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GC×GC Handbook

Fundamental Principles of Comprehensive 2D GC

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Prof. Luigi Mondello Università degli Studi di Messina

1. Principle of GCxGC

Comprehensive gas chromatography, namely GCxGC, is the last destination reached by separation science. It falls into the category of multidimensional techniques, mainly due to the association of two different mechanisms of separation. Compared to the more known MDGC (Multidimensional Gas Chromatography), GCxGC is more "comprehensive" toward the separation process involving sample analytes; in other words, this means that in GCxGC, every portion of the eluate coming from the primary (1D) column undergoes a further 2D separation, instead of MDGC, where the most common method utilized, "heart-cutting" [1-4], transfers only selected portions of eluate from the first to the second dimension. Subjecting the entire sample to a double separation process becomes a necessary condition to achieve a GCxGC separation. Also, analytes separated in the first column must remain separated when passing to the second column. These two analytical phenomena, that make GCxGC a unique separation technique, can be achieved through the "core" of all GCxGC instrumental set-ups: the modulator, which acts as a living interface between the two columns or dimensions of separation.

Basically, a comprehensive GC apparatus exploits two different stationary phases the most common set is non-polar, with conventional dimensions, and a polar, characterized by "fast" features, located in the same or in separate GC ovens. The modulator is placed between the 1D column exit and the 2D column inlet and its functions are to trap, isolate, focus and reinject the bands of 1D eluate in the 2D column. Samples are normally injected at the head of 1D column, they undergo separation, then, by means of the modulator, are diverted to the second dimension, where analytes undergo further separation; finally, they reach the detector, located at the exit of the 2D column.

The exhaustive transfer of a primary dimension eluting peak into the secondary column can be achieved with an appropriate modulation time, which is the time employed by the modulator for sampling (trapping and releasing) 1D peaks. Commonly, the modulation time, being in the order of seconds, is not sufficient to transfer an entire peak, but more reasonably slices of it, generating a series of 2D retention times. Such a separation mechanism adds a new dimension to the visual information that can be obtained by the analyst: the GCxGC chromatogram.

Compared to the conventional GC plots, in comprehensive GC the chromatogram is built up no longer on two (retention time vs. signal),

but on three axes, adding an extra dimension consisting of 2D retention times, as can be seen in figure 1.1 [5]. Therefore, the look of GCxGC chromatograms appears completely different from conventional GC profiles, showing a bidimensional plane where analyte spots are scattered about.

It is obvious that the strengthening of the separation power, with peaks coming from two dimensions of separation, leads to the formation of a crowded data plot, fact that has imposed in the last years serious troubles in data handling, for both analytical (quantitative analysis) and electronic (bigger file size) aspects. This last issue represents the topic of a consistent slice of the latest published papers. From time to time, researchers have tried to provide a solution to this problem, proposing software systems based on graphical measurements or chemometrics/statistical analysis (i.e. parallel factor analysis, generalized rank annihilation, etc.).

This handbook provides a detailed description of comprehensive GC technology and intends to be a clear reference guide for GCxGC beginners or simply curious readers interested in this amazing newborn branch of chromatography.

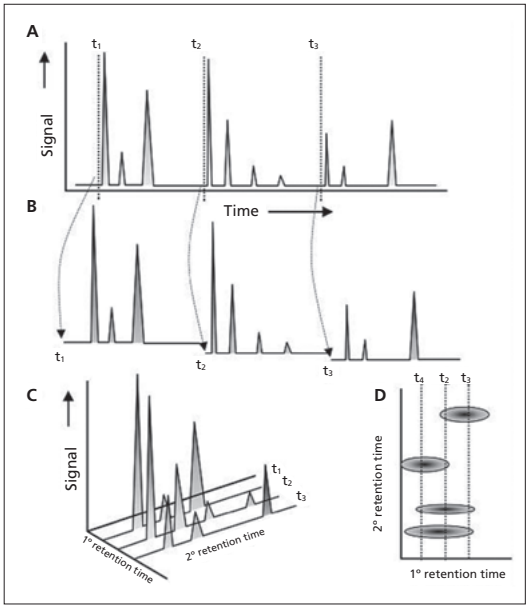

Figure 1.1

A and B: determination of the 2D retention times of the modulated peaks. C: orthogonality. D: GCxGC bidimensional plot.

Górecki et al.: *The evolution of comprehensive two-dimensional gas chromatography (GCxGC)*. J. Sep. Sci. 2004. 27. 359-379. © Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

2. Basic Design of GCxGC Instrumentation

Basically, comprehensive 2D GC exploits the same type of instrumentation utilized in monodimensional GC, with the novel introduction of the modulator within its apparatus. A simple scheme of a GCxGC system is shown in figure 1.2, where the modulator is, in this case, a dual-jet cryogenic modulator [6]. The apparatus is composed of the GC injector, the primary column, the modulator, the secondary column and the detector.

Columns can be placed in the same or in two separate GC ovens; the latter option seems to be preferred when asking for two independent temperature controls. In fact, as it will be explained ahead in the following sections, the two columns are coated with different types of stationary phase, hence, the limits of operating temperatures very often vary from each other.

Figure 1.2 anticipates also how cryogenic modulators generally work: a double pulsed cold jet of a cooling gas (liquid carbon dioxide or nitrogen) is sprayed on the analytes eluting from the first dimension. The alternation of the two cold jets in two different sites at the beginning of the second column, works as a "trap and release" tool. Figure 1.2 emphasizes that the column set mostly utilized is based on a conventional-bore column as first dimension, and a narrow-bore column as the second one.

A detailed description of each instrumental part of a GCxGC instrument will be now given.

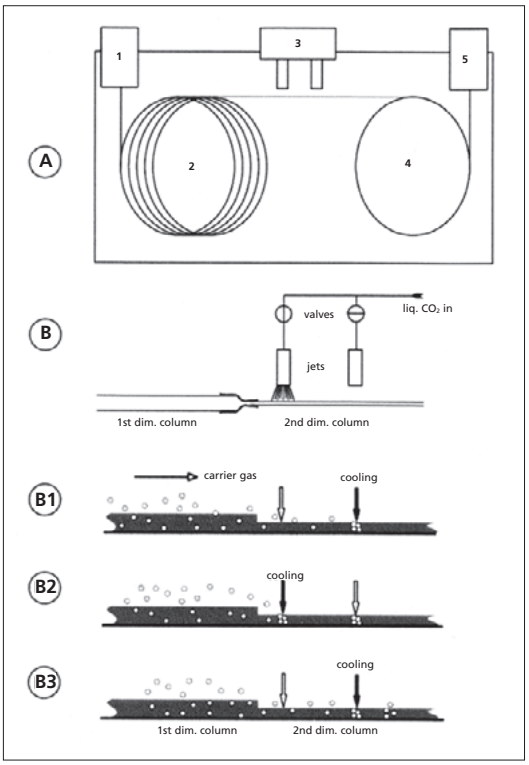

Figure 1.2

A simple scheme of a basic GCxGC apparatus. 1: Injector; 2: 1D-column; 3: Modulator; 4: 2D-column; 5: Detector. Bottom: a cryogenic modulator.

From Beens and Brinkman, *Analyst*, 2005, 130, 123-127. Reproduced by permission of the *Royal Society of Chemistry*.

2.1 Modulators

Due to the fundamental function of the modulator for a successful GCxGC separation, much effort has been devoted to the development of such devices in the last years. To summarize, modulators can be grouped in

two main categories: valve based and thermal modulators, the latter sub-grouped in heater-based and cryogenic modulators.

Table 1.1 summarizes the types of modulators developed up until now and reports their main characteristics. The introduction of the modulators dates back to 1991, when Liu and Phillips developed a single-stage, resistively heated, modulator [7]. This device was based on the use of a segment of capillary column, having a thick film of stationary phase, which worked as a trap for the 1D effluent. The capillary, positioned as interface between the columns, was externally coated with conductive paint, allowing for electrical heating. The heating was responsible for analyte release into the 2D column. However, this modulator design presented various drawbacks, the main being the capillary burning out. This was the main reason why this type of single-stage thermal modulators was abandoned, giving scope to modified new versions based on dual-stage modulation.

Dual-stage modulation was based on the use of two successive segments of thick film capillaries, which, alternatively, trap and release the 1D effluent: i) from the first into the second segment of the modulator; ii) from the second segment of capillary into the 2D column. The function of trapping is performed by the thick film, the one of releasing by the electrically-heated conductive paint. Dual-stage modulation was capable of overcoming band broadening and analyte breakthrough that characterized single-stage modulation, assessing a new design for successive development of thermal modulators. Afterwards, modifications were made on the trapping system, e.g. by placing microsorbent traps inside capillaries of deactivated Silcosteel ® [8]; by using a segment of stainless steel tubing coated with non-polar stationary phase, with a double jet of cold air for trapping and resistive heating at very low voltage for release [9]. Figure 1.3 shows one of the first commercial modulators: the Rotating Thermal Modulator, namely Sweeper [10-12]. In this modulator design, again a capillary with a thick film of stationary phase is used to retain the 1D-eluate, but the release of analytes is achieved by means of a slotted heater that moves along the capillary following the direction of the carrier gas. The temperature reached by the heater is around 100°C above the set GC oven temperature. The sweeper has been described by other authors in their GCxGC investigations [13-14], though scientific debate has arisen on the elution temperatures allowed by the sweeper, which was considered a limit of this type of modulator [15]. In any case, more than the temperature range, the movement of

the rotating heater has been considered as the flaw of sweeper.

Another modification of the sweeper was the semi-rotating modulator, first proposed in 2002, and then followed by other two versions, the latest being presented in 2008 [16]. The modulator consisted of a 180° rotating nozzle from which CO_2 was sprayed onto the column. The shaft was made of stainless steel tubing fitted inside the casing. In the last version, the modulator performance (repeatability of retention times) was improved by introducing a magnetic motor with a gear-head and a pre-programmed micro-controller. The semi-rotating modulator, due to the use of liquid CO_2, is comprised in the group of cryogenic modulators, which are as largely diffused as the heater-based type.

In the Longitudinally Modulated Cryogenic System (LMCS), a moving trap, cooled by means of liquid CO_2, slides up and down around the final part of the 1D-column [17]. Analytes are trapped and focused at a temperature of about -50°C and released at the GC oven temperature. Every time that the cold trap moves away from the trapped position, analytes move into the gas phase. Modulation, due to the longitudinal movement of the cold trap, takes place in two steps. The range of applications of LMCS is generally wider than that of heater-based modulators; however, the development of new modulator designs didn't stop at this point. A limit of the LMCS is its capability to trap very volatile analytes, due to the insufficiently low temperature that can be obtained with liquid carbon dioxide.

A further modification on cryogenic modulators was carried out by Ledford in 2000: the quad-jet modulator, consisting of two cold jets of carbon dioxide (C1 and C2) and two hot jets of air (H1 and H2), for trapping and desorption, respectively [18]. In the first cycle, C1 was on and H1 was off. In the next cycle, C1 was off, while H1 and C2 were on. Today, the quad-jet modulator is commercialized by LECO corporation.

Another interesting design of cryogenic modulators is the one developed by Harynuk and Gorecki in 2002 [9]. It consists of two empty deactivated capillaries of Silcosteel housed in a cryochamber cooled with liquid nitrogen. Analytes are trapped because of freezing inside the capillaries, followed by resistive heating which causes desorption of analytes onto the 2D column. This modulator resulted to be particularly suited for very volatile molecules, although complete insulation could not be guaranteed within the cryochamber.

Much useful for trapping very volatile analytes resulted to be the modulator designed

by Beens et al. in 2001 [19]. This modulator was developed for overcoming the complexity of the quad-jet design, where two extra hot jets were employed for releasing trapped analytes. In this new design, the warm air of the GC oven was used instead of the two hot air jets. The double-jet modulator has become a commercial product as well (Thermo Scientific).

Figure 1.4 shows the scheme of a single jet, single-stage modulator, designed to further simplify the GCxGC apparatus and to reduce the consumption of CO_2 [20]. As can be seen in the enlargement relative to the CO_2 nozzle, seven capillaries have been utilized to spray CO_2 over a short segment of capillary column. A needle valve was inserted in order to finely adjust the flow of the liquid gas. The trapped fraction was released in a very short time (< 15 ms), so to avoid analyte breakthrough and band broadening. The modulated peaks were routinely from 60 to 200 ms wide.

Within the thermal modulation systems, it cannot be forgotten the so called "loop modulator", designed by Ledford and co-workers for Zoex Corps (NE, USA). It is a two-stage modulator which employs hot and cold jets of gas (liquid nitrogen) directed toward a capillary tubing. Two cold spots are formed and the tubing between them is called "delay loop". While the cold jet runs continuously, the hot jet is pulsed periodically, causing the heating of the cold spots with consequent mobilization of trapped analytes. The delay loop is typically 60-100 cm long and the material entrapped in it takes seconds to pass from the first to the second cold spot. The two-stage thermal modulation is practically obtained through switching on and off the hot jet, therefore causing trapping and release of analytes. The hot jet avoids also the cold jet tip frosting.

An alternative design of modulators, which combines the use of a cryotrap with a valve (mechanical or pneumatic), is the stop-flow version. A scheme of such a modulator is reported in figure 1.5. In this case pneumatic switching was used. When working in stop-flow the carrier gas flow in the first dimension is stopped for a certain period of time, while the modulator releases the trapped material onto the second dimension [21]. During the 1D stop-flow, the flow of carrier gas into the second dimension is supplied from an auxiliary source. The main advantage of this modulator consists of the independent use of the two dimensions, therefore overcoming the necessity of operating in "fast" conditions in the second dimension.

Another group of modulators (pneumatic)

comprises diaphragm valve-based and differential flow modulators. This type of modulators have independent flows in the two dimensions.

Diaphragm-based modulators have not had the same success as the thermal systems, mainly due to the limits of temperature imposed by the valve itself. The development of such modulators was dictated by the attempt to avoid the use of the expensive and cumbersome apparatus for cryogenic gas supply. Basically, they work with a rotating valve which transfers a small amount (ca. 2%) of the 1D eluate into the 2D column [22]. Therefore, another limit can be ascribed to the lack of suitability for trace analysis.

Differential flow modulation, although using valves, provides more effective transfer of modulated peaks. A simple scheme of this modulation technique is presented in figure 1.6. The valve is provided with a loop and can be set in two positions: "fill" and "flush". When the valve is switched in the "fill"mode, the 1D eluate flows into the sample loop and an auxiliary flow of carrier gas enters the 2D column. In "flush" position, 1D effluent flows to the exhaust, while the auxiliary flow empties the loop content into the secondary column. The flow in the sample loop is generally higher than that of the primary column, justifying the name given to the technique. The differential flow allows the entire volume of the first column to be diverted in the second column. The basic design of the differential flow modulator has undergone various modifications, such as housing the valve outside the flow path [23]; the introduction of an in-line fluidic modulator [24]; the use of two switching valves [25, 26].

Table 1.1

Different types of modulators

	Thermal		Valve-based
	Heater	Cryogenic	
Single-stage	X		
Dual-stage	X		
Rotating thermal modulator (Sweeper)	X		
Semi-rotating		X	
LMCS		X	
Quad-jet		X	
Cryochamber		X	
Double-jet		X	
Single jet, single stage		X	
Single jet, dual-stage		X	
Stop-flow		X	X
Diaphragm			X
Differential flow			X

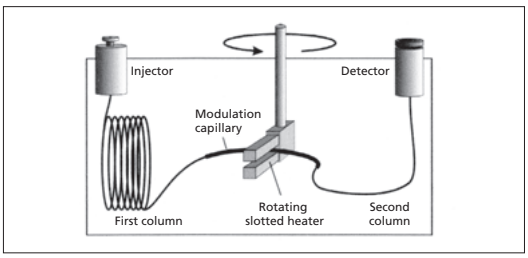

Figure 1.3

The sweeper, a Rotating Thermal Modulator.

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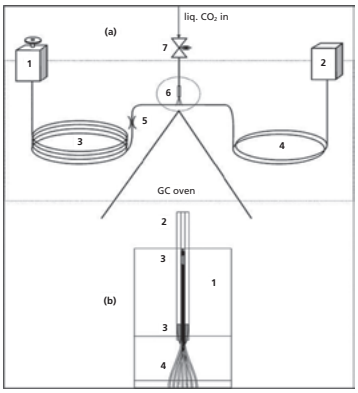

Figure 1.4

A single-jet, single-stage modulator, with the seven capillaries enlarged.

From Adahchour et al., *Analyst*, 2003, 128, 213-216. Reproduced by permission of the *Royal Society of Chemistry*.

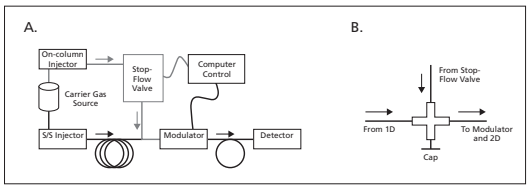

Figure 1.5

A stop-flow modulator.

Oldridge et al.: *Stop-flow comprehensive two-dimensional gas chromatography with pneumatic switching*. J. Sep. Sci. 2008. 31. 3375-3384. © Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

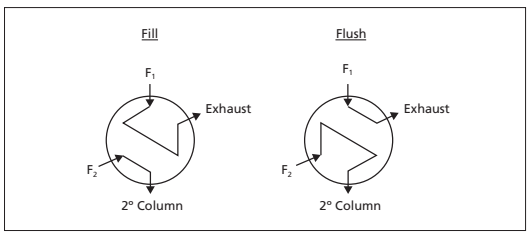

Figure 1.6

A scheme of the differential flow modulation design.

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2.2 Column Combination

As earlier reported in this chapter, one of the fundamental issues of a GCxGC separation is the combination of two different separation mechanisms, that is to say, the combination of two columns which are different in both dimensions and nature of the stationary phase. Several papers have been published in the last years, reporting applications based on the use of the most various column sets. The choice of the most proper column set is a hard task for the GCxGC user, if one considers that on this fundamental decision depends the success of the separation. The difficulty of this choice derives from the coupling of different flow geometries and chemical interactions occurring in the two columns.

First of all, it must be emphasized that an effective 2D separation originates from a sufficient number of cuts from the primary column. An expedient to reach this goal is to slow down the 1D linear velocity, so to generate broader peaks but prejudicing peak shape and analysis time duration. This operational mode, implies, of course, different gas linear velocities and fast temperature programs in the two dimensions, taking into account that, if for the first dimension slow parameters have to be preferred, on the contrary, the second

dimension must work faster in order to perform several consecutive fast 2D separations for each 1D modulated peak portion.

Obviously, different performances do mean different column dimensions, being generally a normal-bore the primary column (e.g. 25 m x 0.25 mm I.D. x 0.25 µm d_f) and a narrow-bore (e.g. 1 m x 0.10 mm I.D. x 0.10 µm d_f) the secondary column. However, second dimensions of 46 cm x 0.100 mm I.D. x 0.1 µm d_f have been used, with the aim of improving the quality of the primary separation, while making faster the secondary separations [12].

As concerns the stationary phase, the most common column set is non polar (100% dimethylpolysiloxane, 5% diphenyl - 95% dimethylpolysiloxane, etc.) x polar (polyethyleneglycol, 50% diphenyl – dimethylpolysiloxane, etc.). The non-polar column interacts mostly through dispersive forces, while hydrogen bonding, pi-pi and dipoledipole interactions occur in the polar phases. In other types of stationary phases the prevailing interactions are dipole-dipole. However, the non polar x polar set is not a strict rule at all; the choice is mainly dictated by the nature of the sample. It is worth to remember that all the variety of stationary phases available for monodimensional analyses can be utilized for GCxGC separations as well. For instance, an ionic liquid (LC-50) x non polar (5% diphenyl - 95% dimethylpolysiloxane) set has been demonstrated to be a valid approach for the analysis of planar PCBs [27]. Chiral columns have been used as 1D or 2D columns [28, 29].

Some papers have reported the use of column combinations consisting of three (GCx2GC) or four (2GCx2GC) columns [30-32]. In GCx2GC, one column as first dimension and two columns as second dimension are used, by means of a flow splitter located after the modulator. The three stationary phases differed from each other. In the 2GCx2GC configuration, two different column sets (non polar x polar and polar x non polar) were housed in the same GC oven, having in common the modulator, the injector and the detector. The two column sets worked independently from each other.

A final consideration on the choice of column sets has to be dedicated to the increasing diffusion of simulation/calculation programs for predicting the best parameters for a good 2D separation [33]. Some of these predictive programs are based on the measurement of retention indices on 2D columns, using alkanes, fatty acid methyl esters, etc. Retention indices are of course subjected to variation depending upon thermodynamic parameters, therefore software for GCxGC conversion of retention indices have been proposed [34].

3. Detectors for GCxGC (GC detectors)

From the GCxGC instrumental description so far reported, it is easy to understand that the main requirement of a GCxGC detector is the speed of acquisition. If, on one hand, a lack of selectivity and sensitivity could be somehow compensated by the enhanced power of the comprehensive separation, on the other hand the very fast, practically isothermal, 2D separations of the modulated peaks require acquisition rates of at least 100 Hz. In fact, 2D peak widths at the base range from 50 to 200 ms. Basically, great part of the commonly used GC detectors have been adapted to a GCxGC system as well, in many applications [35].

The most popular detector in comprehensive GC, since its introduction, has certainly been the FID. This universal detector, which is also characterized by a small internal volume, has proved to be efficient not only for quantitative purposes, but also for peak identification due to the structure-retention relationships on the 2D-plot [36]. It must be recalled that the FID gives a response proportional to the number of carbons present in the molecules that are burned out and ionized. For this reason, the FID has been the elective detector in petrochemical analysis, where predominant components are hydrocarbons. For instance, GCxGC-FID gave a better performance than the PIONA (paraffins, iso-paraffins, olefins, naphthenes, aromatics) analyzer [37]. Also, in another study, GCxGC-FID demonstrated to be more effective than LC-GC in the group-type quantitation of heavy gas oils, with a final improvement of the detection limit [38].

ECD (Electron Capture Detector) is another detector employed in many GCxGC applications. It is known that ECD is sensitive to halogenated compounds, being an electron emitter which attracts high electron affinity molecules. Indeed, such a detector is specifically designed for samples as pesticides, herbicides, and, more in general, compounds having halogens in their structure. However, ECDs are usually characterized by a slow acquisition rate, and hence not suitable for GCxGC. An interesting attempt to overcome this drawback was the development of miniaturized ECDs (mECD), characterized by a smaller internal volume and a higher acquisition speed (50 Hz) [12]. Although improved, even mECD demonstrated to be less effective than FID detector, especially because of the 2D band broadening observed. Today, mECD is relegated to the analysis of organohalogens, so to have a highly selective detector.

AED (Atomic Emission Detector) falls into the group of element-selective detectors, due to its capability to determine up to 23 elements, such as S, Pb, N, H, C, etc. It works through

the formation of a plasma which atomizes the sample, causing the emission of atomic spectra. Although very selective, this detector fails in acquisition speed, which is only 10 Hz. Nevertheless, some research groups applied the AED to the analysis of crude oil and petroleum by-products, concluding that the sensitivity of this detector toward S-containing compounds was very good, but the additional use of TOF-MS was necessary for identification purposes [39]. But still in this case, band broadening resulted to be a very important drawback.

Literature reports also some applications where another S-selective detector has been used: the SCD (Sulphur Chemiluminescence Detector). It must be emphasized that the interest of GCxGC users toward the specific element of sulphur has to be attributed to its presence in many sample-types such as petrochemicals. The SCD apparatus consists of a plasma burner which produces chemiluminescent species through a quite complex combustion reaction. Basically, the SCD signal derives from the formation of SO_2^* which generates light when falling back into the neutral state.

Compared to AED, the SCD possesses a higher acquisition speed (100 Hz). Unfortunately, the cell volume is bigger (ca. 500 mL), consequently band broadening remains a disadvantage of such detectors. However, a literature report demonstrated that, more than the physical dimensions of the detector, it is the electronic system which affects the speed of acquisition and band broadening [40].

A further element-selective detector has been utilized in comprehensive GC: the NCD (Nitrogen Chemiluminescence Detector). Due to its higher acquisition speed, comparable to that of FID [41], this detector has demonstrated a good performance in the analysis of diesel samples [42], and in particular in nitrogen-containing compounds group-type analysis. The basic principles of operation are very similar to those of SCD, with the main difference being the excited species (NO_2^*). According to Adam et al. [41] GCxGC-NCD is a promising technique for nitrogen speciation in diesel samples, providing more reliable results compared to the monodimensional GC-NCD. From their investigation, the authors observed an overestimation of the carbazole/acridine group (51% vs. 34%) and an underestimation of quinoline/indoles (43% vs. 59%), when comparing the data obtained from GC-NCD and GCxGC-NCD, respectively. Above all, it is worthy to remember that the interest of petrochemical research toward nitrogen-containing compounds is due to their catalyst-poisoning character.

Finally, the use of another selective detector cannot be neglected, namely the NPD (Nitrogen Phosphorous Detector), which should be more correctly named Thermoionic Detector (TID) because of its ionization mechanism. It is very similar to the FID detector, with the difference that there is not a flame working as a source, but an alkali salt inside a ceramic cement matrix. Theoretically, the NPD should be very suitable for GCxGC separations, due its high acquisition speed (up to 200 Hz). In fact, it has been successfully applied to the analysis of methoxypyrazines in wine, giving better results compared to the TOF-MS [43].

However, the acquisition speed is not the only parameter positively affecting a GCxGC separation: in NPD, the gas flows (N_2, air, H_2) have to be finely adjusted in order to optimize not only peak widths but also peak symmetry. An example is reported in figure 1.7 where a comparison between the FID and NPD on the same mixture is shown, highlighting the peak tailing which occurs for NPD.

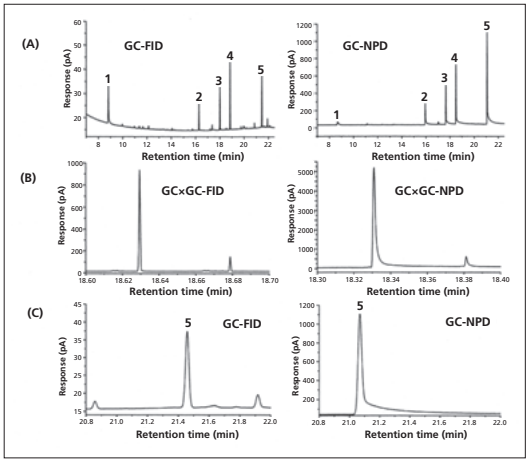

Figure 1.7

Peak tailing observed when using NPD as selective detector in GCxGC. On the left side, the better performance of the FID detector.

Mühlen et al.: *Detector technologies for comprehensive two-dimensional gas chromatography*. J. Sep. Sci. 2006. 29. 1909-1921. © Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

4. Detectors for GCxGC (MS detectors)

Also mass spectrometers can be hyphenated to GCxGC systems and used as detectors. The challenge is to further increase the potential of comprehensive GC, with its group-type pattern analysis, through the addition of another dimension of separation characterized by high identification power. A mass spectrometer is always the preferred choice when making qualitative analysis, allowing to perform acquisition of mass spectra, with consequent library search or raw interpretation of the fragmentation tables. This coupling, when first introduced in 1999 by Frysinger and Gaines [44], wasn't a great success. The reason is quite easy to be understood if one considers the main requirement of a 2D separation: speed.

High acquisition rates must be properly set at the detector dimension, a feature possessed by modern FID detectors, that have practically dominated the scene of GCxGC since its introduction up until recently. The poor performance of those quadrupole mass spectrometers (qMS), available about ten years ago, led to failure for both quantification and identification purposes. Among the various mass spectrometers, it is typically the time-of-flight mass spectrometer (ToF-MS) to be characterized by high acquisition rate (50-200 mass spectra per second). Therefore, it comes out from a survey of the GCxGC literature, that ToF-MS is the most used detector when structural information is desired from a GCxGC analysis. ToF-MS design is based on a simple consideration: if the ionized species start from the same position at the same time, their acceleration or velocity can be directly correlated to their mass-to-charge ratio (m/z); this means that they will reach the detector at a time depending upon their masses. In other words, it is the heaviest ion to determine the velocity of the spectral acquisition. Some ToF-MS instruments are provided with dedicated software for GCxGC data processing, capable of performing signal deconvolution. This type of instrumentation operates at acquisition rates of up to 500 Hz, which are compatible with the narrow (50-200 ms peak widths) 2D peaks.

However, the high cost of ToF-MS and the easier availability of qMS in the laboratories has somehow restricted the use of GCxGC-ToF-MS to routine/industrial applications, such as fuel analysis in petrochemical plants. As previously mentioned, GCxGC literature has occasionally reported on the feasibility of using a qMS as detector. The acquisition rate of quadrupole MS is linked to some entities, namely the scan speed, the interscan delay and the scan mass range. Scan speed is measured in amu/s, while the interscan delay (time between the end

of a scan and the beginning of a new one) is measured in ms.

Recently, Shimadzu has introduced a new qMS (GCMS-QP2010 Ultra) characterized by a scan speed of up to 20,000 amu/s, thanks to a technology called ASSP (Advanced Scanning Speed Protocol) which automatically optimizes the rod bias voltage, without compromising sensitivity and avoiding spectral skewing.

The accuracy of the qualitative and quantitative data obtainable by a mass spectrometer depends, of course, on peak reconstruction, i.e. on the number of data points sampled. A relation exists between the acquisition rate and i) the number of data points which define a peak; and ii) the scan mass range. The higher the acquisition rate, the higher the number of data points and the lower the mass range, respectively [45]. This finding suggests that, for instance, a more restricted mass range can be selected, which covers target analytes, in order to increase the acquisition rate. For example, Adahchour et al. [45] have found a good compromise between an acquisition rate of 33 Hz and a mass range of 50-245, estimated suitable to the type of sample (fragrance allergens). The recent rapid-scanning qMS systems used have been demonstrated effective toward the production of high purity spectra, which pass successfully the identification process derived from library matching (high similarity score). In fact, it cannot be forgotten that in this case the quality of the spectrum is greatly enhanced by the powerful capability of separation possessed by comprehensive GC. Furthermore, these features improve the analytical performance not only from a qualitative point of view but also quantification results tend to be reliable and linear, with LoDs at the pg level.

5. Method Optimization

It is a matter of fact that method optimization in comprehensive GC requires more skill than monodimensional techniques. Many parameters are involved in such a powerful analytical technique, such as modulation, choice of the column set, gas flows, temperature programs, etc. Trying to tune all these issues with the aim of obtaining the best results from a GCxGC analysis may be a tough job. Familiarity with conventional GC instrumentation and with the basic principles of gas chromatography can help the analyst very much.

One first useful consideration is that the instrumentation used in GCxGC is the same utilized in conventional monodimensional GC. Apart from the modulator and the software needed for data handling, GC ovens, injection systems, stationary phases, detectors are the same as those used in GC. The main differences lay in the operational conditions; first of all, the combination of two different columns in one or separate ovens, as previously described. The most diffused column set, defined as the truly orthogonal, is the non-polar x polar, e.g. 5% diphenyl – 95% polydimethylsiloxane x polyethylene glycol. The opposite column set is generally considered as "reversed". Although the orthogonal column set is the most commonly used, the analyst cannot know a *priori* if the sample under investigation could be better separated on a reversed column set. In other words, although the analyst may have a rough idea of the composition of the sample to be analyzed, therefore exploiting a likely suitable column set, the first parameter to be optimized is the choice of the two stationary phases. The best column set should produce a separation, as effective as possible, in terms of both spatial distribution in the 2D plane, sensitivity and reduction of wrap-around effect. The latter is the phenomenon which occurs when the retention time of a 2D peak exceeds the modulation time duration; it is a common case when analytes possess great affinity for the stationary phase, i.e. polar compounds toward wax columns.

Directly related to the columns chosen, gas flows and temperatures are other important parameters to be discussed in method optimization. First of all, it must be remembered that the two columns combined have not only different chemical nature but also different dimensions. In a GCxGC column set, generally the first column is longer and wider than the secondary column, normally a narrow bore column. This implies a slow diffusion of the carrier gas within the first column, in consideration of the resistance exerted by the 2D column. The decrease of diffusion causes a consequent increase of the GCxGC analysis

time. In most cases, the analyst sets a 1D gas linear velocity that allows to perform fast consecutive isothermal 2D separations for each 1D modulated peak. Therefore, oven temperature rates in the primary column are low, in the order of 2-5°C/min. The trend observed in the literature is that only the flow rate of the first dimension is practically optimized up to ideal levels, while the second dimension works at a linear velocity that is far from the ideal. However, some studies have been dedicated to such a topic.

For instance, Beens discussed the possible use of a wider 2D column [33], comparing the efficiency of two different column sets. The results of this study are presented in table 1.2, where a comparison is made between two different sets of columns. As can be seen, it is difficult to find a compromise between the linear velocities operated in the two columns, maintaining a good level of efficiency (low HETP) in a reasonable analysis time. Another important aspect is the temperature program rate, if considering that slow rates (i.e. 1-3°C/min) are used in 1D separations, generating broadened peaks prior to modulation. This means that modulated fractions reach the 2D column at relatively low temperatures, possibly ending up in band broadening and loss of sensitivity. Beens found out that the use of a wider 2D column allows to obtain higher efficiency in terms of separation capability at the same analysis time of a conventional set. Other expedients to optimize the linear velocity in the GCxGC configuration are: i) the reduction of the head pressure, with a consequent loss of resolution in the first column; ii) the use of a longer 2D column, that would imply an increase of the modulation time; iii) partial diversion of the flow to waste before modulation by means of a splitter.

For instance, Tranchida et al. introduced the concept of split-flow GCxGC, adding to the column set an uncoated capillary connected to a split valve [46]. A scheme of this apparatus is shown in figure 1.8. When using the system with the split valve closed, the 2D linear velocity was around 330 cm/s and the bidimensional plot presented a consistent amount of unoccupied space. When the split valve was open, with a split ratio of 35:65, the 2D linear velocity became lower (210 cm/s) and the separation was improved.

Similar benefits have been obtained by means of the "stop-flow" technique, which is based on an auxiliary gas and a solenoid valve. The technique derives its name from the fact that, during part of the modulation time, gas flow in the primary column is stopped, allowing to double the number of cuts per peak and to use

longer secondary columns.

The temperature program set in the 1D column affects the elution temperature and, therefore, the retention times of the 2D peaks. This will have implications also on the modulation parameters.

Dallüge et al. optimized a GCxGC method trying different 1D temperature program rates [47]. An example of this trial is reported in figure 1.9 where a mix of pesticides was analyzed at 2°C/min and at 10°C/min, respectively. The faster temperature rate produced a higher resolution (narrower peaks) in the 1D, although the modulation frequency was lower, therefore not preserving the same degree of separation performed by the primary column. A slower temperature rate (2°C/min in the figure), as can be easily understood, allowed for modulation times more than twice higher compared to the temperature rate of 10°C/min.

A fulfilling discussion on temperature program optimization should take in consideration another important aspect, that is the use of a single or double GC oven in a GCxGC analysis. Certainly, the use of a single oven greatly affects this parameter. In fact, when placing the column set in a single oven, it is the thermal stability of one of the two columns to dictate the maximum operating temperature that can be set in the temperature ramp. Loss of sensitivity and wrap-around effects can occur in such situations. On the other hand, the introduction of twin-oven GCxGC configurations allow to work with a definitely higher degree of flexibility.

As clearly indicated in the previous sections, the core of a GCxGC system is undoubtedly the modulator, of which the various parameters must be set carefully in order to ensure a preservation of the separation achieved in the first dimension. In order to this, a 1D peak must be modulated at least 3-4 times with modulation periods of about 4-6 seconds. An example of the importance of this parameter is reported in figure 1.10 where two different modulation times, 9.9 s and 5 s, respectively, are compared for their separation efficiency. It is clearly visible how the 1D separation is compromised when the modulation time becomes too long, with a consequent reduction of the number of peak slices. Beyond the modulation time, when optimizing the conditions to be set at the modulator, it must be taken into account also the entrapment temperature, that greatly depends upon the type of modulator used. For instance, when using a thermal sweeper the entrapment can take place at the GC oven temperature, while remobilization can take place by thermal desorption (100°C above

the GC oven temperature). Of course, if the modulator in question is a cryogenic type, temperatures of circa -100°C lower than the GC elution temperature, for a specific analyte, are considered sufficient for effective entrapment. However, very volatile compounds can require even lower temperatures (-120°C/ -140°C), sometimes causing a delay in analyte release. If using a loop-type modulator, the most appropriate length of the delay loop must be chosen: too short loops can cause analyte breakthrough (overexposure to the hot jet), too long loops can cause lack of exposure to the cold jet. More specifically, when using a too short loop a double peak formation can occur because the analyte band exposed to the hot jet reaches the other part of the modulator ahead of the cold jet, which splits in two the peak; on the other hand, when having a too long loop, the analyte band is not able to reach the cold part of the modulator in a reasonable time.

A final remark should be given to a test mixture developed for comprehensive GC, the so-called "Phillips mix" [48]. The scope of the mix is similar to that of the Grob test mix, addressed to the verification of the performance of a column set. The mix is composed of several different groups of chemicals, e.g. alkanes, alkenes, alkynes, aldehydes, alcohols, methyl esters, carboxylic acids, etc. Dimandja et al. have reported also the Programmed Temperature Retention Indices (PTRIs) for each constituent of the mix.

	P_{in} (kPa)	^1N	^2N	$^1\bar{u}$ (cm/s)	$^2\bar{u}$ (cm/s)	PR_{opt}* (°C/min)
30m,	56	86,000	9,000	16	64	4
0.32mm	88	100,000	8,000	24	106	5
+	132	98,000	6,500	35	140	6
1.5m,						
0.18mm						
15m,	112	44,000	15,000	10	80	4
0.25mm	224	65,000	10,000	18	160	7
+	400	40,000	4,000	30	280	12
1.5m,						
0.10mm						

** optimum programming rate*

Table 1.2

Comparison of separation parameters for two sets of columns.

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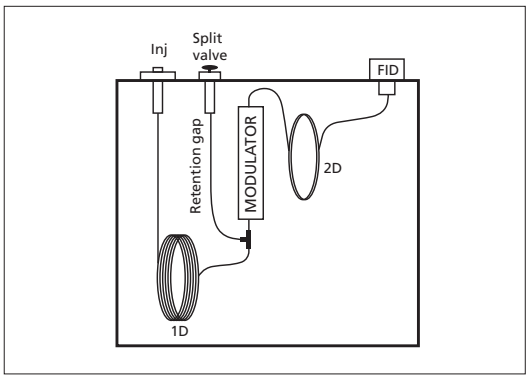

Figure 1.8

Scheme of the split flow GCxGC setup.

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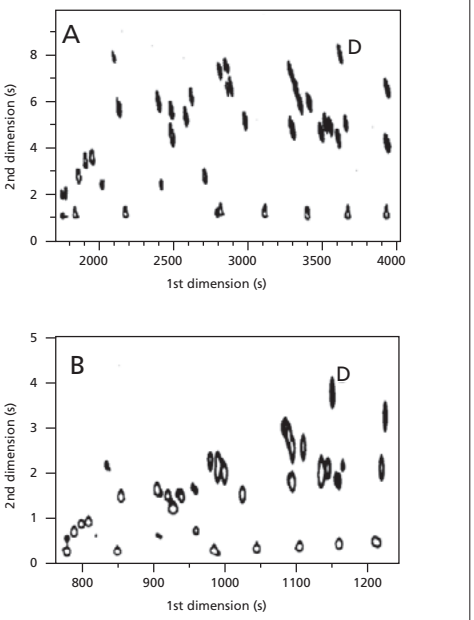

Figure 1.9

Effect of different 1D temperature-programming rate on the 2D retention time. A: 2°C/min, 9.9 s modulation time; B: 10°C/min, 5 s modulation time.

Dallüge et al.: Optimization and characterization of comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection (GCxGC-TOF MS). J. Sep. Sci. 2002. 25. 201-214. © Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

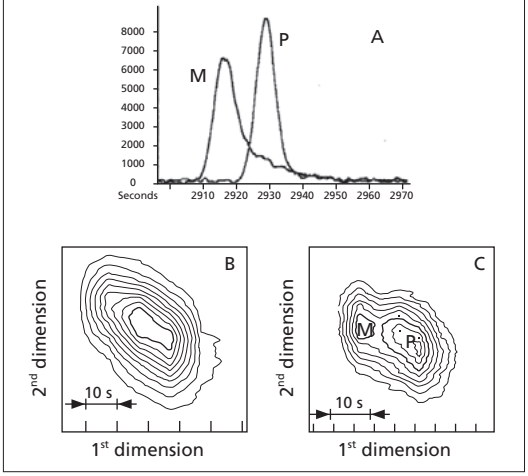

Figure 1.10

Influence of modulation time on separation of metalaxyl (M) and prometryn (P). A: 1D chromatogram. B: GCxGC contour plot, 9.9 s modulation time, loss of 1D separation. C: GCxGC contour plot, 5 s modulation time, 1D separation preserved.

Dallüge et al.: Optimization and characterization of comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection (GCxGC-TOF MS). J. Sep. Sci. 2002. 25. 201-214. © Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

6. Data Processing

It is a matter of fact that GCxGC data files have much bigger sizes compared to the monodimensional files. This is not surprising, if one considers a situation such as that depicted in figure 1.11 where a short fraction (1 min) of a GCxGC chromatogram is represented in its crowded form when passing from the first to the second dimension. If we imagine an entire profile of a complex sample, it is easy to understand that the amount of data coming from the 1D separation, once expanded to the orthogonal plane, becomes very high. Let's remind that each modulation produces continuously short, fast and practically isothermal 2D chromatograms, leading to final sizes of even 500 Mb. File sizes are also strictly linked to acquisition frequency. These dimensions become somehow prohibitive when, for instance, GCxGC has to be used for routine screening. Therefore, the big innovation introduced by comprehensive GC immediately posed this first challenge: how to collect and save data files. The second issue, not less important, has been to find a convenient and easy way to handle and process the data acquired. Dealing with two dimensions of separation became problematic for a double matter of visualization and data interpretation (how to make quantitative and qualitative analysis?). From here the need to develop new software, capable of facing up with all the above cited requirements. Since the beginning, the general trend was to develop home-made software through the adaptation of already-existing chemometric and mathematical programs. As reported by Beens [33], the GCxGC process results in a matrix of data, constituted by columns and rows, which are the number of data points of one 2D chromatogram and the number of modulated fractions, respectively. Indeed, it is exactly this matrix that has to be converted in a visual plot, e.g. a coloured contour plot with spots corresponding to the apices of each separated constituent. This implies, of course, that the software must be able to recognize which 2D peaks correspond to the same compound. This is relevant especially when considering quantitative analysis: the first attempts to quantify all the separated spots present in the bidimensional plane, were based on the most elementary strategy, that is the summation of all the single peak areas attributable to one constituent. The procedure, although tedious and very time-consuming, gave very good results. The next objectives were to construct a software for such an operation, giving appearance in the market to a variety of software packages, such as *Hyperchrom* (Thermo Scientific, MA, USA), *ChromaTOF* (Leco Corps, MI, USA), *GC Image*

(Zoex, TX, USA) and others. The *Hyperchrom* is provided along with the TRACE GCxGC instrument, whereas the *ChromaTOF* is part of the Pegasus ToF-MS instrument. *GC Image*, firstly introduced by the University of Nebraska (USA), is now property of the Zoex corporation.

Today, *GC Image* is successfully used along with other 1D software (*GCsolution* and *GCMSsolution*) with the Shimadzu GCxGC system. Another interesting software has been developed by Chromaleont srl (Messina, Italy), namely the *ChromSquare* software, dedicated to data processing of both GCxGC and LCxLC analyses. An example of the capabilities of this software has been reported in a recent paper by Tranchida et al.[49] and here depicted in figure 1.12. The figure focuses on two peaks of a perfume formulation, namely estragole and α-terpineol. Part (a) shows the chromatogram expansion, part (b) the integrated peak areas, part (c) the identification of the two compounds and part (d) the library matching results for estragole. For a more detailed description of the process, the reader is referred to [49]. In the study achieved by Tranchida et al. a mutual interaction between monodimensional and comprehensive software was carried out, generally exploiting the latter for graphical transformation and visualization of the bidimensional plot, whilst the 1D software for its well-established capabilities such as library searching with the support of the retention index filter option.

Another software package is the *Transform*, part of *Noesys* produced by ITT (NY, USA), which has been extensively used in many GCxGC studies. In this case, the visualization of the GCxGC chromatogram is typical, because along with the coloured bidimensional plane, the 1D chromatogram is displayed on one axis, while the 2D chromatogram is displayed on the other axis, corresponding to each selection made on the bidimensional plane with the cursor .

An accurate overview of the software packages present in the market cannot neglect the initial use of available software and not specifically developed for comprehensive GC applications. Many researchers, especially in the first applications, exploited classical software such as *Visual Basic*, *MatLab*, *LabView*, to create customized programs for data processing. The ongoing trend is undoubtedly the incorporation of chemometrics into GCxGC data handling software, mainly based on multivariate analysis (MVA), with the most promising methods being PARAFAC (parallel factor analysis) and GRAM (generalized rank annihilation method). The first model is frequently associated to a chemometric technique denominated "alternating

least squares" (PARAFAC-ALS), that is iterative and alternates algorithms on a subset of data in order to resolve trilinear signals in more complex samples. The technique results more suitable for ToF-MS data. On the other hand, GRAM, that is more direct and faster, can be applied to a restricted number of samples (the standard and the analytes matrix). In any case, it seems worth to remember that all the methods falling into the MVA area have specific limits and requirements. For example, there must be separation at the peak apex between adjacent compounds and retention times must be reproducible between different runs. Unfortunately, due to the several factors controlling a GCxGC separation (modulation, GC instrumental conditions, MS spectral skewing), retention time shifts may occur in between runs. A successful attempt to overcome this drawback has been proposed by Pierce et al. who designed an alignment algorithm for the correction of the retention time shift [50].

A final observation should concern the "wrap-around" phenomenon occurring when the 2D retention time of a peak exceeds the modulation period, dragging itself to the next modulation cycle. This event causes the formation of broader peaks, the width of which can mask other peaks deriving from successive modulations. Therefore wrap-around can negatively affect the GCxGC qualitative and quantitative analysis if the compounds hidden in the wrap-around peak are among target analytes; otherwise, the analysis is generally not compromised. However, due to the anomalous and larger peak widths detectable in wrap-around, the latter should be automatically recognized by the software.

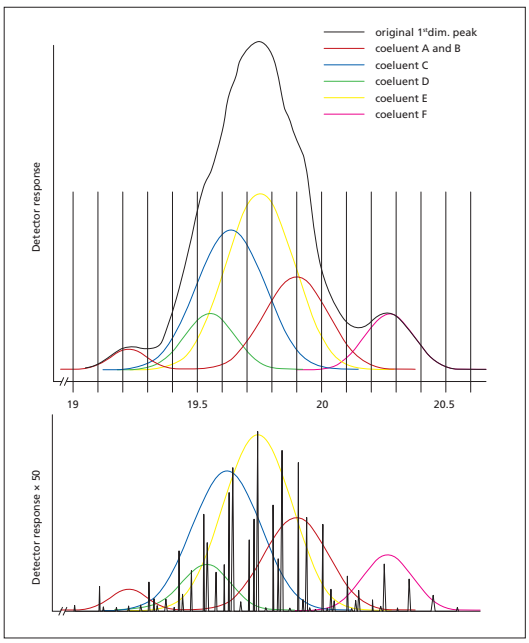

Figure 1.11

A 1.5 min GCxGC separation of a 1D peak, with six co-eluting compounds. The lower part of the figure shows the actual separated fast peaks from the 2D. Co-eluting 1D peaks are superimposed.

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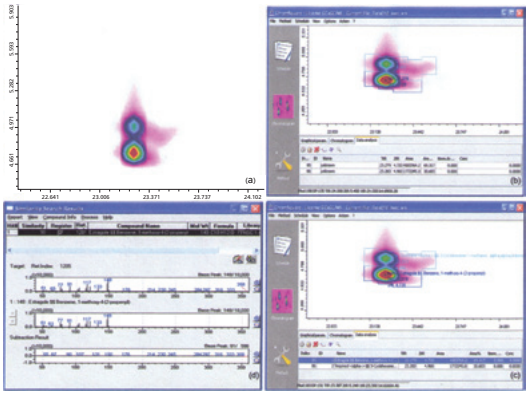

Figure 1.12

***Chromsquare* software windows. See text for detailed description.**

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