SepSolve Analytical

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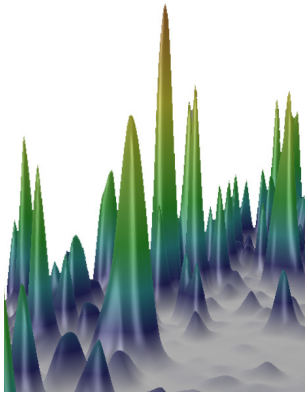
Technical note: Comprehensive two-dimensional gas chromatography



This paper provides a short overview of the theory and practice of the rapidly-developing field of two-dimensional gas chromatography (GC×GC). Included in the discussion are a summary of the detectors used, an assessment of the options available for modulating the first-column eluate, and some recent developments in methodologies for interpreting the results.

Contents

1. Basic principles

Comprehensive two-dimensional gas chromatography (GC×GC) is a high-performance analytical technique with an increased separation capacity that enhances the analysis of complex samples, such as petrochemicals, fragrances and environmental extracts.[1]

GC×GC involves coupling two columns with different stationary phases, to allow separation of a mixture based on two different separation mechanisms (Figure 1). The sample is therefore separated in two dimensions.[2] This provides GC×GC with the capacity to resolve an order of magnitude more compounds than traditional gas chromatography.[3]

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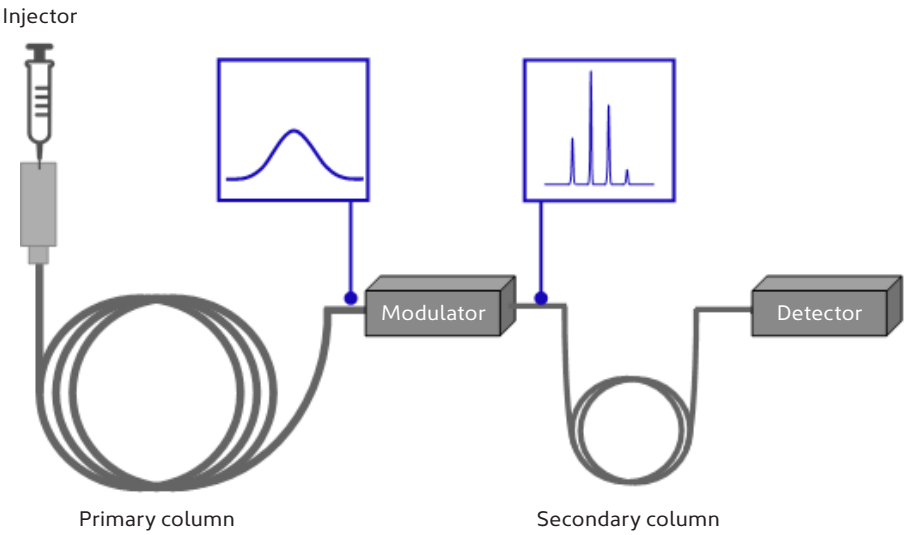

Figure 1

Schematic of a GC×GC system.

As with a conventional GC, the sample is introduced (by a range of mechanisms, such as headspace, thermal desorption, solid-phase micro-extraction or liquid injection) into a heated port and swept through the column by a carrier gas. The first dimension (^{1}D) typically consists of a long (20–30 m) non-polar capillary column, while the second dimension (^{2}D) employs a shorter (1–5 m) polar column; this is deemed normal-phase GC×GC. However, reversing the column polarity has been shown to provide better group-type separation in certain cases.[4] Configuring the column set in such a way is known as reversed-phase (or inverse-phase) GC×GC.

GC×GC provides the ability to separate out previously unresolved co-elutions found in many complex mixtures. When applied to samples such as petrochemicals or environmental extracts, commonly used fractionation processes that are applied prior to analysis can be minimised or eliminated.[5] A complex sample can be injected as a single extract without involving time-consuming fractionation processes. This gives fast screening of the entire sample, allowing many classes of organic contaminants to be monitored simultaneously.

2. Detectors

GC×GC has been coupled with a range of detectors, but due to the narrow peak widths generated in the secondary column, a detector with a data acquisition rate of 30–200 Hz is often used.[6] A popular detector used with GC×GC is flame ionisation detection (FID). FID is an affordable and rugged detector well-suited for quantitative analysis of hydrocarbons, since the response is directly proportional to the number of carbons present in the analyte molecules. However, confident identification can be difficult because retention times (^1t_R and ^2t_R) must be used to characterise the components. Instead, coupling to a mass spectrometer provides an additional level of information on the sample composition by allowing identification of specific peaks based on chemical structure.

In a literature review by Seeley and Seeley,[7] the majority (67%) of published works were obtained using TOF MS, with use of FID and single quadrupole MS also being significant (16% and 11% respectively). However, a number of papers have also been published using selective detectors, such as sulfur chemiluminescence detection (SCD) and electron capture detection (ECD), as well as isotope ratio MS, MS/MS and, most recently, vacuum ultraviolet (VUV) spectroscopy.[8]

3. Modulation

The most critical part of the GC×GC system is the modulation device. Peaks eluting from the first column are sampled and re-injected as narrow chromatographic bands into the secondary column where they are further separated.[2] Separations within the secondary column are fast – normally under 10 seconds in length. To preserve the separation achieved in the primary column, it is recommended that each peak eluting from the primary column is sampled three or four times.[9]

This process of focusing primary column effluent into narrow bandwidths results in improved signal-to-noise ratios for the analyte peaks, generally providing a ten-fold improvement in sensitivity with respect to 1D GC. Ineffective modulation results in broad, tailing peaks in the second dimension, which limits peak capacity.

The two main types of commercially-available modulator – thermal and flow devices – are described in the following sections.

3.1 Thermal modulation

Thermal modulators use broad temperature differentials (by way of hot and cold jets) to retain or desorb analytes eluting out of the primary column.[10] These devices use two-stage operation. In the first stage, the cold jet traps and focuses the eluate at the head of the secondary column (Figure 2A). The hot jet then desorbs the analytes from the stationary phase (Figure 2B), and they continue on to the next cooling stage of the modulation process. Commercial devices use either a quad jet approach (where there are two pairs of jets to trap/desorb the analytes on two different sections of the column) or a delay loop (where the column circles back between the hot/cold jets). Both of these approaches ensure that there are two attempts to focus the analytes.

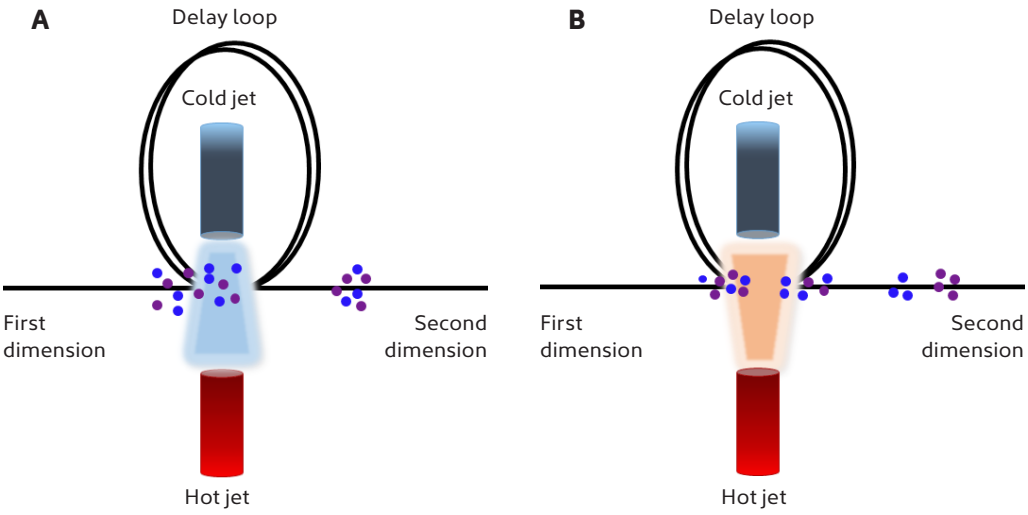

Figure 2

Schematic of a thermal (delay loop) modulator. (**A**) The cold jet traps the first-column eluate, and then (**B**) a pulse of hot air arrives from the hot jet, deflecting the cold jet, and forcing analytes onto the second dimension for further separation.

This process allows the primary column eluate to be focused into narrow injection bands, which increases secondary column resolution and therefore peak capacity. Currently, thermal modulators are the most widely used in GC×GC.[11] The main drawback of thermal modulation is that volatile components cannot be trapped by the cold jet, even when liquid cryogen is used to cool it. Typically, thermal modulators using liquid cryogen can modulate C_4 and above, while those relying on a chiller unit to cool the jets may only be able to modulate C_8 and above.

3.2 Flow modulation

Flow modulators use precise control of carrier and auxiliary gas flows to fill and flush a sampling channel or loop.[12] In the first generation of flow modulators – deemed 'forward fill/flush' – any over-filling of the sample loop flowed directly on to the second dimension, causing poor peak shape and reduced peak capacity. In addition, breakthrough of analytes from the primary column to the secondary column frequently occurred during the flushing stage.

To overcome this, 'reverse fill/flush' dynamics were developed – as adopted in the INSIGHT™ flow modulator by SepSolve Analytical. This approach improves peak shape and limits the baseline rise between modulations by directing any overfill to a bleed line.[13] The sample loop is filled in the forward direction from the first column (Figure 3A), and then rapidly flushed in the reverse direction onto the second column (Figure 3B). The total modulation period (P_M) is the time taken for the fill and flush modes to complete.

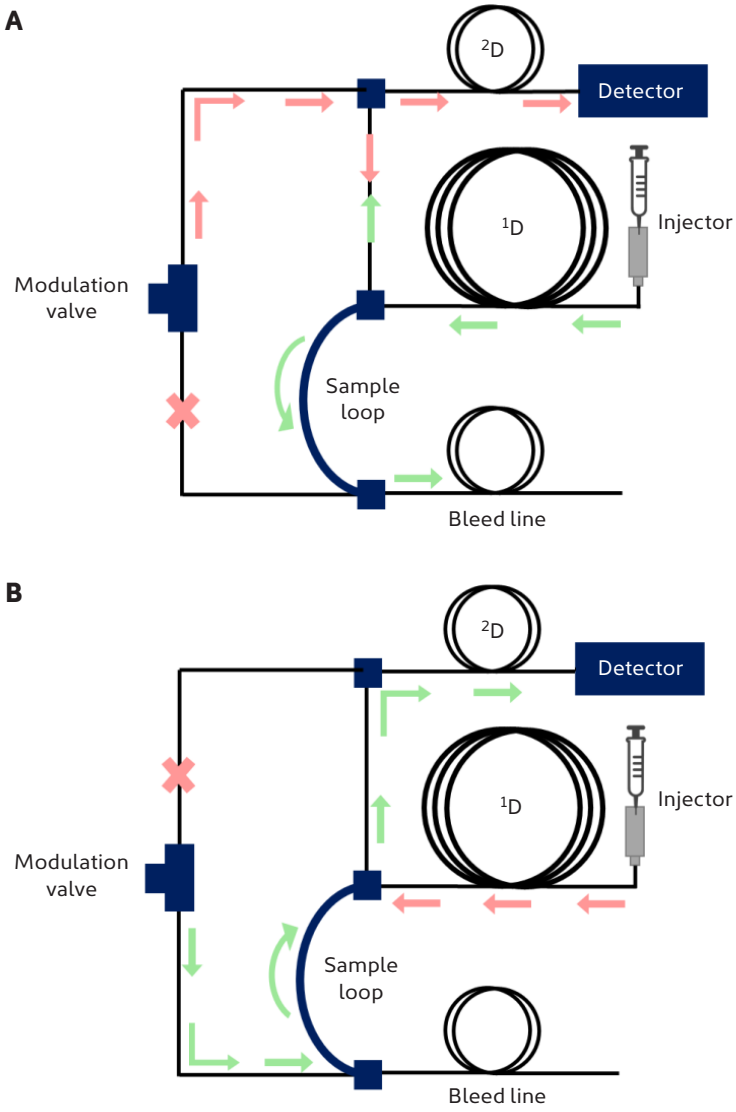

Figure 3

Schematic of a flow modulator using reverse fill/flush dynamics: (**A**) fill mode and (**B**) flush mode.

A key benefit of flow modulation is that it does not suffer from the same volatility restrictions associated with thermal modulation – enabling volatiles from C_1 to be efficiently modulated and expanding the range of applications that can be tackled by GC×GC. There is also an obvious cost benefit, since no liquid cryogen or chiller unit is required.

Additionally, flow modulators are known to exhibit excellent repeatability, due to the precise control of flow in each dimension. Thermal modulators, on the other hand, may show retention time fluctuations due to small variations in column position between the jets, or variation of cryogen flow to the cold jets, making it more difficult to compare large sample batches.

Flow modulators require a high flow rate in the second dimension to compress the primary column eluate, making it challenging to couple directly to mass spectrometric detectors.[14] Typically, the flow is split after the secondary column

to two detectors to harness the power of parallel detection. When using a second detector with different capabilities, two complementary datasets can be captured in a single run, which is often advantageous. For example, robust quantitation can be achieved using FID, while confident identification can be performed by TOF MS.

It has recently been demonstrated that optimisation of GC×GC parameters can allow flow rates compatible with mass spectrometers (~4 mL/min) to be achieved and to avoid the need for splitting.[15] This means that the entire flow can be directed to the mass spectrometer to avoid compromising sensitivity. Some modern TOF MS systems, such as SepSolve's current offering, have sustained a carrier gas loading of 9 mL/min for trace flow-modulated GC×GC.

4. Visualisation of results

The modulated, linear detector output from GC×GC can be represented as a three-dimensional landscape (known as a surface plot) by stacking the fast secondary separations side-by-side (Figure 4). The results can be evaluated using this type of chart, but it is typically simpler to compare samples using two-dimensional colour (or contour) plots. In a colour plot, the x-axis represents the retention time in the primary column (^1t_R), the y-axis represents the retention time in the second dimension (^2t_R), and the colour gradient represents the intensity of the peak, whereas in a 3D surface plot the additional z-axis represents the peak intensity. A colour plot can therefore be thought of as a bird's-eye view of the surface plot.

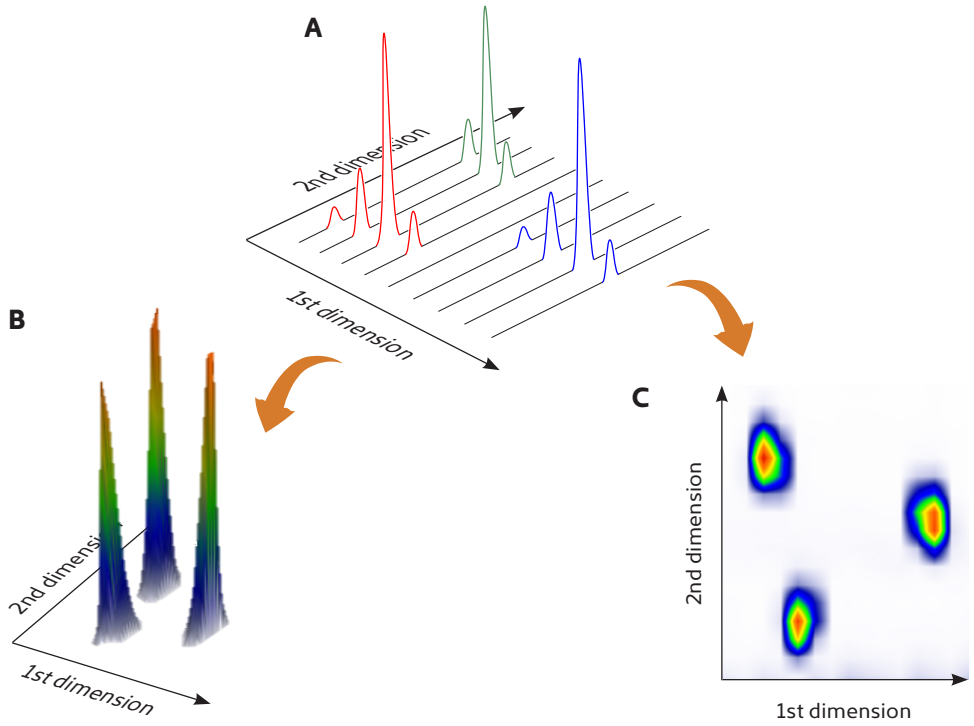

Figure 4

In GC×GC, the eluate from the 1st-dimension column is split into fractions that are individually fed into a much faster-eluting 2nd-dimension column. The resulting chromatograms (**A**) are 'stacked' to form surface plots (**B**), which in turn can be viewed 'from above' as colour (contour) plots (**C**).

5. Structured ordering

An advantage of GC×GC chromatograms is the structured ordering or 'roof-tiling' effect (Figure 5). Compounds from the same chemical class typically elute together in bands, allowing fast, tentative identification of the major components present in the mixture.

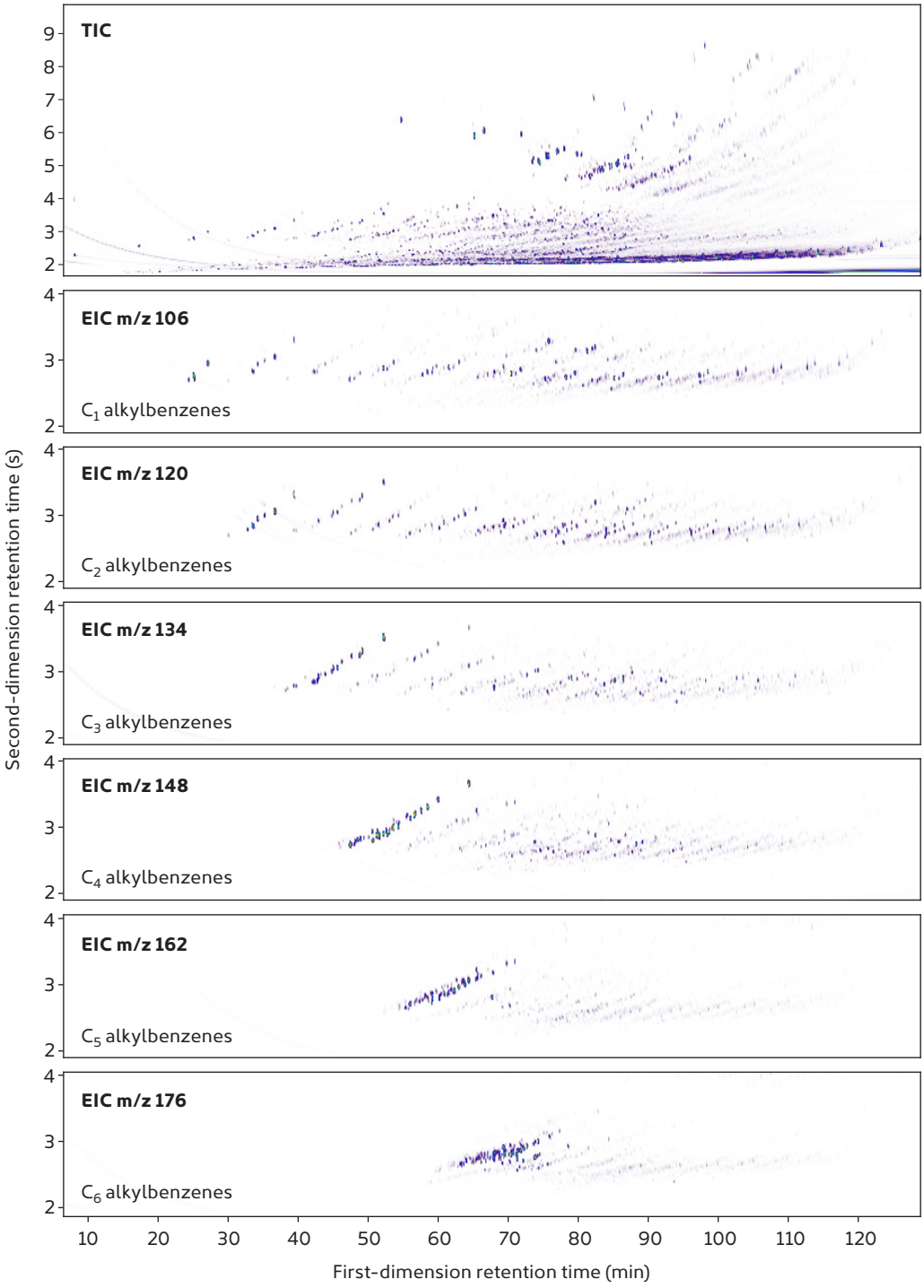

Figure 5

The 'roof-tiling' phenomenon in a GC×GC colour plot of diesel.

In contrast, when a complex mixture is analysed by 1D GC it is difficult to make assumptions about the chemical structure of eluates based solely on their retention times, as they are only separated based on a single chemical property. For example, compounds from many different chemical classes may have similar boiling points, so this alone would not allow classification of different chemical families. However, if these components are further separated based on polarity, as in normal-phase GC×GC, classification of chemical families is easier due to the chemical similarities measured by two distinct properties.

This type of structure allows characteristic patterns to emerge, enabling experienced analysts to quickly identify the main chemical classes within a complex mixture.

6. Software for GC×GC

GC×GC data is acquired by a detector in a linear (1D) format, so specialist software is required to 'fold' the data (based on the known modulation time) in order to view colour and surface plots.[16] There are now a number of commercially-available software packages for GC×GC data processing – some are specific to a particular instrument, such as ChromaTOF® (LECO) and Chrom^{Square} (Shimadzu), while others are capable of processing third-party data files from a range of instrumentation, such as ChromSpace® (Markes International) and GC Image™ (GC Image LLC).

At SepSolve, we champion the use of ChromSpace, due to its robust peak merging, deconvolution and simplified workflows. It is often the case that two dimensions of separation are still not sufficient to fully separate the most complex of samples, so deconvolution can play a large role in this type of analysis. Figure 6 demonstrates the deconvolution of three peak profiles from a single TIC peak in a petrochemical sample.

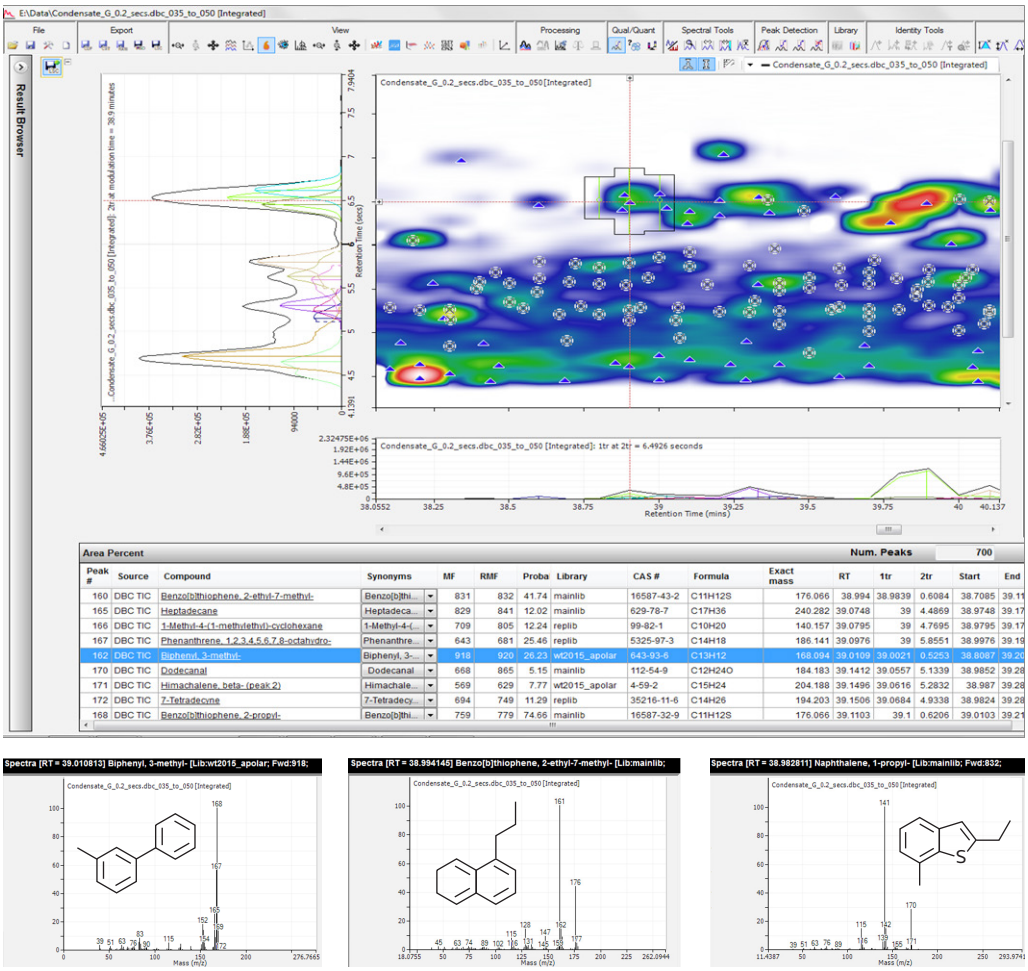

Figure 6

Deconvolution applied to GC×GC–TOF MS of a petrochemical sample in ChromSpace software. The spectra shown are derived from three co-eluting compounds (boxed area).

7. Conclusions

GC×GC technology has progressed significantly in the past 10 years, with advances in modulation and software making the technique more applicable to routine applications. The technology is already established in a number of diverse fields and is likely to provide further insights into challenging samples for years to come. At SepSolve Analytical, we hope to encourage this progress, by providing simple, robust and low-cost flow modulation in a commercial package, alongside a diverse range of detectors.

For more information on GC×GC or the INSIGHT flow modulator, please contact SepSolve.

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8. References

[1] G.S. Frysinger, R.B. Gaines and C.M. Reddy, GC×GC – A new analytical tool for environmental forensics, *Environmental Forensics*, 2002, 3: 27–34, http://dx.doi.org/10.1006/enfo.2002.0077.

[2] W. Bertsch, Two-dimensional gas chromatography. Concepts, instrumentation, and applications – Part 1: Fundamentals, conventional two-dimensional gas chromatography, Selected applications, *Journal of Separation Science*, 1999, 22: 647–665, http://dx.doi.org/10.1002/(SICI)1521-4168(19991201)22:12<647::AID-JHRC647>3.0.CO;2-V.

[3] J.B. Phillips and J. Beens, Comprehensive two-dimensional gas chromatography: A hyphenated method with strong coupling between the two dimensions, *Journal of Chromatography A*, 1999, 856: 331–347, http://dx.doi.org/10.1016/S0021-9673(99)00815-8.

[4] R. Van Der Westhuizen, A. Crouch and P. Sandra, The use of GC×GC with time-of-flight mass spectrometry to investigate dienes and Diels–Alder polymerisation products in high-temperature Fischer–Tropsch-based fuels, *Journal of Separation Science*, 2008, 31: 3423–3428, http://dx.doi.org/10.1002/jssc.200800260.

[5] O. Panic and T. Gorecki, Comprehensive two-dimensional gas chromatography (GC×GC) in environmental analysis and monitoring, *Analytical and Bioanalytical Chemistry*, 2006, 386: 1013–1023, http://dx.doi.org/10.1007/s00216-006-0568-1.

[6] P. Marriott and R. Shellie, Principles and applications of comprehensive two-dimensional gas chromatography, *Trends in Analytical Chemistry*, 2002, 21: 573–583, http://dx.doi.org/10.1016/S0165-9936(02)00814-2.

[7] J.V. Seeley and S.K. Seeley, Multidimensional gas chromatography: Fundamental advances and new applications, *Analytical Chemistry*, 2013, 85: 557–578, http://dx.doi.org/10.1021/ac303195u.

[8] B. Gruber, T. Gröger, D. Harrison and R. Zimmerman, Vacuum ultraviolet absorption spectroscopy in combination with comprehensive two-dimensional gas chromatography for the monitoring of volatile organic compounds in breath gas: A feasibility study, *Journal of Chromatography A*, 2016, 1464: 141–146, http://dx.doi.org/10.1016/j.chroma.2016.08.024.

[9] R.E. Murphy, M.R. Schure, J.P. Foley, Effect of sampling rate on resolution incomprehensive two-dimensional liquid chromatography, *Analytical Chemistry*, 1998, 70: 1585–1594, http://dx.doi.org/10.1021/ac971184b.

[10] M. Edwards, A. Mostafa and T. Gorecki, Modulation in comprehensive two-dimensional gas chromatography: 20 years of innovation, *Analytical and Bioanalytical Chemistry*, 2010, 401: 2335–2349, http://dx.doi.org/10.1007/s00216-011-5100-6.

[11] J. Krupčík, P. Májek, R. Gorovenko, P. Sandra and D.W. Armstrong, On retentivity tuning by flow in the second column of different comprehensive two dimensional gas chromatographic configurations, *Journal of Chromatography A*, 2011, 1218: 3186–3189, http://dx.doi.org/10.1016/j.chroma.2011.03.042.

[12] J.V. Seeley, F. Kramp and C.J. Hicks, Comprehensive two-dimensional gas chromatography via differential flow modulation, *Analytical Chemistry*, 2000, 72: 4346–4352, http://dx.doi.org/10.1021/ac000249z.

[13] J.F. Griffith, W.L. Winniford, K. Sun, R. Edam and J.C. Luong, A reversed-flow differential flow modulator for comprehensive two-dimensional gas chromatography, *Journal of Chromatography A*, 2012, 1226: 116–123, http://dx.doi.org/10.1016/j.chroma.2011.11.036.

[14] J.V. Seeley, S.K. Seeley, E.K. Libby, Z.S. Breitbach and D.W. Armstrong, Comprehensive two-dimensional gas chromatography using a high-temperature phosphonium ionic liquid column, *Analytical and Bioanalytical Chemistry*, 2008, 390: 323–332, http://dx.doi.org/10.1007/s00216-007-1676-2.

[15] F.A. Franchina, M. Maimone, P.Q. Tranchida and L. Mondello, Flow modulation comprehensive two-dimensional gas chromatography–mass spectrometry using ≈4 mL min^{-1} gas flows, *Journal of Chromatography A*, 2016, 1441: 134–139, http://dx.doi.org/10.1016/j.chroma.2016.02.041.

[16] K.M. Pierce, J.C. Hoggard, R.E. Mohler and R.E. Synovec, Recent advancements in comprehensive two-dimensional separations with chemometrics, *Journal of Chromatography A*, 2008, 1184: 341–352, http://dx.doi.org/10.1016/j.chroma.2007.07.059.

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