

Profiling volatile organic compounds in exhaled breath by TD–GC–TOF MS

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Introduction

The accurate identification and measurement of biomarkers in human breath has the potential to flag a range of physiological and pathological conditions. To identify biomarkers in breath it is essential that sensitivity is maintained whilst recording full spectral information. Pre-concentration techniques using solid adsorbents to entrain exhaled breath followed by analysis by thermal desorption–gas chromatography–time-of-flight mass spectrometry (TD–GC–TOF MS) can provide the required sensitivity for both targets and unknowns.

In order to link exhaled substances to diseases, it is important that trace VOCs are reliably identified and accurately measured. In this study we use an innovative TOF design that produces ‘classical’ spectra, allowing direct comparison with established commercial or user-created libraries. Novel data-processing software is also demonstrated that allows real-time background subtraction, deconvolution, library-searching and comparative analysis.

Experimental

Sampling: A disposable Bio-VOC™ sampler was used to collect 2 × 129 mL of end-tidal air from the breath of three participants, and the breath samples were then transferred directly to a sorbent tube.

TD: Instrument: UNITY–ULTRA-xr™ (Markes International). Sorbent tubes: ‘Material emissions’ (Markes International part no. C3-AAXX-5304). Samples are stable on these tubes for several weeks, allowing transport to the analytical laboratory without risk of degradation.

GC: Column: DB-624™, 60 m × 0.25 mm × 1.4 μm.

TOF MS: Instrument: BenchTOF-HD™.

Software: Instrument control & data processing by TOF-DS™ (1DGC) and ChromSpace® (GC×GC).

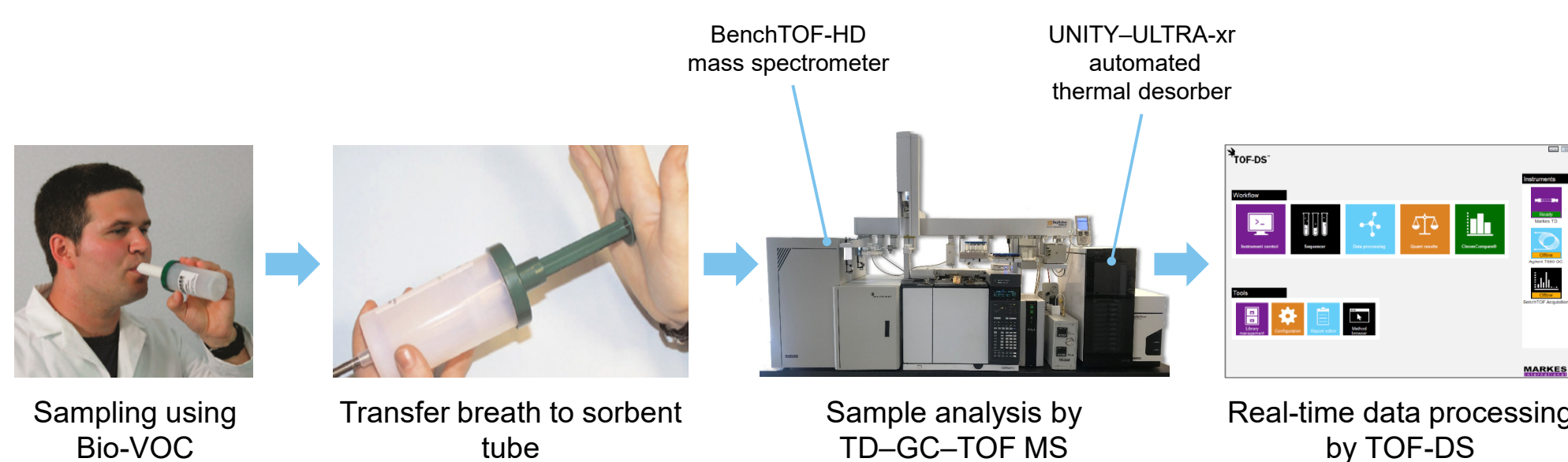


Figure 1: Breath analysis workflow.

Results and discussion

Figure 2 shows the analyses of end-tidal breath from the three participants, with key compounds identified.

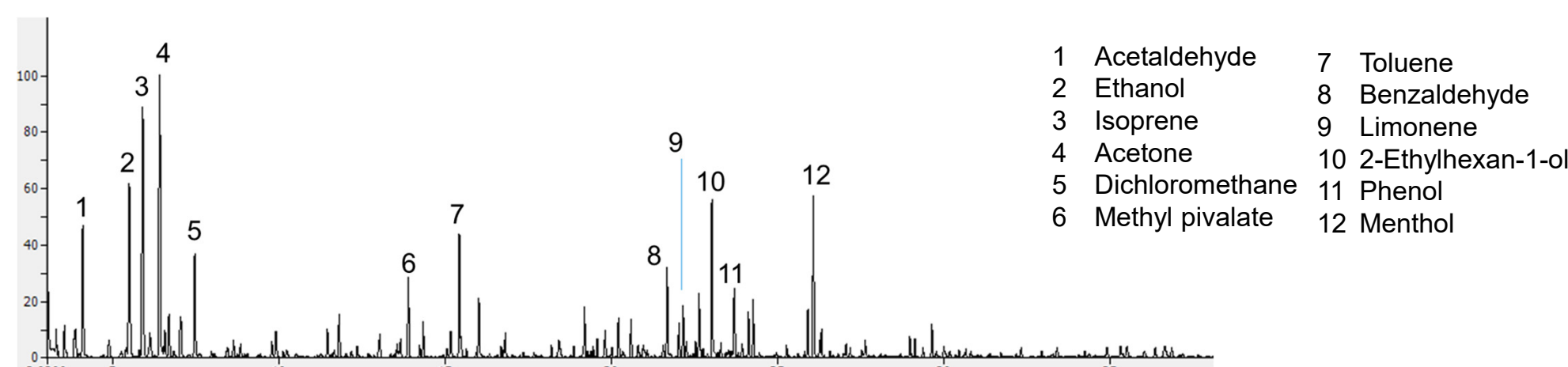


Figure 2: TD–GC–TOF MS chromatogram of end-tidal breath

Real-time data processing

To maximise the number of positive identifications for trace-level compounds while improving sample throughput, TOF-DS software was used to baseline-compensate, integrate, deconvolve and library-search the data while the sample was still acquiring.

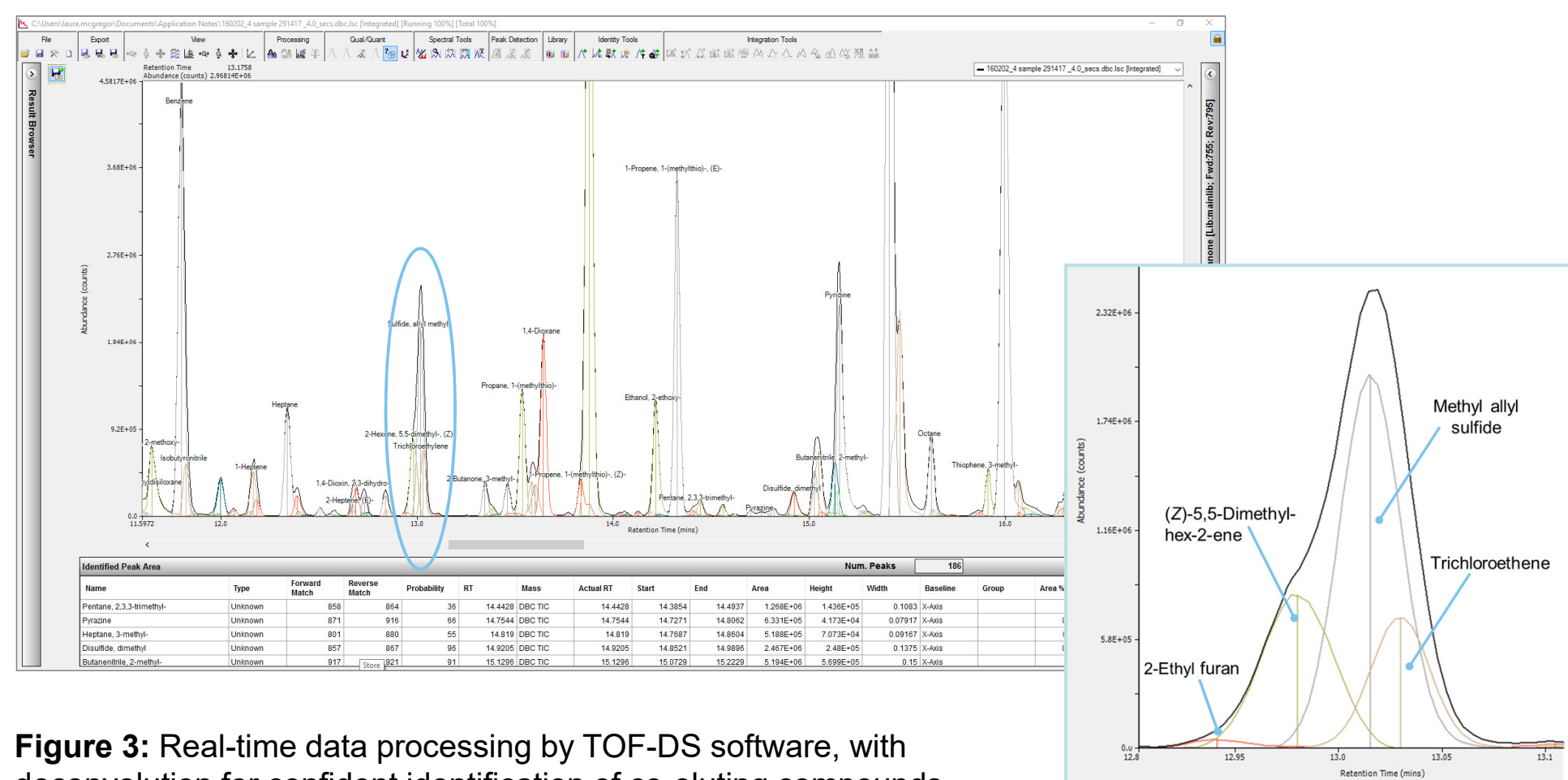


Figure 3: Real-time data processing by TOF-DS software, with deconvolution for confident identification of co-eluting compounds

Deconvolution enables masked and co-eluting peaks to be confidently identified. The expansion (Figure 3) shows a particularly good example, with four components being confidently identified within a single peak. This would have been very challenging to achieve using conventional sampling and analysis methods (particularly in the case of the trace-level component 2-ethylfuran), showing the power of this TD–GC–TOF MS approach.

Comparison of breath profiles

The ChromCompare module in TOF-DS can be used for automated chemical profiling of complex chromatograms (Figure 4). ChromCompare provides rapid and objective comparisons based on relative abundances of target compounds.

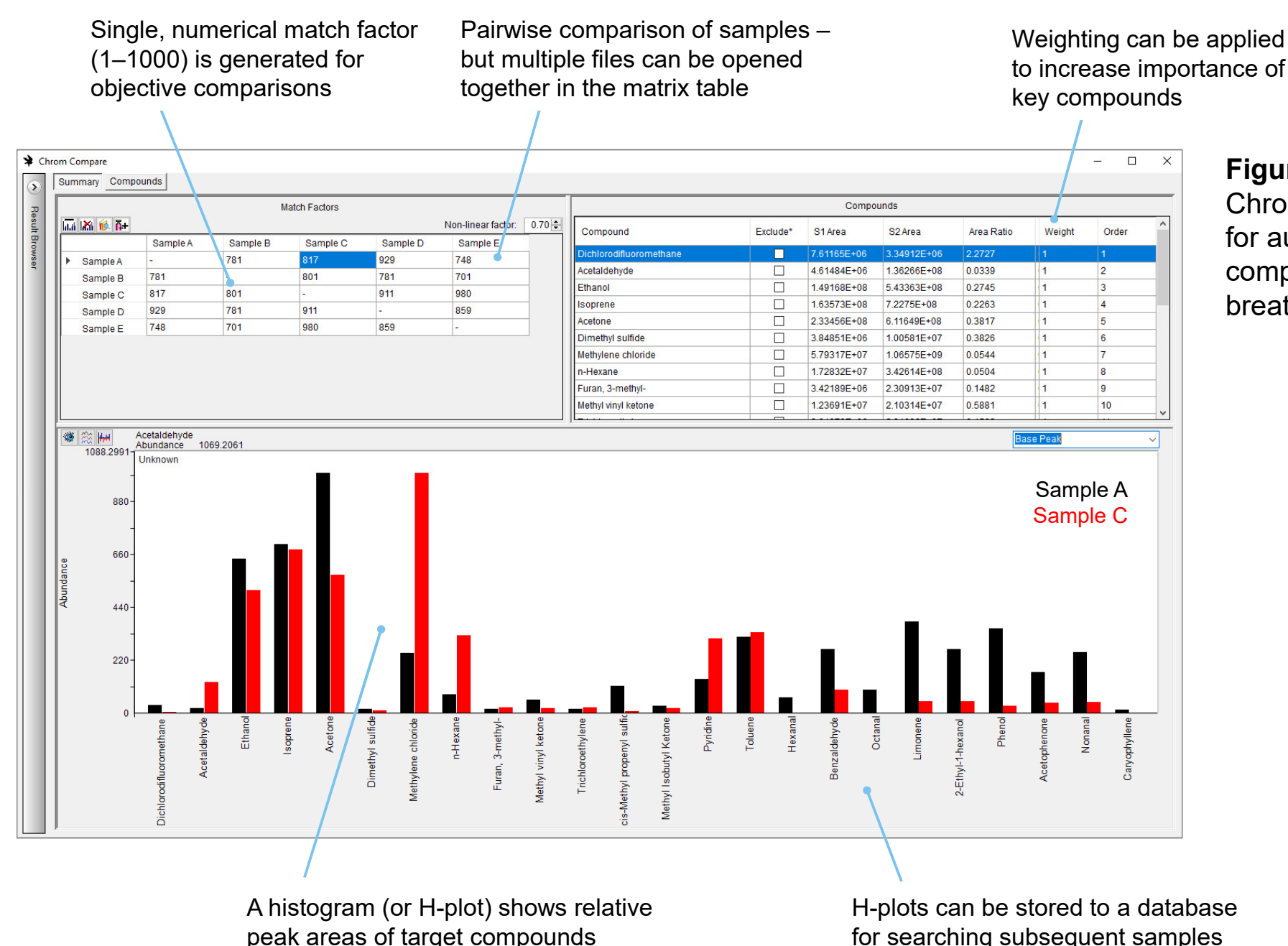


Figure 4: Use of ChromCompare for automated comparison of breath profiles.

Improved biomarker discovery with TD–GC×GC–TOF MS

Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC–TOF MS) can provide the enhanced separation necessary to resolve complex biological matrices, where diagnostic compounds are rarely of high abundance. By adopting a comprehensive approach, measurement of the maximum possible number of compounds is achieved in a realistic run time.

Here, we use the INSIGHT flow modulator (SepSolve Analytical) for consumable-free GC×GC and efficient modulation of volatiles.

INSIGHT's reverse fill/flush dynamics ensure sharp and symmetrical peaks are achieved, with no compound breakthrough.

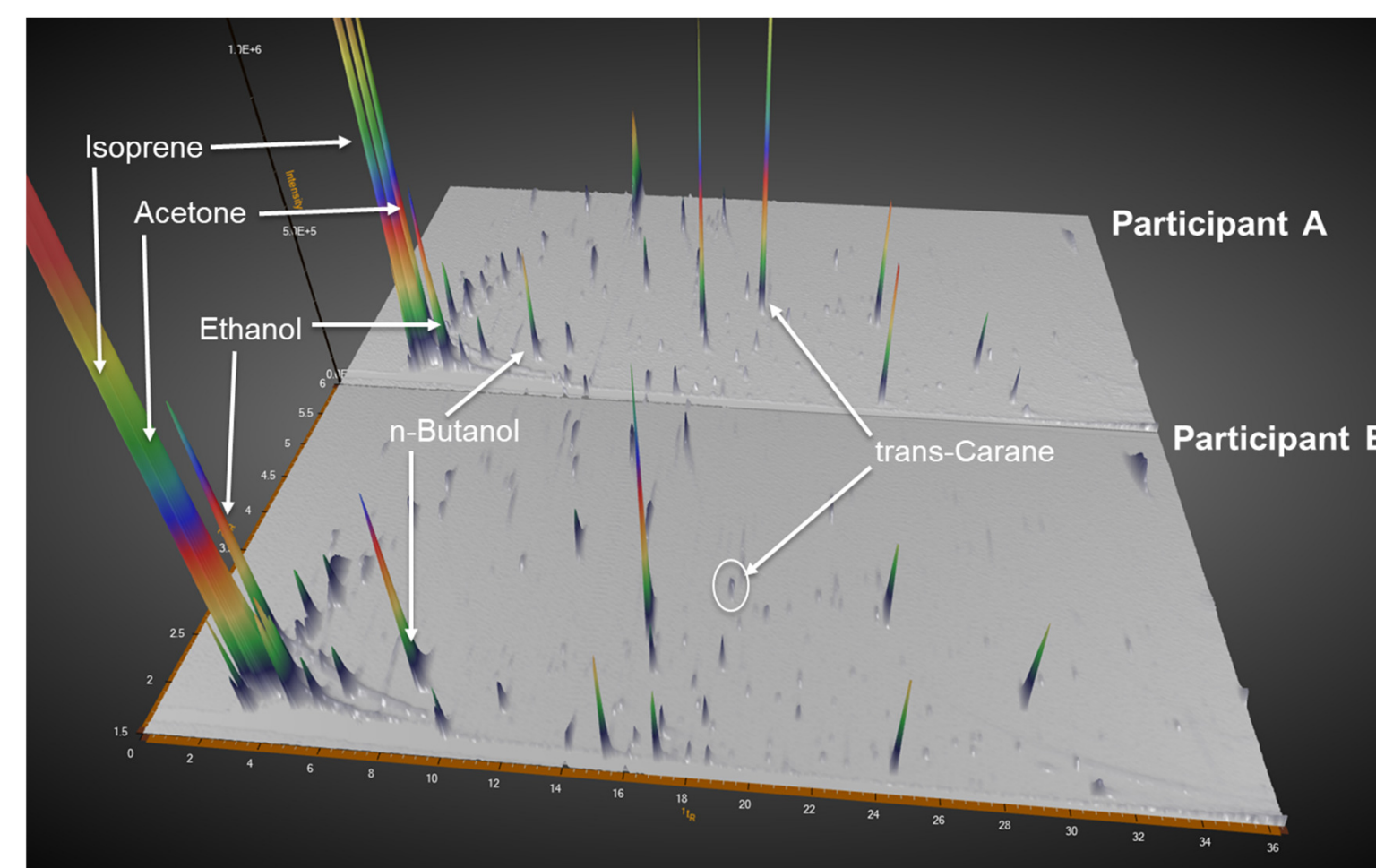


Figure 5: GC×GC–TOF MS surface charts of breath VOCs from two participants.

Conclusions

TD–GC–TOF MS provides a high-performance approach to breath analysis:

- ▶ Easy collection of a wide range of VOCs from end-tidal breath onto packed sorbent tubes.
- ▶ Sampling simplicity and the ease with which TD is automated make it ideally suited to complex clinical trials with large participant numbers.
- ▶ BenchTOF allows the entire sample to be screened with high sensitivity in a single run, and provides the flexibility to upgrade to more advanced methodology, such as GC×GC.
- ▶ The use of TD also boosts sensitivity by pre-concentrating the sampled VOCs, improving identification of important trace-level biomarkers that may otherwise be overlooked.
- ▶ TOF-DS software speeds up data analysis using real-time processing, with no user intervention required.