for analysis and identification.

In this study, an alternative extraction technique was needed to sample VOCs from complex culture media samples that are associated with respiratory diseases and for monitoring liver health.

Here, we evaluate HiSorb, a high-capacity sorptive extraction technique, for sampling and analysis of biomarkers in culture media. Initial method development was performed manually, with analysis of HiSorb probes on a TD100-xr™ thermal desorber. To improve sample throughput and reproducibility of results, the Centri® sample extraction and enrichment platform (Figure 1) was used for full automation of the entire HiSorb workflow (Figure 2), providing good sensitivity for target compounds in complex culture media matrices.

Figure 1: Centri automated sample extraction and enrichment platform.

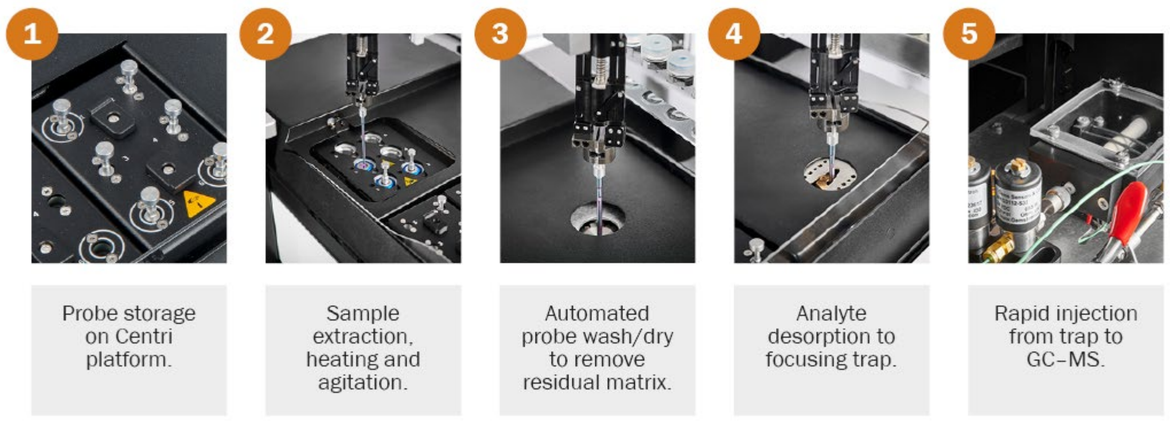

Figure 2: Fully automated high-capacity sorptive extraction workflow on Centri.

Experimental

Robust and reusable HiSorb probes (Figure 3) support a high-capacity sorptive phase and can be used for immersive extraction of VOCs and semi-volatile organic compounds (SVOCs) in liquids, as well as headspace sampling of both liquids and solids.

Culture media (50 µL) were placed into 10-mL sample vials, which were then crimp-capped for sampling and analysis.

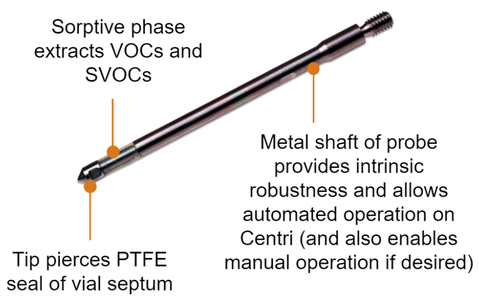

Figure 3: HiSorb probe.

Using headspace mode, HiSorb was used to extract the following VOCs:

- Limonene (added to hepatocyte culture media – Figure 4), which is elevated in the breath of patients with liver cirrhosis as a result of slower enzymatic processing in the liver cells and has the potential to be used as an exogenous VOC (EVOC) biomarker for a breath-based, non-invasive liver function test.
- Aldehydes, alkanes and ketones (from the bronchial air–liquid cell model – Figure 5), which have been identified as potential biomarkers for lung damage and inflammation.

Figure 4: Hepatocyte culture medium, which can mimic all stages of non-alcoholic fatty liver disease, is used to monitor limonene conversion to products in healthy and diseased liver cells.[1]

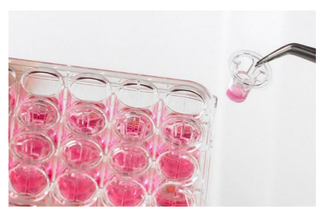

Figure 5: The bronchial air–liquid cell model developed by Epithelix has been used for detecting biomarkers related to acute lung injury by treating cell cultures with lipopolysaccharide).[2]

Method development – biomarker quantification

The first stage of method development, using HiSorb manually with analysis on TD100-xr, provided good results (Figure 6, left). Due to the high number of samples in the next stage, automation was key, so the Centri system was used, which improved results (Figure 6, right), enabling confident detection and identification below 1 ng/µL in culture media with excellent reproducibility (Table 1).

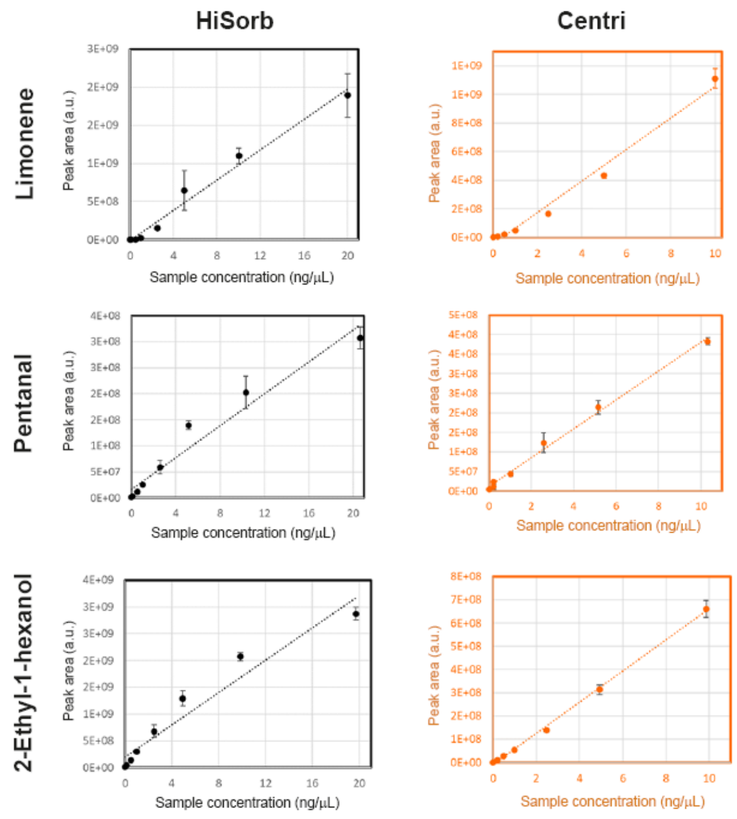

Figure 6: Peak area *vs.* concentration calibration curves for compounds relevant to liver disease and lung damage. Left: curves obtained with manual HiSorb. Right: curves obtained with automated Centri. Top: limonene, middle: pentanal, bottom: 2-ethyl-1-hexanol.

Table 1: Lung damage biomarker panel in order of variation. 2.5 ng/µL samples in 50 µL phosphate-buffered saline (PBS) analysed with Centri–TD–GC–MS. Quality control (QC) pass depended on replicate measurements having a coefficient of variation (CV) <15%.

Compound	CV (%)	Passed QC?	Compound	CV (%)	Passed QC?
Decane	4	✓	Hexanal	6	✓
2-Ethyl-1-hexanol	4	✓	Undecane	7	✓
Butanal	5	✓	*p*-Xylene	7	✓
Nonanal	5	✓	*E*-2-Pentenal	7	✓
2-Hexanone	5	✓	*o*-Xylene	7	✓
Limonene	5	✓	*E*-2-Butenal	8	✓
2-Ethylbutanal	6	✓	3-Methylthiophene	8	✓
Heptanal	6	✓	Pentanal	8	✓
Octanal	6	✓	1-Propanol	9	✓
Decanal	6	✓	n-Hexane	13	✓

Detecting biomarkers for lung damage

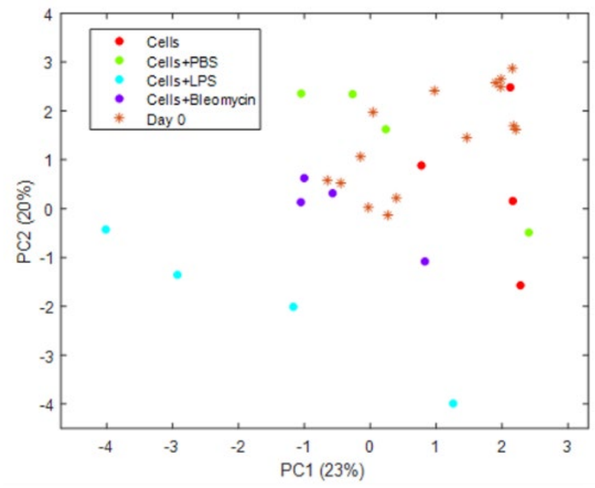

Figure 7: Principal components analysis of different treatments combining detection of all lung damage biomarkers. Red circles: cells with no treatment; green circles: cells with PBS treatment (negative control); blue circles: cells with lipopolysaccharide (LPS) treatment (positive control); purple circles: cells with bleomycin 10 ng/µL treatment and orange stars: all samples prior to treatments. The most obvious cluster consists of the cells with the positive control LPS treatment, and the cells with the test bleomycin concentration treatment are tending towards this result.

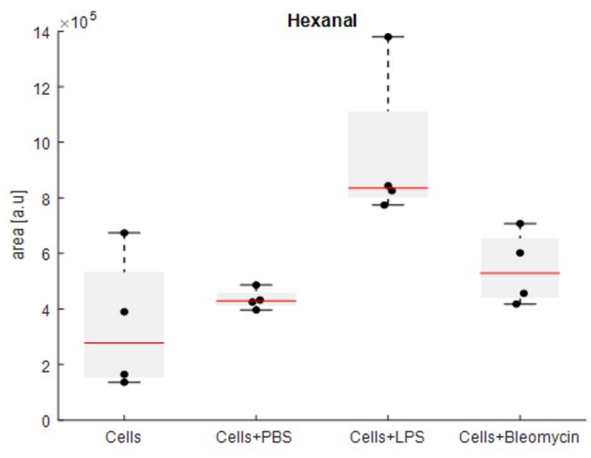

Figure 8: Example analysis for lung damage biomarker signal for cells with four treatments: (1) no treatment, (2) negative control PBS, (3) positive control LPS and (4) test concentration of bleomycin. In this example, the signal increases in response to the positive control LPS treatment, and a small increase is also observed for the test bleomycin concentration.

Conclusions

- Good sensitivity down to 1–2.5 ng/µL and acceptable reproducibility (RSD < 20%) obtained for 20+ biomarker compounds in phosphate-buffered saline (PBS) using manual HiSorb technology, despite the small volumes used.
- Automation of sampling steps gave better sensitivity, such that lung damage and liver disease biomarkers were detected at 1 ng/µL and below, and better reproducibility (RSD < 15%).
- Method developed for detecting exogenous VOC (EVOC) probes for liver disease. The next steps are to analyse the headspace of hepatocyte cells in different stages of cirrhosis treated with limonene using HiSorb technology with Centri automation.
- Analysis of bronchial cells treated to induce acute lung injury using HiSorb probes with Centri automation started – biomarkers for lung damage detected.

References

1. For more information on the hepatocyte cell culture model, Liver-on-a-chip, developed by CN-BIO, see https://cn-bio.com/liver-on-chip/.

2. For more information on the *in vitro* bronchial air–liquid interface model, MucilAir™, developed by Epithelix, see https://www.epithelix.com/products/mucilair.

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