COMPREHENSIVE TWO-DIMENSIONAL GASCHROMATOGRAPHY

The state-of-separation-arts

Part II: Applications

Foods and Fragrances 1

JAN BEENS

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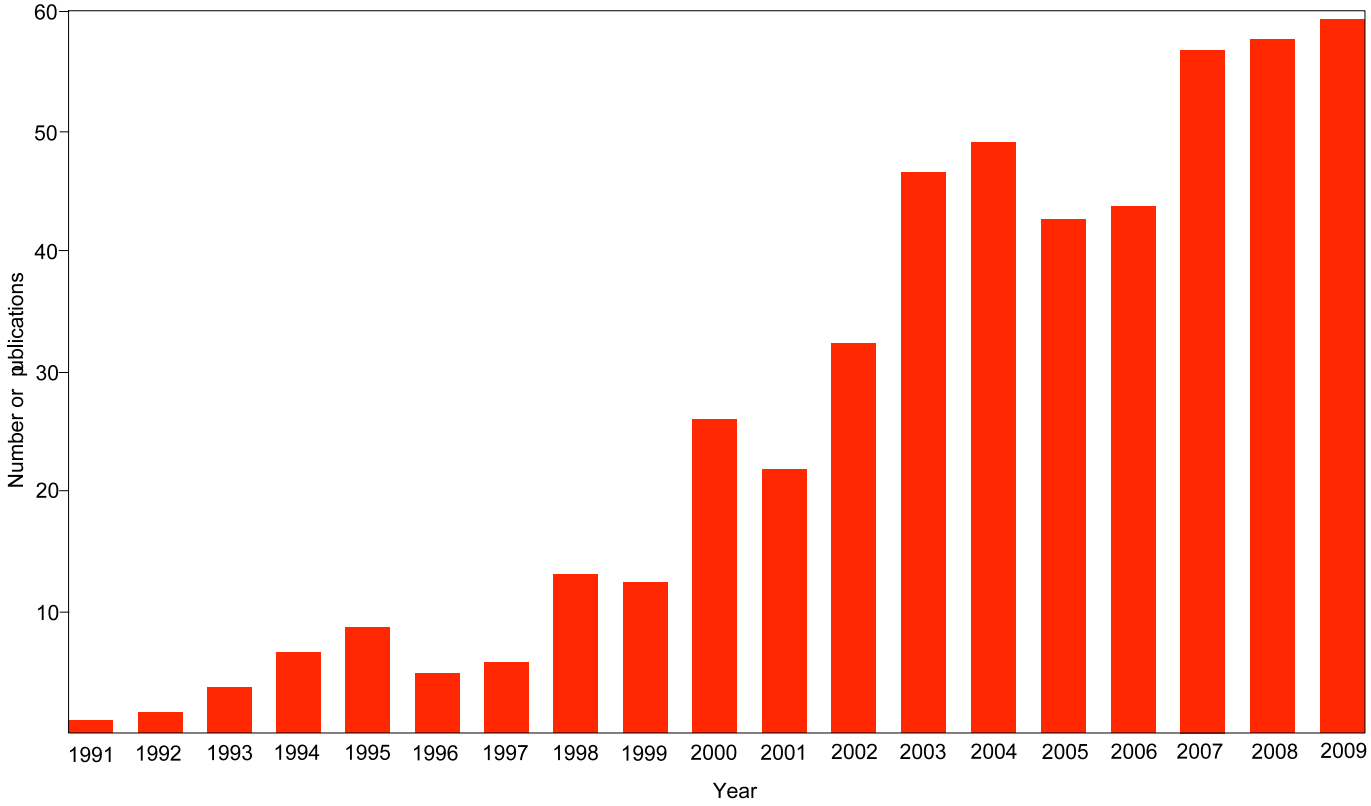
PART II

—APPLICATIONS—

Foods and Fragrances



The number of published papers on GC×GC.

From the very first publication of the technique of GC×GC by Liu and Phillips on, it was clear that interesting separations, containing hundreds to thousands of separated peaks, suddenly became possible.

During the decade following this publication a steadily increasing number of papers have been published about GC×GC, of which the majority demonstrates a specific application. The figure above depicts the growth in interest in this technique quite nicely by the growth of the number of published papers.

In this Part II (referred to as chapter 12), other applications that have been demonstrated and reported so far and not yet covered in previous chapters are collected and depicted. On first page the sample and the GC×GC conditions through which these separations have been derived are presented. On the next page the colour or contour plot of the separated sample appears.

The areas in which GC×GC successfully has been applied have been reviewed in a number of papers [1-5]. The applications that are described in this chapter are listed below.

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Incense smoke

T.C. Tran, P.J. Marriott, *Characterization of incense smoke by solid phase microextraction—comprehensive two-dimensional gas chromatography (GC×GC)*, Atmospheric Environment 41 (2007) 5756–5768

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 0.25 µm BPX5
Second: 1 m × 0.1 mm ID, 0.1 µm BP20
Modulation capillary:

Carrier gas: hydrogen @ 1.5 mL/min

***Temperatures*:**
Main oven: 40°C (2 min), 5°C/min → 260°C
Second oven:

Injector: splitless
Temperature: 250°C
Injection volume: fiber

Modulator: LMCS

Modulation time: 6 s

Detector: ToF MS
Temperature:
Make up gas flow:

Data acquisition: not specified

Sample description and separation:

One stick of each incense sample was powdered and transferred to a 4mL glass vial in order to sample the fragrance/essential oil H/S. A fiber was directly exposed to the smoke of burning incense.

A total of 324 compounds were tentatively identified, with more than 100 compounds in incense powders and more than 200 compounds in the incense smoke, by using GC coupled to quadrupole mass spectrometric detection. The smoke stream comprised compounds originating from the incense powder, and combustion products such as PAH, N-heterocyclics, and furans.

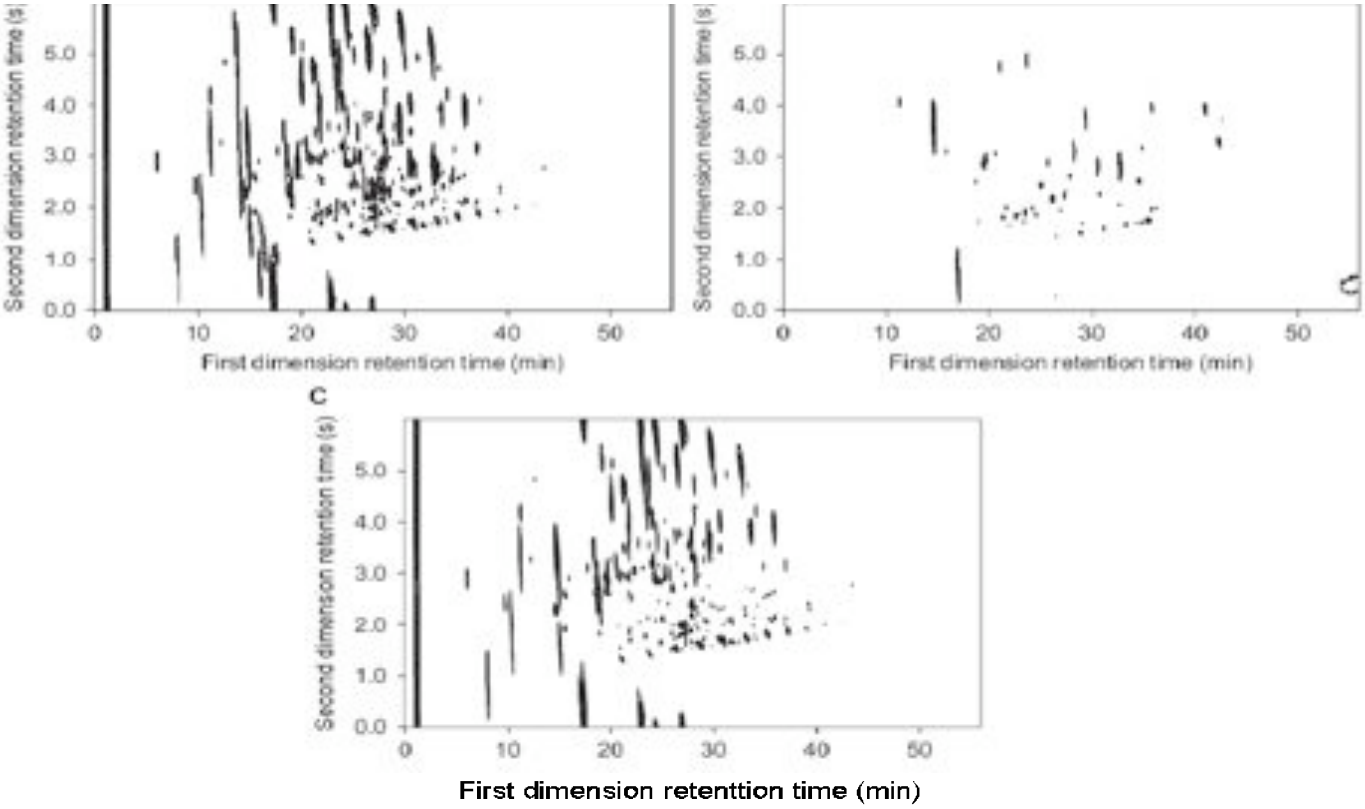


Figure 12.57. GC×GC–FID chromatograms of lotus-scented incense (a) smoke (b) powder and (c) subtracted chromatogram obtained by subtraction of powder H/S chromatogram from smoke chromatogram (a and b).

Contaminants in mussels

A.D. Booth, P. Sutton, C.A. Lewis, A.C. Lewis, A. Scarlett, W. Chau, J. Widdows, S.R. Rowland, *Unresolved complex mixtures of aromatic hydrocarbons: thousands of overlooked persistent, bioaccumulative, and toxic contaminants in mussels*, Environ. Sci. Technol. 2007, 41, 457-464

Instrumental conditions:

***Columns*:**
First: 10 m × 0.18 mm ID, 0.25 µm HP-5
Second: 1 m × 0.1 mm ID, 0.1 µm BP10
Modulation capillary:

Carrier gas: helium, constant flow @ 1.5 mL/min

***Temperatures*:**
Main oven: 40°C (0.2 min), 10°C/min → 160°C (1 min), 3°C/min → 270°C
Second oven: 50°C (0.2 min), 10°C/min → 170°C (1 min), 3°C/min → 280°C (12 min)

Injector: split/splitess
Temperature: 300°C
Injection volume:

Modulator: quad-jet cryogenic, hot pulse 1s

Modulation time: 2.5 s

Detector: ToF-MS
Temperature: ion source 250°C
Make up gas flow: 100 spectra/s

Data acquisition: not specified

Sample description and separation:

Mussels (*Mytilus edulis*) exhibiting a range of scope for growth values were collected from sites around the UK coast. Tissue extracts exhibiting impaired health contained large amounts of aromatic hydrocarbon UCMs compared to the extracts from healthy mussels. The UCMs (up to 125 μg/g dry tissue) contained thousands of previously unidentified branched alkyl homologues of known aromatic hydrocarbons such as branched alkylbenzenes (BABs), tetralins (BATs), and indanes and indenes (BINs).

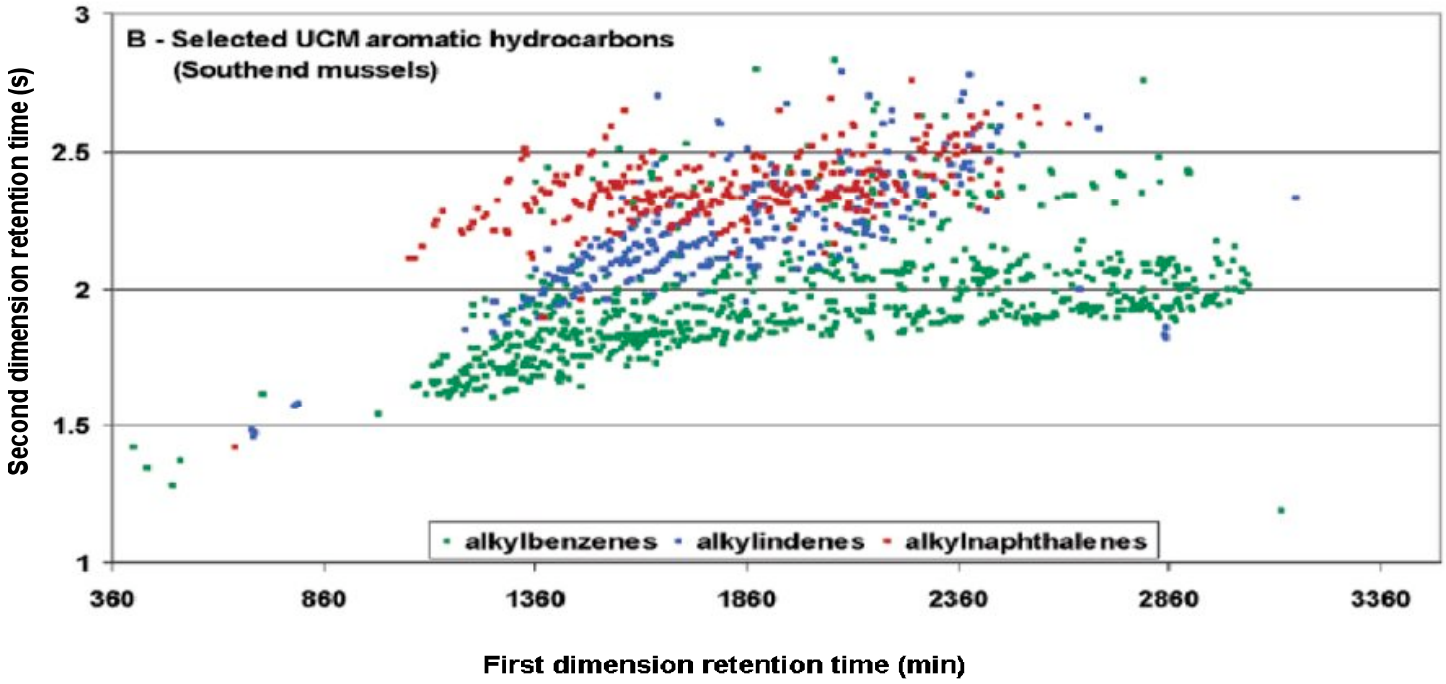


Figure 12.58. Apex plots of GC×GC–ToF MS analysis showing peak marker identifiers for each component in the mixture for which the mass spectrum contained base peak ions with mass:charge (m/z) ratios 91, 105 (■ alkylbenzenes), 129, 143 (■ alkylindenes), 141, 155 (■ alkylhaphthalenes).

Enantioselective separation of (mature) teatree leaf

R. Shellie, P. Marriott, C. Cornwell, *Application of comprehensive two-dimensional gas chromatography (GC×GC) to the enantioselective analysis of essential oils*, J. Sep. Sci. 24 (2001) 823-830

Instrumental conditions:

***Columns*:**
First: 25 m × 0.25 mm ID, 0.25 μm EtTBS-β-CD
Second: 0.8 m × 0.10 mm ID, 0.1 μm BP20
Modulation capillary:

Carrier gas: hydrogen, constant flow @ 95.2 kPa

***Temperatures*:**
Main oven: 45°C (6 min), 2°C/min → 200°C
Second oven:

Injector: split
Temperature: 220°C
Injection volume: 0.2 μL

Modulator: LMCS

Modulation time: 4 s

Detector: FID
Temperature: 250°C
Make up gas flow:

Data acquisition: 100 Hz

Sample description and separation:

The sample was an essential oil, commercial type (steam distilled from mature *Melaleuca alternifolia* plant material). Individual components were identified by comparison with authentic reference standards. They are identified in the figure with by a (+) or (–) sign or 'a' and 'b' where the correct assignment of isomers was not confirmed.
This investigation has seeked to achieve an enantioselective separation whilst maintaining all of the benefits of GC×GC. By using a capillary column containing a CDD in stead of the typical non-selective first dimension column, enantioselectivity has been introduced into the overall separation mechanism.

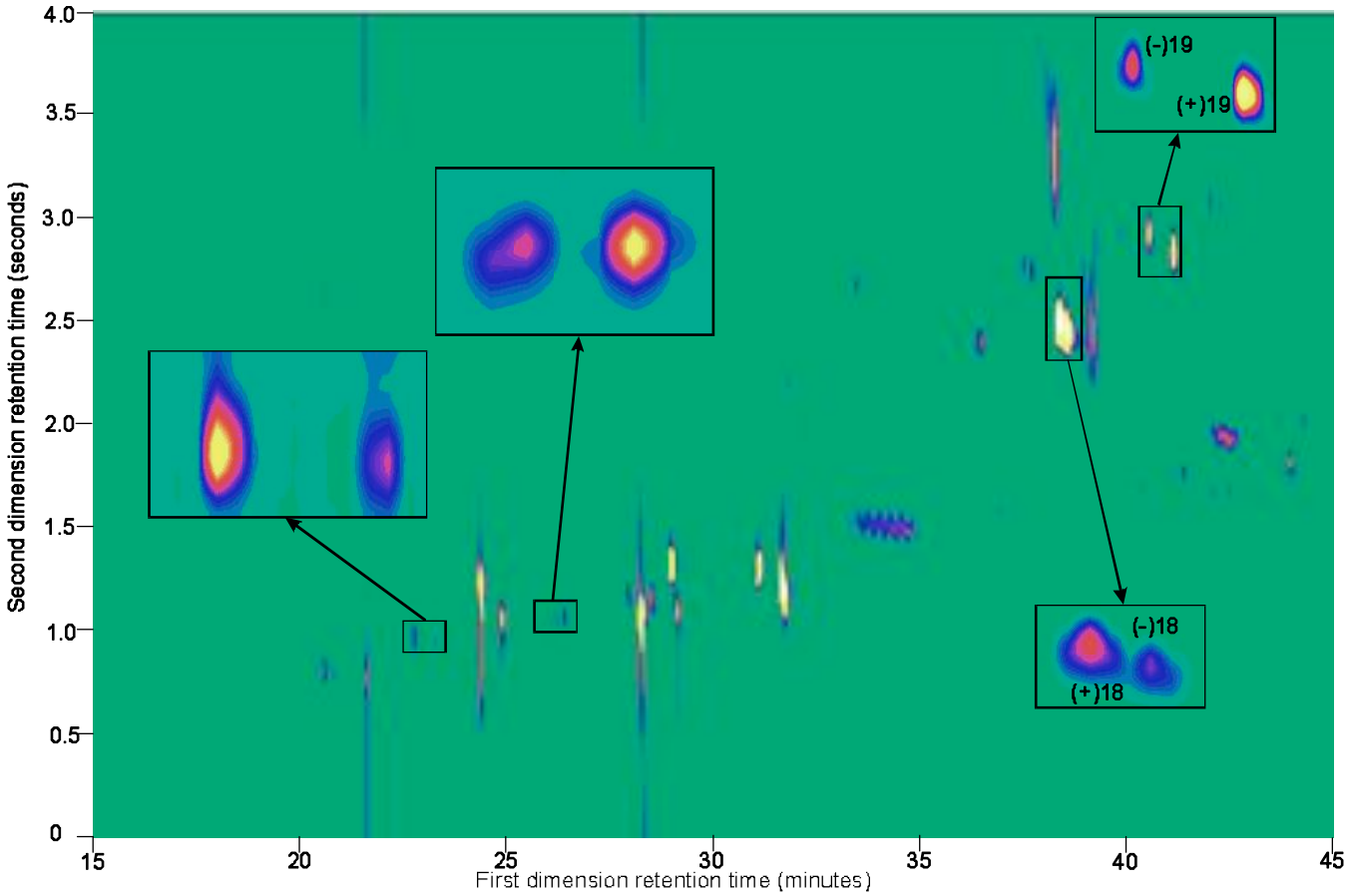


Figure 12.59. Enantioselective GC×GC separation of mature tea tree leaves. 12. trans sabinenehydrate, 18. terpinen-4-ol, 19. α-terpineol.

Lavender essential oil

R. Shellie, L. Mondello, P. Marriott, G. Dugo, *Characterisation of lavender essential oils by using gas chromatography–mass spectrometry with correlation of linear retention indices and comparison with comprehensive two-dimensional gas chromatography*, J Chromatogr. A 970 (1-2) (2002) 225-234

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 0.25 μm BPX5
Second 1 m × 0.1 mm ID, 0.1 μm BP20
Modulation capillary:

Carrier gas: hydrogen, 52 kPa @ constant pressure

***Temperatures*:**
Main oven: 60°C, 2°C/min → 210°C, 20°C/min → 260°C
Second oven:

Injector: split, ratio 1:100
Temperature:
Injection volume: 1 μL

Modulator: LMCS
Modulation time: 5 s

Detector: FID
Temperature:
Make up gas flow:

Data acquisition: 100 Hz

Sample description and separation:

A *Lavender angustifolia* essential oil was diluted 1:10 (V/V) with n-hexane.

At least 203 individual component contour peaks were counted in the Fig. The intensity of components here ranges between ~3 pA (height of the tallest pulse for the minor components), to >4000 pA (for the most abundant component linalool, Y). For simplicity only one contour level is shown at 12 pA (~3 pA above the baseline response), however more contour levels can be plotted which would provide information about the relative heights of individual peaks. The superior resolution that GC×GC offers over single column methods can be further appreciated by comparing the responses for the components marked Y and Z. The component Z, which has a maximum peak height of 10 pA would be difficult (if not impossible) to detect buried beneath the signal of the major component Y (4054 pA) in a single column analysis. Using GC–MS to analyse components Y and Z, spectral de-convolution may be useful, however if these two compounds produce similar MS fragmentation patterns, then the smaller component might (and most probably will) still be missed.

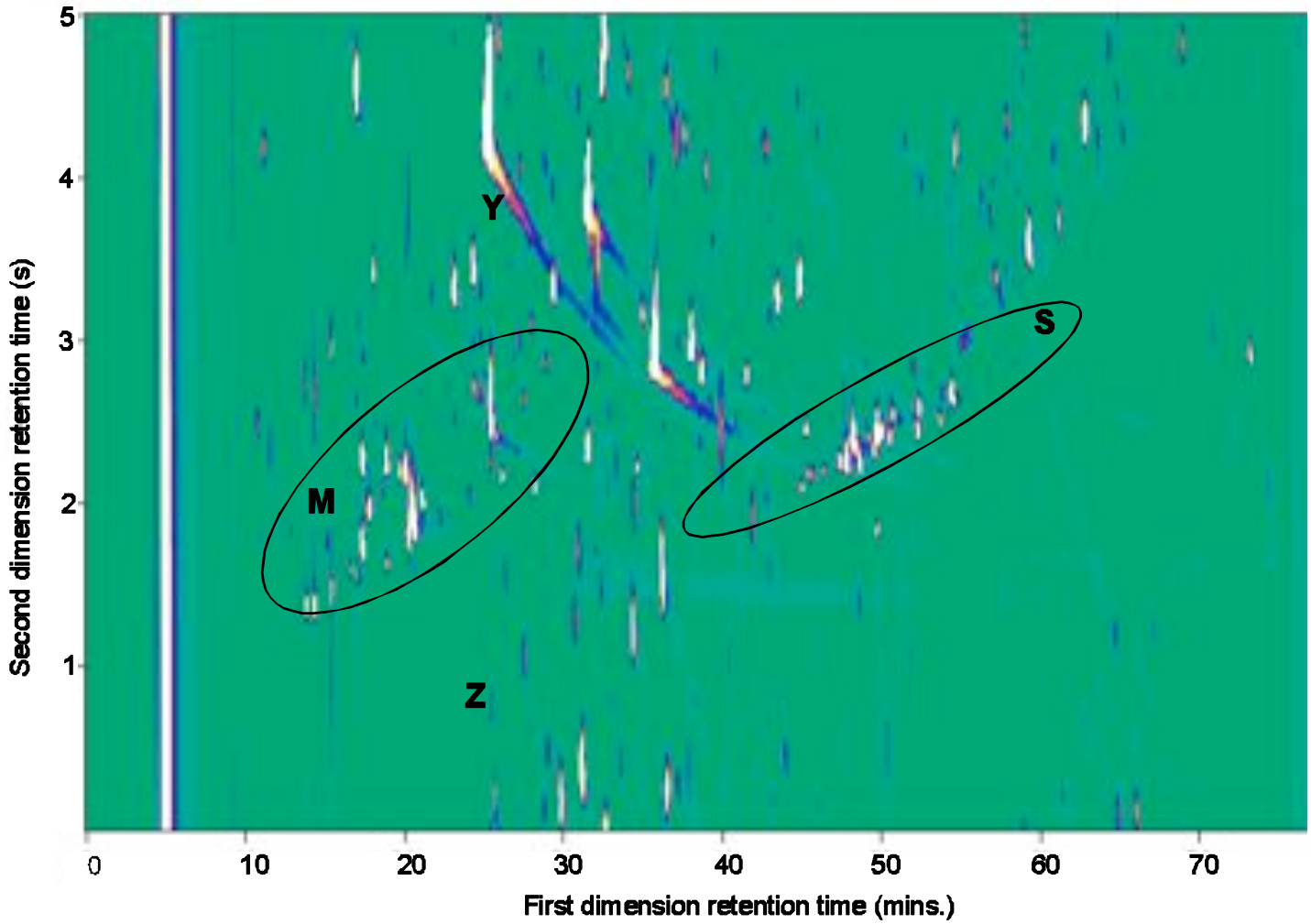


Fig. 12.60. The two-dimensional separation space for the GC×GC analysis of a lavender sample. Z is a minor component, which is completely unresolved from major component Y in the first dimension. M. monoterpene hydrocarbons; S. sesquiterpene hydrocarbons.

Fresh ginger and ginger sweet

Y. Shao, P. Marriott, R. Shellie, H. Hügel, *Solid-phase micro-extraction–comprehensive two-dimensional gas chromatography of ginger (Zigiber officinale) volatiles*, Flavour Fragr. J. 18 (2003) 5-12

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 1.0 µm BPX5
Second: 0.8 m × 0.10 mm ID, 0.1 µm BP20
Modulation capillary:

Carrier gas: hydrogen, constant flow @ 1.6 mL/min

***Temperatures*:**
Main oven: 40°C, 2°C/min → 180°C
Second oven:

Injector: split
Temperature:
Injection volume:

Modulator: LMCS

Modulation time: 4 s

Detector: FID
Temperature:
Make up gas flow:

Data acquisition:

Sample description and separation:
The volatiles from the headspace of the sample were extracted with different SPME fibres. The fibres of 100 µm PDMS appeared to give the highest efficiency based on the amount of recovered or injected solute. No additional carry over was observed when applying these fibers for the samples.

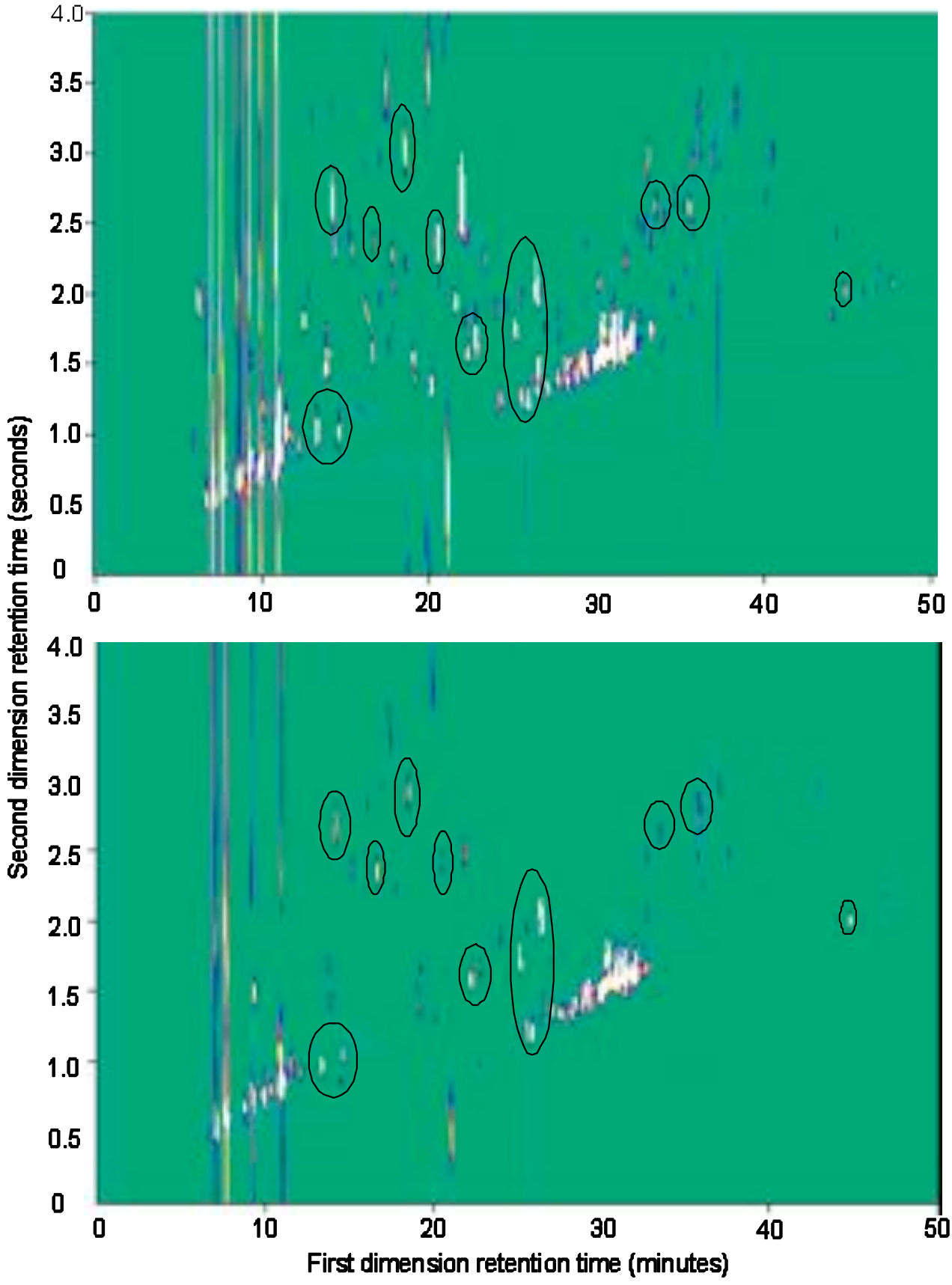


Figure 12.61. GC×GC separation of ginger headspace volatiles.
(upper) ginger sweet. (lower) Fresh ginger.
The circled peaks are the same components in the two samples, illustrating the excellent reproducibility of the GC×GC analysis.

Ginseng

R.A. Shellie, P.J. Marriott, C.W. Huie, *Comprehensive two-dimensional gas chromatography (GC×GC) and GC×GCquadrupole MS analysis of Asian and American ginseng*, J. Sep. Sci. 26 (2003) 1185–1192

Instrumental conditions:

***Columns*:**	
First:	30 m × 0.32 mm ID, 0.25 μm HP-5
Second	1 m × 0.1 mm ID, 0.1 μm BP20
Modulation capillary:	
Carrier gas:	hydrogen, constant flow @ 140 kPa
***Temperatures*:**	
Main oven:	80°C, 10°C/min → 120°C, 2°C/min → 170°C, 10°C/min → 200°C (10 min
Second oven:	
Injector:	split, ratio 1:25
Temperature:	
Injection volume:	
Modulator:	LMCS
Modulation time:	4 s
Detector:	FID
Temperature:	
Make up gas flow:	
Data acquisition:	100 Hz

Sample description and separation:

For the detection of the presence of *P. quinquefolius* (American ginseng) among a mixture of other medicinal herb(s), 28 distinctive bands, labeled in the Figure, can be used for such authentication/quality control purposes, *vide infra*.

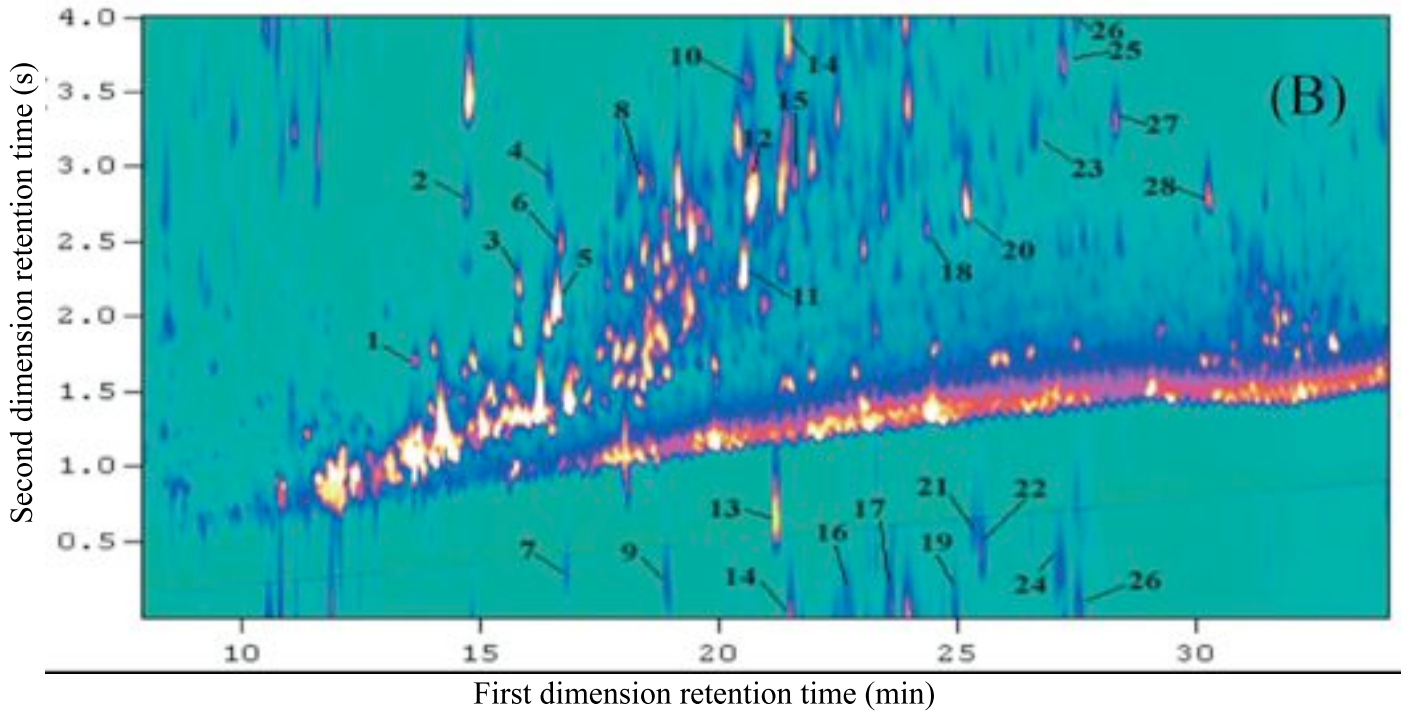


Figure 12.62. Part of the 2-D contour plot of P. quinquefolius. (American ginseng) For identification of the numbers: see paper

Flavours in herbal mixtures (ginseng mixtures)

X. Di, R.A. Shellie, P.J. Marriott, C.W. Huie, *Application of headspace solid-phase microextraction (HS-SPME) and comprehensive two-dimensional gas chromatography (GC×GC) for the chemical profiling of volatile oils in complex herbal mixtures*, J. Sep. Sci. 27 (2004) 451-458

Instrumental conditions:

***Columns*:**
First: 30 m × 0.32 mm ID, 0.25 µm HP-5
Second: 1.5 m × 0.10 mm ID, 0.05 µm BPX50
Modulation capillary:

Carrier gas: hydrogen @ 0.5 mL/min
***Temperatures*:**
Main oven: 80°C, 10°C/min → 120°C, 2°C/min → 170°C, 10°C/min → 200°C (2 min)
Second oven:

Injector: splitless
Temperature: 300°C
Injection volume: SPME fiber

Modulator: LMCS

Modulation time: 4 s

Detector: FID
Temperature: 280°C
Make up gas flow: N_2 @ 10 mL/min

Data acquisition: 100 Hz

Sample description and separation:

The HS-SPME–GC×GC separation was performed as a chemical profiling (fingerprinting) of essential/volatile oils contained in herbal materials. More than 20 marker compounds belonging to *Panax quinquefolius* (American ginseng) can be observed within the contour plots of ginseng itself, a mixture of ginseng and another important herb (*P. quinquefolius/ Radix angelicae sinensis*), as well as a mixture of ginseng and three other herbs (*P. quinquefolius/R. angelicaesinensis/R. astragali/R. rehmanniae preparata*). In particular, the presence of *Panax* in the herb formulation could be readily identified through its specific peak pattern in the GC×GC plot.

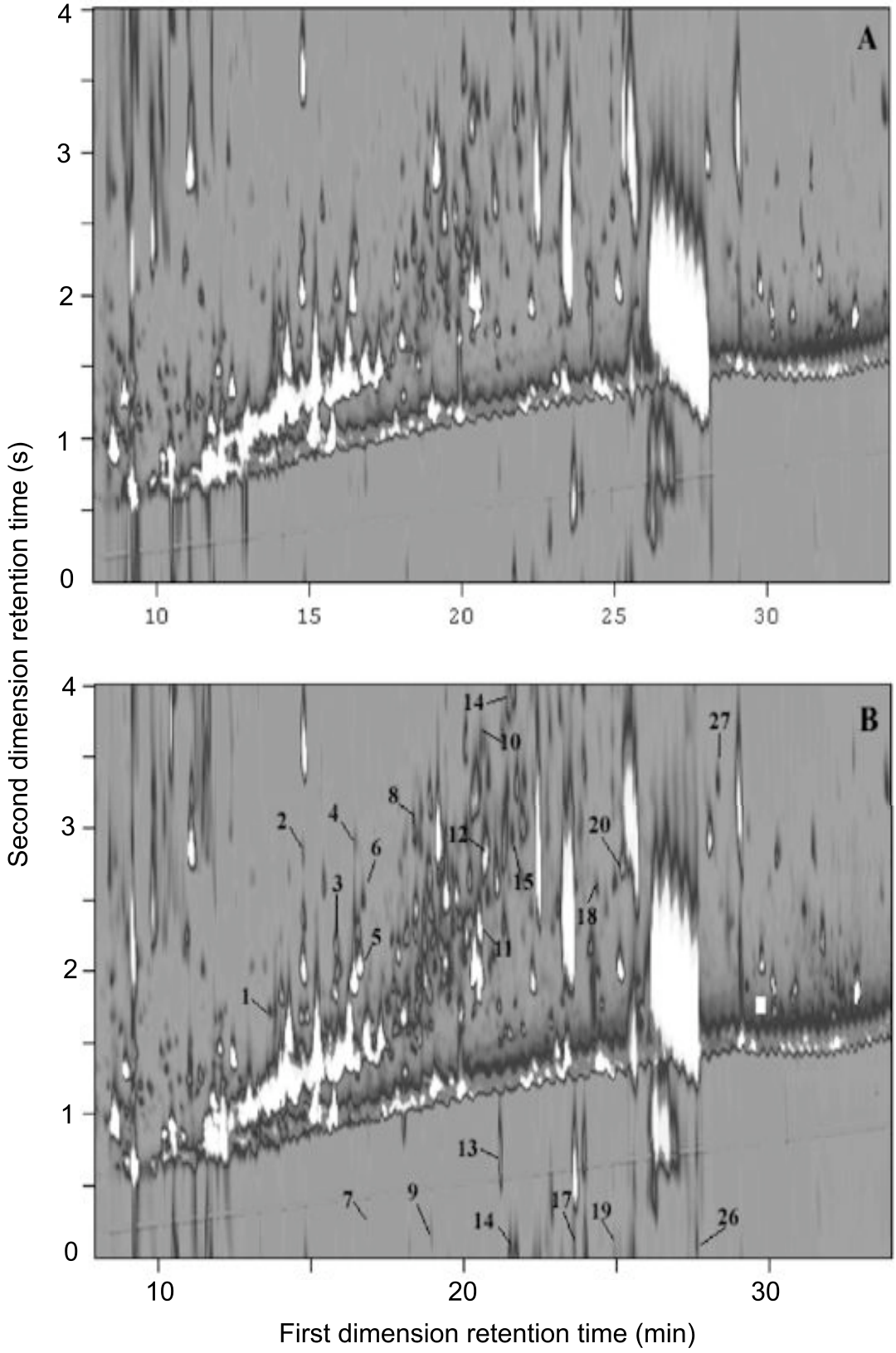


Figure 12.63. Section of the contour plot of (A) a 1:1:1 (w/w) mixture of R. angelicae sinensis/R. astragali/R. rehmanniae preparata (33 mg for each herb) and (B) 1:1:1:1 (w/w) mixture of P. quinquefolius/R. angelicae sinensis/R. astragali/R. rehmanniae preparata (25 mg for each herb). For identification: see referenced paper.

Vetiver essential oil

P. Marriott, R. Shellie, J. Fergeus, R. Ong, P. Morrison *High resolution essential oil analysis by using comprehensive gas chromatographic methodology*, J. Flavour Fragr. 15 (2000) 225-239

Instrumental conditions:

***Columns*:**
First: 25 m × 0.25 mm ID, 0.25 µm BPX5
Second 2 m × 0.1 mm ID, 0.1 µm BP20
Modulation capillary:

Carrier gas: hydrogen, constant flow @ 47.4 psi

***Temperatures*:**
Main oven: 60°C (1 min), 10°C/min → 120°C, 1°C/min → 240°C
Second oven:

Injector: split, ratio 1:25
Temperature:
Injection volume:

Modulator: LMCS

Modulation time: 4 s

Detector: FID
Temperature:
Make up gas flow:

Data acquisition: 100 Hz

Sample description and separation:

The sample used was a Vetiver whole oil (*Vetiveria zizanioide*s) of the Java type, provided by Australian Botanical Products, diluted with hexane.

Of major importance in the separation is that since components are baseline resolved, the low or trace abundant components can be readily recognized even when they co-elute with major components on the first column. The colour plot of minor peaks, *e.g*., as identified by (a) or (b) in the Fig., can be clearly and unambiguously found and so now it is readily apparent how many solutes overlap at any point on the first column, and importantly now they can be measured.

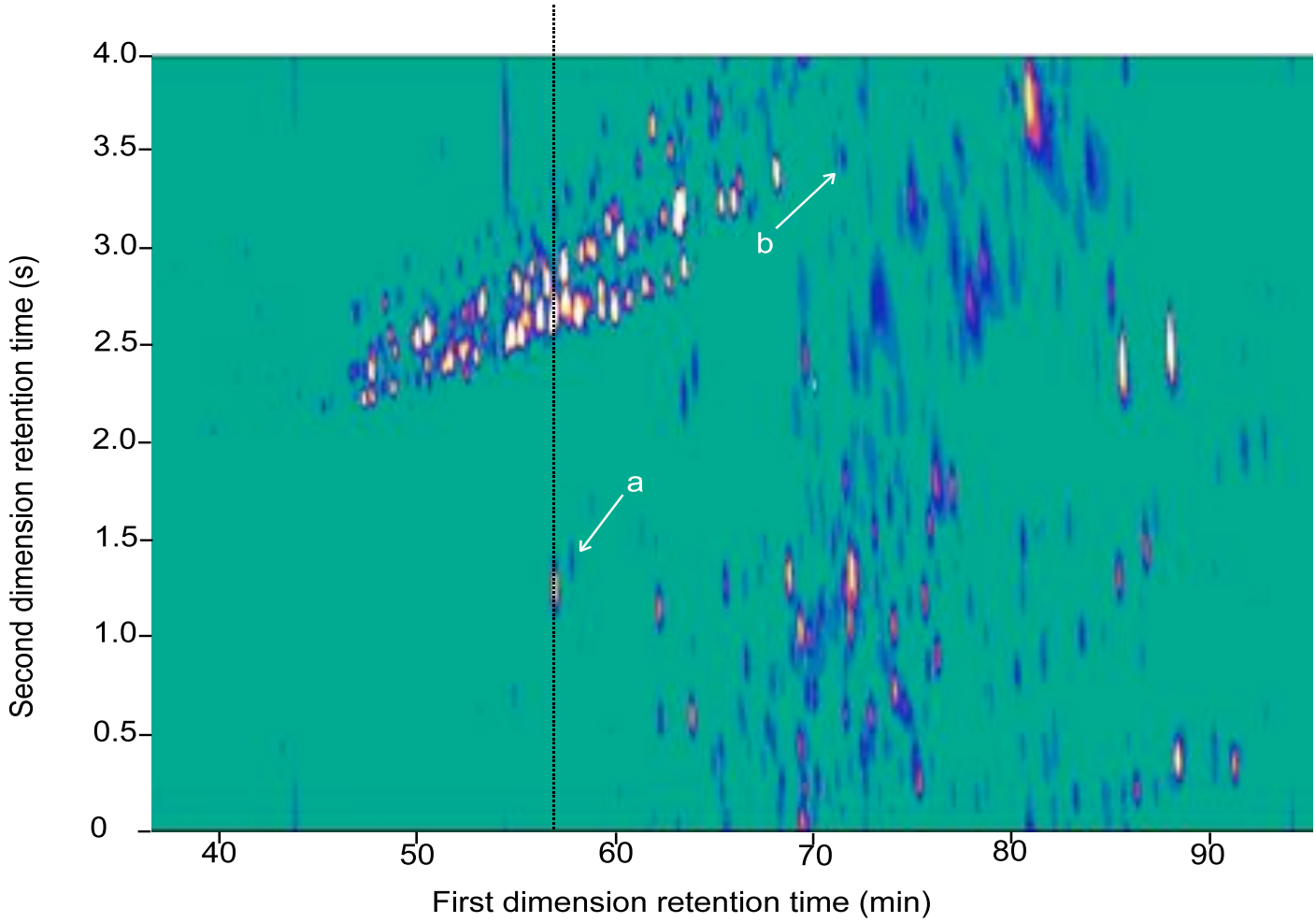


Figure 12.64. Separation of vetiver oil. Upper part: 1D–GC separation, Lower part: GC×GC separation.

Tea extract

M. Adahchour, Vrije Universiteit, Amsterdam, *unpublished results*,

Instrumental conditions:

***Columns*:**
First: 25 m, 0.25 mm ID, 0.25 μm DB1
Second: 1 m, 0.10 mm ID, 0.1 μm BPX50
Modulation capillary:

Carrier gas: helium @ 180 kPa

***Temperatures*:**
Main oven: 30°C (2 min), 4°C/min → 220°C (5 min)
Second oven:

Injector: PTV, splitless
Temperature: → 270°C
Injection volume: 1 μL

Modulator: LMCS

Modulation time: 6 s

Detector: ToF-MS
Temperature:
Make up gas flow:

Data acquisition: 50 spectra/s

Sample description and separation:

The sample is the neutral basic fraction of a Solvent-Assisted Flavour Evaporation (SAFE) extraction of a commercial tea product.

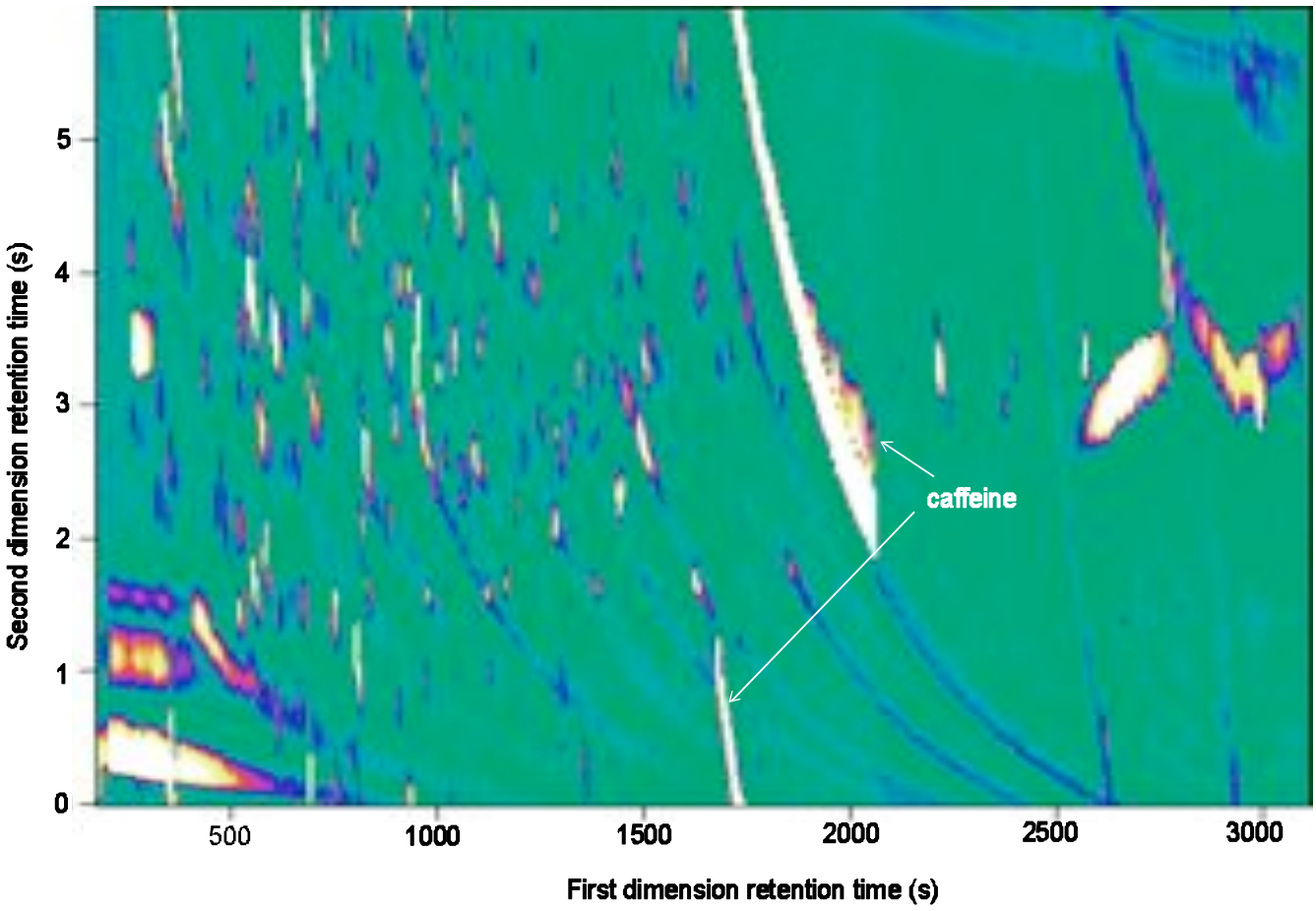


Figure 12.65. TIC colour plot of a GC×GC separation of a tea extract. The white arrows indicate the long first dimension tailing peak of caffeine.

Dairy sour cream, CFD extract

M. Adahchour, L.L.P. van Stee, J. Beens, R.J.J. Vreuls, M.A. Batenburg, U.A.Th. Brinkman, *Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection (GC×GC–ToF MS) for the trace analysis of flavour compounds in food*, J. Chromatogr. A, 1019 (2003) 157-172

Instrumental conditions:

***Columns*:**
First: 25 m × 0.25 mm ID, 0.25 µm DB1
Second: 1 m × 0.10 mm ID, 0.1 µm BPX50
Modulation capillary:

Carrier gas: helium, constant pressure @180 kPa

***Temperatures*:**
Main oven: 30°C (2 min), 4°C/min → 220°C (5 min)
Second oven:

Injector: PTV, splitless
Temperature: → 270°C
Injection volume: 1 µL

Modulator: LMCS

Modulation time: 6 s

Detector: ToF-MS
Temperature:
Make up gas flow:

Data acquisition: 50 spectra/s

Sample description and separation:

The sample is a total fraction of a Cold Finger Distillation (CFD) of a dairy sour cream.

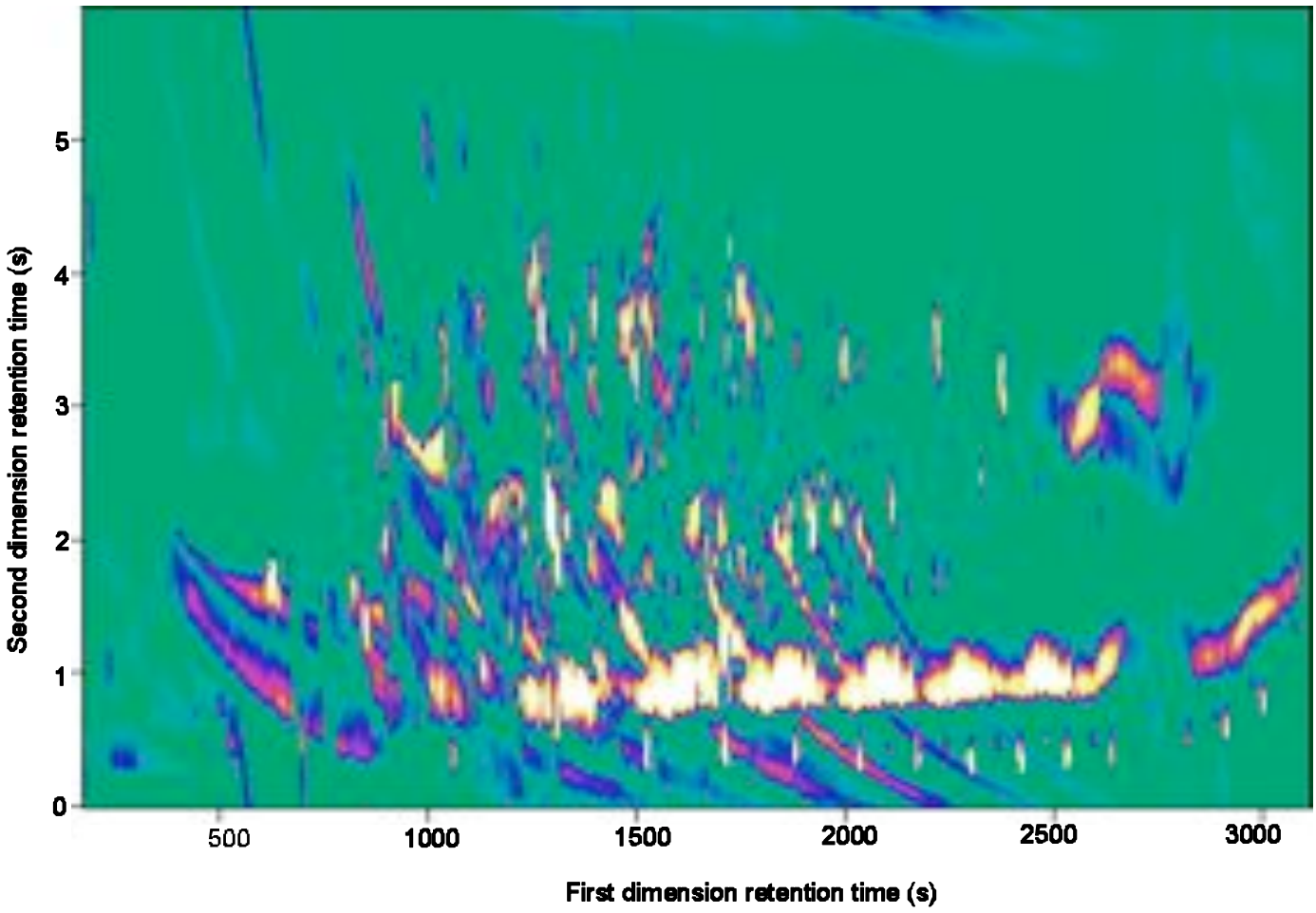


Figure 12.66. TIC colour plot of a GC×GC separation of extract 5. The curved lines are long 1D tailing peaks of polar compounds. The band of individual spots on the right end bottom part of the plot are silicon-containing compounds originating from the extract procedure.

Non-dairy sour cream, SAFE extract

M. Adahchour, L.L.P. van Stee, J. Beens, R.J.J. Vreuls, M.A. Batenburg, U.A.Th. Brinkman, *Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection (GC×GC–TOF MS) for the trace analysis of flavour compounds in food*, J. Chromatogr. A, 1019 (2003) 157-172

Instrumental conditions:

***Columns*:**
First: 25 m × 0.25 mm ID, 0.25 μm DB1
Second: 1 m × 0.10 mm ID, 0.1 μm BPX50
Modulation capillary:

Carrier gas: helium @ 180 kPa

***Temperatures*:**
Main oven: 30°C (2 min), 4°C/min → 220°C (5 min)
Second oven:

Injector: PTV, splitless
Temperature: → 270°C
Injection volume: 1 μL

Modulator: LMCS

Modulation time: 6 s

Detector: ToF-MS
Temperature:
Make up gas flow:

Data acquisition: 50 spectra/s

Sample description and separation:

The sample is the neutral basic fraction of a Solvent-Assisted Flavour Extraction of a non-dairy sour cream

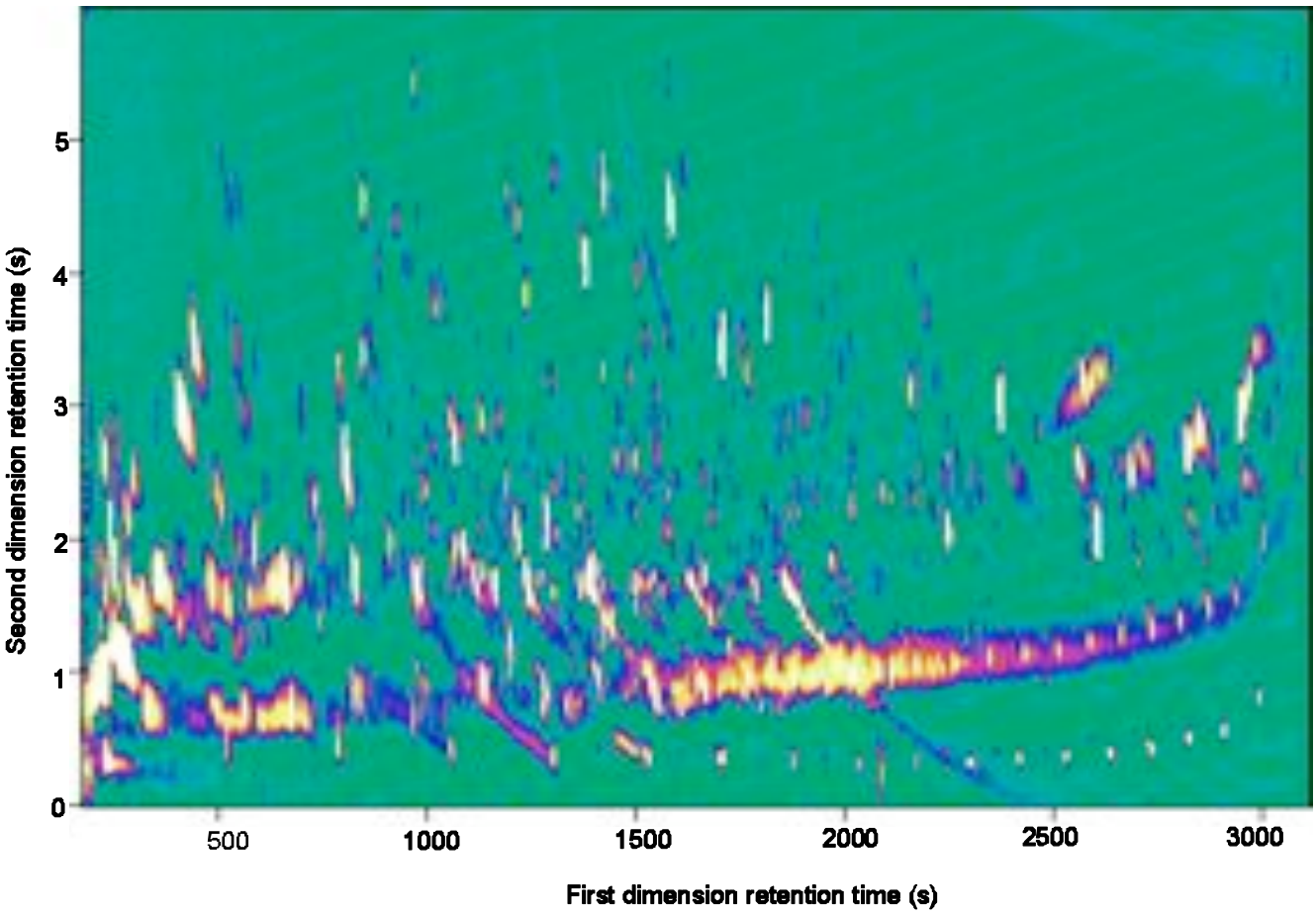


Figure 12.67. TIC colour plot of a GC×GC separation of extract 12. The curved lines around1000 and 2000 seconds (1D retention) are the long 1D tailing peaks of an alcohol and organic acids respectively. The band of individual spots on the right end bottom part of the plot are silicon-containing compounds originating from the extract procedure.

Non-dairy sour cream, CFD extract

M. Adahchour, L.L.P. van Stee, J. Beens, R.J.J. Vreuls, M.A. Batenburg, U.A.Th. Brinkman, *Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection (GC×GC–TOF MS) for the trace analysis of flavour compounds in food*, J. Chromatogr. A, 1019 (2003) 157-172

Instrumental conditions:

***Columns*:**
First: 15 m × 0.25 mm ID, 0.25 μm DB1
Second: 1 m × 0.10 mm ID, 0.1 μm BPX50
Modulation capillary:

Carrier gas: helium @ 180 kPa

***Temperatures*:**
Main oven: 40°C (2 min), 5°C/min → 240°C (5 min)
Second oven:

Injector: PTV, splitless
Temperature: → 270°C
Injection volume: 1 μL

Modulator: LMCS
Modulation time: 6 s

Detector: ToF-MS
Temperature:
Make up gas flow:

Data acquisition: 50 spectra/s

Sample description and separation:

The sample is the neutral basic fraction of a Cold-Finger Distillation of a non-dairy sour cream. Although the colour plot is seemingly chaotic and unordered, inspection by library search of the spectra of the ToF MS reveals the presence of a number of clusters:

- on the bottom line, although vaguely visible, a regular line of silica-containing species, probably originating from the extraction technique;
- in the polygon designated with A, branched paraffins;
- in the polygon designated with B, series of ketones, aldehydes, alcohols and esters;
- along curve C: substituted dihydrofuranones, from methyl-substituted around ^1t_R = 500 s up to ^1t_R = 2000 s substitutions with 6 C atoms;
- along curve D: substituted tetrahydropyranones, from methyl-substituted around ^1t_R = 500 s up to ^1t_R = 200 s substitutions with 6 C atoms.

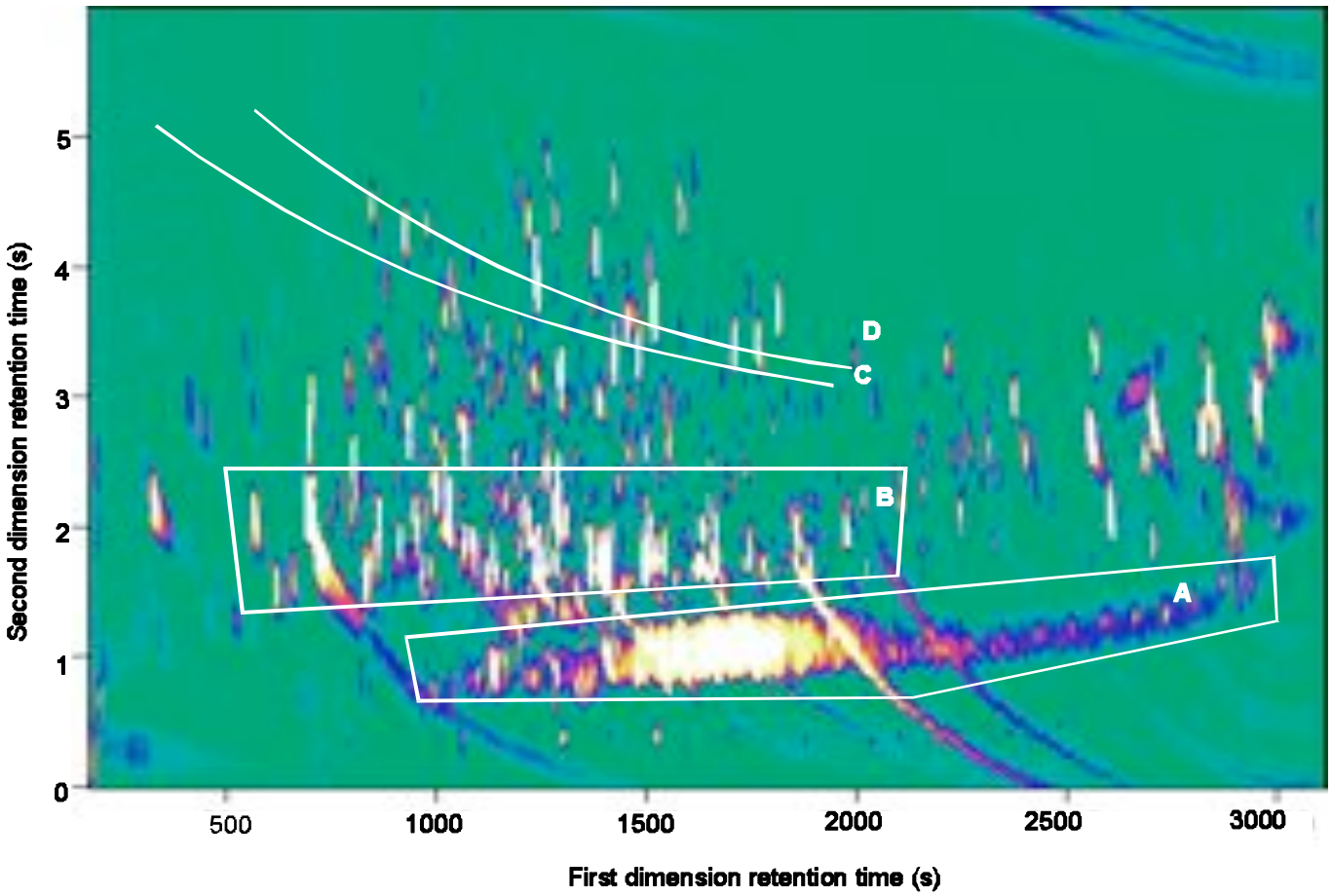


Figure 12.68. TIC colour plot of a GC×GC separation of extract 14. The curved lines around1000 and 2000 seconds (1D retention) are the long 1D tailing peaks of an unsaturated alcohol (2-octyn-1-ol) and two organic acids respectively. A. Alkanes, B. Ketones, aldehydes, alcohols and esters, C. Substituted dihydrofuranones, D. Substituted tetrahydropyranones.

Dairy sour cream, SAFE extract

M. Adahchour, L.L.P. van Stee, J. Beens, R.J.J. Vreuls, M.A. Batenburg, U.A.Th. Brinkman, *Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection (GC×GC–TOF MS) for the trace analysis of flavour compounds in food*, J. Chromatogr. A, 1019 (2003) 157-172

Instrumental conditions:

***Columns*:**
First: 15 m × 0.25 mm ID, 0.25 μm DB1
Second: 1 m × 0.10 mm ID, 0.1 μm BPX50
Modulation capillary:

Carrier gas: helium @ 180 kPa

***Temperatures*:**
Main oven: 40°C (2 min), 5°C/min → 240°C (5 min)
Second oven:

Injector: PTV, splitless
Temperature: → 270°C
Injection volume: 1 μL

Modulator: LMCS

Modulation time: 6 s

Detector: ToF-MS
Temperature:
Make up gas flow:

Data acquisition: 50 spectra/s

Sample description and separation:

Although the colour plot is seemingly chaotic and unordered, inspection by library search of the spectra of the ToF MS reveals the presence of a number of clusters:

- on the bottom line, a regular line of silica-containing species, probably originating from the extraction technique;
- in the polygon designated with A, branched paraffins;
- in the polygon designated with B, series of ketones, aldehydes, alcohols and esters;
- along curve C: substituted dihydrofuranones, from methyl-substituted around ^1t_R = 500 s up to ^1t_R = 2000 s substitutions with 6 C atoms;
- along curve D: substituted tetrahydropyranones, from methyl-substituted around ^1t_R = 500 s up to ^1t_R = 2000 s substitutions with 6 C atoms.

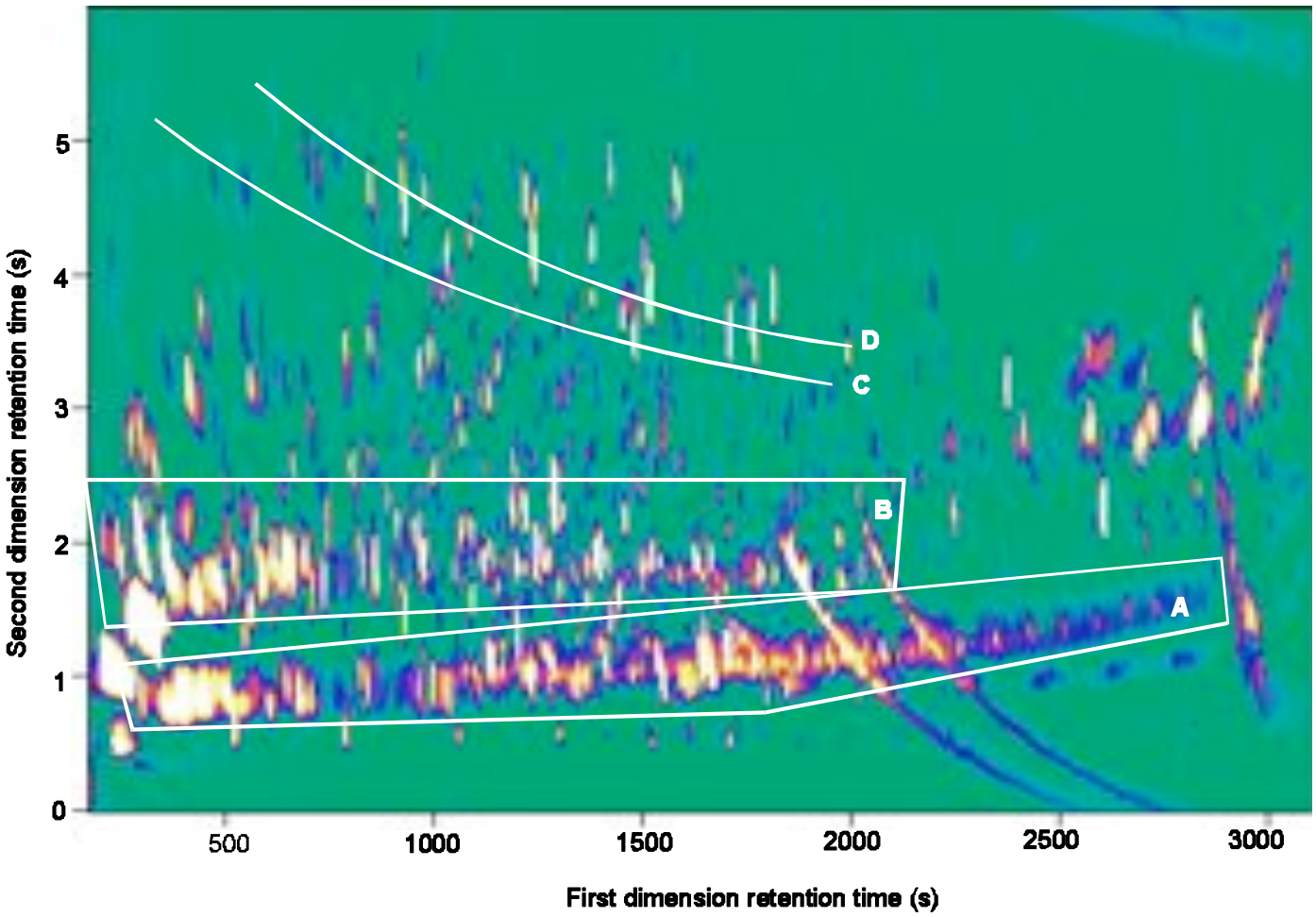


Figure 12.69. TIC colour plot of a GC×GC separation of extract 16. The two curved lines around 2000 seconds (1D retention) are the long 1d tailing peaks of two organic acids. A. Alkanes, B. Ketones, aldehydes, alcohols and esters, C. Substituted dihydrofuranones, D. Substituted tetrahydropyranones.

Fatty acids in milk

B. Vlaeminck, J. Harynuk, V. Fievez, P. Marriott, *Comprehensive two-dimensional gas chromatography for the separation of fatty acids in milk,* Eur. J. Lipid Sci. Technol. 109 (2007) 757–766

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 0.25 µm BPX80
Second: 0.25 m × 0.1 mm ID, 0.1 µm BPX35
Modulation capillary:

Carrier gas: helium, constant flow @ 1 mL/min

***Temperatures*:**
Main oven: 90°C , 2°C/min → 250°C
Second oven:

Injector: splitless
Temperature: 250°C
Injection volume: 1µL

Modulator: LMCS

Modulation time: 6 s (3 s for non-polar × polar column set)

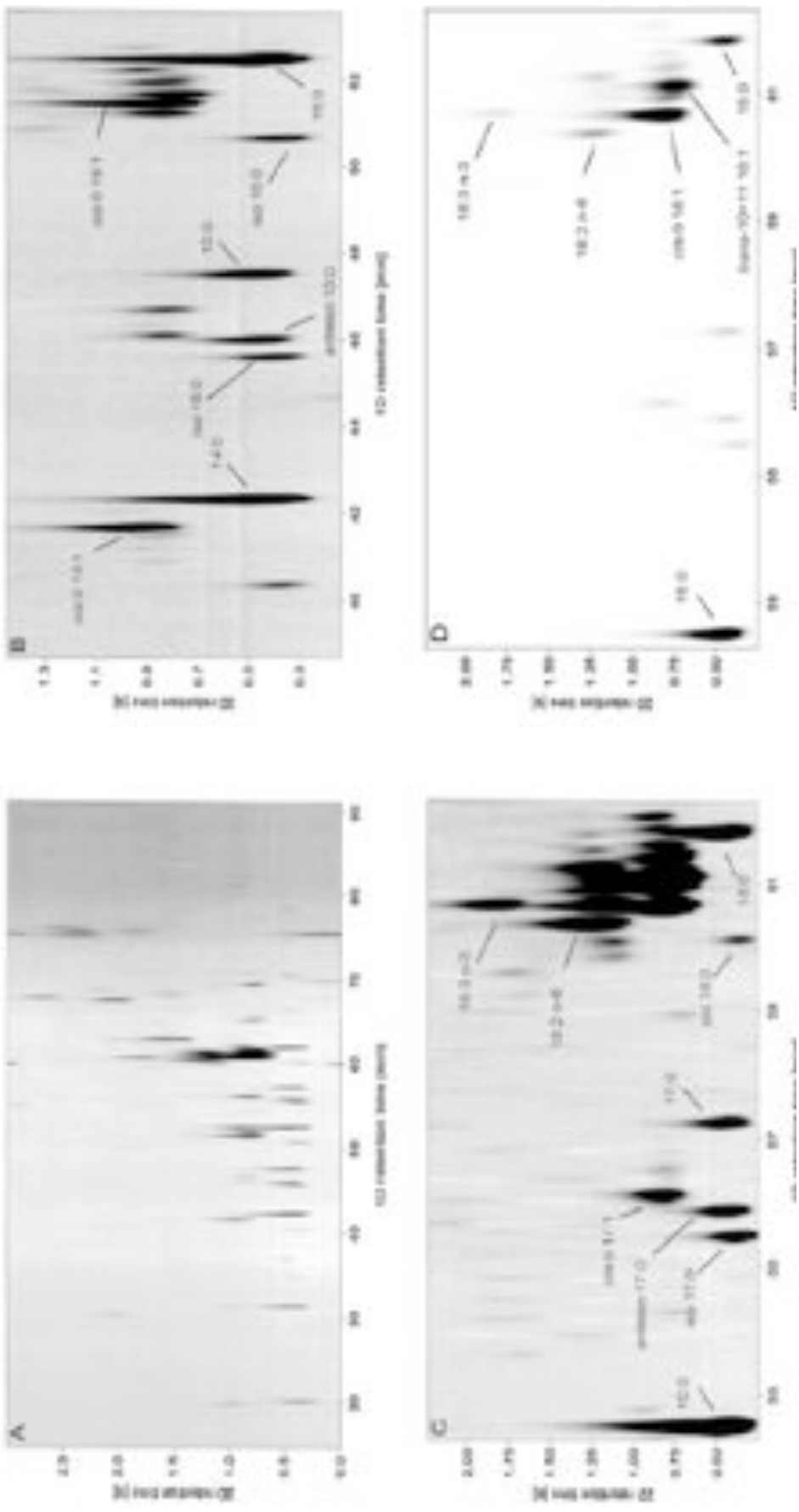
Detector: FID
Temperature: 260°C
Make up gas flow:

Data acquisition: 100Hz

Sample description and separation:
Milk fat was extracted from milk samples of cows receiving a control diet or the control diet supplemented with marine algae.

The peaks in the contour plot showed a well-ordered structure of fatty acids according to their number of carbon atoms and degree of unsaturation, facilitating identification of known and unknown compounds. Based on these relations, identification of carbon number and degree of unsaturation of several 22-fatty acids was possible. The large difference between the 22-fatty acids from milk fat of cows fed the control and the marine algaecontaining diet suggest that rumen hydrogenation of 22:6 *n*-3 results in a similar complex profile of hydrogenation intermediates as observed for 18:2 *n*-6 and 18:3 *n*-3.

Figure 12.70. GC×GC chromatogram of the FAME from milk fat (A) and close-up of the 14–16-region (B) and 16–18-region (C) separated on a nonpolar/polar column set (BPX5×BP20). (D) is identical to (C) but presented at a less sensitive response scale, to highlight major components. The GC×GC chromatogram was shifted with 2.5 s to aid in visualization of the data.



Odour-active compounds in hop

G.T. Eyres, P.J. Marriott, J.-P. D.ufour, *Comparison of odor-active compounds in the spicy raction of hop (Humulus lupulus L.) essential oil from four different varieties,* J. Agric. Food Chem. 55 (2007) 6252-6261

Instrumental conditions:

***Columns*:**
First: 25 m × 0.25 mm ID, 0.25 µm BPX5
Second: 0.8 m × 0.1 mm ID, 0.1 µm BP20
Modulation capillary:

Carrier gas: helium, constant flow @ 2 mL/min

***Temperatures*:**
Main oven: 60°C (0.2 min), 3°C/min → 225°C, 10°C/min → 250°C (20 min
Second oven:

Injector: split/splitless
Temperature: 250°C
Injection volume: 1µL

Modulator: loop, hot period 375 ms

Modulation time: 6 s

Detector: ToF MS
Temperature:
Make up gas flow:

Data acquisition: 100 spectra/s, 100-415 *m/z*

Sample description and separation:

The "spicy" character of hops is considered to be associated with "noble hop aroma".. Odorants in four samples of the spicy fraction of hop essential oil were characterized using GC–O and CharmAnalysis. Odour-active compounds were tentatively identified using GC×GC–ToF MS. An intense "woody, cedarwood" odour coincided with a complex region where between 8 and 13 compounds were coeluting. The peak responsible was determined by (i) correlating peak areas with Charm values in eight hop samples and (ii) heartcut MDGC–O. The compound responsible was tentatively identified as 14-hydroxy-*â*-caryophyllene. Other important odorants identified were geraniol, linalool, *â*-ionone, and eugenol.

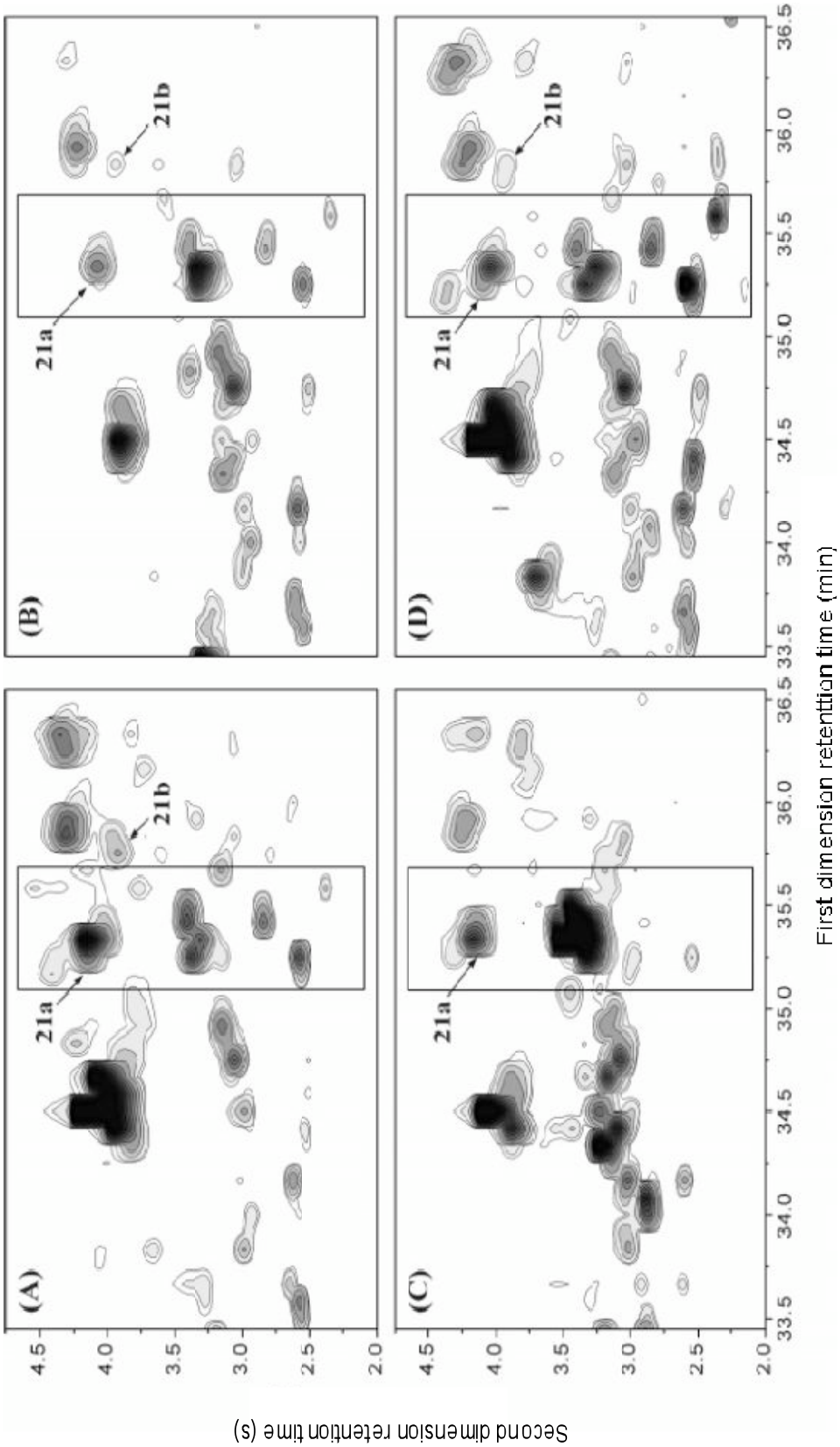


Figure 12.71.

Selected regions GC×GC–FID contour plots of the spicy fractions of (a) Cascade hops, (b) Target hops, (c) HHE hops, and (d) Saaz hops. The outlined regions correspond to where the woody, cedarwood odour (peak 21a) was perceived during GC–O. The peaks determined to be responsible for the odours perceived in this region during GC–O (peaks 21a and 21b) are labeled.

Extract of garlic powder

M. Adahchour, J. Beens, R.J.J. Vreuls, A.M. Batenburg, E.A.E. Rosing, U.A.Th. Brinkman, *Application of solid-phase micro-extraction and comprehensive two-dimensional gas chromatography (GC×GC) for flavour analysis*, Chromatographia, 55 (2002) 361-367

Instrumental conditions:

***Columns*:**
First: 30 m × 0.32 mm ID, 0.25 µm HP-1B
Second: 1.5 m × 0.10 mm ID, 0.05 µm BPX50
Modulation capillary:

Carrier gas: helium @ 180 kPa

***Temperatures*:**
Main oven: 30°C (2 min), 4°C/min → 220°C (5 min)
Second oven:

Injector: split, split ratio 1:100
Temperature: 300°C
Injection volume: SPME fiber

Modulator: moving cryogenic

Modulation time: 8 s

Detector: FID or ToF-MS
Temperature:
Make up gas flow:

Data acquisition: 200 Hz or 50 spectra/s

Sample description and separation:

An SPME extract of garlic powder was separated by GC×GC–FID. To enable identification of some interesting components the same separation was performed using a ToF MS as a detector.
As can be seen in the inset B in the Fig., the amount of separated compounds is very high. This is especially true since a large amount of very low intensity peaks, not visible in 1D-GC and generally ignored as “chemical noise”, are now made visible.

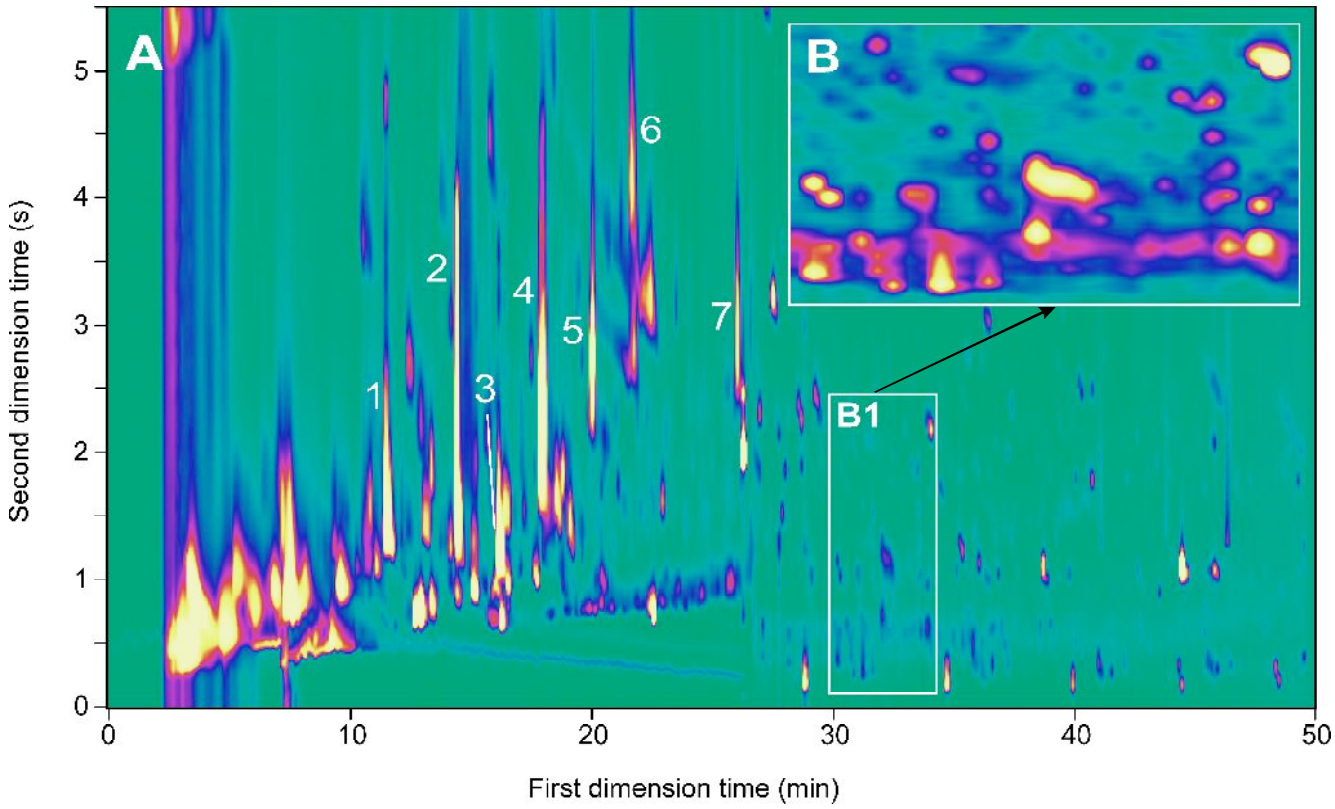


Figure 12.72. Colour plot of a GC×GC separation of an SPME extract of garlic powder. The region enclosed in B1 is inserted in B with a higher graphical sensitivity to indicate the large amount of separated peaks in this area with low concentrations.

1. Methyl-2-propenyl-disulfide, 2. dimethyl-trisulfide, 3. 1-methyl-3-(1-methylethyl-)benzene, 4. diallyl-disulfide, 5. methyl-2-propenyl-trisulfide,

6. 3-vinyl-1,2-dithiocyclohex-4-ene, 7. di-2-propenyl-trisulfide.

Sweet sagewort

C. Ma, H. Wang, X. Lu, G. Xu, B. Liu, *Metabolic fingerprinting investigation of Artemisia annua L. in different stages of development by gas chromatography and gas chromatography–mass spectrometry*, J. of Chromatogr. A, 1186 (2008) 412-419

Instrumental conditions:

***Columns*:**
First: 50 m × 0.20 mm ID, 0.5 µm DB petro
Second: 2.6 m × 0.10 mm ID, 0.10 µm DB17HT
Modulation capillary:

Carrier gas: helium, constant pressure @ 600 kPa

Temperatures
Main oven: 60°C, 3°C/min → 260°C (15 min)
Second oven:

Injector: splitless
Temperature: 260°C
Injection volume:

Modulator: quad cryogenic jets

Modulation time: 4 s

Detector: ToF-MS
Temperature: ion source: 220°C, transfer line: 260°C
Make up gas flow:

Data acquisition: 50 spectr/s, 35-400 amu

Sample description and separation:
Sweet sagewort (*Artemisia annua* L.*)* is an annual herb native of Asia, it has been used for many centuries for the treatment of fever and malaria. Three hundred and three components were tentatively identified and terpene compounds are the main
components of *Artemisia annua* L. volatile oil. Artemisinic acid is tentatively qualified.

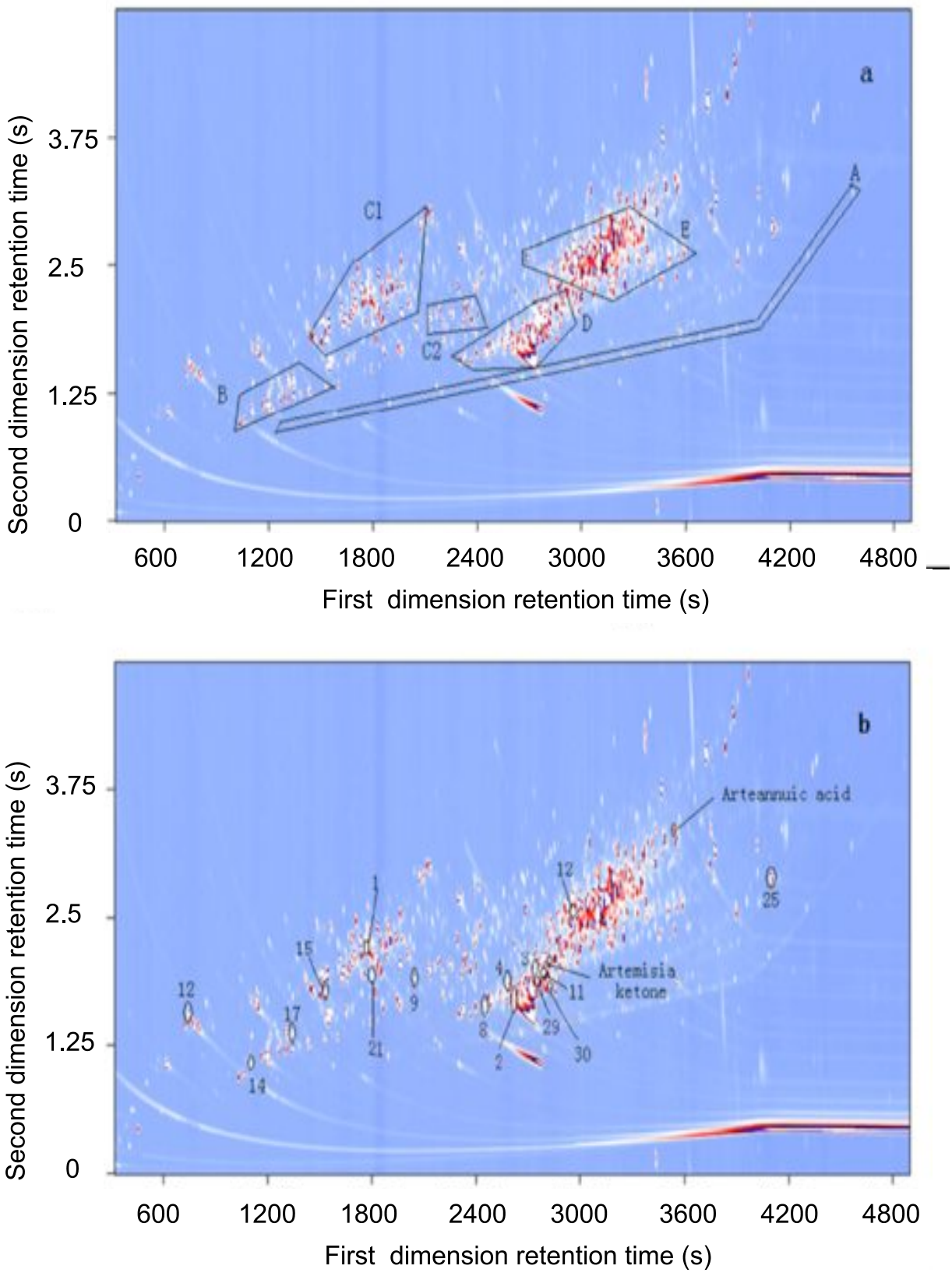


Figure 12.73. Colour plot of the analysis of sweet sagewort. For identification of the numbering, see referenced paper.

Gemander (Teucrium chamaedrys)

(M.Z. Özel, F. Göğüş¸ A.C. Lewis, *Determination of Teucrium chamaedrys volatiles by using direct thermal desorption–comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry*, J. Chromatogr. A, 1114 (2006) 164–169

Instrumental conditions:

***Columns*:**
First: 30 m × 0.32 mm ID, 0.25 μm DB5
Second: 1.9 m × 0.10 mm ID, 0.10 μm DB17
Modulation capillary:

Carrier gas: helium 370 kPa

Temperatures
Main oven: 60 °C (0.5 min), 5°C/min → 280°C (2min)
Second oven: 75 °C (0.5 min), 5°C/min → 300°C (2min)

Injector: direct thermal desorption from injector liner at low temperature,
Temperature: after closure and 2 min purge (N_2), heated 40°C/min → 150°C
Injection volume:

Modulator: quadjet cryogenic

Modulation time: 4 s

Detector: ToF-MS
Temperature:
Make up gas flow:

Data acquisition: 45 spectra/s 45-350 amu

Sample description and separation:
The direct qualification and quantification of the volatile components of *Teucrium chamaedrys* was studied using a direct thermal desorption and GC×GC–TOF/MS
The TOF/MS enabled high probability identifications for 68 compounds. The quantitative results were determined through the use of internal standards and the desorption of differing amounts of raw material in the injector liner. The highest yield of volatile compounds (0.39%) was obtained at 150 °C thermal desorption temperature using 1.0 mg of dried sample when studied over the range 1.0–7.0 mg. Lowest yield of 0.33% was found for the largest sample size of 7.0 mg. Relative standard deviation (RSD) for 10 replicates at each size sample
were in the range 3.9–21.6%.

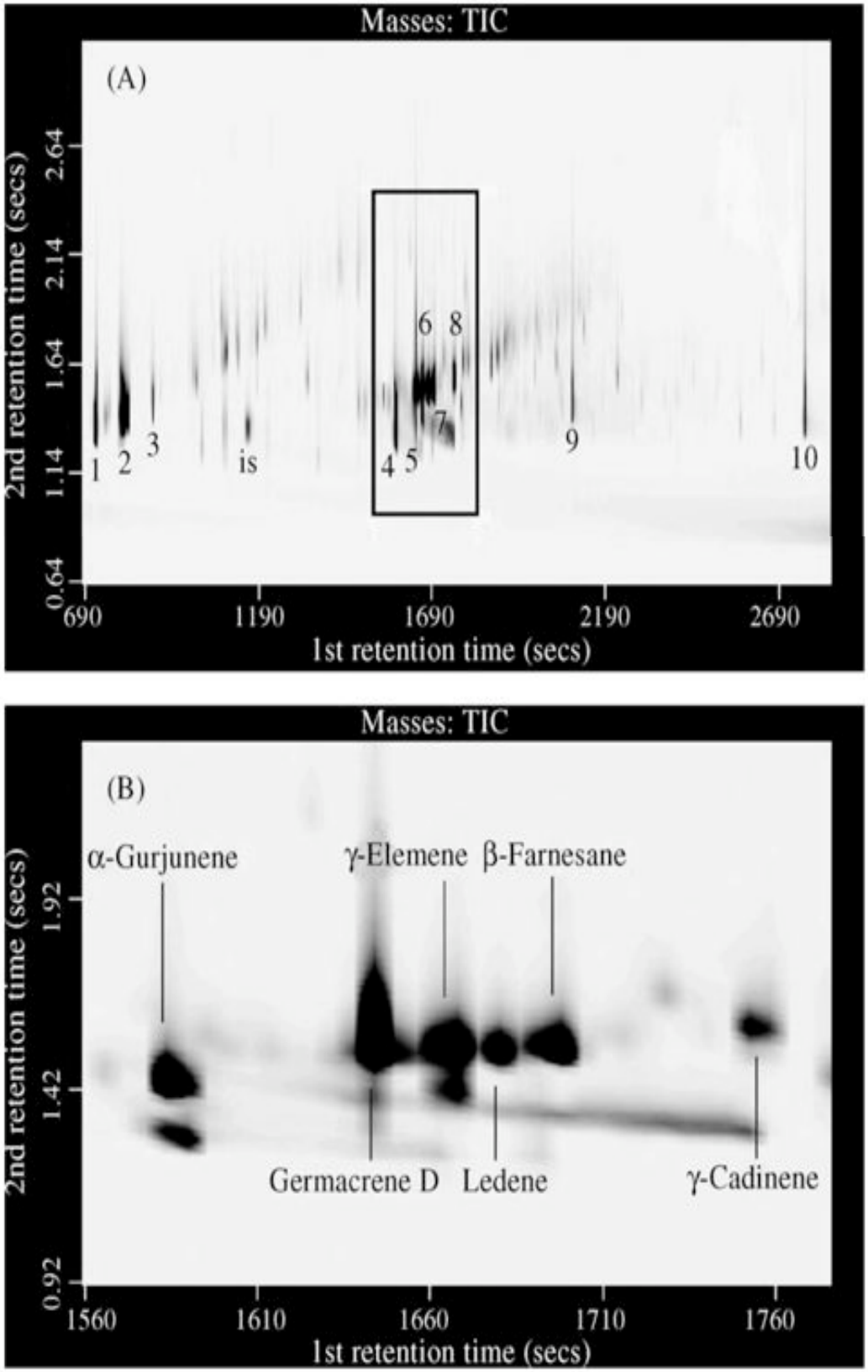


Figure 12.74. Top: GC×GC–TOF/MS total ion current (TIC) plot of T. chamaedrys volatiles at 150°C. Bottom: Expansion of the selected region from (A). 1: α-pinene; 2: β-pinene; 3: limonene; is: internal standard; 4: α-gurjunene; 5: germacrene D; 6: γ-elemene; 7: α-farnesene; 8: γ-cadinene; 9: hexahydrofarnesyl acetone; 10: heptacosane.

Essential oils in lemon

L. Mondello, A. Casilli, P. Q. Tranchida, P. Dugo, G. Dugo, *Comprehensive two-dimensional GC for the analysis of citrus essential oils*, Flavour Fragr. J. 20 (2005) 136–140

Instrumental conditions:

***Columns*:**
First: 10 m × 0.25 mm ID, 0.25 μm, Rtx5
Second: 1 m × 0.10 mm ID, 0.10 μm, BPX50

Carrier gas: hydrogen @ 128.3 kPa

***Temperatures*:**
Main oven: 50°C, 2.5°C/min → 280°C
Second oven:

Injector: split 1:30
Temperature: 270°C
Injection volume: 1 μL

Modulator: LMCS, CO_2-cylinder pressure modified to exit at 150 atm.

Modulation time: 6 s

Detector: FID
Temperature: 270°C
Make up gas flow:

Data acquisition: 50 Hz

Sample description and separation:

The lemon essential oil volatile fraction is considered one of the most complex amongst all citrus oils. With GC×GC it was possible to separate a large number of compounds. Four groups, *viz.* monoterpene alcohols, aldehydes and esters and sesquiterpene hydrocarbons, could be classified and within these groups a number of individual compounds.

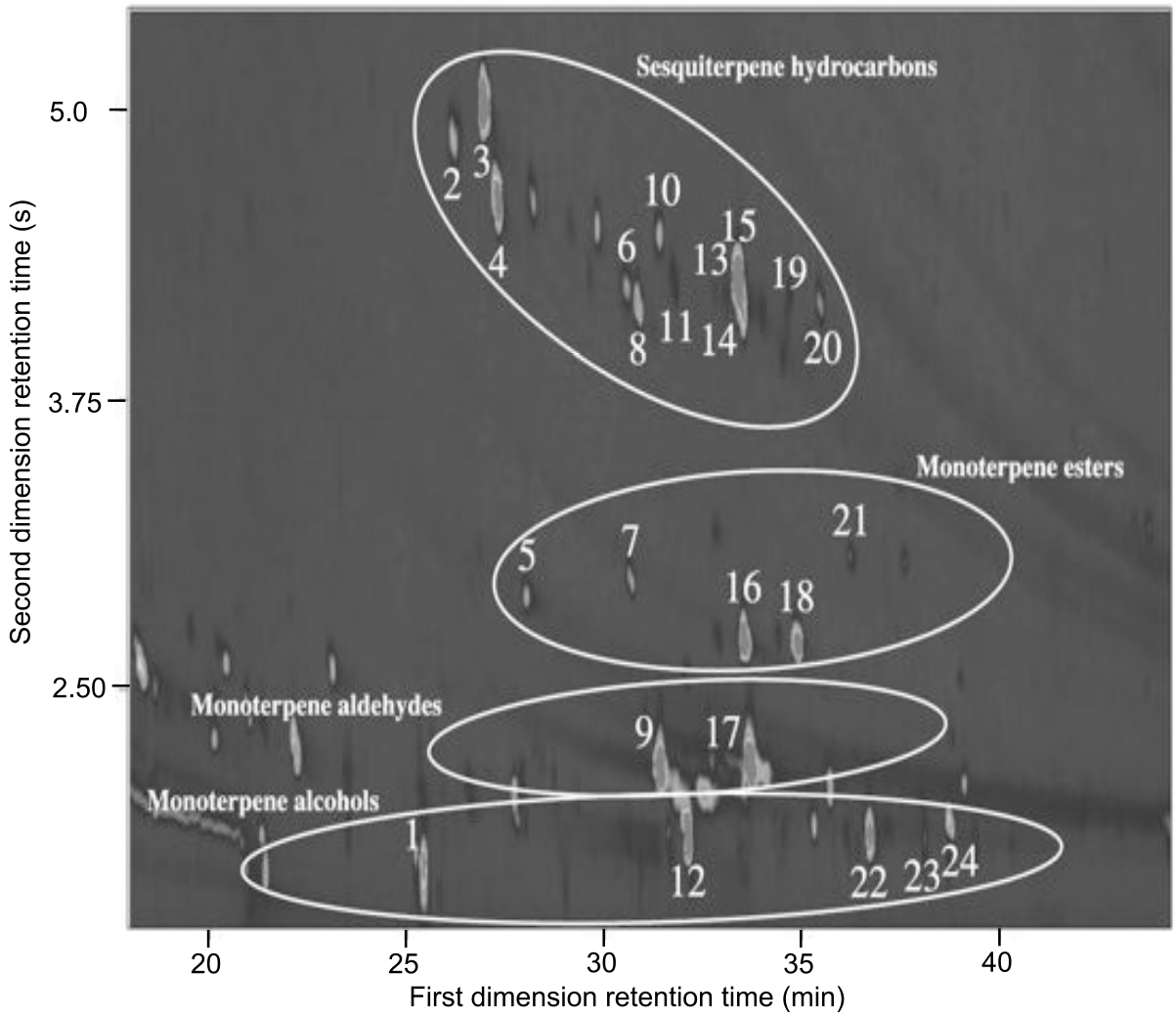


Figure 12.75. Section of the GC×GC separation of lemon.

Roasted coffee beans (*Arabica*)

L. Mondello, A. Casilli, P.Q. Tranchida, P. Dugo, R. Costa, S. Festa, G. Dugo, *Comprehensive multidimensional GC for the characterization of roasted coffee beans*, J. Sep. Sci. 27 (2003) 442-450

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 0.25 µm Supelcowax-10
Second: 1 m × 0.10 mm ID, 0.10 µm SPB-5
Modulation capillary:

Carrier gas: hydrogen, constant linear velocity 70 cm/s @ 131.2 kPa

Temperatures
Main oven: 60°C (5 min), 1.5°C/min → 230°C (2 min)
Second oven:

Injector: splitless (2 min) → split, ratio 1:20
Temperature: 250 °C
Injection volume: SPME fiber

Modulator: LMCS (extended CO_2 pressure for trapping and focusing of low volatiles

Modulation time: 5 s

Detector: FID
Temperature: 280°C
Make up gas flow:

Data acquisition: 50 Hz

Sample description and separation:

Roasted Arabica (Coffea arabica) coffee beans.
Two g coffee beans in 10 mL vial. SPME triple phase 50/30 µm fiber (divinylbenzene/Carboxen/polydimethylsiloxane). After preliminary equilibrium, the SPME fiber was exposed to the head space of the coffee beans for 40 min. Thermally desorption in the GC injection port for 0.1 min at 250°C.
The identification was done by comparison with 1D-GC–MS.

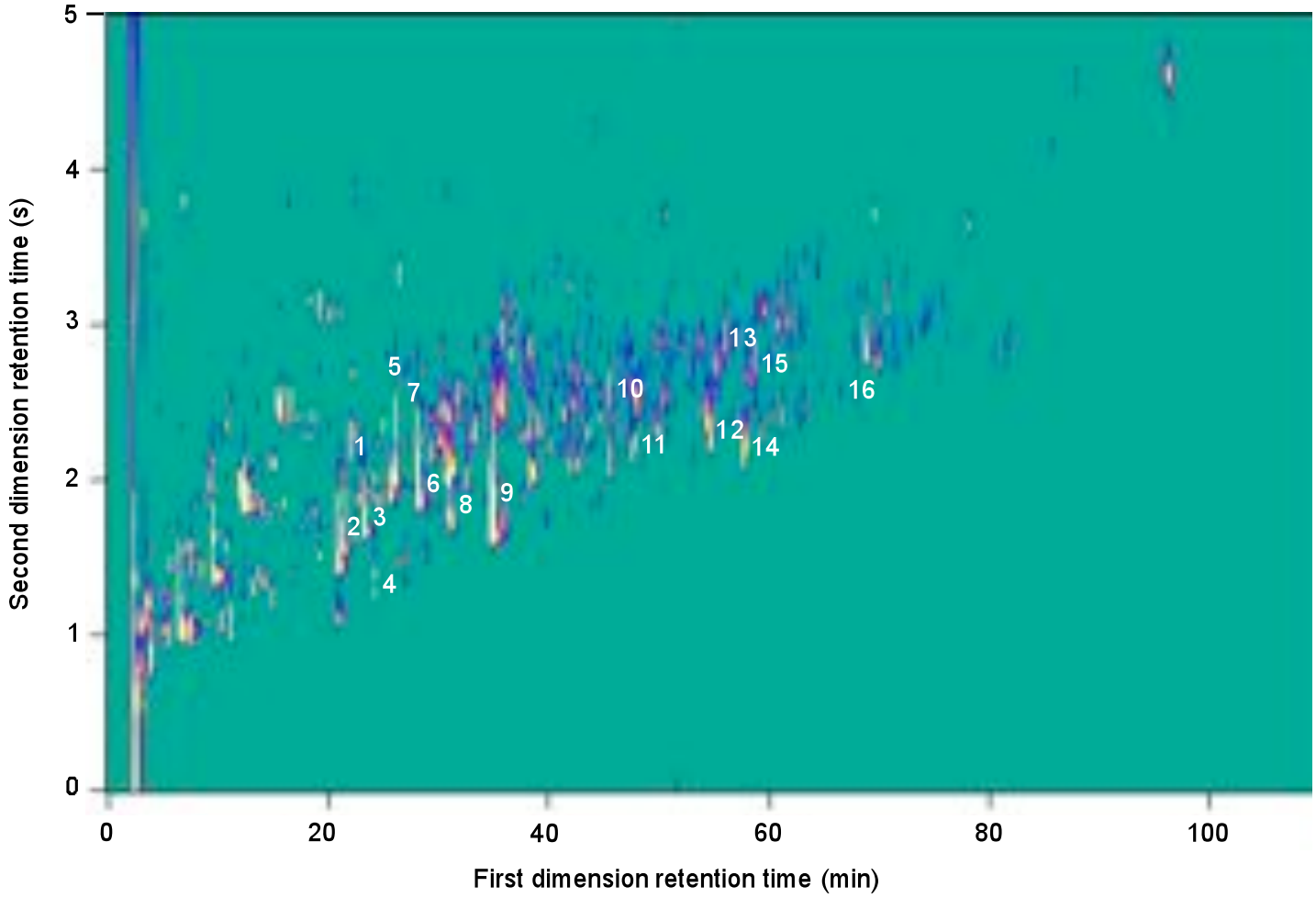


Figure 12.76. GC×GC chromatogram of roasted Arabica (Brasile) coffee beans.

1. furfural. 2. furfuryl formate, 3. 2-acetylfuran, 4. 2-ethylpyrrole, 5. furanmethanol acetate, 6. 5-methylfurfural, 7. 2-acetyl-5-methylfuran,

8. γ -butyrolactone, 9. furanmethanol, 10. 1-(2-furanylmethyl)pyrrole. 11. 2-methoxyphenol, 12. maltol, 13. 1-(1H-pyrrole-2-yl) ethanone,

14. 4-ethyl-2 methoxyphenol, 15. 2-methylbenzofuran, 16. 3,5-dimethyl benzoic acid.

Roasted coffee beans (*Robusta*)

L. Mondello, A. Casilli, P.Q. Tranchida, P. Dugo, R. Costa, S. Festa, G. Dugo, *Comprehensive multidimensional GC for the characterization of roasted coffee beans*, J. Sep. Sci. 27 (2003)

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 0.25 µm Supelcowax-10
Second: 1 m × 0.10 mm ID, 0.10 µm SPB-5
Modulation capillary:

Carrier gas: hydrogen, constant linear velocity 70 cm/s @ 131.2 kPa

Temperatures
Main oven: 60°C (5 min), 1.5°C/min → 230°C (2 min)
Second oven:

Injector: splitless (2 min) → split, ratio 1:20
Temperature: 250 °C
Injection volume: SPME fiber

Modulator: LMCS (extended CO_2 pressure for trapping and focusing of low volatiles

Modulation time: 5 s

Detector: FID
Temperature: 280°C
Make up gas flow:

Data acquisition: 50 Hz

Sample description and separation:

Roasted Robusta (Coffea canephora ex Froehner) coffee beans.

Two g coffee beans in 10 ml vial. SPME triple phase 50/30 µm fiber (divinylbenzene/Carboxen/polydimethylsiloxane). After preliminary equilibrium, the SPME fiber was exposed to the headspace of the coffee beans for 40 min. Thermally desorption in the GC injection port for 0.1 min at 250°C.

The identification was done by comparison with 1D-GC–MS.

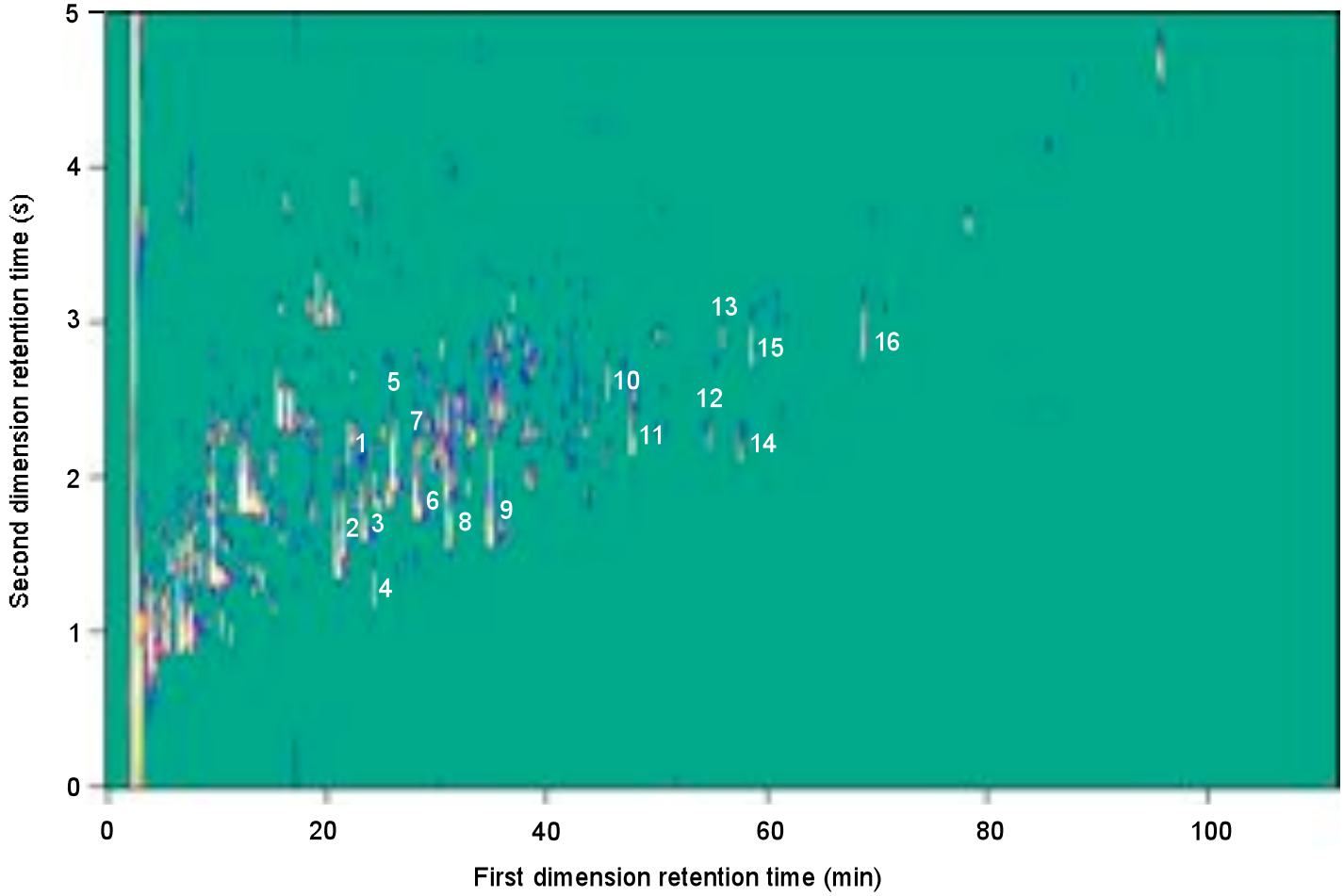


Figure 12.77. GC×GC chromatogram of roasted Robusta (Coffea canephora ex Froehner) coffee beans. 1. furfural. 2. furfuryl formate, 3. 2-acetylfuran, 4. 2-ethylpyrrole, 5. furanmethanol acetate, 6. 5-methylfurfural, 7. 2-acetyl-5-methylfuran,

8. γ -butyrolactone, 9. furanmethanol, 10. 1-(2-furanylmethyl)pyrrole. 11. 2-methoxyphenol, 12. maltol, 13. 1-(1H-pyrrole-2-yl) ethanone,

14. 4-ethyl-2 methoxyphenol, 15. 2-methylbenzofuran, 16. 3,5-dimethyl benzoic acid.

FAMEs in menhaden oil

L. Mondello, A. Casilli, P.Q. Tranchida, P. Dugo, G. Dugo, *Detailed analysis and group-type separation of natural fats and oils using comprehensive two-dimensional gas chromatography*, J. Chromatogr. A 1019 (2003), 187-196

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 0.25 μm, BPX5
Second: 1 m × 0.10 mm ID, 0.10 μm SBP wax

Carrier gas: hydrogen, constant pressure @ 200 kPa

***Temperatures*:**
Main oven: 200°C, 2°C/min → 250°C
Second oven:

Injector: split, ratio 1:100
Temperature: 260°C
Injection volume: 1 μL

Modulator: LMCS

Modulation time: 4 s

Detector: FID
Temperature: 280°C
Make up gas flow:

Data acquisition: 100 Hz

Sample description and separation:

Identification has been performed by comparison with 1D-GC–MS analyses and using linear retention indices maps.

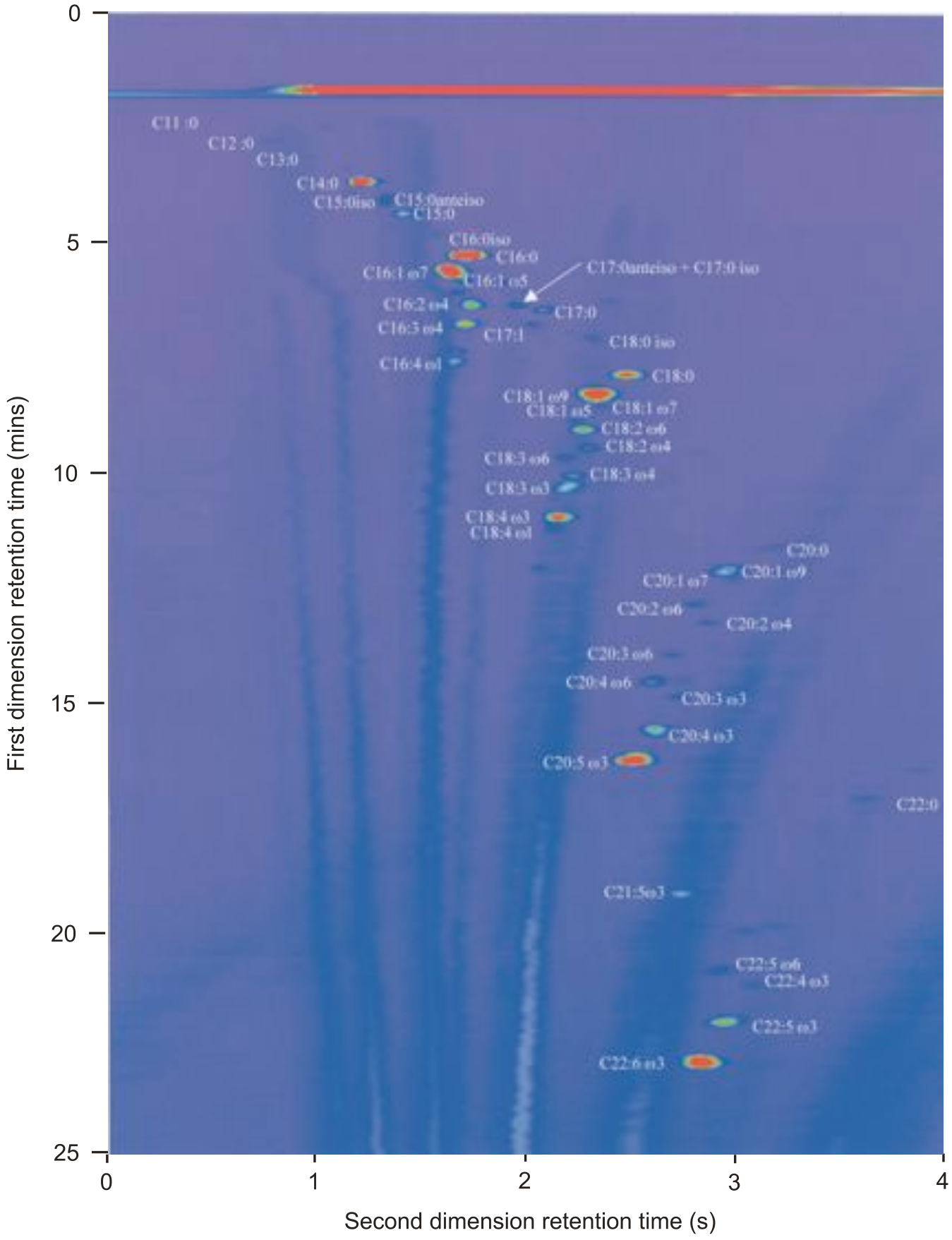


Figure 12.78. GC×GC separation of FAMEs in menhaden oil.

Triacylglycerides in edible oils

S. de Koning, H-G. Janssen, U.A.Th. Brinkman, *Characterization of triacylglycerides from edible using single and multidimensional techniques*, LC-GC Europe, November 2006

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 0.25 μm, CP-WAX
Second: 3 m × 0.1 mm ID, 0.1 μm VF-23

Carrier gas: helium, constant pressure @ 350 kPa

***Temperatures*:**
Main oven: 160°C (0.2 min), 30°C/min → 165°C (120 min)
Second oven: 165°C isothermal

Injector: split/splitless injector (split ratio 1:100)
Temperature:
Injection volume:

Modulator: quad-jet cryogenic modulator

Modulation time: 6 s

Detector: FID
Temperature:
Make up gas flow:

Data acquisition: 100 Hz

Sample description and separation:

It was shown, somewhat unexpected, that an isothermal analysis at 165 °C without any second-dimension oven offset offered a close to baseline separation of the *cis/trans* isomers. To bring this separation to perfection, the column length was increased from 2 to 3 m. The optimum column inlet pressure was calculated using the programme developed by Beens *et al*. [92]. In order to achieve the best possible separation in the second dimension this resulted, for the present column set, in a column head pressure of 350 kPa. The next figure shows the contour plot of the GCFAME×GCFAME analysis of a test sample. It is evident that chain length, number and position of the double bond and *cis/trans* orientation can be clearly distinguished. This will greatly improve the reliability of the quantification of the *trans* isomers.

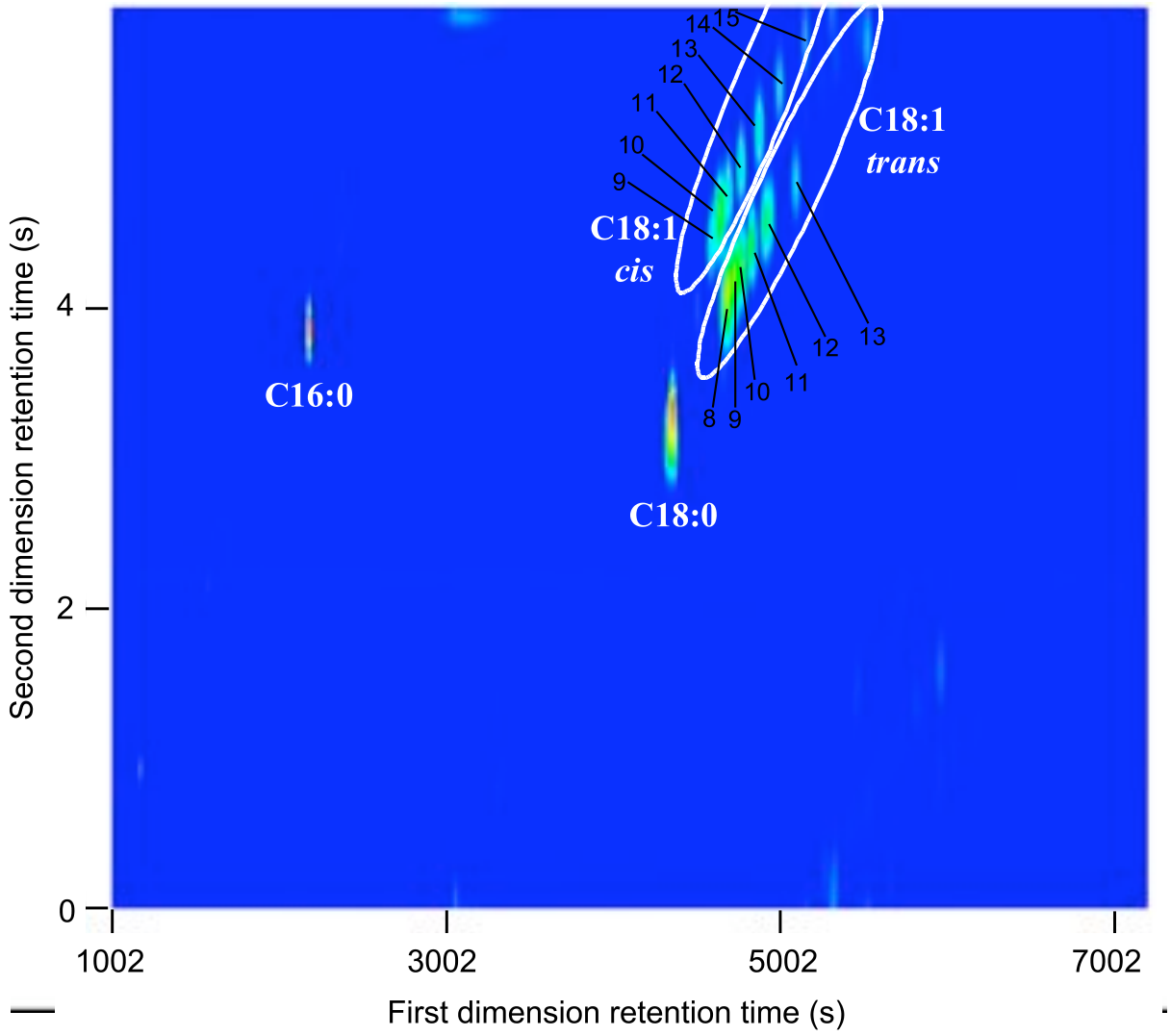


Figure 12.79. $GC_{FAME} \times GC_{FAME}$–FID analysis of a test sample. The numbers added to the cis C18:1 and trans C18:1 spots indicate the position of the double bond.

Lipids in lanolin

E. Jover, M. Adahchour, J.M. Bayona, R.J.J. Vreuls, U.A.Th. Brinkman, *Characterization of lipids in complex samples using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry*, J. Chromatogr. A 1086 (2005) 2-11

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 0.25 μm, Supelcowax-10
Second: 1 m × 0.10 mm ID, 0.10 μm, SBP-5

Carrier gas: hydrogen @ 128.3 kPa

***Temperatures*:**
Main oven: 50°C, 2.5°C/min → 280°C
Second oven:

Injector: split 1:30
Temperature: 270°C
Injection volume: 1 μL

Modulator: LMCS

Modulation time: 6 s

Detector: FID
Temperature: 280°
Make up gas flow: N_2 @ 50 mL/min

Data acquisition: 50 Hz

Sample description and separation:

The lanolin sample was methylated by the so-called methylated-plus-silylation method. To find the different groups the ions m/z 74, 103 and 147 have been selected. They are depicted in separate inserts.

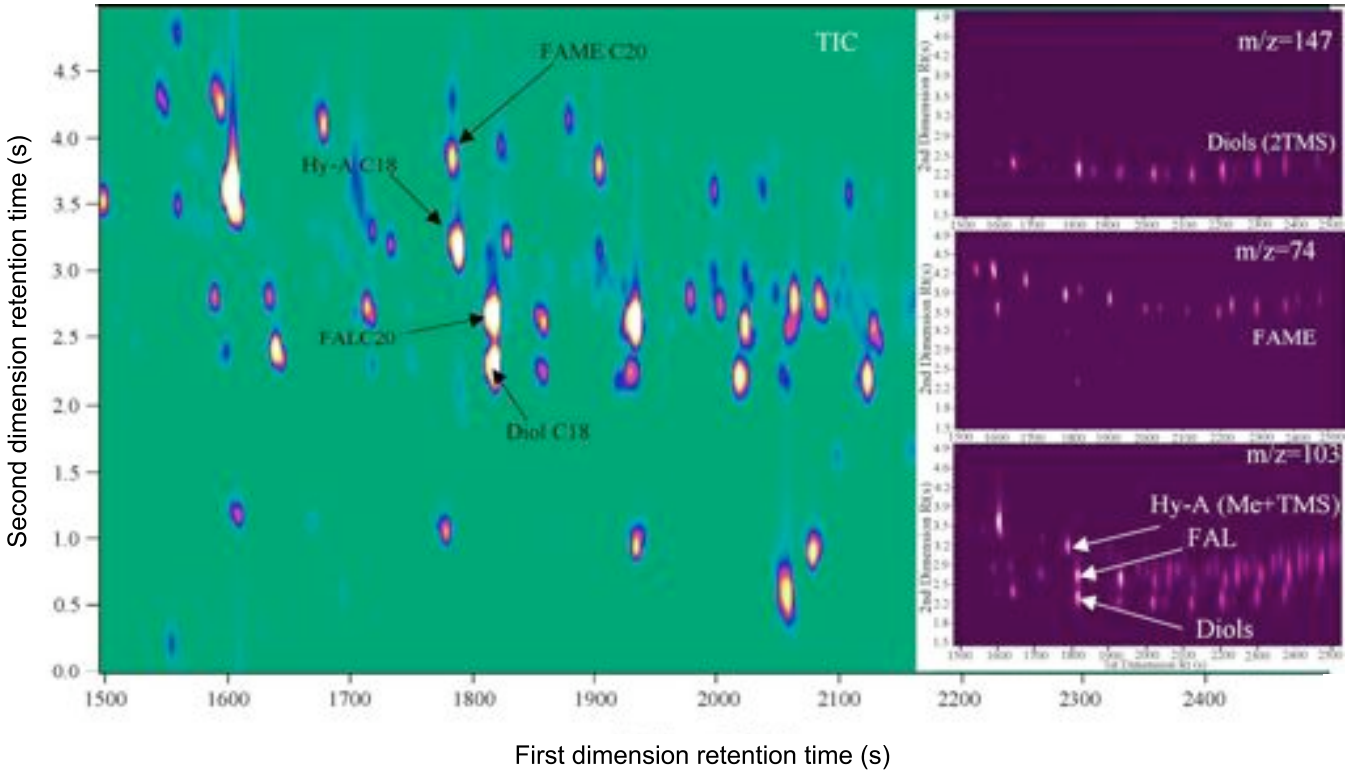


Figure 12.80. TIC contour plot obtained by GC×GC–ToF-MS of methylated-plus-silylated lanolin. Inserts show fragmentograms m/z 74, 103 and 147

Chinese liquor Moutai

S. Zhu, X. Lu, K. Ji, K. Guo, Y. Li, C. Wu, G. Xu, *Characterization of flavor compounds in Chinese liquor Moutai by comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry,* Analytica Chimica Acta 597 (2007) 340–348

Instrumental conditions:

***Columns*:**
First: 60 m × 0.25 mm ID, 0.25 μm HP-Innowax
Second: 1.2 m × 0.1 mm ID, 0.4 μm DB1701
Modulation capillary:

Carrier gas: helium @ 600 kPa

***Temperatures*:**
Main oven: 50°C, 2°C/min → 230°C (10 min)
Second oven:

Injector: split 1:30
Temperature: 250°C
Injection volume: 0.5 μL

Modulator: quad-jet cryogenic

Modulation time: 4 s

Detector: ToF MS
Temperature: ion source 220°C
Make up gas flow:

Data acquisition: 50 spectra/s

Sample description and separation:

The liquor was extracted after adding NaCl, with diethyl ether/pentane 2:1.
According to the automated data processing by ToF MS software, combined with the ordered chromatogram and the retention index database developed by our group, a total of 528 components are identified in a Moutai liquor sample, including organic acids, alcohols, esters, ketones, aldehydes, acetals, lactones, nitrogen-containing and sulfur-containing compounds. In addition, the contribution of some important aroma compounds to the flavour of Moutai liquor has also been studied.

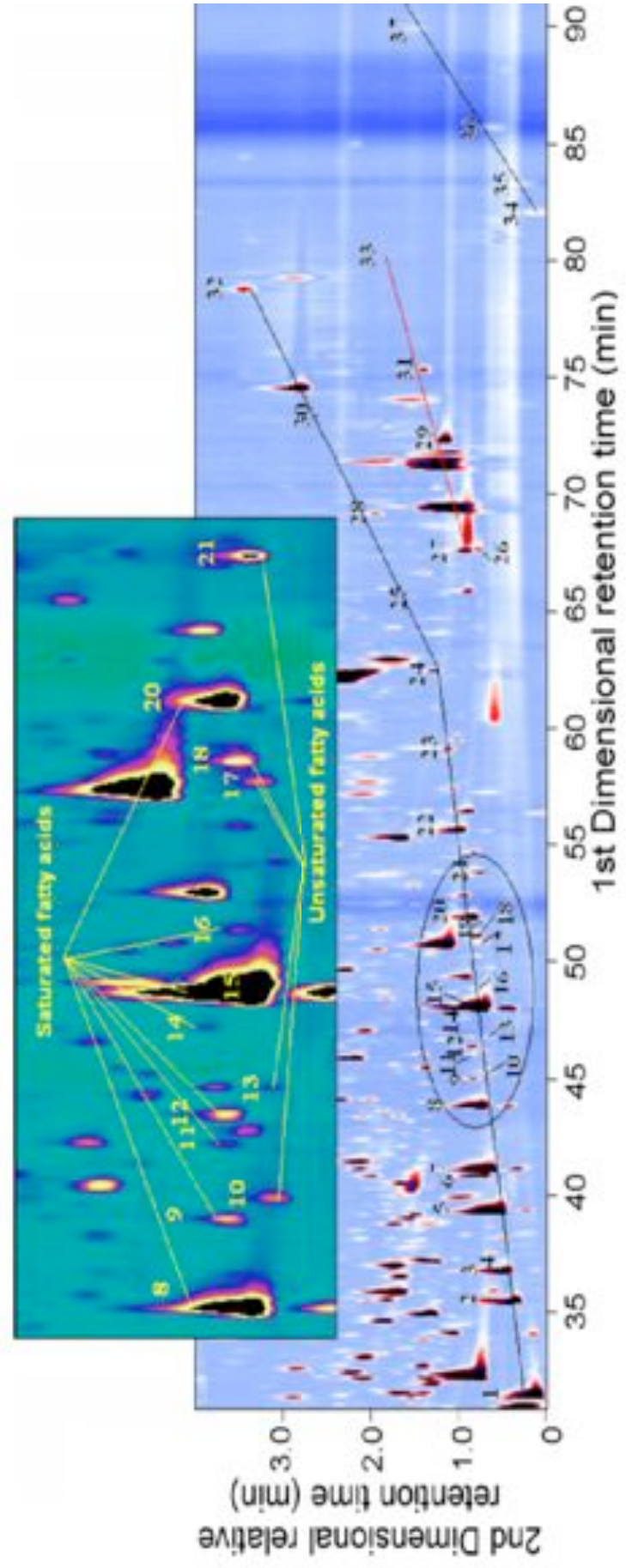


Figure 12.81. GC×GC–ToF MS contour plot of organic acids in Moutai liquor.

Enantiomeric amino acids in beer

M. Junge, H. Hügel, P.J. Marriott, *Enantiomeric analysis of amino acids by using comprehensive two-dimensional gas chromatography,* Cirality 19 (2007) 1-7

Instrumental conditions:

***Columns*:**
First: 25 m × 0.25 mm ID, 0.16 µm Chirasil-L-Val
Second: 3 m × 0.1 mm ID, 0.1 µm BPX50
Modulation capillary:

Carrier gas: hydrogen @ 26.36 psi

***Temperatures*:**
Main oven: 80°C (3 min), 20°C/min → 180°C (5 min)
Second oven:

Injector: split/splitess
Temperature: 200°C
Injection volume:

Modulator: LMCS

Modulation time: 5 s

Detector: FID
Temperature: 250°C
Make up gas flow:

Data acquisition: 100 Hz

Sample description and separation:

A chiral separation of amino acids (AA) derivatised with ethyl chloroformate was achieved. A commercially available enantioselective capillary column (Chirasil-L-Val) has been tested as first-dimension column. The method was demonstrated for chiral analysis of AAs in different beer samples. The major AA in the beer samples was proline with amounts ranging from around 65–95% with minor contents of glycine and the L-enantiomers of alanine, valine, leucine, and isoleucine.

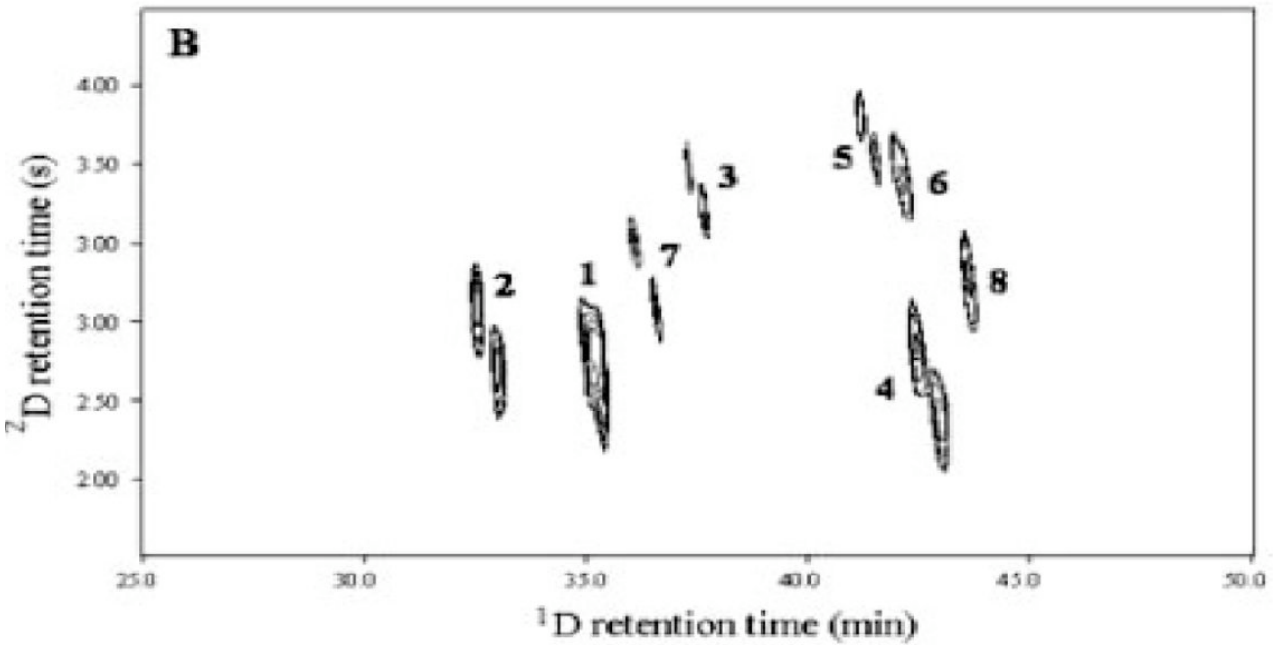


Figure 12.82. Expanded GC×GC chromatogram of alanine, glycine, valine, aaminobutyric acid, isoleucine, allo-isoleucine, leucine, and proline derivatised with ethyl chloroformate on a chiral/polar column set.

Cahaça

Z.L. Cardeal, P.P. de Souza, M.D.R. Gomes da Silva, P.J. Marriott, *Comprehensive two-dimensional gas chromatography for fingerprint pattern recognition in cachaça production,* Talanta 74 (2008) 793–799

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 0.25 μm BPX-5
Second: 1.5 m × 0.1 mm ID, 0.1 μm BPX-20
Modulation capillary:

Carrier gas: not specified

***Temperatures*:**
Main oven: 35°C (5 min), 3 °C/min → 210°C, 40 °C/min → 240°C (10 min)
Second oven: 75°C, 5 °C/min → 325°C (5 min)

Injector: split/splitless
Temperature:
Injection volume:

Modulator: LMCS

Modulation time: 6 s

Detector: ToF-MS
Temperature: 200°C ion source
Make up gas flow:

Data acquisition: 100 spectra/s 45-415 *m/z*

Sample description and separation:

Cachaça samples were studied during the fermentation process and after ageing in different wood materials. The analyses of the aroma compounds were performed after HS-SPME using an 85 μm polyacrylate fibre. Fingerprint monitoring of the distillation process allowed the easy determination of the turning points of the process and comparison of *cabeça* (head), *coração* (core) and *cauda* (tail) fractions. The ageing process in different wood materials was well characterised through fingerprint similarity observations; in the absence of a suitable metric for expressing the overall similarity, here we use a visual and retention time comparison to identify co-incident peaks and those that differ between samples.

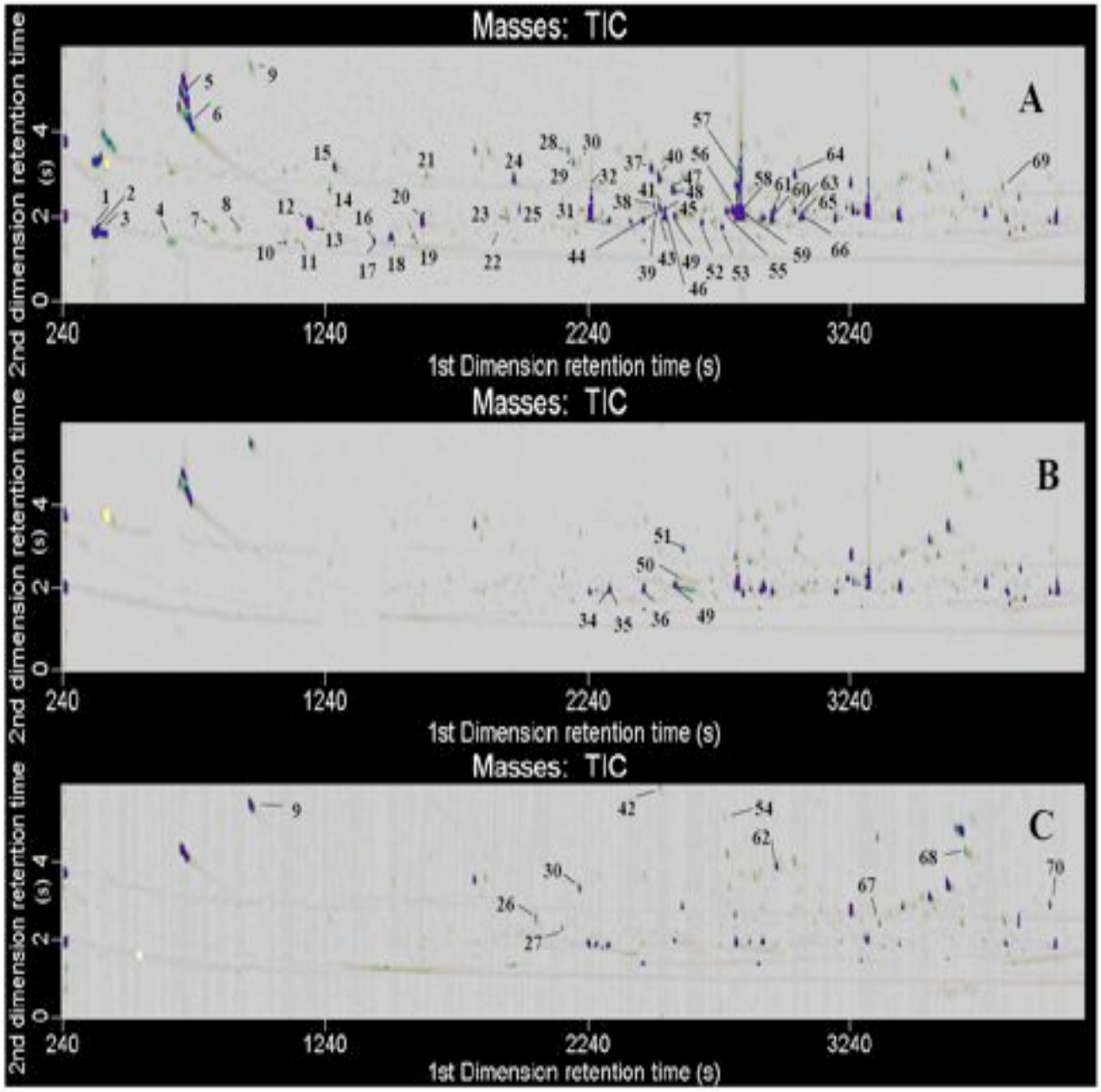


Figure 12.82 .GC×GC–ToF MS contour plots of SPME headspace extracts of the distillation process during cachaça distillation.

Top: Contour plot obtained for the first fraction (cabeça) with high content of ethanol (not shown; mass scanning starts after elution of ethanol) and the more volatile and semi-volatile compounds.

Middle: coração fraction depleted of more volatile compounds and richer in the semi-volatile compounds.

Bottom: cauda fraction corresponding to the end of the distillation with enhanced water content and less volatile compounds. Peak numbering and assignment see referenced paper.

Drugs in racing animals

A.J. Kueh, P.J. Marriott, P.M. Wynne, J.H. Vine, *Application of comprehensive two-dimensional gas chromatography to drugs analysis in doping control*, J. Chromatogr. A, 1000 (2003) 109-124

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 0.25 µm BPX5
Second: 0.4 m × 0.1 mm ID, 0.2 µm BPX50

Carrier gas: not specified

***Temperatures*:**
Main oven: 55°C, 10°C/min → 180°C, 3°C/min → 280°C (10 min)

Injector: splitless
Temperature:
Injection volume:

Modulator: LMCS

Modulation time: 3 s

Detector: FID

Temperature:
Make up gas flow:

Data acquisition: 50 Hz

Sample description and separation:

The analysis was us for the screening of drugs and their metabolites in biological fluids and is described using prolintane metabolites in canine urine as an example, with samples taken at four time intervals after administration. Most drug compounds were found to be retained on the 0.8-m second column for a greater time than the modulation period (3 s) used for initial analysis, under the conditions described. Hence a 0.4-m D2 BPX50 (50% phenyl methyl polysilphenylene) column was then used throughout, with most compounds retained less than 4 s. For the standard drug mixture, three overlapping drugs on the first dimension column (BPX5) were subsequently baseline resolved on the BPX50 column. For prolintane administration samples, the parent drug and metabolites could be effectively resolved from background matrix peaks. Likewise a 23-drug spike standard in horse urine blank gave acceptable resolution of the drugs from matrix peaks.

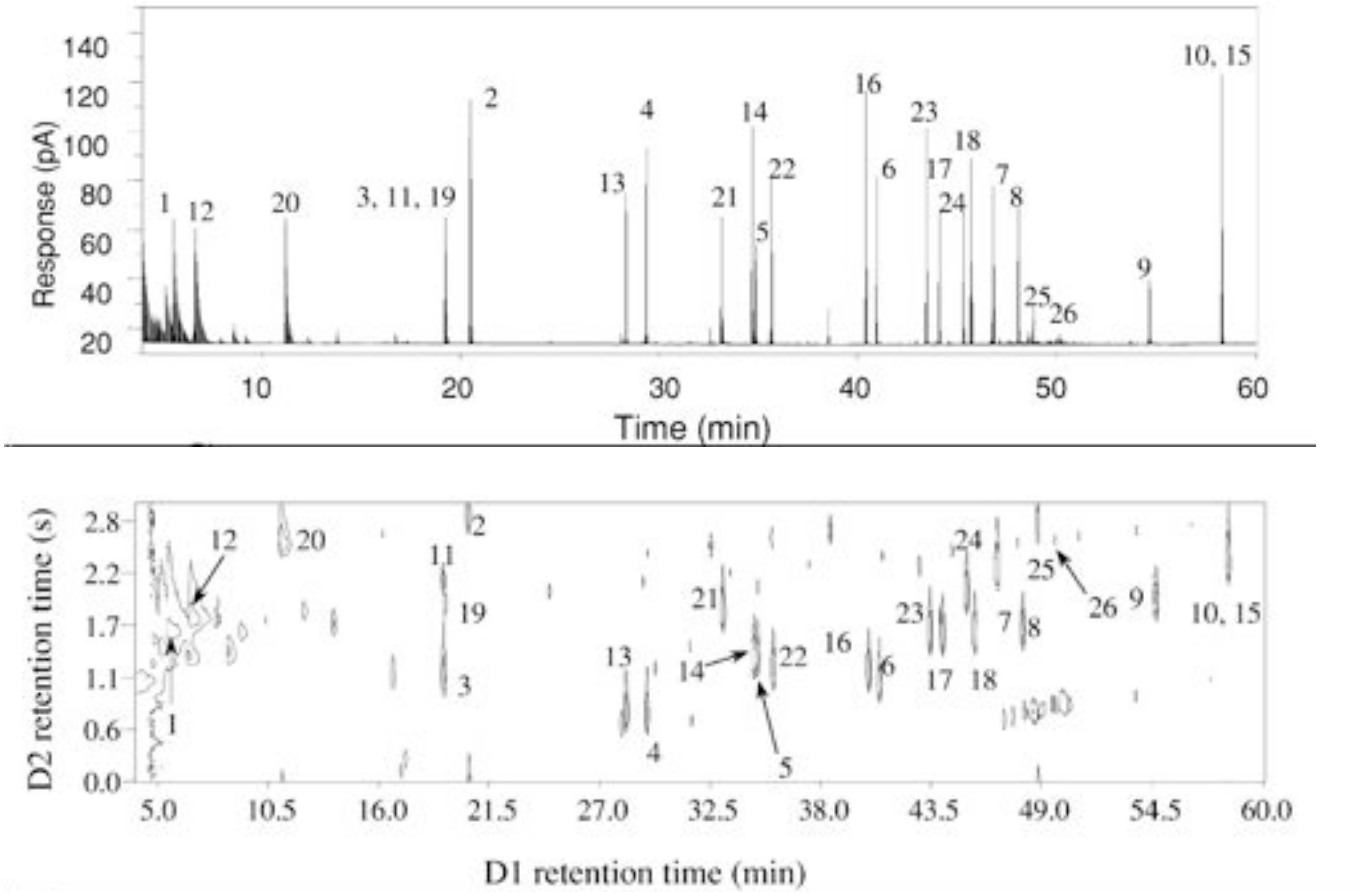


Figure 12.83. Analysis of composite standard MSB 11213. Top: pulsed GC×GC analysis; Bottom: GC×GC contour plot. For identification, see referenced paper.

Sterols in faecal material

T.T. Truong, P.J. Marriott, N.A. Porter, *Analytical study of comprehensive and targeted multidimensional gas chromatography incorporating modulated cryogenic trapping*, J. AOAC Int., 84 (2001) 323-335

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 0.25 µm BPX5
Second: 2 m × 0.1 mm ID, 0.2 µm BPX50

Carrier gas: hydrogen @ 1.2 mL/min, 7.34 psi

***Temperatures*:**
Main oven: 50°C (1 min), 30°C/min → 280°C (50 min)

Injector: pulsed splitless, split vent 50 mL/min @ 60 psi
Temperature: 280ºC
Injection volume:

Modulator: LMCS

Modulation time: 4 s

Detector: FID
Temperature:
Make up gas flow:

Data acquisition: 100 Hz

Sample description and separation:

The standards were: 5β-cholestane-3β-ol (coprostanol, COP), 5β-cholestane-3α-ol (epicoprostanol, EPI), 5-cholesten-3β-ol, cholesterol, CHL), 3β-hydroxy-5α-cholestane, (dihydrocholesterol, DHC), 3β-hydroxy-24-ethyl-5,22-cholestadiene, (stigmasterol, SROL), 24β-ethylcholesterol, (β-sitosterol, B-SIT), 24α-ethyl-5α-cholestane-3β-ol (stigmastanol, SNOL), 5-α-cholestane (internal standard, IS),.
They were derivatised before injection with bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS.

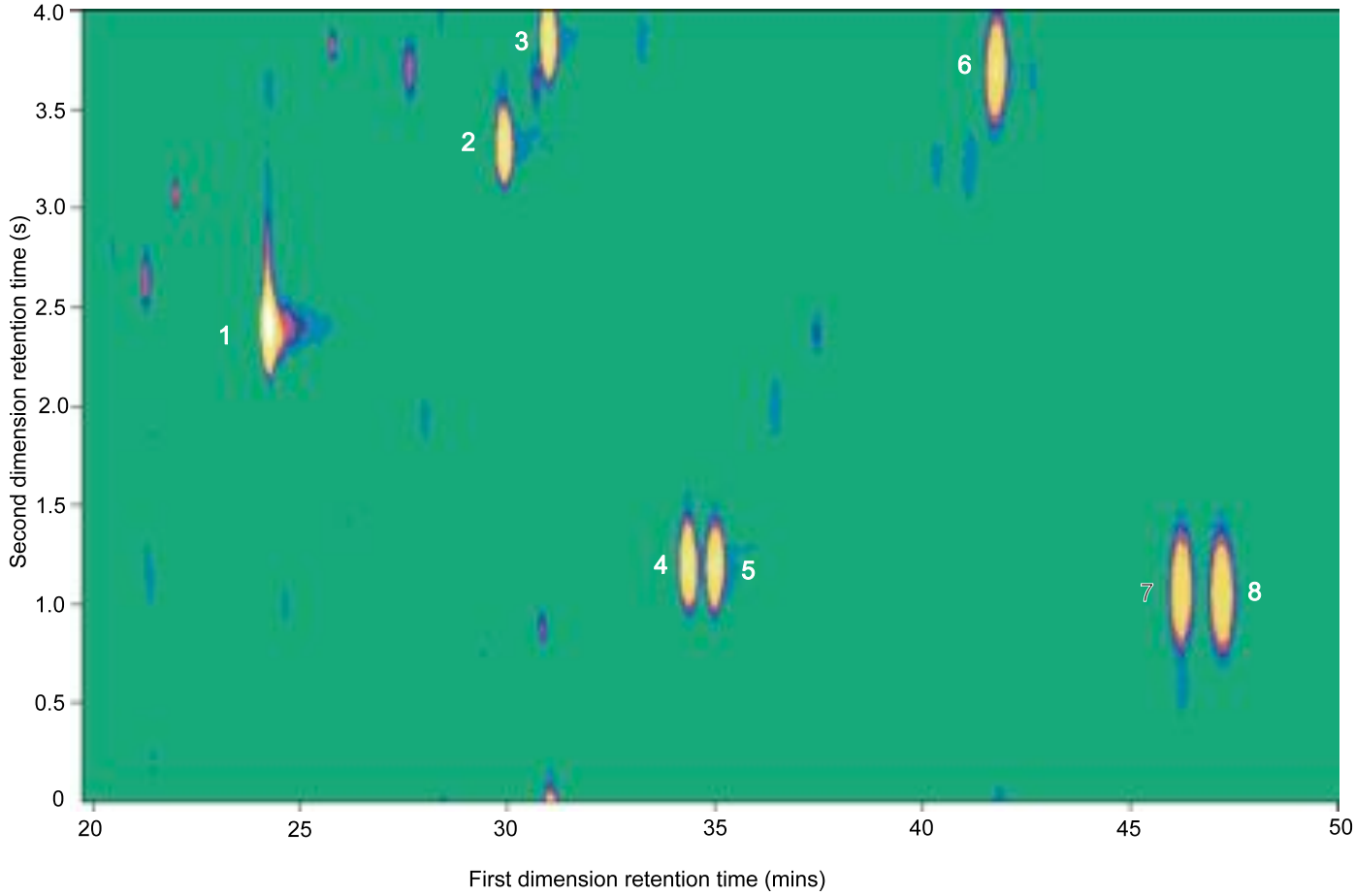


Figure 12.84. Colour plot of a GC×GC separation of faeces. The enantiomer pairs of sterols are clearly separated.

identification: 1. IS, 2. COP, 3. EPI, 4. CHL, 5. DHC, 6. SROL, 7. B-SIT, 8. SNCL.