www.sepsolve.com

Reliable speciation of fatty acid methyl esters by flow-modulated GC×GC–TOF MS/FID with Tandem Ionisation

Jan Nordin[1], Laura McGregor[2], Keith Summerhill[3], Aaron Parker[2], Bob Green[2] and Albert Koulman[3,4]

[1] Chemalys, Dalarö, Sweden. [2] SepSolve Analytical, Peterborough, UK. [3]MRC Elsie Widdowson Laboratory, Cambridge UK. [4] NIHR BRC Nutritional Biomarker Laboratory, University of Cambridge, UK.

Introduction

The large-scale profiling of plasma phospholipid fatty acids as methyl esters (FAMEs) by GC has contributed significantly to our understanding of the role of dietary fat in the aetiology of type II diabetes.[1] With our current GC–FID platform we have been able to profile around 25 fatty acids in over 50,000 participants.[2] We expected that in our GC–FID method, some fatty acids were overlapping with different *trans* fatty acids and branched-chain fatty acids, which are specific markers of dairy fat intake and processed fat intake.

Increasing the resolution without increasing analysis time or affecting reproducibility demanded the introduction of a second dimension. Our standard J&W HP-88 column was retained in the first dimension – enabling direct correlation of new sample data with historic results from conventional GCs – while the second dimension provided enhanced separation of *trans* fatty acids (such as conjugated linoleic acid) and branched-chain fatty acids.

This poster outlines the development and validation of the GC×GC–TOF MS/FID approach for FAMEs, to enable routine analysis of a wider range of fatty acids, without increasing the analysis time.

Experimental

Samples: A 37-component FAME standard was prepared in hexane at 200–400 µg/mL. The butter extract was prepared by saponification, derivatisation and SPE clean-up.

GC×GC: Injector: OPTIC-4™ multi-mode inlet; Injection volume: 1.0 µL; Split 25:1;
Flow modulator: INSIGHT™ (SepSolve Analytical).

FID: H_2 flow: 30 mL/min; Air flow: 400 mL/min; Temperature: 300°C.

TOF MS: Instrument: BenchTOF-Select™. A bespoke three-port splitter (available from SepSolve Analytical) was used to direct the flow to the TOF MS and FID detectors in the ratio 1:4.

Software: Instrument control and GC×GC data processing by ChromSpace®.

Results and discussion

Method validation

GC×GC separation was optimised using the commercial FAME standard (Figure 1). Separation of all 37 components was achieved with an analytical run time of under 30 minutes, which was not possible with conventional one-dimensional GC, leading to overlap of $C_{20:4(\omega-6)}$ and $C_{20:3(\omega-3)}$.

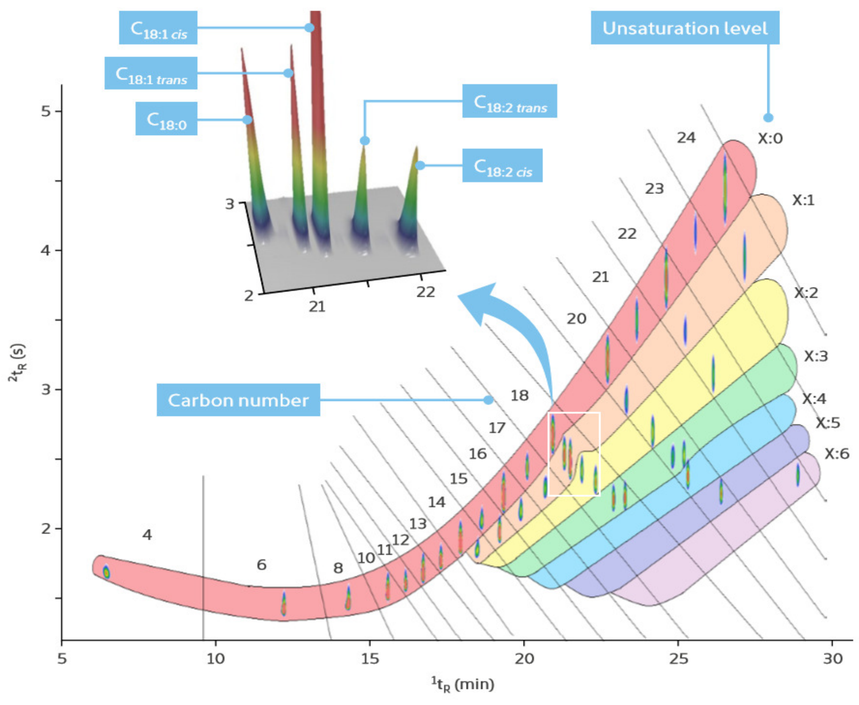

Figure 1: GC×GC–FID colour plot showing the 37-component FAME standard. The coloured bands indicate successively higher levels of unsaturation, from 0 (red, with no C=C bonds) to 6 (violet, with six C=C bonds). The expansion shows separation of *cis* and *trans* isomers.

The INSIGHT flow modulator provided high repeatability (RSDs <1.5%) for replicate analysis of the FAME standard (Figure 2).

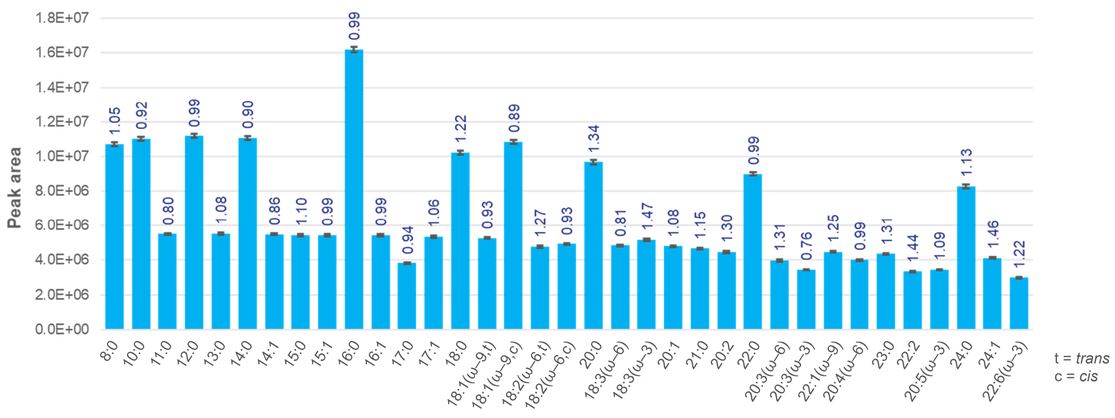

Figure 2: Repeatability of the GC×GC method for replicate analysis of the FAME standard. This was run as a QC sample for ~300 samples in 10 batches. Data labels show RSDs (%) calculated when *n* = 10.

Speciation of FAMEs in real samples

Method validation was performed as part of much larger study (~200 samples) on nutritional biomarkers. Co-elutions in GC–FID are problematic for quantitation, even when peak overlap is slight – especially when analysing complex real samples, such as butter or plasma extracts (Figure 3). The circled regions in Figure 3 highlight where co-elutions would have occurred with a one-dimensional system, causing overestimation of abundances.

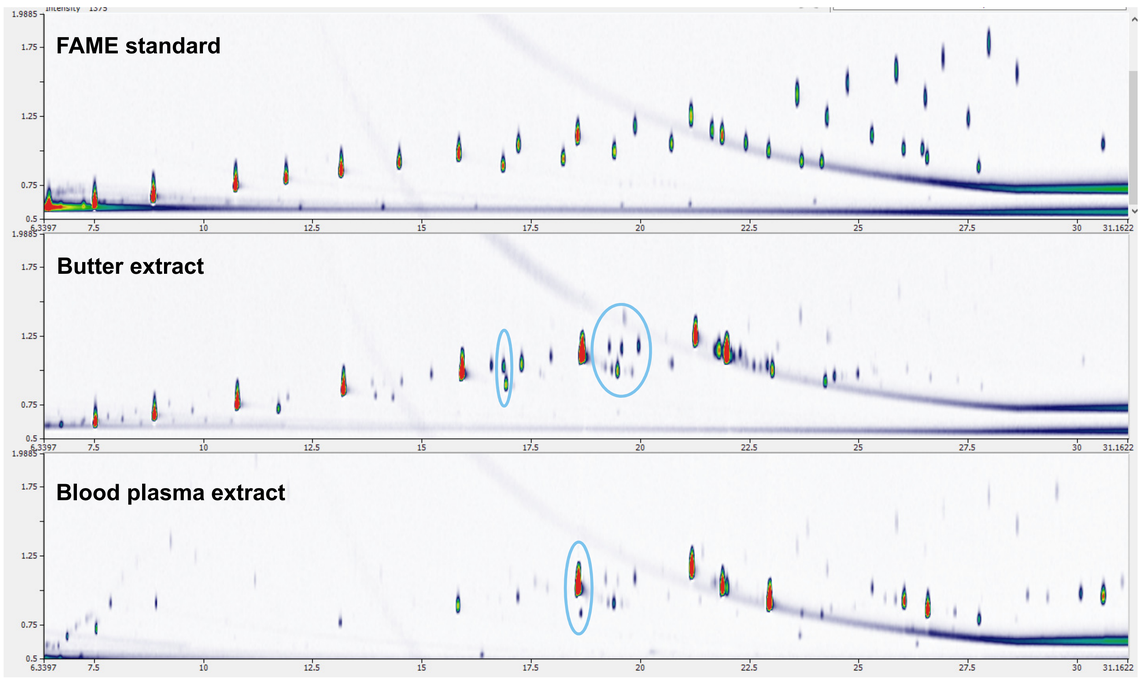

Figure 3: GC×GC–TOF MS colour plots for the FAME standard, a butter extract and blood plasma extract, viewed simultaneously in ChromSpace software.

Adding dimensions with Tandem Ionisation

Despite the benefits of GC×GC with dual TOF MS and FID detection, certain FAMEs can be challenging to identify using standard 70 eV ionisation alone, due to the high degree of fragmentation and subsequent low response for the molecular ion.

The BenchTOF-Select instrument used in this study can address this issue through its Tandem Ionisation® capability, which enables fast switching between conventional 70 eV EI and low-energy 'soft' EI for improved isomer speciation. Figure 4 shows how Tandem Ionisation at 70 eV and 12 eV can assist discrimination between the ω–3 and ω–6 isomers of methyl octadecatrienoate ($C_{18:3}$). The 70 eV spectra are similar, with a high degree of fragmentation and weak molecular ions making it difficult to confirm the carbon chain length. However, the 12 eV spectra show a much stronger molecular ion, as well as structurally significant fragment ions.

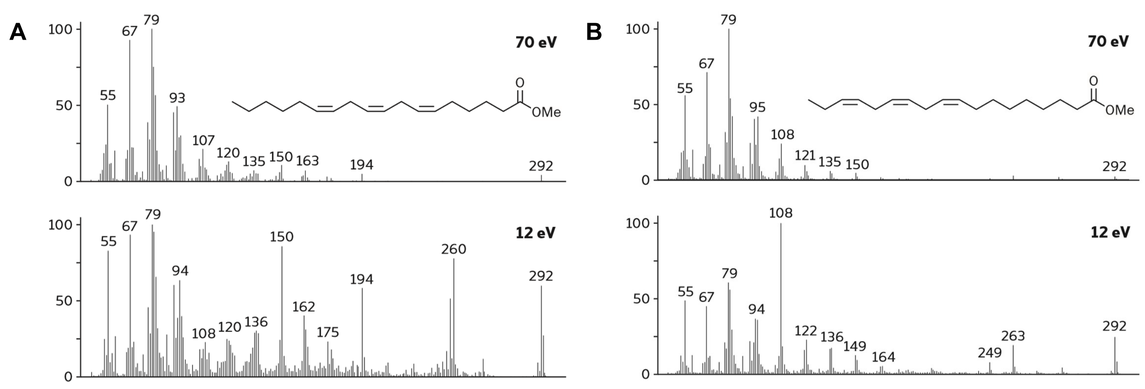

Figure 4: Tandem Ionisation mass spectra at 70 eV and 12 eV for: (**A**) $C_{18:3(\omega-6)}$ and (**B**) $C_{18:3(\omega-3)}$.

Conclusions

This poster has demonstrated that flow-modulated GC×GC–TOF MS/FID provides:

- Simple, robust and affordable separation of FAMEs by INSIGHT–GC×GC.
- Full separation of saturated and unsaturated FAMEs, *cis*/*trans* isomers and ω–3/ω–6 isomers… all in a 30-minute run.
- The ability to identify and quantify targets and unknowns, through simultaneous acquisition of FID and TOF MS data.
- Another level of information with Tandem Ionisation, in cases where 70 eV data alone cannot speciate similar compounds.

References

[1] N.G. Forouhi et al., Differences in the prospective association between individual plasma phospholipid saturated fatty acids and incident type 2 diabetes: The EPIC-InterAct case-cohort study, *The Lancet Diabetes & Endocrinology*, 2014, 10: 810–818.

[2] L.Y. Wang et al., Development and validation of a robust automated analysis of plasma phospholipid fatty acids for metabolic phenotyping of large epidemiological studies, *Genome Medicine*, 2013, 5: 39.

SepSolve Analytical Ltd
T: +44 (0)1733 669222 (UK) +1 888-379-383 (US)
E: hello@sepsolve.com

www.sepsolve.com