Breath Analysis with Thermal Desorption-Field Asymmetric Ion Mobility Spectrometry-Mass Spectrometry: A Rapid and Non-Invasive Means of Screening for Pulmonary-Related Diseases

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1. VOCs in breath

In the lungs, gases are exchanged between circulating blood and inhaled air. Alongside O_2 and CO_2, volatile organic compounds (VOCs) pass from the blood into the lungs, and are then exhaled (Figure 1). For this reason, breath is particularly valuable for diagnosing pulmonary conditions like asthma and lung cancer.

However, as blood circulates throughout the body before returning to the lungs, VOCs in exhaled breath also provide a useful source of biomarkers of diseases elsewhere in the body, as well as being sensitive to potentially important endogenous VOCs, such as drug metabolites.

Breath analysis therefore offers a non-invasive means of biomarker detection, for wide-ranging applications in diagnostics and precision medicine.

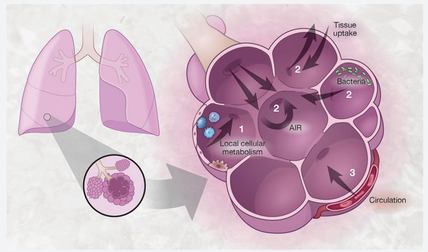

Figure 1. Diffusion pathway of VOCs into breath.

2. Breath collection

Breath samples were collected using the ReCIVA breath sampler (Owlstone Medical Ltd., UK) (Figure 2). Developed in conjunction with a multi-disciplinary group of researchers as part of the Breathe Free Consortium[1], ReCIVA provides an out-of-the-box means of reliably and reproducibly capturing breath samples on sorbent packed thermal desorption (TD) tubes for analysis.

Advantages include:

- Convenient for storage for analysing at a later date.
- Pre-concentration before analysis.
- Variety of sorbents offers extra selectivity to sampling.

Depending on the disease under investigation, the VOCs of interest may be present in different breath fractions.

Pressure and CO_2 sensors in ReCIVA provide real-time monitoring of the patient's breathing, allowing the following fractions to be selected: upper airways only, lower airways only, upper and lower airways, whole breath (including mouth air) and upper airways including mouth air (Figure 3).

Each pair of sorbent tubes is gated independently, allowing different breath fractions to be sampled in a single collection event.

TD tubes as a method of trapping and pre-concentrating VOCs from the gas phase have been widely used in conjunction with GC-MS. However, lengthy chromatographic run times can limit sample throughput. A faster analytical method makes breath analysis a much more viable screening technique in the clinical environment. Here we describe such a technique: thermal desorption-extractive electrospray ionisation-field asymmetric ion mobility spectrometry-mass spectrometry (TD-EESI-FAIMS-MS).

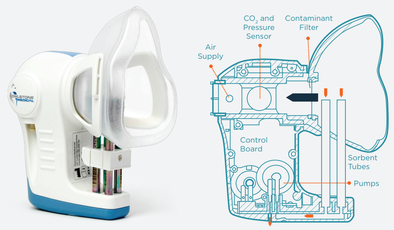

Figure 2. The ReCIVA Breath Sampler.

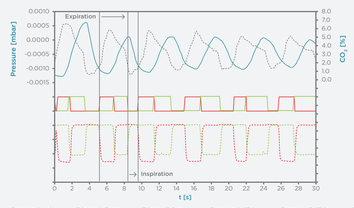

Figure 3. CO_2, pressure and pump activation traces from ReCIVA.

References

1. www.breathe-free.org
2. Smith, RW, Reynolds, JC, Lee, S-L, Creaser, CS, *Anal.Methods*, **2013**,5, 3799

3. Methods

2L of breath was captured onto Tenax/carbograph 5TD sorbent tubes (Markes International, Swansea, UK). TD-EESI-FAIMS-MS analyses were carried out using a TD-100 (Markes International, Swansea, UK) combined with a 6230 TOF-MS (Agilent, Santa Clara, US) fitted with an ultraFAIMS device (Owlstone Medical Ltd, Cambridge, UK) (Figure 4).

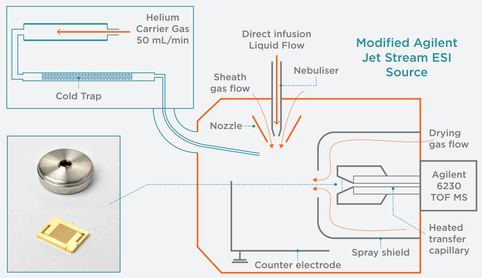

Figure 4. ultraFAIMS chip and source schematic.

Glass was removed from a viewing window in the source housing, and the heated fused silica transfer line (0.25 mm i.d.) from the TD was introduced into the ESI source at an angle of approximately 55° to the nebuliser, set back 10 mm to give the maximum response (Figure 5)[2]. TD settings are shown in Table 1.

The key dimensions of the ultraFAIMS device are the 100 µm electrode gap and 700 µm path length.

The small scale offers short ion residence times meaning an entire CF scan per second can be achieved, compatible with TD time scales.

Figure 5. Photograph of fused silica transfer line in ESI source.

Table 1: TD settings

Pre-desorption settings		Tube/desorption settings		Trap settings	
Dry purge	0.1 s	Desorb time	5 min	Cold trap material	Tenax/carbograph 5TD
Flow rate	20 mL min^{-1}	Temperature	300°C	Trap flow rate	50 mL min^{-1}
Pre-purge	1.0 s	Flow rate	50 mL min^{-1}	Pre-trap fire purge	1 min
Flow rate	50 mL min^{-1}			Cold trap low	20°C
Flow path temperature	170°C			Heat rate	variable - see discussion
				Trap high	300°C
				Trap hold	3 mins

4. Results

Initial experiments focussed on optimizing the TD-EESI-FAIMS-MS set-up using a test mixture of volatile standards in methanol.

- Acetone, D-limonene and 2-butanone were selected because they are commonly found in breath.
- Toluene was selected because it is related to the degradation of the thermal desorption tubes.
- Methyl salicylate and 1-naphthalenemethanol were selected for their high vapour pressure and densities.

The primary ESI spray was introduced in positive ion mode using a 0.4 mL min^{-1} flow, introduced via an LC binary pump. A comparison of water:acetonitrile (50:50) with 0.1% formic acid and water:acetonitrile (80:20) with 0.1% formic acid is shown in Figure 6.

- Sensitivity for the methyl salicylate response was strongly dependent on solvent composition (Fig. 6).
- Repeatability of analyte response did not appear to be affected by solvent composition – ~15% RSD.
- Water:acetonitrile (80:20) was selected for subsequent analysis due to the increased sensitivity.

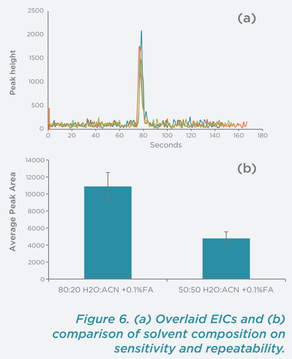

Figure 6. (a) Overlaid EICs and (b) comparison of solvent composition on sensitivity and repeatability.

TD peak width was directly correlated to the temperature ramp of the cold trap.

- The temperature ramp was decreased to give wider peaks (Figure 7a).
- Wider peaks offer the ability to implement increased number of ToF scans, and subsequent FAIMS settings.
- Wider peaks came at the expense of sensitivity (Figure 7b).
- 24 °C s^{-1} was selected as the optimal ramp rate as a compromise between sensitivity and the number of data points across the peak, with 6 seconds the minimum for acceptable FAIMS scan resolution for nested data sets.

Table 2: EESI-TD-ultraFAIMS-MS optimization experiments.

	Start CF (Td)	End CF (Td)	# of CF steps	# ToF spectra s^{-1}
1	-0.9	4.1	4	6
2	-0.9	2.1	3	5
3	-0.9	2.1	6	8
4	-0.9	2.1	4	6

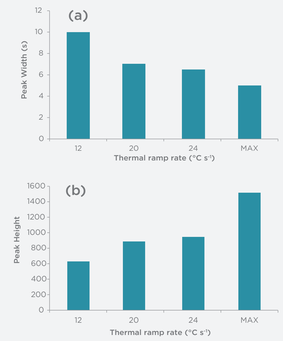

Figure 7. Effect of changing TD ramp rate on peak height and peak width.

Optimisation of nested FAIMS data focussed on:

- FAIMS CF settings to give optimal FAIMS separation.
- Number of data points within the TD peak timescale.
- Optimal sensitivity via number of TOF scans s^{-1}.

Full FAIMS acquisition parameters are shown in Table 2 and data shown in Figure 8.

Breath VOCs were not detected at a CF higher than 2.1 Td so this was eliminated from further optimization.

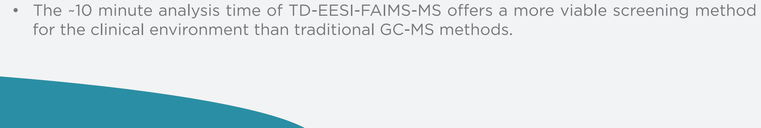

Figure 8. TD-EESI-FAIMS-MS at different FAIMS and ToF scan settings; (a) = 2, (b) = 3, (c) = 4 from Table 2, above.

A CF range of -0.9 to 2.1 Td, scanning the CF in 0.75 Td steps, was selected for analysis of breath.

Breath spectra at each CF are shown in Figure 9.

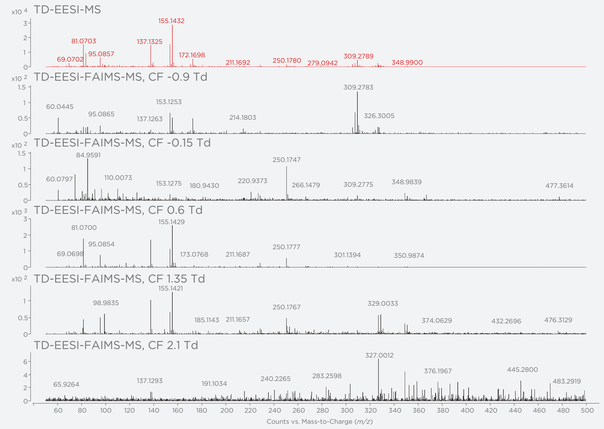

Figure 9. Breath spectra from (red) without FAIMS and (black) at CFs -0.9 Td to 2.1 Td.

Different breath VOC profiles were observed at each FAIMS setting.

This additional selectivity can be utilized for non-targeted profiling of breath or targeted screening methods by applying the CF required to isolate a species of interest.

5. Conclusions

- We describe the first example of nested FAIMS data within the timescale of TD-EESI-MS analysis.
- The orthogonal FAIMS separation provides the additional selectivity required for a matrix as complex as breath.
- The ~10 minute analysis time of TD-EESI-FAIMS-MS offers a more viable screening method for the clinical environment than traditional GC-MS methods.