Mass spectrometry techniques for volatile organic compounds measurement in cell and organoid media

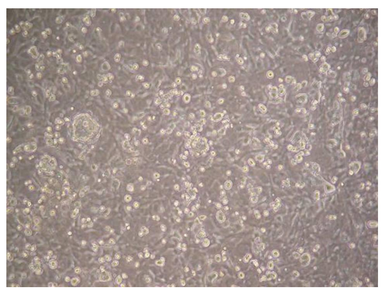
Ilaria Belluomo[1], Katerina V Savva[1], Bhamini Vadhwana[1], Tetsuji Furukawa[1], Antonis Myridakis[1], Qing Wen[1], Yan Mei Goh[1], Oscar Ayrton[1], Stefan Antonowicz[1], Piers R Boshier[1] and George B Hanna[1]

[1] Department of Surgery and Cancer, Imperial College London

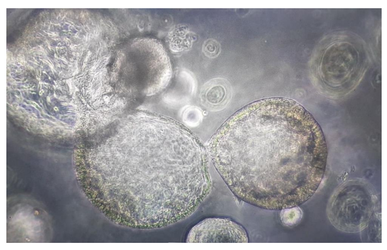
Background

In the last decade, measurement of volatile organic compounds (VOCs) in breath is increasing in importance as a non-invasive diagnostic tool, especially for complex pathologies such as cancer [1]. Many methodological and clinical studies have been carried out to develop sophisticated new mass spectrometry methods and pinpoint differences in VOC levels between patients and controls. However, the origin of VOCs and their imbalance in certain diseases remain poorly understood. In a recent study from our group, cancer tissue headspace (HS) has been analysed ex-vivo to evaluate the in-loco production of VOCs [2]. In this study, we compared two different techniques of sample collection and analysis for VOCs in the HS of cells and organoids to create a new tool that will help clarify the role and production of VOCs in-vitro.

Cells and Organoids



Oesophageal cancer cells are an immortal cell line that constantly proliferates under favourable conditions. Cell lines provide a cost effective model commonly used to study molecular changes associated with physiological and pathological states.

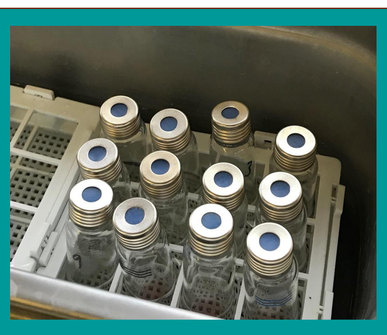


Gastrointestinal organoids, allow an in-vitro physiological model of both oesophagogastric cancer and healthy tissues, for experimental diagnostic and therapeutic research.

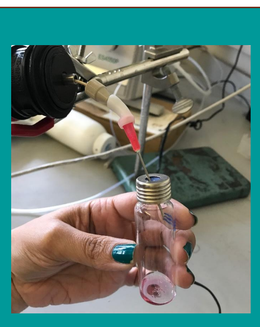
VOCs collection and mass spec analysis

I. Direct Sampling Analysis

Direct sampling mass spectrometry instruments, like the proton transfer reaction time-of-flight mass spectrometry (PTR-ToF-MS), allow direct measurements of VOCs via an heated inlet directly connected to the mass spectrometry instrument. This method provides real-time results and the analysis of each sample within one minute.



Cell media is incubated at 37° C for 30 minutes, to allow the VOCs to pass from the liquid to the HS.

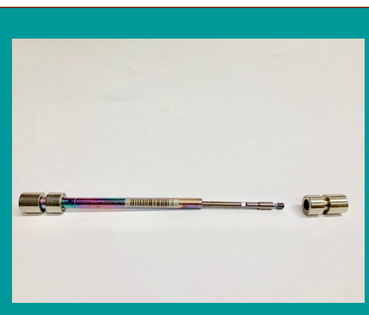


The volatile containing gas is drawn by the instrument inlet, heated at 110°C and directly analysed (real-time results).

II. HiSoRB

The second strategy used for VOC measurement from cell and organoid HS is using the HiSORB, coated probes specifically designed for the extraction and pre-concentration of VOCs from solid and liquid samples. Once VOCs bind the coated surface, the probes are inserted into special tubes that can be analysed via an autosampler with an automated method with PTR-ToF-MS.

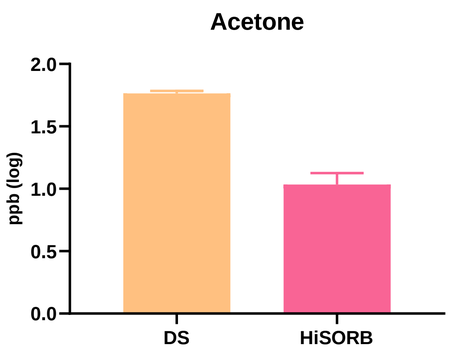
The special coated HiSORB probe is placed in the HS of the samples, incubated at 37° C for 30 minutes.

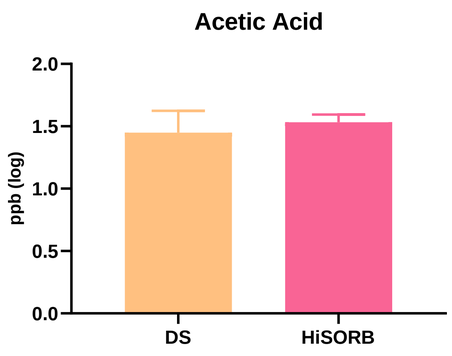


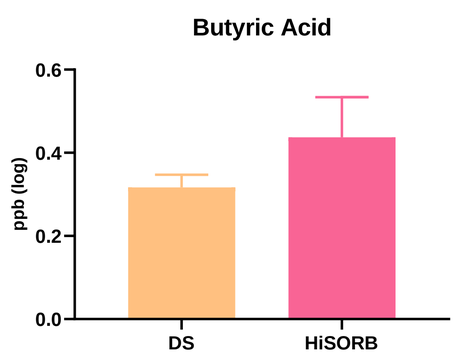
The probe is inserted in a special tube that can be analysed with PTR-ToF-MS through an autosampler.

Results

We compared the preliminary results of VOC measurement in media obtained during the method development experiments with the two different techniques. The VOCs analysed are part of a panel of VOCs involved in several types of cancer or physiological changes, previously identified by our group. Coefficient of variation (CV%) has been evaluated for the repeatability.







	DS	HiSORB
	CV%	
Acetone	4.50	21.15
Acetic Acid	6.67	13.82
Butyric Acid	7.44	24.05

Conclusions

Both methods are effective and easy to perform. Recovery varies in a non-specific manner, depending on the compound and not on the used techniques. CV% is lower using direct sampling, however it is also within an acceptable range using HiSORB. The techniques are comparable in performance, although offering different advantages: HiSORB analysis is an automated process, while direct sampling is faster and provides real-time results.

1. Hanna, G.B., et al., *Accuracy and Methodologic Challenges of Volatile Organic Compound-Based Exhaled Breath Tests for Cancer Diagnosis: A Systematic Review and Meta-analysis.* JAMA Oncol, 2018: p. e182815.
2. Adam, M.E., et al., *Mass-Spectrometry Analysis of Mixed-Breath, Isolated-Bronchial-Breath, and Gastric-Endoluminal-Air Volatile Fatty Acids in Esophagogastric Cancer.* Anal Chem, 2019. **91**(5): p. 3740-3746.