COMPREHENSIVE TWO-DIMENSIONAL GASCHROMATOGRAPHY

The state-of-separation-arts

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THEORY PART 2

5. Columns and column combinations

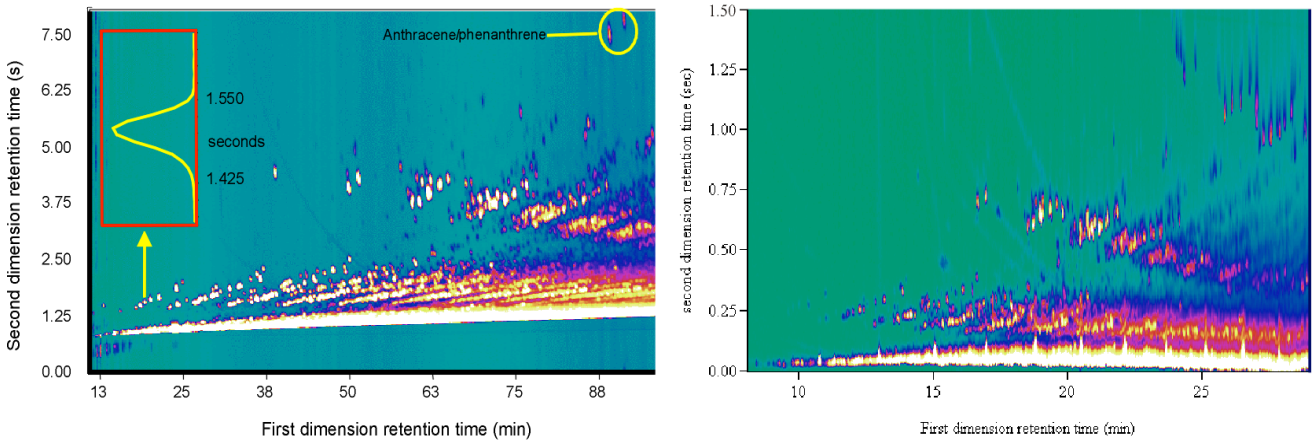
5.1 Column dimensions

As already pointed out in chapter 3, in GC×GC the second column must be such fast in comparison to the first-dimension column that it finishes its separation in the short time span of the selected cut from the first-dimension peak. Assuming at least five cuts over a first-dimension peak and a separation of every cut on the second-dimension column having a peak capacity of at least 30, the speed of separation of the second-dimension must be at least 30 × 5 = 150 times higher than that of the first-dimension column.

Generally, a conventional "normal-bore" capillary column, *e.g*. 10 m, 0.25 mm ID, d_f = 0.25 mm, is used in the first dimension. An important reason in choosing these dimensions is the fact that such a column often is already in use for the 1D-analysis of the sample under consideration. All of the retention characteristics that are known for this column and probably implemented in a 1D-GC method can now be used in the GC×GC separation. A slow temperature program will provide peaks from such a column having widths in the order of ten seconds. The second-dimension column that fits with such a first-dimension column should separate the five consecutive cuts of a first-dimension peak then within the duration of the first-dimension peak, *i.e*. ten seconds. This means that each individual second-dimension separation should be finished within two seconds. Generally a short 0.1mm ID column with a thin film of stationary phase (*e.g*. 0.1 μm) is used as the second dimension, but such a column needs around six to ten seconds in finishing the necessary separation. So, in many GC×GC systems the choice is made to deliberately slowing down the first dimension separation such that it provides peaks with widths of about 30 seconds (see also Fig. 3.9, 3.10 and 3.11) that consequently have lower amplitudes. This means that in many GC×GC systems the first dimension column is not used at its optimum resolution. The increase in separation power and in the enhancement of peak amplitudes that will be accomplished by using GC×GC as compared to 1D-GC is therefore exaggerated in some publications.

A much better fit between the two dimensions is presented in [64]. Here the second dimension consists of a 0.5 m of a 50 μm ID column with a 50 nm film thickness. Cuts from the first dimension now can be separated within one or two seconds and still provide the necessary peak capacity. In order to diminish the linear velocity in this narrow-bore column to an acceptable level, the first dimension flow has to be decreased to a rather low value, just at the lower side of the optimum H/ū-curve of the van Deemter equation. But the temperature programming can be made quite fast allowing the resolution in the first dimension to be

optimal. Fringe benefit of such a combination is the gain in speed of the total system. It was demonstrated that by employing the first column at its optimum, the separation speed could



be enhanced by a factor of four without surrendering separation efficiency (see Fig.5.1).

Figure 5.1. Conventional and fast GC×GC separation of a gasoil.

***Left**: secondary column of 0.1 mm ID, modulation time 7.5 s.**Right**: secondary column of 0.05 mm ID, modulation time 1.5 s*

The proper column dimensions and column combination, needed for a specific separation, can also be predicted by calculating the optimum flows. This can easily be done according to the calculation procedure outlined in chapter 7.3 C*arrier gas flows*.

5.2. Stationary phases

It is clear that only a limited number of stationary phase combinations are suitable in accomplishing an orthogonal separation in GC×GC. In general the separation mechanisms of the two columns must be such different that they in fact are independent. In order to get the maximum advantage of retention behaviour of compounds that is already common knowledge from 1D-GC separations, the first-dimension column is often chosen to be the column that is already in use in the 1D-GC analysis. For the majority of the separations therefore a strictly non-polar poly-(dimethylsiloxane) such as OV-1, DB-1, BPX1, HP-1, CP-Sil 5 or Rtx-1 is used. The somewhat less non-polar phases containing 5% phenyl, such as DB-5, BPX5, HP-5, CP-Sil 8 or Rtx-5 are also quite popular, since very polar compounds will exhibit less tailing on these columns.. The secondary column must have a specific separation mechanism, as polarity or shape-selectivity. Common phases are OV-1709 (14% phenyl), BPX 35 (35% phenyl in the backbone of the siloxane), BPX50 (50% phenyl in the backbone of the siloxane), or Carbowax (polyethylene-glycol).

Shape-selective columns can be used both as the first as well as the second dimension. Such a column as a first dimension (liquid crystalline phase LC−50) and a non-polar column (5% phenyl-polydimethylsiloxane) as the second offer the separation of toxic planar PCBs from other congeners as is described in [65]. A combination of two different shape-selective columns (a permethylated β-cyclodextrin phase in the first, and a liquid crystal phase in the second) is demonstrated in [66], which system enabled the separation of six out of nine atropisomeric PCBs. In two papers the use of a chiral column in GC×GC for essential oil analysis is described. The chiral column as the first dimension column in [67] and the chiral column in the second dimension in [68]. Table 5.2 gives the trade names, composition and manufacturers of all stationary phases mentioned in this book.

Table 5.2. Trade names, composition and manufacturers of stationary phases.

Trade name	Manufacturer Distributor	Composition
OV-1 gum	Ohio Valley [1]	100% methylpolysiloxane
OV-17	Ohio Valley	50%-phenyl-methylpolysiloxane
DB petro	J&W [2]	100% dimethylpolysiloxane
DB-1	J&W	100% dimethylpolysiloxane
DB-17HT	J&W	50%-phenyl-methylpolysiloxane
DB-5	J&W	5% phenyl- methylpolysiloxane
DB-17	J&W	14% cyanopropylphenylpolysiloxane,
DB21 (210)	J&W	50%-trifluoropropyl-methylpolysiloxane
DB624	J&W	6% cyanopropylphenyl-dimethylpolysiloxane
DB-Wax	J&W	polyethyleneglycol
DB-XLB	J&W	proprietary
BP-1	SGE [3]	100% dimethylsilphenylene
BP-20	SGE	polyethyleneglycol
BP-21	SGE	polyethyleneglycol TPA treated
BPX5	SGE	5% phenyl polysilphenylene-siloxane
BPX35	SGE	35% phenyl polysilphenylene-siloxane
BPX50	SGE	50% phenyl polysilphenylene-siloxane
HT5	SGE	5% phenyl-methylpolysiloxane (carborane)
HT8	SGE	8% phenyl-methylpolysiloxane (carborane)
SE30	GE [4]	100% methylpolysiloxane
SE52	GE	5% phenyl-methylpolysiloxane
SE54	GE	50% phenyl-methylpolysiloxane
007-1	Quadrex [5]	100% dimethylpolysiloxane
007-65HT	Quadrex	65% phenyl-dimethylsiloxane
007-1701	Quadrex	14% cyanopropylphenyl-methylpolysiloxane

Rtx-1	Restek [6]	100% methylpolysiloxane
Rtx-5	Restek	5% diphenyl/95% dimethylpolysiloxane
Rtx-wax	Restek	polyethyleneglycol
β-DEX 120	Sigma-Aldrich [7]	nonbonded, 20% permethylated γ-cyclodextrin in poly(35% diphenyl/65% dimethylsiloxane)
SP-2340	Sigma Aldrich	100% cyanopropyl silicone
CP-Sil 5CB	Varian/Chrompack [8]	100% methylpolysiloxane
Chirasil-Dex	Varian/Chrompack	β-cyclodextrin bonded to polysiloxane
CP-Sil 88	Varian/Chrompack	cyanopropyl-based siloxane
CP-Wax-52	Varian/Chrompack	polyethyleneglycol
CycloSil-B	Varian/Chrompack	β-cyclodextrin
VF-23	Varian/Chrompack	70-90% cyano containing polymer
SBP-1	Supelco [9]	100% methylpolysiloxane
SPB-5	Supelco	5% phenyl-methylpolysiloxane
Supelcowax 10	Supelco	polyethyleneglycol
SBPwax	Supelco	polyethyleneglycol
EtTBS-β-CD	Mega [10]	25% 2,3-diethyl-6-*tert* butyldimethylsilyl β-cyclodextrin in PS086
Megawax	Mega	polyethyleneglycol
PS-086	Mega	100% dimethylpolysiloxane
PS-089	Mega	5% phenyl-methylpolysiloxane
PS-225	Mega	14% cyano substituted polysiloxane
HP1B	Agilent [11]	100% methylpolysiloxane
PONA	Agilent	100% methylpolysiloxane
HP5	Agilent	5% phenyl-methylpolysiloxane
Carbowax	Agilent	polyethyleneglycol
LC-50	J&K [12]	50% liquid crystalline-methylpolysiloxane
BGB-176SE	BGB [13]	20% 2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl β-cyclodextrin in SE-52
BGB-1701	BGB	14% cyanopropylphenyl-dimethylpolysiloxane
SOP-50	[14]	50% phenyl-methylpolysiloxane

[1] Ohio Valley Specialty Company, OH, USA. [2] J&W Scientific, Folsom, CA, USA. [3] SGE International, Ringwood, Victoria, Australia. [4] General Electric Company, Silicone Division, USA. [5] Quadrex, New Haven, CT, USA. [6] Restek Corporation, Bellefonte, PA, USA. [7] Sigma-Aldrich, Brussels, Belgium,
[8] Varian/Chrompack, Middelburg, the Netherlands, [9] Supelco, Bellefonte, PA, USA. [10] Mega, Milan, Italy. [11] Agilent, Palo Alto, CA, USA. [12] J&K Environmental, Sydney, NS, Australia. [13] BGB Analytik, Aldiswil, Switzerland. [14]

It is worthwhile noting that, although the most general used combination of columns is the non-polar *versus* polar combination, with that respect that the first dimension is generally the non-polar column, also the reverse combination or a combination of different polar columns might provide interesting separations. It was already considered in previous

publications [69], *viz.* the polar *versus* non-polar combination, designated as *reversed-phase GC×GC*, but never demonstrated in practice. In the paper of Adahchour [70] a very peculiar separation of an oil fraction on a high-polar *versus* low-polar combination has been demonstrated. From this publication it is clear that only the full use of the separation plane is achieved when the polarities of the two dimensions are quite different. When using a Carbowax (polyethyleneglycol) in the first dimension, non-polar compounds will elute at very low retention temperatures (See also Fig. 5.2). These compounds will exhibit quite high retention times at these low temperatures on the low-polarity (BPX35 = 35% phenylene in a siloxane backbone) secondary column. The more polar compounds as mono- and di-aromatics will have higher retention times on the first column and consequently elute at relative higher temperatures, thus providing far lower secondary retention times. So, now the structures as they are achieved in the separation plane are more or less reversed compared to the normal non-polar *versus* polar combination. If it is aimed to get more separation detail in the non-polar part of analytes that are contained in a more polar matrix, such a combination could provide the solution to the analysis problem.

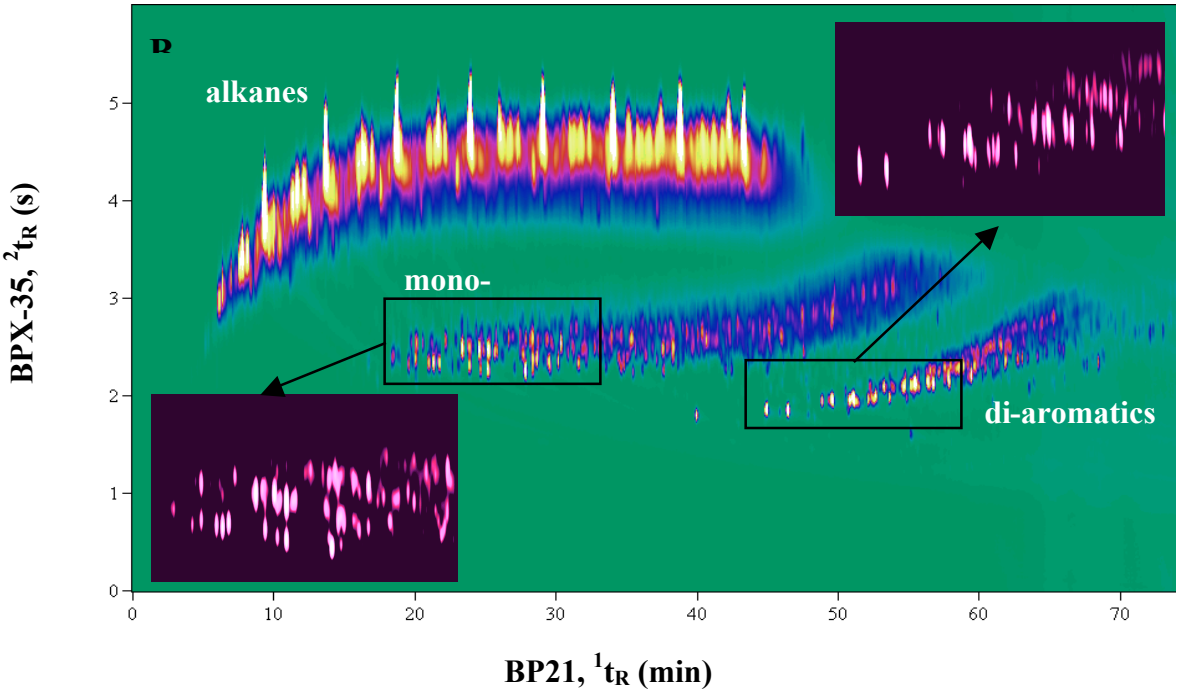


Figure 5.2. GC×GC–FID chromatograms of a diesel oil sample analysed on a BP21 × BPX-35 column set. The two columns were, temperature-programmed independently (20 °C temperature difference). Note that the mono- and di-aromatics have the shortest retention on the second dimension. The alkanes and cyclo alkanes have very large second dimension retention times and exhibit a double wrap-around (alkanes: 15 s and cyclo alkanes 13 s). Also note the high resolution between the non-polar alkanes and cyclo alkanes.

From the group of Górecki, a paper has been presented discussing the effect of film thickness in GC×GC [130] as a means to prevent column overload in the second dimension. Column overloading in GC×GC is a considerable problem, aggravated by the fact that two columns are involved. Broad first-dimension peaks of an analyte help produce smaller fractions of the analyte in the second-dimension, reducing the chance of secondary column overloading. One of the means to generate broad peaks in the first-dimension is to use thick film primary columns. A series of primary columns of various film thickness were tested in the study, and the results indicate that when other conditions are kept constant, 1μm film columns often provide better resolution in both first and second-dimension but at the expense of a much longer separation time; 0.1 μm is clearly inadequate for GC×GC separation; 0.5 and 0.25 μm film columns seem to be the best compromises.

5.3. Dual-secondary column systems

An interesting possibility in GC×GC column combinations is the use of a dual parallel secondary column system by splitting the focused pulse and direct it to two parallel secondary columns instead of a single one. This was first described by Seeley [56, 57], who used an effluent splitter just behind the modulation valve for directing the selected fractions to two parallel and different secondary columns. The resulting technique, dual-secondary column GC×GC (GC×2GC) produces a pair of two-dimensional contour or colour plots in a single run rather than a single one. The non-polar primary column interacts largely through dispersive forces, a polyethylene glycol secondary-column exhibits strong hydrogen bonding interactions, and a trifluoropropyl-methyl polysiloxane secondary-column exhibits strong dipole-dipole interactions. It was demonstrated that the dual secondary column configuration can increase the resolution and qualitative information supplied by GC×GC analysis. The largest improvement was observed for mixtures containing compounds with a wide range of dipole moments and hydrogen bond acidities such as alcohols, ketones, and chlorinated compounds. See also Chapter 12, *Perspectives and future developments*.

5.4. Twin GC×GC system

An even more ingeneous system using two different column combinations at the same time has been presented by Adachour *et al.* in a so-called “twin system” [58]. This twin GC×GC system (or 2GC×2GC) has been designed such to enable the analysis of a sample by means of two different and independent column combinations simultaneously. Both

combinations are incorporated in the same oven, using the same temperature programme, and are fed using a 50:50 column-entrance-split. The effluents of both first columns are modulated by the same cryogenic modulator [40]. It is demonstrated that, employing combinations of a conventional non-polar × polar and a reversed-type polar × non-polar column set, the information content is as high, and the analytical performance is as good as when using two separate GC×GC systems independently. That is, there is an appreciable gain of time and a reduction of costs without any loss in quality. The general usefulness and performance is further illustrated with the analysis of pollutants in a sediment sample in the twin system using an orthogonal and a non-orthogonal column set and the comparison of these with two mutually different GC×GC chromatograms. (Fig. 5.4).

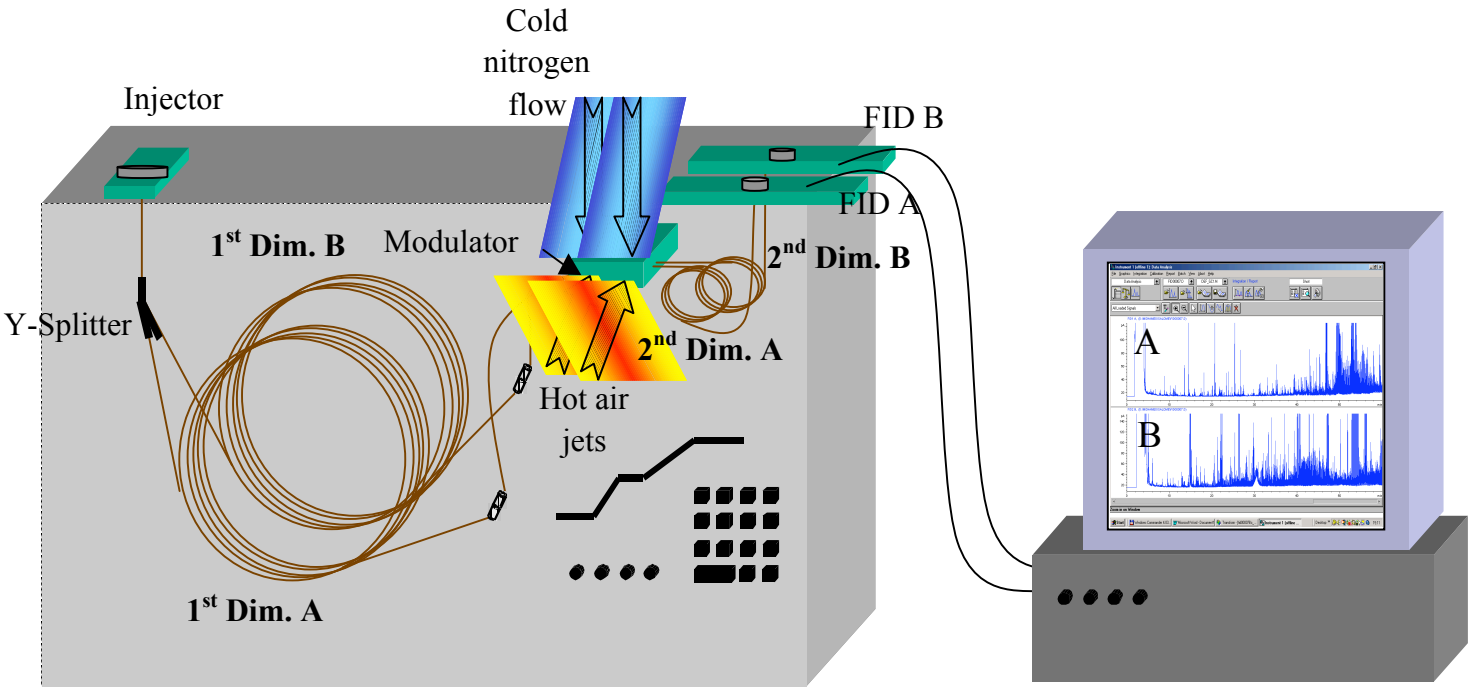


Figure 5.3. Schematic of the twin GC×GC system with its two column sets, A and B. For more details see [58].

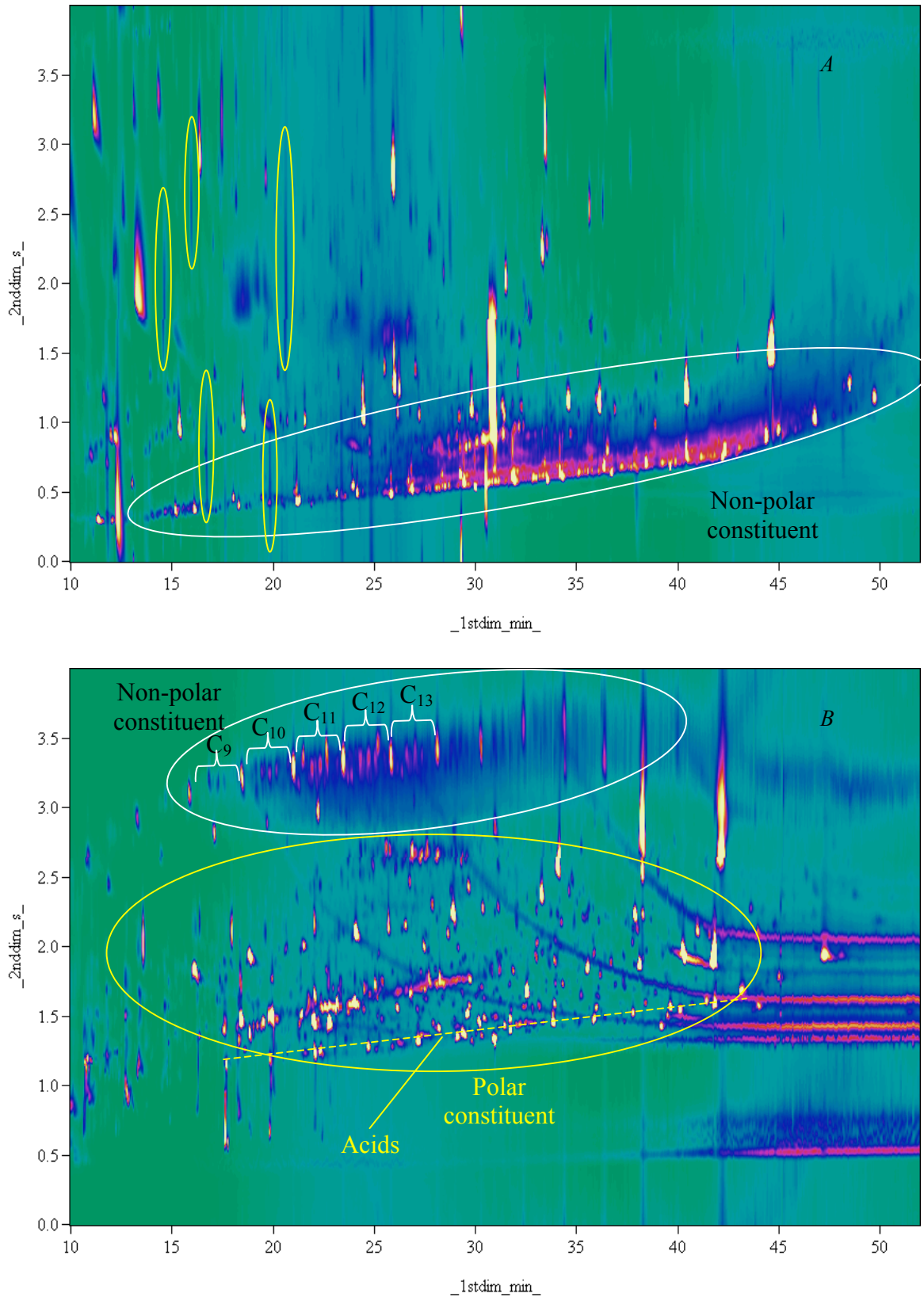


Figure 5.4. Twin GC×GC–FID chromatograms of a marine sediment extract: ***A****, orthogonal;* ***B****, non-orthogonal.*

5.5 The use of retention indices and prediction of GC×GC separation

Since in GC×GC the two columns are coupled in series, the mass flow of the carrier gas through both columns is the same. Consequently, the average linear gas velocity in the two columns, having different internal diameters is quite different. And because the columns, located in the same oven, have the same temperature, both carrier gas velocity and temperature affect the separation simultaneously, but differently. It is therefore rather complicated choosing the appropriate columns and column combination needed for a specific separation that is envisaged. The construction of the column set and the determination of the eventual GC×GC chromatogram is a rather labour-intensive task. Therefore several authors have reported on calculation programs with which a simulation or prediction of the GC×GC separation can be made. Beens *et al*. [69] used linear retention indices in the first and Kováts retention indices in the second column for that purpose. Since in normal use it is not possible including more than one single n-alkane in the same second dimension separation, they made use of the "iso-volatility lines" (see also chapter 10.2 and Fig.10. 4) of continuous injected n-alkanes, enabling the calculation of the Kováts indices. This paper gives a complete calculation method for the simulation of the eventual separation. Western *et al*. [71] also used Kováts retention indices in the first dimension, but proposed using retention indices referring to other types of compounds for the second dimension. Since most (polar) compounds to be separated in the second dimension fall outside the boundaries of the n-alkanes retentions, they proposed using series of 2-methyl ketones and fatty acid methyl ester standards for this purpose. Enabling the elution in the same second dimension separation of multiple members of the same standards family, they made re-injections of the standards mix for separation on a regular time base during the analysis. In a next paper they also introduced linear alcohols to complete the series of possible polar compounds [72].

Analogous to 1D-GC analyses, retention indices can be used for identification purposes. But since in GC×GC two different and independent retention measures are available, the identification is much more assured. The software programs for data processing should incorporate a means of using both retention measures for identification purposes. This has been done by Zhu et al. [73], where they describe a method for the conversion of GC×GC retention indices among different column temperature conditions and based on some thermodynamics parameters. The calculation accuracies are better than 1.0 retention index unit. Furthermore, a retention index database was developed and used to identify the compounds in a cigarette essential oil sample. It showed that identification by the database

was of close agreement with by time-of-flight mass spectrometry, and some isomers could also be distinguished based on the retention index database. See also Chapter 10. *Data processing*.

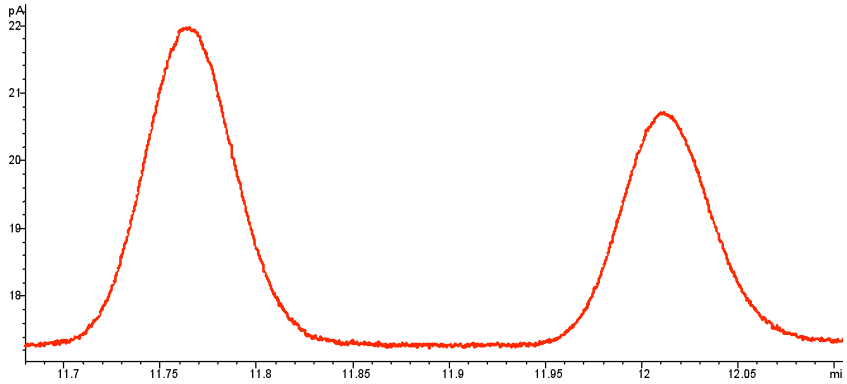
6. GC×GC Optimisation

6.1 The Phillips mix

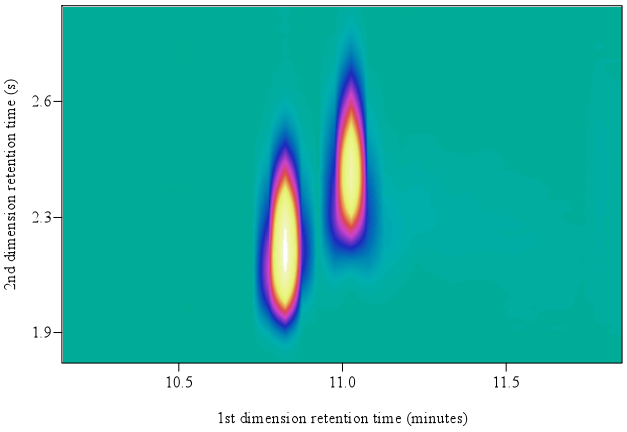
Apart from the choice of stationary phases and column dimensions, the optimisation of carrier flow and column temperature regimes, but also the modulation time and modulation temperature are important issues in GC×GC. Since most of these parameters are interrelated, the optimisation process is far more complicated than that in 1D-GC and is as yet not a straightforward method. Although experienced GC×GC users may find this not particularly challenging, it is not a standardised procedure. In that respect it is worthwhile mentioning the so-called "Phillips Mix" [74], a mixture of compounds selected to test and characterise the performance of the GC×GC set, comparable to the way the "Grob Mix" is used to judge the performance of 1D-GC systems. In order to cover a reasonable range of chemical functionalities and thus provide more detailed information in the 2D-plane, the Phillips mixture contains much more and different compounds.

6.2 Modulation frequency

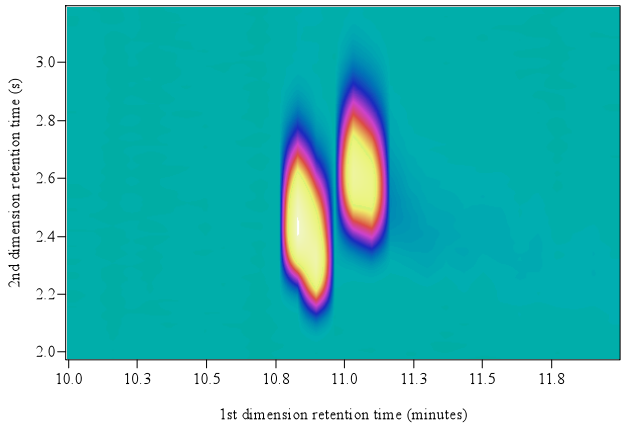
Before optimising the conditions of the instrumentation it should be emphasised that modifications of some of the settings of GC×GC will also influence others. A few examples may illustrate this. In chapter 3 it is mentioned that, for the preservation of the first-dimension separation throughout the total separative process, at least four cuts have to be made across a first-dimension peak. This is nicely illustrated in [76] in the separation of two closely related flavour compounds. *viz.* 1-octene-3-one and 1-octene-3-ol. As illustrated in Fig. 6.1, these two compounds were analysed with a GC×GC under different modulation times. Where the separation of the compounds have been preserved completely with a modulation time of three seconds (number of modulations over the first-dimension peak is four), the separation decreased using longer modulation times (less modulations over the first-dimension peak) and disappeared all together using only one modulation over a first dimension peak. The same phenomenon is also reported in [42].



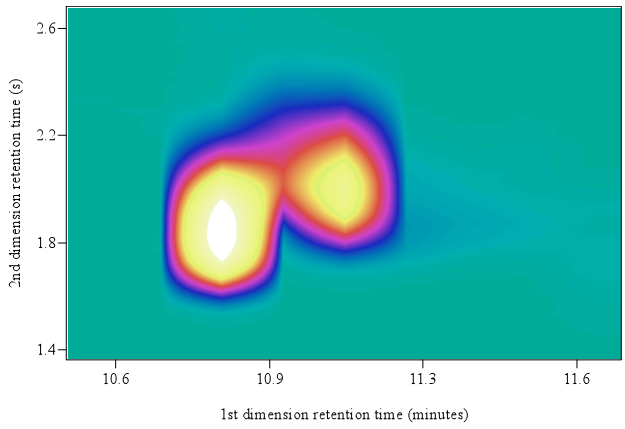
First-dimension separation of 1-octene-3-one and 1-octene-3-ol



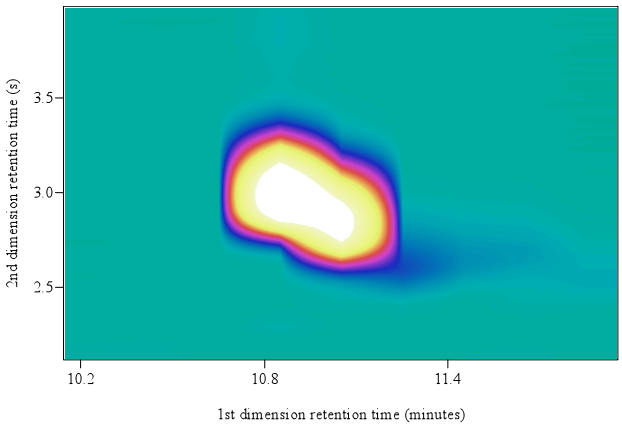
Modulation time 3 seconds



Modulation time 4 seconds



Modulation time 8 seconds



Modulation time 12 seconds

Figure 6.1. The effect of modulation time on preservation of the first-dimension separation.

6.3 Carrier gas flows

As far as carrier flow is concerned, the situation is more complex. Generally, the flow conditions of the first column are set according to the conditions of a 1D-GC column. This means that input pressures generally are chosen such that the void time of the column indicates the correct flow. But in view of the high resistance of the narrow-bore second column, the pressure inside the first column is rather high, and consequently, the diffusion in the first column very low. Beens *et al*. demonstrated that in their paper [75] describing the software programme they developed for calculating the flow and separation parameters of GC×GC column combinations. From these calculations it became apparent that different secondary column dimensions can be used, but using GC×GC always goes with a penalty of analysis time. Irrespective of the column dimensions that are used, the prize that has to be paid for using GC×GC instead of 1D-GC is always a longer analysis time. All combinations that are workable exhibit a longer analysis time than comparable 1D-GC analyses. This is mainly the result of slow diffusion in the first column as a result of an increased average column pressure. Moreover, when a narrow-bore second-dimension column is used, the first dimension separation has to be slowed down in order to be able to perform at least four second dimension runs during a first dimension peak. Practical means to do so include the use of slow temperature-programming or longer first columns. A final parameter to consider in the selection of the column set is the sample capacity of the second-dimension column. Wider columns offer a higher sample capacity (about the inner diameter to the third power). To illustrate the above, two column combinations (one with a 'conventional' narrow-bore second column and one with a wider bore second column) are used in the calculation program to produce the separation parameters as displayed in Fig. 6.2A and B.

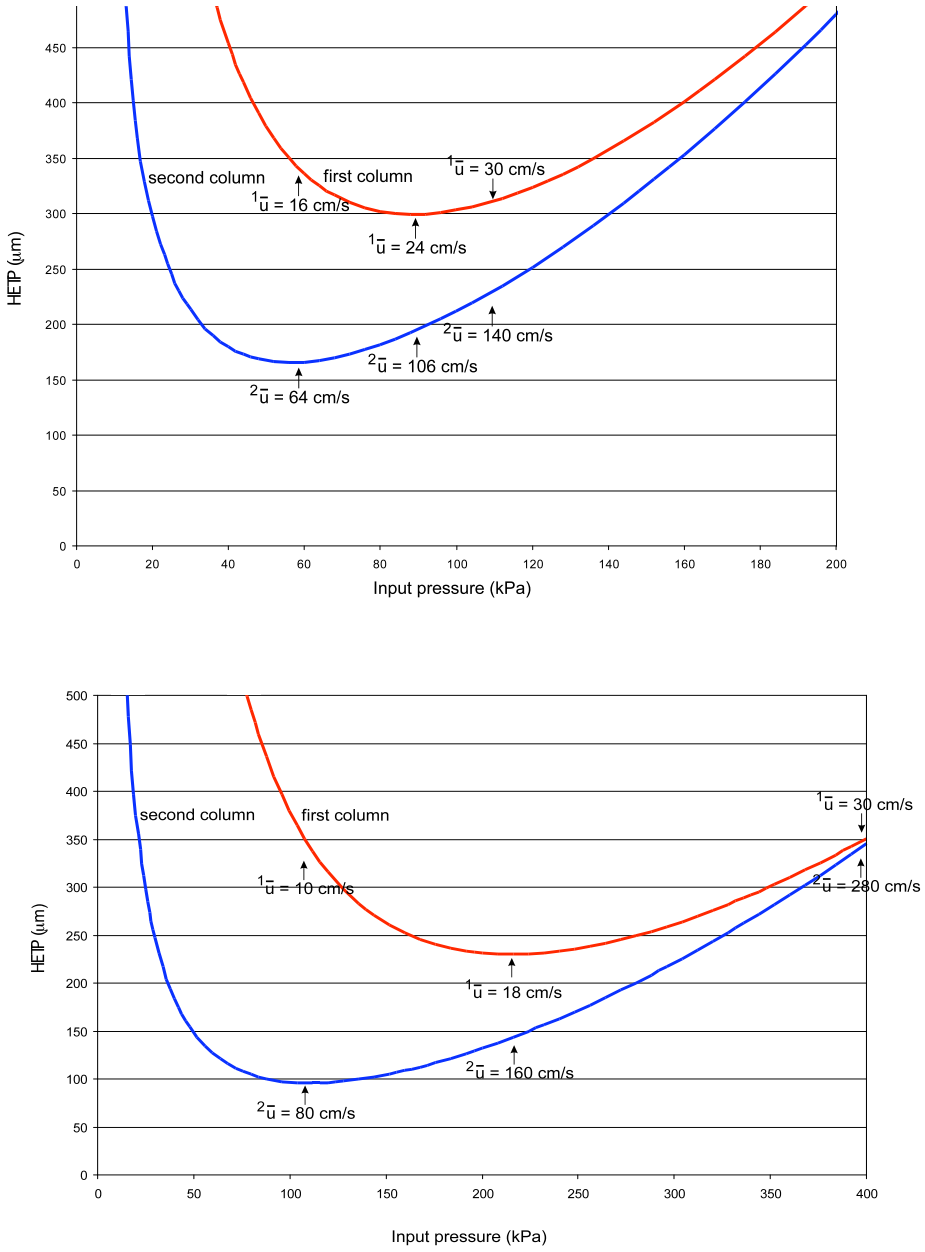


Figure 6.2 HETP versus input pressure for: A (upper) with 'conventional' column dimensions and B (lower) with wide-bore columns

In Fig. 6.2A the dimensions are 15 m × 0.25 mm + 1.5 m × 0.1 mm and the HETP displayed against the input pressure. Red for the first column and blue for the second. As can be seen, by choosing the flow such that the second column is at its optimum (^2N = 15,000), the average linear velocity of the carrier gas in the first column ($^1\bar{u}$) is only 10 cm/s and ^1N = 44,000. Applying the rule that the optimum temperature programming rate is about 10 ºC/void time, gives an optimum programming rate of 4 ºC/min. By choosing the optimum of the first column, $^1\bar{u}$ = 18 cm/s, ^1N = 65,000 and ^2N = 10,000. Optimum programming rate is now 7 ºC/min. Choosing a flow setting as would be done normally for 1D-GC, gives: $^1\bar{u}$ = 30 cm/s, ^1N = 40,000 and ^2N = 4,000 where the optimum programming rate would be 12 ºC/min. In all three cases peaks from the first column would have been too narrow to perform four

modulations across a peak and a slower temperature programming rate would have been needed to fulfil the modulation criterion.

In Fig. 6.2B the dimensions are 30 m × 0.32 mm + 1.5 m × 0.18 mm. In the optimum of the second column (^2N = 9,000), $^1\bar{u}$ = 16 cm/s giving an optimum programming rate of 4 ºC/min, where ^1N = 86,000. In the optimum of the first column (^1N = 100,000) $^1\bar{u}$ = 24 cm/s, giving an optimum programming rate of 5 ºC/min. And even the higher velocity of 30 cm/s in the first column can now be used, where ^1N = 88,000, ^2N = 8.000 and the optimum programming rate 6 ºC/min. As is the case with the narrow bore column set, the peaks of the first column are somewhat too narrow to allow four modulations per peak and the programming rate should be slowed down a bit. From putting these two examples together for easy comparison (Table 6.I), it is clear that the wider bore column set offers more separation at the same analysis time (coloured cells).

Table 6.I Comparison of separation parameters for two sets of columns

	P_{in} (kPa)	^1N	^2N	$^1\bar{u}$ (cm/s)	$^2\bar{u}$ (cm/s)	PR_{opt}* (ºC/min)
30m, 0.32mm + 1.5m, 0.18mm	56	86,000	9.000	16	64	4
	88	100,000	8,000	24	106	5
	132	98,000	6,500	35	140	6
15m, 0.25mm + 1.5m, 0.10mm	112	44,000	15,000	10	80	4
	224	65,000	10,000	18	160	7
	400	40,000	4,000	30	280	12

* *optimum programming rate*

A second side-effect of column flow in GC×GC can be explained as follows: since both columns are connected in series, but have quite different internal diameters, changing the flow have different effects on the average linear velocity in the two columns. Increase of flow will result in a (rather small) decrease of retention in the (temperature-programmed) first column, and consequently a decrease in elution temperature. Since this elution temperature is also the temperature at which the compound, as it elutes from the first column, will be separated in the second column, increase in flow will give an increase in retention in the

second column. On the other hand, an increase in flow will decrease the retention in the (isothermal) second dimension separation. These two competing effects in the second column will generally rule out each other [42].

6.4 Column temperatures

The temperature and its programming have even more different and stronger effects than the carrier flow. Changing temperature programming of the first column will not only influence the elution temperature, but also the peak widths of eluting compounds. This will affect the number of modulations that can be made across a first-dimension peak. On the other hand, a different elution temperature will influence the retention and separation in the second column (see above). The result of a higher temperature-programming rate generally diminishes preservation of the separation already acquired in the first column. In [42, 77] a number of examples on the above-mentioned relationships between the parameters are demonstrated and discussed.

7. Detection systems

The second-dimension separations are such short in time that they are essentially performed under isothermal conditions. Consequently, the width of the peaks eluting from the second dimension increase with its retention times. The first eluting peaks have, dependent upon the modulation system and the second-dimension column that is used, peak widths at base (W_b) ranging from 50 to 200 ms. Detectors should be accordingly fast to enable handling these fast changing signals. The rise time of detectors for GC×GC should be $c_t < \frac{\sigma}{5}$ [78], which means for peaks with $W_b = 50\ ms$, that $c_t < 2.5\ ms$. For a proper description of the peaks at least ten data points are needed over a single peak, thus data acquisition rates should be at least 200 Hz.

Most modern FIDs (Flame Ionisation Detectors) fulfil these fast requirements. But some other detectors, like the ECD (Electron Capture Detector) are not completely suited for this fast chromatography. Some of the ECDs deliver the data at an acquisition speed of 50 Hz or lower and/or have too big internal cell volumes [79], which will give rise to peak broadening. Of all the tested ECDs described in this paper none could produce peaks narrower than 300 ms. Also the reaction volume and the electronics of the SCDs (Sulphur Chemiluminescence Detectors) are such, that they have to be adapted for a proper detection of these narrow peaks [79, 80, 81].

An interesting detector, in fact offering extra information to a GC×GC separation for identification purposes is the **mass spectrometer.** Quadrupole MS in general have scanning rates not exceeding 20 scans/s and are therefore not fast enough [83] to produce comprehensive information in GC×GC. Nevertheless, they can be used in GC×GC combinations for identification purposes. For identifying an unknown peak in the contour or colour plot two mass scans across a second dimension peak fulfils this requirement [84, 117]. The newest types of quad-MS's, however, with an acquisition rate of 33 Hz. using a mass range of up to 200 amu— provides a sufficient number of data points per peak (dppp≥7) to enable identification on the basis of high-quality mass spectra, with proper linearity and quantification. LODs were in the low-pg range for a wide variety of test compounds. [118]. Modern Time-of-Flight MS (ToF MS), however, can acquire spectra at very high frequencies and are therefore utmost suited to act as a detector in GC×GC. Since the separation power of GC×GC is so much higher than in 1D-GC, there is less co-elution and peak overlap, so that the spectra obtained with this technique are much cleaner and the identification is much more

ascertained. This may, however, pose a problem since the spectra collected in the public available libraries, generally are made using 1D-GC separations and are consequently less cleaner than those acquired with GC×GC. In those cases where still peak overlap exists after a GC×GC separation, deconvolution algorithms generally present in ToF MS data processing software, can help in digitally resolving these peaks, provided that the apices of co-eluting peaks are at least one scan apart. This deconvolution will not only provide a kind of digital separation, but will also provide much cleaner resolved spectra and thus a more reliable identification. A number of interesting applications of GC×GC coupled to ToF MS have been described in [31, 42, 82].

Another interesting detector, also offering elemental information on the separated species is the **atomic emission detector (AED)**. Although the data acquisition rate of an AED is only 10 Hz, it can, like the quadropole MS, be used for identification purposes [85].

Meanwhile, both Sulphur Chemiluminescence Detectiors [86, 87] as well as the Flame Photometric Detector [88] have successfully been used as element-specific detectors for GC×GC experiments. It must be admitted though that both detectors in fact are not perfectly suited for this fast technique, so that they broaden the final peaks somewhat. Nevertheless, as is evident from some applications, shown in chapter 13, applications, they can be used. As a result of the concluded increase in peak width that these detectors produce, investigations are underway to overcome these problems.

Also the rather interesting olfactometer (GC-O), an almost indispensable sniffing detector in the edible oil, flavour and fragrance analysis, has been successfully used in a GC×GC system [89].

In general, the capacity of the detector to closely track a rapidly changing chromatographic peak profile depends on a number of factors. These are design of electronics, flow paths and make-up gas introduction, type of detector response mechanism, and the chemistry of the response. These factors are discussed in a comprehensive overview of all detectors that have been successfully applied in GC×GC [90], as a means to appreciate the technical demands of detection in GC×GC. The MS detector, however, is not included in this review.

7.1 Sensitivity enhancement in single channel detectors

It must be realised that for single channel detectors as FID and ECD, higher data acquisition rates give reduced sensitivity (S/N) by the square root of the data acquisition rate and so 200 Hz data acquisition gives about two times less S/N than 50 Hz. The zone compression effect in GC×GC, however, leads to greater mass flux in the detector. If peak response increase is proportional to peak width reduction, a peak amplitude increase of between 20 and 50 is to be expected and is generally observed in GC×GC. According to formula (3) in Chapter 3, the ratio between the peak widths of the first and the second dimension (for the narrowest peak) is 160:

$$^{2}\sigma_{A} = \frac{^{1}\sigma}{160} \qquad (8.1)$$

Consequently, for a proper description of all peaks (ten data points across a peak) the rate at which the data has to be acquired should be different by the same ratio. This means that the S/N of the narrowest second dimension peak will decrease by $\sqrt{160}$ = 12.6 because of the higher data acquisition rate that is needed. The peak amplitude enhancement will of course be smaller for later eluting compounds from the second dimension (broader peaks). And although for these broader peaks a lower data acquisition rate may suffice, a constant high data acquisition rate is used throughout the complete analysis. The net result of the peak amplitude enhancement because of the zone compression and the sensitivity reduction because of the higher data acquisition rate is still positive, *viz*. a factor of 4 – 5, but it is far less then is stated in several papers. Lee *et al*. derived from model calculations and experimental observations the same sensitivity enhancement of a factor of 4 – 5 [91].

The peak enhancement and steep slopes of modulated peaks generated in GC×GC also leads to better lower limits of detection (LOD). An experiment on determining the LOD in GC×GC as compared to 1D-GC and depicted in Fig. 7.1 and 8.2 demonstrates this. Here an aliquot of sample containing 10 pg of octene-3-one has been injected onto the polar first column of a GC×GC system equipped with an FID. The parameters of the column combination were set such that the first column functioned under optimum conditions, thus producing maximum peak amplitudes for 1D-GC. The injected aliquot produced a (non-modulated) peak just at the lower limit of detection, *viz*. *3×S/N*. An injection of the same aliquot in the GC×GC system, *i.e*. using the same GC with columns and conditions but now with modulating the peak four times at a modulation frequency of two seconds, produced much higher peaks. In both the 1D-GC and in the GC×GC-mode, the data acquisition rate was set such that about 10 data points across the peak could be acquired, *viz*. 2 Hz (noise = 0,045

pA) for the 1D-GC analysis and 50 Hz (noise = 0.150 pA) for the GC×GC analysis. The sample had to be diluted sixfold in order to produce a (modulated and so, compressed) peak at the lower limit of detection (Fig. 7.2). This gain in improvement of LOD of a factor of six will, of course, be less for peaks that have higher second dimension retention times and consequently broader peaks.

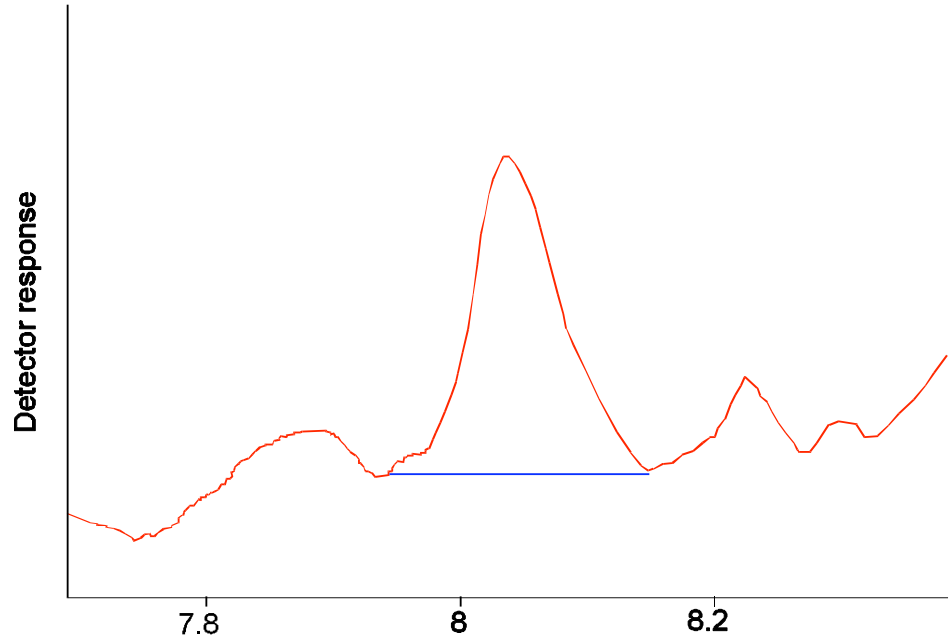


Figure 7.1 Lower limit of detection on an optimised 1D-GC (5 pg 1-octen-3-one).

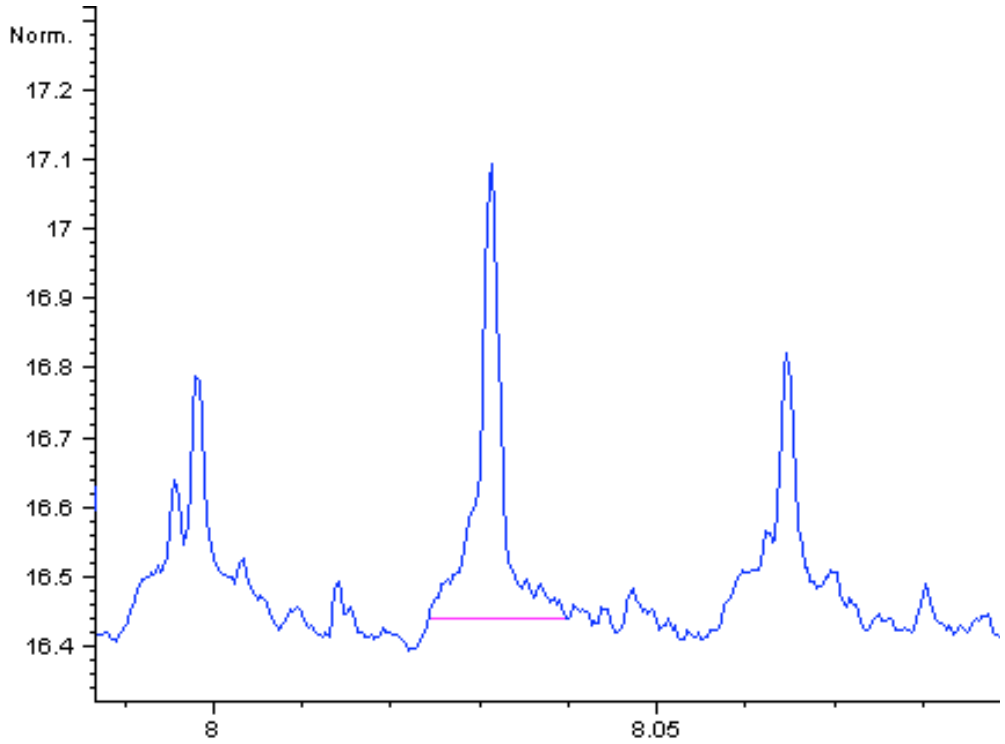


Figure 7.2 Lower limit of detection on GC×GC (0.75 pg 1-octen-3-one).

7.2 Sensitivity enhancement in time-of-flight MS

The situation of decreased sensitivity at increased data acquisition rates is even more dramatic for ToF MS. Dallüge *et al*. [82] measured how the sensitivity loss of a ToF MS is dependent on the increase of the scan speed and found a non-linear relationship as depicted in Fig. 7.2. The final increase in sensitivity is, according to their measurements, merely a factor of 2 – 5.

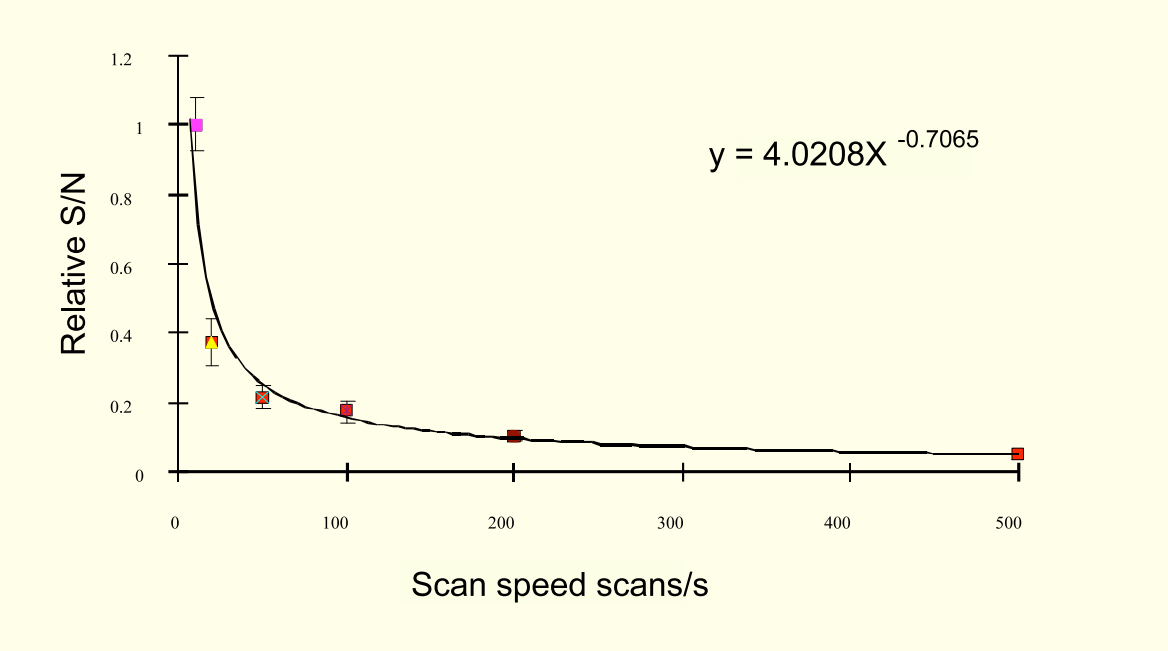


Figure 7.3. The dependency of the sensitivity of ToF MS on the mass spectral acquisition speed.

7.4. Comparison GC×GC versus GC–MS

In [92] a comparison has been made between GC×GC and GC–MS from a theoretical point of view. The same comparison has been made by Schoenmakers et al. [93], but here also the practical implications have been included.

As in other hyphenated systems, GC×GC consists of two columns connected serially (by means of the modulator) such that all sample components eluting from the first instrumental method enter the second and are analysed sequentially. This hyphenation produces two-dimensional data in which each method provides an axis to the plane. Fig. 8.4 illustrates this process and compares it with the hyphenation of GC to MS. The only instrumental difference between the two hyphenated methods is the use of a second column rather than a mass separator as the second dimension.

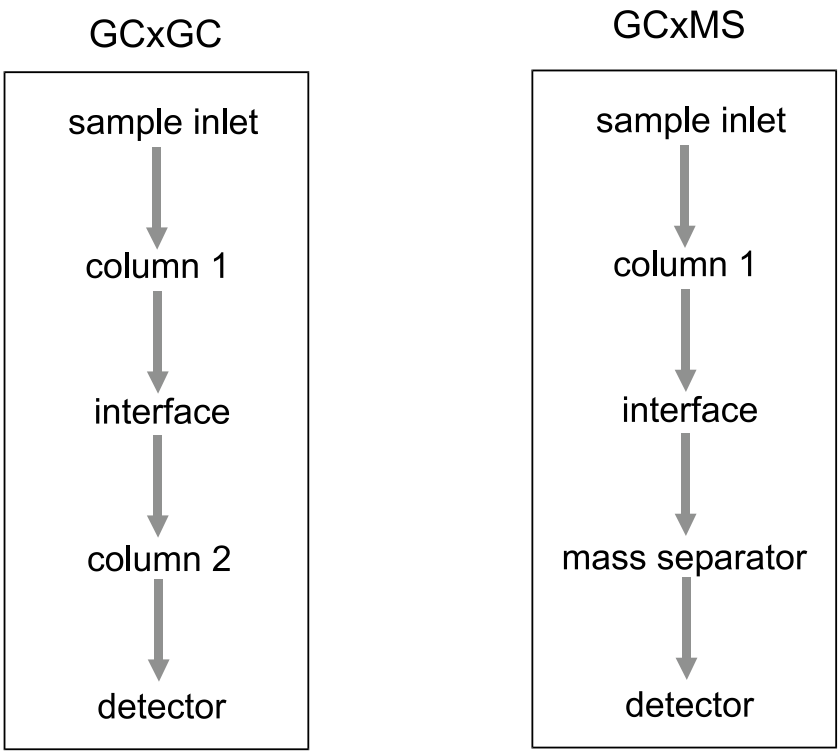


Figure 7.4. GC×GC compared to another hyphenated method, GC–MS

In any hyphenated system designed as illustrated in Fig. 7.4, the second separation must be very must faster than the first in order to keep up with the first-dimension separation. In GC–MS this is almost naturally accomplished. GC is commonly operated at a speed that is about the right factor slower than MS. If we, however, replace the MS for a second column, this column must be of a fast design. Only then this second column can generate the right amount of separated peaks in only a fraction of the duration of the first-dimension peak. This ultra fast chromatography, offering many peaks within a time scale of seconds has been demonstrated already several times. The only problem with this ultra fast GC was in finding appropriate applications for it. GC×GC is just an excellent application for ultra fast GC.

Assuming an (hypothetical) very soft ionisation technique for MS, so that no fragmentation is obtained, or perfect software for distilling essential information from a very complex MS data set, then in [93] it is concluded: "*Under these assumptions, the separation potential of GC–MS is greater than the separation power of GC×GC. However, the separation potential of GC×GC×GC is very much higher than either. In the field of hydrocarbons, it may be much easier to realise GC×GC×GC than to realise the full potential of GC–MS.*"

Blomberg made a practical comparison of GC×GC and GC–MS for the possibility of separating group-types of petroleum fractions [83]. Under the same assumptions as mentioned above of an MS having an ideal soft-ionisation technique or perfect deconvolution software

(which is never accomplished in practice), he derived the following comparison (see Table 7.1) leading to the aforementioned conclusion.

Table 7.1 Separation power of GC×GC (in current practice) and GC–MS (assuming the availability of an ideal soft-ionisation technique or perfect deconvolution software) (Courtesy of Jan Blomberg)

GC×GC (in practice)	GC–MS (in principle)
class level	
alkanes // alkenes + cyclics // aromatics or: alkanes + cyclics // alkenes // aromatics	alkanes // alkenes + cyclics[a] // aromatics
subclass level	
mono-ring // di-ring // tri-ring // … etc. cyclo-pentanes // cyclo-hexanes // … etc.	mono-ring // di-ring // tri-ring // … etc.
cluster level (given class, different carbon number)	
separation thanks to roof-tile effect	ample separation
isomer level (given class, given carbon number)	
linear isomers // methyl branched /…/ multiple branched (*decreasing separation between successive groups*)	linear isomers // methyl branched /…/ multiple branched (*decreasing separation between successive groups*)

[a] GC separation between these classes may be maximised for best results.

[b] Separation of isomers is entirely due to GC dimension and is, therefore, somewhat better in GC×GC than in GC-MS.

8. Injection systems

Up till now the majority of reported GC×GC investigations and developments have been performed using split or programmed-temperature-vaporising (PTV) injection systems. Since the main attention in these investigations was focused onto the separation increase of GC×GC, onto special detection of narrow peaks or onto the complex data processing, investigations on injection systems has been a neglected area.

In view of the fact that the first column in GC×GC generally is a conventional capillary column, there is, however, no reason to expect that any injection system that can be used in normal 1D-GC cannot be deployed equally well in GC×GC. Injection by means of splitless or on-column techniques will not need any specific provision compared to normal 1D-GC. Large-volume-injections (LVI), for increasing the detectability of analytes of very low concentrations, can also successfully be performed in GC×GC [94]. Accordingly, it is to be expected that coupling other separation systems in front of the GC×GC system can be done equally well.

The use of Solid-Phase Micro Extraction (SPME) for GC×GC e.g. has already been described for the analysis of aromatic compounds in water and of flavours in foodstuff [95]. The use of the comprehensive coupling of supercritical fluid chromatography (SFC) with GC has been described by Rohwer *et al*. [96] But also the on-line coupling of Solid-Phase Extraction (SPE) or high performance liquid chromatography (HPLC) must be feasible. The only problem area to be expected here is the overload of the modulator or the second column and the flow regimes.

An obvious use of the high separation power and ability to exhibit patterns in the separation plane of GC×GC is its combination with pyrolysis. When combined with appropriate pattern recognition data processing software, pyrolysis–GC×GC may enhance the commitment of pyrolysis–GC for the characterisation of various high molecular mass products considerably. In view of the fact that pyrolysis in general may also produce compounds of low volatility, the modulation process and construction of modulators may have to be reconsidered, since the majority of the modulators in use are not able to modulate compounds with boiling points well below 100ºC. Pyrolysis–GC was first demonstrated to work on a polyethylene and a polyacrylate sample by Pursch *et al.* [97].

A quite complicated system for the detailed PIONA-analysis of heavy naphtha has been described by Vendeuvre *et al.* [98]. In their paper they demonstrate the hyphenation of an olefin trap to GC×GC in order to temporarily trap and release the olefins from the sample to prevent co-elution of these compounds with saturates, followed by their GC×GC separation.

9. Irregular peak shapes and sample overload in GC×GC

It has been adequately demonstrated throughout the past decade in which GC×GC is in use now that contour and colour plots are excellent means to visualise the separations obtained. In general those plots are produced for any GC×GC separation that is performed. The accommodation of all the separated spots on a plane that is formed by the two retention axes not only demonstrates the enormous increase in peak capacity of the technique, but also often exhibit bands and structures of chemically related compounds on that plane. These bands and structures reveal important information on the composition of the separated samples and can be readily recognised. Nevertheless, the careful inspection of the peak shapes as they arrive from both the first and second column is at least as important as those contour and colour plots. Especially in the development stage of the GC×GC method they can help in understanding the separation phenomena under consideration and in trouble shooting. Many different peak shapes are possible in GC×GC. Some of the most frequent peak shapes observed in GC×GC and their causes will be described in this chapter.

9.1 Peak distortion caused by the primary column

Because the first column generally is a normal sized column and the second column a fast column with a smaller internal diameter and a thin film of stationary phase, overloading the first column, will almost always overload the second column as well. The effect of this double overload results in fronting of these peaks in both dimensions (see peaks of benzothiophene and n-C_{12} in upper and middle trace of Fig. 9.1). The peak pattern of the modulated chromatogram follows nicely the original first dimension chromatogram. In the contour plot the normal peak shape of minor (non-overloaded) peaks, indicated by the arrows in the 1D-GC and GC×GC chromatograms, is added to make a comparison with the overloaded peaks. Finally two modulated peaks are enlarged in the lower trace of Fig. 9.1 to show the overloading effect of these peaks in the second dimension. Generally the apices of overloaded peaks shift to higher retention times.

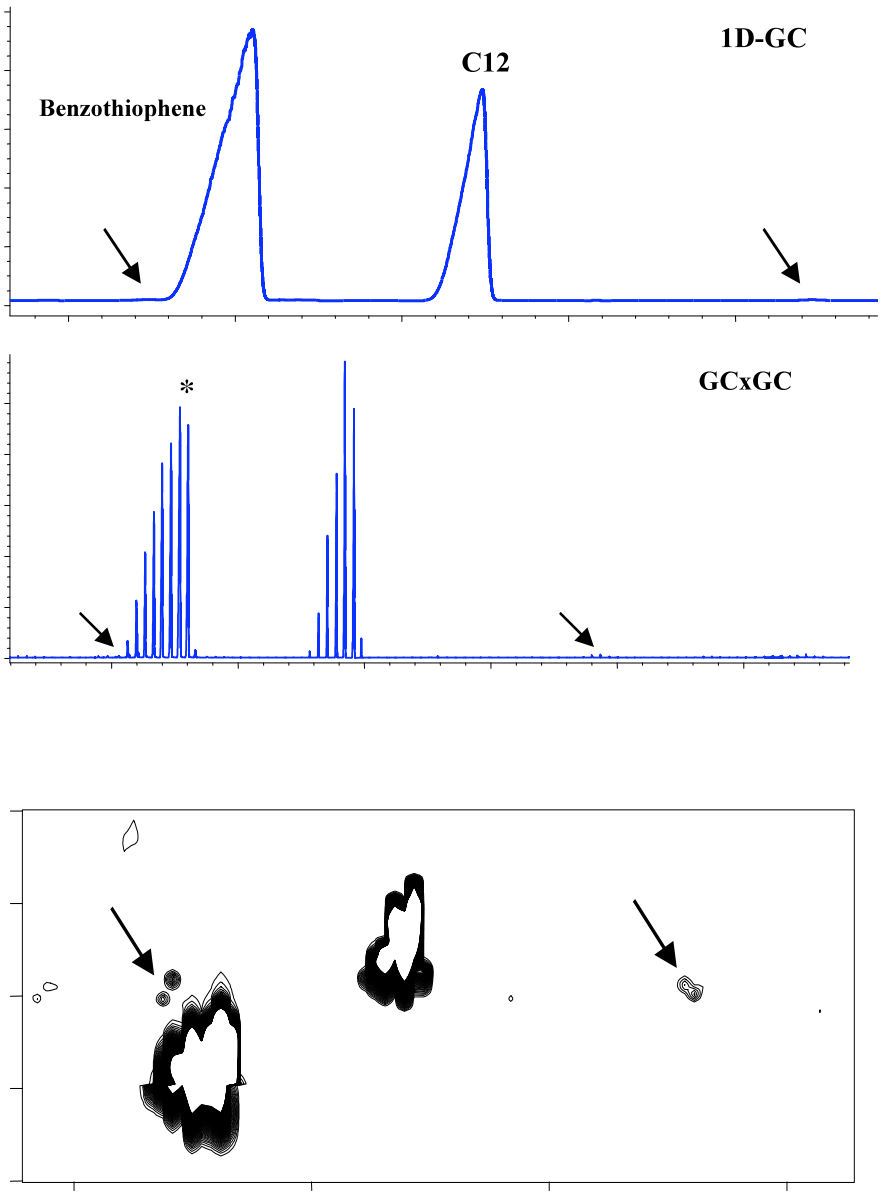


Figure 9.1. Overloaded, fronting peaks of benzothiophene and n-C_{12}. The peaks indicated by the arrows are small, non-overloaded peaks. Upper trace: non-modulated (1D–GC), second trace: modulated (GC×GC), third: contour plot. Lower trace: two consecutive second dimension peaks of benzothiophene as indicated with the asterisk (*).

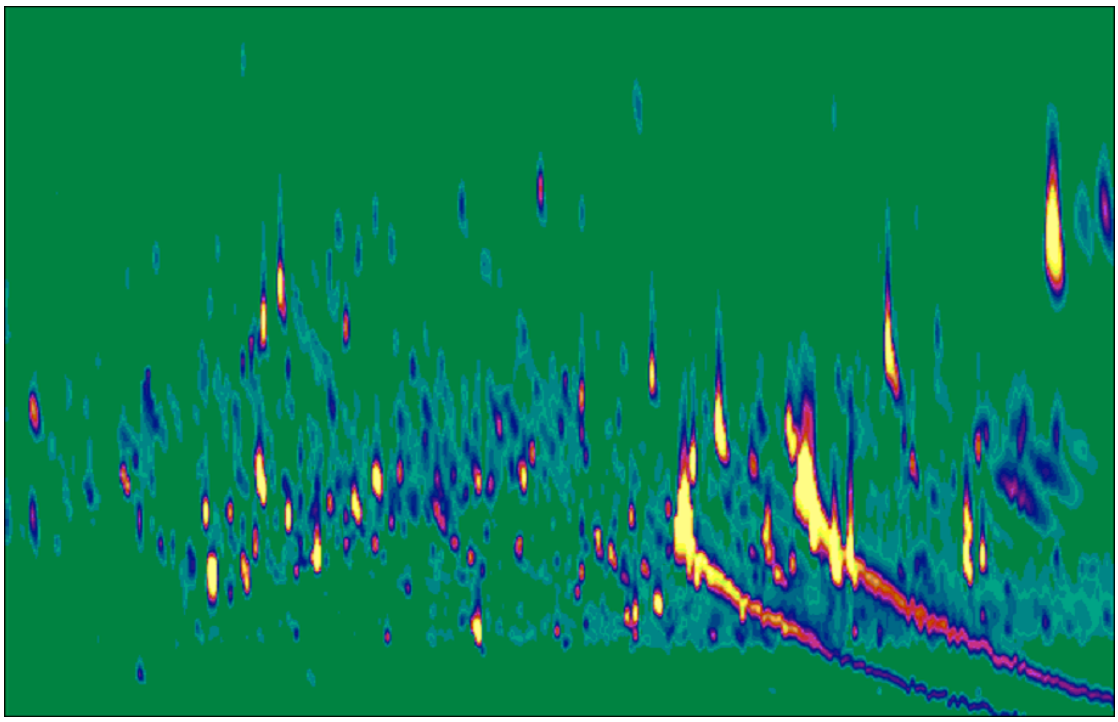


Figure 9.2. Tailing peaks in the first dimension

Fig. 9.2 shows a separation of an extract of carrot [44]. Two large curved "spots" are visible in the lower right corner of the colour plot. These spots represent the (large number of) second dimension peaks from two badly tailing peaks in the first dimension representing two organic acids (*n*-hexadecanoic and octadecadienoic acid). In each next second dimension separation a small part of the tail of this first dimension tail is injected, and will be separated at a slightly higher temperature and thus exhibit a slightly lower retention. The curvature of these decreasing retention times ("iso-volatility line") has been discussed by Beens *et al*. [71] (see also Fig. 10.2). While in 1D-GC such tailing peaks in complex chromatograms generally posses a serious analysis problem, *viz*. quantification of the tail and separation of peaks riding on this tail, in GC×GC this tail is separated from co-eluents and can be quantified quite easily. An example of an analysis in which an important part of the eluted material in the form of tails has been quantified is presented by Lewis *et al*. [37] in the analysis of organic compounds in urban atmospheres.

9.2 Peak distortion in the second column

Peaks in the second dimension can be distorted for several reasons. As already pointed out in Chapter 9.1 they can exhibit a fronting effect by column overload. A second reason can be a tailing effect. In Fig. 9.3 a part of a GC×GC separation of an extract of celeriac, spiked with pesticides [84] is depicted, showing severe tailing in the high intensity spots in the left part of the colour plot. Fading, lower intensity stripes will follow the main spot. From the colour plot (or contour plot) this tailing effect is not very apparent. The individual second dimension chromatogram, however, depicted in the inset, clearly show the individual peak shapes. This tailing phenomenon can be compared with the tailing effect of peaks in 1D-GC separations. Co-elution of components with this tail, *i.e.* peaks riding on the tail, are difficult to quantify. Spectra obtained from these peaks will be mixed spectra, containing fragments of the co-eluting tail.

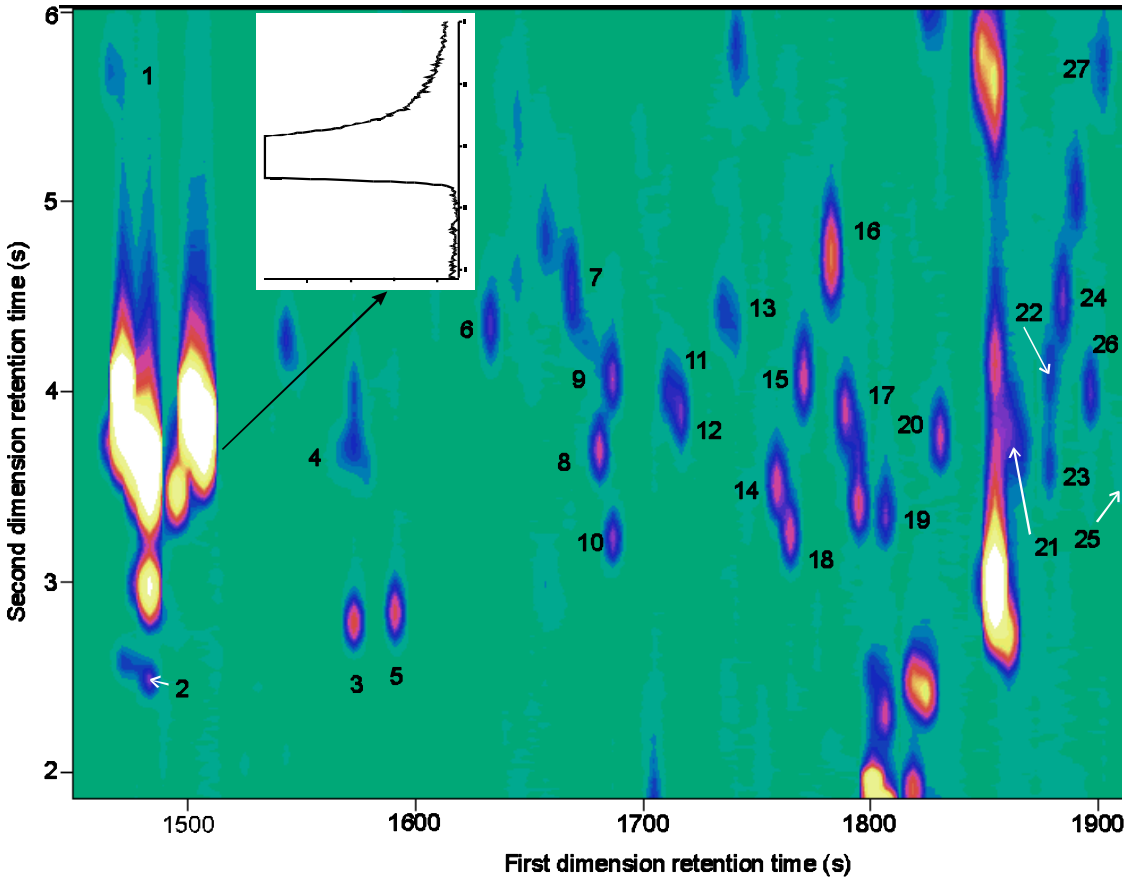


Figure 9.3. Tailing peaks in the second dimension of a GC×GC separation of spiked pesticides in an extract of celeriac. For identification, see [84].

9.3 Peak distortion caused by long second-dimension retention.

When components have retention times on the second-dimension column exceeding the modulation time, they will not elute in their own, but rather in a next second-dimension chromatogram. This effect is called “wrap-around”. It is clearly visible from the fact that the peak width of these components is far larger than surrounding peaks having far shorter second-dimension retention times. In Fig. 9.4, spot number 14, pyrole, having a high affinity to the Carbowax secondary column, exhibits this “wrap-around” effect.

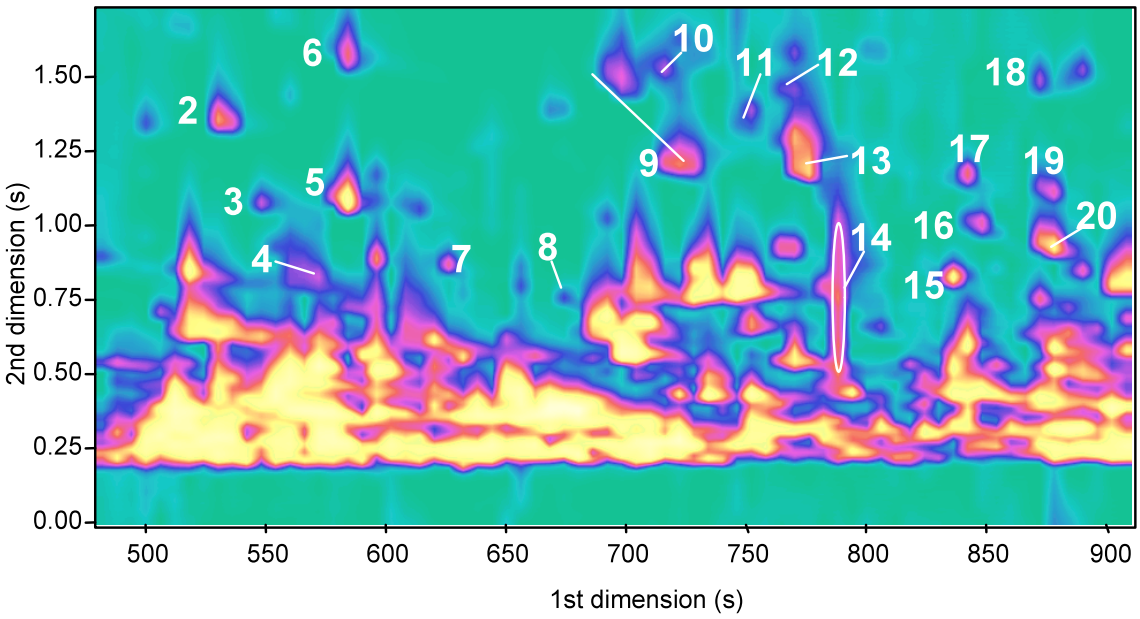


Figure 9.4. Blow-up of part of the GC×GC-ToF colour plot of a separation of cigarette smoke [99]. The large peak width of peak #14 (pyrole), which has a second-dimension retention time exceeding the modulation time of six seconds, exhibits the “wrap-around” effect.

9.4 Peak distortion caused by the modulator

Depending upon the type of modulator several peak distortion effects can be distinguished. Phase-ratio modulators can only accommodate a limited amount of analyte in its (thick film of) stationary phase. Overload of analytes in the modulator will cause breakthrough. Cryogenic modulators in general have accumulation capacities that are far higher than that of phase-ratio modulators. But cryogenic modulators must accumulate analytes at a sufficient low temperature. Here breakthrough will occur at too high modulation temperatures. Breakthrough can be very easy detected in contour or colour plots. In Fig. 9.5, a GC×GC separation of a brunch extract, breakthrough in the modulator of components is depicted by the black stripe in the middle of the plot.

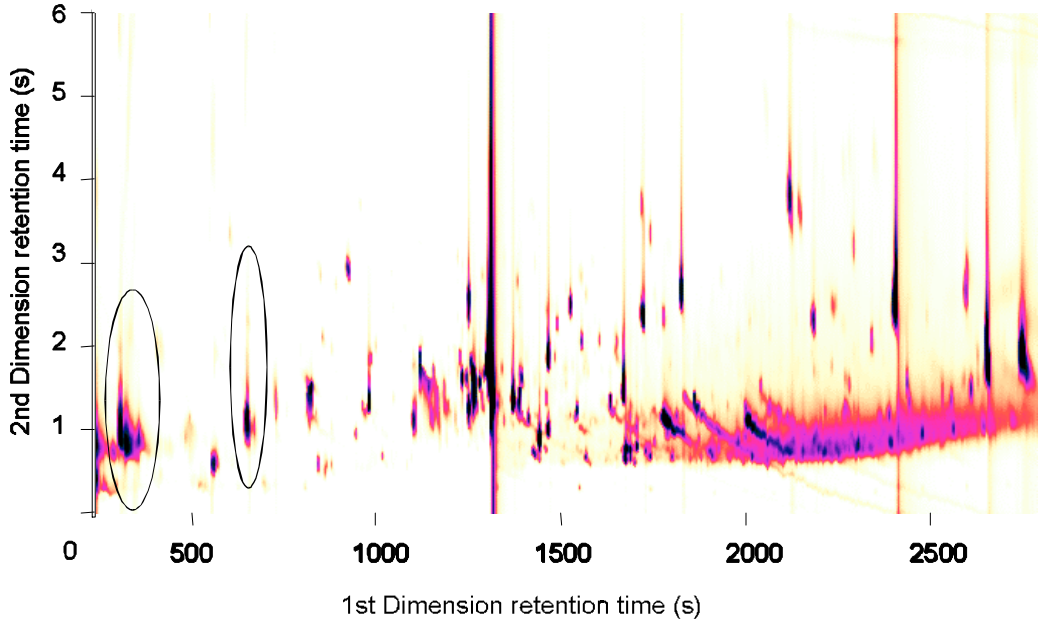


Figure 9.5. Separation of a brunch extract showing modulator breakthrough in the middle of the contour plot [95].

Another cause for breakthrough in a cryogenic modulator with two jets is the fact that there is a very small time delay between switching the CO_2 valves and the effect of this switching on the cooling of the capillary. A part of this time delay is caused by the mechanical switching of the valve that needs some time and another part is the time required for emptying and filling the capillary tubing between the valve outlet and the nozzle. In general this time delay is typically less than 100 ms. If the linear carrier gas velocity is (typically) around 1 m/s or higher and the two jets are situated at least 10 cm apart, than this breakthrough will not occur. In Fig. 9.6 such a breakthrough is depicted. The upper colour plot and the original detector output (non-converted signal) beneath it, represent a correct working dual-jet cryogenic modulator. Since the modulation time is eight seconds, the peaks in the original detector output elute eight seconds apart. Note the tailing peak shape in the first dimension, which is also visible from the profile of the peak maxima in the original detector output. The lower colour plot and original signal show the result of a dual-jet cryogenic modulator where the distance between the two jets is not long enough, or the linear carrier gas velocity is too high.

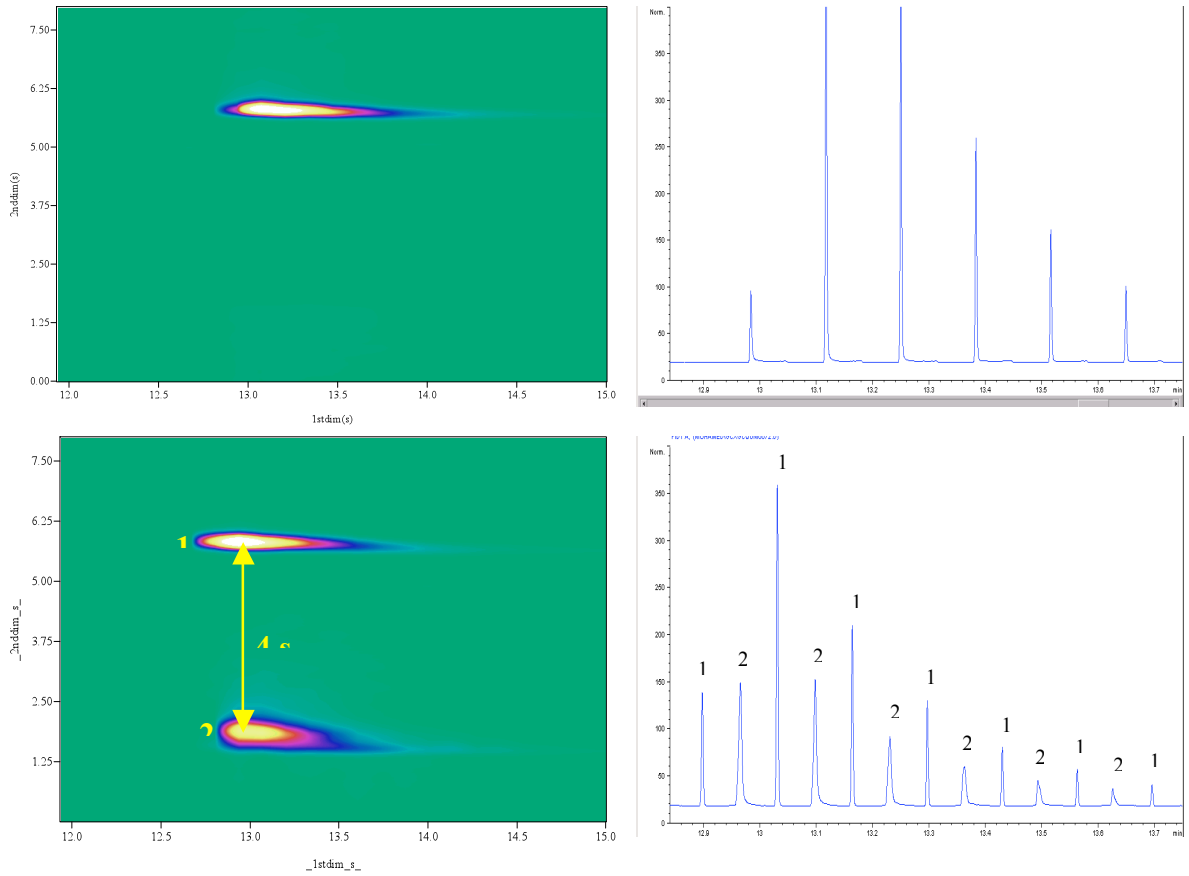


Figure 9.6. Breakthrough effect in a dual-jet cryogenic modulator by a too high carrier gas velocity or a too short distance between the nozzles.

Analytes are trapped by the cooling effect of the first (the upstream) jet. When this cooling is stopped (after four seconds), the accumulated, narrow fraction is remobilized and travels in the direction of the second (the downstream) jet. During this short travel time of the pulse, the second jet has started its cooling function, but just a very short time too late. The pulse had just reached this section of the column, so that a part of the pulse will not be trapped properly and continues travelling through the column. This can be seen from the original detector output showing these fractions as extra peaks, broader than normal modulated peaks and tailing, because of the temperature gradient (from oven temperature down to cooling temperature) that has been experienced underway.

Breakthrough in the modulator and its cause can only be detected by plotting the individual second dimension chromatograms and inspecting the individual second dimension peaks. This is particular true for the improper trapping of analytes in the four-jet cryogenic system of Ledford as described in Chapter 5.1.2 and depicted in Fig. 4.10. Since the construction is such that the two cold and the two hot jets are placed quite close together in the same small chamber, they may influence each other. Applying the hot jet for a too long time, will prevent proper focusing of the analytes by the other cold jet as is shown in Fig. 9.7. The upper colour plot and a second dimension chromatogram (along the red dotted line in the colour plot) beneath it show that when applying the hot jet for 300 ms or longer, will cause a part of a next fraction breaking through. When applying this hot jet for only 50 ms, the fractions are trapped and remobilized correctly (lower colour plot).

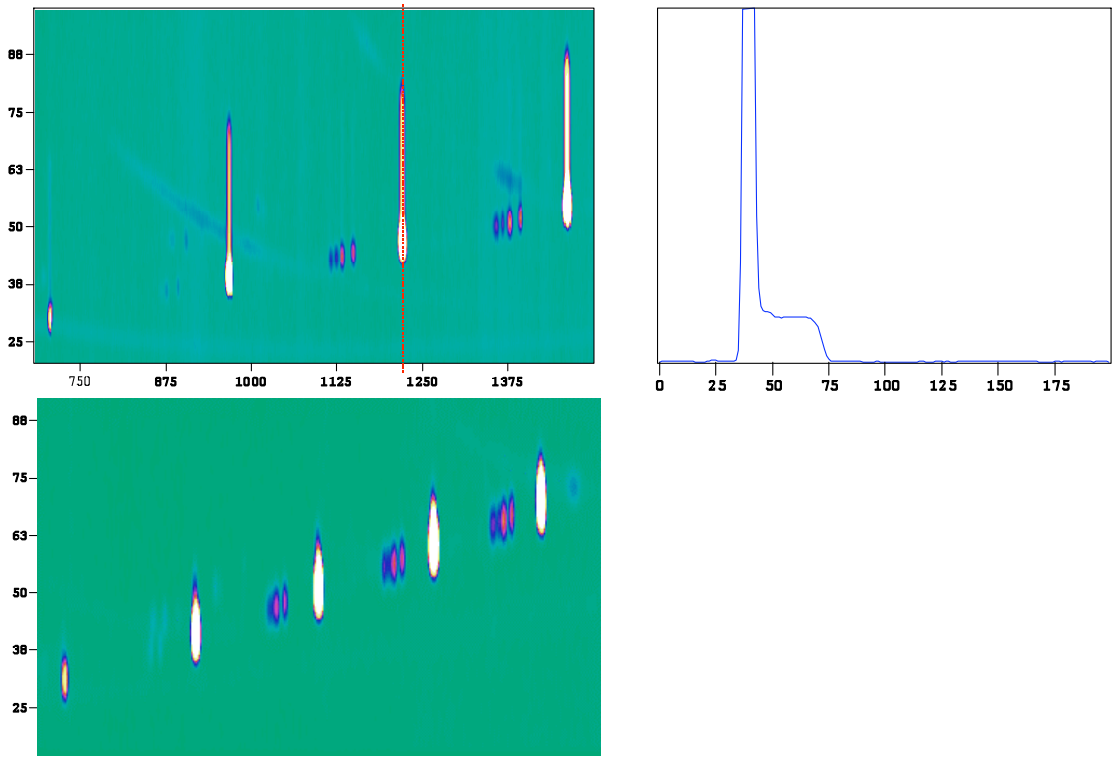


Figure 9.7. Breakthrough of sample in a four-jet cryogenic modulator.

Accordingly, the same modulator can also be too cold, or the cooling time too long. Because of the limited space in which the four jets are located, they can influence each other. When the temperature of the jet is too low (since liquid nitrogen is used, the temperature of the gas can be as low as −180°C) or the cooling time too long, the remobilization will be far more difficult and will need a longer time. Consequently, the narrow accumulated pulses will be elongated as is shown in Fig. 9.8. This phenomenon can suddenly show up during a programmed analysis of which the first part is correct (around modulation number 300). It can simply be stopped by decreasing the cooling period (around modulation number 500).

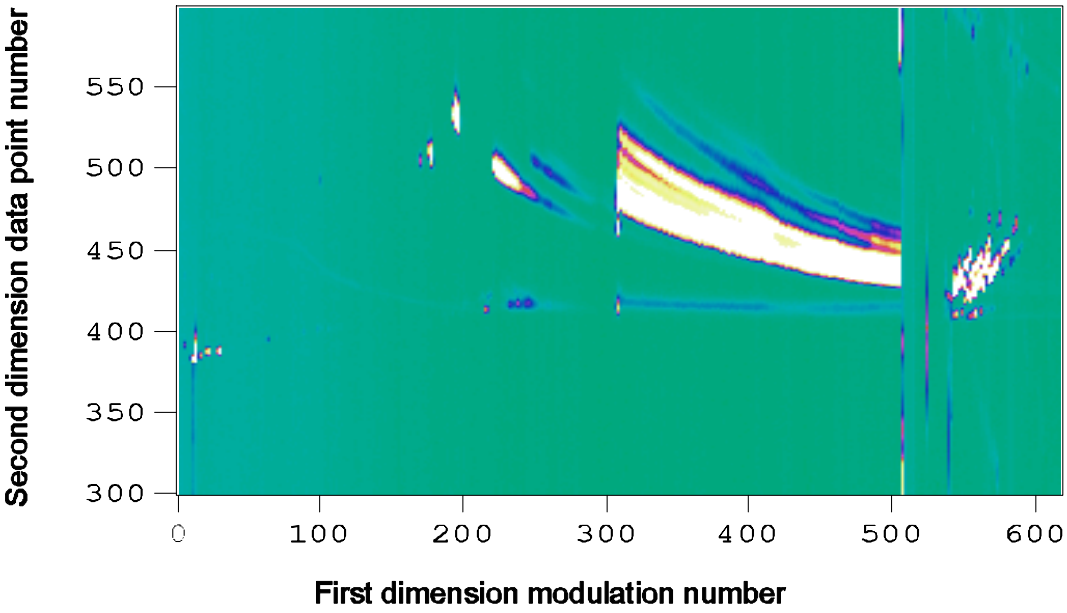


Figure 9.8. Peak distortion from a four-jet cryogenic modulator by a too low jet temperature.

Sample overload of the modulator or of the separation columns has been investigated and described in two papers, *i.e.* in [100] for the LMCS and in [78] for the dual-jet cryogenic modulator. In both papers it was reported that the secondary column would be the main source of overload. This is obviously the result of the thin film of stationary phase in the second column. A secondary column of 0.1 mm ID with a film thickness of 0.1 μm holds four times less stationary phase per unit length then a normal-bore column of 0.25 mm ID with a film thickness of 0.25 μm and furthermore have narrower peaks and accordingly will be overloaded far easier. This overload, visible by fronting peaks, already occurred at amounts of 5 ng/compound in systems with very effective cryogenic modulators, exhibiting a significant zone compression effect (see Fig. 9.9). Accordingly, 50 μm ID columns with a film thickness of only 50 nm are already overloaded around 1.6 ng/compound.

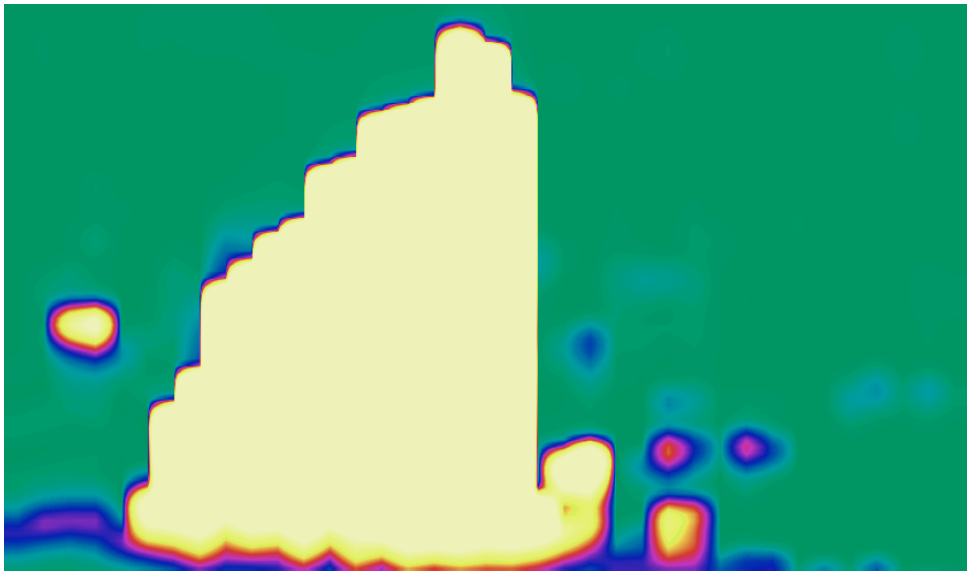


Figure 9.9. Breakthrough as a result of overload of a cryogenic modulator. Note the normal sized spots of peaks that did not overload the modulator.

It is obvious that the phase-ratio-focusing modulators have different sample overload characteristics than the cryogenic focusing systems. Even at such high amounts of compounds that overloading of the primary column occurs, the dual-jet cryogenic modulator still modulated normally and did not produce any sign of break-through of compounds.

Although overload of compounds and consequently broadening and fronting of peaks on the secondary column can be accepted sometimes, provided that the resulting separation is still sufficient, in general this effect restricts the linear dynamic range of a GC×GC system. On the other hand the increase in sensitivity caused by the zone compressing in the modulator amply compensates this effect.

10. Data processing

10.1 Qualitative data processing

Data processing of the large data files produced by GC×GC analyses is far more complicated than that of those derived from 1D-GC analysis. Where there exist, already for a long time, various excellent data processing systems with user-friendly and integrated software for 1D-GC analysis this is not (yet) the case with GC×GC analyses. GC×GC systems are able establishing the separation of several thousands of peaks, which are very narrow, so that they have to be acquired at very high acquisition rates. One GC×GC analysis that lasts half an hour and is acquired at a frequency of 200 Hz consists of almost half a million data points. Coupling such a separation system to a ToF MS, where each data point is represented by a complete spectrum is even far larger and may produce a data file as large as 400 Mb.

Apart from the fact that GC×GC data files are extremely large, they also need far more complex processing procedures than those of 1D-GC analyses. The 1D-GC data files just consist of a series of data points that represent the two-dimensions of a chromatogram, *viz*. retention time and time-integrated area and can therefore be interpreted very easy. A simple plot of the retention times against the time-integrated areas just delivers the straight-forward chromatograms that are all so common to us and that can be recognised immediately by any chromatographer. In order to visualise the separation that is acquired from a GC×GC analysis, we have to manipulate the data file and convert it conveniently. In Fig. 10.1 the modulation process of a first-dimension peak of only 1.5 minutes into a linear series of 15 second-dimension chromatogram is depicted.

It is obvious that the data as acquired from a single-channel GC×GC detector like FID or ECD looks like the lower part of this figure. However, even in this short time frame of 1.5 minute the GC×GC chromatogram as produced is hard to interpret, if at all. Now if the total data file of *e.g.* 30 minutes is presented in this way, and the separated sample is of a complex nature, which is generally the case, the interpretation is totally impossible. Therefore the data file has to be cut in all of the individual second-dimension chromatograms, which should be stacked side-by-side. This, in fact, will produce a matrix of which the number of columns represent the number of data points of one second-dimension chromatogram and the number of rows the number of modulated fractions, or the number of second dimension separations. This conversion process is depicted in Fig. 10.2. Then this matrix has to be transformed into a colour plot or in a contour plot with one axis being the first-dimension retention time, the

second axis being the second-dimension time and the contours or colours the peak amplitude, in order to be visualised meaningfully. This process is visualised in Fig. 10.3.

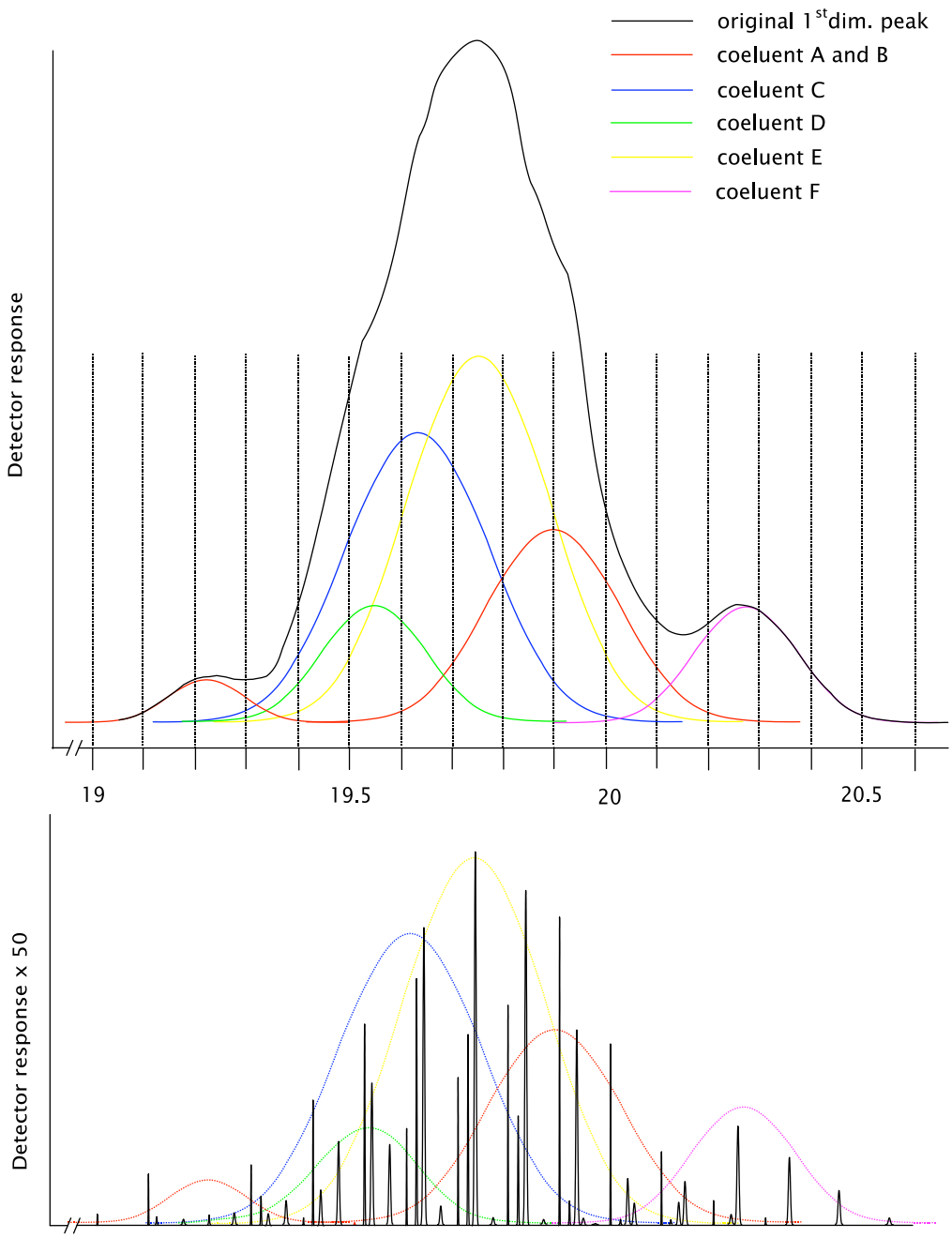


Figure 10.1. A detail (1.5 minute) of a GC×GC separation of a first-dimension peak that consists of six co-eluting components. The upper part of the figure represents the (virtual) first-dimension separation and gives the (virtual) traces of the co-eluting components. The modulation time was six seconds and the modulation times are superimposed on the peaks with dotted lines. The lower part represents the actual separated fast peaks from the second dimension (in black). The co-eluting peaks from the first dimension are superimposed.

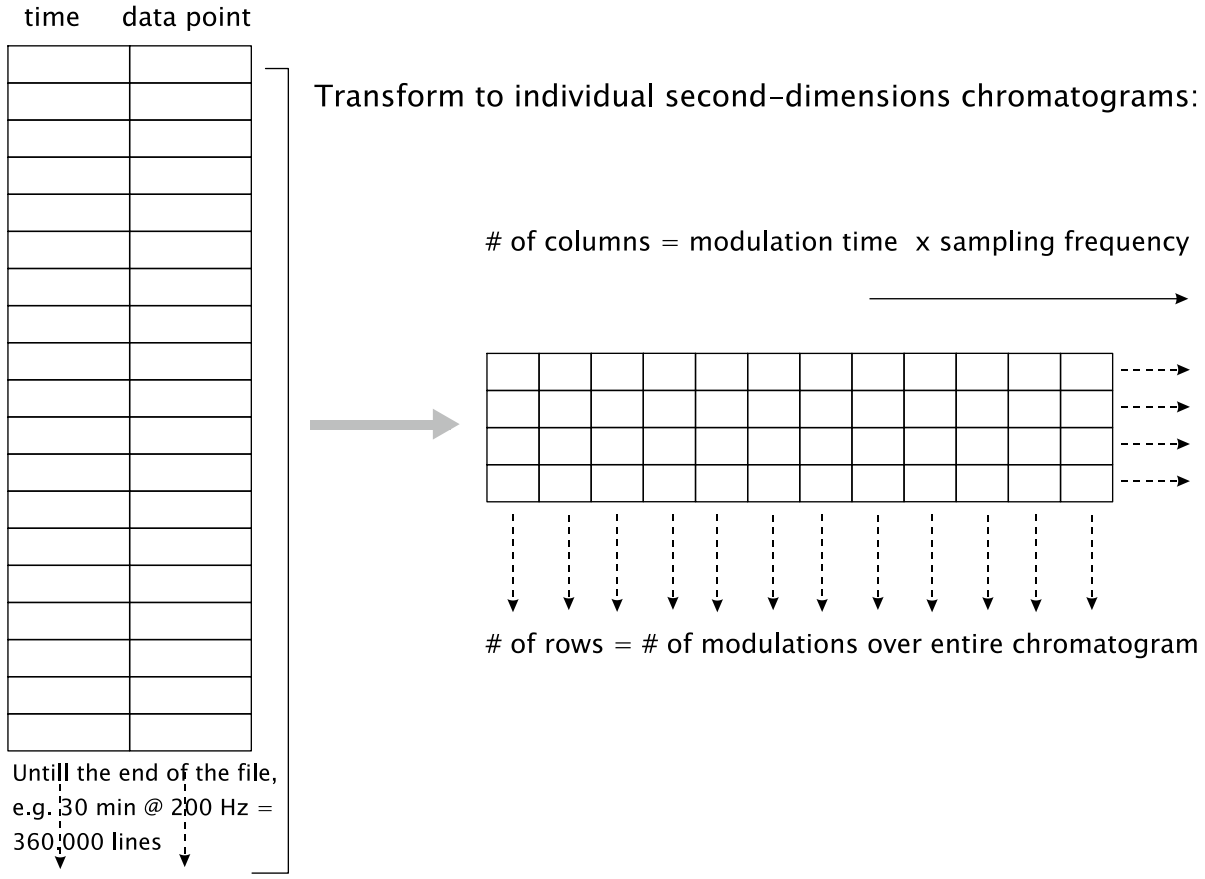


Fig.10.2. The conversion process of the original GC×GC data file into a matrix. (Courtesy of Philip Marriott).

In general one would choose the modulation time such that all compounds transferred from the first-dimension column onto the second column will elute within the small time frame of the next modulation. There is, however, not a free choice of the modulation time. It is mainly determined by the number of modulations that is needed for preservation of the first separation, the choice of conditions and dimensions of the second column and the composition of the sample. So, sometimes very polar compounds will exhibit second-dimension retention times that exceed the modulation time. These peaks will show up in a next second-dimension chromatogram in between the peaks of that next fraction. This is generally designated as "wrap-around" and can even be extended to more than one modulation. It can easily be detected by the peak width of that peak, since peak widths in the second-dimension increase with its retention time, and consequently "wrap-arounds" are far broader than the surrounding peaks. From comparison of its peak width with the peak widths of surrounding peaks it can be decided whether such a peak has made one or more wrap-arounds.

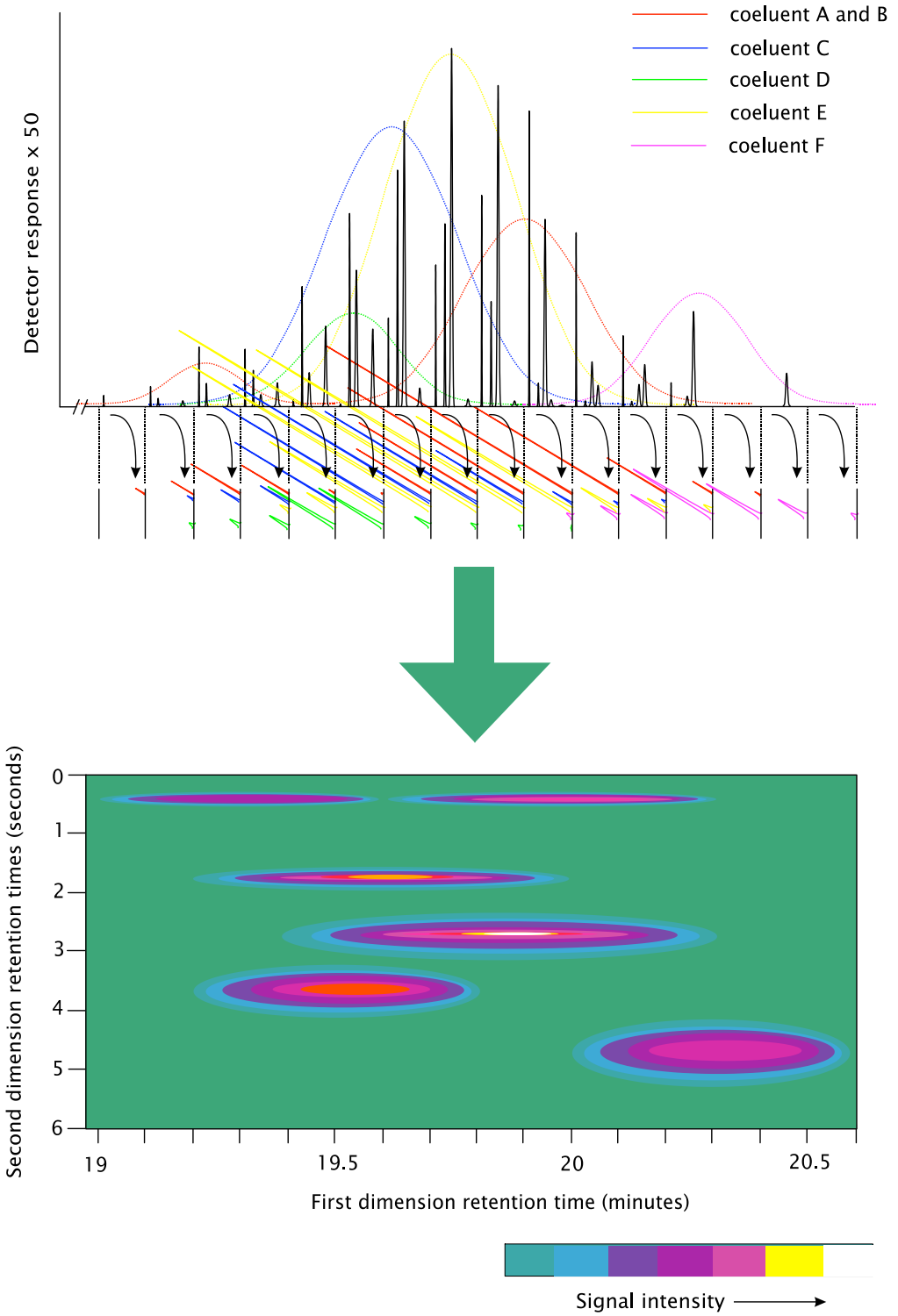


Figure 10.3. The conversion of the raw GC×GC data file via a matrix to a colour plot.

Several research groups have developed their own software modules enabling the above-mentioned processes. The final step in this process is generally produced by commercial available software as *e.g.* Transform, a fractional part of the Neosys software package (Research Systems International, Crowthorne, UK). In future integrated software packages that can be expected to be developed by GC×GC instrument manufacturers, all of these modules will be incorporated.

10.2. The use of apex plots

In order to simplify the visual presentation of the (complex) GC×GC separation and to indicate which of the peaks have been recognised by the data system as being a peak, apex plots are often used. In these plots a single symbol, *e.g.* a square, is used to indicate the co-ordinates of the apex of a second dimension peak in the colour/contour plot. To simplify it further – to reduce the amount of presented data – all apices belonging to the same compound are combined and represented by a single symbol on the co-ordinates of the apex of the highest second dimension peak. This process is depicted in Fig.10.4. It is obvious that apex plots can only be made after the data processing software has searched through the data file and found peaks and peak apices. The thus produced peak table is incorporated in Fig. 10.4.

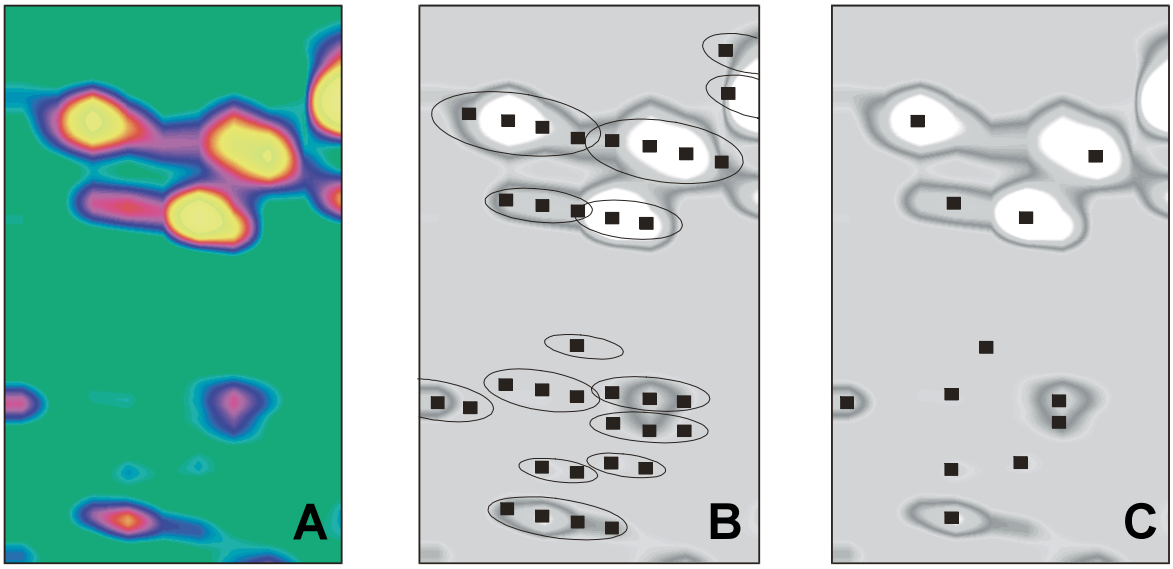


Figure 10.4. Colour plot and apex plots of a section of a GC×GC–FID chromatogram of a crude oil.

(A) colour plot,

(B) colour plot with apex plot overlaid showing all individual second-dimension peaks, ellipses indicate the apexes belonging to one single second dimension peak,

(C) colour plot with apex plot overlaid, individual second-dimension peaks are combined

10.3 Synchronisation of the modulation and the data acquisition process

In general the modulation process as well as the data acquisition process is controlled by individual (and generally separated) time-based systems. Since data acquisition is a process of discrete steps (the discrete number of data points/time), the number of data points

of an individual second-dimension chromatogram, *i.e.* the number of data points of an individual row of the matrix, is also discrete and is an integer number. The duration of a single second-dimension chromatogram, however, is not a discrete time, but can be a time that can not be expressed by an integer number. This means that when converting the series of data points produced by the detector electronics in discrete cuts, with the duration of the modulation time, from time to time a "leap year" construction has to be made to fit the data points into the matrix. This means that either a number of data points have to be ignored or extra points have to be fabricated and inserted. Synchronising both modulator control and data acquisition from one time-base source and controlling the modulation process from the (already) acquired number of data points is mandatory as to avoid this "leap year" effect.

The produced colour plot is a means for visualisation and inspection of the separation and to fit that part of the separation into the frame that contains the peaks/spots of interest. Some separations, however, are such that, although the second-dimension retention times are *e.g.* between seven and nine seconds, the modulation can be repeated every two seconds. In such a colour plot, peaks will only be present in the region between seven and nine seconds. So, the contour plot should be constructed such, that only this part is shown. This means that a "start injection in the second dimension"-pulse should be generated after the first seven seconds and then the modulation should continue with a modulation time of two seconds. These times should be such exactly in length, that even after thousands of these modulations, the time-base is still in phase with the data acquisition. Otherwise, the co-ordinates of the separated spots on the plane of the contour plot will drift away. All these provisions can be incorporated in the GC soft/hardware, if the time-base of the data acquisition system is used as the time-base for the modulation process. And if the modulation action is triggered after that the correct number of data points has been collected [101].

Substantially more complex than GC×GC-single detector data is the processing of GC×GC–ToF-data. The colour plot of the total ion current (TIC) is comparable to the colour plot that can be made of a GC×GC separation with *e.g.* FID. There are, however, colour plots possible of every extracted ion from the mass range that has been collected. In order to select one specific target compound out of these plots, or in finding the complete spectrum for one specific spot in one of those numerous colour plots, very specific and fast software is needed. Besides this, automatic library search, deconvolution and identification of all the peaks in the colour plot should be possible. Quantification should also be possible, but this should be used with care, especially for non-calibrated compounds. Since fragmentation in the EI or CI-source of the ToF is quite different for different compounds, it is unpredictable how quantification of non-calibrated peaks should be performed. An example of the extensive processing of data in GC×GC–ToF is presented in [44]. A schematic of the total data

processing necessary for GC×GC–ToF has been extracted from this paper and presented in Fig. 10.5. Up till now the ToF-manufacturers software do not provide modules for performing these processing steps automatically.

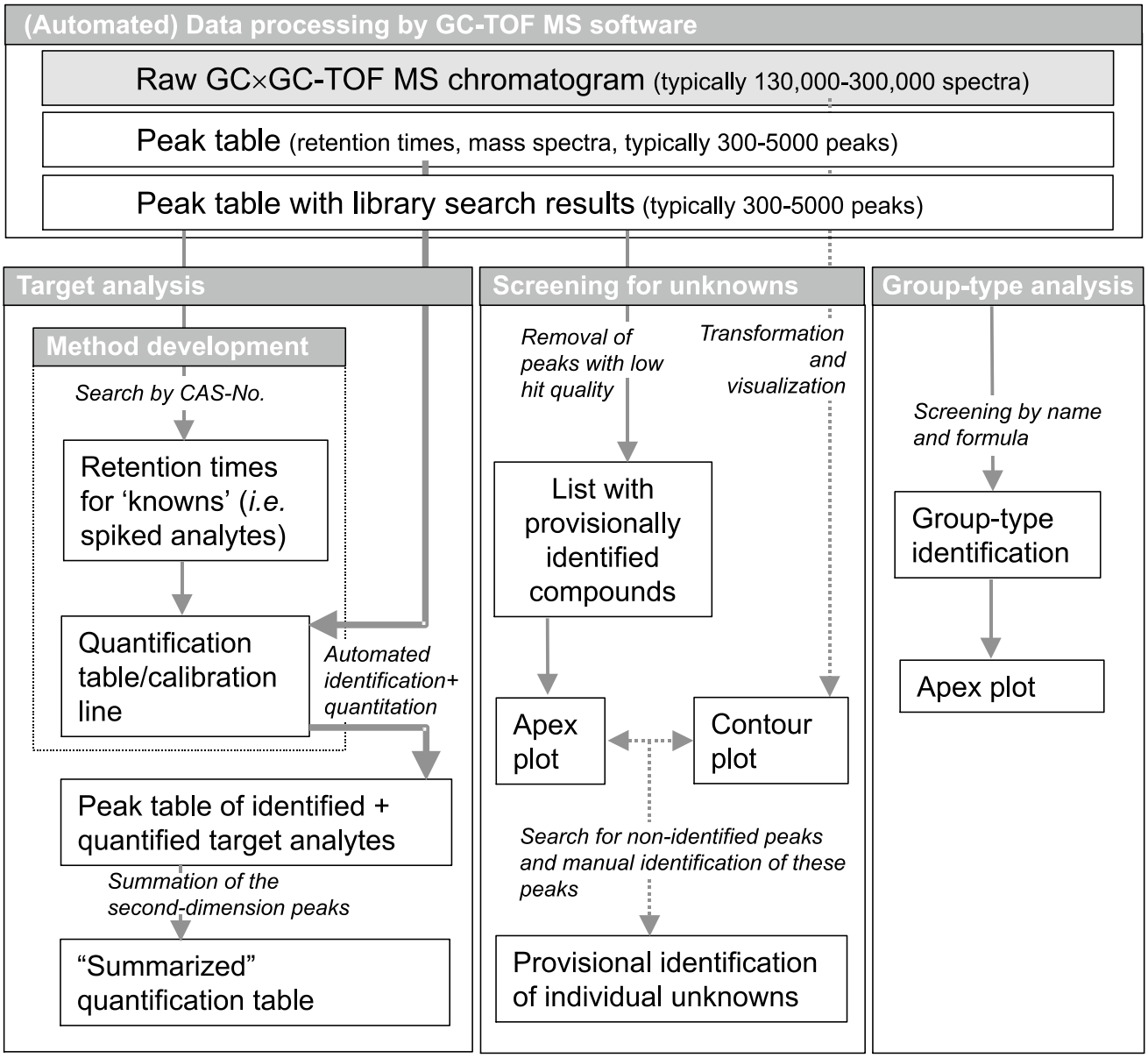


Figure 10.5. Flow diagram of the (automated) data processing of GC×GC–TOF data.

10.3.1 Modulation phase distribution.

Even when the process of modulation and data acquisition is synchronised as is described in the previous chapter, then in a modulated chromatogram, we may observe first-dimension peaks modulated into different types of peak pulse patterns. There is a strong time relationship between peak distribution and the pulsing process. The modulation process

normally generates these randomly, but it can be explained how they are generated and why different pulse patterns arise. Let us assume a first-dimension peak to be symmetrical in shape. The modulation process randomly distributes narrow fractions of this peak into a series of second-dimension chromatograms. This random distribution can have any form between the limits “in-phase” and “180° out-of-phase” as depicted in Fig. 10.6.

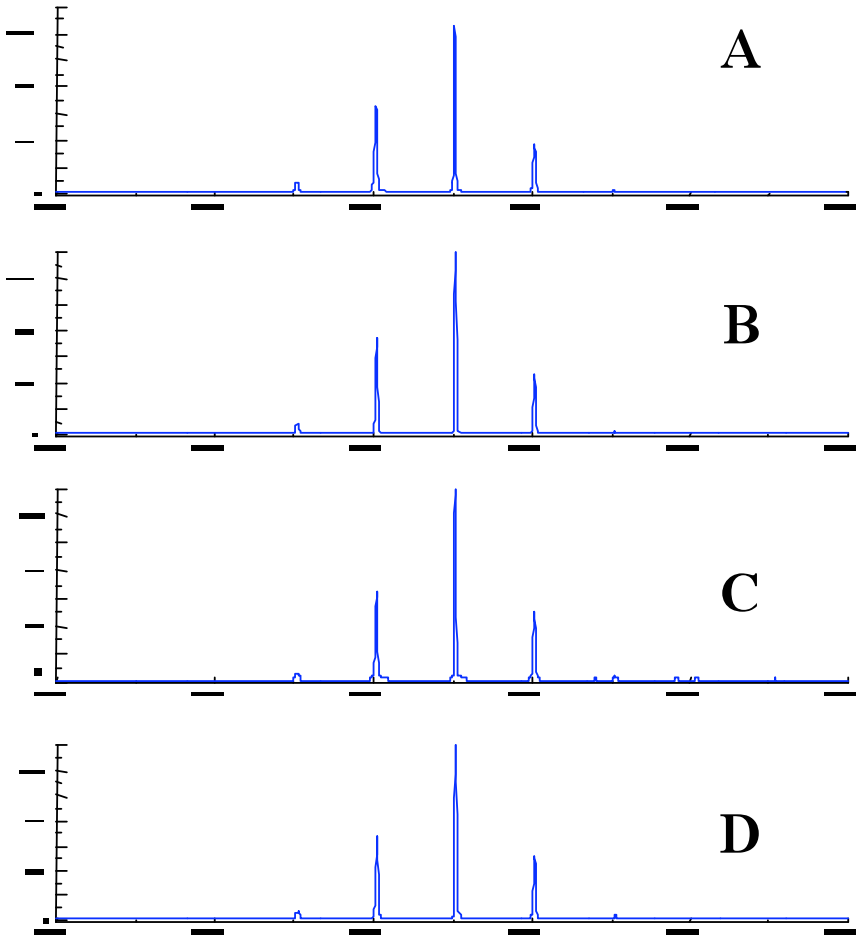


Figure 10.6. Modulation phase shift. (Courtesy of Philip Marriott).

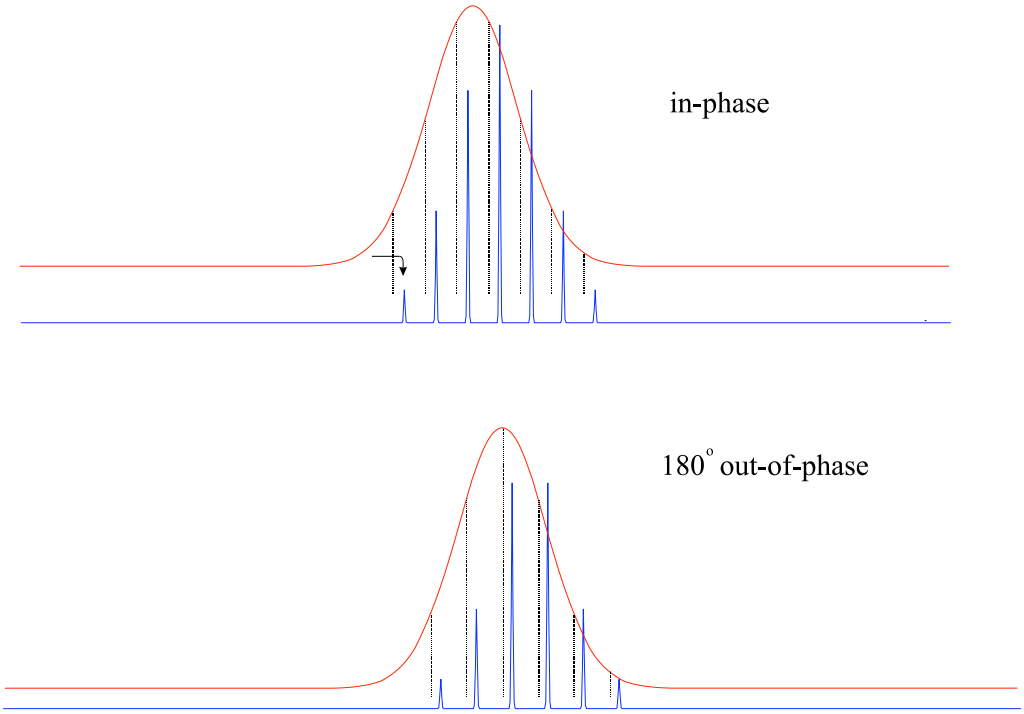


Figure 10.7. Reproducibility of chromatographic and modulation process. (Courtesy of Philip Marriott).

When both the chromatographic and the modulation processes are highly reproducible, then the (random) distribution patterns will also be highly reproducible. See Fig. 10.7, a highly reproducible distributed pulse pattern of a four-fold repeated injection of a single 1D-peak, with a modulation time of three seconds. Note that the peak pulse patterns in all four analyses are exactly the same. If we now shift the start of the modulation process, compared to the chromatographic process half a second each time, the modulation pattern will change, but eventually, after six of these half-a-second modifications, *i.e.* three seconds coincide with the original pattern. The pattern after three modifications is then of course 180° out-of-phase with the original one. This is clearly demonstrated in Fig. 10.8. Note that second-dimension chromatograms A and F have the same phase and that B and E are 180° out-of-phase.

An extended description of these distribution patterns and their background is given in a paper on phase relationships in GC×GC [102] and on modulation ratio in [103].

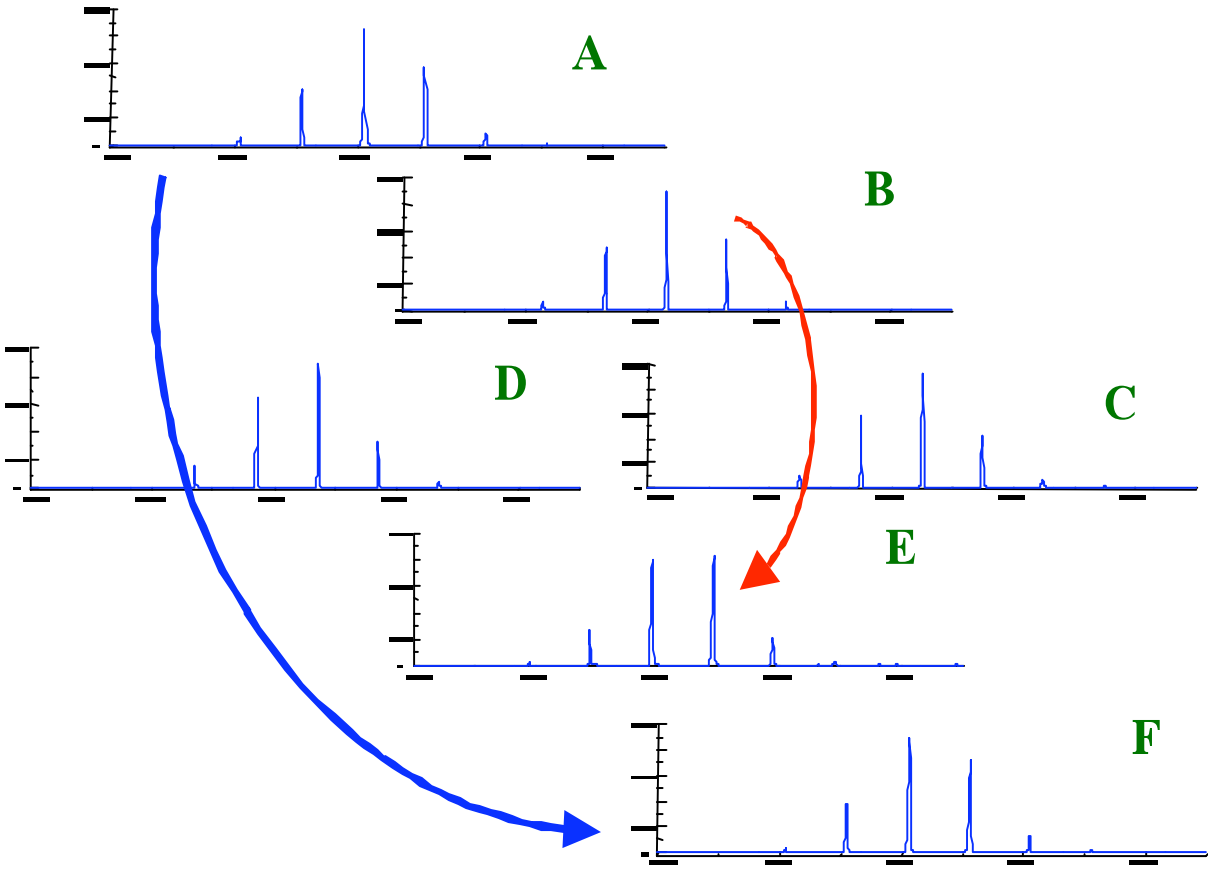


Figure 10.8. Shift in peak distribution patterns caused by a change in time of the modulation process.

Pattern A and F are similar and in phase, Pattern B and E are similar and 180° out-of-phase. (Courtesy of Philip Marriott).

10.4 Reproducibility of retention times in GC×GC.

Several authors have reported on the reproducibility of retention times in the two dimensions of GC×GC [44, 74, 103,]. Shellie *et al*. [106] demonstrated run-to-run, day-to-day, instrument-to-instrument, and column set-to-column set reproducibility from the experimental design. A total of 60 GC×GC analyses were conducted. The cryogenic system (LMCS) produced reproducible modulation starting times and consistent modulation phase profiles for individual components in all experiments, and retention time variations in both dimensions were negligible. The average run-to-run reproducibility of 43 components for six replicate injections was found to be 0.12% RSD in the first dimension, and 0.74% RSD in the

second dimension. The general conclusion of all authors is that reproducibility of retention times in the first dimension is (as could be expected) comparable with that in 1D-GC. Furthermore that reproducibility of retention times in the second dimension is very much dependent on the reproducibility of the modulation timing and, not so much on the chromatographic conditions.

10.5 Quantitative data processing

Now that we have visualised the GC×GC separation, we have only produced a qualitative result. Nevertheless, for quite some (especially complex) samples the structured colour plots provide a fast fingerprint of the sample. The structures present in the colour plot may provide quite some information and give a general insight in the composition of the separated sample. For a quick visual inspection of *e.g.* off-quality specifications of a sample, or a comparison of the similarity of various samples this sometimes may suffice.

However, in general the quantification of some or all of the separated components must be executed by integration of the separated peaks. The visualisation software that is generally used for producing colour plots, like *e.g.* Transform produce plots in which the individual second-dimension peaks that are descending from the same first-dimension peak are depicted into one spot (see also Fig. 10.3, lower part). This visualisation suggests that a spot in the colour plot is an entity and not a series of individual peaks. Accordingly, some quantification software is based on the assumption that the spots are an entity and have peak volumes [107]. Especially in those GC×GC separations that have been performed with relative long modulation times, and accordingly a rather wide first-dimension distance between similar second-dimension peaks, this may introduce quantitative errors. In order to produce a single spot in the colour plot from a series of similar second-dimension peaks, quite some interpolation has to be done. Several investigators report results obtained by using calculation procedures for determination of peak volumes [29, 108, 115]. Different results have been obtained, with RSDs ranging from 1% to 15%.

The most straightforward quantification of the separated components in GC×GC can be done by the integration of individual second-dimension peaks. This can easily be performed with existing chromatographic software and is highly accurate. Unlike the integration of poorly resolved or co-eluting peaks in crowded 1D-chromatograms, in GC×GC there are numerous “empty spaces” in a separation plane and baseline between peaks is available at various places. This is nicely demonstrated in Fig. 10.9, a separation of an extract of a river sediment as such. It is obvious that in the 1D–GC analysis the large envelope co-eluting with the polyaromatic species that are the target compounds in this analysis makes it completely

impossible to identify, but even worse, to quantify these compounds. From the first eluting peaks in the very beginning of the chromatogram until the very end the signal never returns to baseline. In the GC×GC analysis, however, there is baseline, not only between the large envelope of hydrocarbons in the bottom of the colour plot, but also between the polyaromatic species that are well separated now from the envelope and from each other. Moreover, because of the zone compression, peaks have relative steep up and down slopes and peak start and stop can be easily determined.

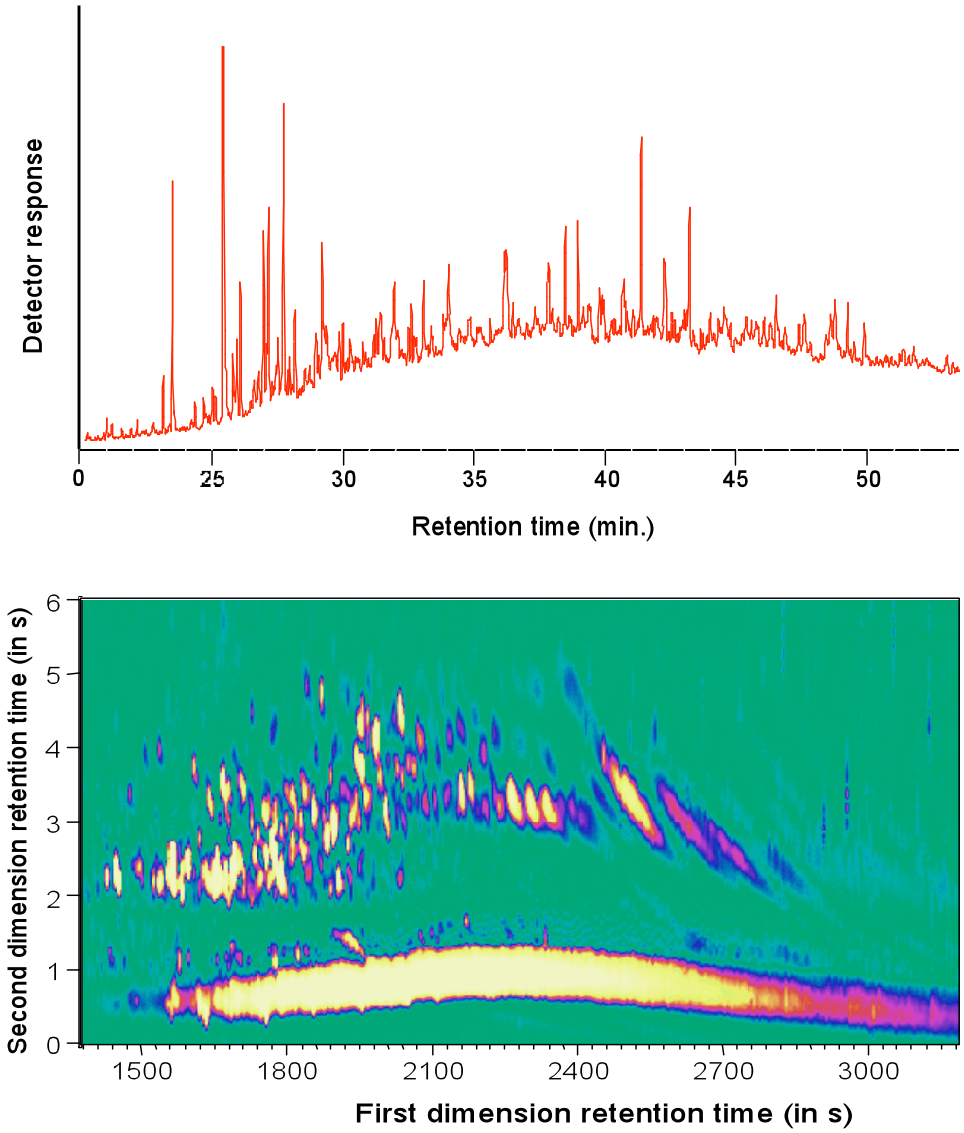


Figure 10.9. Separation of an extract of a river sediment.

Top: 1D-GC separation

Bottom: GC×GC separation. The lower intense (yellow) envelope is a band of hydrocarbons, the separate spots in higher second dimension retention times are (oxygenated and/or halogenated) polyaromatics.

The software then should be able to recognise which peaks belong to the same component and its areas should be accumulated. Several papers have been published on methods using this "summed integrated area" principle [29, 107, 113, 114]. Beens *et al.* [29] has been the first demonstrating that the quantitative results of GC×GC by using the "summed integrated peak area"-method are at least as good as those derived from 1D-GC analyses. Table 10.1 reflects the quantitative results they derived by using university-made quantification software ("Tweedee") written in a Matlab® environment. Van Deursen *et al*. [31] reported results derived with GC×GC−ToF MS and demonstrated that the ToF MS is not very well suited for quantification of large groups of non-calibrated isomeric (hydrocarbon) compounds, which have different degrees of ionisation and fragmentation. They propose the use of a ToF MS for method development (qualitative results), but deploy a FID for quantification.

Table 10.1. Quantitative comparison of GC×GC and 1D-GC results [29]

Compound	1D-GC	GC×GC	Δ	Rel. Δ (%)	SD (n = 14)	RSD
n-C_8 (impurities)	0.08	0.081	0.001	1.23	0.002	2.33
ethyl-benzene	4.50	4.46	-0.04	0.81	0.04	0.80
para + meta xylene	15.79	15.73	-0.06	0.41	0.09	0.54
ortho xylene	6.14	6.18	0.04	0.66	0.03	0.49
n-nonane	5.88	5.85	-0.03	0.46	0.03	0.55
iso propyl-benzene	9.49	9.45	-0.04	0.38	0.02	0.24
n-propyl benzene	5.52	5.52	0.01	0.13	0.07	1.28
tert. butyl-benzene +	2.93	2.96	0.03	0.88	0.03	1.08
sec. butyl-benzene	5.75	5.71	-0.04	0.72	0.03	0.68
para cymene	3.85	3.91	0.06	1.48	0.02	0.53
impurity	0.57	0.57	0.004	0.71	0.01	2.33
trans decalin	5.80	5.82	0.02	0.31	0.02	0.33
n-undecane	3.93	3.94	0.01	0.23	0.05	1.33
1,2,4,5 tetrame-benzene	3.14	3.16	0.02	0.57	0.02	0.68
1,2,3,4 tetrame-benzene	4.90	4.88	-0.02	0.39	0.03	0.57
naphthalene	1.08	1.04	-0.04	3.65	0.02	2.20
n-dodecane	4.42	4.43	0.01	0.32	0.02	0.41
n-tridecane	3.60	3.60	0.002	0.06	0.01	0.41
1-me-naphthalene	4.71	4.68	-0.03	0.58	0.03	0.67
average				0.71	0.03	0.92

[a] not separated in 1D-GC

An elegant chemometric method for quantification of target compounds, using so-called "multiway analysis methods" is proposed by Mispelaar *et al.* [119]. In their paper they claim that with regard to accuracy and precision, integration performs slightly better than Parallel Factor (PARAFAC) analysis. (See also Chapter 11.5, *The use of chemometrics in GC×GC data processing.*) In terms of speed and possibilities for automation, multiway methods in general are far superior to traditional integration.

But they all share the same conclusion that the accuracy of GC×GC quantitative results is at least the same as those acquired from a1D-GC analysis, if not better.

Depending on the purpose for performing GC×GC analyses, in processing data files four different methods for quantification of data can be distinguished:

1. Identification and quantification of individual target compounds,
2. Identification and quantification of non-target compounds,
3. Identification and quantification of groups of compounds, and
4. Pattern recognition and fingerprinting of the total separated sample.

In the following sub-chapters the data processes will be described briefly.

10.5.1 Target analysis

Analyses in which only a number of selected (and known) compounds have to be analysed, whether or not contained in a complex matrix, are considered to be target analyses. In general the exact co-ordinates of the spots representing these targets are already known, *e.g.* from GC×GC–ToF MS analyses. It is obvious that only those spots in the contour or colour plots of the sample have to be found, identified and quantified that are part of the targets. So, the data processing software should enable the identification and quantification of these peaks and ignore all other separated components. This procedure could also incorporate the use of retention indices or retention times. Generally this is part of a (routine) method that already exists and has been developed *e.g.* with the help of a ToF MS, but by itself can be performed with a single channel detector as FID or ECD. In Fig. 10.10 a colour plot is shown, demonstrating the target analysis of a number of selected pesticides in an extract of leek.

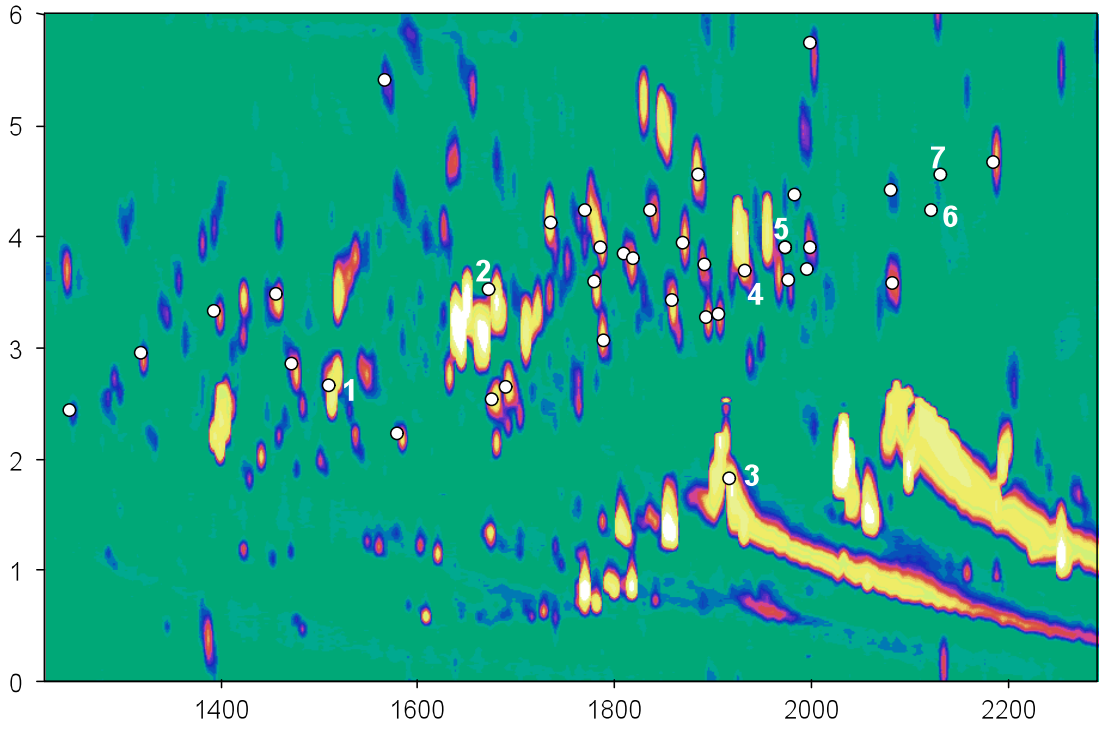


Figure 10.10. Detail of the GCxGC–ToF MS chromatogram of an extract of leek, spiked with a mixture of pesticides at a level of 0.16 ng/µl. White spots indicate the apices of the pesticide peaks. 1. Chlorpropham, 2. Pyrimethanil, 3. Fenpropimorph, 4. Bromophos-methyl, 5. Chlorfenvinphos(a), 6. Flusilazole, 7. Bupirimate.

10.5.2 Non-target analysis

If an unknown sample has to be characterised as completely as possible by identifying any separated component that can be identified, then it is almost always mandatory to use an MS detector or a selective detector for identification purposes. The data processing software should therefore be able to handle the large amount of data that is present and enable the use of *e.g.* deconvolution and library search routines for identification. It is obvious that such software should include data reduction modules, *e.g.* integration of individual peaks and reduction of the data to peak and apex tables or in making apex plots. An example of such a non-target analysis is given in Fig. 10.11, in the characterisation of an extract of cigarette smoke [109]. This paper extensively describes the data processing steps that are necessary for performing such an analysis. This data processing is schematically depicted in Fig. 10.4.

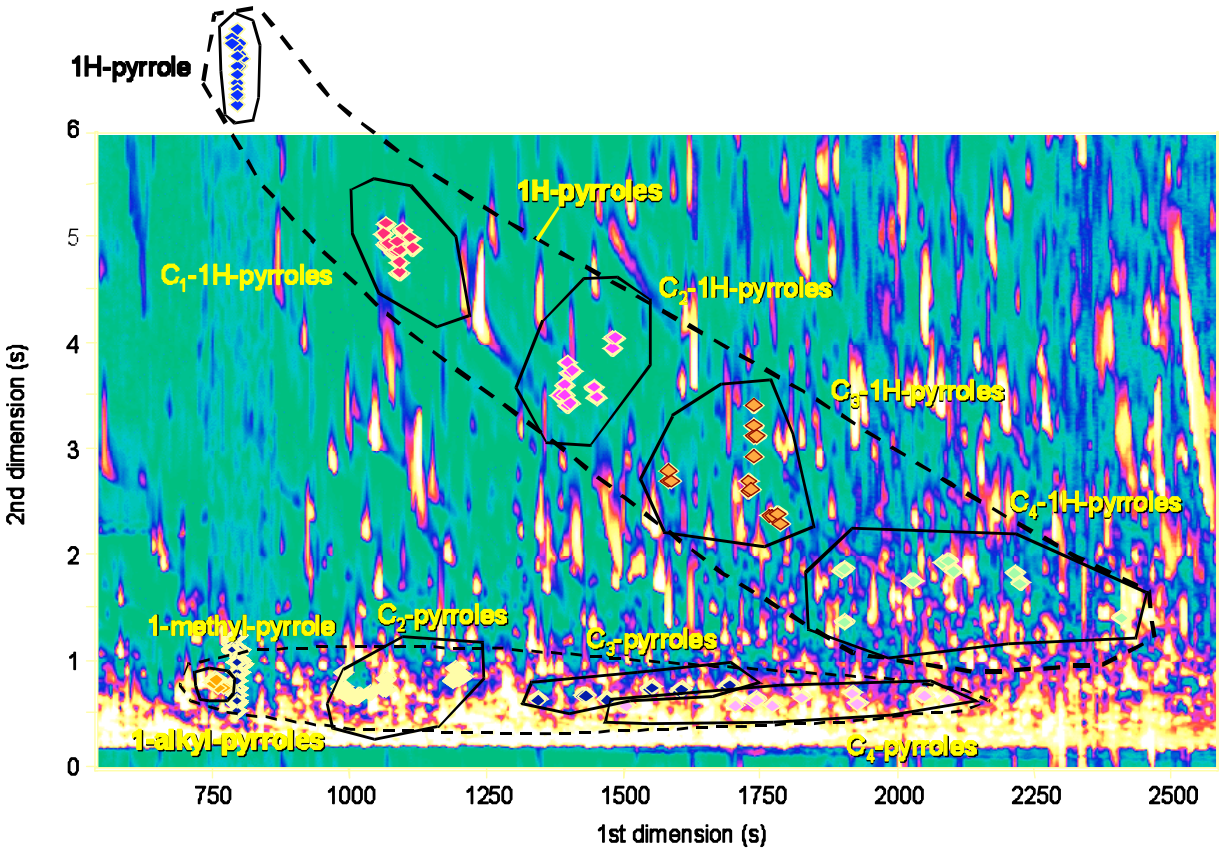


Figure 10.11. Colour plot of the GC×GC–ToF separation of cigarette smoke.
Only the 1-alkyl-pyroles (horizontal band) and the substituted 1H-pyroles are indicated.
The identified compounds are designated by their apex coordinates.
Note that 1H-pyrole itself exhibited wrap around and has been transferred to its exact retention time.

10.5.3 Group-type analysis

In Chapter 3.2.2, *structured separations*, it is described how orthogonal columns can provide structured separations and bands of components along the separation plane. Generally samples that contain not too many different types of compounds, but large numbers of members of the types present, are amongst petrochemical based samples and edible and essential oils. In Fig. 10.12 such a separation is depicted, clearly demonstrating the different groups that are present. It is not necessary nor possible to separate all the different isomers, let alone that they can be identified. Group-type characterisation of these samples generally suffices. The data processing software for identification of all constituents of a chemical class and quantification of these is very different from target and non-target analysis software. It should include graphical means to define and enable the visualisation of the retention windows, *e.g.* the possibility of drawing polygons on the screen where the contour or colour plot is depicted to define the borders of areas, in which all peaks should be summed.

Furthermore it should contain tools to indicate/visualise the peaks found by the integration software.

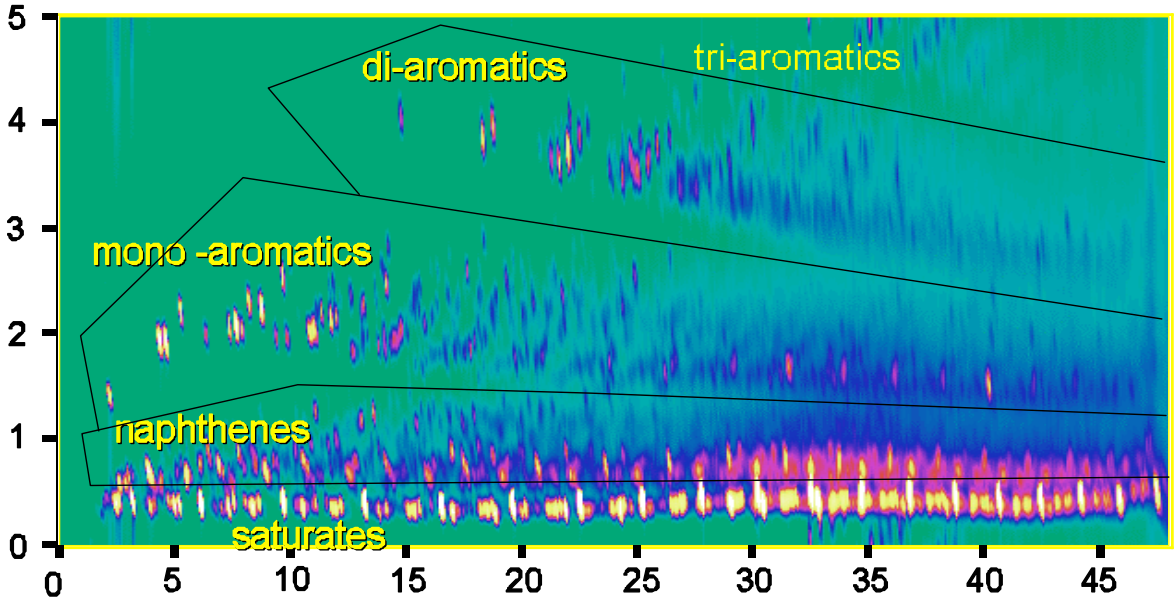


Figure 10.12. Group-types in colour plot of the GC×GC separation of a gasoil.
The different groups are indicated by the polygons.

10.5.4 Pattern recognition and fingerprinting

Sometimes it is not necessary to identify separated compounds of groups of compounds nor to quantify them. For specific samples as oil spills, fire debris samples, off-spec. samples, food adultery samples, it is sufficient performing a pattern recognition data processing step in order to judge whether they comply with reference samples. These pattern recognition and fingerprinting data processes can only be performed by chemometric means. See also Chapter 11.5, *The use of chemometrics in GC×GC data processing.*

It is obvious that the quantification software that is to be developed now should include all of the aforementioned options.

10.6 The use of chemometrics in GC×GC data processing.

In view of the large amount of data that is available from a GC×GC separation it is tempting to use chemometrics for enhancement of the quality or the amount of information to be extracted from the available data set. In that respect the signal to noise ratio can be enhanced by reduction and averaging of the noise, or the retention time stability can be made more secure. Moreover, sample product properties or process conditions can be correlated

with the patterns that arise from a GC×GC separation, thus providing information on these properties and enabling predictions on these items.

The various second-dimension retention times of an individual compound in adjacent second-dimension separations have a relationship that can be estimated by multivariate analyses (MVA). Considering the fact that, although a second-dimension separation is essentially performed isothermally, each next second-dimension separation will be executed at a somewhat higher temperature. Accordingly, each next second-dimension peak descending from the same first-dimension component will have a somewhat shorter second-dimension retention time. This decrease in second-dimension retention time follows a curve that can be estimated rather simple. An example of a GC×GC plot in which these curves are nicely depicted is given in Fig. 10.13. Here the descending retention time curves of a series of n-alkanes, designated as "iso-volatility" lines, are demonstrated. These curves were obtained by a continuous injection (non-heated injection port) of a series of n-alkanes [71]. With continuous rising second-dimension temperatures the retention factors of the n-alkanes will decrease and finally tend to approach the retention factor $k = 0$ as the vapour pressure $p^0_i \rightarrow \infty$ and the dead time $t_0 \rightarrow 0$.

MVA techniques can also be used for the construction of models that predict product properties and process variables from the analytical data as acquired from GC×GC separations. This can be done by linking the patterns obtained from MVA of GC×GC separations and physical or chemical properties of the sample.

The Generalised Rank Annihilation Method (GRAM) [56] enables deconvolution of chromatograms with reduced resolution. Although GC×GC generally provides high-resolution results, even with this technique co-elution still frequently occurs. GRAM enables deconvolution of these co-eluents. This method, however, requires a highly run-to-run retention time precision in the second column, a rather minimal signal intensity ratio and a minimum sampling frequency [103].

Another chemometric approach, trilinear Partial Least Squares (tri-PLS) data analysis, was applied to the quantification of the aromatic and naphthenic contents in a naphtha fraction [120]. This technique, however, needed that the complete GC×GC separation was performed at a constant temperature for the second-dimension, *i.e.* the temperature of the second column did not change during the entire run.

Van Mispelaar *et al*. demonstrated the use of MVA methods for processing GC×GC data sets for quantification purposes and designated this technique as *chromametrics* [121].

As an effort to reduce the ambiguity of GC×GC retention times, Micyus *et al*. [122], developed a simple algorithm that determines absolute secondary retention times when wrap-around (*see* above) occurs. The efficacy of this algorithm is tested and found to be particularly

useful during the initial stages of method development since accurate values of absolute secondary retention times are vital for proper compound identification and also helpful in optimizing experimental conditions and using chemometric methods. In another effort, Ni *et al*. [122] developed the so-called affine transformation model to remove the pattern variations resulting from small variations in acquisition conditions. The approach can be used for applications such as pattern matching *e.g*., fingerprinting and normalizing retention times to retention indices when their reliable generation is developed.

In a recent paper [130] Hollingworth *et al.* discusses extension of conventional techniques for image comparison by utilizing specific characteristics of GC×GC data and developing new methods for comparative visualization and analysis. The paper describes techniques that register (or align)
GC×GC datasets to remove retention-time variations; normalize intensities to remove sample amount variations; compute differences in local regions to remove slight misregistrations and differences in peak shapes; employ color (hue), intensity, and saturation to simultaneously visualize differences and values; and use tools for masking, three-dimensional visualization, and tabular presentation with controls for graphical highlights to significantly improve comparative analysis of GC×GC datasets. Experimental results indicate that the comparative methods preserve chemical information and support qualitative and quantitative analyses. Although not too much evidence is incorporated in the paper, the development of these techniques will continue and might produce proper pattern comparison and recognition techniques.

An overview of the different chemometric techniques and methods used in GC×GC is presented by Sinha *et al.* [123]. They finally conclude that "there is still much research to be done to bridge the gap between 2D separations and chemometrics. One major area that has the potential to meet some of these challenges and advance this field is the development of novel algorithms for retention time alignment of 2D data. Although most of the chemometric work to date has been done with GC×GC data, the inherent characteristics of all 2D separations, e.g. LC×CE and CE×CE, make them amenable to chemometric analysis. Some of the most exciting new developments may come by applying pattern recognition with 2D separations and mass spectrometric detection. The amount of information resulting from these separations is so great that only with the implementation of chemometric techniques will the desired information be garnered efficiently and completely."

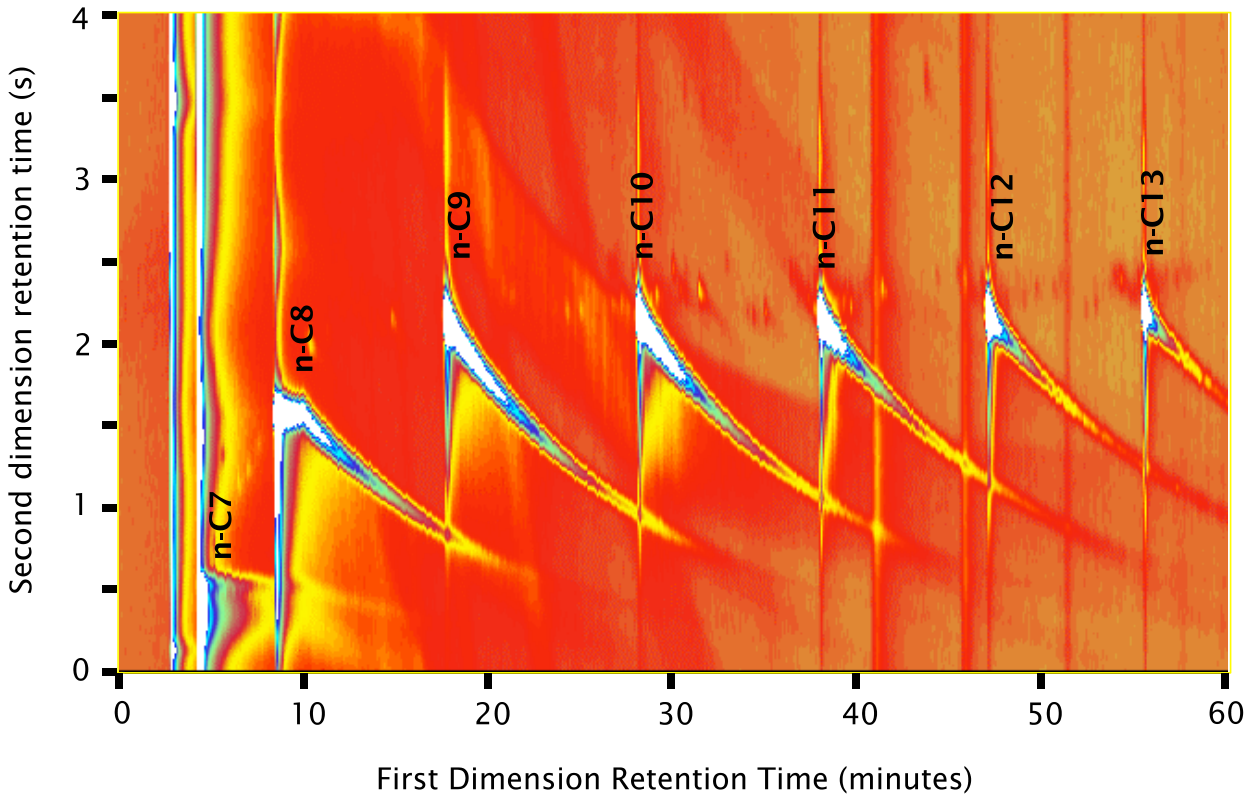


Figure 10.13. "Iso-volatility" curves of seven n-alkanes obtained from a GC×GC separation after continuous injection.

11. Perspectives and future developments.

As already stated in the introduction of this book, it is now 140 years ago that the first experiments on chromatography have been applied by Goppelsröder and exactly 100 years after Tswett performed his first separation experiments, which he called chromatography. Gas chromatography has now been used for almost fifty years and although the speed at which new developments have been introduced in GC is diminishing in the last decade, a new and exciting technique, comprehensive two-dimensional GC has given GC a revival. The number of developments in GC×GC over the last decade is quite substantial and GC as a separation technique has made a leap ahead. Nevertheless, this technique is up till now only accepted and applied in a limited number of research laboratories. There is, however, a sharp increase in interest exhibited for GC×GC in the last few years as is clearly shown by the number of papers on this technique presented at and the number of participants in symposia dedicated to GC×GC. It is therefore to be expected that GC×GC will now breakthrough as a general accepted and implemented analytical technique. At this moment in time three manufacturers work on the further development and adaptation of commercial instrumentation and software. Further interesting developments in this field will most probably follow. These new developments may include both hardware and software items.

11.1 Hardware developments

As far as the hardware is concerned, modulators as they have been developed up to now work quite satisfactory. They indeed produce very narrow bands for re-injection, and some of them are very easy in use and very robust. It is nevertheless, in view of existing patent claims, to be expected that some new forms or even principles for modulation will be developed in the near future. Especially now that it has been proven that single-stage modulation is a feasible technique [47]. Next developments in hardware will probably include the columns. Combining the two dimensions into one physical capillary, so that connecting the columns is no longer necessary, has already been demonstrated [125]. But the major adaptations in hardware should be performed in detector technology. As already pointed out in Chapter 8, at this moment in time only the FID and the ToF MS are, without any adaptations, suited for the proper detection of the ultra fast peaks that are generated in GC×GC. Other selective detectors like ECD, SCD and NCD (Nitrogen Chemiluminescence Detector) have either a too large detector cell volume or too slow electronics [82, 83]. Although, from 2006 on, some Chemiluminescence Detectors have been adapted such, that

they have been used for GC×GC successfully [135]. In this paper two NCDs have been compared and one of them fulfils the GC×GC requirements. The FPD (Flame Photometric Detector), PFPD (Pulsed Flame Photometric Detector), PDECD (Pulsed Discharge Electron Capture Detector), TID (Thermoionic Detector) and PID (Photoionic Detector) should also be adapted accordingly before they are able to be used as detectors in GC×GC.

An interesting option that never has been published to be the purpose of an experiment is the programming of the temperature of the second column. Normally in GC×GC the second separation is such fast that is essential isothermal. This means that peaks eluting from the second dimension will increase in peak width in every second dimension separation. If the temperature of the second column could be programmed very rapidly during the short duration of the secondary separation, all peaks would exhibit the same (narrow) peak width. It would enhance the detectability of those (generally polar) compounds that exhibit enhanced retention times on the second column. It would moreover simplify data processing considerably since all peaks would have more or less the same slopes and peak widths. It will not enable a faster modulation though, because, although the retention time of the second dimension would decrease, this spared time of the second dimension is necessary for cooling the second column to the original temperature again before the next modulation (and temperature programming) could be started.

Already in the first publication on GC×GC [17] Phillips envisaged a three-dimensional form of his technique: GC×GC×GC. Since, even with the extreme high resolution that GC×GC can offer, not all of the individual constituents of complex samples can be separated, comprehensive three-dimensional GC (GC×GC×GC) would be an interesting option. Such a construction can only work if the speeds of the three dimensions are sufficiently different from each other (see Fig. 11.1). This could be performed by combining *e.g.* a relative long, thick film, 0.53 mm ID capillary column in the first dimension with a short 0.25 mm ID column in the second dimension and a short 0.05 mm ID column in the third dimension. The first column must be non-polar for producing the “boiling point” separation, the second column *e.g.* a BPX 50 column for the separation according to aromaticity of the separated components, and the third *e.g.* a Carbowax column for the H-bonding separation. Using two modulators could be necessary in view of the fact that two different modulation times have to be used and the speed in the three columns is substantially different. From an instrumental point of view such an arrangement should not be too difficult to sustain, but the tuning of the system and the data processing will be much more

complicated than that of GC×GC. A visualisation of a hypothetical separation from such a GC×GC×GC system is depicted in Fig. 11.1.

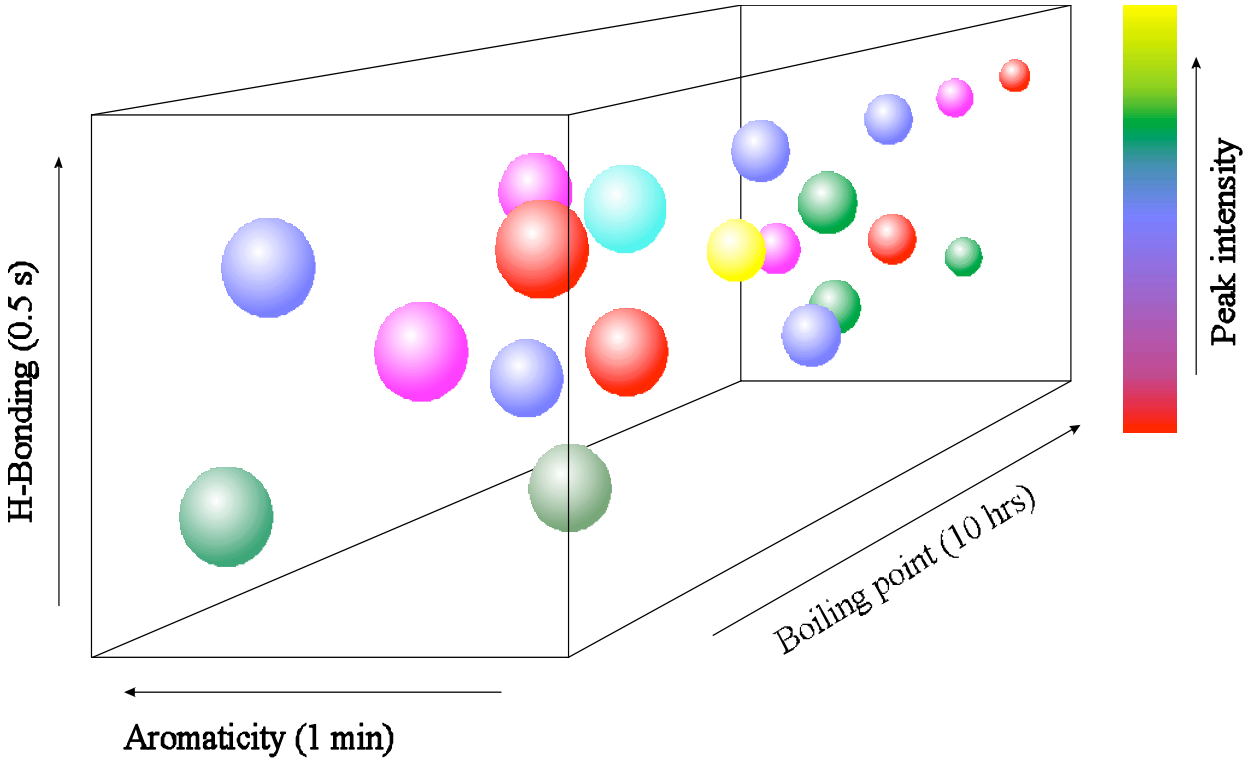


Figure 11.1. Colour plot of a hypothetical comprehensive three-dimensional GC separation. Compounds are separated according to three different properties: first dimension – boiling point; second dimension – aromaticity; third dimension – H-bonding ability.

Now, we can extract two different colour plots from the produced GC×GC×GC data file, *viz.* one of the boiling point separation against the H-bonding separation by taking the side-view. The other of the boiling point against the aromaticity separation by taking the top-view. These two colour plots are depicted in Fig. 11.2. Such dual colour plots can also be obtained from a single analysis from a GC×2GC system as described in Chapter 6.3, or from two separate GC×GC systems with different secondary columns. It is obvious, that, if we are able to identify each individual spot in both those very simple contour plots, or in other words, if we are able to indicate what the two pairs of retention times of the individual compounds are, then we know all the coordinates of the compounds in a 3D-space. And then we could draw a figure as presented in Fig. 11.1 exhibiting the three-dimensional separation. But, in general we are not able to identify all individual separated spots in a GC×GC colour plot. Such a separation is only performed on complex samples, and the majority of the

separated peaks can not or only tentatively be identified. This makes the transformation from two (different) colour plots into one three-dimensional plot impossible.

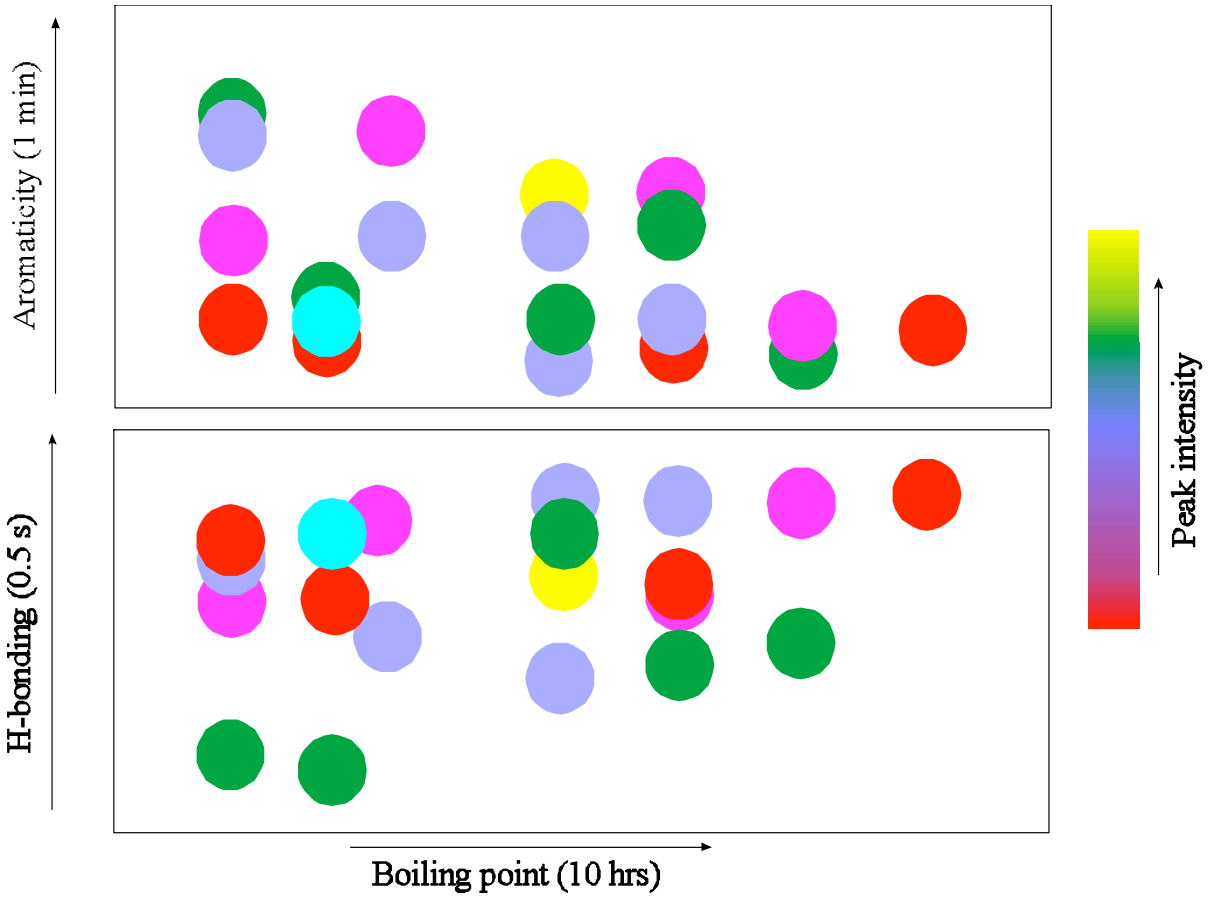


Figure 11.2. Dual colour plots extracted from the GC×GC×GC separation of Fig. 11.1. Upper colour plot: distribution between boiling point and aromaticity of the compounds, Lower colour plot: distribution between boiling point and the H-bonding ability of the compounds.

If we would hyphenate such a GC×GC×GC system with a ToF Ms, which is almost inevitable in view of the complexity of the sample, extremely large data files will be produced (> 10 Gb) containing over 10,000,000 complete spectra. Processing these data will only be possible with extremely powerful computers with very intelligent data reduction and processing software, and the visualisation will put a severe assessment to our ability of spatial perception.

Deviant from some prevailing opinions, we do not consider an MS to add an extra dimension onto a separation system. So, a GC×GC×GC–Tof MS is a three-dimensional separation system, although hyphenated to a detection system that can add extra information on the (more or less) separated species. Especially the deconvolution algorithms can provide this extra information on analytes that are not fully separated, but only if they are already partially separated. Therefore there will be a clear difference in the designation of the

acronyms for multidimensional separation systems containing the multiplication sign (×) and hyphenated systems indicated by the hyphen (–). For a complete list of definitions and acronyms used in the techniques described in this book, see the appropriate Chapter, Nomenclature, to be found as a separate pdf on Chromedia.

As already stated in Chapter 9, *Injection systems*, probably all pre-separation systems that can be coupled on-line to a 1D–GC system can also be coupled to a GC×GC system. These include SPE–GC×GC, LC–GC×GC, SFC–GC×GC. These on-line coupled systems probably have to be combined with a (ToF)MS as a detector for compound identification or confirmation. In the near future it is to be expected that all of these forms of hyphenated systems will be demonstrated.

11.2 Software developments

As far as software is concerned for processing the large data sets derived from GC×GC separations and extracting all of the information that is contained in these data sets, this is the area in which most of the pitfalls exist. Up till now most of the processing steps that have been performed for obtaining the published results, have been done by home-written software programmes and modules. Experienced and well-educated personnel with knowledge and understanding of the processes involved have accomplished all of these processing steps. As a consequence of this, there is a rather perspicuous insight in what is needed in this field in order to provide routine-personnel the appropriate tools for performing these complicated tasks. At the same moment quite some research groups and manufacturers are in the process of developing software tools both with conventional as well as chemometric data processing features. If the two groups of research workers and manufacturers can co-operate in the development stage of new software, it is to be expected that a number of different and commercially available software tools will be produced for automated processing GC×GC data in the near future.

11.3 GC×GC applications

Up till now very many areas where GC×GC successfully has been applied on real-life samples have been published. The latest being the analyses of metabolomics [132, 133, 134], of which the latter has been combined with principal component analysis. Nevertheless still some areas have not yet been explored to exploit this technique. It is to be expected that these will be covered in the near future.

Concluding, although it is difficult to foresee what the real direction of new developments will be in separation science, we may well end by quoting the last sentence of the review on GC×GC by John Phillips [33], who all started this technique: *"The future of GC×GC is bright× bright*."

Appendix. Nomenclature and conventions

In the First Symposium on Comprehensive Two-Dimensional Gas Chromatography, held in March 2003 in Volendam, the Netherlands, the participants, after an extensive discussion, unanimously agreed upon the following nomenclature and conventions for further use in this technique [P. Schoenmakers, P. Marriott. J. Beens, *Nomenclature and Conventions in Comprehensive Multidimensional Chromatography*, LC–GC Europe, 16 (2003) 335-339

1 General nomenclature

Table 1: Examples of abbreviations involving the multiplex (×) sign.

Abbreviation	Full term
GC×GC	Comprehensive two-dimensional gas chromatography
GC×GC–FID	Comprehensive two-dimensional GC with flame-ionization detection
GC×GC–MS	Comprehensive two-dimensional GC with mass spectrometric detection
LC×LC	Comprehensive two-dimensional liquid chromatography
LC×SEC	Comprehensive two-dimensional (liquid×size-exclusion) chromatography
LC×GC	Comprehensive two-dimensional (liquid×gas) chromatography
SFC×GC	Comprehensive two-dimensional (supercritical-fluid×gas) chromatography
GC×GC×GC	Comprehensive three-dimensional gas chromatography
LC–GC×GC	On-line liquid chromatography–Comprehensive two-dimensional gas chromatography
SFC–GC×GC	On-line supercritical-fluid chromatography–Comprehensive two-dimensional gas chromatography

Table 2: Definitions of "orthogonality" in various fields of science.

Field	Definition of orthogonality
Mathematics*	Of two vectors or functions: perpendicular; having an inner product equal to zero. Of a set of vectors or functions: such that the inner product of any two is zero if and only if the two are distinct.
Statistics*	Of a set of variates: statistically independent. Of an experimental design: such that the variates under investigation can be treated as statistically independent.

Analytical Chemistry	Of two separation dimensions: such that the elution times in both dimensions can be treated as statistically independent.

* From the Oxford English dictionary (on-line edition).

2 Other Definitions

In Table 3 a number of other relevant definitions are collected. A number of these are specifically derived from the field of GC×GC, but their use is not exclusive for this technique. For example, the word modulator may equally well be used to describe the intermediate stage in a number of other comprehensive two-dimensional separation methods. One result from the discussion was that a modulator does not necessarily need to incorporate a focusing effect. The latter is easily achieved by thermal modulation in GC×GC, but it is not usually encountered in the comprehensive two-dimensional combination of two liquid-phase separation methods.

Table 3: Nomenclature suggested for comprehensive two-dimensional (gas) chromatography.

Term	Definition
Modulator	Interface device between the two columns in a comprehensive two-dimensional separation system that accumulates or samples narrow bands from the eluate of the first column for fast re-injection into the second column.
Modulation time or Modulation period (P_M)	The duration of a complete cycle of modulation in a comprehensive two-dimensional separation system (equals the length of a second dimension chromatogram*, i.e.*, the time between two successive injections into the second column).
Modulation frequency (f_M)	Number of modulations per unit of time.
Modulation number (n_M)	The number of modulations for a given first-dimension peak.
Single-stage modulation	Accumulation and focusing during one series of processes at one location in the modulator.
Dual-stage modulation	Accumulation and focusing during two successive series of processes at two locations in the modulator.
Focusing effect	Reduction of the band width (in time, distance and/or volume units) (= band width without modulation/band width with modulation).

Sensitivity enhancement	Ratio between peak height with and without modulation (peak-amplitude enhancement) (note: sensitivity refers to the signal, not to the noise!).*
Zone compression	The effect of reducing a chromatographic peak (width) in space or time to give a higher concentration within a chromatography column.
Separation space	The region within the two-dimensional GC×GC plot in which compounds are, or may be, distributed.
Wrap-around	The occurrence of second dimension peaks in subsequent elution sequences, caused by second-dimension retention times that exceed the modulation time of a comprehensive two-dimensional system [46].
Modulation phase (F_M)	The pattern of modulated peaks caused by the time relationship between peak distribution and the pulsing process of the modulator in a comprehensive two-dimensional separation system [98].
In-phase modulation	The modulation phase that produces a symmetrical sequence of peaks with a single maximum pulse peak [4].
Out-of-phase modulation	Any modulation phase that produces a non-symmetrical peak-pulse distribution [98].
Column set	The combination of columns used for a given GC×GC experiment.
Column set cross sectional area ratio	The relative change in cross sectional area for the ^1D to ^2D columns of the column set ($^1D/^2D$).
Chromatographic structure	The observed ordering of chemically related compounds in the plane of a comprehensive two-dimensional separation.
Colour plot	Two-dimensional plot representing a comprehensive two-dimensional separation, in which the colour represents the signal intensity of the separation system.**
Contour plot	Two-dimensional plot representing a comprehensive two-dimensional separation, in which similar signal intensities are connected by means of a line.**
Apex plot	Two-dimensional plot representing a comprehensive two-dimensional separation, in which peak apices of second-dimension peaks are displayed by a symbol on the co-ordinates of the maximum of the second dimension signal intensity.**

* A reduction in the detection limit may also be achieved. This reduction is proportional to the product of the sensitivity enhancement and the noise reduction.
** The x-axis represents the first-dimension retention time, the y-axis the second-dimension retention time of the separation system.

3 Symbols

The next table lists a number of symbols that are recommended for use in comprehensive two-dimensional separations in general and GC×GC in particular. The use of the superscript prefix 1 or 2 is suggested to distinguish between the first- and second-dimension columns.

Table 4. Non-exhaustive list of symbols suggested for use in GC×GC (and other comprehensive two-dimensional separation methods).

Symbols	Definition
${}^1d_c$, ${}^2d_c$	Internal diameters of the first- and second-dimension columns (respectively) used in a comprehensive two-dimensional system.
${}^1D$, ${}^2D$	First dimension and second dimension of a GC×GC system.
${}^1t_R$, ${}^2t_R$	Retention times of a peak in the first and second dimension of a comprehensive two-dimensional system respectively.
${}^1t_M$, ${}^2t_M$	Hold-up times (or "dead" times) of the first and second columns of a comprehensive two-dimensional system respectively.
${}^1k$, ${}^2k$	Retention factors of a peak eluting from the first-and second-dimension columns of a comprehensive two-dimensional system respectively.
${}^1I$, ${}^2I$	Retention indices of a peak eluting from the first- and second-dimension columns of a comprehensive two-dimensional system respectively.
${}^1N$, ${}^2N$	The numbers of theoretical plates of the first and second columns of a comprehensive two-dimensional system respectively.
${}^1\sigma$, ${}^2\sigma$	Standard deviations of a peak eluting from the first-and second-dimension columns of a comprehensive two-dimensional system respectively.
${}^1w_b$, ${}^2w_b$	Peak widths at base of a peak eluting from the first-and second-dimension columns of a comprehensive two-dimensional system respectively.
${}^1R_s$, ${}^2R_s$	Resolution values of a peak pair eluting from the first and second column of a comprehensive two-dimensional system respectively.

^{1}n, ^{2}n	Peak capacities of the first and second columns of a comprehensive two-dimensional system respectively.
$^{1}d_f$, $^{2}d_f$	Film thicknesses of the first and second columns of a comprehensive two-dimensional system respectively.
$^{1}\hat{u}$, $^{2}\hat{u}$	Average linear velocities in the first and second columns of a comprehensive two-dimensional system respectively.
$^{1}T_e$, $^{2}T_e$	Elution temperatures for a peak eluting from the first dimension and second dimension of a comprehensive two-dimensional system respectively.

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