COMPREHENSIVE TWO-DIMENSIONAL GASCHROMATOGRAPHY

The state-of-separation-arts

Part II: Applications
Foods, Fragrances 2
and various other compounds

JAN BEENS

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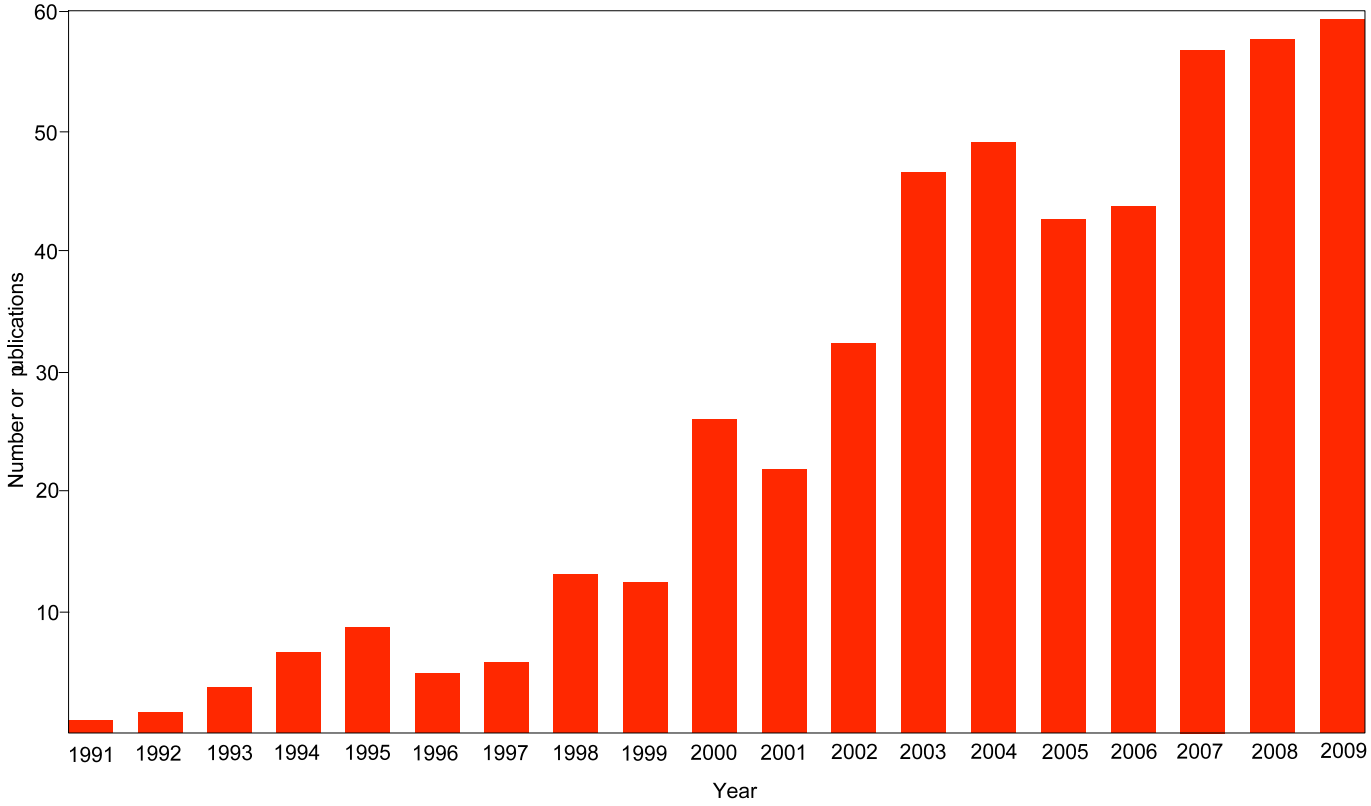
PART II

—APPLICATIONS—

Foods, Fragrances 2

And other compounds



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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Wound induced plant volatiles

R.M.M. Perera, P.J. Marriott, I.E. Galbally, *Headspace solid-phase microextraction–comprehensive two-dimensional gas chromatography of wound induced plant volatile organic compound emissions*, Analyst 127 (2002) 1601-1607

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 0.25 μm BPX5
Second: 2 m × 0.1 mm ID, 0.2 μm BPX20

Carrier gas: hydrogen @1.5 mL/min

***Temperatures*:**
Main oven: 30°C, 5°C/min → 250°C (50 min)

Injector: splitless
Temperature:
Injection volume:

Modulator: LMCS

Modulation time: 4 s

Detector: FID
Temperature:
Make up gas flow:

Data acquisition: 100 Hz

Plant emissions of volatile organic compounds from mechanically wounded *Agrostis stolonifera, Pennisetum clandestinum, Eucalyptus leucoxylon* and *Trifolium repens* have been sampled by headspace-solid phase microextraction and analysed. The produced fingerprint of the volatile organic compounds in a 2D separation space that may be approximately interpreted as a boiling point–polarity space, and may then be presented as a two-dimensional contour plot. This allows identification of sample-dependent variations in component distributions in the 2D plot, which will contain information about plant differences and could therefore facilitate recognition of different plant materials and displays the gross differences in volatiles between each plant species.

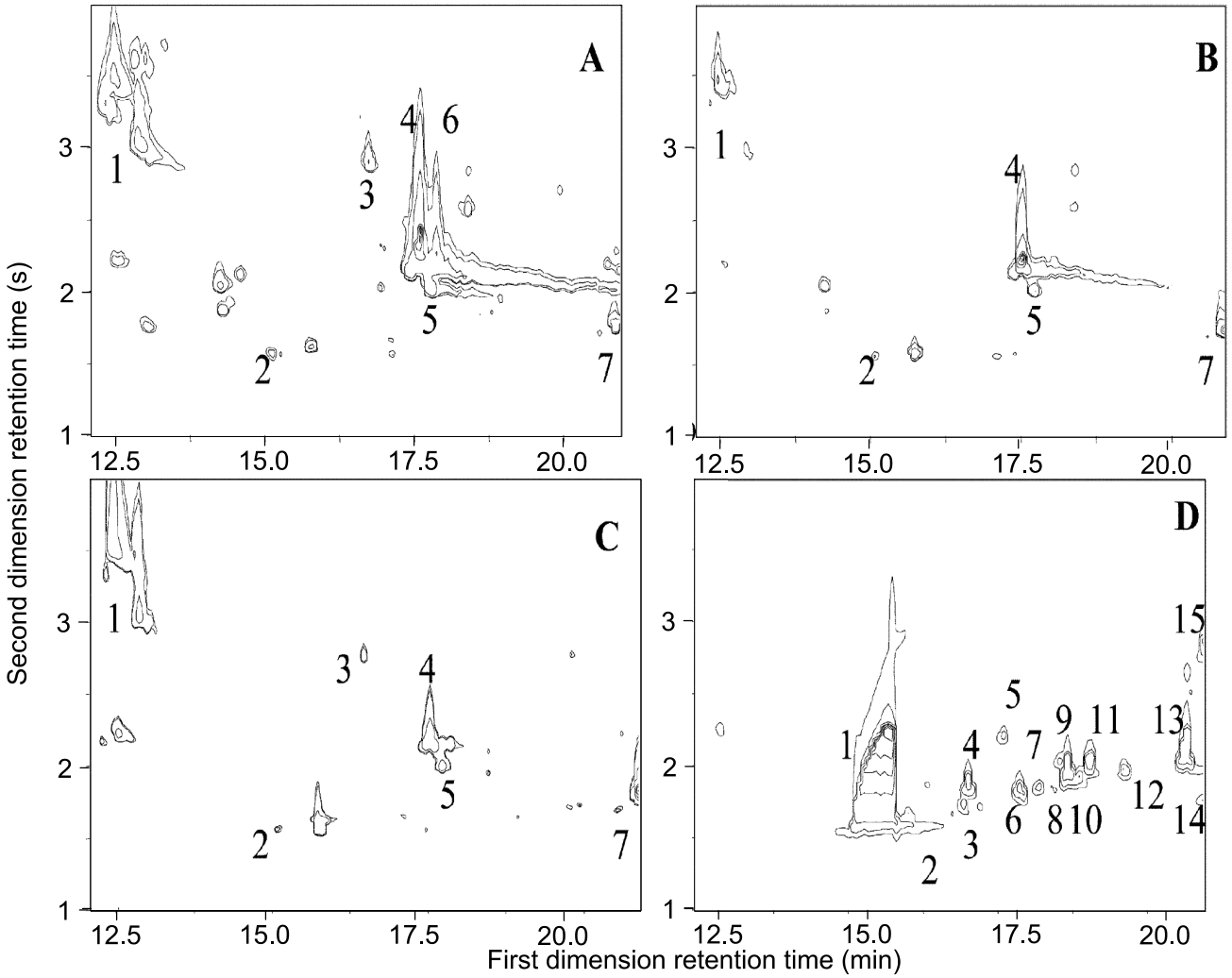

Figure 12.85. Expanded windows showing a possible fingerprint region for GC×GC analysis of the headspace volatiles from

(A) Agrostis stolonifera; (B)Pennisetum clandestinum; (C) Trifolium repens and (D) Eucalyptus leucoxylon.

For identification: see referenced paper.

Traditional Chinese medicine (*Cablin Patchouli*)

C. Ruan, G. Xu, X. Lu, R. Hua, H. Kong, K Xiao, Q. Yang, *Quality evaluation of volatile oils of traditional Chinese medicines by using comprehensive two-dimensional gas chromatography (GC×GC),* Chromatographia Suppl. 57 (2003) 265-270

Instrumental conditions:

***Columns*:**
First: 2 m × 0.1 mm ID, 3.5 μm DB1
Second: 0.4 m × 0.1 mm ID, 0.1 μm OV-17
Modulation cap.:

Carrier gas: helium

***Temperatures*:**
Main oven: 45°C, 3.5°C/min → 220°C (4 min)
Second oven:

Injector:
Temperature: 250°C
Injection volume:

Modulator: quad-jet cryomodulator

Modulation time: 4 s

Detector: FID
Temperature:
Make up gas flow:

Data acquisition: 100 Hz

Sample description and separation:

Sample: Patchouli 2. (Patchouli is a commonly used TCM with a selective prohibition effect on dermatophytes.)

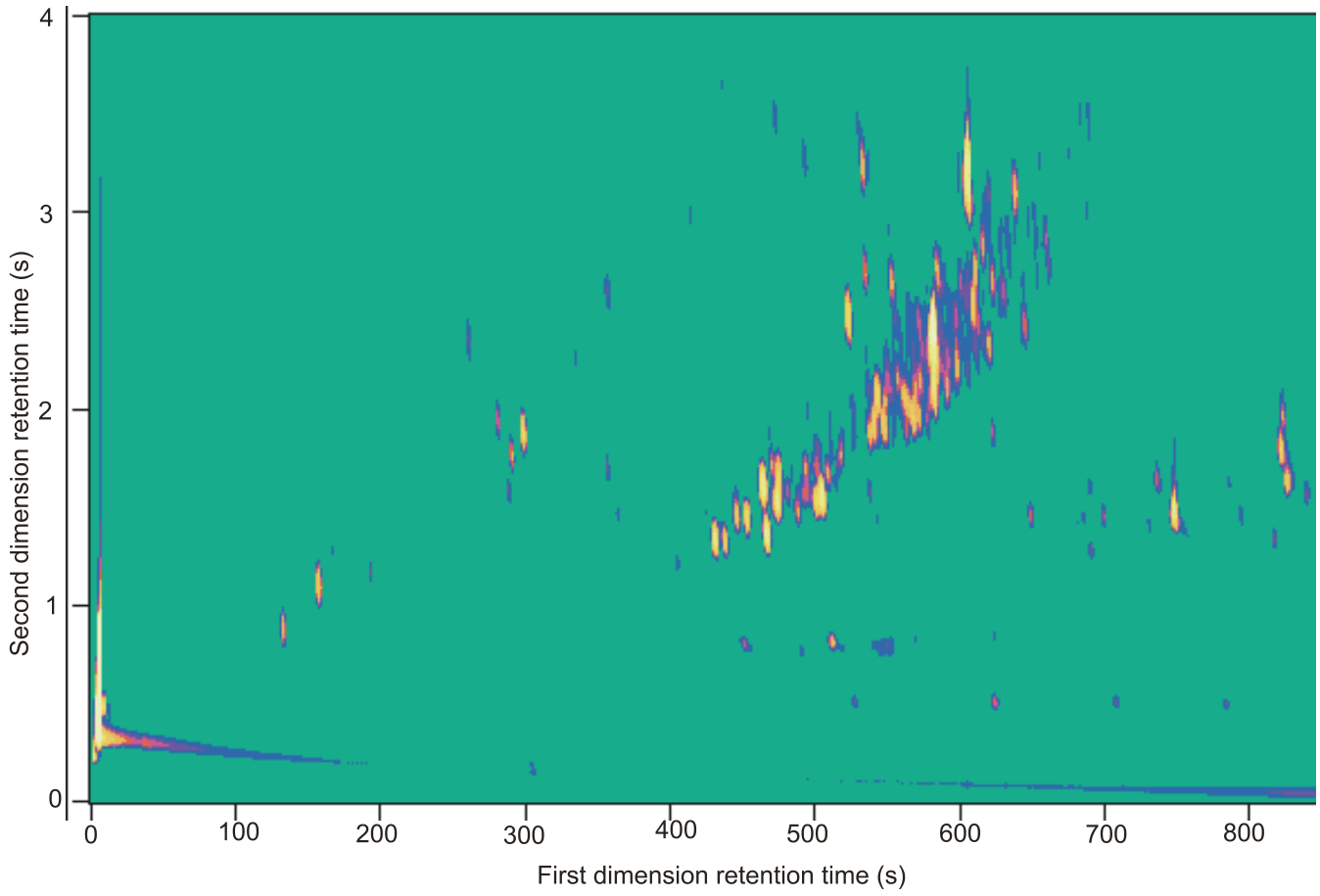

Figure 12.86. GC×GC separation of Cablin Patchouli.

Allergenes in orange sweet

D. Cavagnino, Thermo Electron, Milano, Italy, *unpublished results*

Instrumental conditions:

***Columns*:**
First: 15 m × 0.32 mm ID, 0.25 µm Rtx-5
Second: 0.8 m × 0.1 mm ID, 0.1 µm Megawax
Modulation cap.:

Carrier gas: helium

***Temperatures*:**
Main oven: 50°C, (1 min) 3°C/min → 250°C (3 min)
Second oven:

Injector: split/splitless, split 1:100
Temperature:
Injection volume: 0.8 µL

Modulator: dual cryogenic jet modulator

Modulation time: 5 s

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

Data acquisition: 20 Hz, range m/z 55–239

Sample description and separation:

The detector used was a quadrupole MS with a limited mass range of m/z 55-239, so that 20 scans per second could be achieved. This amount of scans was sufficient for a positive identification of all the target compounds.

The target compounds (27 allergenes) were spiked at a level of 20 ppm in an extract of orange sweet.

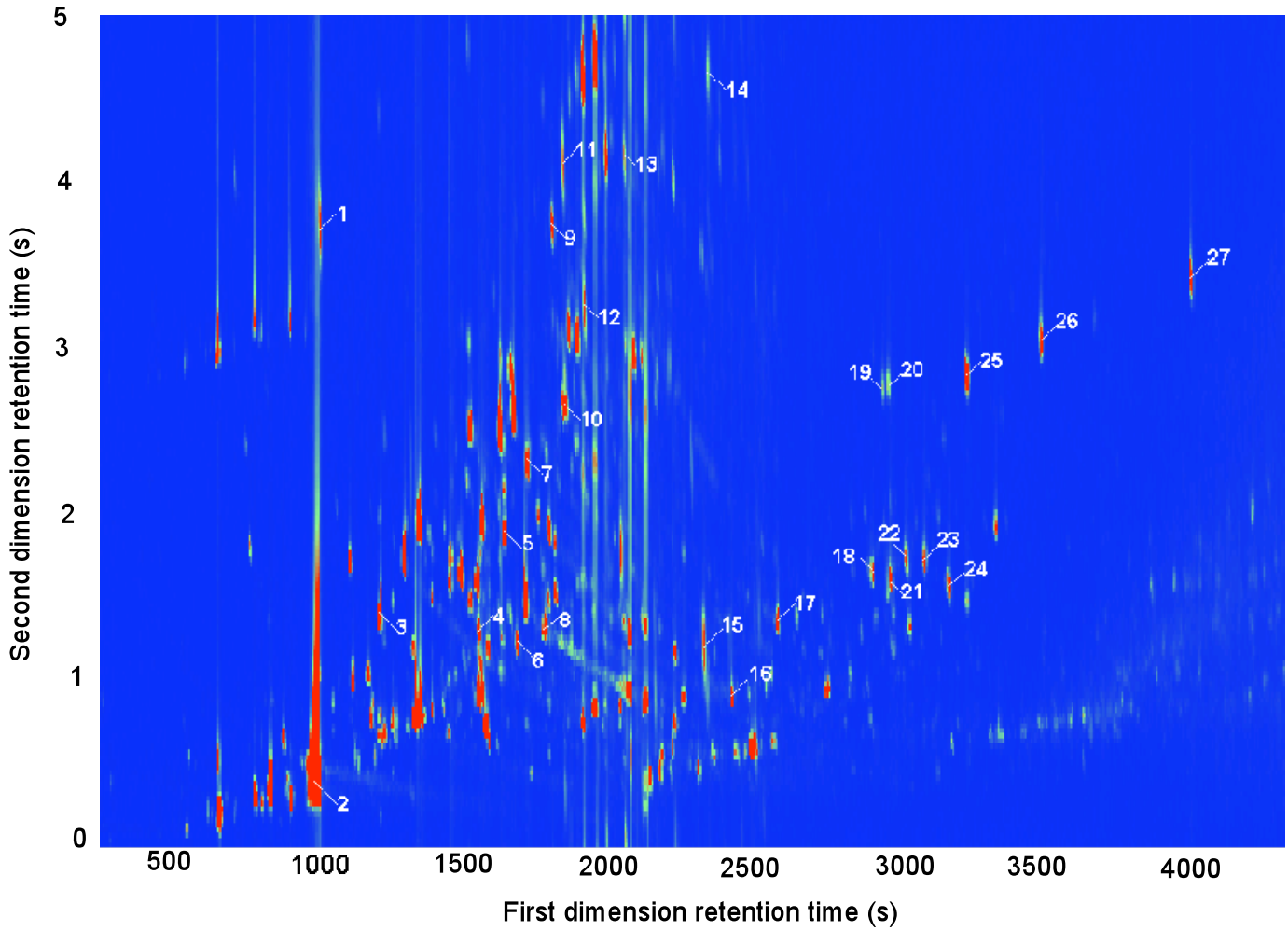

Figure 12.87. Colour plot of the TIC of the GC×GC separation of spiked allergenes in orange sweet 1. Benzyl alcohol, 2. limonene, 3. linalol, 4. folione, 5. citronellol, 6. geranial, 7. geraniol, 8. neral, 9. cinnamaldehyd, 10. hydroxycitronellal, 11. anisic alcohol, 12. cinnamic alcohol, 13. eugenol, 14. isoeugenol, 15. coumarin, 16. iso α methylione, 17. lilyal, 18. amyl cinnamaldehyd, 19. lyral1, 20. lyral 2, 21.benzyl salicylate, 22. farnesol1, 23. farnesol2, 24. hexyl cinnamaldehyd, 25. benzyl benzoate, 26. benzyl salicilate, 27. benzyl cinnamate.

Allergenes in fragrances

M.S. Dunn, N. Vulic, R.A. Shellie, S. Whitehead, P. Morrison, P.J. Marriott, *Targeted multidimensional gas chromatography for the quantitative analysis of suspected allergens in fragrance products*, J. Chromatogr. A, 1130 (2006) 122-129

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 0.25 μm SPB-1
Second: 1.0 m × 0.1 mm ID, 0.1 μm SupelcoWax-10
Modulation cap.:

Carrier gas: hydrogen, constant pressure @ 100 kPa

***Temperatures*:**
Main oven: 100°C (2 min), 5°C/min → 230°C (7 min)
Second oven:

Injector: split 50:1
Temperature:
Solvent vent:
Splitless time:
Injection volume: 1 μL

Modulator: LMCS

Modulation time: 4 s

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

Data acquisition: 100 Hz

Sample description and separation:

An evaluation of comprehensive two-dimensional gas chromatography was performed to assess its suitability for the analysis of volatile fragrance components, recognized by the European Commission's Scientific Committee on Cosmetics and other Non-food Products (SCCNFP) as possible skin sensitizers. The 24 volatile components listed by the SCCNFP were baseline-resolved or better within one 30 min analysis. Highquality calibration data for standard mixtures were obtained, with $R_2 < 0.998$ over the concentration range 2–1000 mg/L. However, the analysis of small spiked amounts of target compounds in truly complex fragrances was problematic, due to uncertainty in component assignment.

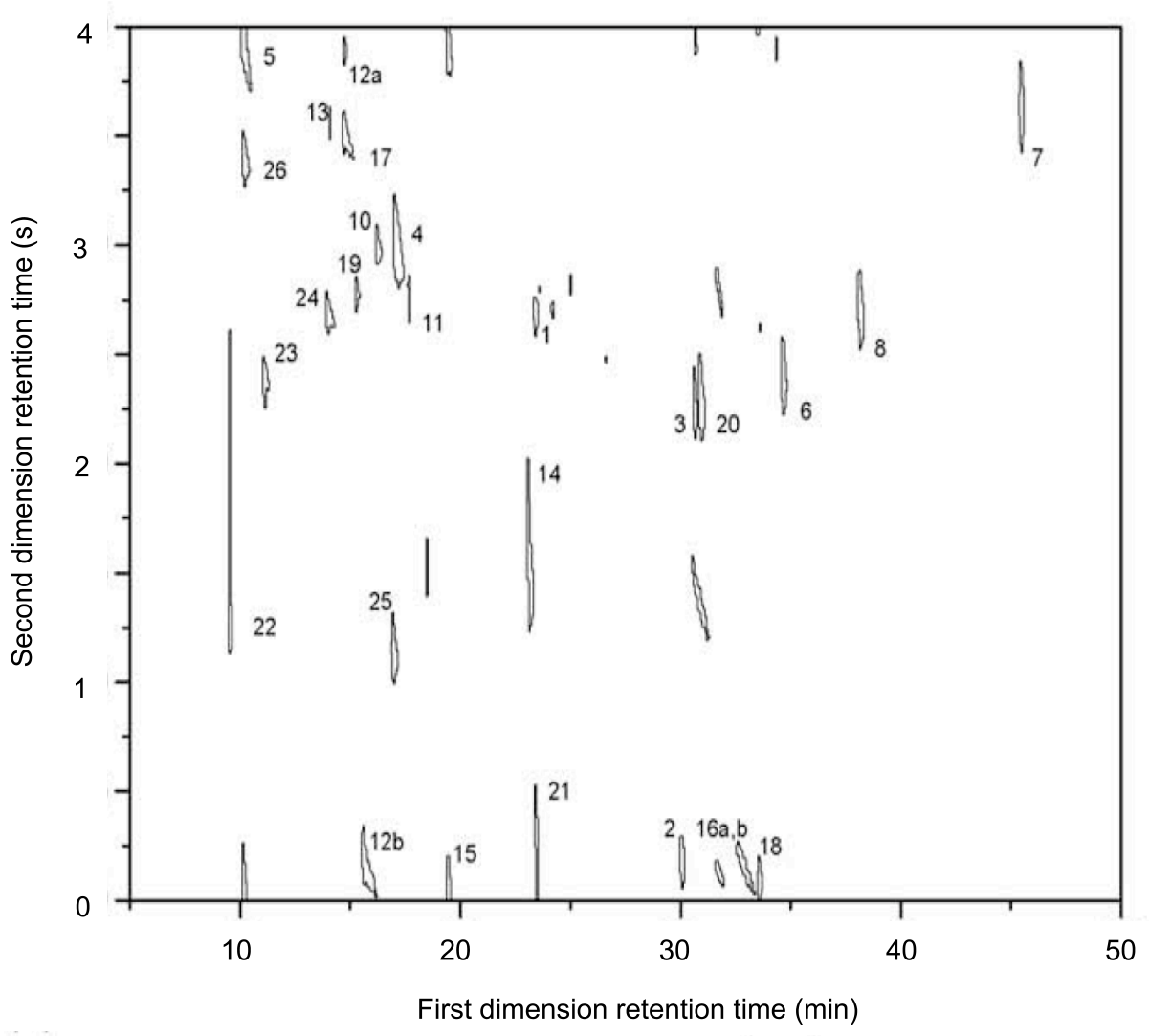

Figure 12.88. Contour plot of the GC×GC separation of the 25 suspected allergenes

Suspected allergens in fragrances

C. Cordero, C. Bicchi, D. Joulain, P. Rubiolo, *Identification, quantitation and method validation for the analysis of suspected allergens in fragrances by comprehensive two-dimensional gas chromatography coupled with quadrupole mass spectrometry and with flame ionization detection*, J. of Chromatogr. A, 1150 (2007) 37–49

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 1 μm OV-1
Second: 1 m × 0.1 mm ID, 0.1 μm OV-1701
Modulation capillary:

Carrier gas: helium, constant flow @ 1.2 mL/min

***Temperatures*:**
Main oven: 60°C (1 min), 3°C/min → 350°C (5 nin)
Second oven:

Injector: split 1:50
Temperature: 250°C
Injection volume: 1 μL

Modulator: dual-jet cryogenic

Modulation time: 4 s

Detector: qMS (EI mode)
Temperature: ion source 250°C
Make up gas flow:

Data acquisition: 18.52 scans/s, 40-240 *m/z*

Sample description and separation:

The effectiveness of the quadrupole MS operating at different scanning speed (1000 and 11,111 amu/s) was evaluated in identifying (full scan mode acquisition – TIC) and in quantifying (single ion monitoring – SIM) the target analytes in complex mixtures. In full scan acquisition mode the mass range was reduced to 40–240 amu to increase the scan acquisition rate while in SIM mode the influence of different dwell-times (40, 10 and 5 ms) was tested. The number of scans for each single modulated chromatographic GC×GC peak and the total number of scans for the 2D peak, together with half height peak width of each allergen in the standard mixture in both TIC and SIM modes were determined.

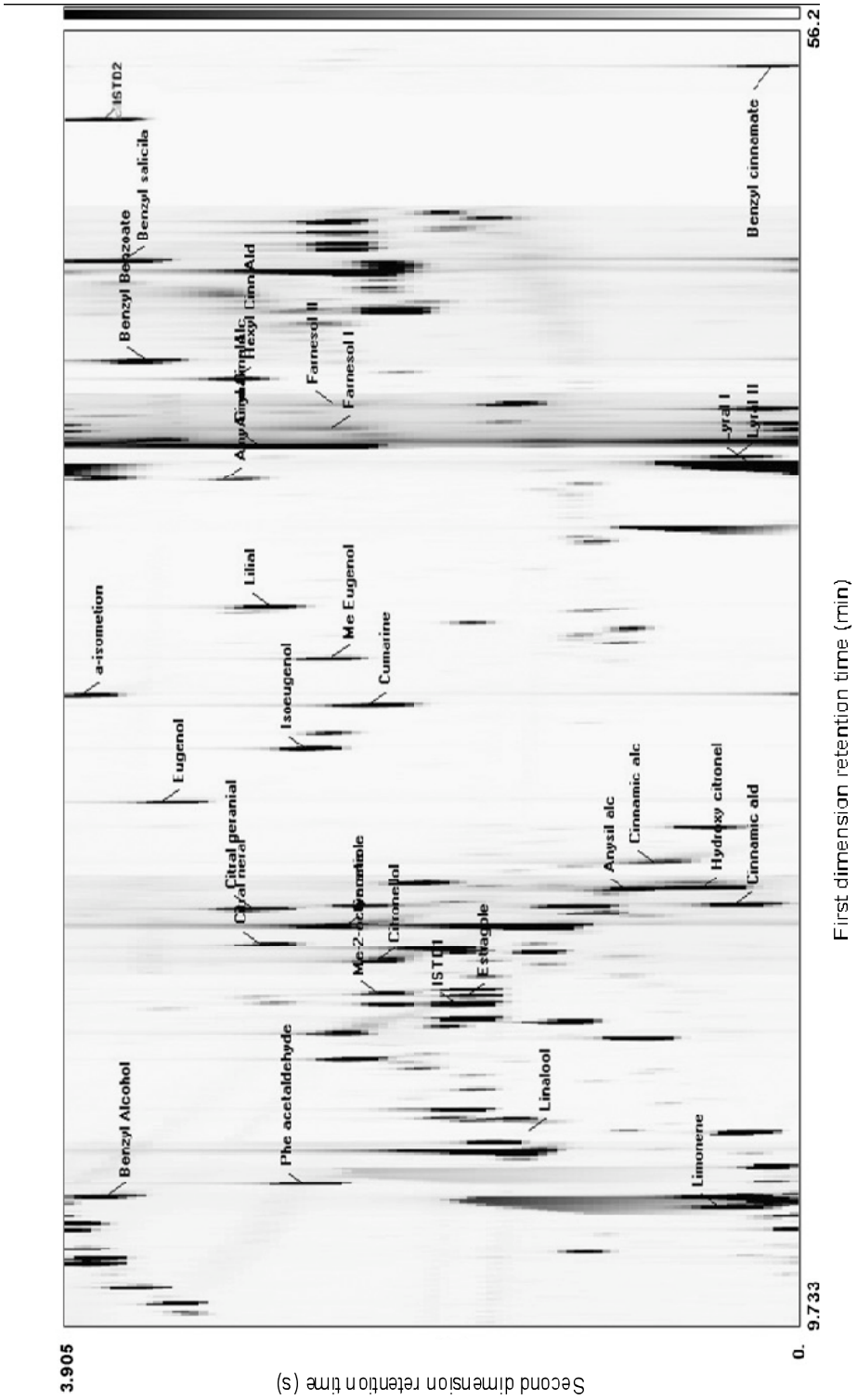

Figure 12.89. Contour plot of a GC×GC–SIM qMS analysis of a QC sample spiked with 10 ppm of allergen standard mixture (CC2dD10). For chromatographic conditions see referenced paper.

Preorbital secretion of the Steenbok

B.V. Burger, T. Snyman, W.J.G.Burger, G. Weibchen, *Affordable Comprehensive Two-Dimensional GC and GC–MS,* presented at First International Symposium on Comprehensive Multidimensional Gas Chromatography, Volendam, The Netherlands, March 6-7 2003

Instrumental conditions:

***Columns*:**
First: 20 m × 0.25 mm ID, 0.5 μm PS-089
Second: 0.4 m × 0.20 mm ID, 0.14 μm OV-1705
Modulation capillary: 0.1 m × 0.2 mm ID, 3 μm PDMS.

Carrier gas: hydrogen @ 68.5 cm/s at 40°C

***Temperatures*:**
Main oven: 40°C (5 min), 2°C/min → 250°C
Second oven:

Injector: split
Temperature: 220°C
Injection volume: 1 μL

Modulator: thermal modulator array

Modulation time: 6 s

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

Data acquisition: 50 Hz

Sample description and separation:

The sample is a dichloromethane extract of the volatile organic constituents of the preorbital secretion of the steenbok, *Raphicerus campestris*. The antelope uses the exudate for territorial marking. In this analysis the system was heavily overloaded to allow detection of the minor constituents. Furthermore, the combination of an non-polar first column with a second column of intermediate polarity, resulted in the elution of the polar compounds as very broad and tailing peaks, as exemplified by tetradecanoic acid eluting at about polar compounds as very broad and tailing peaks, as exemplified by tetradecanoic acid eluting at about 65 min.

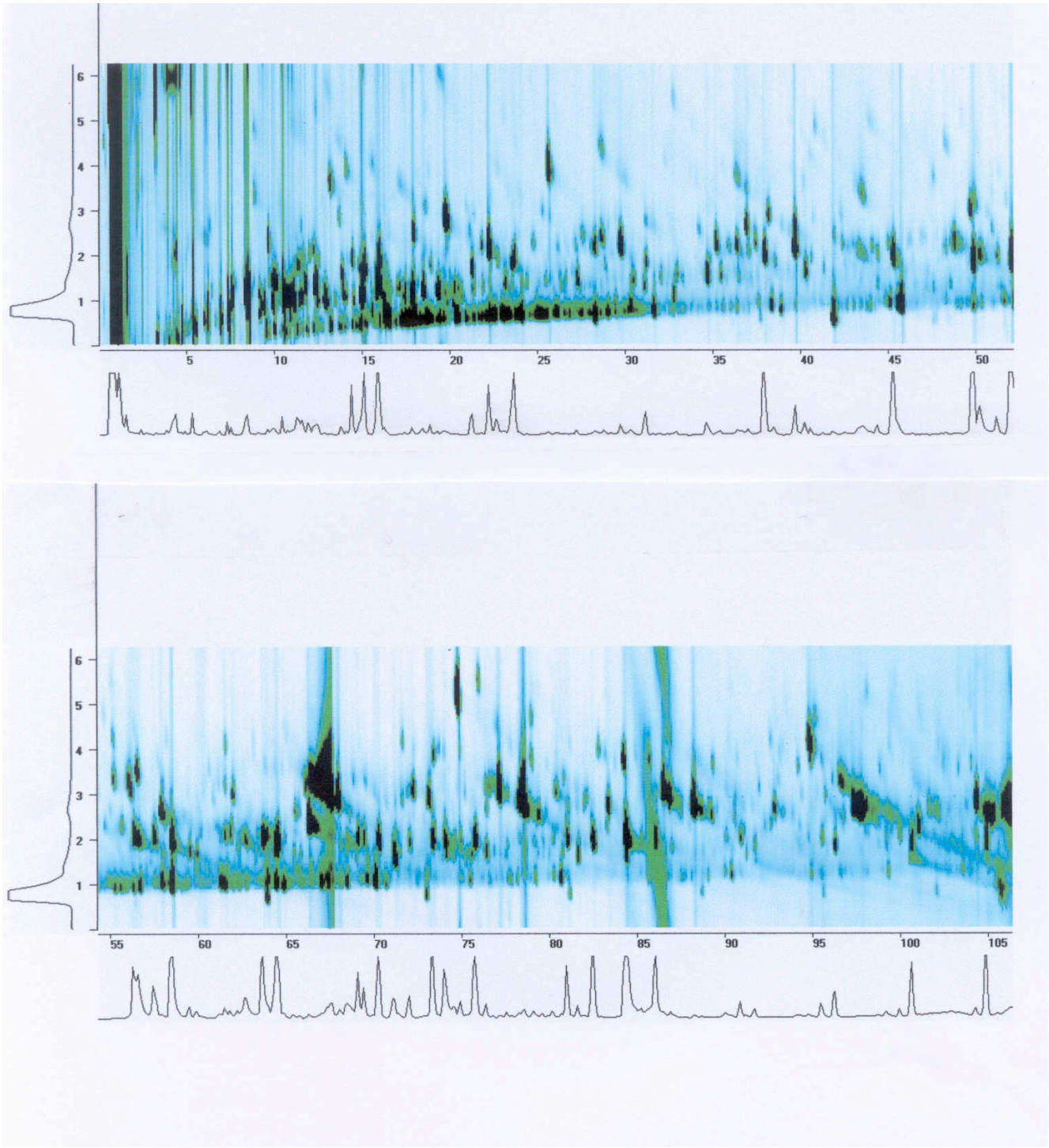

Figure 12.90. Colour plot of the GC×GC analysis of the preorbital secretion of a steenbok.

Colony odour of meat ants

E. van Wilgenburg, D. Ryan . P. Morrison, P.J. Marriott, M.A. Elgar, *Nest- and colony-mate recognition in polydomous colonies of meat ants (Iridomyrmex purpureus*), Naturwissenschaften (2006) 93: 309–314

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 0.25 μm BPX5
Second: 1.0 m × 0.10 mm ID, 0.2 μm BPX50
Modulation capillary:

Carrier gas: hydrogen

***Temperatures*:**
Main oven: 50°C (0.5 min), 20°C/min → 200°C, 5°C/min → 340°C (5 min)
Second oven:

Injector: split/splitles
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:

Meat ants of four colonies were collected for the hydrocarbon extraction from the same population. Two of these colonies consisted of a single nest and two were polydomous (with 13 and 19 nests per colony). For the polydomous colonies, ants from two nests of the same colony were collected. Cuticular lipids of 22 ants per nest were extracted by immersing each ant in 1 mL of pesticide-grade hexane for 10 min. Identification of the cuticular compounds from a pool of 15 ants from four colonies was achieved on a GC×GC system as above with a time-of-flight mass spectrometer (GC×GCTOFMS) using the same columns as those described above.

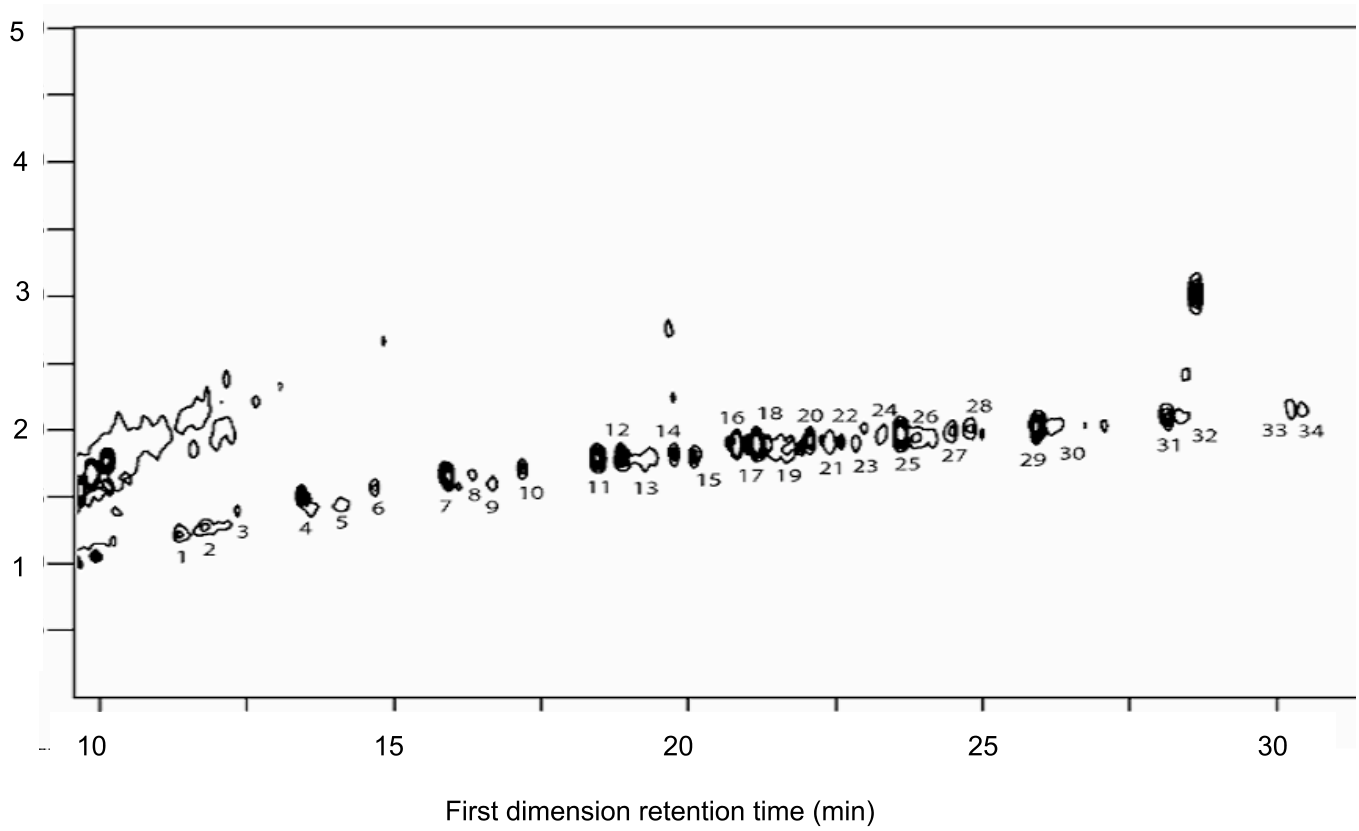

Figure 12.91. GC×GC-FID chromatogram of the hydrocarbons obtained from a single ant of I. purpureus. 1. C_{21}; 2. 9- 10 -11- MeC_{21} + 10,14-diMeC_{21}; 3. C_{22}; 4. C_{23}; 5. 9,13- 10,14-diMeC_{23}; 6. C_{24}; 7. C_{25}; 8. 11- 13-MeC_{25}; 9. 11,15- 10,14-diMeC_{25}; 10. C_{26}; 11. C_{27}; 12. 11- 13-meC_{27}; 13. 7,11- 7,15-diMeC_{27}; 14. C_{28}; 15. 12- 14- 16- 18-MeC_{28}; 16. ?; 17. C_{29}; 18. 11- 13- 15-MeC_{29}; 19. 11,15- 13,17-diMeC_{29}; 20. 7,15-diMeC_{29}; 21. C_{30}; 22. 11- 12- 13- 14- 15-MeC_{30}; 23. 11,15- 13,17-diMe_{30}; 24. C_{31}; 25. 11- 13- 15-MeC_{31}; 26. 11,15- 13,17-diMeC_{31}; 27. C_{32}; 28. 12- 13- 14- 15- 16-MeC_{32}; 29. 11- 13- 15- 17-MeC_{33}; 30. 11,15- 13,17-diMeC_{33}; 31. 11- 13- 15- 17-MeC_{35}; 32. ?; 33. 11- 13MeC_{37}; 34.?; and 35.?.

Positional isomers in wine

Y. Shao, P. Marriott, *Separation of positional isomers by the use of coupled shape-selective stationary phase columns,* Anal. Bioanal. Chem. 375 (2003) 635-642

Instrumental conditions:

***Columns*:**

First:	24 m × 0.25 mm ID, 0.5 µm EtTBS-β-CD connected to 30 m × 0.25 mm ID, 0.25µm CycloSil-B
Second:	1 m × 0.1 mm ID, 0.1 µm BPX50
Modulation capillary:	16.5 cm × 0.15 mm ID, 25 µm BPX5

Carrier gas: hydrogen, constant flow @ 2.5 mL/min

***Temperatures*:**

Main oven: 56°C (16 min) 10°C/min →70°C (6 min), 25°C/min → 100°C (18 min), 5°C/min →180°C, 3°C/min → 200 °C (5 min)

Second oven:

Injector:	split
Temperature:	220°C
Injection volume:	1 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 successful separation of 2- and 3-methylsubstituted positional isomers of butanol, butyl acetate, and butanoic acid and its ethyl ester, is demonstrated. These compounds are of interest in the study of wine flavour, however the separation of the 2- and 3-methyl isomers may present problems, and more so in the presence of the wine matrix components, when single capillary column gas chromatography (GC) is used. The strategy to achieve separation was based on the use of shape-selective cyclodextrin derivative (CDD) capillary columns.

PDMS/DVB fibre solidphase microextraction (SPME) samples were taken from the headspace of the solution.

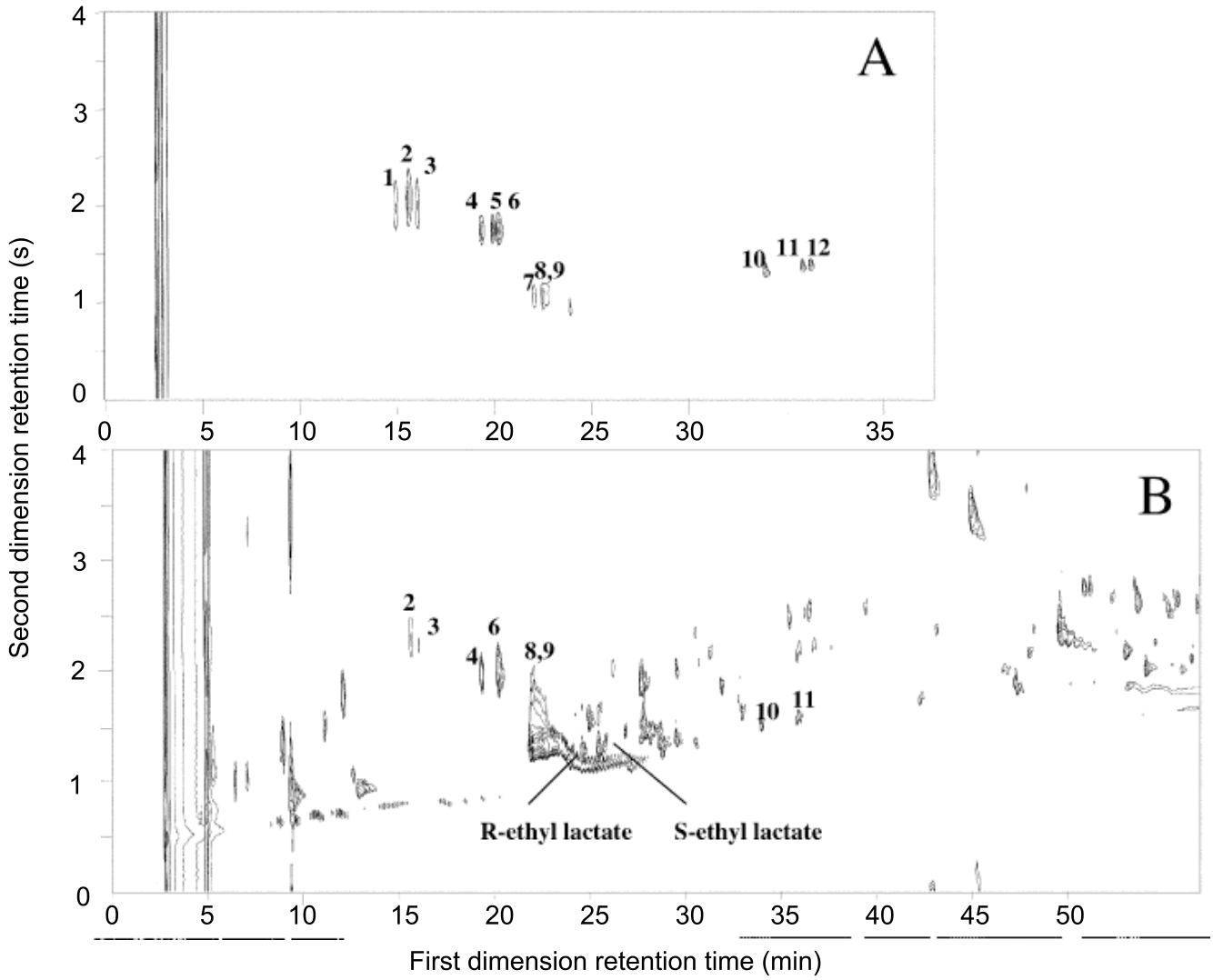

Figure 12.92. Top: GC×GC analysis of the standard mixture with 65 µm PDMS/DVB fibre solidphase microextraction (SPME) from the headspace of the solution.
Bottom: SPME extract of 0.5 mL of Shiraz red wine headspace. Note the correspondence of the position of the target analytes with results in A.

Ephidrine enantiomers in herbal products

M. Wang, P.J. Marriott, W-H. Chan, A.W.M. Lee, C.W. Huie, *Enantiomeric separation and quantification of ephedrine-type alkaloids in herbal materials by comprehensive two-dimensional gas chromatography*, J. Chromatogr.A, 1112 (2006) 361–368

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 0.25 μm Cyclodex-B
Second: 1 m × 0.10 mm ID, 0.1 μm BP20
Modulation capillary:

Carrier gas: hydrogen @ 1.2 mL/min

***Temperatures*:**
Main oven: 70°C, 10°C/min → 100°C, 2°C/min → 160°C (10 min)
Main oven (for enantiomers): 70°C, 10°C/min → 100°C (20 min), 1.5°C/min → 160°C (10m)

Injector: split 1:20
Temperature:
Injection volume: 2 μL

Modulator: LMCS

Modulation time: 6 s

Detector: FID
Temperature: 220ºC
Make up gas flow: N_2 @ 10 mL/min

Data acquisition: 200 Hz

Sample description and separation:

Naturally occurring ephedrine-type alkaloids and their synthetic analogues (enantiomeric counterparts) were adequately resolved. Detection limits in the order of 0.1–1.3 μg/mL and linearity of calibration with $R2 \geq 0.999$ over approximately the range of 0.5–100 μg/mL for the quantitative determination were obtained. The products tested contained mostly (−)-ephedrine, (+)-pseudoephedrine, (−)-*N*-methylephedrine and (−)-norephedrine, with concentrations in the range of 40–2100, 0–1300, 15–300 and 0–30 μg/g of the product, respectively, and repeatability of analysis was generally in the range of 1–5%.

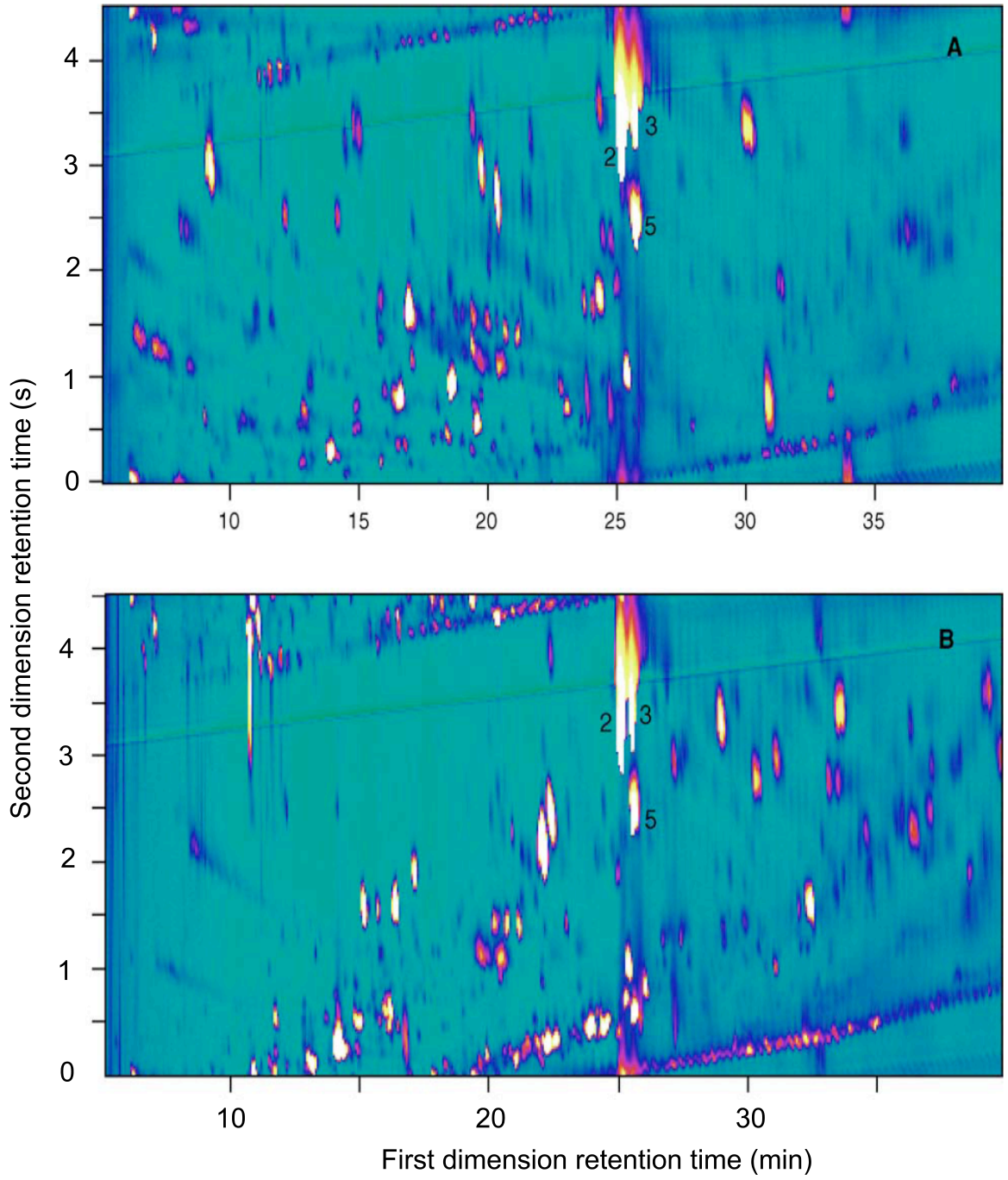

Figure 12.93. Colour plot of a section of a GC×GC separation of an extract of a 1:1 (w/w) mixture of: Top: four herbs (10 mg each) containing Ma Huang, Gan Cao, Gui Zhi and Xing Ren and Bottom: eight herbs (10 mg each) containing Ma Huang, Bai Shu, Fang Feng, Xi Xin, Be He, Du Huo, Qian Hu and Chuan Qiong.

Alkaloid assignments: 2, (−)-ephedrine; 3, (+)-pseudoephedrine; 5, (−)-N-methylephedrine.

Fatty acids in micro algae and aquatic meiofauna

L. Akoto, F. Stellaard, H. Irth, R.J.J. Vreuls, R. Pel*, Improved fatty acid detection in micro-algae and aquatic meiofauna species using a direct thermal desorption interface combined with comprehensive gas chromatography–time-of-flight mass spectrometry*, J. of Chromatogr. A, 1186 (2008) 254–261

Instrumental conditions:

***Columns*:**	
First:	30 m × 0.25 mm ID, 1 µm HP-5 MS
Second:	1 m × 0.1 mm ID, 0.1 µm BPX-50
Modulation capillary:	
Carrier gas:	helium, constant flow @ 1.2 mL/min
***Temperatures*:**	
Main oven:	70°C (1 min), 3°C/min → 300°C (15 nin)
Second oven:	
Injector:	PTV **(**Optic 2 with automatic liner exchange (DTD) inserts)
Temperature:	
Injection volume:	40µL sample to be placed in the liner
Modulator:	quad-jet cryogenic
Modulation time:	4 s
Detector:	ToF MS
Temperature:	ion source 280°C
Make up gas flow:	
Data acquisition:	50 spectra/s 70-800 *m/z*

Sample description and separation:

GC×GC –ToF MS is used to profile the fatty acid composition of whole/intact aquatic microorganisms such as the common fresh water green algae *Scenedesmus acutus* and the filamentous cyanobacterium *Limnothrix* sp. strain MRI without any sample preparation steps. It is shown that the technique can be useful in the identification of lipid markers in food-web as well as environmental studies. For instance, new mono- and diunsaturated fatty acids were found in the C_{16} and C_{18} regions of the green algae *S. acutus* and the filamentous cyanobacterium *Limnothrix* sp. strain MRI samples. These fatty acids have not, to our

knowledge, been detected in the conventional one-dimensional (1D) GC analysis of these species due to either co-elution and/or their presence in low amounts in the sample matrix.

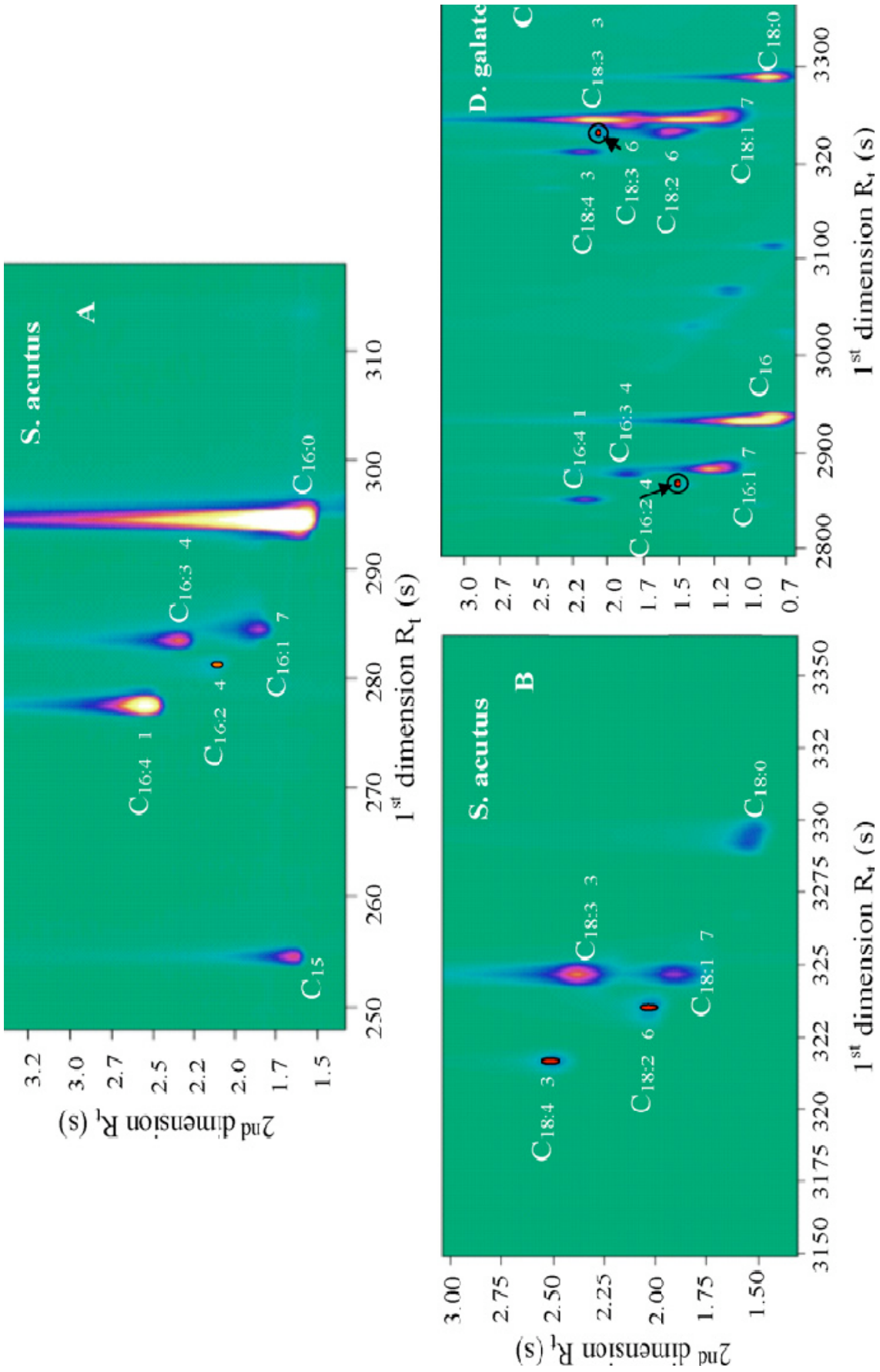

Figure 12.94. 2D extracted ion (m/z = 55, 79 and 74) elucidation of the C16 and C18 regions of S. acutus and Daphnia galatea samples.

Cycling yeast metabolites

R.E. Mohler, B.P. Tu, K.M. Dombek, J.C. Hoggard, E.T. Young, R.E. Synovec, *Identification and evaluation of cycling yeast metabolites in two-dimensional comprehensive gas chromatography–time-of-flight-mass spectrometry data*, J. of Chromatography 1186 (2008) 401-411

Instrumental conditions:

***Columns*:**
First: 20 m × 0.25 mm ID, 0.5 μm Rtx-5 MS
Second: 2 m × 0.18 mm ID, 0.2 μm Rtx-200 MS
Modulation capillary:

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

***Temperatures*:**
Main oven: 60°C (0.25 min), 8°C/min → 280°C (10 nin)
Second oven: 70°C (0.25 min), 8°C/min → 290°C (10 nin)

Injector: splitless
Temperature:
Injection volume: 1 μL

Modulator: quad-jet cryogenic

Modulation time: 1.5 s

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

Data acquisition: 100 spectra/s

Sample description and separation:

A yeast metabolome exhibiting oscillatory behavior was and in-house developed data analysis software methodology, referred to as a signal ratio method (S_{ratio} method). 44 identified unique metabolites were found to exhibit cycling, with a depth-of-modulation amplitude greater than three. After the initial locations are found using the S_{ratio} software, and identified, the refined mass spectra and peak volumes were subsequently obtained using PARAFAC. The S_{ratio} reported is a rapid method to determine the depth-of-modulation while not constraining the search to specific cycling frequencies. The phase delay of the cycling metabolites ranged widely in relation to the oxygen consumption cycling pattern.

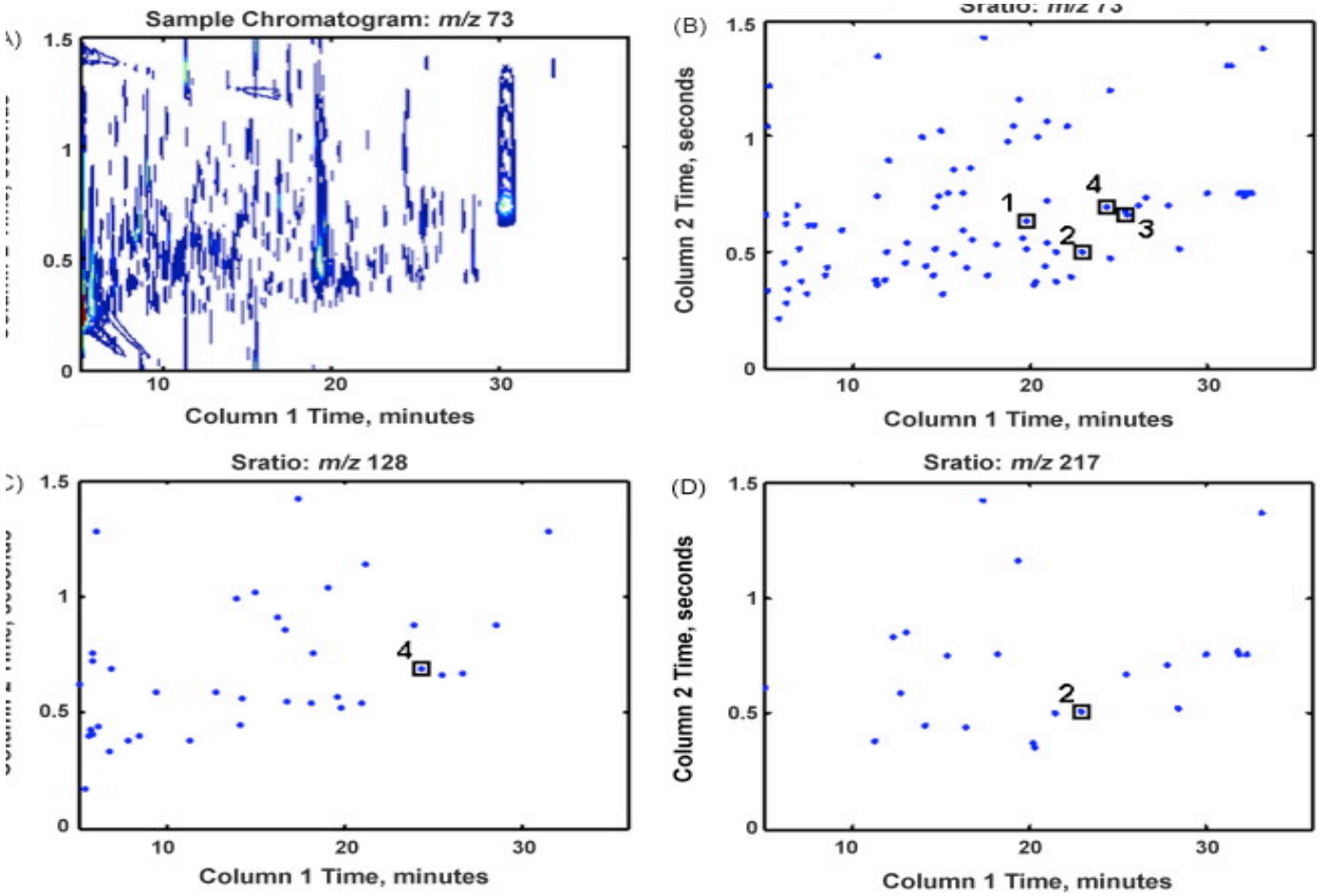

Figure 12.95. (A) GC×GC–ToF MS data for typical yeast extract sample at m/z 73. (B) The results at m/z 73 after all 72 GC×GC–ToF MS chromatograms at this m/z have been analyzed by the S_{ratio} method. (C) As in (B), with S_{ratio}s at m/z 128 determined. (D) As in (B), with S_{ratio}s at m/z 217. The m/z of 128 and 217 provide more selective information than the global m/z 73. The locations of the metabolites for the more in-depth discussion are enclosed in the boxed regions labeled 1–4, which were identified as methyl citrate, myo-inositol, glucose-6-phosphate and cystathionine, respectively.

Fungicides in vegetables

W. Khummueng, C. Trenerry, G. Rose, P.J. Marriott *Application of comprehensive two-dimensional gas chromatography with nitrogen-selective detection for the analysis of fungicide residues in vegetable samples*, J. Chromatogr. A, 1131 (2006) 203-214

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 0.25 μm, BPX5
Second: 1 m × 0.15 mm ID, 0.15 μm, BPX50
Modulation capillary:

Carrier gas: hydrogen @ 1 mL/min

***Temperatures*:**
Main oven: 50°C, 10°C/min → 210°C, 5°C/min → 260°C (5 min)
Second oven:

Injector: splitless
Temperature: 50°C, 15°C /s → 280°C
Injection volume:

Modulator: LMCS
Modulation time: s

Detector: NPD
Temperature: 300°C
Make up gas flow:

Data acquisition: 50 Hz

Sample description and separation:

The analyzed sample consisted of a vegetable extract. External calibrations of fungicides were performed over a concentration range from 1 to 1000 μg/L. The peak area calibration curves generally had regression coefficients of $R^2 > 0.9980$, however for iprodione which was observed to undergo on-column degradation, an R^2 of 0.990 was found. The limit of detection and limit of quantitation were less than about 74 and 246 ng/L, respectively. The intra-day and inter-day RSD values were measured for solutions of concentration 0.100, 0.500 and 1.50 mg/L. For the 0.500 mg/L solution, intra- and inter-day precision of peak area and peak height for most of the pesticides were about 2% and 8%, respectively. Excellent linearity was observed for these standards, from 0.001 to 25.00 mg/L. The standard mixture peak positions were identified using GC×GC with quadrupole mass spectrometry (qMS).

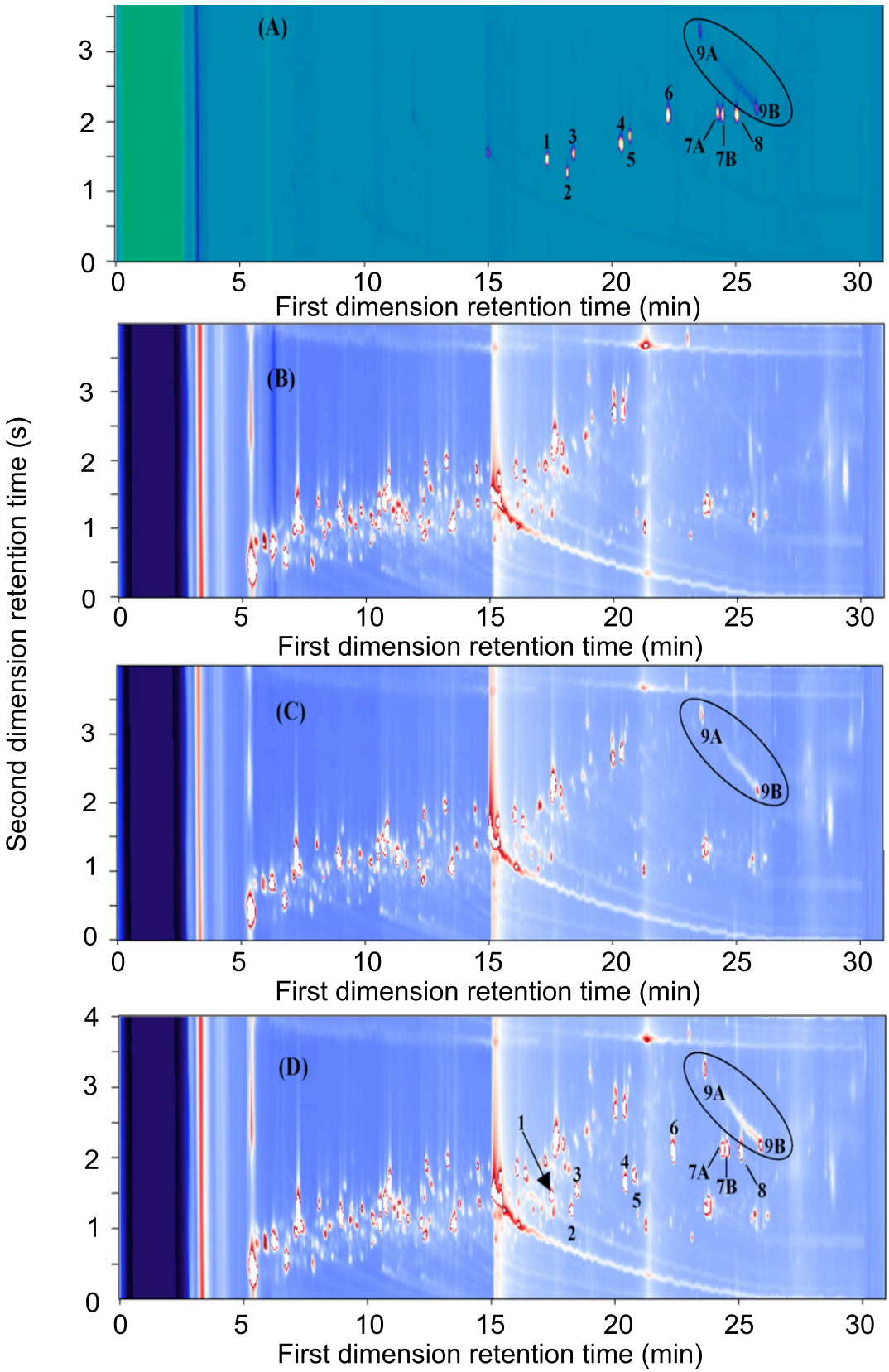

Figure 12.96. GC×GC-NPD colour plots of: (A) Fungicide standard mixture; (B) blank vegetable extract; (C) blank vegetable extract spiked with iprodione standard; (D) blank vegetable extract spiked with standard fungicide mixture. Circled region shows iprodione and iprodione decomposition products.

Metabolites in yeast cells

R.E. Mohler, K.M. Dombek, J.C. Hoggard, E.T. Young, R.E. Synovec, *Comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry analysis of metabolites in fermenting and respiring yeast cells*, Anal. Chem. 78 (2006) 2700-2709

Instrumental conditions:

***Columns*:**
First: 20 m × 0.25 mm ID, 0.5 µm Rtx5MS
Second: 2 m × 0.18 mm ID, 0.2 µm Rtx200MS
Modulation capillary:

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

***Temperatures*:**
Main oven: 60°C (0.25 min), 8°C/min → 280°C (10 min)
Second oven: 70°C (0.25 min), 8°C/min → 290°C (10 min)

Injector: splitless
Temperature: 280°C
Injection volume: 1µL

Modulator: quad-jet cryomodulator

Modulation time: 1.5 s

Detector: ToF-MS
Temperature: transfer line 280°C
Make up gas flow:

Data acquisition:

Sample description and separation:

GC×GC-ToF-MS coupled with rapid chemometric analysis were used to identify chemical differences in metabolite extracts isolated from yeast cells either metabolizing glucose (repressed (R) cells) via fermentation or metabolizing ethanol by respiration (derepressed (DR) cells). Principal component analysis (PCA) followed by parallel factor analysis (PARAFAC) in concert with the TOF software located and identified the differences in composition between the two types of cell extracts. By selecting three selective mass channels (*m*/*z* 73, 205, 387), 26 metabolites that differentiate repressed cells from derepressed cells were identified.

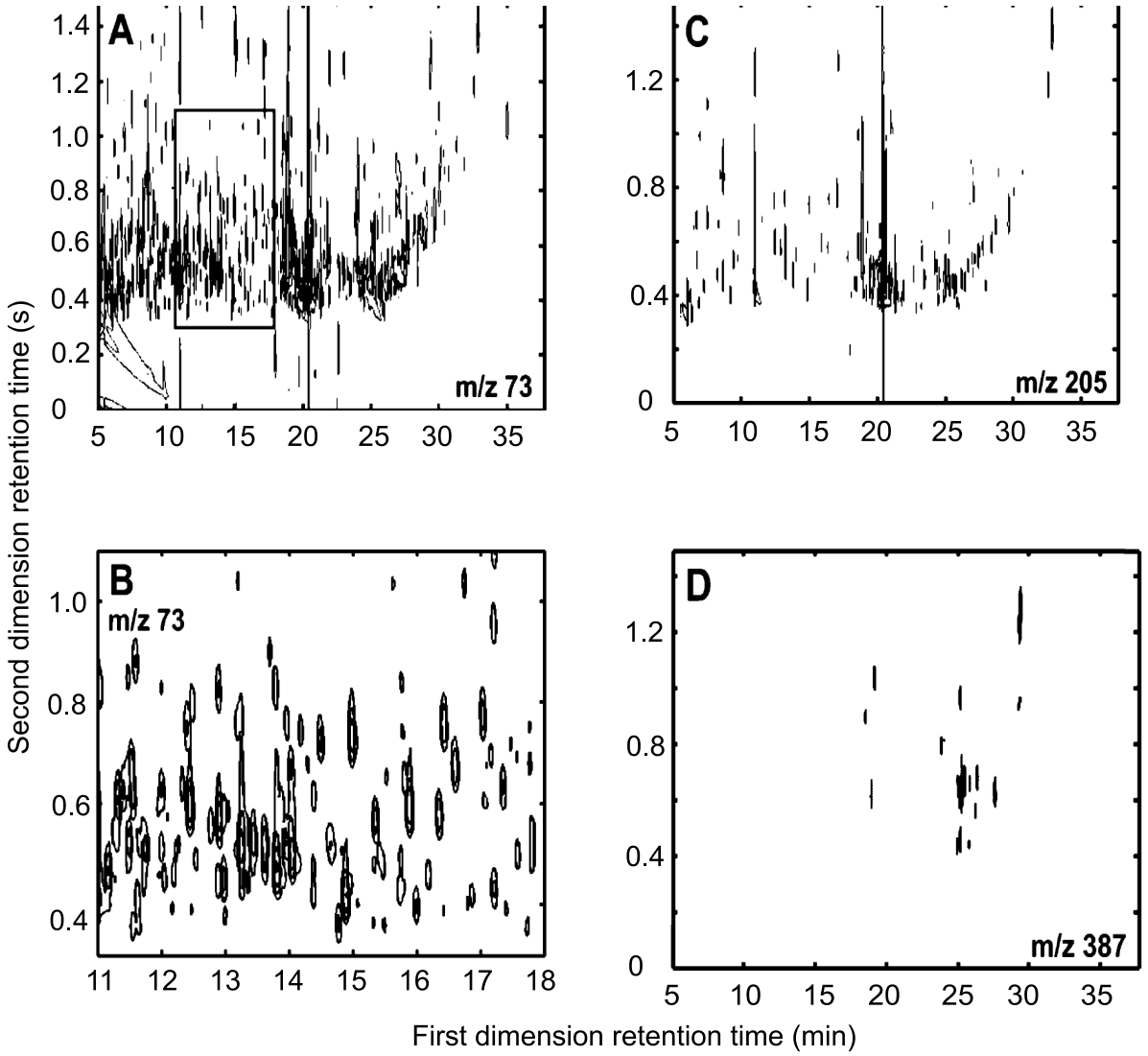

Figure 12.97. Contour plots of a repressed sample extract at selected mass channels.
(A) Mass channel (m/ z) 73, (B) boxed region of (A) emphasizing the separation resolution achieved. (C) Mass channel 205, which is fairly selective for carbohydrates, and (D) mass channel 387, which is selective for sugar phosphates.

Nonylphenols

M. Moeder, C. Martin, D. Schlosser, J. Harynuk, T. Górecki, *Separation of technical 4-nonylphenols and their biodegradation products by comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry*, J Chromatogr A 1107 (2006) 233–239

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 0.25 μm, VF-5MS
Second: 1.6 m × 0.1 mm ID, 0.1 μm VF-23MS

Carrier gas: hydrogen, constant flow @ 1.2 mL/min

***Temperatures*:**
Main oven: 65°C, 15°C/min → 180°C, 1°C/min → 190°C, 20°C/min → 260°C (6 min)
Second oven:

Injector: splitless
Temperature: 280°C
Injection volume: 1μL

Modulator: liquid nitrogen cryogenic modulator with delay loop [95]

Modulation time: 5 s

Detector: ToF-MS, 70 eV, 50-355 u
Temperature:
Make up gas flow:

Data acquisition: 100 spectra/s

Sample description and separation:

Identification of components is facilitated as shown for isomeric 4-nonylphenols and metabolites of their biodegradation by *Clavariopsis aquatica*, an aquatic fungus. GC×GC–TOF-MS analysis enabled the separation of about 40 alkylphenol isomers included
in technical 4-nonylphenol. During biodegradation the variety of emerging compounds increased with longer reaction time. The comprehensive analysis indicated a broad spectrum of hydroxylated, carboxylated nonylphenolisomers and additionally, chlorinated aromatic compounds produced and released from the fungal culture.

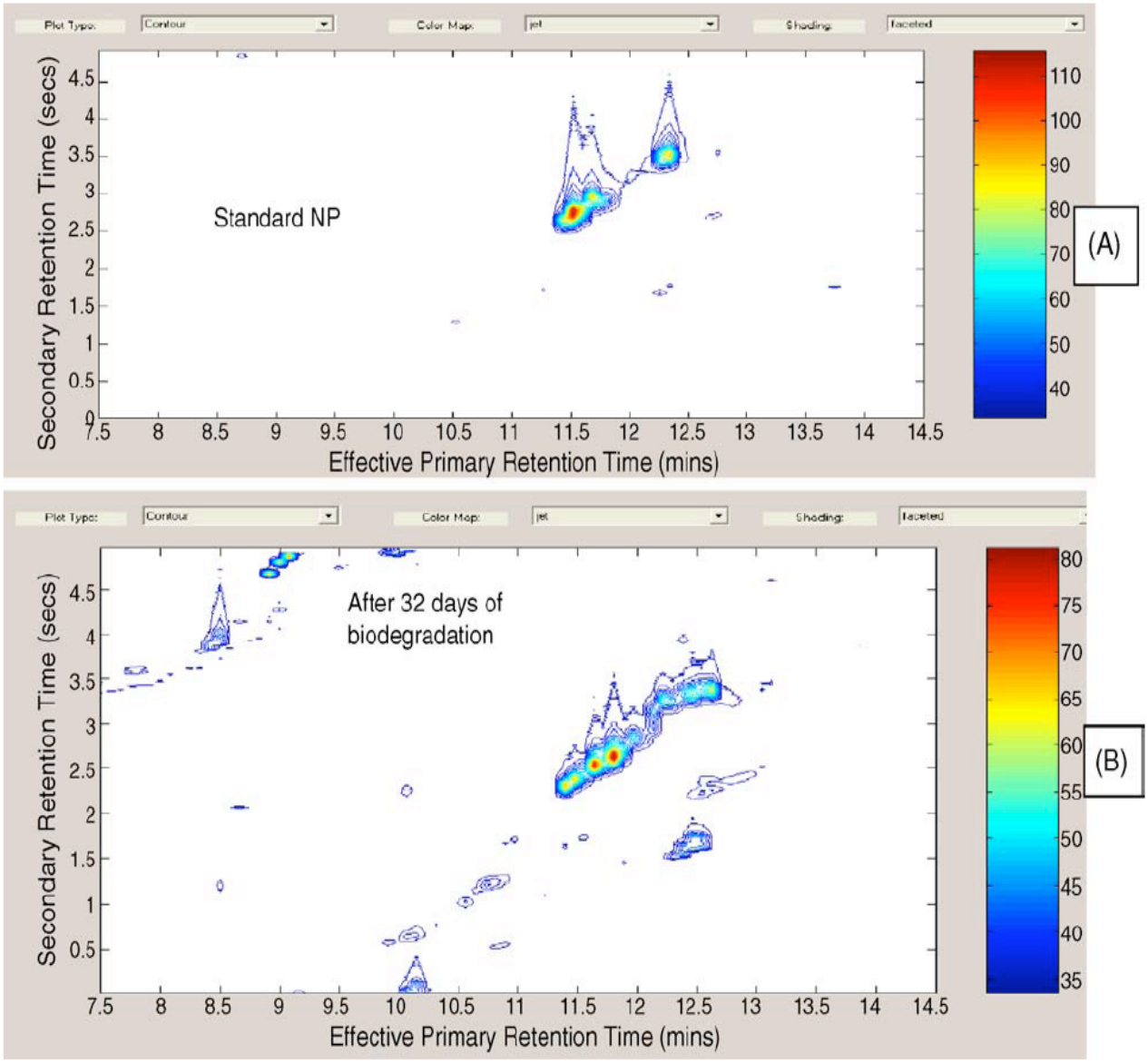

Figure 12.98. Top: GC×GC contour plot of ion m/z 191 extracted from analysis of technical nonylphenol.

Bottom: GC×GC contour plot of ion trace m/z 191 from analysis of the nonylphenol mixture after 32 days of biodegradation by Clavariopsis aquatica.

Tensides

J. Hübner, R. Taheri, D. Melchior, H.-W. Kling, S. Gäb, O.J. Schmitz, *Analysis of tensides in complex samples with comprehensive two-dimensional gas chromatography coupled with timeof-flight mass spectrometry*, Anal Bioanal Chem (2007) 388:1755–1762

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 0.25 µm DB5
Second: 1 m × 0.1 mm ID, 0.1 µm DBX50
Modulation capillary:

Carrier gas: helium, constant flow @ 1.4 mL/min

***Temperatures*:**
Main oven: 70°C (1 min), 40°C/min → 150°C, 5°C/min → 320°C (10 min)
Second oven: 90°C (1 min), 40°C/min → 170°C, 5°C/min → 330°C (12 min)

Injector: split 1:10
Temperature: 320°C
Injection volume:

Modulator: quad-jet cryogenic

Modulation time: 2.5 s

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

Data acquisition: not specified

Sample description and separation:

The first aliquot of the sample was silylated, diluted and analysed; then, in order to detect anionic tensides (FAES, FAS) too, the second aliquot was hydrolysed before being silylated for analysis. Because of their amphoteric character, the betaines can only be analysed by gas chromatography after thermal decomposition in the injector, which leads to the corresponding amidoamines among other products. Quantitative determination was achieved of alkyl polyglucosides, fatty alcohol ethoxylates, fatty alcohol sulfates, fatty alcohol ether sulfates and cocamidopropyl betaines in shower gel and cleaning agents.

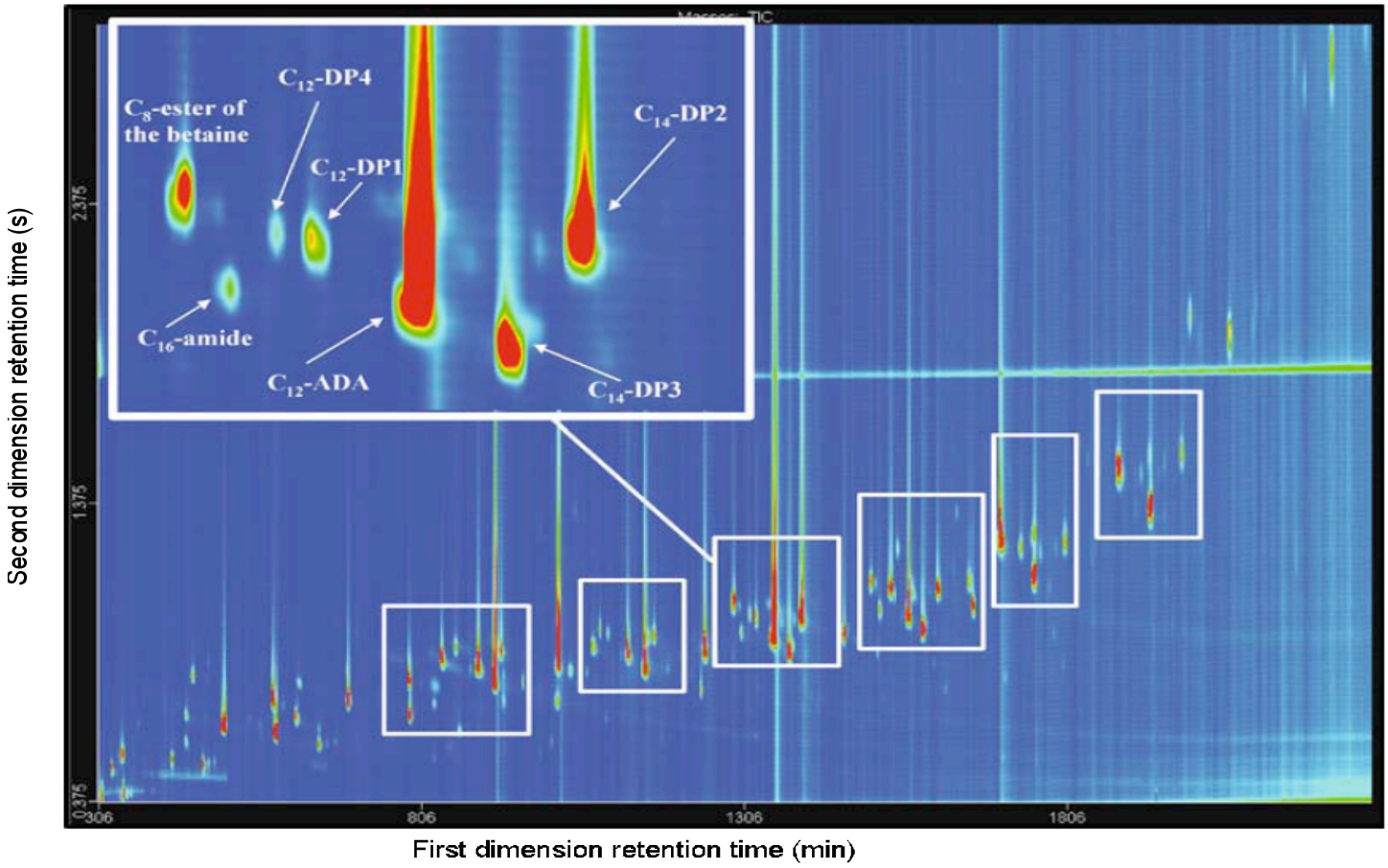

Figure 12.99. TIC of betaines of thermal decomposition products of Dehypon PK-45®. The expanded part shows the repeated signal pattern in detail. (CnADA: amidoamines with n C atoms in the alkyl chain; Cn-DP1–4: decomposition products 1, 2, 3 and 4 with n C atoms in the alkyl chain).

Invertebrates metabolomics

K. Ralston-Hooper, A. Hopf, C. Oh, X. Zhang, J. Adamec, M.S. Sepůlveda, *Development of GCxGC–ToF MS metabolomics for use in future ecotoxicological studies with invertebrates,* Aquatic Toxicology (2007), doi:10.1016/j.aquatox.2008.03.002

Instrumental conditions:

***Columns*:**
First: 10 m × 0.18 mm ID, 0.2 µm Rtx-5 matrix
Second: 1.1 m × 0.18 mm ID, 0.18 µm DB17
Modulation capillary:

Carrier gas: helium @ 1 mL/min

***Temperatures*:**
Main oven: 50°C, 5 °C/min → 300°C (5 min)
Second oven: 75°C, 5 °C/min → 325°C (5 min)

Injector: split 1:20
Temperature: 280°C
Injection volume: 2 µL

Modulator: quad-jet cryogenic

Modulation time: 4 s

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

Data acquisition: 50 spectra/s 50-900 *m/z*

Sample description and separation:

A comparison was made of 31 metabolite profiles between *Diporeia* collected from Lake Michigan (declining 32 populations) to those residing in Lake Superior (stable populations), and also between 33 *Diporeia* exposed to a chemical stressor (atrazine) and controls. Overall, 76 and 302 34 total metabolites were detected from the lake comparison and atrazine studies, 35 respectively. Many of the identified metabolites included fatty acids, amino acids, and 36 hydrocarbons. Furthermore, we observed unique and almost non-overlapping 37 metabolite profiles in both studies.

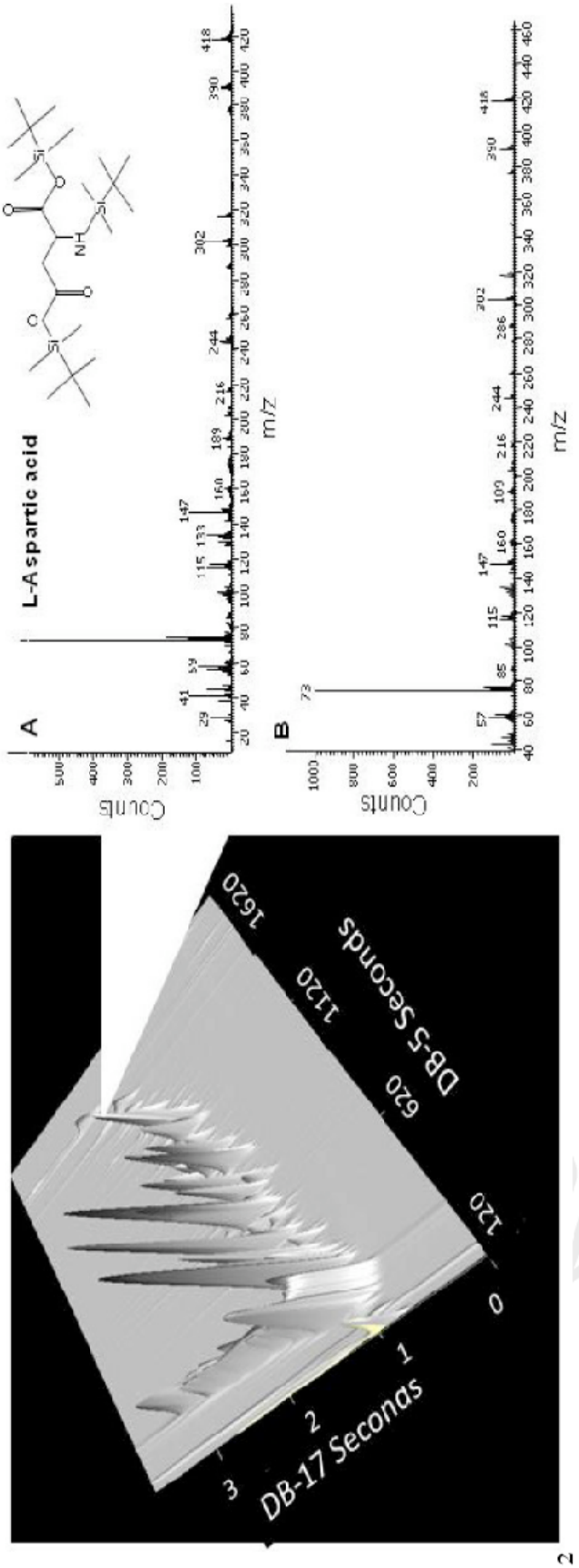

Figure 12.100. Two-dimensional chromatogram and mass spectra for down-regulated 393 metabolite L-aspartic acid for the atrazine-exposed and control comparison.
Left: Actual 394 mass spectra for L-aspartic acid and
Top: matched mass spectra from the NIST library.

Styrene/Butadiene/Acrylonitrile copolymer after pyrolysis

M. Pursch, W. Winniford, K. Sun, P. Eckerle, B. Gerhart, J. Griffith, S. Green, J. Luong, H. Cortes, *Comprehensive GC for industrial applications*, presented at First International Symposium on Comprehensive Multidimensional Gas Chromatography, Volendam, The Netherlands, March 6-7 2003

Instrumental conditions:

***Columns*:**
First: 25 m × 0.25 mm ID, 0.25 µm DB5
Second: 1.2 m × 0.10 mm ID, 0.2 µm CP-Wax
Modulation capillary:

Carrier gas: hydrogen @ 39 kPa

***Temperatures*:**
Main oven: 45°C (6 min), 10°C/min → 250°C (12 min)
Second oven:

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

Modulator: dual cryojets, liquid nitrogen

Modulation time: 6 s

Detector: FID
Temperature: 250ºC
Make up gas flow: 20 mL/min

Data acquisition: 200 Hz

Sample description and separation:

In-column Pyrolysis GC×GC was utilized to separate an emulsion copolymer made up of styrene and butadiene. An aqueous emulsion was injected to an in-column pyrolyzer coupled to the GC×GC system. Fragments that are produced by pyrolysis can be indicative of polymer composition. In this example it is seen that a huge amount of peaks are generated. Group type separation is observed and several aromatic/olefinic components are present in the pyrogram. GC×GC–TOF MS would be needed for exact identification, however the pyrolysis pattern in the two-dimensional separation space can be used as a screening tool for fast and very informative material characterization.

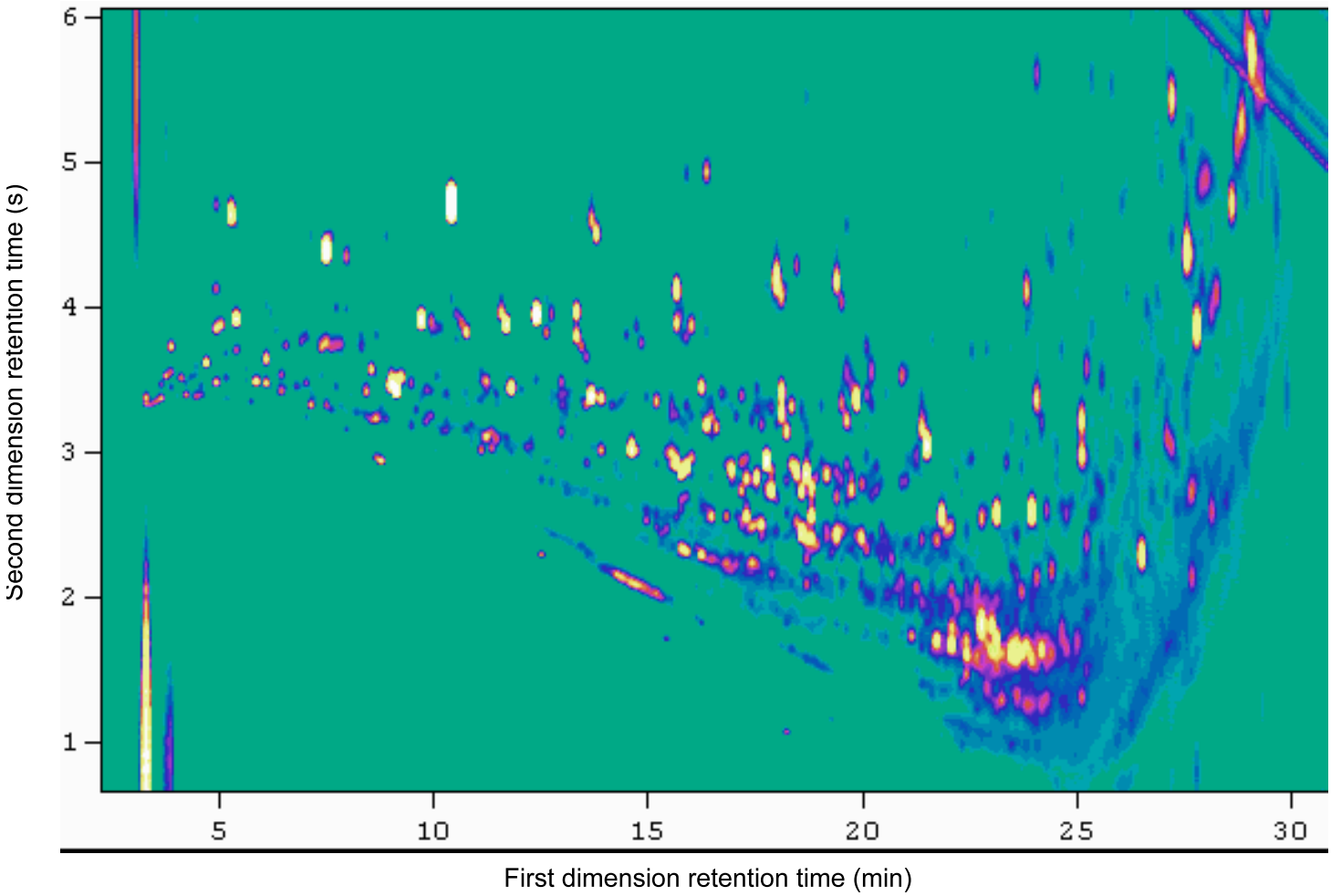

Figure 12.101. Colour plot of the pyrolysis product of styrene/butadiene/acrylonitrile copolymer.

Pyrolysis of polyethylene

M. Pursch, W. Winniford, K. Sun, P. Eckerle, B. Gerhart, J. Griffith, S. Green, J. Luong, H. Cortes, *Comprehensive GC for industrial applications*, presented at First International Symposium on Comprehensive Multidimensional Gas Chromatography, Volendam, The Netherlands, March 6-7 2003

Instrumental conditions:

Columns:
First: 30 m × 0.32 mm ID, 0.25 µm DB5
Second: 1 m × 0.10 mm ID, 0.1 µm DB-Wax
Modulation capillary:

Carrier gas: hydrogen, constant pressure @ 8 psi.

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

Second oven:

Injector: split
Temperature: 280°C
Injection volume:

Modulator: LMCS, −60°C

Modulation time: 5 s

Detector: FID
Temperature: 250°C
Make up gas flow: nitrogen, 30 mL/min

Data acquisition: 100 Hz

Sample description and separation:

Polyethylene, ~1 µg.

Pyrolysis System: CDS Instruments, Pyroprobe 2000
Pt coil with quartz tube and wool
Heat Rate: 50-600°C (20 s) at 20°C /ms

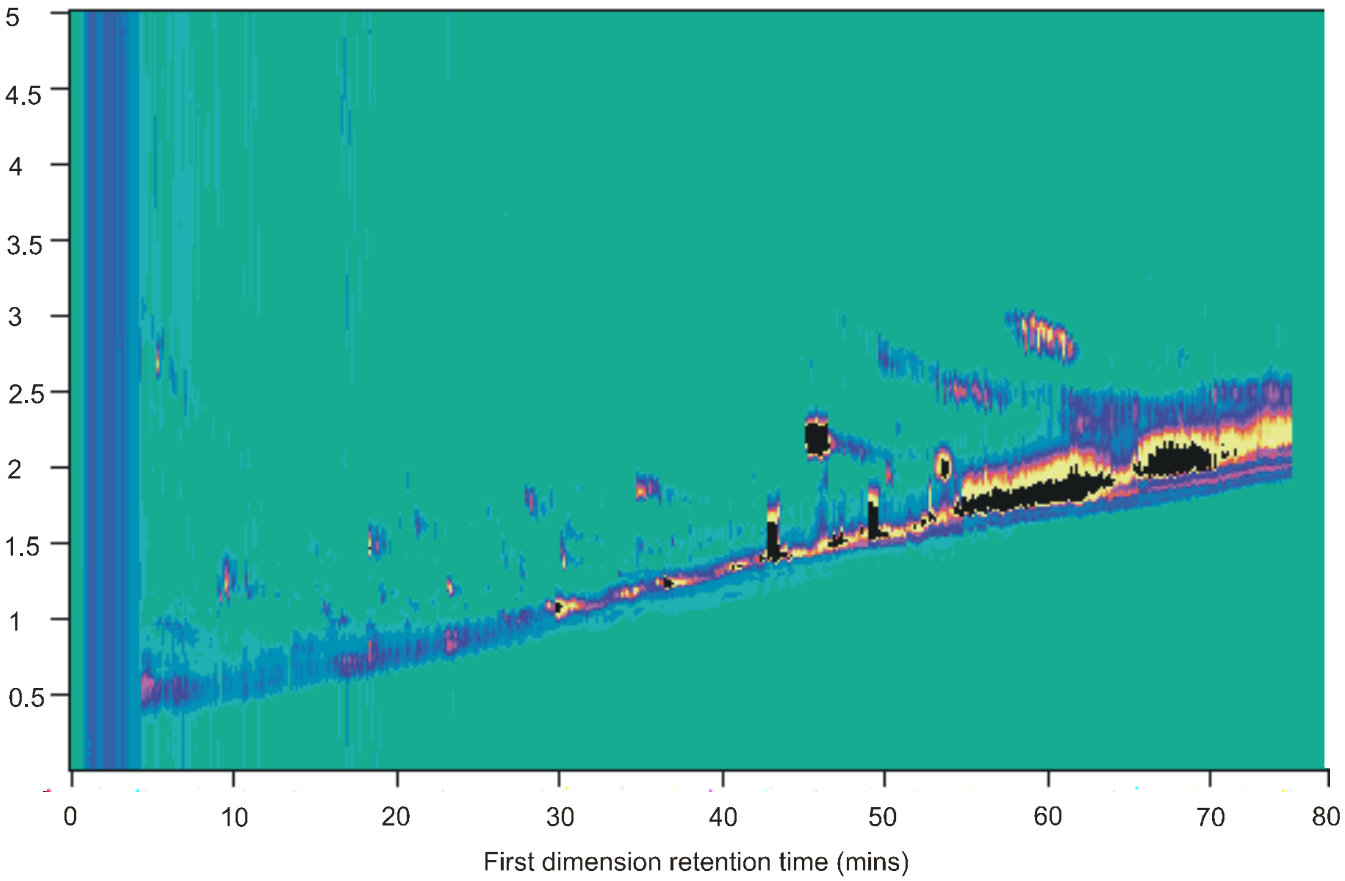

Figure 12.102. GC×GC plot of the pyrolisis product of polyethylene.

Industrial phenol additives

F.C-Y. Wang, *The applications of comprehensive two-dimensional gas chromatography in the petroleum industry*, presented at the First International Symposium on Comprehensive Two-Dimensional Gas Chromatography, Volendam, the Netherlands, March 2003

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 1.0 μm, SPB-5
Second: 3 m × 0.25 mm ID, 0.25 μm BPX50

Carrier gas: helium, constant pressure @ 45psi.

***Temperatures*:**
Main oven: 90°C, 1.5°C/min → 180°C
Second oven:

Injector: split, ratio 1:100
Temperature: 300°C
Injection volume: 0.2 μL

Modulator: quad-jet cryomodulator

Modulation time: 10 s

Detector: FID
Temperature:
Make up gas flow:

Data acquisition: 100 Hz

Sample description and separation:

This sample of technical phenol-additives consists of isomers of alkylated phenols, carrying 6 through 12 C-atoms in the alkyl part. As is evident from the Fig., in the group-type separation compounds containing the same number of C-atoms are nicely grouped together and separated from other groups. Within each group, a large number of isomers consist.

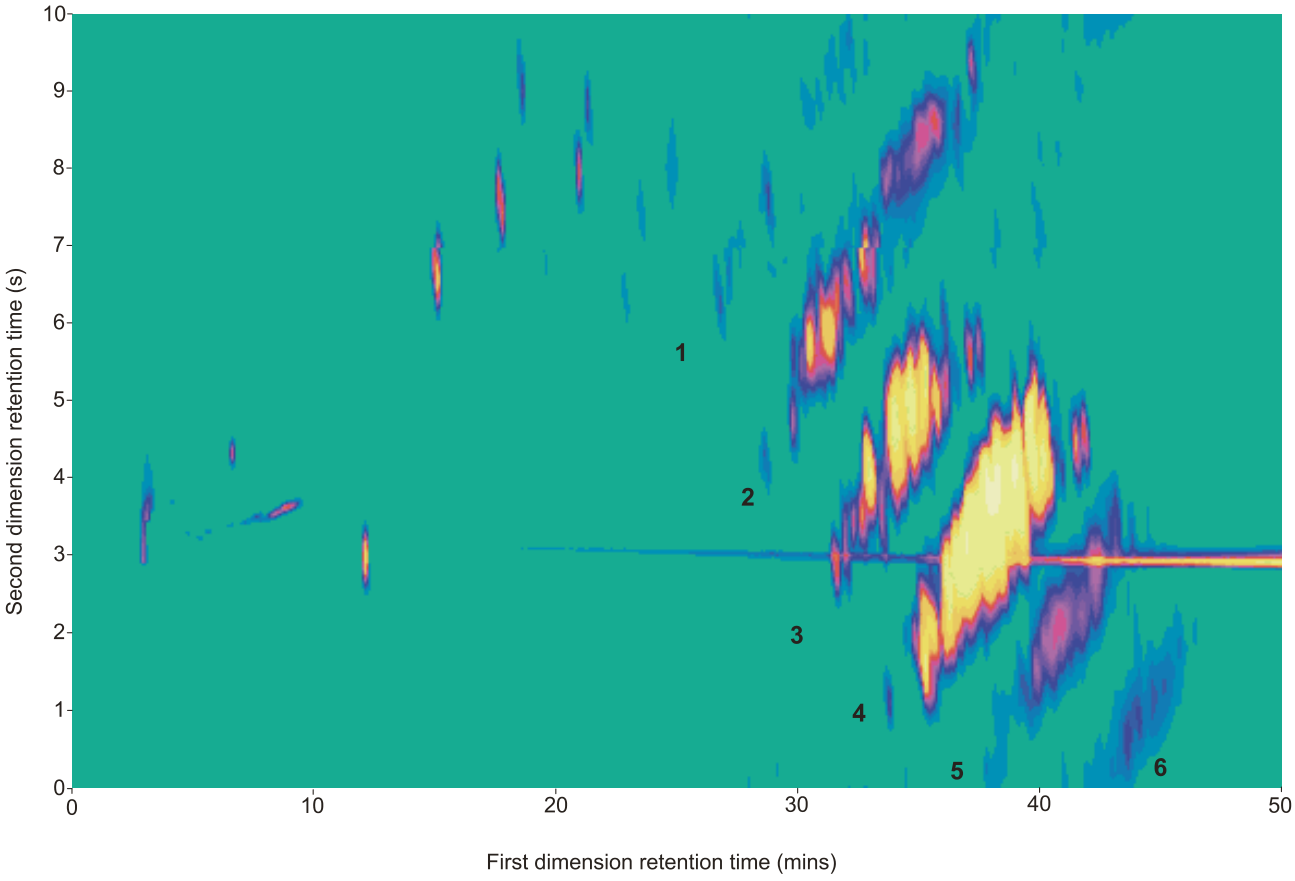

Figure 12.103. Colour plot of the GC×GC separation of an industrial alkyl phenol mixture. The different groups are separated according to the number of carbon atoms in the alkyl part. Identification: 1. C_6-phenol, 2. C_7-phenol, 3. C_8-phenol, 4. C_9-phenol, 5. C_{10}-phenol, C_{11}-phenol.

Industrial Alcohol Solvent

F.C-Y. Wang, *The applications of comprehensive two-dimensional gas chromatography in the petroleum industry*, presented at the First International Symposium on Comprehensive Two-Dimensional Gas Chromatography, Volendam, the Netherlands, March 2003,

Instrumental conditions:

***Columns*:**
First: 30 m × 0.25 mm ID, 1.0 μm, SPB-5
Second: 3 m × 0.25 mm ID, 0.25 μm BPX50

Carrier gas: helium, constant pressure @ 45 psi.

***Temperatures*:**
Main oven: 90°C, 1.5°C/min → 180°C
Second oven:

Injector: split, ratio 1:100
Temperature: 300°C
Injection volume: 0.2 μL

Modulator: quad-jet cryomodulator

Modulation time: 10 s

Detector: FID
Temperature:
Make up gas flow:

Data acquisition: 100 Hz

Sample description and separation:

Industrial primary alcohol solvent. The major portion is $C_{13}OH$, with all isomers in the alkyl part. However, the solvent also contains $C_{11}OH$, $C_{12}OH$ and small amounts of $C_{14}OH$. The compounds are ordered in clusters with the same number of carbon atoms. The degree of branching of the alcohol increases with decreasing first and second dimension retention times.

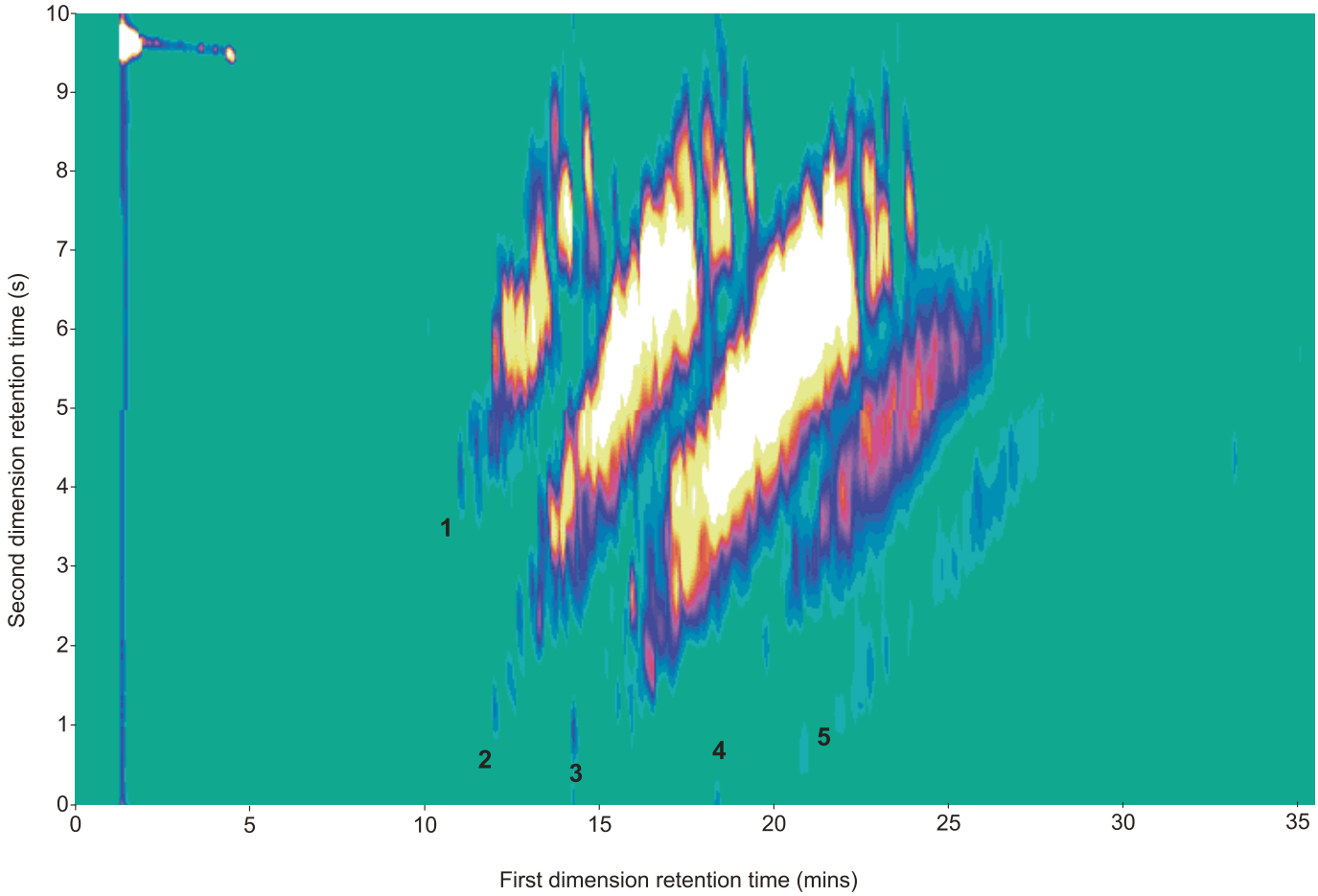

Figure 12.104. Colour plot of a GC×GC separation of an industrial alcohol mixture.

The different groups are separated according to the number of carbon atoms in the alkyl chain. 1. $C_{11}OH$, 2. $C_{12}OH$, 3. $C_{13}OH$, 4. $C_{14}OH$, 5. $C_{15}OH$

Glycol-derivatives mixture

J. Blomberg, *A crosslight on GC×GC*, presented at the First International Symposium on Comprehensive Two-Dimensional Gas Chromatography, Volendam, the Netherlands, March 2003,

Instrumental conditions:

***Columns*:**
First: 10 m, 0.25 mm ID, 0.25 µm DB1
Second: 0.8 m, 0.10 mm ID, 0.1 µm BPX50
Modulation cap.:

Carrier gas: helium @ 200 kPa

***Temperatures*:**
Main oven: 40°C (5 min), 2.5 °C/min → 275°C (10 min)
Second oven: 65°C (5 min), 2.5 °C/min → 300°C (10 min)

Injector: PTV, split, ratio 1:100
Temperature: → 350°C
Injection volume: 0.2 µL

Modulator: Sweeper

Modulation time: 7.5 s

Detector: FID
Temperature: 375 °C
Make up gas flow: nitrogen, 25 mL/min

Data acquisition: 50 Hz

Sample description and separation:

The sample is an industrial product, containing a large number of different isomers of polyethyleneglycol. The separation of glycol-derivatives mixture, indicates that a large number of isomers are separated. Because of the clustering, identification of the various spots is relative easy. Compounds containing the same number of C-atoms, have more or less identical first dimension retention times, where compounds with the same chemical/stereometrical composition have more or less the same second dimension retention.

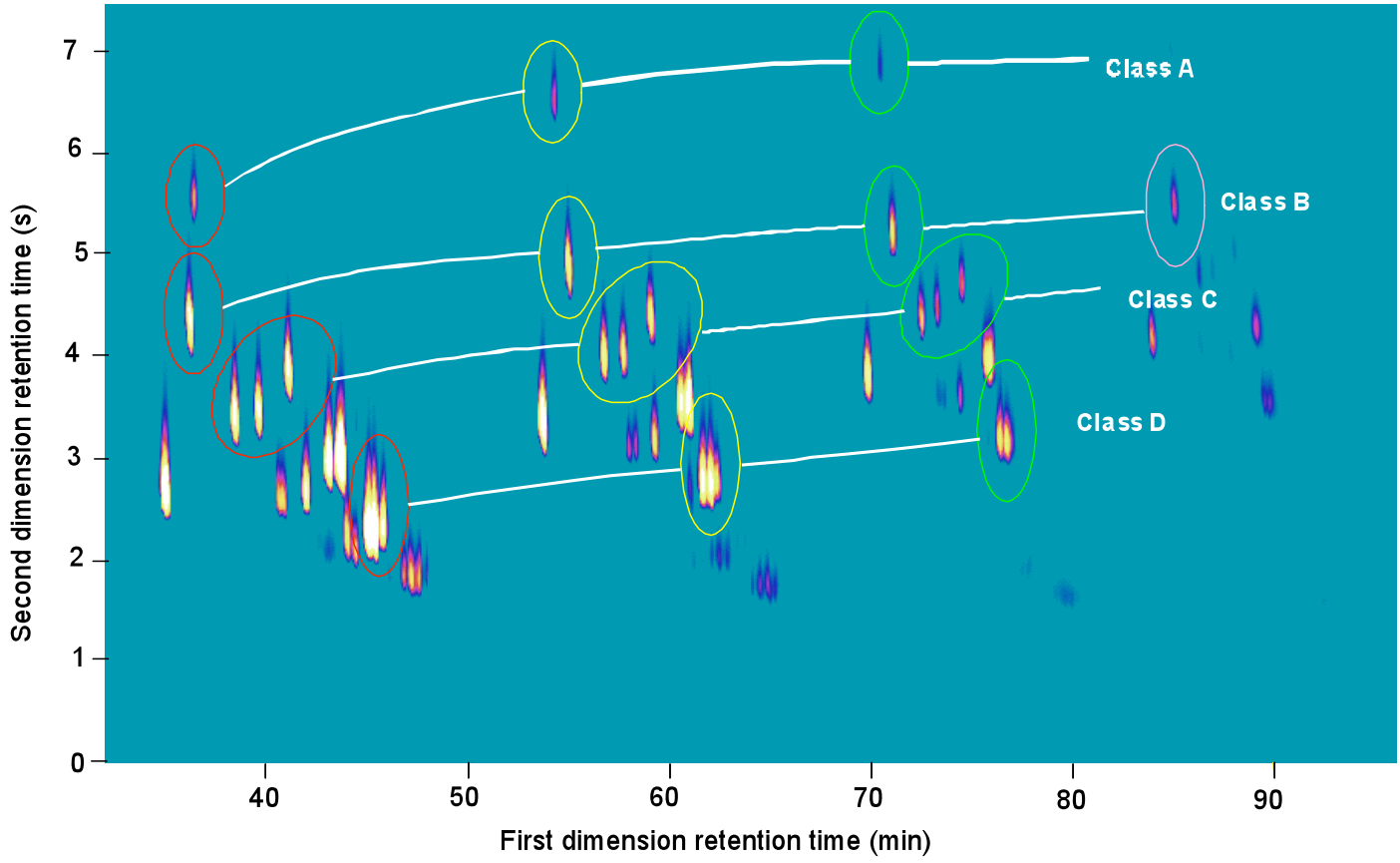

Figure 12.105. GC×GC colour plot of the separation of glycol-derivatives mixture, showing separation of all possible isomers.
The coloured polygons designate the components with the same number of C-atoms, where yellow is the highest number, green one C-atom less, and pink again one C-atom less. The white curves designate the clusters with the same chemical/stereometrical composition.

Molecular interconversion behaviour

P. Marriott, K. Aryusuk, R. Shellie, D, Ryan, K. Krisnangkura, V. Schurig, O. Trapp, *Molecular interconversion behaviour in comprehensive two-dimensional gas chromatography,* J. Chromatogr. A 1033 (2004) 135-143

Instrumental conditions:

***Columns*:**
First: 25 m × 0.22 mm ID, 0.25 μm BP21
Second: 0.8 m × 0.1 mm ID, 0.1 μm BP20

Carrier gas: hydrogen (?) @ 20 psi

***Temperatures*:**
Main oven: 70°C (2.5 min), 5°C/min → 100°C

Injector:
Temperature:
Injection volume:

Modulator: LMCS

Modulation time: 3 s

Detector: FID
Temperature:
Make up gas flow:

Data acquisition:

Sample description and separation:
Dynamic comprehensive two-dimensional gas chromatography (DGC×DGC) provides information on dynamic molecular behaviour (interconversion), with the interconversion process occurring on both columns. The extent of interconversion depends on prevailing temperature, retention time, and the phase type of the stationary phase. Polyethylene glycol-based phases (e.g. BP20) were found to result in high interconversion kinetics, although terephthalic acid-terminated polyethylene glycol (e.g. BP21) had a lesser extent of interconversion. Much less interconversion was seen for phenyl-methylpolysiloxane and cyclodextrin phases. The interconversion of E-Z isomerisation about partial-double bonds of two oximes (acetaloxime and butyraldoxime) is shown at specific temperatures (temperature programming).

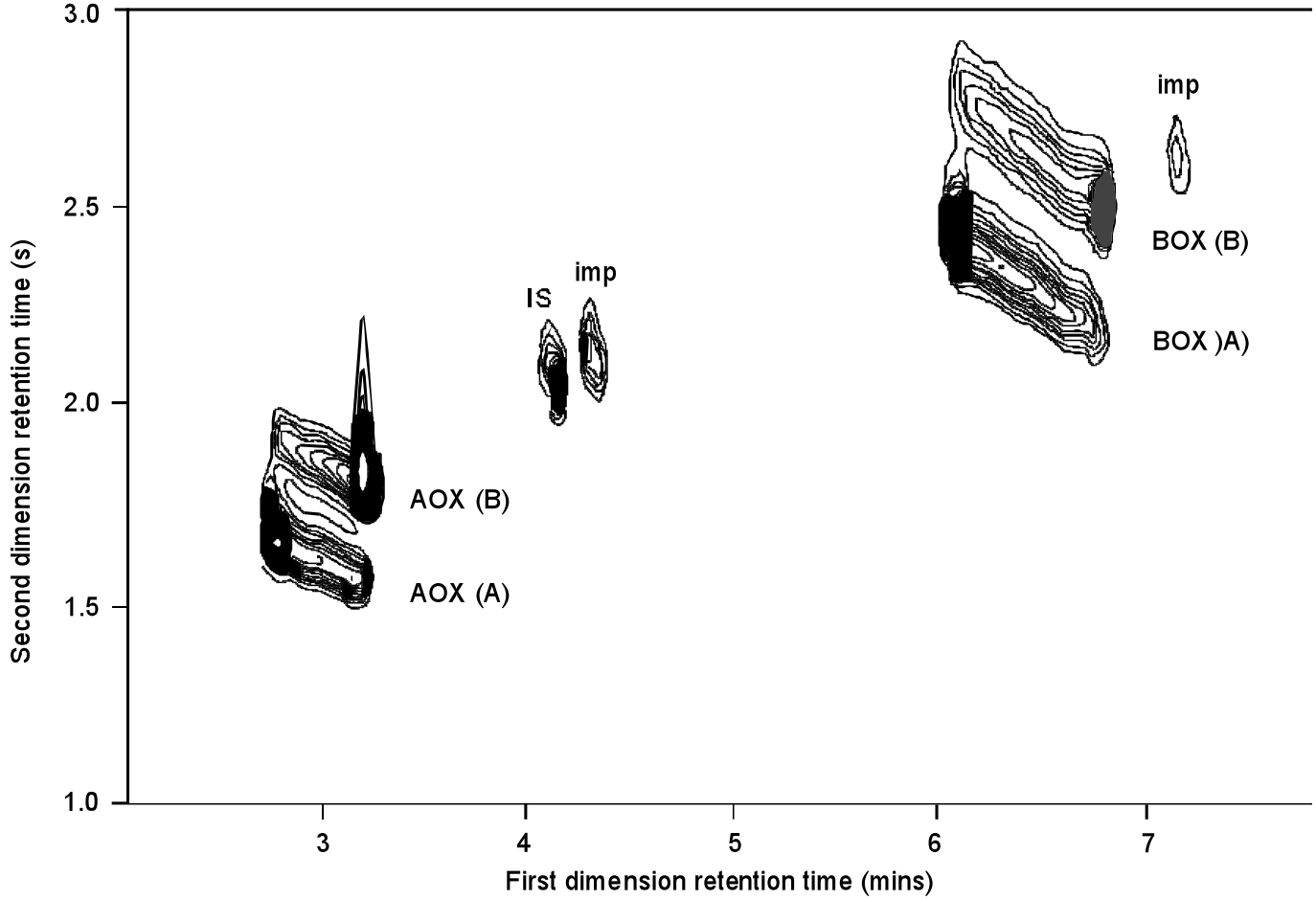

Figure 12.107. Contour plot of the interconversion of the E and Z isomers of actealdoxiem and butyraldoxime.
AOX = acetaldoxime, BOX = butyaldoxime, IS = internal standard (alcohol), imp = impurity in the starting material.

List of abbreviations

FID	flame ionization detector
(μ)ECD	(micro) electron capture detector
SCD	sulphur chemiluminescence detector
NCD	nitrogen chemiluminescence detector
FPD	flame photometric detector
PFPD	pulsed flame photometric detector
AED	atomic emission detector
EAD	electronic antenna detector
ToF-MS	time-of-flight mass spectrometer
ENCI	electronegative chemical ionization
EI	electron impact
Quad-MS	quadrupole mass spectrometer
PID	pulsed ionization detector
PTV	programmed temperature vaporizer
LMCS	longitudinal modulating cryogenic system
EPC	electronic pressure controller
SPME	solid phase micro extraction