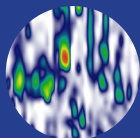![SepSolve Analytical logo](SepSolve Analytical)

GC×GC
Applications Guide

The benefits of comprehensive
two-dimensional gas chromatography
for a range of applications

Contents

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Introduction

GC×GC first attracted attention in the late 1970s, but for many years advances in conventional GC–MS meant that its superior separation capacity was not seen as being worth the additional technical complexity.

Thanks to greatly improved hardware and software, this has changed, and as a result GC×GC is now being routinely used in a variety of fields. This guide demonstrates just some of the most important applications.

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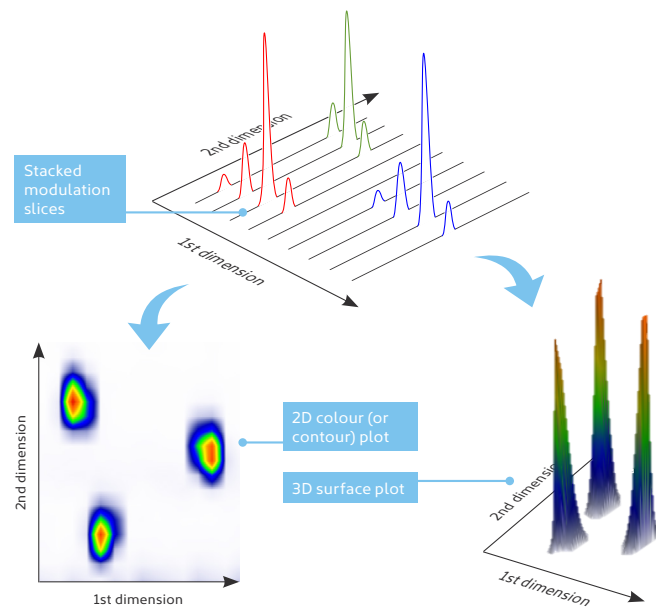Analytical

GC×GC essentials

GC separation in two dimensions

Comprehensive two-dimensional gas chromatography (GC×GC) is an advanced analytical technique with an increased separation capacity suitable for the analysis of complex samples.

The process involves the use of two columns with different stationary phases. This allows separation of mixtures that co-elute on the first column, so enabling an order of magnitude more compounds to be resolved. The entire sample is subjected to both separations, and so it is deemed a 'comprehensive' technique.

One of the most critical parts of the GC×GC system is the modulation device, which samples narrow bands of first-column eluent and then injects them into the faster-eluting secondary column. The individual chromatograms from the secondary column ('modulation slices') are then stacked side-by-side to give the characteristic GC×GC contour and surface plots.

The INSIGHT™ flow modulator from SepSolve now removes previous objections to GC×GC by improving the repeatability for routine analysis and minimising the cost of ownership.

Stacked modulation slices

2nd dimension

1st dimension

2nd dimension

1st dimension

2D colour (or contour) plot

3D surface plot

2nd dimension

1st dimension

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

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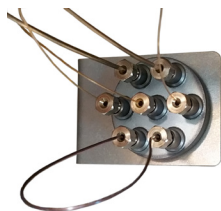Analytical

Complementary technologies

A number of technologies offered by SepSolve assist GC×GC applications

Flow modulation with INSIGHT™

INSIGHT™ overcomes the cost and logistical issues of thermal modulators, and can separate volatiles from C_1 to C_{40+} with no modulation breakthrough.
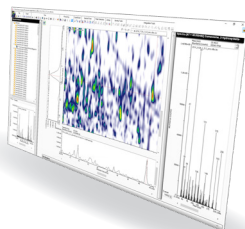
Learn more

ChromSpace® software

ChromSpace® improves GC×GC workflow for high-throughput labs, with sophisticated peak merging and flexible data navigation.

Learn more

Sample preparation robots

SepSolve offers a range of CTC autosamplers to increase the productivity, reproducibility and flexibility of GC×GC analyses.

Learn more

TOF mass spectrometers

BenchTOF™ time-of-flight instruments are perfect for GC×GC, because of their high acquisition speed and ability to monitor trace targets and unknowns in a single run.

BenchTOF™ instruments also now offer Tandem Ionisation® capability, for simultaneous acquisition of regular 70 eV spectra and low-eV soft ionisation data.

Learn more

Thermal desorption

Thermal desorbers such as Markes International's TD100-xr™ (pictured) are the natural partner to many GC×GC applications, because of the sensitivity boost offered and compatibility with a range of sample introduction approaches.

Learn more

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Food and drink

Whether used for routine quality-control or for research into aroma profiles, GC×GC is rapidly coming to be valued by analysts for handling the vast range of compounds encountered in foods and beverages.

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Edible oils

Full separation of FAMEs

The analysis of fatty acid methyl esters (FAMEs) in food is important in order to measure nutritional content. Most important is the qualitative and quantitative measurement of *cis/trans* fatty acids. However, conventional 1D GC struggles to resolve all components.

GC×GC is capable of full separation and identification of FAMEs based on both carbon number and degree of unsaturation. Here, INSIGHT™ flow-modulated GC×GC–FID provides sharp, symmetrical peaks and excellent repeatability across large sample batches.

Analytical details

▶ **Sample:** 37-component FAME standard in dichloromethane (200 ppm) with 200:1 split. ▶ **GC×GC:** Injection: 1.0 µL. Split 25:1. Modulation period: 3.8 s. ▶ **FID**. ▶ **Software**: ChromSpace®.
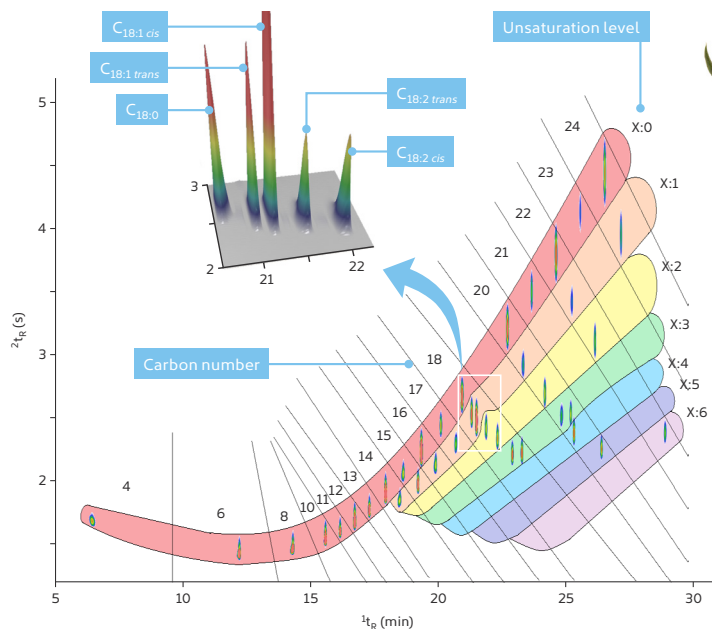
This excellent separation of FAME isomers reveals an additional benefit of GC×GC – the structured ordering of chemical classes. Here FAMEs are separated by their carbon number as well as their degree of unsaturation, simplifying the identification process.

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Whisky

Uncovering 'hidden' aroma compounds

Hundreds of compounds from a wide range of chemical classes are known to contribute to the aroma of whisky, and these include alcohols, phenolics, fatty acids, esters, lactones, aldehydes and nitrogen-containing compounds. It is important to be able to confidently identify these volatiles, for quality control and authentication purposes, as well as in the engineering of new aromas.

Here we demonstrate the high performance of a GC×GC–TOF MS system using INSIGHT™ flow modulation and the powerful ChromSpace® software platform for the separation and identification of trace volatiles in whisky.

Analytical details

► **Sample:** Whisky, diluted 3:1 with water.
► **SPME:** Fibre: DVB/Car/PDMS StableFlex™ (Sigma-Aldrich). Incubation: 35°C for 10 min.
► **GC×GC:** Split 5:1. Modulation period: 4.0 s. ► **TOF MS:** BenchTOF-HD™. Mass range: m/z 40–350. ► **Software**: ChromSpace®.
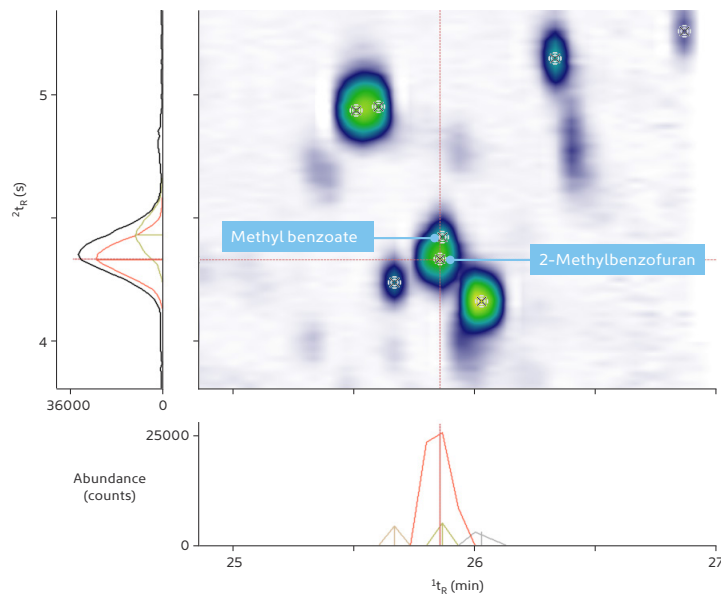
Expansion of a whisky sample, showing deconvolution applied in ChromSpace® software. Two co-eluting aroma-active species were confidently identified against the NIST database (match factors >890).

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Hop oils

Flow-modulated GC×GC for routine hop-oil analysis

The distinctive flavours of beers result in large part from plant-derived monoterpenes (C_{10}) and sesquiterpenes (C_{15}), which originate from the essential oils of various plants, including hops. However, the sheer number of terpenes that may be present is a major challenge for the analyst, as is the effect of both minor and major components upon the aroma of the finished beer.

Addressing these issues, flow-modulated GC×GC–TOF MS enhances the separation of hop-oil constituents (even those boiling below C_5), while Tandem Ionisation® adds an extra dimension of information, by simultaneously acquiring hard and soft EI spectra, and improving terpene speciation with no inherent loss in sensitivity.

Analytical details

► **Sample:** Hop oil in hexane (10% v/v).
► **GC×GC:** Injection: 0.5 µL. Split 100:1. Modulation period: 3.8 s. ► **TOF MS:** BenchTOF-Select™. Mass range: m/z 40–300. Tandem Ionisation®: 70/12 eV.
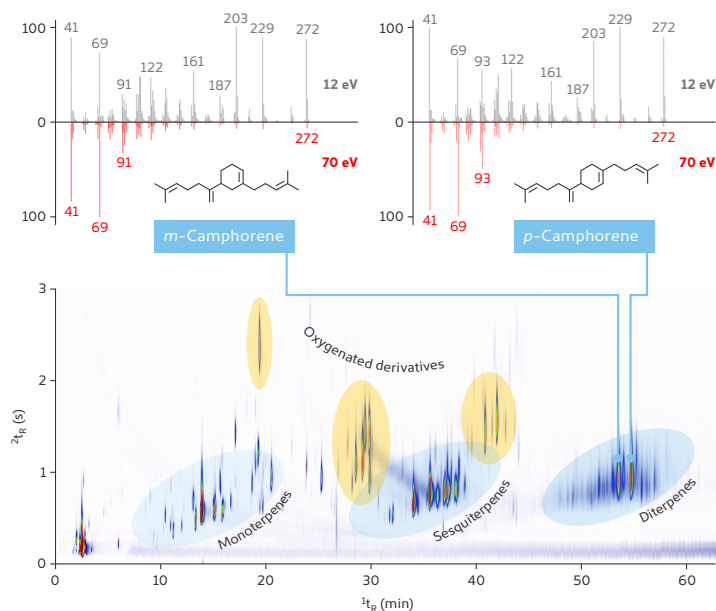
The enhanced separation of flow-modulated GC×GC–TOF MS allows confident identification of hop-oil compounds, including many that would co-elute on a 1D GC–MS system. In addition, Tandem Ionisation® allows speciation of isomers that cannot be identified using 70 eV data, thanks to enhanced structurally-significant fragments and molecular ions.

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Coffee

Expanding separation capacity with GC×GC

Roast coffee extracts are highly complex and vary depending on factors such as coffee bean origin and degree of roasting. The overall flavour and aroma of coffee results from a complex combination of hydrocarbons, aldehydes, acids, esters as well as sulfur- and nitrogen-containing compounds, with the latter being of particular importance to the aroma of roasted coffee.

GC×GC with BenchTOF™ detection provides the enhanced separation capacity to allow the entire composition to be screened in a single analysis – as illustrated in this example of a roasted coffee extract.

Analytical details

▶ **Sample:** Dichloromethane extract of roasted coffee. ▶ **GC×GC:** Injection: 1.0 µL. Split 5:1. Modulation period: 6 s. ▶ **TOF MS:** BenchTOF-HD™. Mass range: m/z 35–400.
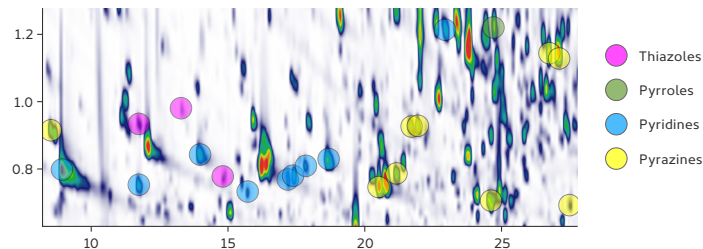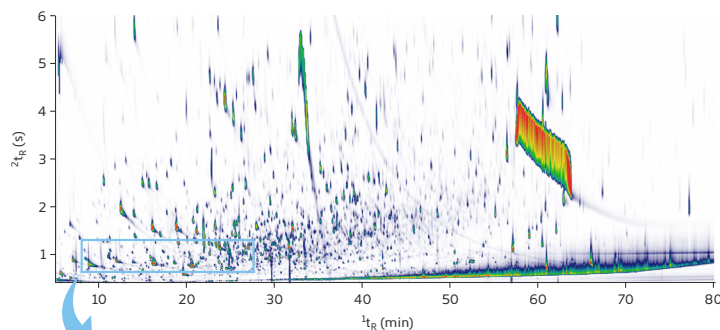
A number of nitrogen-containing compounds including pyridines, pyrazines and thiazoles contribute to the aroma of coffee, and are identified in this GC×GC run of a ground coffee extract. Such detailed analysis would be extremely difficult to achieve in a regular GC–MS run.

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Milk

Sorptive extraction of volatiles

Milk, as a liquid product produced on a vast scale, is highly susceptible to contamination, and reliable analysis of its volatile components is therefore valuable for quality control. Traditionally, solid-phase micro-extraction (SPME) has been used for flavour profiling, and although fast and simple, it can be limited in terms of sample capacity, reproducibility and sensitivity.

In this example sorptive extraction is used for immersive sampling of milk. A high-capacity PDMS phase results in higher sample loadings than SPME methods, and combined with pre-concentration by thermal desorption (TD) offers greater sensitivity across a wide analyte range.

Analytical details

►**Sample:** Filtered cow's milk, sampled onto a PDMS HiSorb™ probe (Markes International). ►**TD:** TD100-xr™ (Markes International). Trap: 'General-purpose hydrophobic'. ►**GC×GC:** Split 100:1. Modulation period: 3.8 s. ►**TOF MS:** BenchTOF-Select™. Mass range: m/z 40–300. Tandem Ionisation®: 70/14 eV. ►**Software**: ChromSpace®.
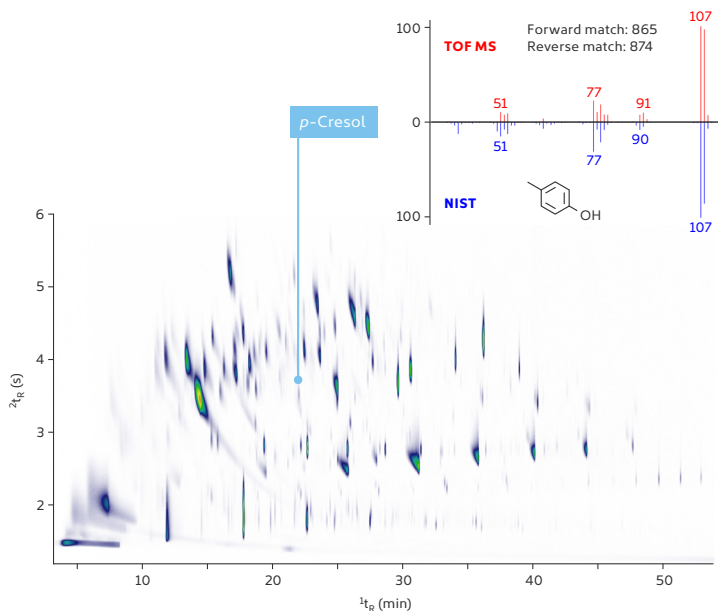
High-capacity sorptive extraction of cows' milk combined with analysis by TD-GC×GC–TOF MS allows identification of *p*-cresol – known to cause a 'cowy, barnyard' odour taint.

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Fragranced products and tobacco

Rising concern over potentially hazardous chemicals in consumer products is driving regulations in many countries – developments that benefit from the detailed knowledge of constituents provided by GC×GC.

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Allergens in consumer products

Keeping ahead of cosmetic regulations

The EU Directive 2003/15/EC restricts the use of allergenic compounds in cosmetic products, and names 26 allergens that should be labelled if present above certain levels. It is anticipated that this list of allergens will extend to over 100 compounds by 2018.

Flow-modulated GC×GC using INSIGHT™ ensures that laboratories are ready for such demands, by allowing enhanced separation with simultaneous detection by TOF MS and FID, for confident identification and robust quantitation in a single run.

Analytical details

▶ **Sample:** 84-component allergen standards in MTBE (3.2–400 µg/mL).
▶ **GC×GC:** Injection: 1.0 µL. Split 25:1. Modulation period: 4.0 s. A splitter was used to direct the flow to the TOF MS and **FID** detectors in the ratio 1:4. ▶ **TOF MS:** BenchTOF-Select™. ▶ **FID**. ▶ **Software**: ChromSpace®.

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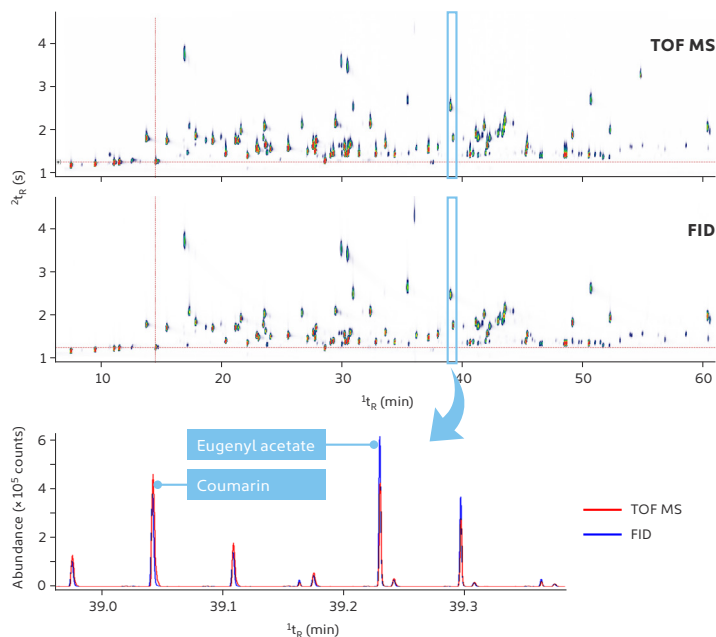
Excellent retention time correspondence between TOF MS and FID datasets for two peaks in an allergen standard permits simplified translation between the two detection methods.

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Fragranced products

Improved identification of isomers

Fragranced products frequently contain large numbers of monoterpenes, sesquiterpenes and oxygenated derivatives in a complex matrix, with substantial numbers of isomers. Traditionally, GC coupled with MS or FID has been used to analyse such samples, relying on the combination of mass spectra and Kovats retention indices to confirm the identity of challenging compounds.

The inherent ability of GC×GC to handle complex matrices makes it a better option for such samples, by eliminating the need for extensive sample preparation. Analysis is further enhanced by using TOF MS with Tandem Ionisation® capability, as shown in this example.

Analytical details

▶ **Sample:** Allergen standards in acetone (2 ppm). ▶ **GC×GC:** Injection: 1.0 µL. Split 50:1. Modulation period: 2.5 s. ▶ **TOF MS:** BenchTOF-Select™. Mass range: m/z 40–600. Tandem Ionisation®: 70/12 eV. ▶ **Software**: ChromSpace®.

Farnesol
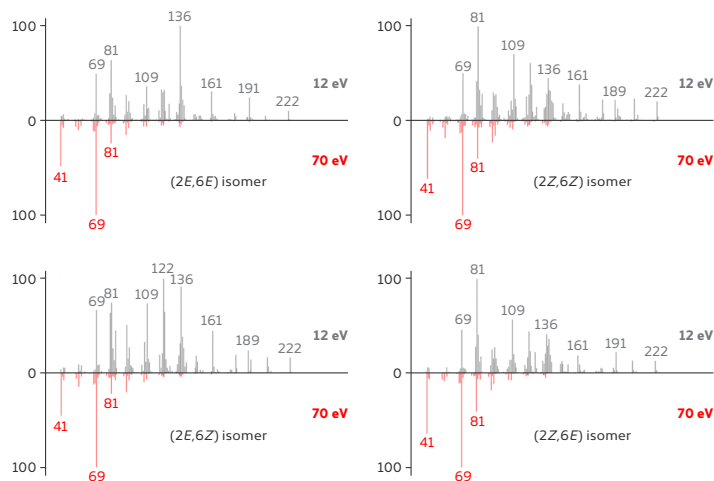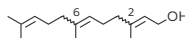
In a single analysis, Tandem Ionisation® provides regular 70 eV spectra for conventional library searching *and* low-eV spectra with enhanced molecular ions. The resulting capability for isomer speciation is seen here for four isomers of farnesol.

Cigarette smoke

Enhancing separation and identification by GC×GC

Tobacco smoke contains thousands of components across multiple chemical classes and wide concentration ranges, meaning there is much we can still learn about these complex samples.

Flow-modulated GC×GC–TOF MS provides improved chemical fingerprinting of complex samples such as tobacco smoke, and in this example is complemented by the sensitivity boost of thermal desorption (TD) for collection of whole cigarette emissions.

Analytical details

►**Sample:** Cigarette smoke (~50 mL), drawn directly onto a Tenax® TA sorbent tube. ►**TD:** TD100-xr™ (Markes International). Trap: 'General-purpose hydrophobic'. Overall split: 200:1.
►**GC×GC:** Modulation period: 8.0 s.
►**TOF MS:** BenchTOF-Select™. Mass range: m/z 35–400. Tandem Ionisation®: 70/14 eV.
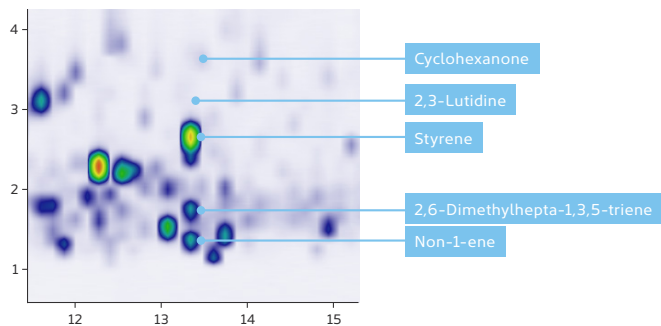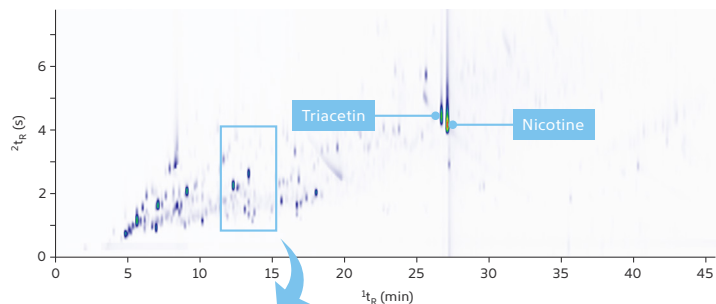
Minimal breakthrough and tailing is achieved even for the high-loading nicotine and triacetin in this TD–GC×GC–TOF MS analysis of whole cigarette smoke, while an expansion highlights five components that would co-elute in a 1D separation.

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Defence and forensic

The enhanced separation of GC×GC is ideal for military and forensic applications, where complex sample matrices can interfere with detection and/or confident identification.

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Tear gas

Detecting trace-level lachrymators on clothing

Increasing illegal use of tear gas and self-defence sprays has meant that forensic analysts have to assess crime-scene materials for their presence – and to use their 'chemical fingerprints' to link suspects with a particular event.

The complex nature of forensic samples makes GC×GC a natural choice for such in-depth analysis, and sensitivity is further improved when it is coupled with a highly sensitive TOF MS instrument, as shown here.

Analytical details

▶**Sample:** Solvent extract of a section of clothing exposed to tear gas. ▶**GC×GC:** Injection: 1.0 μL (splitless). Modulation period: 6 s. ▶**TOF MS:** BenchTOF-Select™. Mass range: m/z 40–450.

TOF MS

Forward match: 771
Reverse match: 831

NIST

EIC
m/z 137

Dihydrocapsaicin
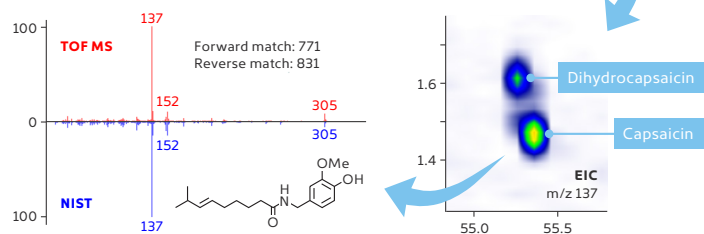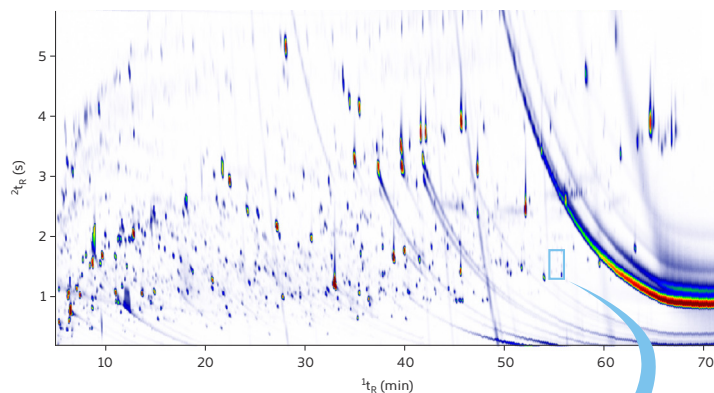
Capsaicin

The lachrymators capsaicin and dihydrocapsaicin were identified and quantified in a complex clothing extract using GC×GC–TOF MS, despite being present at only 64 and 72 ppb respectively, and undergoing extensive fragmentation.

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Cannabis

Enhanced separation of complex drug extracts

Cannabis, as well as being a drug of abuse, is increasingly being used medicinally, which has led to a growing need for robust testing of cannabis products. However, this can be challenging due to matrix complexity and the variety of target compounds – including the cannabinoids that define potency, aroma-active terpenoids, and contaminants causing safety concerns, such as pesticides.

GC×GC–TOF MS is ideal for finding 'needle in a haystack' targets in cannabis products, with new software improving lab efficiency by automating the detection of target compound groups.

Analytical details

▶ **Sample:** QuEChERS-prepared cannabis extracts (courtesy of Frank Dorman, Pennsylvania State University, US).
▶ **GC×GC:** Injector: Split 5:1. Modulation period: 4.0 s. A splitter was used to direct the flow to the TOF MS and **FID** detectors in the ratio 1:4. ▶ **TOF MS:** BenchTOF-Select™. Tandem Ionisation®: 70/12 eV. m/z 45–550. ▶ **Software**: ChromSpace®.

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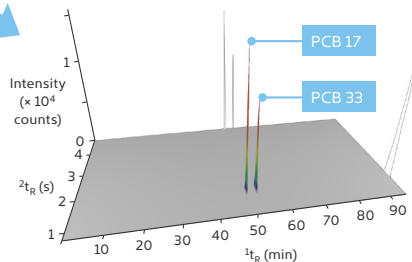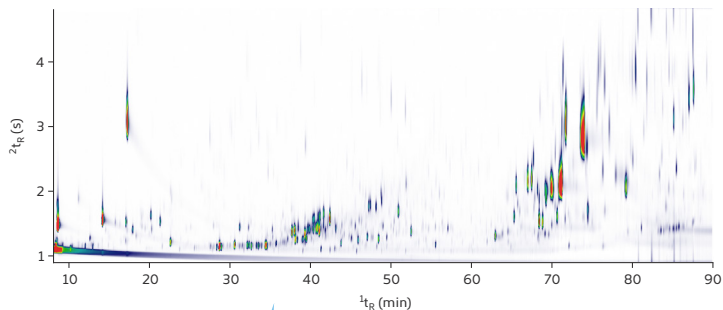
Simple scripting tools in ChromSpace® enable rapid identification of contaminants in cannabis extracts.

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Environmental monitoring

Although the separating power of GC×GC is usually not necessary in conventional environmental air monitoring, it is indispensable where pollutants are contained in complex matrices such as soil, sediment and river water.

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Total petrochemical hydrocarbons

Fast and affordable environmental monitoring

Petroleum contamination of soil or groundwater is of concern for the environment and human health. Various methods have been developed for the analysis of C_8-C_{40} extractable petroleum hydrocarbons in environmental media, but these typically use time-consuming and costly off-line fractionation to separate aliphatics and aromatics, which are then analysed separately by GC–FID.

SepSolve's product package for TPH analysis uses the enhanced separation capacity of GC×GC and an optimised method to fully separate aliphatics and aromatics in a single run – eliminating the need for off-line sample fractionation and multiple analyses.

Analytical details

▶**Sample:** Contaminated soil extract in hexane. ▶**GC×GC:** Injector: Split 5:1. Modulation period: 4.0 s. Dual-channel configuration using two modulators and column sets in the same GC oven. ▶**FID**. ▶**Software**: ChromSpace®.
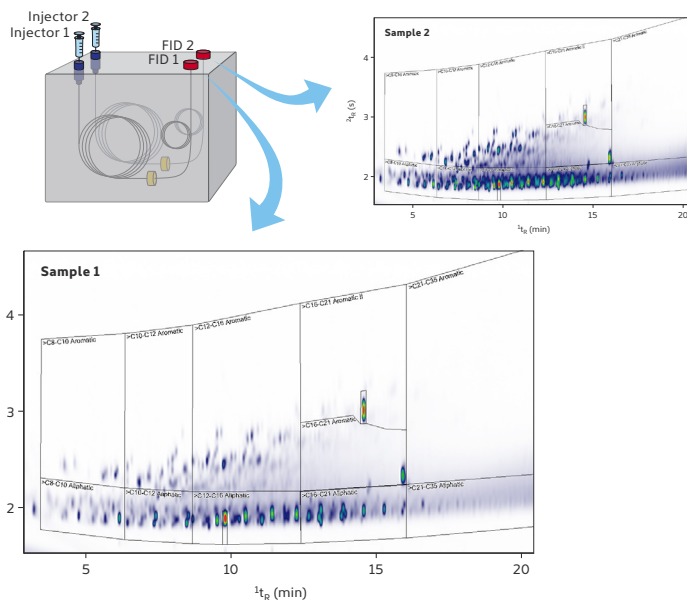
Dual-channel GC×GC–FID enables simultaneous analysis of two contaminated soil samples, and in this example is followed by stencil-based processing for fast identification of key analyte groups.

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Organochlorine pesticides

Dual detection for another level of information

GC with electron capture detection (ECD) is the method of choice for the analysis of organochlorine pesticides in the environment. However, due to the high complexity of environmental matrices, extensive sample fractionation and analysis on parallel columns is usually required.

GC×GC with dual detection by MS and ECD offers the best of both worlds, allowing qualitative and quantitative analysis of environmental extracts in a single run with minimal sample preparation. While dual detection can be troublesome to configure in thermally-modulated GC×GC systems, optimal alignment of retention times can easily be achieved with flow modulation.

Analytical details

▶**Sample:** Organochlorine compound standard in 1:1 hexane–toluene (100 ppm).
▶**GC×GC:** Injection: 1 µL. Split: 50:1. Modulation period: 4.0 s. ▶**TOF MS:** BenchTOF-Select™. Mass range: m/z 35–500. Tandem Ionisation®: 70/14 eV.
▶**ECD:** Temp.: 300°C. N$_2$: 100 mL/min.
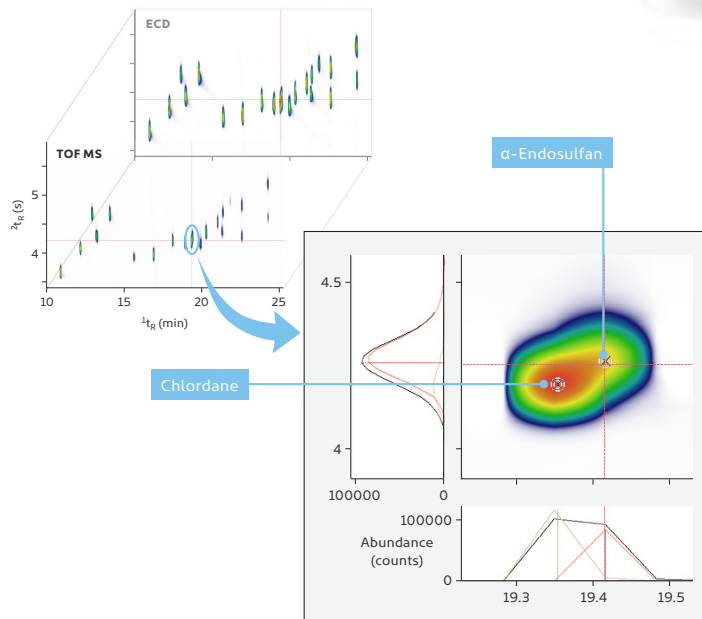
Parallel detection by TOF MS and ECD allows the identity and purity of co-eluting peaks in this pesticide mix to be verified – as demonstrated here by deconvolution in ChromSpace®.

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Petrochemical and geochemical

The study of petrochemical composition was one of the earliest applications to benefit from GC×GC, and it remains invaluable today for providing a high degree of component separation and useful information on molecular structure through the well-known 'roof-tiling' effect.

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Petrochemical fingerprinting

Parallel detection by GC×GC–FID/TOF MS

Precise characterisation of petrochemical samples is crucial for quality control, as well as for understanding the reactions that take place during refining processes. In order to achieve this, analysts need to combine a high degree of separation with robust quantitative and qualitative analysis.
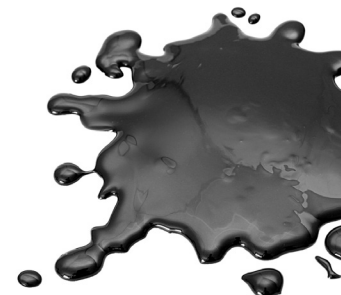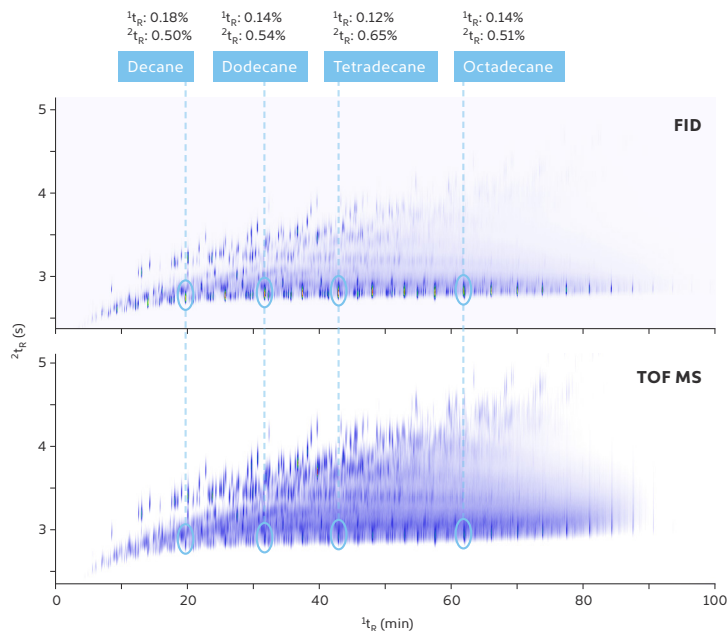
This challenge is addressed by the use of GC×GC with dual detection by FID (which has long been the 'gold standard' for petrochemical quantitation) and TOF MS (which offers robust analyte identification, and options for peak deconvolution and retrospective data-searching).

Analytical details

► **Sample:** Pump diesel (undiluted).
► **GC×GC:** Injection: 1 µL. Split: 300:1. Modulation period: 4.0 s. ► **FID**.
► **TOF MS:** BenchTOF-Select™. Mass range: m/z 45–450. Tandem Ionisation®: 70/14 eV. ► **Software**: ChromSpace®.

	Decane	Dodecane	Tetradecane	Octadecane
^1t_R:	0.18%	0.14%	0.12%	0.14%
^2t_R:	0.50%	0.54%	0.65%	0.51%

A high degree of component separation and highly structured groupings of compounds are the two main reasons why GC×GC is favoured for analysis of petrochemicals such as this diesel sample. Flow modulation now allows easy splitting for dual detection, with excellent retention time alignment between FID and TOF MS datasets (mean RSDs indicated for 24 injections over the course of 3 days).

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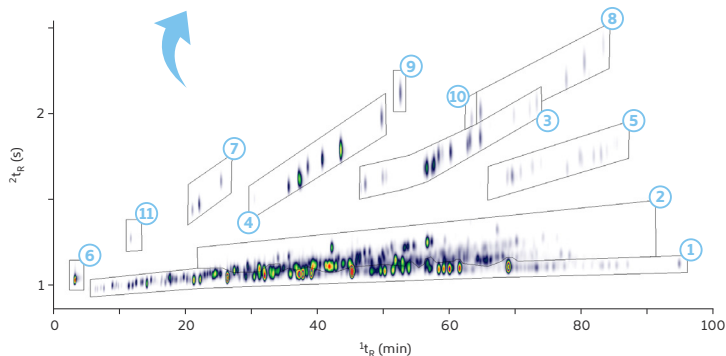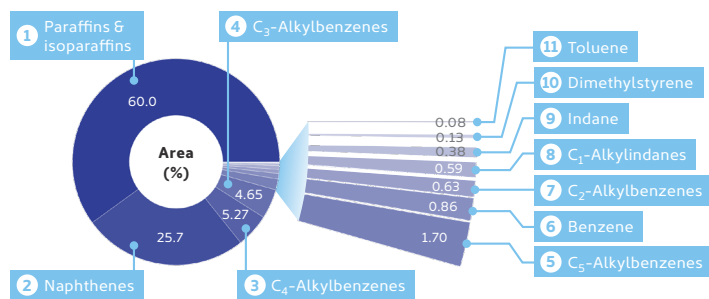
Hydrocarbon group-type analysis

Stencils for fast and efficient identification of compound classes

Precise characterisation of petroleum-derived fuels is important, but it can be a tedious and challenging task to identify each of the individual components present in these complex samples.

Group-type analysis using GC×GC overcomes these issues by summing the response for hydrocarbons according to their chemical class. Stencils in ChromSpace® software also allow automated calculation of percentage areas, enabling quick review of sample composition.

Analytical details

►**Sample:** Naphtha (undiluted).
►**GC×GC:** Injection: 0.1 µL. Modulation period: 2.0 s. ►**FID**. ►**Software**: ChromSpace®.

1 Paraffins & isoparaffins — 60.0
4 C_3-Alkylbenzenes
2 Naphthenes — 25.7
3 C_4-Alkylbenzenes — 5.27
4.65

Area (%)

11 Toluene — 0.08
10 Dimethylstyrene — 0.13
9 Indane — 0.38
8 C_1-Alkylindanes — 0.59
7 C_2-Alkylbenzenes — 0.63
6 Benzene — 0.86
5 C_5-Alkylbenzenes — 1.70

^2t_R (s)

^1t_R (min)

The application of pre-defined stencils allows rapid group-type speciation of the components in this naphtha sample, as well as reporting of summed peak integrals. At the same time, INSIGHT™ GC×GC provides high peak capacity, with peak widths typically <100 ms in this case.

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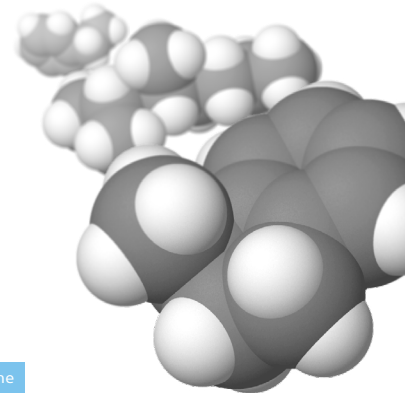
Scripting in petrochemical analysis

Enhanced confidence in class boundaries for MS and FID data

Despite the enhanced separation of GC×GC, overlap between classes may remain, making it imperative to accurately identify the boundaries between them.

The use of parallel detection by mass spectrometry (MS) and flame ionisation detection (FID) enables confident classification and robust quantitation of hydrocarbon classes in a single run. Using a combination of EICs and filtering scripts, the MS data can be used to create accurate stencil regions, which can in turn be applied to the FID data.

Analytical details

► **Sample:** Pump diesel (undiluted).
► **GC×GC:** Injection: 0.1 µL. Modulation period: 3.0 s. ► **FID**. ► **TOF MS:** BenchTOF-HD™. Mass range: m/z 45–450.
► **Software**: ChromSpace®.

TOF MS

C_2-Alkyldecalins

FID

^2t_R (s)

^1t_R (min)
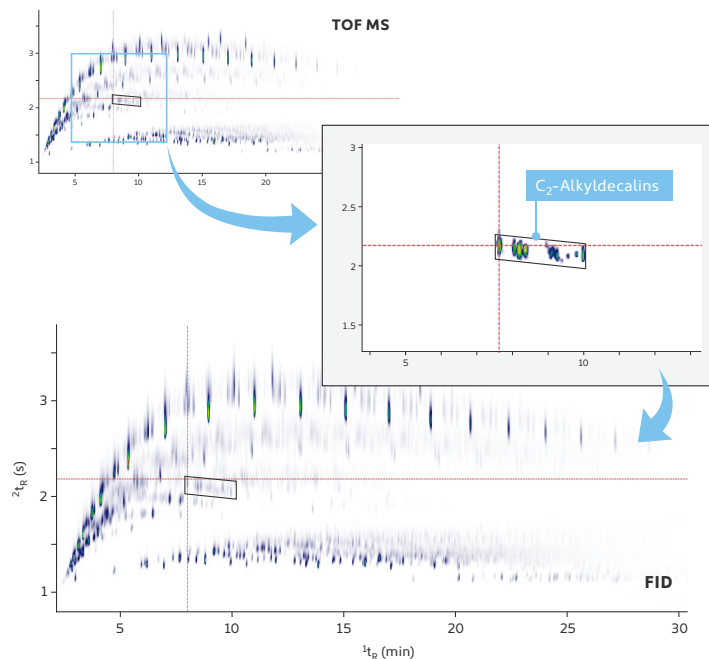
Filtering scripts (as shown here for the C_2-alkyldecalins) or EICs enable accurate and close-fitting class boundaries to be defined using MS data. The excellent retention time correspondence achieved using parallel detection with the INSIGHT™ flow modulator means that the resulting stencils can then easily be applied to FID data for quantitative analysis.

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Biological monitoring

The growing use of small-molecule biomarkers for health-related research is another area benefiting from GC×GC, because of its ability to separate structurally similar compounds in highly complex matrices.

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Volatiles in blood

## Using scents to discriminate between fresh and aged blood

Training search-and-rescue dogs on blood odour is very important to ensure they can accurately locate disaster victims. Therefore, analysts need to be able to reliably profile the diversity of compounds present in blood, and also monitor how this profile changes over time.

This challenge is easily addressed by GC×GC–TOF MS, with the INSIGHT™ flow modulator enabling efficient modulation of VOCs in this complex matrix – a task that is impossible using thermal modulators.

Analytical details

▶ **Sample:** Headspace of fresh blood injected onto a Tenax® GR–Carbograph™ B sorbent tube. ▶ **TD:** TD100-xr™ (Markes International). Trap: 'General-purpose hydrophobic'. ▶ **GC×GC:** Modulation period: 2.5 s. ▶ **TOF MS:** BenchTOF-Select™. Mass range: m/z 35–400. Tandem Ionisation®: 70/14 eV.

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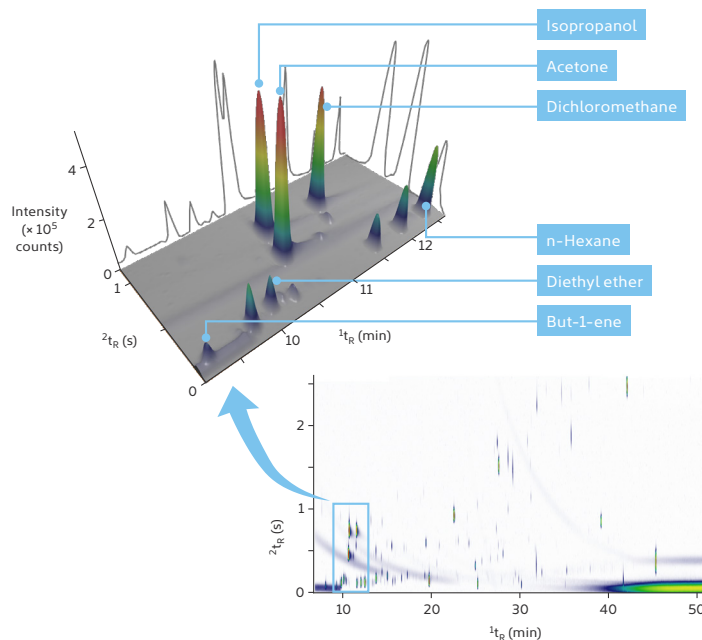
A range of volatiles including hydrocarbons, ketones, terpenes, heterocyclics and aromatics are detected in this GC×GC–TOF MS analysis of blood, with INSIGHT™ providing efficient modulation of compounds boiling below C_5.

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About SepSolve Analytical

SepSolve Analytical is dedicated to helping analysts select the best equipment for GC and GC×GC analysis.

The wide range of products offered include SepSolve's INSIGHT™ flow modulator for GC×GC, and sample preparation equipment, robotic autosamplers, thermal desorbers and mass spectrometers with novel soft EI capability from leading manufacturers including GL Sciences, CTC Analytics and Markes International.

SepSolve's application experience is extensive, placing it in an excellent position to advise on getting the best from an analysis – in everything from environmental monitoring to petrochemical analysis and food aroma profiling.

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