Large-scale breath metabolomics by two-dimensional gas chromatography (GCxGC)

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Optimisation of a high-throughput method for breath analysis by thermal desorption combined with flow modulated comprehensive two-dimensional gas chromatography with dual flame ionisation and quadrupole mass spectrometric detection (TD-GCxGC-FID/MS).

Context

- GC×GC affords unparalleled separation power over conventional GC-MS (Figure 1).
- GC-MS is still considered 'gold standard'.
- Previous GCxGC breath studies are small scale or involve use of expensive detectors and modulators with high consumable costs.

Aims

Provide clinical and analytical researchers with a method that makes breath analysis by GCxGC a routine/high-throughput, cost-effective and reliable option for large scale biomarker discovery.

Study

- East Midlands Breathomics Pathology Node (EMBER).
- Established UK breathomics centre, developing in-clinic analytical technologies.
- Discovery of biomarkers in breath in patients presenting with undifferentiated acute breathlessness.

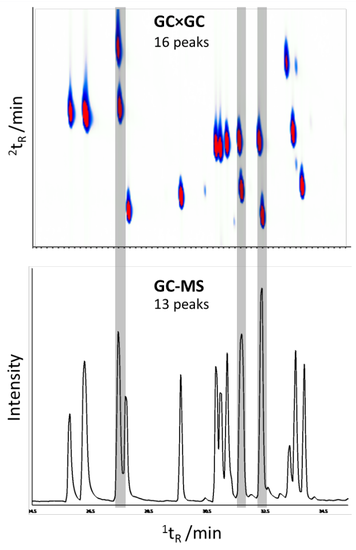

Figure 1: (top) 2D GCxGC chromatogram showing the separation of peaks in the 2nd dimension which co-elute in the (bottom) GC trace.

Methods

- Patients were recruited with self-reported acute breathlessness. Indicator diagnoses of interest were asthma, COPD, pneumonia and heart failure.
- Breath samples were collected at 448 patient visits, using the ReCIVA breath sampler (*Owlstone Medical*). ×1 breath, air supply, room air per patient.
- Sorbent tubes were dry purged, spiked with internal standard and analysed by TD-GC×GC-FID/MS at the University of Leicester, UK.
- Instrumentation included a thermal desorption autosampler coupled to a GC×GC with flow modulator and purged splitter plate, with dual FID and qMS (Figure 2).

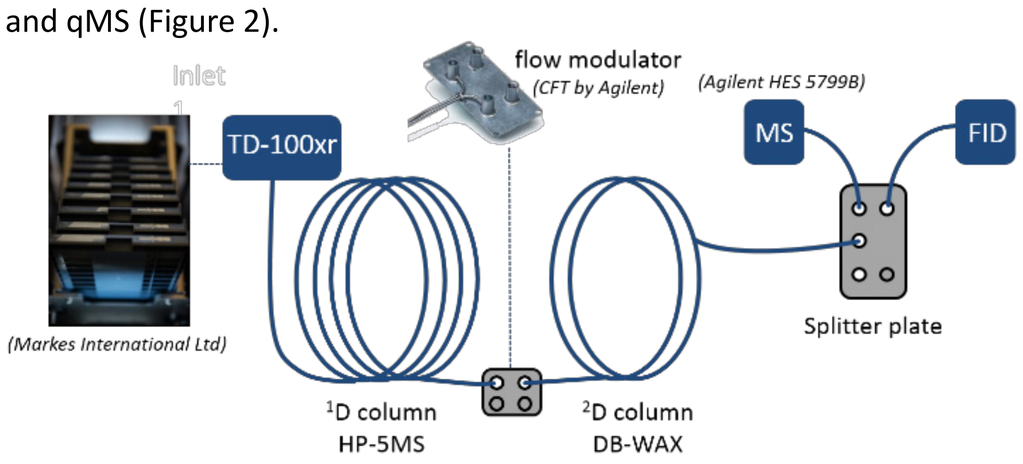

Figure 2: Analytical setup showing thermal desorption autosampler with flow modulation comprehensive two-dimensional gas chromatograph coupled to a splitter plate for dual flame ionisation and mass spectrometric detection.

Samples were analysed in batches with regular trap blanks and multi-component reference mixtures to monitor retention behaviour, separation and detector response.

6 D^2 phases were tested
- Stabilwax, Rtx-200, Rtx-5MS, Rtx-1MS, BPX-50 and SLB-IL111.

Flow modulation was achieved using a microfluidic device based on Agilent's capillary flow technology.

Results

Full method development included optimisation of D^1 and D^2 flow rates, modulation period (P_M), load and flush times, D^2 stationary phase and column lengths. For example, optimal load time was 0.220 s, ±50 ms resulted in significant peak fronting and tailing (Figure 3).

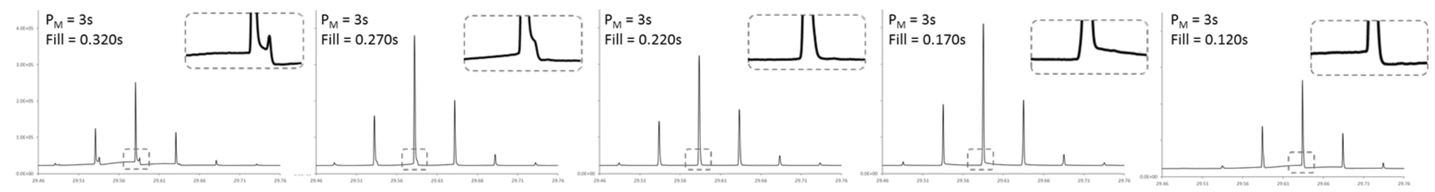

Figure 3: 1D representation of modulated peaks of α-pinene showing the effect of modulation and flow parameters on peak shape. Effect of load and inject time on modulated peak shape.

- Reference mixes were used to monitor column degradation. Δ^2t_R gradually increased with initial ^2t_R e.g. m = -0.00023 decane to m = -0.01194 anthracene, as expected within an orthogonal system (Figure 4).

This informed when to replace D^2 columns, for chromatographic alignment and for retention indices.

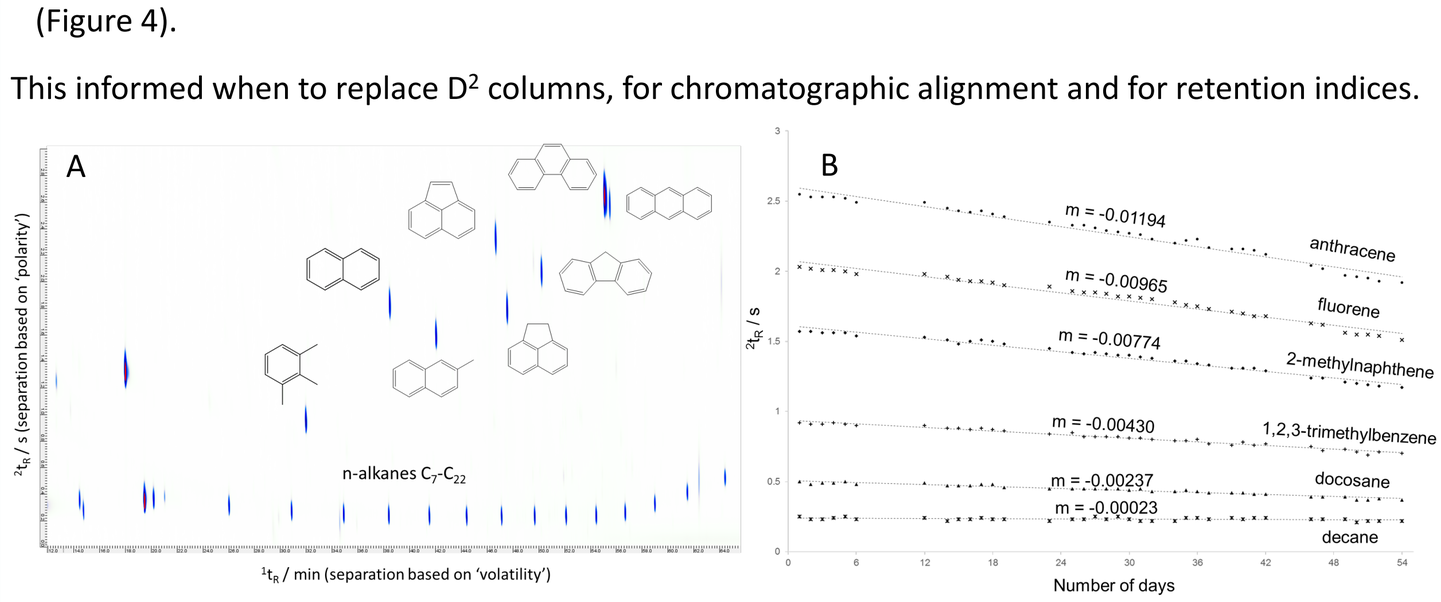

Figure 4: (A) 2D chromatogram of n-alkane and aromatic mixture. (B) Change in ^2t_R over two months.

Figure 5 shows the separation of breath VOCs by GCxGC using the optimised method. Few published GCxGC chromatograms exist which clearly depict

- excellent separation of the complex breath matrix,
- underlying chromatogram structure (ordering of chemically related compounds),
- the types of groups present and relative abundances.

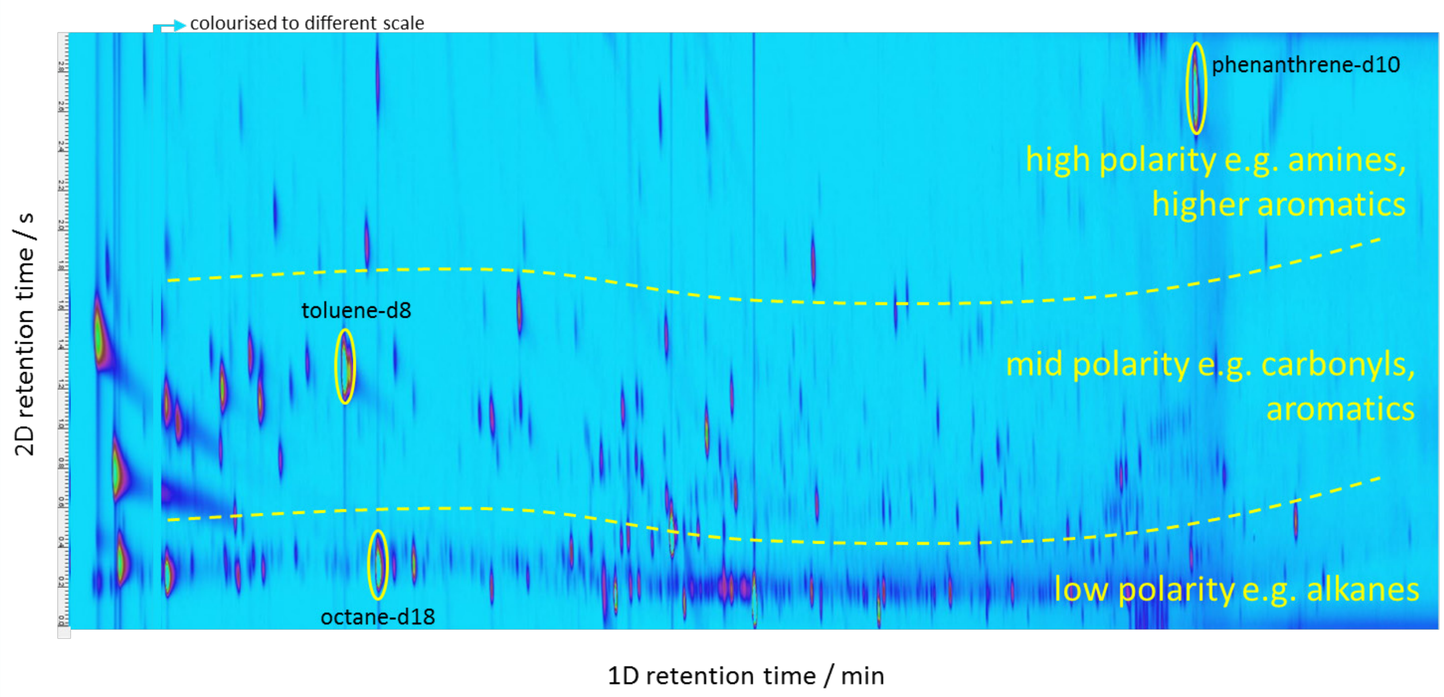

Figure 5: GCxGC chromatogram colour plot showing separation of hundreds of VOCs in breath.

- Reliable flow modulation removes the cost and complications experienced with thermal modulation.
- FID was compatible with high D^2 flow rates and provided high acquisition rates and dynamic range.
- Supported by a fast scanning qMS replaced the need for an expensive spectrometer.

Conclusions

- Optimised GC×GC method provides highly resolved chromatographic data.
- Low cost flow modulator and qMS makes advanced technique accessible.
- Collection protocol and automated analysis clinically viable and meets large recruitment targets.
- Demonstrates potential of GC×GC for routine breath analysis for large scale metabolomics studies.

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