

# Flavour profiling of milk using high-capacity sorptive extraction and TD-GC×GC-TOF MS/FID



This study demonstrates the high performance of PDMS sorptive extraction probes, coupled with thermal desorption pre-concentration and INSIGHT™ flow-modulated GC×GC-TOF MS/FID analysis, for the separation and identification of flavour compounds in milk. As well as comparing the profiles of ten milk samples, we focus on the ability of the analytical setup to physically separate components that would co-elute in a one-dimensional GC system.

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## Introduction

Milk, as a liquid product produced on a vast scale, is highly susceptible to contamination – from chemicals used in agriculture, from animal feed, or from the transport, processing and packaging processes. Reliable analysis of the volatile components of milk is therefore valuable to ensure high quality.

Traditionally, solid-phase micro-extraction (SPME) has been used for flavour profiling of foods and beverages, and although fast and simple, it can suffer from limited sensitivity and reproducibility. In addition, immersive sampling is often avoided because it can reduce the fibre lifetime, or cause matrix interference (resulting from capillary effects).

High-capacity sorptive extraction using HiSorb™ probes can tackle these issues, by providing a relatively large volume of PDMS stationary phase that results in higher sample loadings.<sup>[1]</sup> The probes are also robust, easily rinsed free of matrix, and can typically be reused at least 50 times without any reduction in performance. Used in conjunction with pre-concentration on Markes' thermal desorption (TD) instruments, this approach offers excellent sensitivity, as well as the ability to re-collect a portion of the sample onto a clean sorbent tube for repeat analysis.

The aroma profiles of foods and beverages are often highly complex, with important compounds such as trace-level off-odours sometimes masked by higher-loading components. Comprehensive two-dimensional gas chromatography

(GC×GC) coupled with time-of-flight mass spectrometry (TOF MS) is therefore an excellent choice, with the enhanced separation capacity of GC×GC complementing the ability of (a) TOF MS to provide confident identification of targets and unknowns, and (b) FID to provide robust quantitative analyses.

In this study, we demonstrate the value of high-capacity sorptive extraction with TD–GC×GC–TOF MS/FID to investigate the flavour profiles of a range of milk products. In this case the GC×GC method employs INSIGHT™ reverse-fill/flush flow modulation, lowering running costs and avoiding the logistical issues associated with liquid cryogen.

For more information on GC×GC please read our [white paper](#).

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## Experimental

**Samples:** Ten milk products were analysed in this study:

- ▶ Cow's milk – Whole
- ▶ Cow's milk – Semi-skimmed
- ▶ Cow's milk – Skimmed
- ▶ Cow's milk – Extended shelf-life
- ▶ Infant formula (Brand A)
- ▶ Infant formula (Brand B)
- ▶ Goat's milk
- ▶ Soya milk
- ▶ Coconut milk
- ▶ Almond milk

**Sample preparation:** 10 mL of each sample was placed in a 20 mL headspace vial with 2 g of NaCl. To prepare the infant formula, 10 mL of water was added to 2 g of powder.

**Immersive sorptive extraction:** PDMS sampler: Inert HiSorb™ probe (Markes International); Time: 60 min; Temperature: 35°C.

**TD:** Instrument: TD100–xr™ (Markes International); Focusing trap: 'General-purpose'. HiSorb probes were inserted into empty inert-coated stainless-steel TD tubes.

**GC×GC:** Flow modulator: INSIGHT™ (SepSolve Analytical). P<sub>M</sub>: 5.0 s.

**TOF MS:** Instrument: BenchTOF-Select™; Tandem Ionisation®: Simultaneous acquisition of 70 eV and 12 eV data; Mass range: m/z 35–500.

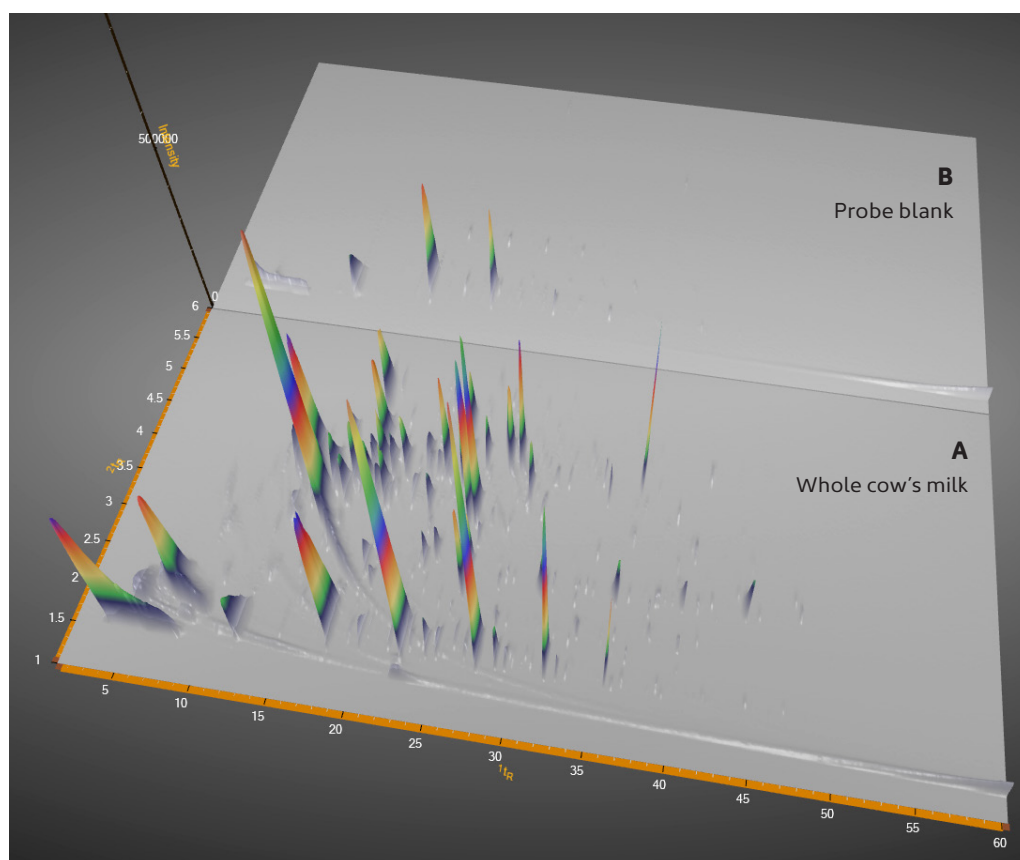
**Software:** ChromSpace® GC×GC software for full instrument control and data processing.

Please contact SepSolve for full analytical parameters.

## Results and discussion

### 1. Overall separation of the extracted components

The GC×GC–TOF MS colour plot obtained from whole cow's milk is provided in Figure 1, showing the excellent separation achieved. Analysis of a probe blank shows that the only major peaks are siloxanes from the PDMS sorbent. It is worth noting that the second separation in GC×GC ensures that the aroma/flavour compounds of interest are well-separated from the siloxane interferences – unlike in one-dimensional GC.

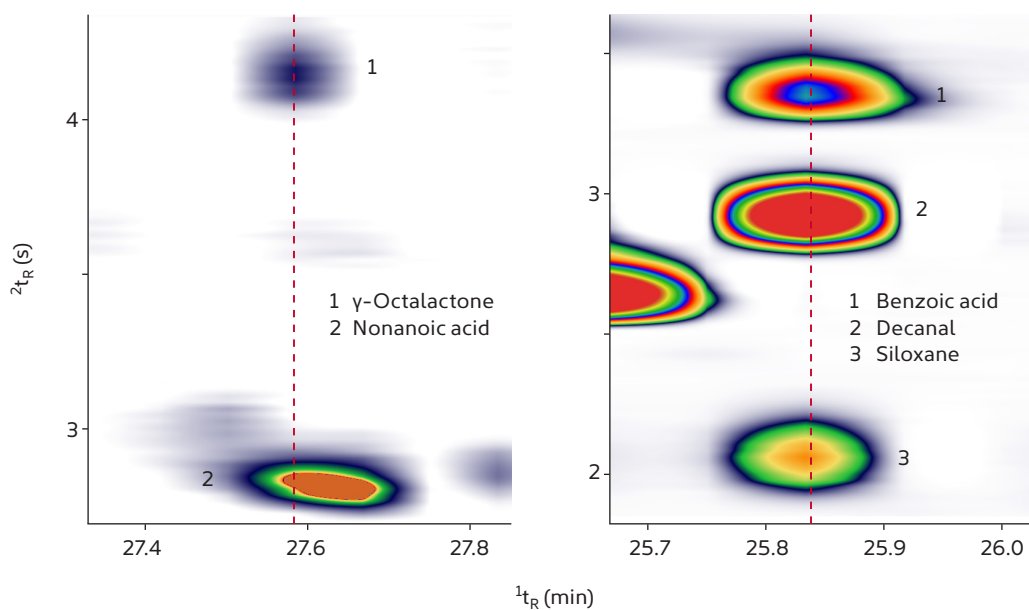


**Figure 1**

GC×GC–TOF MS surface plots of (A) whole cow's milk, and (B) a probe blank, viewed in ChromSpace.

### 2. Separation of co-eluting components

To highlight the added separating capability of GC×GC, Figure 2 highlights two regions of the colour plots for almond milk and whole cow's milk where components would have co-eluted in a one-dimensional separation. In the almond milk plot, it is highly likely that the minor peak for  $\gamma$ -octalactone would have been overlooked in a one-dimensional separation.

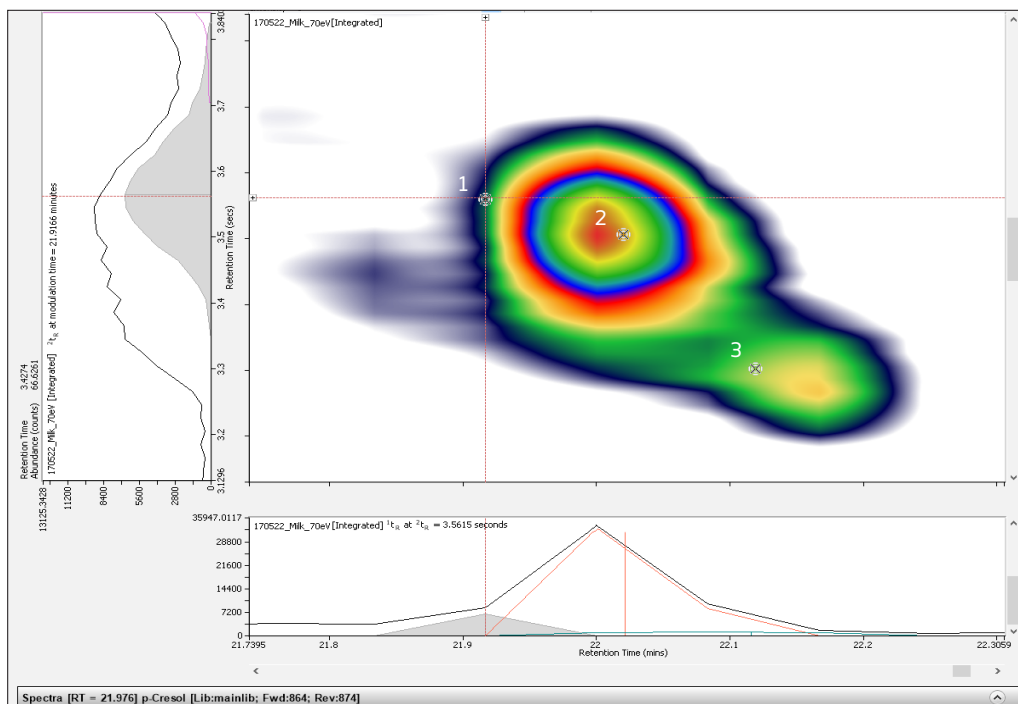


**Figure 2**

Enhanced separation of flavour components in (left) almond milk and (right) whole cow's milk by GCxGC-TOF MS.

### 3. Deconvolution of co-eluting components

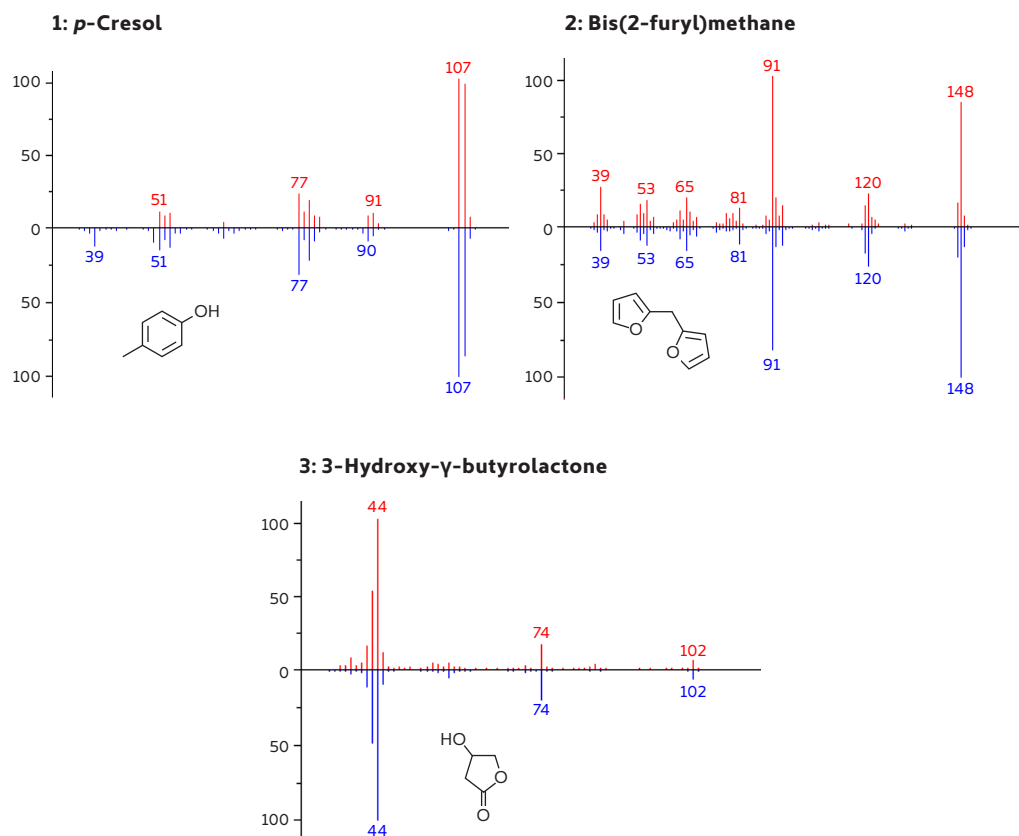
Despite the enhanced peak capacity of GCxGC, co-elutions may still remain. This is easily addressed by the use of deconvolution software for TOF MS, which enhances method optimisation by allowing such peaks to be distinguished – as shown by the example in Figure 3.



**Figure 3**

Deconvolution of *p*-cresol (component 1; grey-shaded profiles) from two closely-eluting peaks in the GCxGC-TOF MS profile of the extended shelf-life milk, viewed in ChromSpace.

Confident identification of these components is achieved by use of a BenchTOF™ time-of-flight instrument, which inherently provides excellent matches to quadrupole-acquired spectra, such as those in the NIST database (Figure 4). Such confident identifications are imperative in situations where minor but aroma-active components are present – for example, in this case *p*-cresol is known to impart a ‘cowy, animal’<sup>[2]</sup> taint to milk.



**Figure 4**

BenchTOF spectra (top, red) and NIST 14 spectra (bottom, blue) for components 1–3 identified in Figure 3.

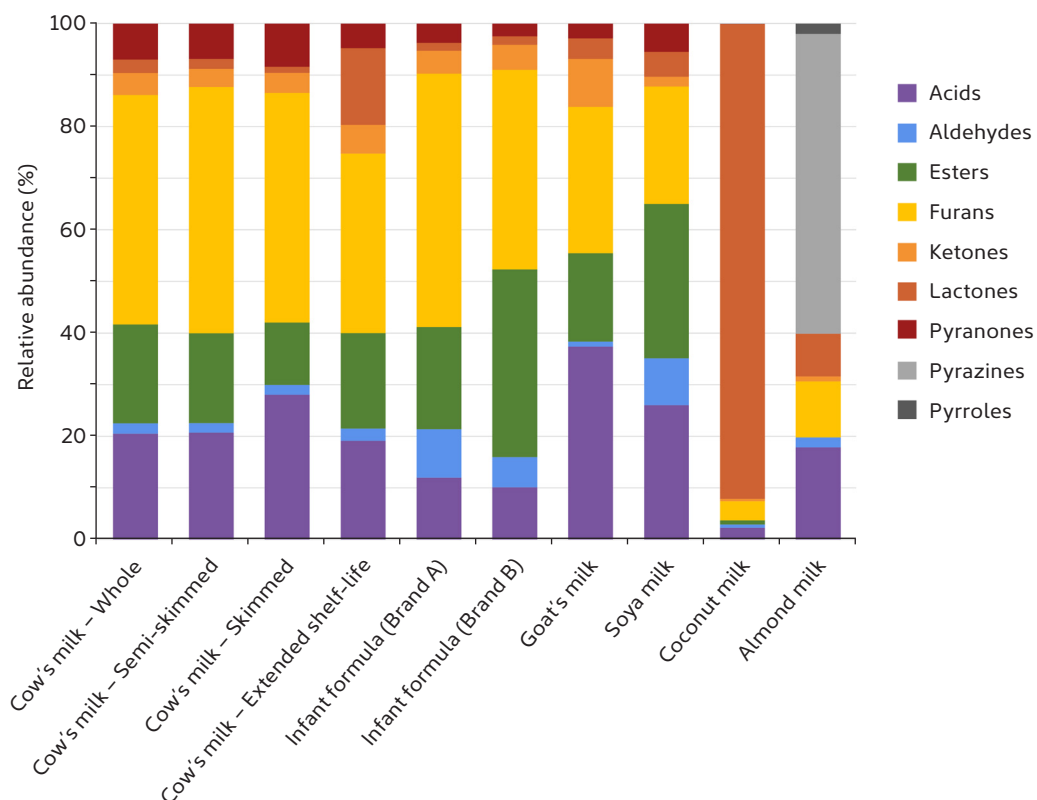
#### 4. Comparison of milk products

To illustrate how the GC $\times$ GC–TOF MS data can be applied to targeted GC $\times$ GC–FID analysis, a stencil of 59 major components was created in ChromSpace®. The stencil was created using TOF MS data files for confident identification, and was then applied to the FID data files to collate the peak area data for these target components across the ten milk samples. The resulting peaks were grouped by chemical class, and the corresponding relative abundances are displayed in Figure 5.

Some interesting findings are:

- ▶ The four cow’s milks have very similar compositions, albeit with a higher proportion of acids in the skimmed milk and a higher proportion of lactones in the extended shelf-life milk.
- ▶ The two infant formula milks have lower proportions of acids and higher proportions of aldehydes than the cow’s milks.

- ▶ The soya and goat's milks have lower volatile content than the other products. However, the goat's milk has a relatively high proportion of ketones, with one of the more abundant of these, benzyl ethyl ketone, reported to impart an 'earthy' taste.<sup>[3]</sup>
- ▶ The coconut milk contains a far higher proportion of lactones than the other samples –  $\delta$ -octalactone and  $\delta$ -decalactone, for example, impart a 'creamy, coconut', flavour.<sup>[3]</sup> This was also the only sample to contain traces of vanillin.
- ▶ The almond milk was the only sample to contain a selection of pyrazines, which impart a 'roasted, nutty' flavour.<sup>[3]</sup>



**Figure 5**

Comparison of the compositions of the milk products, grouped by chemical class.

## Conclusions

In this study, we have shown that the combination of high-capacity sorptive extraction with TD pre-concentration and INSIGHT™ GC×GC–TOF MS/FID analysis is a powerful approach to characterising the complex flavour profiles of milk products, with minimal sample preparation.

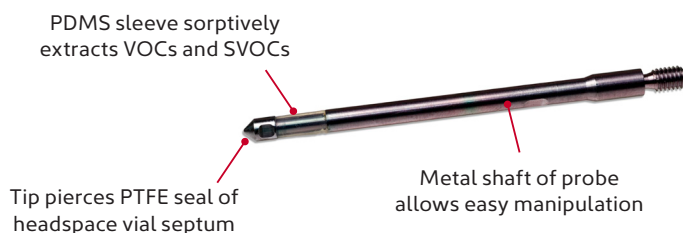
As well as being easier to carry out than SPME, sampling with PDMS probes followed by TD pre-concentration offers improved sensitivity while retaining the ability to sample a wide range of chemical classes. The probes and tubes are also reusable, which minimises the cost per sample.

Running costs are further lowered by the use of flow-modulated GC×GC, which provides high chromatographic resolving power without the need for expensive liquid cryogen. Confident identification is also assisted by peak deconvolution software and the use of a TOF MS instrument able to generate ‘reference-quality’ spectra. After robust method optimisation using GC×GC–TOF MS, chemical profiling can easily be performed using GC×GC–FID systems across multiple quality-control laboratories.

For more information on this application, or any of the techniques or products used, please contact SepSolve.

## References and notes

- [1] High-capacity sorptive extraction operates on a similar principle to SPME, but with a larger area of polydimethylsiloxane (PDMS) sorbent to increase sampling capacity. Solid or liquid samples are placed inside standard 20 mL or 10 mL vials, sealed with a crimped septum cap, and an inert HiSorb™ probe (from Markes International) is inserted into the vial for either immersive or headspace sampling. The vial and probe are agitated and heated using the HiSorb™ Agitator, following which the probe is washed, dried, and inserted into a conventional TD tube for direct desorption and automated TD–GC–MS analysis.



- [2] R.J. McGorin, Character-impact flavor compounds, in: *Sensory-directed Flavor Analysis*, R. Marsili (ed.), 2006, CRC Press.
- [3] The Good Scents Company Information System (search facility), [www.thegoodscentscompany.com/search2.html](http://www.thegoodscentscompany.com/search2.html) (accessed on 28 September 2017).

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