COMPREHENSIVE TWO-DIMENSIONAL GASCHROMATOGRAPHY

The state-of-separation-arts

JAN BEENS

in co-operation with www.chromedia.org

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Foreword

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In the late fifties of the past century gas chromatography on packed columns was a reasonably well established technique. It could separate complex mixtures into a few dozens of compounds. A flavour isolated from coffee was found to consist of some 25 peaks. A few of these peaks had shoulders, so probably the number of compounds was slightly higher than 25. With the introduction of capillary columns by Golay in 1958 the separation power of GC increased dramatically. The same coffee flavour now showed some 250 peaks! A few of these peaks had shoulders, so probably the number of compounds was slightly higher than 250. The next step was comprehensive GC×GC, a revolutionary technique introduced by the late Prof. John Phillips in 1991. Coffee flavours have been analysed using this technique. Comprehensive GC×GC chromatograms of the flavour now contain some 2000 peaks. A few of these have shoulders, so probably …....

Gas chromatography is a mature technique. The above anecdote illustrates how it grew from a technique that was thought to be almost perfect to a technique that performs 100 times better and still is 'only' almost perfect….. How long will GC continue to develop? This question is difficult to answer. Probably it will continue to develop as long as there is a need to separate mixtures into more compounds and detect lower levels of the analytes. Is GC×GC a step to far? Is it more than we need? Clearly the answers to these questions are NO. GC×GC does significantly increase our ability to separate mixtures into more compounds and detect lower levels, but there are numerous situations where we still need an even better performance.

In its development GC has seen a number of huge revolutions, such as the introduction of the capillary column, the introduction of fused-silica and the invention of the flame-ionisation detector. Also the introduction of comprehensive GC×GC classifies as a revolutionary improvement. Next to these revolutionary inventions there have also been a huge number of small, incremental improvements. It is this combination of revolutionary innovations and the massive number of smaller improvements that eventually gave GC the unsurpassed peak capacity, sensitivity and reliability it has to date. It is interesting to mention already at this point that the author of the current contribution has witnessed all these innovations occurring! With some 50 years of experience in analytical chemistry, and

chromatography in particular, there are not many innovations that did not happen in Dr. Jan Beens' active research period.

Comprehensive GC×GC is basically an extension of heart-cut two-dimensional GC. However, whereas in heart-cut two-D GC only one or a few peaks are transferred to a second column, in comprehensive two-D GC all peaks are transferred to a second column for a further separation. A full explanation of how this can be done, what are the advantages of the method and when this newest technique should be used, can be found in Dr. Beens contribution. What is particularly interesting is to see that comprehensive GC×GC offers the same features that contributed to the success of all revolutionary improvements in GC: hugely increased peak capacity, improved sensitivity and better reliability and ruggedness. Additionally, however, GC×GC also offers one additional advantage: it improves the performance of GC in terms of *selectivity*. Normal GC can separate many peaks, but is not great in terms of selectivity. The key-factor determining the retention order is volatility, or boiling point. Factors like stationary phase- or compound polarity play just a minor role and their effects are very often completely overshadowed by the volatility effect. GC×GC effectively allows decoupling the volatility and polarity contributions to retention. In that way allowing to visualise small retention time differences as a result of different polarities on a background of hugely different volatilities. This makes GC×GC ideally suited for group-type separations. Indeed the technique has been introduced originally for group-type separation of mineral oil fractions. This also exactly is the reason why Dr. Beens, together with Dr. Jan Blomberg, entered the GC×GC area. Dr. Beens was working on the development of hyphenated systems for LC-GC group-type separations. When he became aware of the pioneering work of Prof. Phillips he immediately realised the power of GC×GC and included the technique in his scientific research.

Comprehensive GC×GC, as a stand-alone method, but certainly in combination with selective detectors or mass spectrometric detection is ideally suited for trace level analysis, for group-type separation as well as for profiling- and fingerprinting applications. Because of the massive amount of data and information generated it is nowadays also combined with chemometrics. This combination opens-up entirely new application areas for GC. Metabonomics and life-sciences applications, but also mineral oil analysis, are areas that will certainly benefit from this. Comprehensive GC×GC has now become accepted in numerous application areas. Armed with the knowledge and tips and tricks presented in the current contribution researchers trying to apply the technique are likely to become successful, probably after applying solutions proposed by Dr. Beens and described in detail here.

The broad, deep and long experience of Dr. Jan Beens together with his great teaching and explanation skills make him the ideal author for a contribution on GC×GC. Dr. Beens has many decades of experience in chromatography and has worked with several chromatographic

techniques. I recommend the readers to make use of his experience. And I am sure most of them will respond enthusiastically to the solutions and ideas presented. Finally, GC×GC is a revolutionary development in GC. It is not a step too far, neither in terms of complexity nor in terms of over-resolving samples. With the knowledge provided by Dr. Beens in this contribution you will find it an easy technique to use and rewarding in what it gives you.

Hans-Gerd Janssen,
Amsterdam 2010

1. How chromatography started

Tswett was the first to publish a paper on chromatography about 100 years ago, in 1901, when he made his observation of the first separation of coloured bands from the analysis of plant pigments.

He separated herbal pigments by using calcium carbonate as a solid absorber and petroleum ether with a small amount of alcohol as the mobile phase. He crushed fresh picked leaves in a mixture of petroleum ether and a small amount of alcohol into a greenish coloured extract.

M

Михаил Семёнович Цвет

(Mikhail Semenovich Tswett)

The extract was shaken with distilled water to remove the alcohol. This cleaned extract was fed onto a glass column filled with solid calcium carbonate. As a result of the different absorption characteristics of the components in the mixture, a series of bands with different colours was generated.

The method was described on 30 December 1901 at the XI Congress of Naturalists and Physicians (XI съезд естествоиспытателей и врачей) in St. Petersburg. The first printed description was in 1903, in the Proceedings of the Warsaw Society of Naturalists, biology section. In his next paper [1] in 1906, he again described the separation and termed the technique for the first time "**chromatography**" (derived from the Greek words $\chi\rho\omega\mu\alpha$ = colour and $\gamma\rho\alpha\varphi\iota$ = to write)

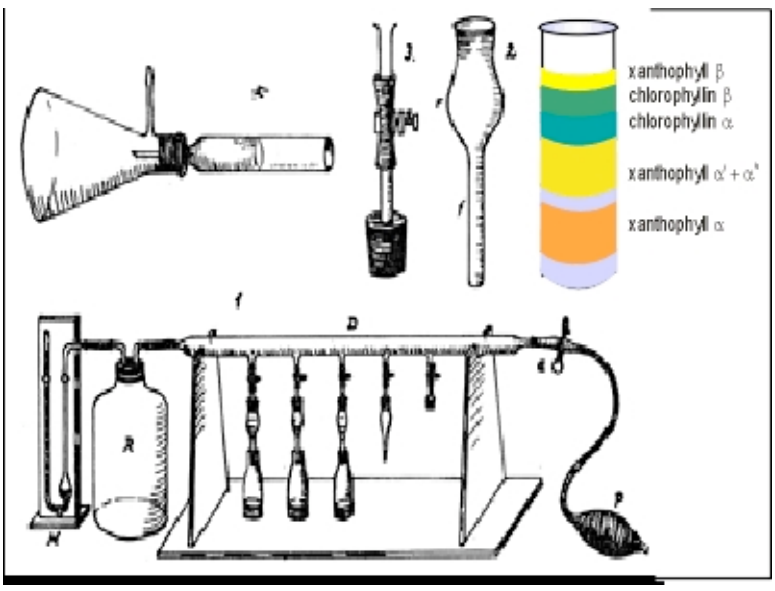
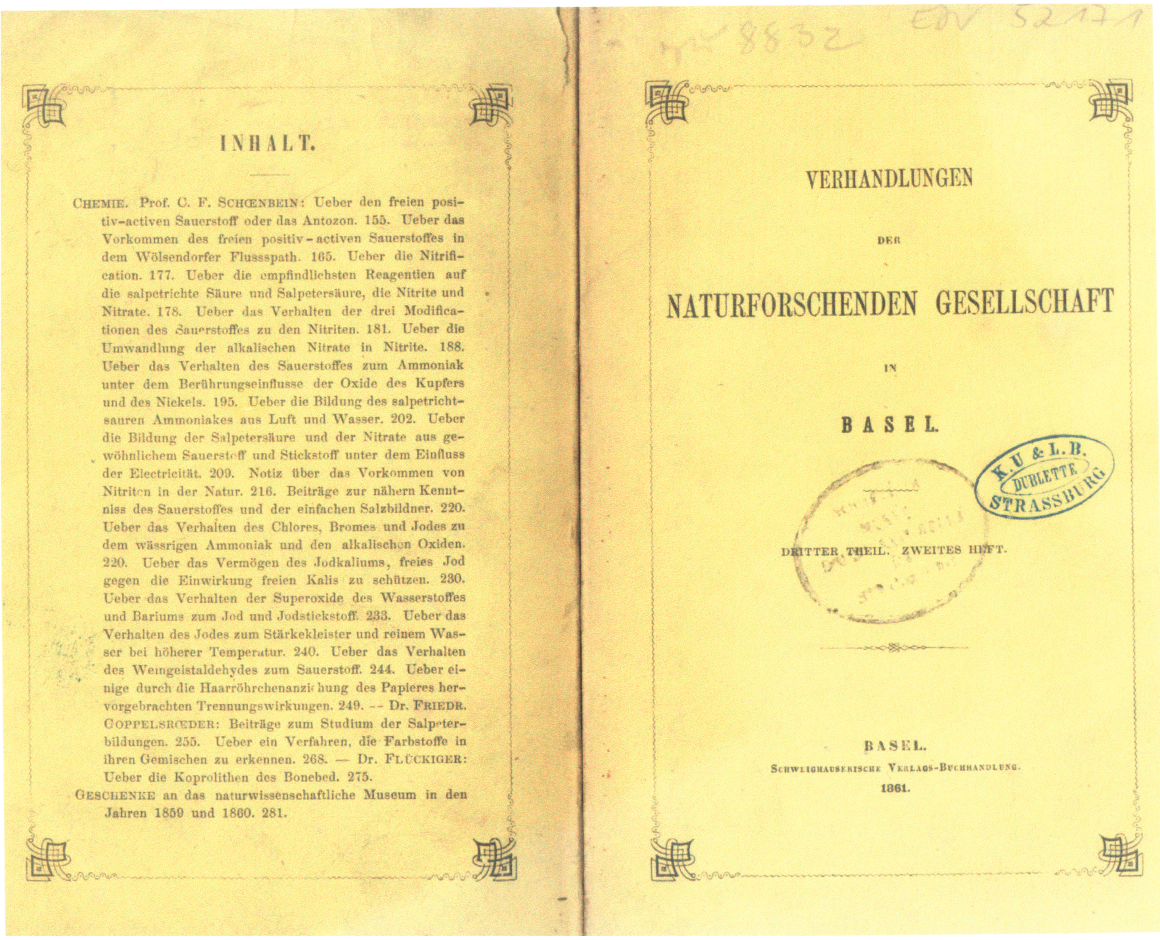


Figure 1.1 The instrumentation of Tswett.

However, already more than 40 years earlier, in 1861, F. Goppelsröder, a Swiss scientist and professor at the University of Basel, reported at the Congress of "*Naturforschenden Gesellschaft*" held in Basel, "*Ueber ein Verfahren, die Farbstoffe in ihren Gemischen zu erkennen*." (*About a procedure to recognise dyes in their mixtures*) [2] (Fig.1.2 and 1.3).



INHALT.

VERHANDLUNGEN

DER

NATURFORSCHENDEN GESELLSCHAFT

IN

BASEL.

DRITTER THEIL. ZWEITES HEFT.

BASEL.
SCHWEIGHAUSERISCHE VERLAGS-BUCHHANDLUNG.
1861.

Figure1.2. Report of the Naturalists Corporation

Ueber ein Verfahren, die Farbstoffe in ihren Gemischen zu erkennen.

Von Dr. Friedr. Goppelsröder.

Eine Reihe höchst interessanter Versuche Schönbein's haben auf das Deutlichste bewiesen, welches ungleich grosse Wanderungsvermögen in porösen Medien die verschiedenen Körper zeigen, und müssen Jedermann zu der festen Ueberzeugung leiten, dass auf dem angebahnten Versuchsfelde noch ein reicher Schatz interessanter Thatsachen zu finden sei. Ich meinestheiles wurde sogleich von der Ansicht beseelt, dass wir es hier in nicht ferner Zeit mit einer Art von Analyse zu thun haben würden.

Figure 1.3. The Introduction of Goppelsröder's presentation.

Soon thereafter, he published a paper "*Note sur une méthode nouvelle propre à déterminer la nature d'un mélange de principes colorants" (Memorandum on a new method for determining the nature of a mixture of dyes)* in Bulletin de la Société Industrielle de Mulhouse [3]. It was mentioned in "Jahresbericht über die Fortschritte der Chemie ... für 1862" (Giessen) ("Annual Review of Progress in Chemistry ....., 1862"), a kind of *"Chemical Abstracts"* before their day: *"F. Goppelsröder (....) has shown that Schönbein's observation – whereby solutions of various substances are aspired with very different rates and intensities by filter paper – can be used to separate and distinguish different dyes contained in the solution."*

Friedrich Goppelsröder

Christian Friedrich Schönbein

In his paper Goppelsröder wrote: "*A strip of filter paper (is dipped) several millimetres into an aqueous solution of blue litmus. The solution is seen to rise quickly above the level of the liquid by capillary aspiration. A blue band is formed but, because the water and dye both rise to the same extent, no separation between solvent and dissolved substance is observed. A different situation arises when the experiment is repeated with litmus coloured with sulphuric acid. In this case, three zones are formed on the paper above the liquid. The first (i.e. the highest) contains only water, the second is due to dilute sulphuric acid, and the third contains water, acid, and dye. Clearly, a partial separation of the three components in the mixture has occurred and is due to their different abilities to rise within the pores of the paper*." Goppelsröder adds the following capital remark: "*I saw in these observations the key to a new analytical method*".

Goppelsröder dried the filter paper used in the analysis, cut out a relevant zone, eluted the product with a solvent (*e.g.* ethanol), and submitted the products obtained for further analysis. In this way, he detected picric acid, a yellow substance that migrates upfront (*i.e.* upwards) from within a mixture that, in his experiment, was unpurified fuchsin prepared from aniline and in solution in ethanol.

Goppelsröder also isolated pure azulene – a blue hydrocarbon of curious structure that has always fascinated chemists – by repeated capillary analysis (preparative paper chromatography). The pink-coloured impurity was probably fuchsin. He coloured small skeins of silk either in pink with the impurity or in blue with pure azulene. He concludes: *"I am convinced that this method will prove to be very practical for the rapid determination of the nature of a mixture of dyes, especially if appropriately chosen and characterised reagents are used...."*

From 1861 onwards, Goppelsröder spent his time improving his method. He describes his results in the introduction to the " *Studien über die Anwendung der Capillaranalyse*....." [3] in some detail. So, more than 40 years before the publication of Tswett, these papers on a "new analytical technique", which we nowadays would call paper chromatography, were already published. This probably means that, although Tswett has been rewarded with a Nobel price for the invention of the analytical technique of chromatography, we must reconsider who the real inventor of chromatography is.

2. Multidimensional gas chromatography

Already since the very beginning of GC, it became apparent to chromatographers that single-column separations could not provide enough separation efficiency of clearly separating every analyte in a complex sample. And since complex samples are defined in this respect being all those samples containing over 100 compounds, most of the real-live samples must be considered to be complex. Giddings applied statistical calculations on the number of theoretical plates that are needed for separation of a mixture consisting of about 100 compounds [5]. In this statistical approach he implemented the fact that components are almost never evenly distributed along the chromatographic line, but tend to fall haphazardly across the chromatogram, frequently overlapping, even in cases where the separation space is substantially larger than theoretically needed for resolution. From these calculations he derived a number of theoretical plates of $N > 5.10^8$ to reach a probability that 95 of the 100 compounds being adequately isolated for analytical measurements. The failure of not sufficiently resolving all of the compounds present becomes increasingly pronounced as the number of components increases significantly above 100.

Multidimensional chromatographic separations already have been employed from the very early beginnings of chromatography. Consden, Gordon and Martin [6] already did the first publication in this area in 1944, where they described an orthogonal separation on a paper sheet. Flat bed separations were demonstrated a few years later using combinations of chromatography and electrophoresis [7] and electrophoresis and electrophoresis [8]. Thin-layer chromatography (TLC) has been involved in many different multidimensional separation systems. It was hyphenated with other chromatographic techniques using columns, such as GC−TLC and LC−TLC [9].

Multidimensional gas chromatography (MDGC) was developed already from the sixties on a large scale with many different constructions and applications. One of the most frequently used multidimensional systems is the so-called heartcut technique, a technique in which a relative narrow fraction from one column is focused and reinjected on a second column with different characteristics for further separation. An example is given in Fig. 2.1 in which a small amount of phenol, coeluting with the hydrocarbon matrix from the first non-polar column, is separated on a second polar column.

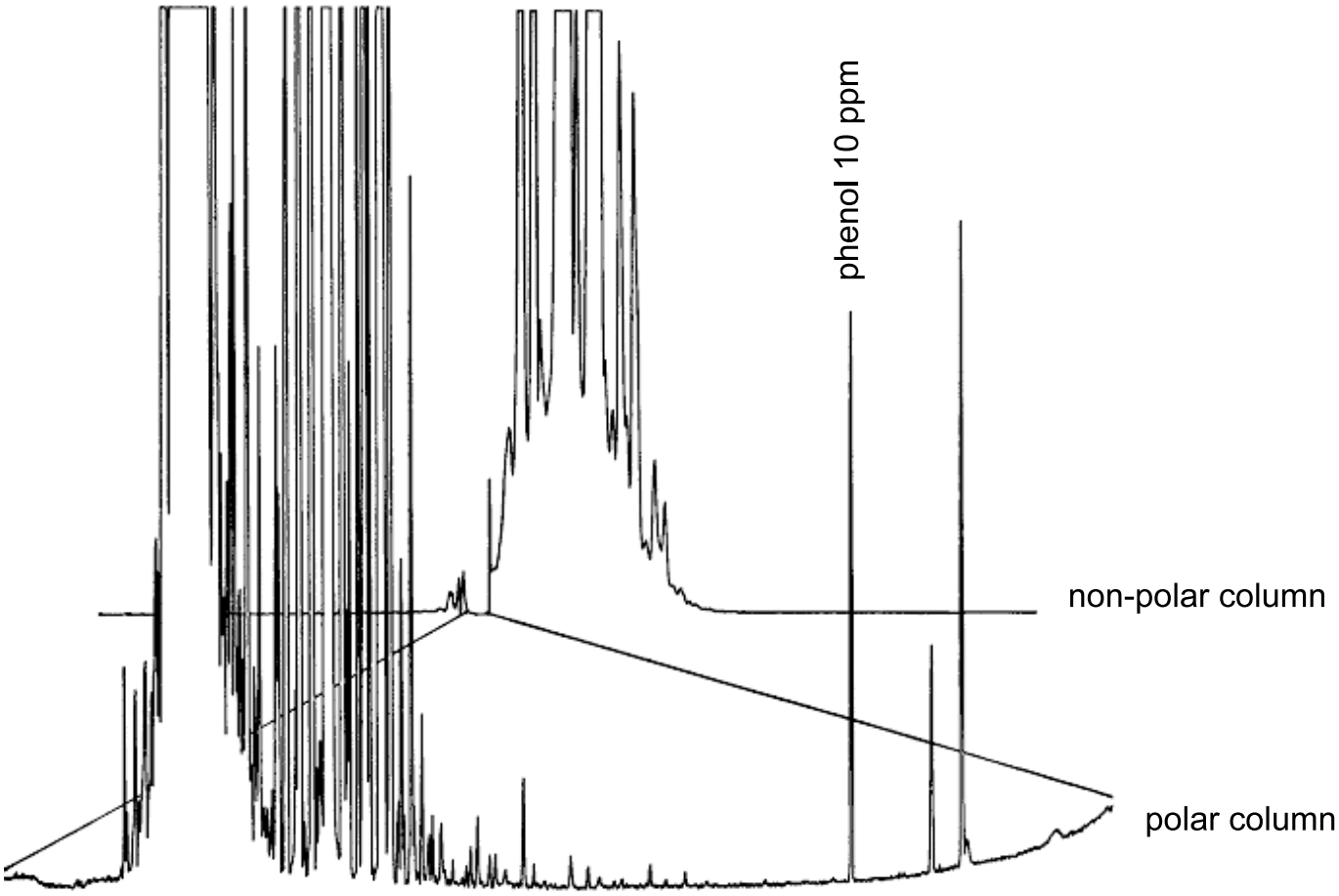


Figure 2.1. Analysis of 10 ppm phenol from a hydrocarbon matrix by heartcutting.

Many of these, mainly valve-based multidimensional systems are still in use in *e.g.* the oil industry [10]. A demonstration of the ultimate capabilities in separation power and selectivity of MDGC was the introduction of the so-called PIONA-analyser in 1971 in the petroleum industry, where PIONA stands for Paraffins, Iso-paraffins, Olefins, Naphthenes and Aromatics [11]. This system has exploited the unique separation of naphthenes (cyclo alkanes) from paraffins (alkanes) as a function of carbon number on a column packed with zeolites of a very specific pore size (13X molecular sieves) [12]. It incorporates four different packed columns, three sample traps in a set-up with six switching valves. It enables the group-type separation of gasolines and naphthas in the aforementioned five group-types per carbon number. In later years the technique has been expanded to samples having boiling points up to 270ºC [13] and implemented in a commercial instrument [14]. Other investigators developed comparable systems with capillary columns, some of which incorporated a mass spectrometer, but these were never commercialised. Lately, with the introduction of oxygenates in gasolines, all of these analyser systems experienced the shortcoming that they are not able to separate oxygenates from the hydrocarbon matrix. A

new multi-column system has been developed, the Reformulyser, which overcomes this shortcoming. Figure 2.2 depicts the schematic diagram of the Reformulyser. Although the Reformulyser utilises some packed columns, the system is still in use in the majority of refinery laboratories.

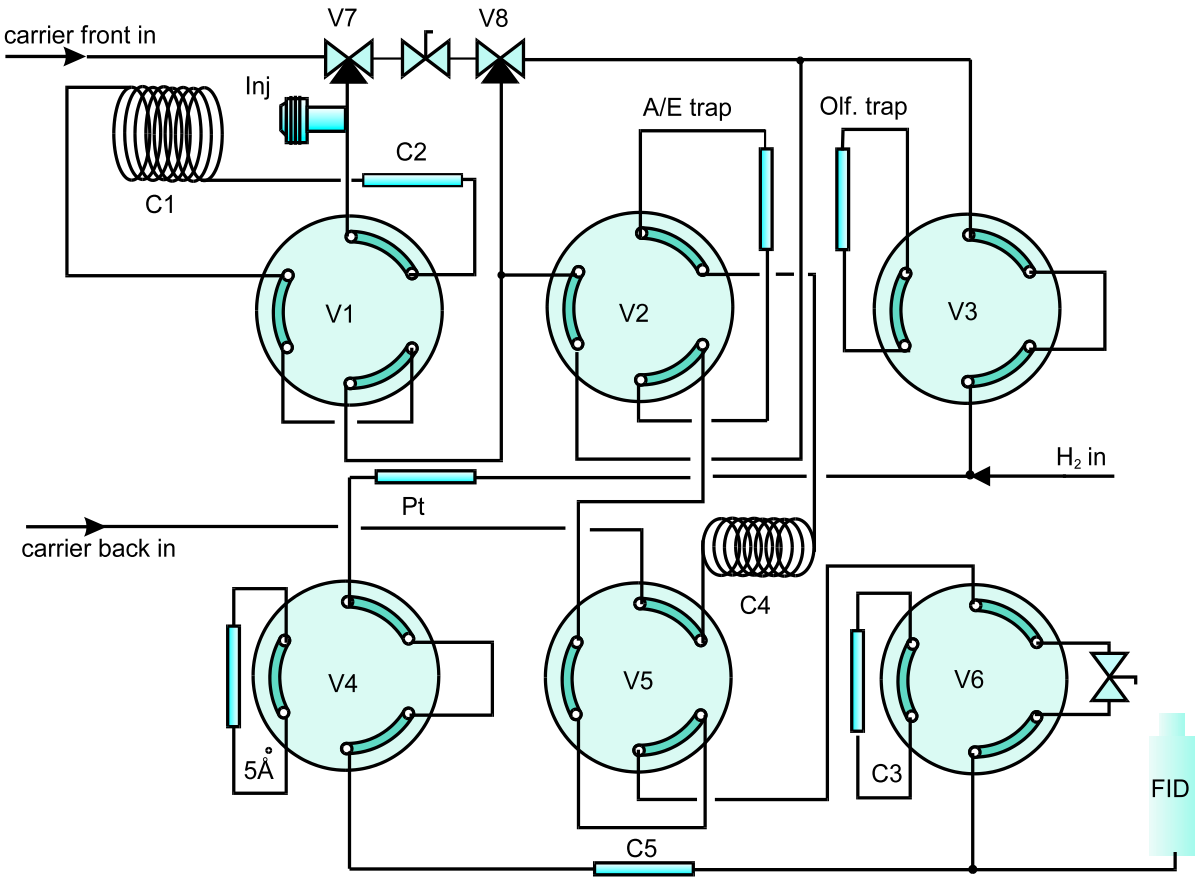


Figure 2.2 Schematic diagram of the Reformulyser.

Inj = split injector, C1 = polar capillary column, C2 = packed column to retain the alcohols, C3 = packed Porapak column for the separation of the oxygenates, C4 = capillary non-polar column, C5 = packed mol.sieves 13X column, A/E trap = Teenax trap to retain the aromatics, Olf. trap = trap to retain the olefins, Pt = olefins hydrogenator, 5Å =mol.s ieves 5Å trap to retain the n-alkanes, V1 through V6 = six-way valves

However, after the introduction of fused silica capillary columns in the early eighties and the conversion of packed to capillary column systems, the chromatographic landscape changed drastically. Not only was the instrumentation adapted enabling the use of these capillary columns, but also most of the users accepted capillary technology and adapted their methods to it. But at the same time the development of multidimensional GC with capillary columns just faded away.

MDGC has always been an attractive way of increasing peak capacity or increasing selectivity in GC systems. Especially in capillary GC, the multidimensional techniques are by no means simple in construction or operation. Even dual column systems enabling the single heart-cutting technique of transferring a single fraction from a first column onto a second one for further separation are technologically but also from an operational point of view much more demanding than single column systems. Only a few research groups and a few instrument manufacturers took tentative steps in developing multidimensional GC further. The majority of the GC users, however, were seemingly satisfied with the existing situation of the separation ability offered by capillary GC, especially when combined with quadrupole MS for the characterisation of their samples. And although it will be admitted by those who have the expertise in fully employing MDGC that it does hold the promise that many of the pioneers on the technique already predicted in the early days, it did not find a wide-spread acceptance. There has, nevertheless, been made some progress in MDGC in the past 20 years, but the new applications, and especially new techniques have appeared only very sparsely and were not very exiting.

This situation did not change drastically until John Phillips and his co-workers in 1991 [15] demonstrated a new two-dimensional GC technique, which he designated by the term Comprehensive Two-Dimensional GC. Since then this technique has been adapted by a number of research groups and is further developed into an exiting new form of two-dimensional GC that can be considered as a leap ahead in MDGC. And although there has always been a time lag between the introduction of a new technique and the general acceptance of it, it is now about time that this exciting technique should be introduced in many more laboratories.

3. Comprehensive two-dimensional gas chromatography

Multidimensional GC can be performed in many different ways. The main argument in using a multidimensional technique is probably to separate components that co-elute on a single column. When such a group of co-eluting components is transferred from one column to a second one, having different separation characteristics, the co-eluents hopefully will be separated. This heart-cutting technique can be performed several times during an analysis, but all at the cost of complexity of the set-up and of total analysis time.

An example of such a multiple heart-cutting multidimensional analysis is depicted in Fig. 3.1. The sample, a mixture of PCBs, is first separated on a non-polar column. Throughout the chromatogram several co-eluents exist, and seven narrow fractions, containing some of the co-eluents are cut at the exit of the column, collected and re-injected onto a more polar column, *viz.* 28/31, 47/48/48/52/69, 99/101/113, 118/122/143, 132/153, 138/160/163/164 and 156/157/201. On this column all of the co-eluents are separated now. An extreme example of such a multi heart-cutting analysis is given in [16]. Here a two-column system is used where the columns were connected by means of a liquid CO_2 coldtrap for focusing the chosen fraction and re-injected – each time only one heart cut fraction – onto the second column. In view of the complexity of the sample – flue-cured tobacco essential oil – 23 fractions have been selected and transferred from the first to the second column. The first-column separation lasted 110 minutes and had to be repeated for each of the next 22 fractions. Since each of these 23 secondary separations needed another 60 minutes for the second separation, the total analysis time must have been around 70 hours! And although this number of heart-cuts is extremely – and unusually – high and the amount of information collected is enormous, quite some information is lost during the analysis. The major reason being the fact that the fractions collected from the first dimension have to be relatively broad – to ensure that the fraction is covering the compound of interest completely – and have to be focused before re-injection into the second column, thus destroying the separation already accomplished in the first column.

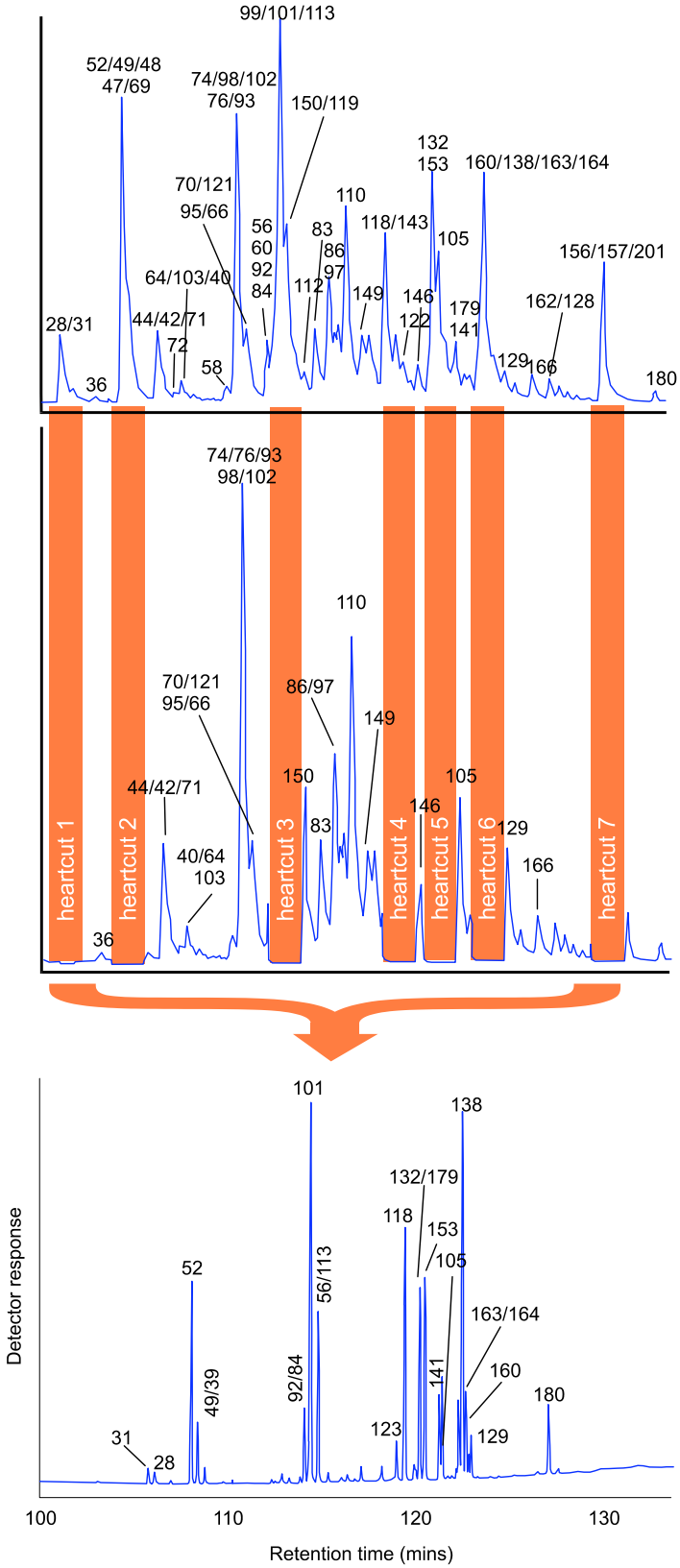


Figure 3.1 Multiple two-dimensional heart-cutting analysis for the separation of PCBs in a complex PCB mixture. The upper chromatogram shows the co-elution in the 1D-GC analysis; the middle chromatogram depicts the places of the seven heart-cuts that are taken (red bars) and the lower chromatogram is the separation of these seven heart-cuts.

A theoretically more comprehensive way of performing multiple heart-cuts is illustrated in Fig. 3.2. This hypothetical system has been described by several authors [17-19] and is in fact a true comprehensive multidimensional system. The first column can be a conventional capillary column, connected by means of a stream selection system to one of the secondary columns. The secondary columns can all be identical, but must have an independent separation mechanism from the first column. The number of secondary columns must be that large that at least four or five cuts can be made over each peak eluting from the first column. The effluent of the secondary columns is fed to their own detector. By stacking the data of these detectors, a data array can be produced, from which a contour or colour plot can be derived. Such a plot will accommodate all the separated components on a separation plane.

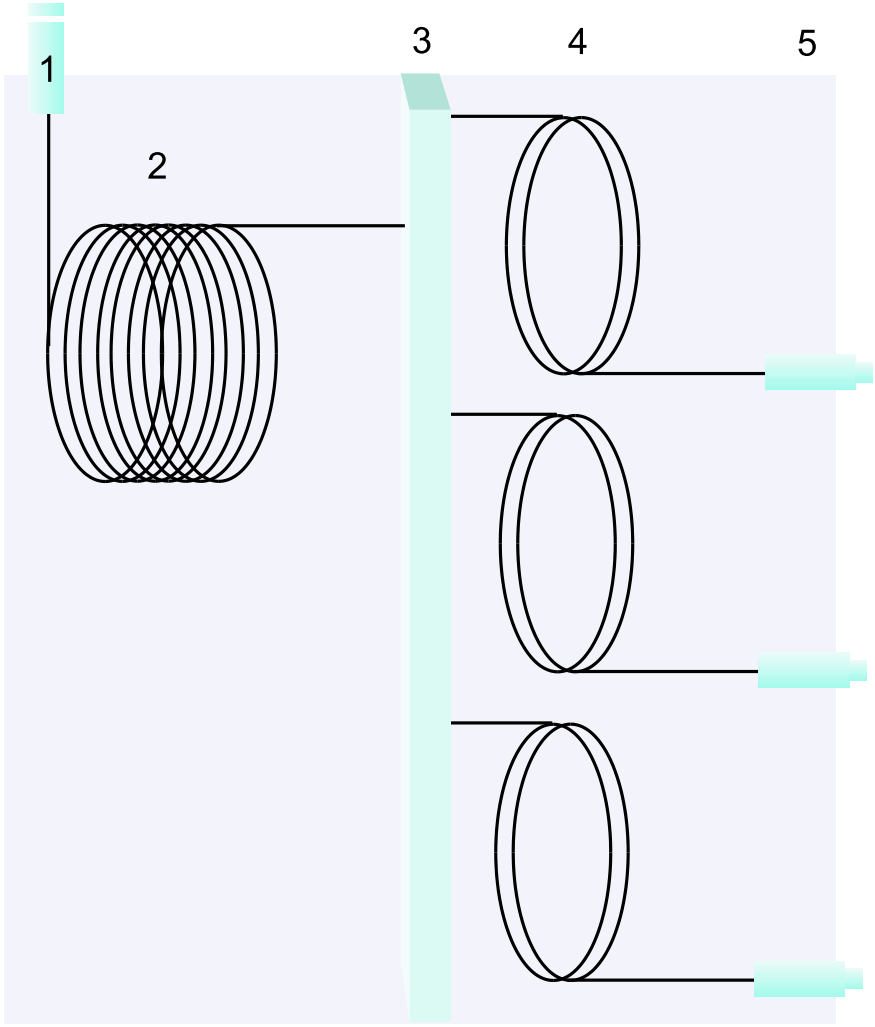


Figure 3.2. Schematic diagram of a hypothetical comprehensive two-dimensional GC.
1. injector, 2. first column, 3. (infinite large) array of stream selectors, 4. (infinite large) array of secondary columns, 5. (infinite large) array of detectors.

By using comprehensive two-dimensional GC as developed by John Phillips, far more than the 23 selected fractions as in the tobacco multiple-heart-cut example, will be selected,

focused and further separated on one single secondary column. In fact a very long series of adjacent fractions throughout the complete first-dimension separation will be transferred to this second column. Any of these individual fractions will be further separated during the very short duration of collecting the next fraction. So, total analysis time of such a separation using the same first-dimension column would have been 110 minutes in stead of 70 hours. It must be admitted though that the second-dimension separation of course would produce a much lower separation efficiency. Nevertheless, in view of the thousands of first-dimension fractions that would have been taken for further separation, the total separation power of such a comprehensive system is certainly higher than the multi heart-cutting system. Fig. 3.3 depicts the general set-up of such a comprehensive two-dimensional system.

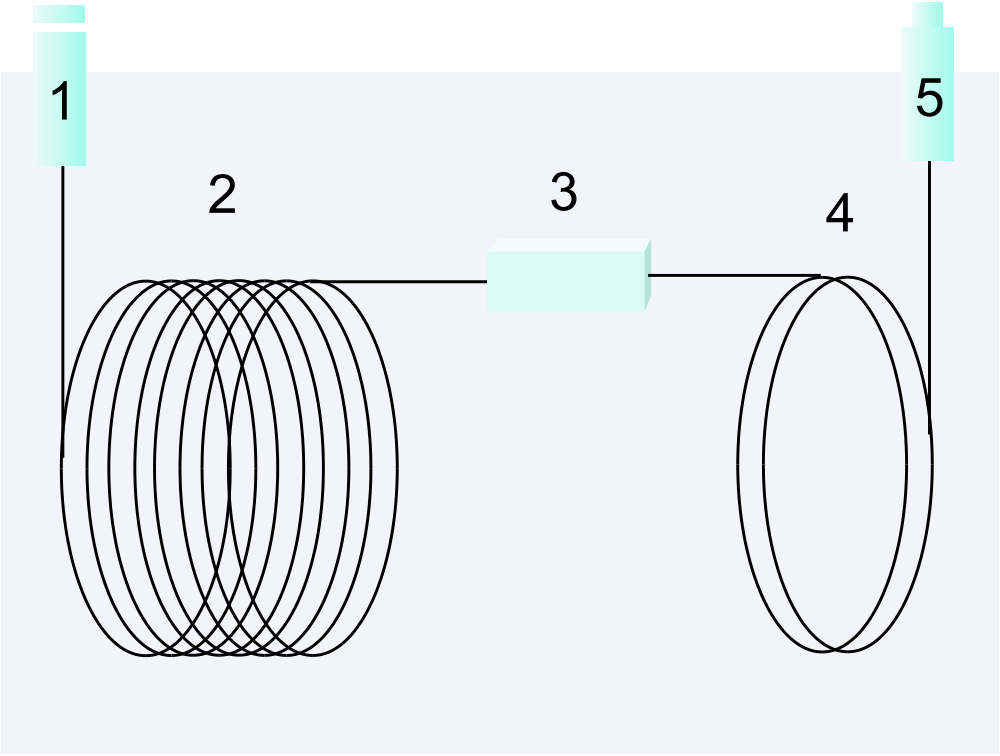


Figure 3.3. General set-up of a GC×GC system.

1. injector, 2. conventional first column, 3. modulator (interface between columns), 4. ultra fast second column, 5. detector

Comprehensive two-dimensional GC (GC×GC) can formally be regarded being equivalent to planar bed separation schemes such as thin-layer chromatography (TLC), except that both dimensions of separation are gas chromatographic. Comparably it provides a two-dimensional separation in which sample substances are distributed over a retention plane formed by the operation of two independent columns rather then on one or two retention lines. Separation and analysis in two dimensions is much more powerful than in one. A retention plane has much more peak capacity than a retention line and so can accommodate much more

complex mixtures. Component identification is moreover potentially more reliable because each substance has two identifying retention measures rather than one. Separations are likely to be more structured in two dimensions, leading to recognisable patterns characteristic of the mixture source and therefore an extra and reliable identification source is acquired.

In GC×GC two independent GC separations are applied to the entire sample. The sample is first separated on a normal-bore high-resolution capillary GC column in the programmed temperature mode. All of the effluent of this first column is then focused in very many, very narrow fractions at regular, short and adjacent intervals and subsequently injected onto a second capillary column, which is short and narrow allowing very rapid separations.

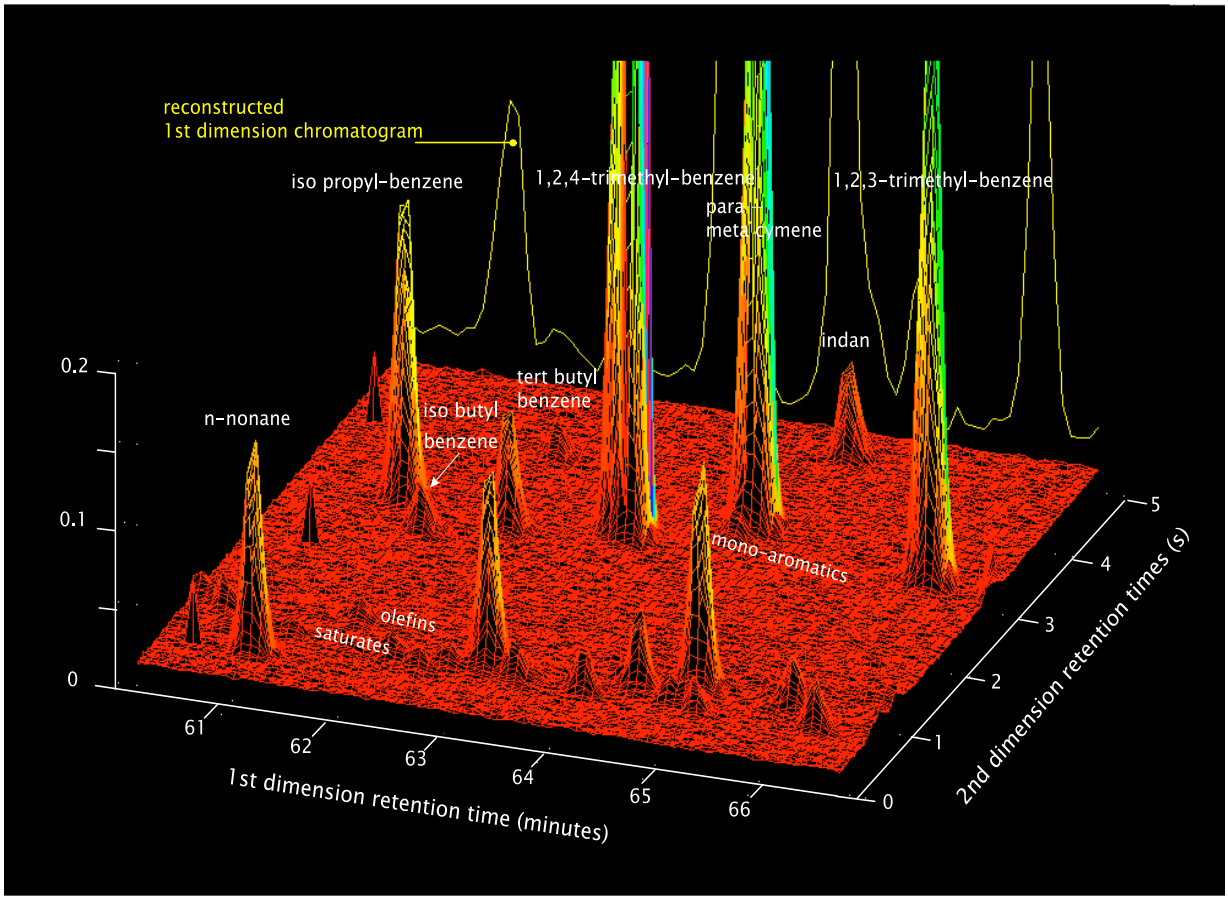


Figure 3.4. Three-dimensional representation of a seven minute section of a GC×GC separation of a gasoline. The yellow trace in the background is the reconstructed (virtual) 1D-GC separation from the first column to demonstrate the difference in separation power.

The resulting chromatogram has two time axes (retention on each of the two columns) and a signal intensity corresponding to the peak height. So in fact a three-dimensional space develops, in which the separated peaks appear as mountains. In Fig. 3.4 a seven-minute section of such a 3D-space is depicted, containing a number of separated gasoline compounds separated in a GC×GC analysis of over an hour.

Since every peak eluting from the first-dimension column is cut into four or five fractions, the separation of each of these fractions on the second-dimension column must be finished within a few seconds. Considering the fact that the peak width at baseline of the peak as it elutes from the first dimension (^1W_b) is $4\times{}^1\sigma$, the separation on the second column must be finished within $^1\sigma$. An example of the precedence of cutting of a first-dimension peak in GC×GC and the resulting second-dimension chromatograms is depicted in Fig. 3.5.

These short-timed pulses put stringent requirements to the re-injection and the separation on the second column and on the detection device. Assuming a peak capacity of the second-dimension column of 30, which is very common, the average peak width of peaks eluting from the second column then is:

$$^2\overline{W_b} = \frac{^1\sigma}{30} \tag{3.1}$$

in which $^1\sigma$ is the standard deviation of a peak eluting from the first column. Since the separation in the second column is such short that it essentially can be considered of being an isothermal separation, the widths of its peaks increase approximately linearly with its retention times [20]. With series expansion it can be estimated that the first eluting peak (the narrowest) has a width of

$$^2W_A = \frac{^2\overline{W_b}}{1.3} = \frac{^1\sigma}{40} \tag{3.2}$$

in which $^2\overline{W}_b$ is the average width at baseline of a peak eluting from the second dimension and ^2W_A is the width of the first peak eluting from the second dimension. So, σ of this narrowest 2D-peak is:

$$^2\sigma_A = \frac{^1\sigma}{160} \tag{3.3}$$

Assuming negligible extra-column contribution to band broadening from detector volume and electronics (see also Chapter 8, *Detection systems*), the ultimate peak widths of peaks from the second column can be estimated by applying the rule of additivity of variances [21]:

$$\sigma_t^2 = \sigma_i^2 + \sigma_c^2 \tag{3.4}$$

in which σ_t is the final bandwidth in terms of standard deviation, σ_i the input band width and σ_c the chromatographic band broadening. Now it can be estimated that the bandwidth of the injection in the second dimension must be:

$$^2\sigma_i \leq \frac{^2\sigma_A}{5} = \frac{^1\sigma}{800} \tag{3.5}$$

such that the bandwidth of the injected pulse does not have a significant contribution to the final bandwidth. The bandwidth of the pulse at which the focused fraction is re-injected onto the second column must thus be extremely narrow. Considering the fact that in general the second column is a 100 µm ID column of a length in the order of a meter, and in view of the existing linear carrier gas velocity, the injection band width should ideally be less than 10 ms. Broader injection bands will have a significant influence on the final band width, and thus on the separation efficiency of the second column. This focusing and re-injection process is performed with a modulation system. The dimensions of the second column are chosen such (length and diameter as above and a thin film stationary phase), that it provides the necessary speed. This means that it finishes the separation of all the constituents of the pulsed fraction within a few seconds and before the next fraction is re-injected. Finally, the detector must have a small rise time and permit a high data-sampling rate (≥ 100 Hz). Most modern FID detectors fulfil these requirements. When using a mass spectrometer however, only the Time-of-Flight (TOF) spectrometers with the ability of acquiring up to 200 scans/s or even higher are suited. A discussion and overview of modulators is given in paragraph 5, of columns and column combinations in paragraph 6 and of detection systems in paragraph 8.

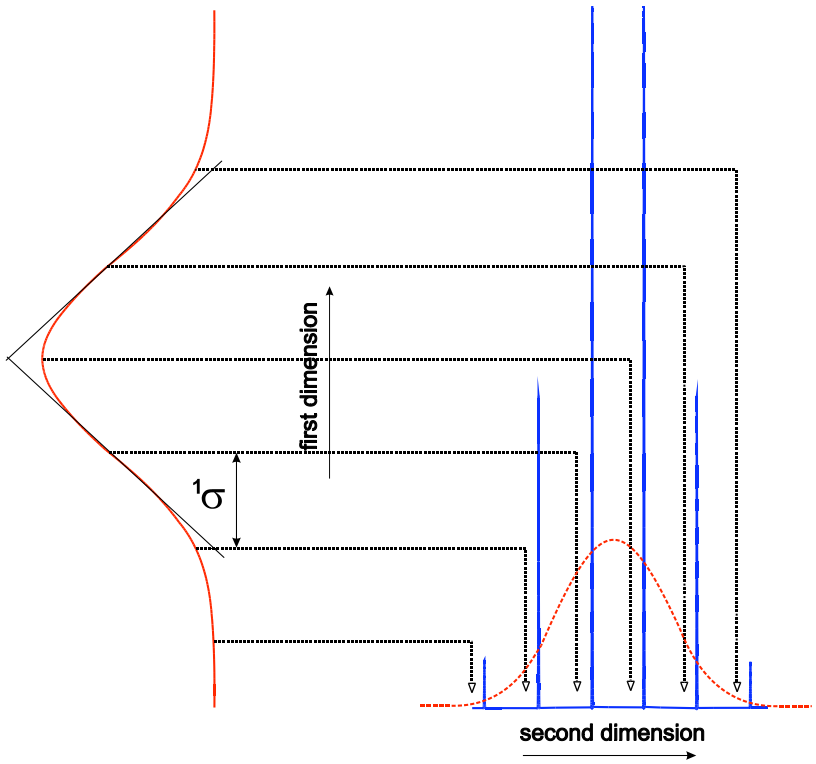


Figure 3.5. Precedence of cutting a first-dimension peak in six fractions onto the second column.

3.1 Comprehensive separations

In conventional two-dimensional GC (2D-GC), *e.g*. in the heart-cutting technique, one or only a limited number of fractions from the first-dimension separation are selected, transferred and subjected to the second-dimension separation. The remainder of the first-dimension separation is left unchanged and not subjected to the second separation. In GC×GC, all of the effluent of the first-dimension column is divided into narrow fractions, focused and subjected to the second-dimension separation. It is for this reason that the term *comprehensive* has been introduced for this technique. It refers to the fact that every component of the sample as it enters the first separation step, is subjected to the separative operation of the total system. No part of the sample is vented off or transferred to the detector without being subjected to both separation steps. As a consequence, all of the sample's components that enter the first separation step must eventually leave at the end of the second separation step and reach the detector. Only thus the final data can represent a comprehensive overview of the sample's constituents. This not only expands the obtained separation to the complete sample under consideration and enhances the reliability of the identification, but it moreover facilitates quantification considerably. If in any one single chromatographic data file, depicted in a chromatogram, being it 1D or 2D, all compounds are represented, then quantification methods like internal normalisation are possible. No quantitative comparisons with external standards are necessary then.

3.2. Orthogonality in multidimensional gas chromatography

In [5] Giddings defined the conditions that a separation system has to fulfil in order to be truly multidimensional:

1. the components of a mixture are subject to two or more separation steps or mechanisms, in which their displacements are dependent on different factors, and
2. when two or more components are substantially separated in any single step, they always remain separated until the completion of the total separative operation.

The first condition in fact prescribes that the distribution of components in one dimension is not correlated with the distribution of components in the other dimension. Given such a separation system, then the dimensions are orthogonal. This condition is fulfilled in GC×GC for the following reasoning. In GC retention is inversely proportional to the product of both the pure-compound vapour pressure and the activity coefficient of the compound in the

stationary phase at infinite dilution. For isothermal elution the net retention is proportional to the retention factor:

$$t'_{R_i} \div k_i \div \frac{1}{p_i^0 \gamma_i^\infty} \tag{3.6}$$

in which k_i is the retention factor of compound i, p_i^0 is the vapour pressure of the pure compound and γ_i^∞ is the activity coefficient for the column's stationary phase. For temperature programmed analysis, the relevant parameter is net retention factor at the time (temperature) of elution:

$$k_{e_i} \div \frac{1}{p_i^0(T_e)\gamma_i^\infty} \tag{3.7}$$

in which $k_{e,i}$ is the retention factor of compound i at the time of elution and T_e is the temperature of the column at the moment of elution.

In principle, all kinds of stationary phases can be used in the first dimension of GC×GC, but generally non-polar phases are used. Then ideally, in the absence of molecular interactions (and entropic effects), all analytes will have an activity coefficient of unity. Since a linear temperature program is used, the retention factors at the time of elution are roughly equal (*i.e.* equal $k_{e,i}$ values). With all activity factors approximately equal, solutes are eluted from the first-dimension column with approximately equal vapour pressures ($p_i^0(T_e)$). The compound-dependent vapour pressure *versus* temperature relationship determines the elution temperature (T_e), which in turn determines the retention time. This is a well-known phenomenon, commonly referred to as *boiling-point separation*. In a linear temperature gradient, all components have approximately identical retention factors at the relative time of elution, as is evident from the constant peak width in a conventional temperature-programmed one-dimensional chromatogram. This means that compounds eluting at the same first dimension retention time have similar vapour pressures (the same $p_i^0(T_e)$ values), but possibly different activity coefficients to the stationary phase in the second dimension.

The second column is operated very rapidly in comparison with the first column and in comparison with the temperature gradient in the column oven. As a result, the temperature of the second column is only increased a few tens of a degree per separation and thus the separations that are obtained on this column are essentially isothermal separations. Since all components that are injected in the second dimension came from the same first dimension modulation as they were separated according to the aforementioned boiling-point-separation, they will have the same $p_i^0(T_e)$ values. The separation of these components is therefore essentially determined by the γ_i^0 only, *i.e.* by molecular interactions between the analytes and

the stationary phase (polarity) of the second column. This second column generally contains a more-polar stationary phase, such that it retains polar substances more strongly than less polar substances. In other words, the aim is creating large differences in activity coefficients on this column. The net effect is that the second column distinguishes between substances only on the basis of their polarity.

Thus, GC×GC offers virtually orthogonal separation mechanisms in the two dimensions, *viz.* analyte volatility and analyte polarity. Since volatility and polarity are uncoupled now, it results in independent separations in the two columns. This fact enables the comparison of GC×GC and two-dimensional bed separations (2D-TLC). The conceptual link here is the relationship between independence and perpendicularity. Graphically this can be expressed within a Cartesian plane, with perpendicular axes, one axis being volatility and the other axis polarity. If the retention times of a solute on the two columns are independent, it can be represented as in Figure 3.6. In practice, this means that we can use the entire plane of a GC×GC chromatogram in separating peaks, rather than a limited zone around, for example the diagonal. But the most important consequence of the orthogonality is that the eluting components will be arranged according to their chemical structures on the separation plane. In other words, the plane will exhibit a great deal of arrangement in bands and clusters of members of the same chemical family.

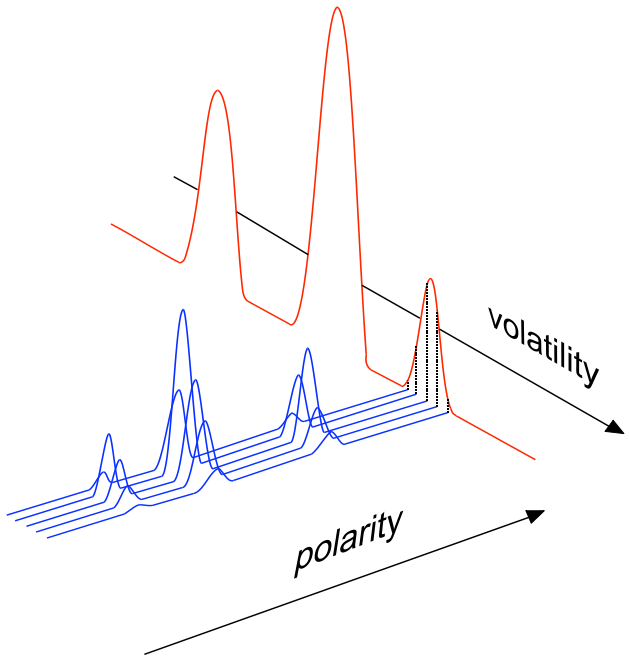


Figure 3.6. The orthogonality of GC×GC.

If now from every peak as it elutes from the first dimension, the cuts that are made are narrower than about 25% of the peak width (at least four cuts over a peak), we can conclude

that any substantial separation that has been achieved in the first column will be preserved during the second separation [22, 23]. This means that if four or more cuts are made over a first-dimension peak and further separated, then the second condition as stipulated by Giddings, preservation of the separation of the first dimension, is also fulfilled.

Apart from the fact that the two separation steps of GC×GC should be orthogonal as to fulfil Giddings' definition of a truly two-dimensional system, it has some important consequences, viz. A. An increase in separation power and B. A structured separation.
An extensive study on the effect of orthogonality using different sets of columns and column combinations has been performed by Cordero *et al.* [24]. They applied several sets of column combinations from non-polar/polar to polar/non-polar and subjected the spreading of the compounds to Factor Analysis estimating the correlation coefficients and spreading angles of the sample components in the two-dimensional chromatographic plane

3.2.1 Increase in separation power.

As already demonstrated by Blomberg *et al*. [25], the separation power, expressed as peak capacity, is enhanced dramatically in GC×GC. Normally, in single-column GC (1D-GC) increasing peak capacity comes with a penalty of substantially increased analysis time and ultimately becomes impractical. Peak capacity is proportional to the square root of the column length or analysis time. Increasing peak capacity by a factor of ten thus requires a hundred times longer analysis time.

The peak capacity in GC×GC can be estimated by arithmetic multiplication of the peak capacities of the first and second dimension separation [5]. Blomberg *et al*. therefore preferred to use the acronym GC×GC to alternatives as C2D-GC in assigning the technique, because it emphasises this main separation characteristic [25]. This acronym now has found a general acceptance in the analytical world and will be used throughout this book (See also Chapter "Definitions and Nomenclature" to be found as a separate pdf on Chromedia). In Fig. 3.8 this phenomenon of arithmetic multiplication of the peak capacities is further visualised by Giddings [5]. The primary column, typical 30 m long with an inner diameter of 0.25 mm and a film thickness of 0.25 μm will generate about 130,000 plates when used at its optimum performance. Peaks eluting from such a column used under optimum conditions will have widths of about five seconds. In a programmed analysis of 1.5 hours, this column then would exhibit a peak capacity of around 1100. Peak capacity being the maximum number of peaks that can be separated by the column if they all were just baseline separated ($R_s = 1.5$). The second dimension chromatograms, because of the relatively short elution times, are essentially

run under isothermal conditions. In this direction the peak capacity is relatively modest, *e.g.* 30. This has not only been demonstrated in practice, but can also be derived from theoretical calculations for a realistic secondary column of 1 m and 0.10 mm ID run under typical GC×GC conditions. With the help of the formulas for calculations of σ and R_s, as they can be found in most GC textbooks, a series expansion of peaks was generated.

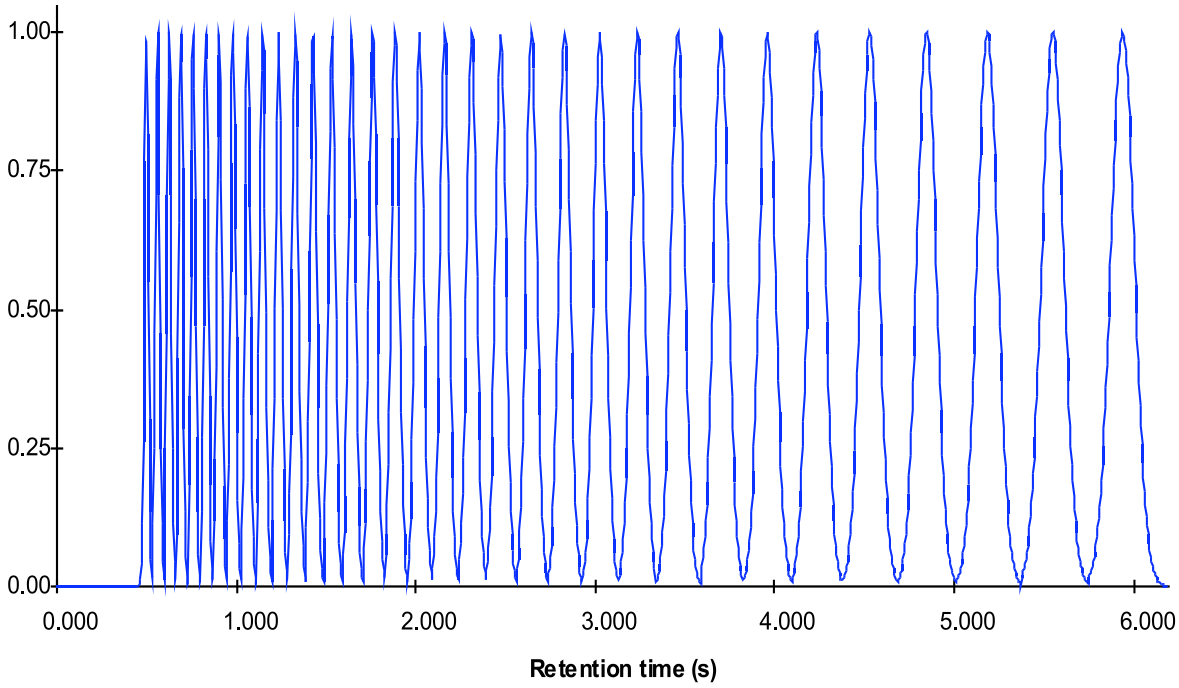


Figure 3.7. Calculated peak capacity (n = 34) in a six-second isothermal analysis for a realistic second dimension column of 1m, 0.1 mm ID, with a He flow @ 2 m/s. First peak W_b = 65 ms, last peak W_b = 400 ms

Fig. 3.7 gives the result of these calculations in a drawing of such a separation of 34 (gaussian) peaks with R_s = *1.5* eluting from a 1 m, 0.10 mm ID column under isothermal conditions in about six seconds. Consequently, a realistic peak capacity for GC×GC of 1100 × 30 = 33,000 is awesomely high in comparison with even the best one-dimensional separation system.

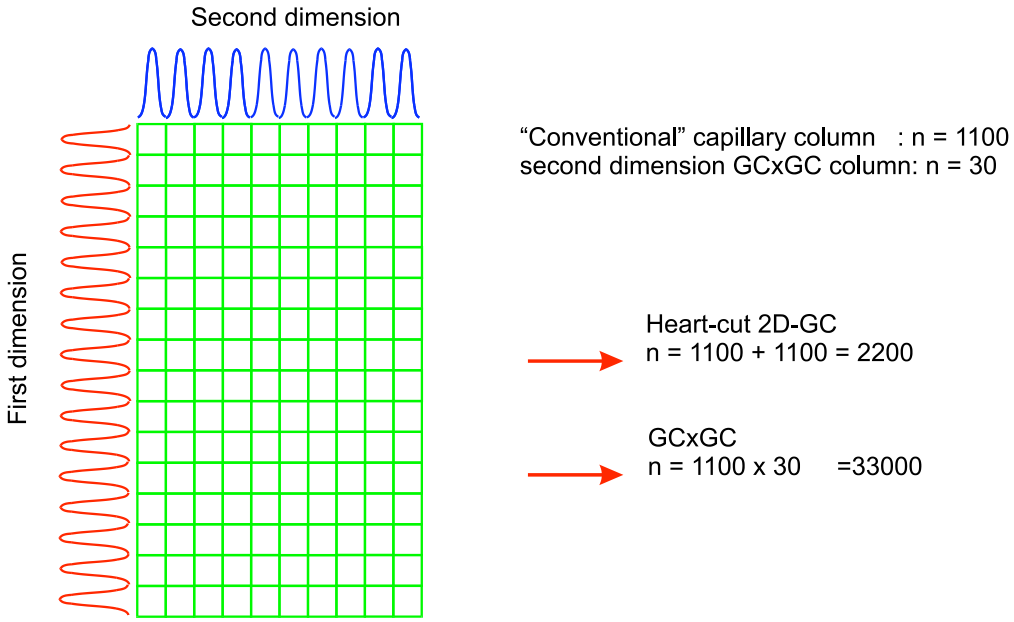


Figure 3.8. The increase in peak capacity of GC×GC.

Blumberg [26] discussed the same matter in a paper and concluded that GC×GC only can provide an order of magnitude gain in peak capacity. From his (theoretical) approach underlying this conclusion he starts from the simplifying assumption that the columns in both dimensions as well as the thermo-modulator have the same diameter and share the same flow. This is not very realistic, since in general, the ID of the second column is about three times smaller than that of the first column, but ideally five times smaller (see also Chapter 6, *Columns and column combinations*). These differences in ID are necessary for obtaining the necessary difference in speed in the two dimensions (as is explained in the beginning of this chapter). A further assumption in the paper of Blumberg is that the secondary column has a vacuum outlet, which is only true in those cases where MS-based detectors are used. Furthermore, vacuum outlet conditions only influence peak capacity significantly if these vacuum conditions are valid throughout the length of the column, which is hardly true for the narrow-bore columns (ID = 0.01 mm) that are used as the second dimension in GC×GC. A second conclusion that is made in the same paper is more meaningful, but valid for any separation system, *viz*. that the peak capacity gain of GC×GC reduces the peak saturation of a separation system only if the peak distribution along the second dimension (or in 1D-GC, along its separation line) is substantially uniform.

If the full separation plane that is created by the increase in peak capacity is used by employing two independent separating columns, a very powerful separation can be achieved, even from very simple samples. And although high-resolution capillary columns have a great

potential in separating samples, from the calculations of Giddings as mentioned in Chapter 2, it is clear that frequent peak overlap and co-elution occurs. From our experiences in using GC×GC as a separation technique for real-live samples from a large variety of sources and used in many different applications, it became evident that there hardly are any separations in which no co-elution in 1D-GC analyses will occur. This is nicely demonstrated in Fig. 3.9, the separation of a very simple sample seemingly containing only eight compounds, of which two pairs are not fully resolved in a five-minute 1D-GC analysis. The upper trace in the figure depicts the 1D-GC separation, indicating that the first and the last peak pairs are only partly resolved. The middle chromatogram is acquired after modulation in a GC×GC system using the same (first) column and a modulation frequency of 0.25 Hz (one modulation every four seconds). The peak pattern in this chromatogram more or less follows the original 1D-GC chromatogram trace, but it is apparent that more and different peak patterns arise from small co-eluting compounds. The final colour plot clearly demonstrates the separation power of GC×GC. Not only are the partly resolved pairs fully resolved now, it suddenly becomes evident that underneath the large peaks there are some tiny peaks that were hidden completely in the 1D-GC analysis. In stead of eight peaks, we end up with twelve separated peaks. This phenomenon of co-elution is even more pronounced in the separation of less simpler samples. And as is already stated before, samples containing hundred or more compounds should be considered of being complex samples.

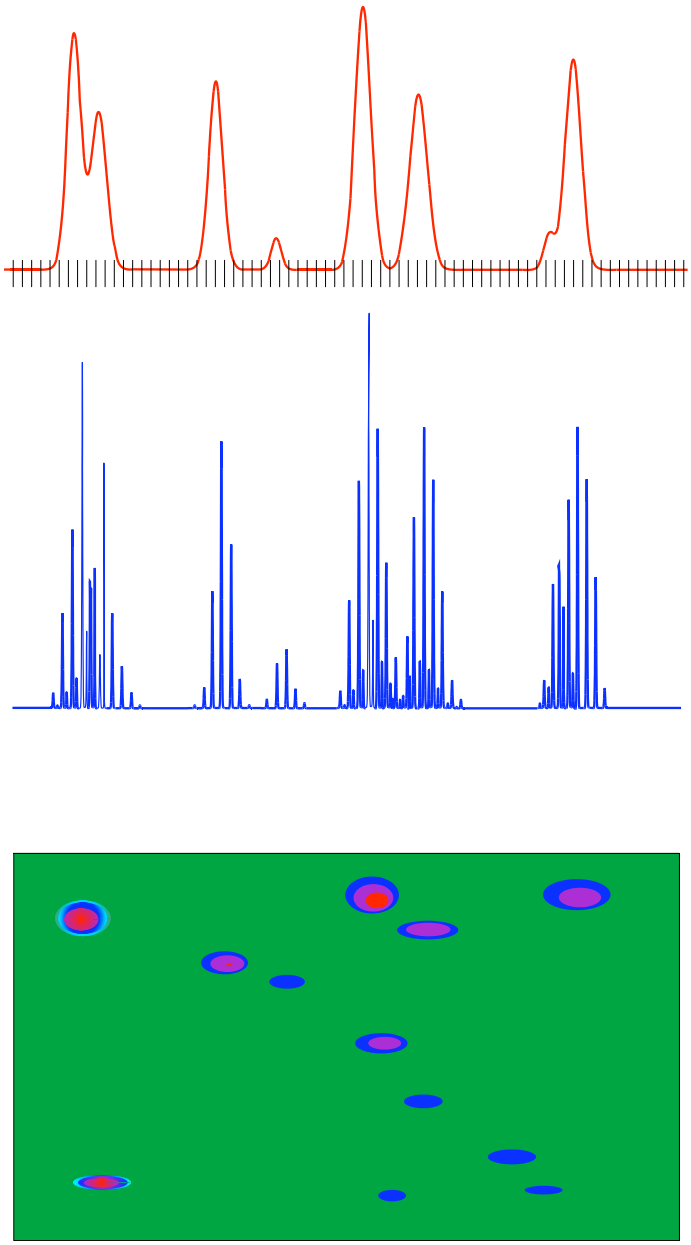


Figure 3.9. The GC×GC separation of a very simple mixture demonstrating the increase in separation power of the technique.

Upper (red) trace: 1D-GC separation of five minutes

Middle (blue) trace: Modulated GC×GC chromatogram acquired after four-second modulations

Lower colour plot: Final GC×GC colour plot after transformation.

3.2.2. Structured separation.

In 1995 Giddings [27] introduced the concept of sample dimensionality, an intrinsic property of analytical samples (other than the number of components) that determines their amenability to multidimensional techniques. The sample dimensionality is defined as the number of independent variables that must be specified to identify the components of the sample. In other words, if two chemical properties of the components of a mixture vary independently, then we may assign to such a mixture a dimensionality of two. Accordingly, the hypothetical sample in Fig. 3.10, containing compounds differing in shape, colour and size, has a dimensionality of three. If such a sample contains a reasonable amount of compounds, then there is no chance of separating all its constituents in a 1D-system. Either the separation is performed according to size, but then colour and shape will interfere. Or the separation is performed according to colour, but then size and shape will interfere. Or the separation is performed according to shape, and size and colour will interfere. The only possibility of separating all of the constituents is in using an orthogonal 2D-separation system. And if there is a match between the dimensionality of

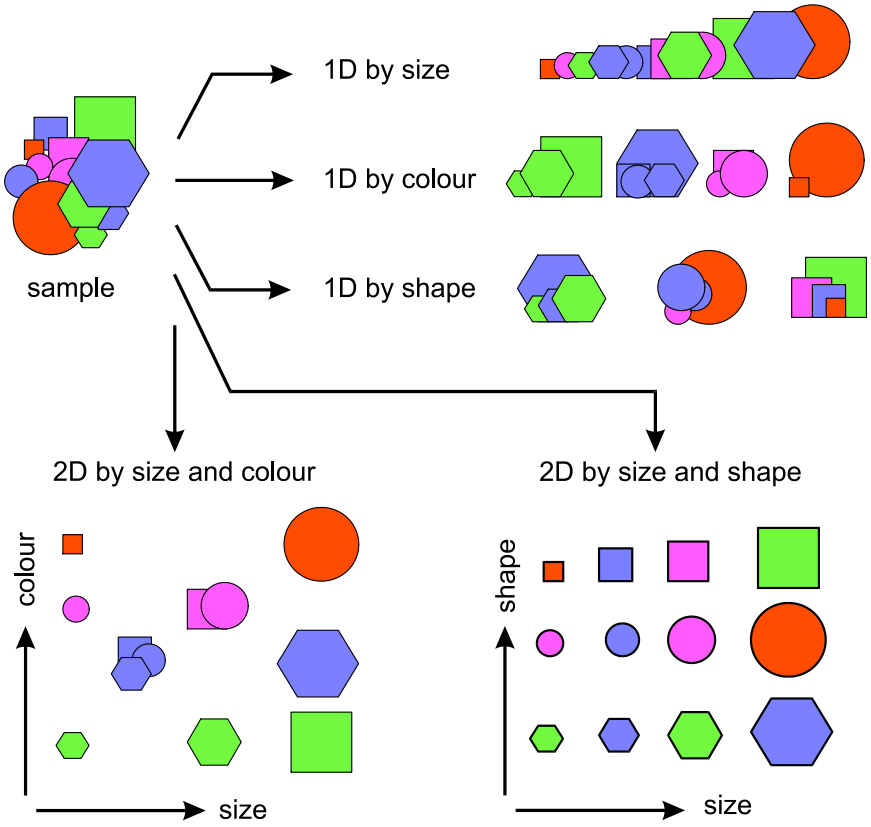


Figure 3.10. Match between separator and sample dimensionality in GC×GC. (Courtesy of Jan Blomberg).

the sample and of the separation system, we can use the total separation space that is available very efficiently to accommodate separated analytes and structures will appear in the colour plot as is depicted in Fig. 3.10.

Accordingly, a petroleum fraction, a mixture of hydrocarbons containing large amounts of isomers of the chemical groups of alkanes, alkenes, cyclo alkanes, and aromatics, has a dimensionality of four. If we now, according to the orthogonality of the GC×GC separation, arrange the hydrocarbons of a petroleum fraction on a plane on which the horizontal axis is the volatility (may be easier: boiling point) and the vertical axis is the polarity of the compounds, we automatically get a highly structured plane as a result. The same happens if we introduce retention index on a strictly non-polar column (DB–1) for the horizontal axis – the boiling point separation – and retention time on a medium polar column (OV1701, 14% cyanopropylphenyl-methyl-polysiloxane) for the vertical axis (Fig. 3.11). Walraven in 1968 [28] already showed the 'roof-tile'-structures of retention on two orthogonal columns. Since there is a match between the different groups of hydrocarbons and the dimensionality of the separation system, these structures are formed by the dimensionality of the sample.

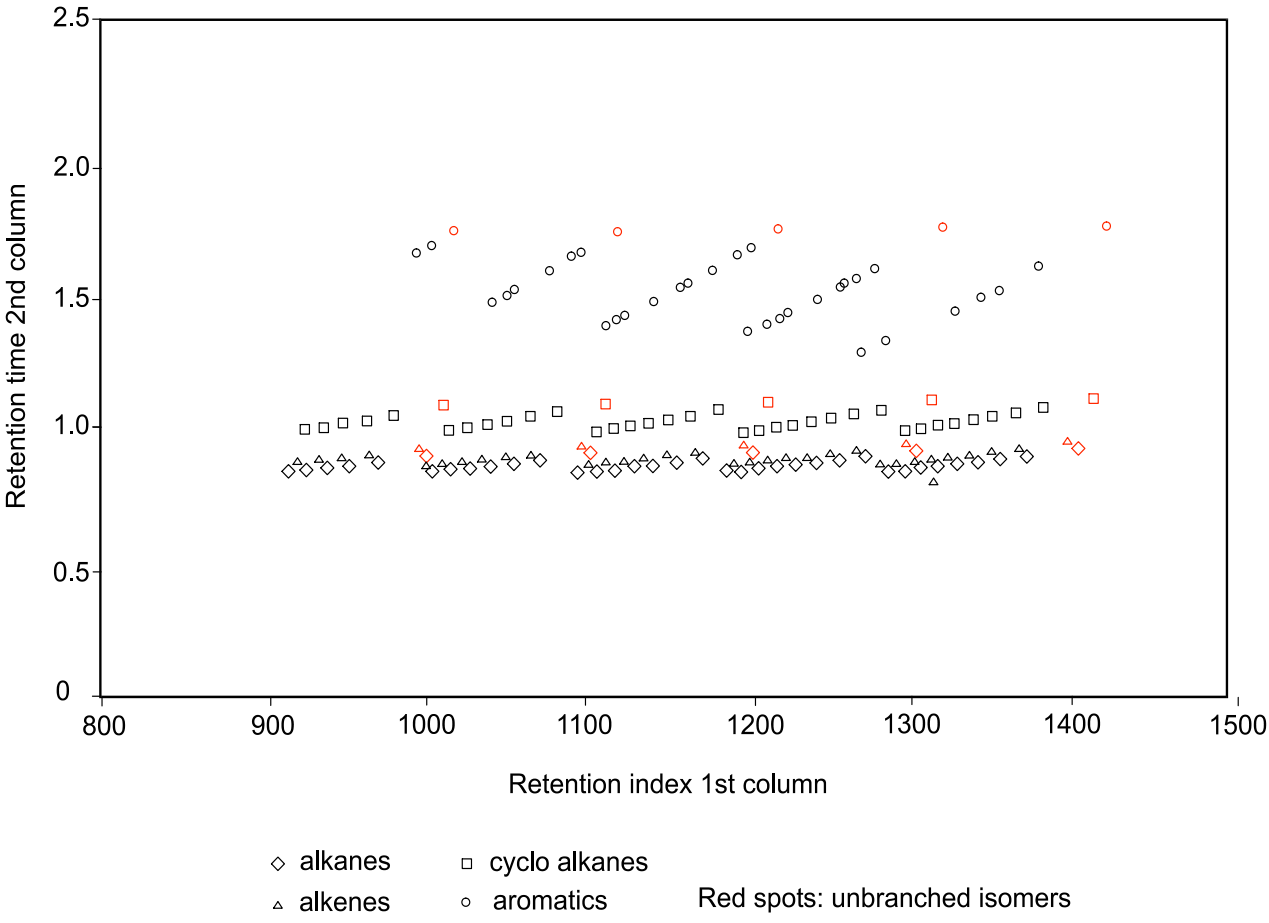


Figure 3.11. The arrangement of chemical classes on two independent columns.

These structures are obtained in many GC×GC separations, provided that the dimensionality of the sample allows such a structuring and the separations of the two columns in use are independent. It was already predicted by Giddings [27] from his fundamental considerations on orthogonality and the dimensionality of samples in the discreteness of molecular structures. The most striking examples of these structures or clusters of

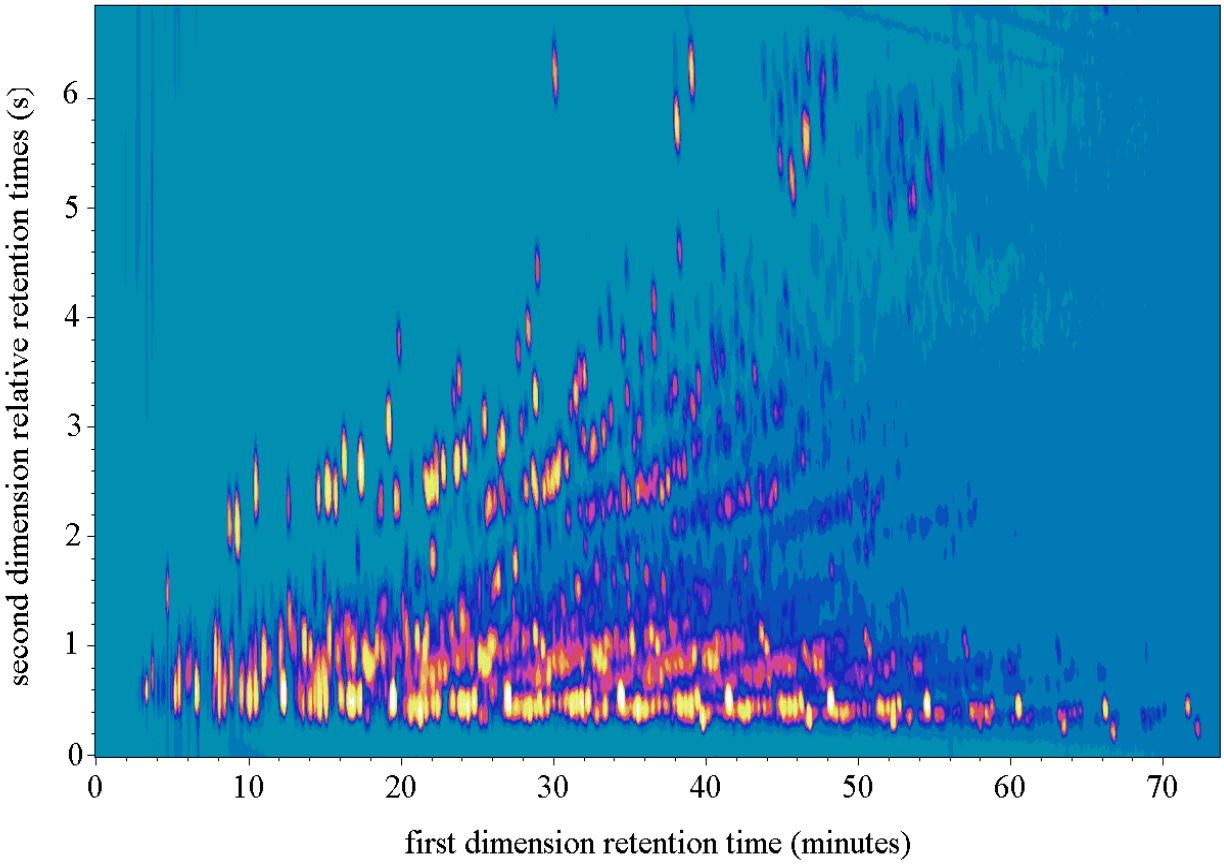


Figure 3.12. Colour plot of a GC×GC-separation of a kerosene. (Copied from [31])

components belonging to the same chemical family are found in the separations of *e.g.* petroleum products. Blomberg and Beens reported a number of these separations and indicated how this clustering can help in the group-type identification of complex petroleum products but also in identifying the spots within the clusters [25, 29, 30]. Identification is thus not only facilitated but is also made very reliable. An example of such an ordered chromatogram, representing the GC×GC separation of a kerosene is given in Figure 3.12.

The abundant crowded band of spots at the lower end of the figure represents the saturates, where each high intensity *n*-alkane can be seen as a white spot at regular time intervals. In between those *n*-alkanes are the branched alkanes situated, from alkanes with a

low degree of branching just in front of the *n*-alkane, up to the highly branched ones with ever lower retention times. Above the alkanes, "roof-tiles" of cyclic alkanes are present, each tile representing different isomers with the same number of carbon atoms, again with the lowest branching on the right side of the tile (highest retention time) and the ever higher branched ones more to the left side of the tile. About in the middle of the plane the different groups of aromatics with one ring (mono-aromatics) can be found. From toluene at a retention time of about five minutes, through the xylenes (three spots) around ten minutes, the mono-aromatics with nine C-atoms from 15 through 20 minutes. The rest of the mono-aromatics again are ordered in "roof-tiles" with the same number of C-atoms from 10 (20 to 30 minutes) through 15 C-atoms (50 to 60 minutes). And again, also within these tiles the different branching of the isomers are arranged in accordance with the arrangements in the cyclic alkanes tiles, *i.e.* the lowest branching on the right side of the tile (highest retention time) and the ever higher branched ones more to the left side of the tile. Finally, in the top of the plane the two-ring aromatics can be clearly seen. From naphthalene at a retention time of 30 minutes, through the two methylnaphthalenes (around 40 minutes) up to a cloud of C_3-naphthalenes (13 C-atoms) at 55 minutes. Confirmation of the aforementioned (tentative) structuring has been performed by Time-of-Flight mass spectrometry coupled to GC×GC in the work of van Deursen *et al*. and Cramers *et al*.[31, 32].

Since the various groups are well separated from each other and can easily be found and indicated, the data of the various group types can simply be extracted from the underlying data file of Fig. 3.12. From this extracted data a virtual 1D-GC chromatogram can be reconstructed by integration and accumulation of the areas of all the separated peaks in the same second dimension. The resulting 1D-chromatogram nicely demonstrates the distribution of the different substituted species. The resolution with which these virtual first-dimension peaks can be reconstructed is of course dependent on the number of modulations across a peak. This is very similar to the data acquisition rate of a normal chromatogram but generally giving less data points per peak. A representation of such a reconstruction of the group of di-aromatics from the extracted portion of the data file is presented in Figure 3.13.

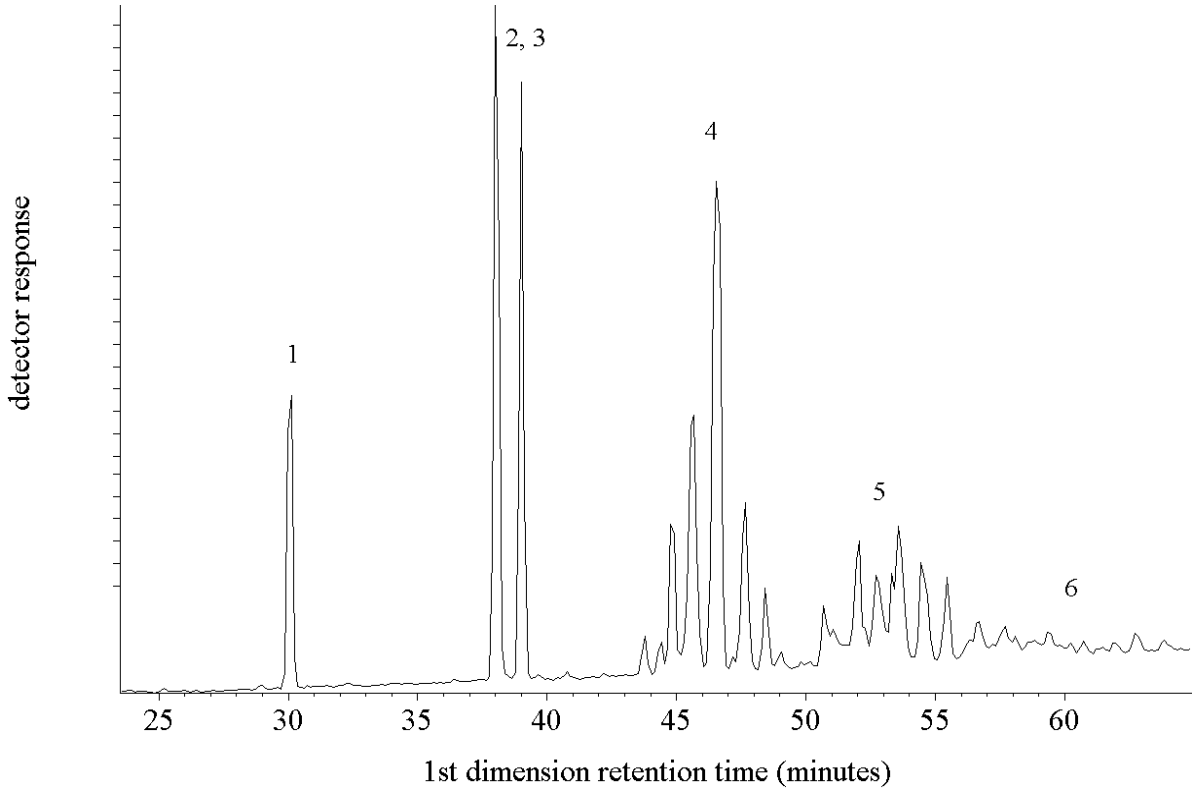


Figure 3.13. Reconstructed (virtual) 1D-chromatogram of the selected group of di-aromatics.

1. naphthalene, 2. 2-me-naphthalene, 3. 1-me-naphthalene, 4. C_2-naphthalenes, 5. C_3-naphthalenes, 6. C_4- and higher naphthalenes. (Copied from [31])

Likewise, in Fig. 3.14 the reconstructed virtual 1D-chromatogram of the mono-aromatics is represented. And finally in Figure 3.14 the reconstruction of the virtual 1D-separation of the total sample, showing the complexity of the sample and the inability of 1D-GC of separating all of the constituents adequately.

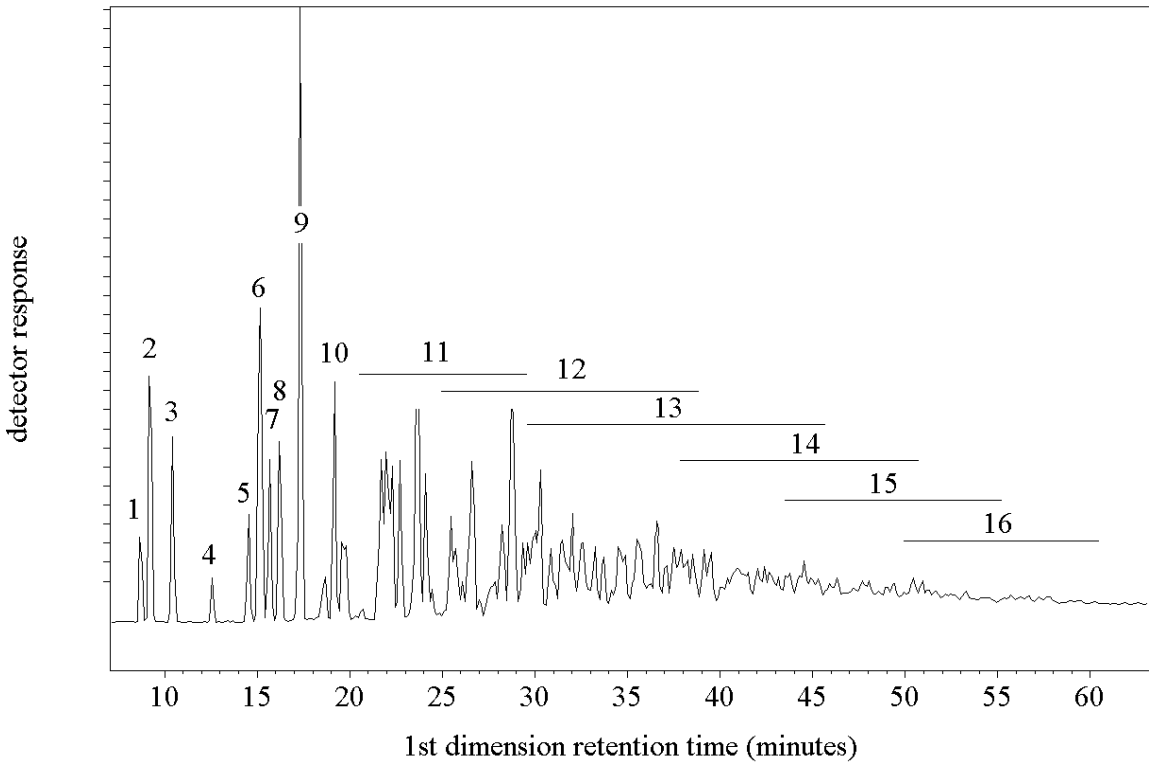


Figure 3.14. Reconstructed (virtual) 1D-chromatogram of the mono-aromatics.
1. eth-benzene, 2. para + meta xylene, 3. ortho xylene, 4. isopropyl-benzene, 5. n-propyl-benzene, 6. 1-me-3- + 1-me-4-ethyl-benzene, 7. 1,3,5-trime-benzene, 8. 1-me-2-eth-benzene, 9. 1,2,4-trime-benzene, 10. 1,2,3-trime-benzene, 11. C_{10}-mono-aromatics, 12. C_{11}-mono-aromatics, 13. C_{12}-mono-aromatics, 14. C_{13}-mono-aromatics, 15. C_{14}-mono-aromatics, 16. C_{15}-mono-aromatics. (Copied from [31])

Many more sample-types separated by GC×GC exhibit structures as bands or clusters in their contour and colour plots. All those samples containing chemical families with a reasonable number of structurally related members may form structures, provided that the dimensionality of the sample allows such a structuring and the separations of the two columns in use are independent. A variety of examples of structured separations have already been reported, as is shown in Chapter 13, Applications. (see separate pdf)

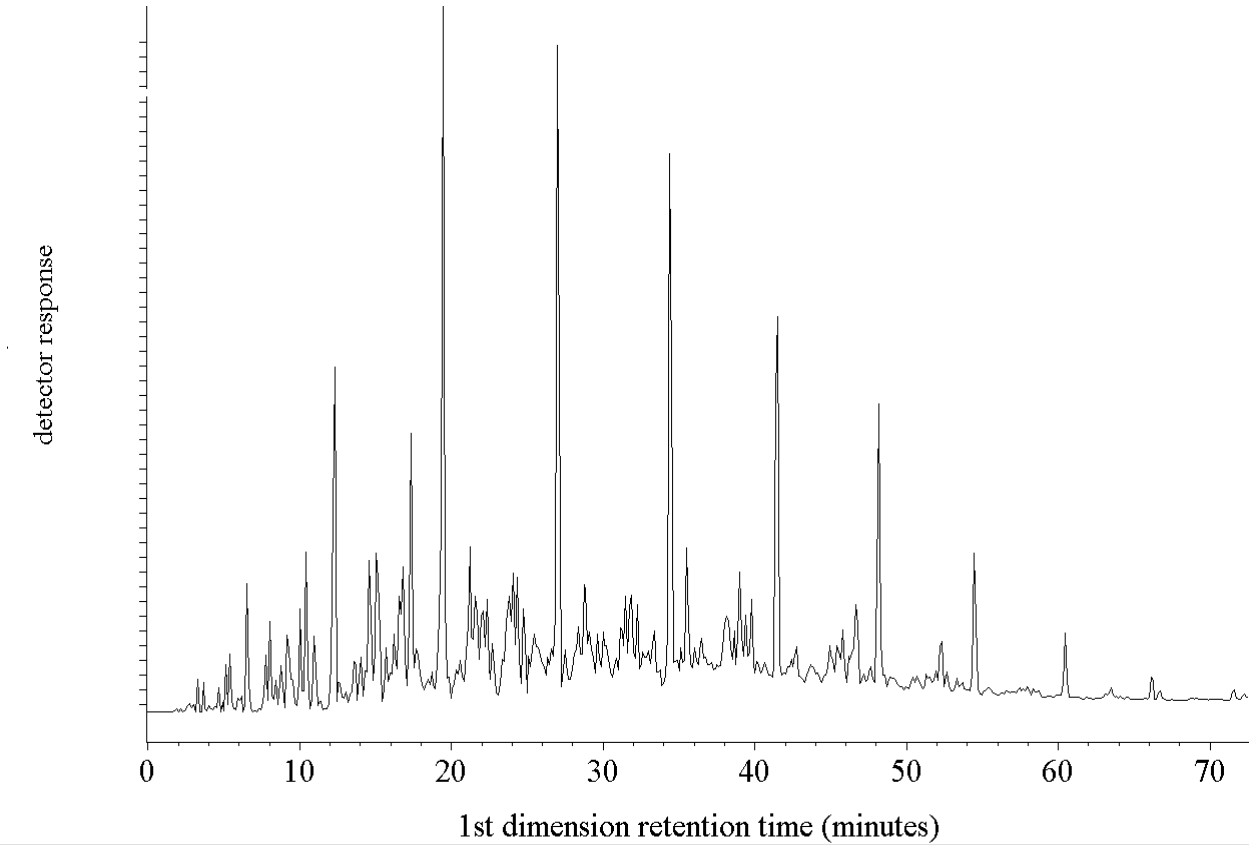


Figure 3.15. Reconstructed (virtual) 1D-chromatogram of the total kerosene sample. (Copied from [31])

4. Modulators

As in any multidimensional system, the interface between the dimensions is a key component in the instrumentation. This interface was designated as the *modulator* by its inventor, John Phillips in his paper on the possibilities of retention alteration in GC by thermal modulation [35]. In this paper he describes the chromatographic column as a signal processor, dispersing chemical signals. The detector, in this respect, is not the source of the signal, but merely a chemical-to-electronic transducer, converting the physical form of the signal. Encoding (demodulation) of these signals is a direct result of the sample introduction and consequently the modulation signal has the form of an injection pulse. Because multiple chemical signals travel through the column simultaneously, the detector output signal carries multiple copies of the modulation. If only a single injection pulse is present, demodulation is a unit operation and can be ignored. When multiple modulation signals are present, demodulation is required for extracting the information from the analytical signal. By introducing thermal gradients in between two columns as a means of retention alteration on a regular time base, a multiple modulation signal is introduced onto the analytical signal [34]. Phillips' observations of the "Christmas-tree effect" of the first existing capillary columns in old-fashioned GC ovens that were originally designed for packed columns provided the idea of using thermal gradients as a means of retention alteration or thermal modulation. This effect of slowing down and acceleration of parts of a chromatographic peak by deliberately applying temperature differences over the column was in fact implemented in the first dual-stage thermal modulator.

Converted into practical consequences, the modulator serves three functions:

- it discretely samples small and adjacent parts from the first dimension effluent,
- it focuses these fractions into narrow pulses, and
- it launches these narrow pulses into the second dimension.

Two principally different types of modulators exist: those that conserve mass and those that only sample parts of the first dimension effluent. A different classification, yet providing the same two groups, can be made in those focusing the sampled fractions in space, and those focusing the fractions in time.

As already argued in Chapter 2, the bandwidth of the accumulated fraction as it is injected into the second column must be 800 times narrower than ${}^{1}\sigma$. This accumulation of bands and focusing in space in GC can only be performed by thermal means. The modulators

focusing in time, on the other hand, all are valve-based systems. So, a third classification, again providing the same two groups is: thermal modulators and valve-based systems.

4.1. Thermal modulators

Two different forms of thermal modulation exist, *viz*. *A*. accumulation and focusing of the analytes in a thick film capillary (modulation capillary) and remobilization by means of applying extra heating.

B. accumulation and focusing by cryogenic means and remobilization by returning to the original temperature. Both these types use, although in a different way a compression in space of the accumulated fraction by thermal means. This has to be done in order to make the fraction suitable in acting as a narrow injection pulse for the fast second dimension column. Bandwidths of the injected pulses are, dependent on the type of modulator, in the order of <10 to 200 ms. A major result of this space compression and the use of a fast second column is the fact that peaks elute from this column as very narrow peaks, which have, in view of the conservation of mass, enhanced signal intensities. A graphical example of the enhanced peak amplitudes is given in [29] (Fig. 4.1). This peak amplitude enhancement is, depending on the focusing effect, in the order of 20 – 150. To what extend this enhancement affects the sensitivity is also dependent on the data acquisition rate and is further discussed in Chapter 8.1 *Sensitivity enhancement*.

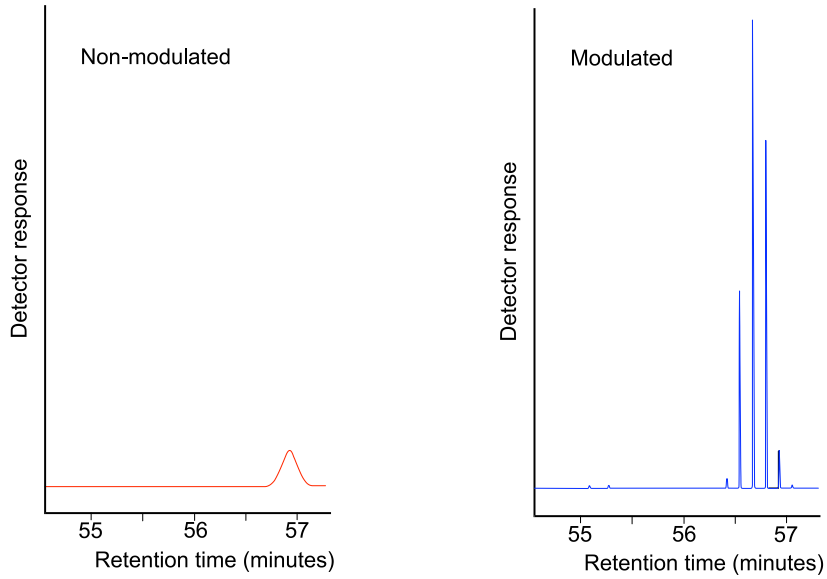


Figure 4.1. Peak intensity enhancement through compression in space by a GC×GC modulator. GC conditions: first dimension: 10 m × 0.25 mm ID, 0.25 µm DB–1, second dimension: 1.5 m × 0.10 mm ID, 0.10 µm OV1701. Modulation with a Sweeper at 5s/modulation. (Copied with permission from [29])

Since the number of fractions that can be sampled from a first-dimension peak is dependent on the widths of the first-dimension peak and the speed of the second-dimension separation (and thus modulation frequency), this number may vary between two and *e.g.* ten. It is obvious that the peak amplitude enhancement is dependent on the amount of material that has been accumulated in one fraction. The lower the number of fractions that are sampled from a first-dimension peak, the higher the peak amplitude enhancement. Fig. 4.2 depicts how the number of modulations affects the peak amplitude enhancement.

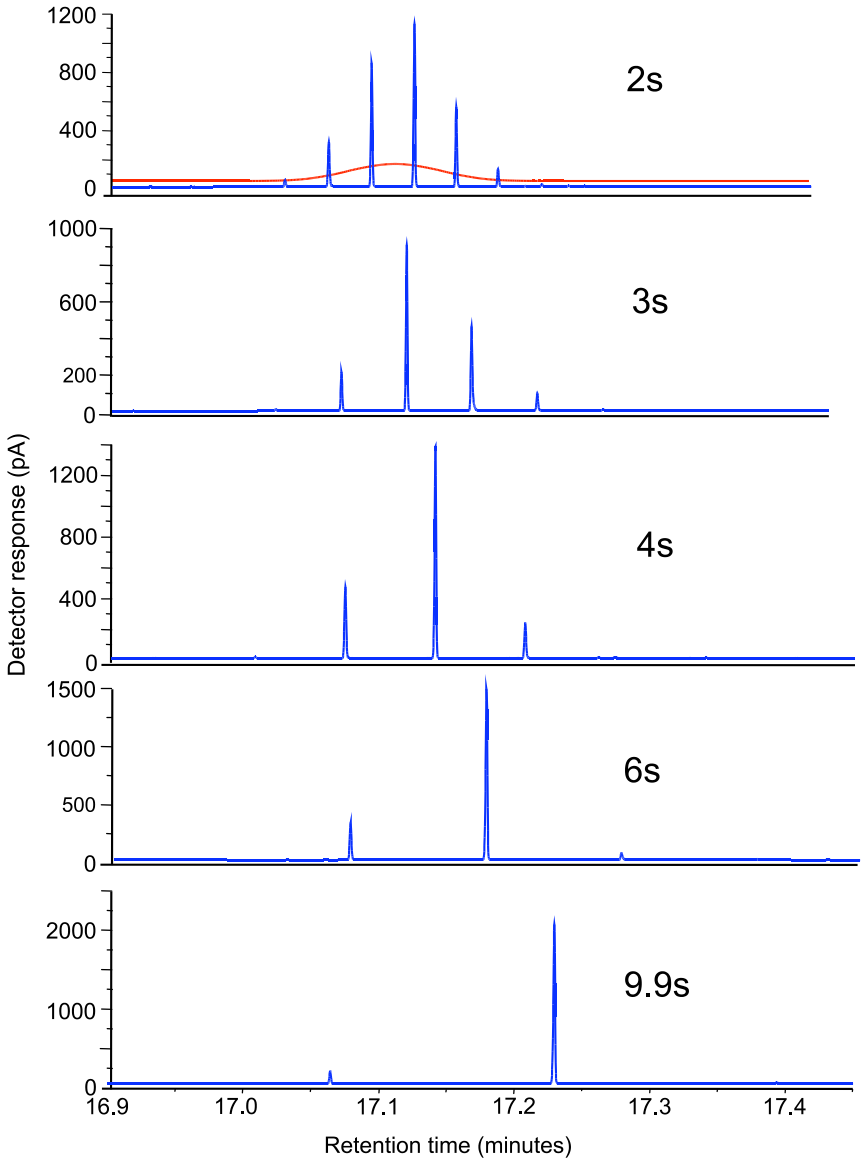


Figure 4.2. The effect of the number of modulations on peak amplitude enhancement.
The red trace in the upper chromatogram is the original, non-modulated 1D-GC trace.
The blue traces are the modulated chromatograms after modulation times of 2, 3, 4, 6 and 9.9 seconds respectively. Note that the maximum peak amplitude enhancement, for one modulation per first dimension peak is about a factor of 150.
(Courtesy of Philip Marriott)

An important result of this peak amplitude enhancement is nicely demonstrated in a practical application by Lewis *et al*. [35]. In this paper a GC×GC analysis is described of an urban air sample in which, apart from the major peaks, hundreds of small peaks were made visible and could be quantified. A section of this separation is presented in Fig. 4.3. In the upper trace representing the previously performed 1D-GC separation shows about 15 peaks, whilst in the GC×GC separation around 120 spots are recognisable. In the 1D-GC analysis, the major part of these peaks are invisible and they only produce a small and irregular baseline offset underlying the major peaks. This baseline offset, which is observed more frequently in 1D-GC analyses is generally designated as "chemical noise", and ignored.

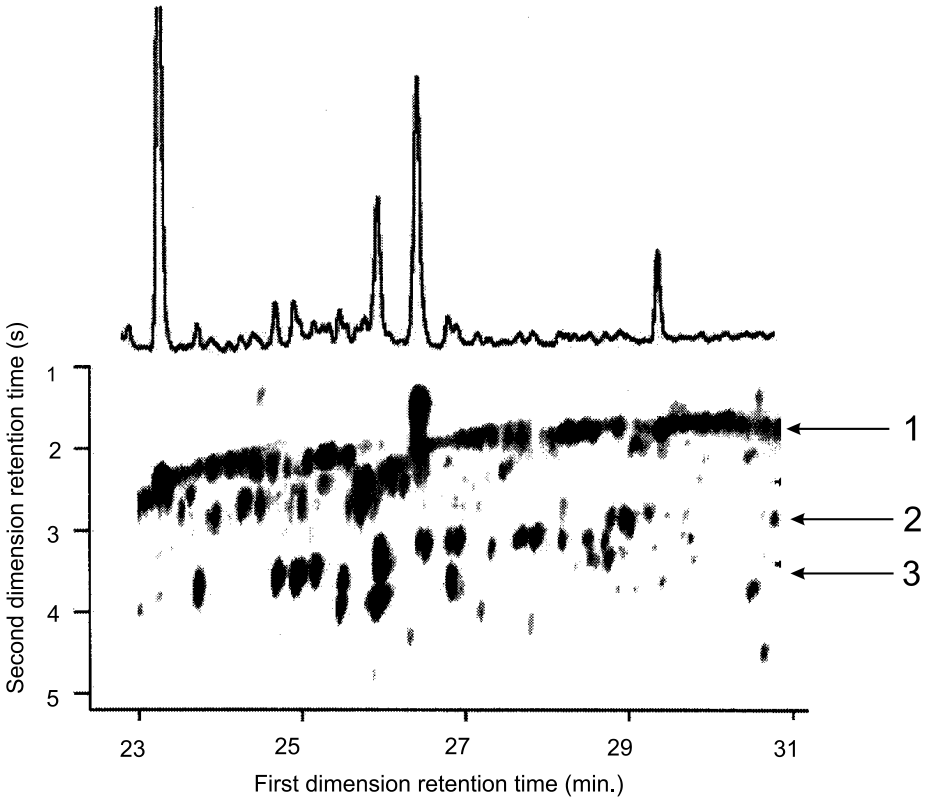


Figure 4.3. Section of 1D-GC (upper trace) and GC×GC (lower plot) separation of an urban air sample. In the GC×GC plot three structures are clearly present: 1. aliphatics, 2. carbonyls, 3. aromatics.

4.1.1. Phase-ratio focusing

As from the first existing modulator, the dual-stage heated modulator of Phillips *et al*. [15], the

focusing effect was obtained by phase-ratio focusing of the compounds in a separate modulation capillary containing a thick film of stationary phase. The retention factor k can be calculated from the partitioning coefficient K and the phase ratio β according to:

$$k = \frac{K}{\beta} \tag{5.1}$$

The phase ratio β is defined as $$\beta = \frac{r}{2d_f} \tag{5.2}$$

in which r is the radius of the column and d_f is the film thickness. In the first dimension in general the film thickness is 0.25 μm, giving ${}^{1}K = 250$, and in the modulation capillary the film thickness is 3 μm, giving ${}^{m}K = 8$, thus giving for the retention factor ratio

$$\frac{{}^{1}K}{{}^{m}K} = 30 \tag{5.3}$$

This will retain the analytes thirtyfold, which is sufficient to accumulate a next modulation fraction.

By applying heat, these accumulated and focused fractions are remobilized and injected as narrow pulses into the second dimension. This modulation process is further explained in Fig. 4.4.

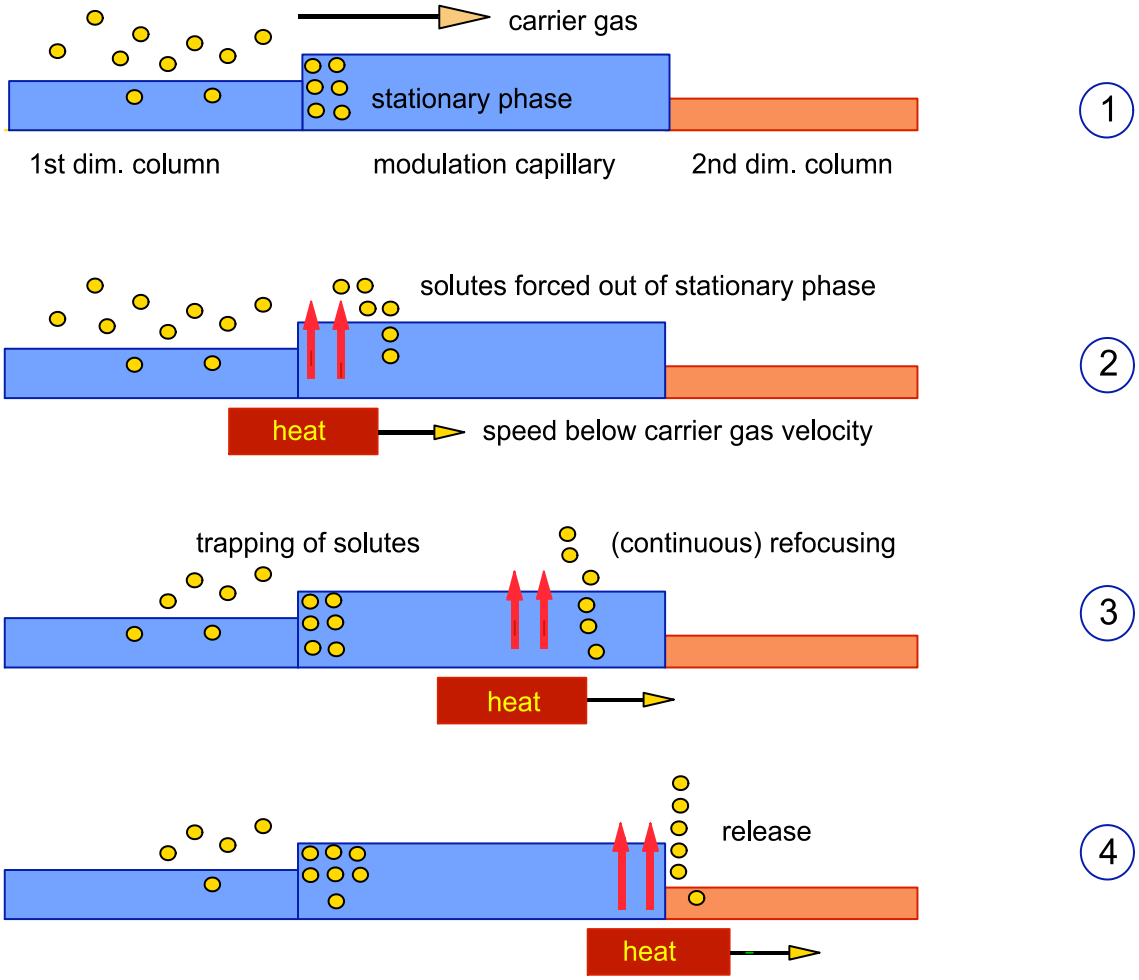


Figure 4.4. The modulation process of a heated modulator.

***Step1**. The compounds in the effluent of the first dimension are retained in the thick film of stationary phase of the modulation capillary by phase-ratio focusing.*

***Step 2**. By applying heat, the analytes are remobilized.*

***Step 3**. When they reach the end of the modulation capillary, the beginning of this capillary has cooled down to oven temperature again, where the subsequently eluting analytes from the first dimension are retained.*

***Step 4**. The focused fraction has been transferred across the junction between the first and the second dimension and thus deposited onto the second column to be separated.*

The remobilization of the focused fraction in the original Phillips dual-stage heated modulator (schematically shown in Fig. 4.5) was performed by resistive heating, by means of the application of an alternating pulsed current through the outside metal-painted coating of two pieces of the modulation capillary. Focused fractions with a bandwidth of 16 – 20 ms were produced [33].

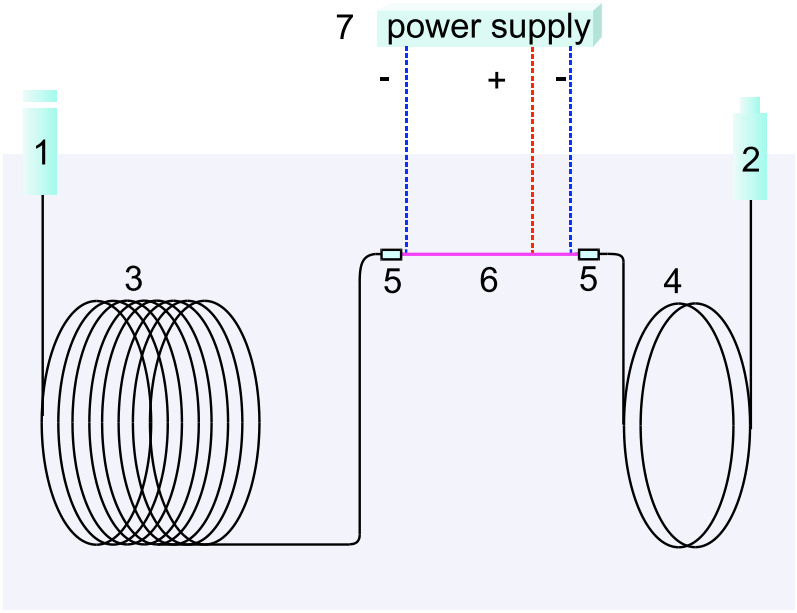


Figure 4.5. Schematic diagram of a GC×GC with a dual-stage heated modulator.

1.injector, 2. detector, 3. first dimension column, 4. second dimension column, 5. column connection, 6. metal-painted modulation capillary, 7. pulsed current input for ohmical heating.

A more or less similar modulation system has been developed by de Geus *et al*. [37] by depositing a 200-400 nm layer of aluminium or silver onto the modulation capillary, or by winding copper wire around the modulation capillary. All of these modulators share a major drawback: they were not very robust since the very thin metal coating frequently burnt out.

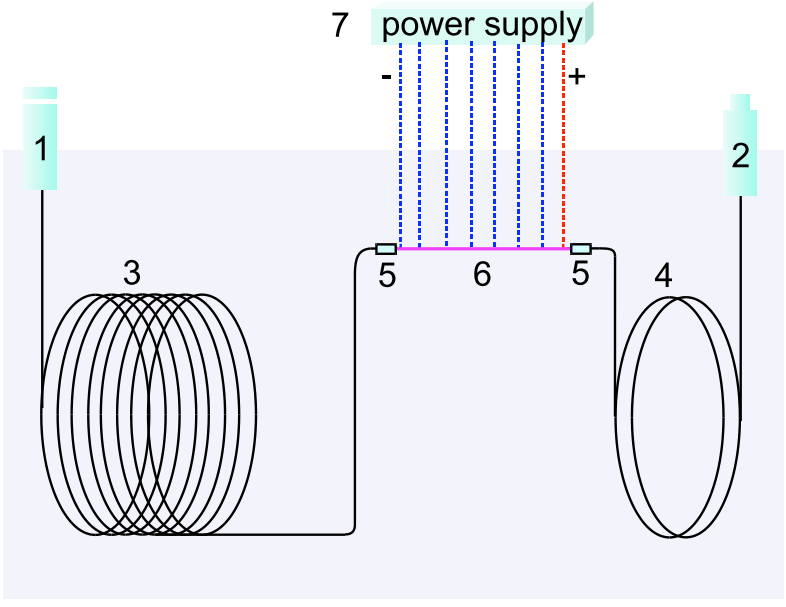


Figure 4.6. Schematic diagram of a GC×GC with a multi-stage heated modulator.

1.injector, 2. detector, 3. first dimension column, 4. second dimension column, 5. column connection, 6. metal capillary-enclosed modulation capillary, 7. electrical input for resistive heating.

A multi-stage heated modulator, heated by means of the application of a current through the enclosing steel capillary around the modulation capillary has been described by Burger *et al.* [37] (schematically shown in Fig. 4.6). Since the heat capacity of the metal enclosing capillary is much higher than that of the metal painting of the original Philllips design, the cooldown time is extended substantially and the slope of the thermal gradient across the length of the capillary is very low. Therefore, the focusing effect is far lower and the narrowest peaks were only obtained when using seven or more subsequent stages. Nevertheless, the obtained width of focused fractions in this modulator appeared to be 145 ms at half height [37].

A more robust, but moving system was then developed as an alternative, the so-called Sweeper [38] (schematically shown in Fig. 4.7 and 5.8), which was also the first commercially available modulator for GC×GC. A slotted heater rotates and moves very close around the thick-film modulation capillary for remobilization of the retained analytes and sweep them across the junction between the two dimensions. The temperature of the slotted heater has to be kept about 100°C above the modulation (and thus oven) temperature in order to remobilize the retained fractions. This limits the upper end of the application range, being 100°C below the maximum allowable temperature of the modulation capillary. This modulator focuses the fractions into pulses with a bandwidth of about 60 ms [46].

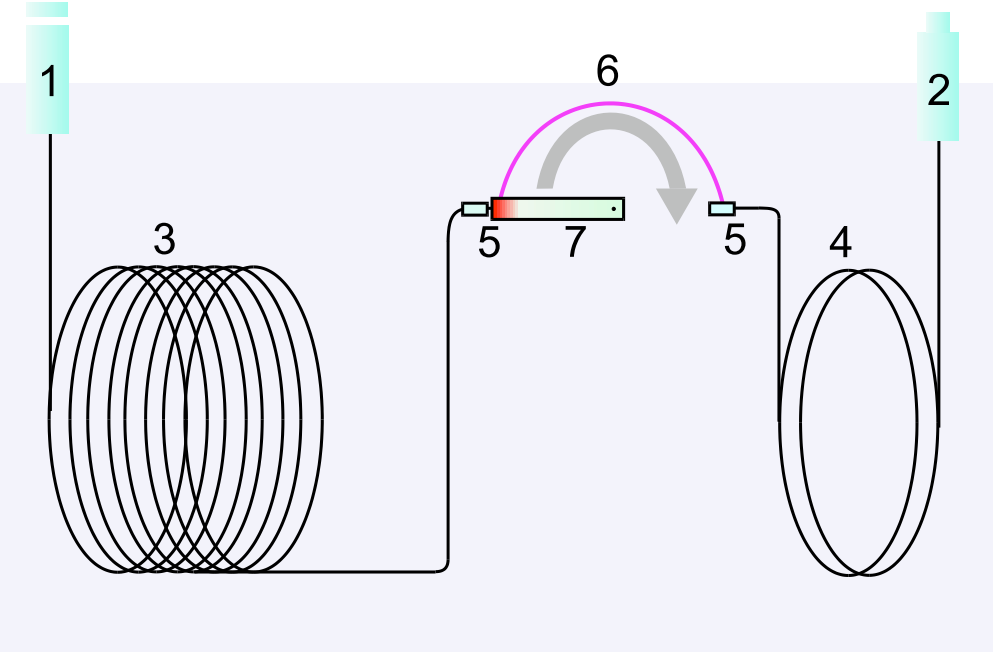


Figure 4.7. Schematic diagram of a GC×GC with a Sweeper modulator.

1. *injector, 2. detector, 3. first dimension column, 4. second dimension column, 5. column connection, 6. modulation capillary, 7. slotted heater, moving from the first to and over the second column junction.*

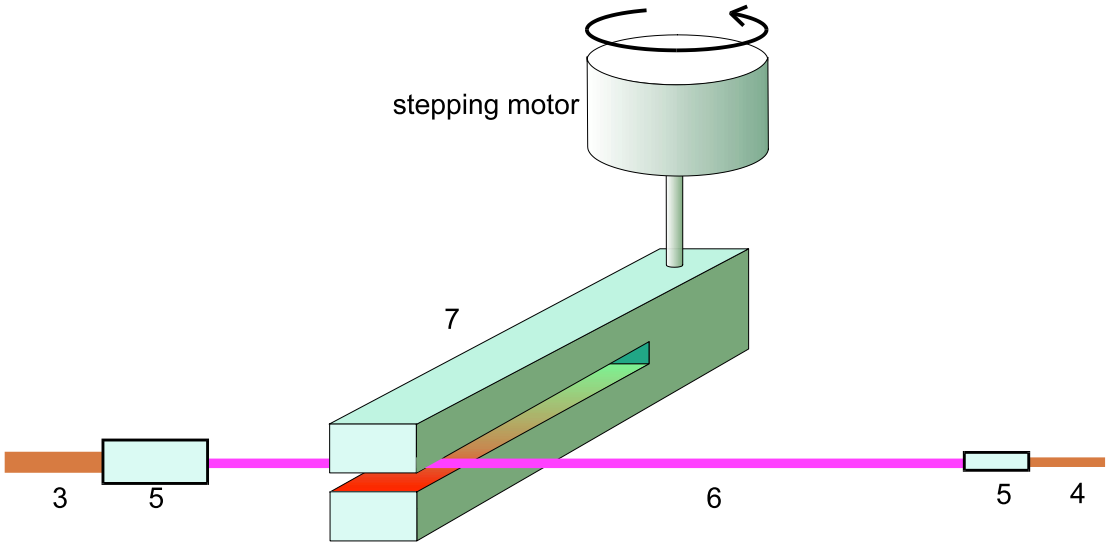


Figure 4.8A. Details of the movement of the slotted heater across the modulation capillary. 3. First dimension column, 4. second dimension column, 5. column connectors, 6. modulation capillary, 7. slotted heater. The right column connector (#5, right hand side) being very small in diameter, allowing the slotted heater to pass over it, for delivering the narrow pulse onto the second column.

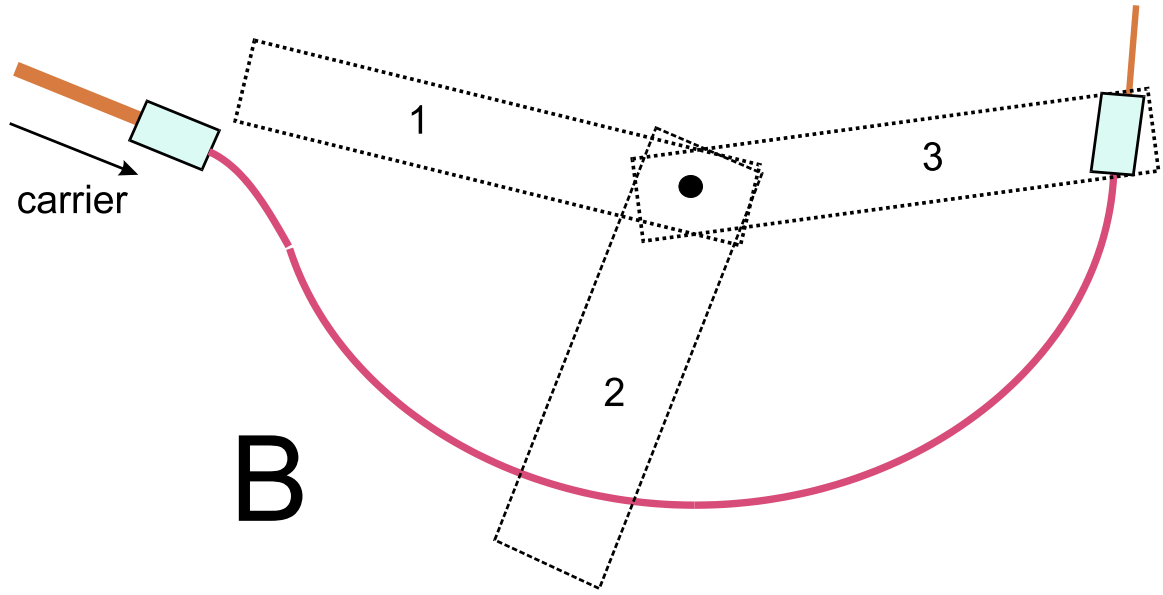


Figure 4.8B. Schematics of the movement of the slotted heater across the modulation capillary.

1. Rest for accumulation of the analytes, 2. movement for focusing and transfer, 3. rest to deliver across the junction onto the second column.

4.1.2 Cryogenic focusing.

Although the idea of using cryogenic means for trapping and focusing fractions in GC is not very new, it was Marriott *et al.* [40] who recognised that this technique could be efficiently used in the modulation process in GC×GC. Instead of retaining and focusing analytes eluting from the first-dimension column by phase-ratio focusing in a thick-film-coated capillary, it can also be accomplished by cooling a short length of the thin-film secondary column. Using the throttling effect of expanding liquid carbon dioxide, a few millimetres of the head of the secondary column is cooled. Analytes are trapped and focused in this short section very efficiently in very narrow bands. In general the temperature difference of the cooled section compared to the rest of the column should be about 100ºC to generate this focusing effect. Following the rule of thumb that a temperature decrease of about 16ºC will double the retention time of an analyte, a difference of 100ºC will increase the retention of those compounds that remain in the gaseous phase by a factor of about 60. This focusing effect is even more efficient than the phase-ratio focusing, as can also be concluded from the bandwidths of the pulses thus produced being substantially narrower. After the cooling is stopped and the cooled section is heated up again to oven temperatures very rapidly, the fraction is remobilized as a narrow chemical pulse and will continue to travel through the remaining part of the second column for further separation. This cryogenic modulation process is depicted in Fig. 4.9:

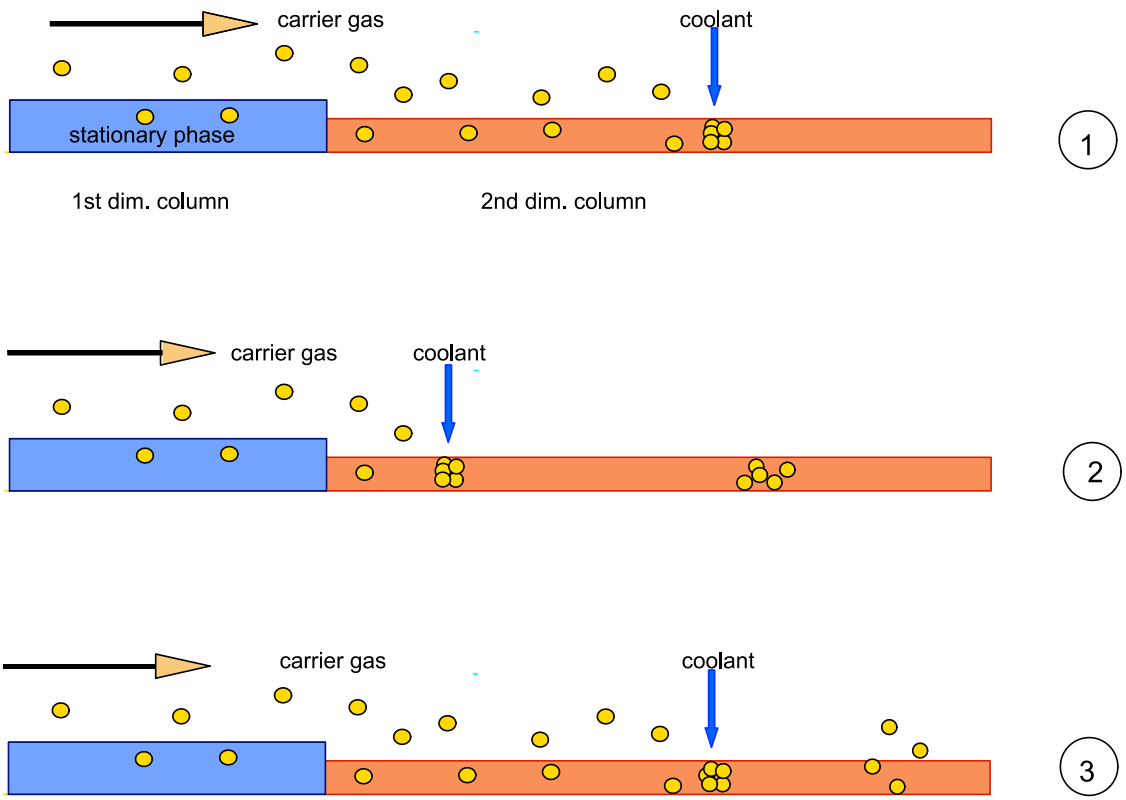


Figure 4.9. The modulation process of a cryogenic modulator.

Step1. *The compounds in the effluent of the first dimension are trapped and focused in the cooled section of the head of the second column.*

Step 2*. By stopping the cooling, this fraction will be remobilized and injected as a narrow pulse. Meanwhile the (continuous) eluting material from the first column is temporarily stopped to interfere with the injected narrow band.*

Step 3*. Repetition of step 1.*

The first cryogenic modulator was demonstrated by Marriott *et al*. in their Longitudinal Modulating Cryogenic System (LMCS, schematically shown in Figure 4.10) [40]. The cooling is performed by the Joule–Thompson effect of expanding liquid CO_2 into the gaseous state. This does not cool the second column directly, but rather indirectly via a sleeve that moves up and down a few centimetres around the vertical mounted and stretched second column. In order to prevent the formation of ice from water of the surrounding air and freezing of the fragile secondary column to the moving sleeve, a stream of dry nitrogen is passed through the inner pipe. The heating of the cooled section and remobilization of the focused fraction is performed by moving the LMCS from the T-position to the R-position. The surrounding oven air is able to very rapidly heat up the short cooled section of the second

column to the prevailing temperature such that immediate remobilization of the focused fraction is accomplished. Generally, the LMCS produces secondary pulses with bandwidths of 20 – 50 ms [46].

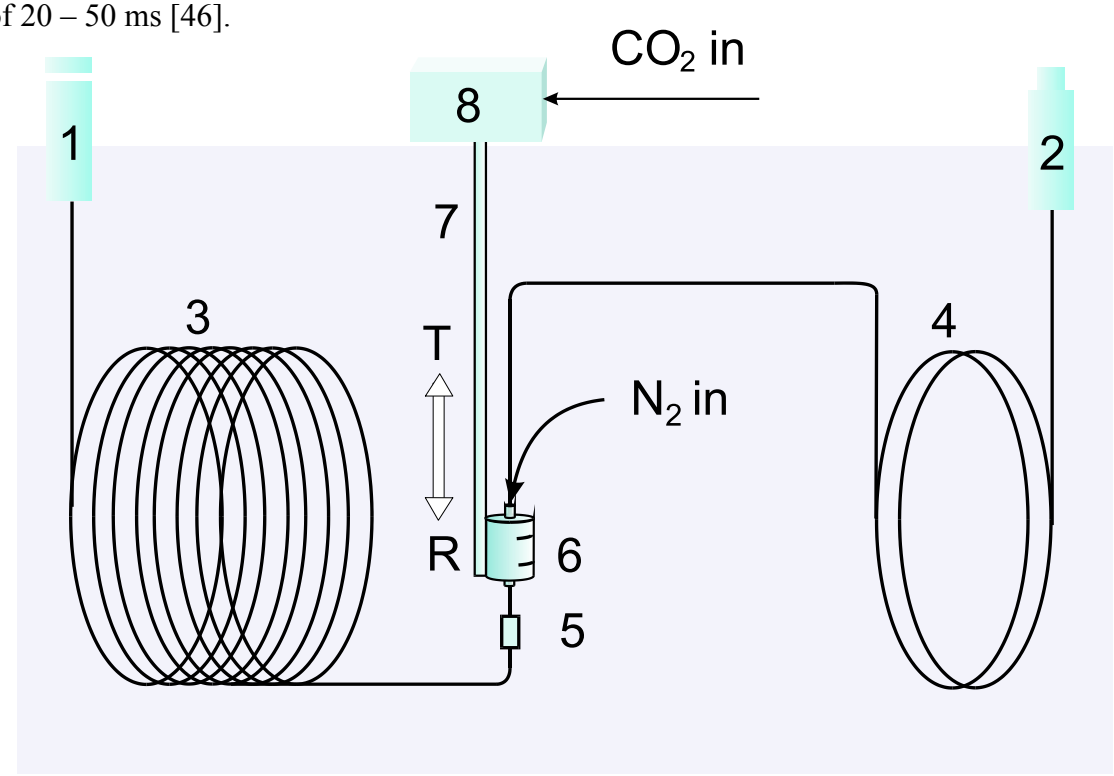


Figure 4.10. Schematic diagram of a GC×GC with a LMCS modulator.

1. injector, 2. detector, 3. first dimension column, 4. second dimension column, 5. column connection, 6. cooling chamber (in the remobilization position = R) 7. (in the trap position = T), 8. up en down movement control of the LMCS

As is shown in the inset in Fig. 4.10 the sheath gas nitrogen, which is used for preventing ice-formation inside the inner pipe, leaves the system through the bottom. The expanded (cold, gaseous) CO_2 leaves the cooling chamber through two slits. The prevailing direction of the oven air may direct this (very cold) gas such that it may influence the temperature of a part of (one of) the two columns. In this respect the following comments have been made in Ref. [42]: "*At very low modulator temperatures (more than 185°C difference), the retention times in the first as well as the second dimension increased, the first-dimension peak became deformed and its width increased. Cooling of an extra piece of the second-dimension column most likely causes this by the large amount of CO_2 leaving the modulator. A temperature difference of 100°C was used for all further experiments. No peak deformation or retention time shifts were observed for the tested pesticides*."

A modification of this LMCS, spraying the CO_2 directly onto the capillary inside the cooling chamber was reported by Beens *et al*. [40] (schematically shown in Figure 4.11). The exit of the CO_2 was situated at the upper right side of the chamber and accordingly the escaping flow of gas was divided into three streams: through the top, through the bottom and via the side exit of the chamber. No peak deformation or retention time shifts were observed on this type of modulator. Because of the rather high linear velocity of the CO_2 exit flow, water from the surrounding oven air was prevented entering the cooling chamber and no extra flow of sheath gas was needed for preventing the formation of ice and freezing of the column

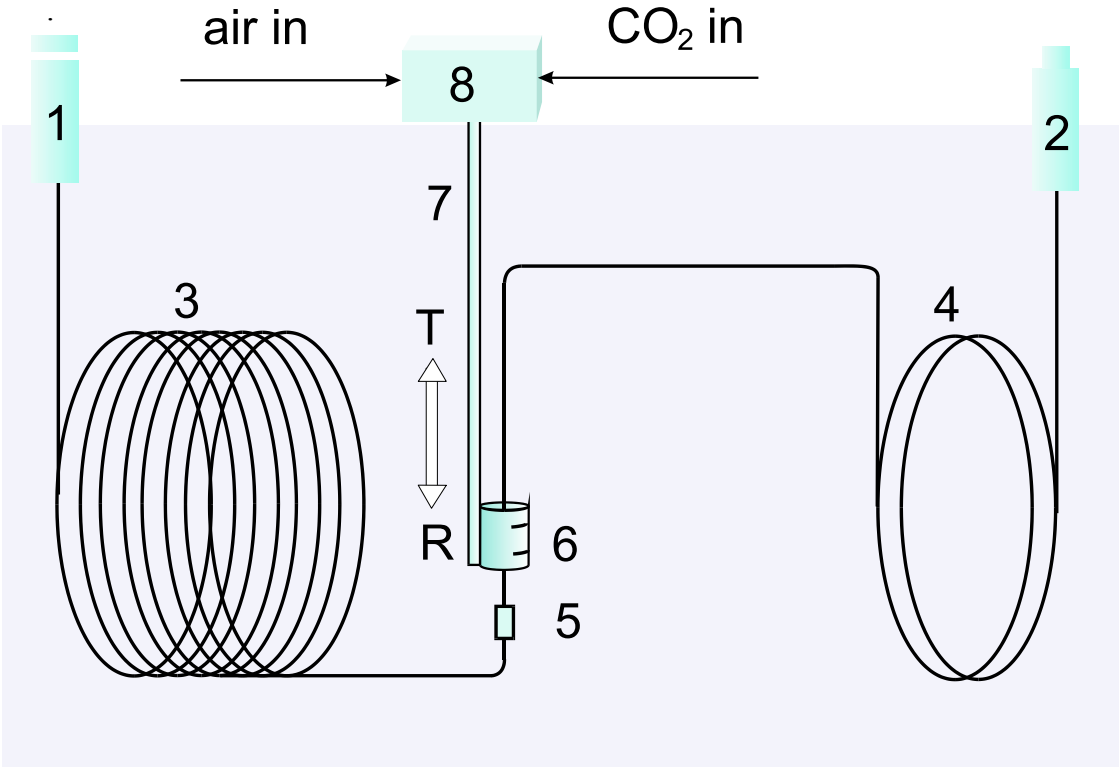


Figure 4.11. Schematic diagram of a GC×GC with moving cryogenic modulator.
1. injector, 2. detector, 3. first dimension column, 4. second dimension column, 5. column connection, 6. cooling chamber (in the remobilization position = R) 7. (in the trap position = T), 8. up en down movement control of the cooling chamber

However, all of these moving modulators share one major disadvantage: they move uncomfortably close in the vicinity of the thin and fragile secondary column. Proper installation is therefore complex and very critical. In view of the numerous number of motions the modulators make along the tiny capillary at changing oven temperatures and the differences in thermal expansion of fused silica and steel, column abrasion and breakage will quite often occur.

A non-moving, four-jet system has been described by Ledford *et al.* [41] (schematically shown in Figure 4.12). Gaseous nitrogen is cooled to very low temperatures through a heat exchanger immersed in liquid nitrogen and blown through two nozzles onto two short sections of the head of the second column. Alternately a hot pulse of nitrogen or air is blown onto one of these sections by one of the two jets perpendicular to the cold jets, for remobilization of the trapped fraction (see insert in Figure 22). Since all of these jets are placed close together inside a small chamber, the jets of cold and hot gas may influence each other. Therefore the duration of the heating and cooling periods of the jets need proper optimisation.

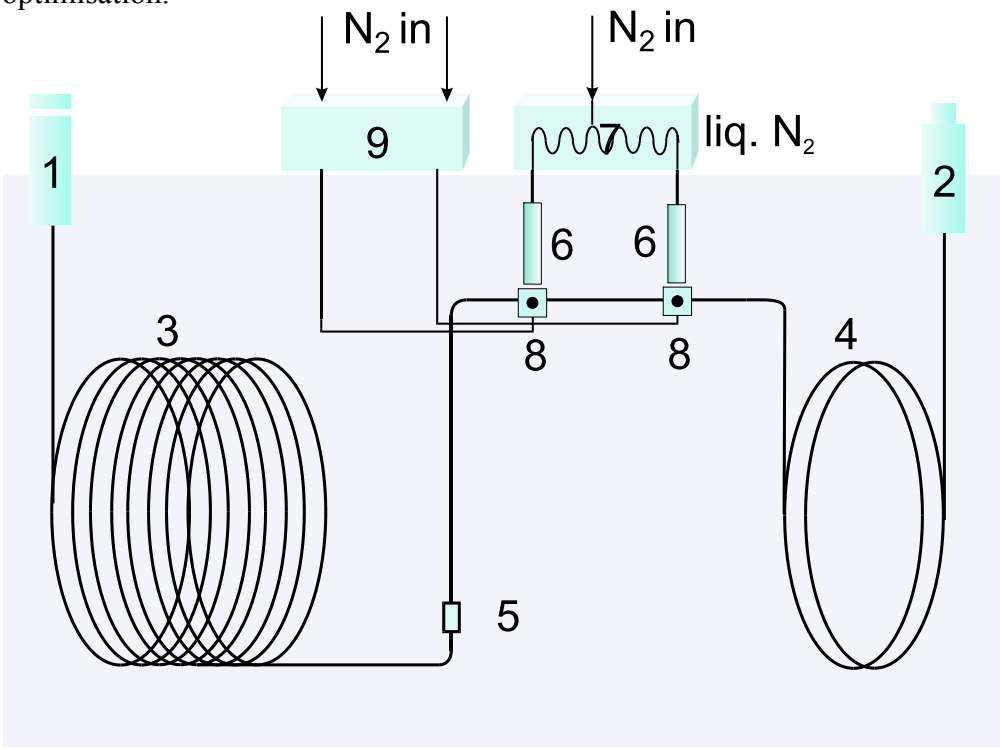


Figure 4.12. Schematic diagram of a GC×GC with a four-jet modulator.
1.injector, 2. detector, 3. first dimension column, 4. second dimension column, 5. column connection, 6. (continuous) cooling jets, 7. insulated storage vessel with liquid nitrogen and heat exchanger, 8. (modulating) hot gas jets perpendicular to the cooling jets, 9. heating device

If the temperature of the cooling gas is too low or the time during which it is applied is too long, the analytes will not be properly remobilized (see also Chapter 10.4 *Peak distortion caused by the modulator).* On the other hand, a too high temperature and/or too long hot gas pulse duration will result in break-through of (a part of) the trapped fraction or thermal degradation and bleed of the column's stationary phase.

The focusing effect of this modulator is such effective (cooling temperature of the cold jet down to about −180°C) that it even enables the modulation of compounds with boiling points as low as pentane. The produced pulses have bandwidths of < 10 ms.

Two jets, two valves. A simpler system has been described by Beens *et al*. [43, 45] (schematically shown in Figure 4.13). Two jets, controlled by two valves directly and alternately spray liquid CO_2 onto two short sections of the head of the second column (see also the photograph in fig. 4.14). The expansion of the CO_2 cools these sections very efficiently with a steep temperature gradient along the length of the capillary. Analogous to the LMCS approach of Marriott, the surrounding oven air heats up these cooled sections after the cooling is stopped, in order to remobilize the trapped fractions. Marriott *et al*. also calculated the time required for heating up such a short section of a 100 µm OD fused silica column and found that theoretically this could be done within 15 ms. This short time duration for heating up the section of the column for remobilization of the trapped fraction is not really needed. Compounds contained in the fraction descend from the same first dimension peak (fraction) and since the separation in the first column is a 'boiling point separation' all of these compounds will have the same vapour pressure at the prevailing temperature. Consequently, as soon as they are remobilized, they will be remobilized simultaneously. Comparable to the four-jets system of Ledford, this modulator produces pulses for re-injection into the second column with band widths smaller than 10 ms. Since the temperature of the cooled sections of the column are kept about 100 ºC below the oven temperature, compounds having boiling points below that of n-heptane (boiling point = 98ºC) will not be properly modulated, and will breakthrough partly unless a thicker film of stationary phase is used in the second column.

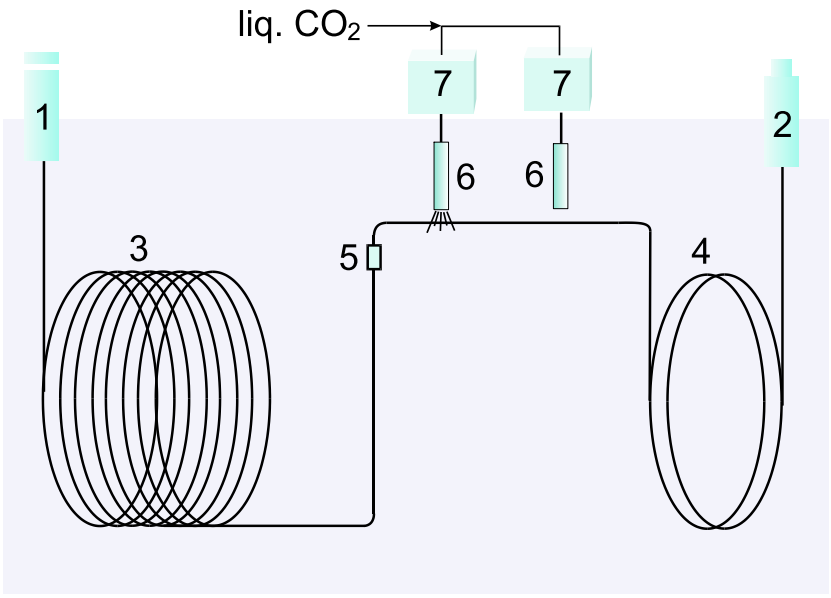


Figure 4.13. Schematic diagram of a GC×GC with a dual-jet modulator.

1. injector, 2. detector, 3. first dimension column, 4. second dimension column, 5. column connection, 6. cooling jets 7. two-way valves for opening and closing the CO_2 flow.

Figure 4.14. Photograph of a GC×GC system with a cryogenic dual-jet modulator.

The two brass cryogenic spraying nozzles are depicted within the yellow ellipse, the secondary column is located just a few millimetres below the nozzles.

Single jet, single stage. An even more simple system is described by Adahchour *et al*. [46] (schematically shown in Fig. 4.15), only having a single jet. This modulator is very comparable to the previous described one, except that it only has one jet and modulates in a single stage. The jet sprays its CO_2 almost continuously onto a short section of the head of the second column, except for a very short period of time during which the trapped and accumulated fraction is heated up and remobilized. Considering the fact that it takes between 15 and 20 ms, depending on the wall thickness of the second column, for heating up the short section and its contents, this time is just long enough for remobilization of the focused fraction. Since the average linear carrier gas velocity in the second column generally is above 1 $m.sec^{-1}$, it only takes a few milliseconds to transfer the remobilized fraction further into the column and away from the cooling zone. Meanwhile, *i.e.* during all the other time, it cools, traps and accumulates the analytes from the first column. The single-stage modulation process of the system is depicted in Fig. 4.16. Bandwidths of the focused fractions also are very comparable with that of the dual-jet system, being <10ms.

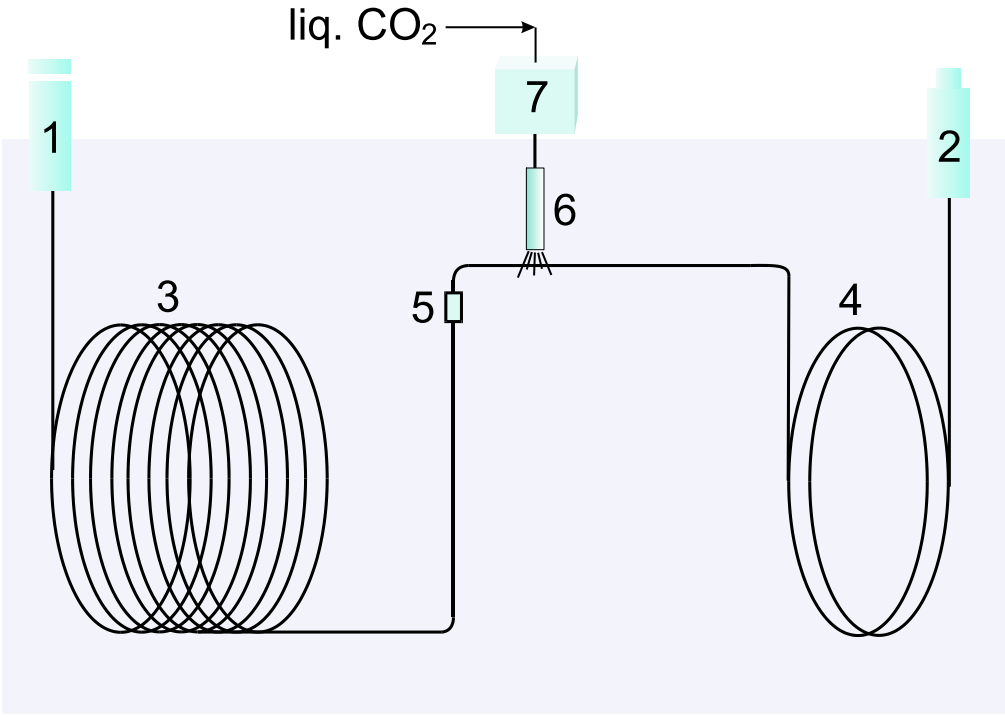


Figure 4.15. Schematic diagram of a GC×GC with a single-jet, single-stage modulator.

1. *injector, 2. detector, 3. first dimension column, 4. second dimension column, 5. column connection, 6. cooling jet, 7. two-way valve for opening and closing the CO_2 flow.*

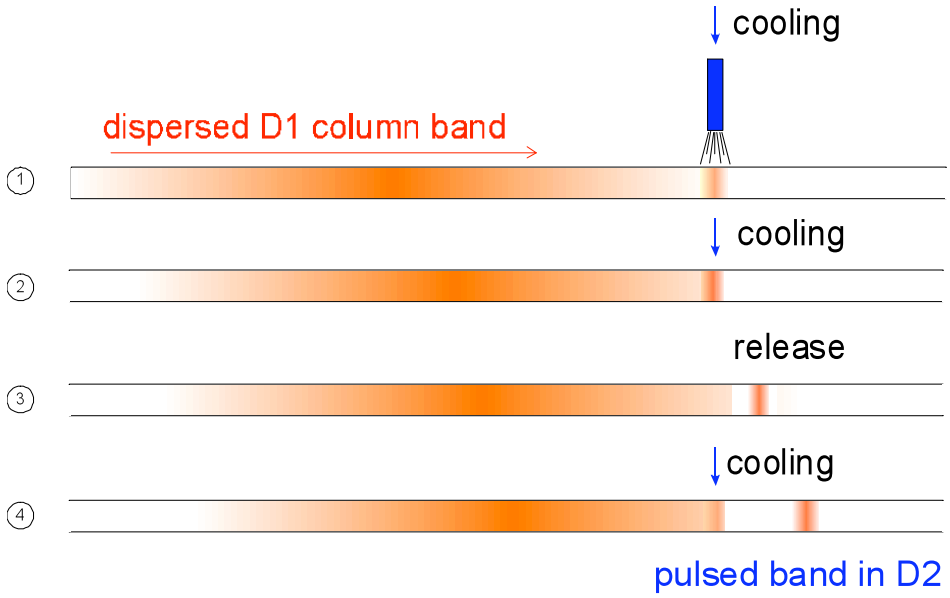


Figure 4.16. The single-stage modulation process of a single cryogenic jet modulator.

Step 1, 2 *Eluate from the first column is accumulated and focused in a narrow band in the cold spot,*

Step 3. *As the cooling stops for a short moment, the narrow pulse is released and launched,*

Step 4. *Cooling is re-established after this short moment so that breakthrough of the remainder of the D1 dispersed band is prevented.*

A rotating cryogenic modulator, using CO_2 as the coolant and not touching the second column has been reported by Hyötyläinen et al. [47]. Two nozzles continuously spray liquid CO_2 onto one out of two short sections of the second dimension column. The nozzles are mounted on a vertical bar that can be revolved over an angle of 45º. It thus cools one of two specific parts of the vertical mounted second column alternately. After the nozzle has been turned away from the first spot, this column section is fast heated by means a small heater coil around the capillary for remobilization of the trapped fraction, whilst the other nozzle has started cooling the other section.

A modification of this modulator was presented in [48] (see Fig. 4.17). The rotating vertical bar now carries only one jet, continuously spraying liquid CO_2. It sprays on one of two sections of the horizontally mounted second dimension column by a rotation action over 180º. It is obvious that the amount of CO_2 used in this modulator is about twice as low as in the previous one. The bandwidths of the focused fractions, though, are very comparable.

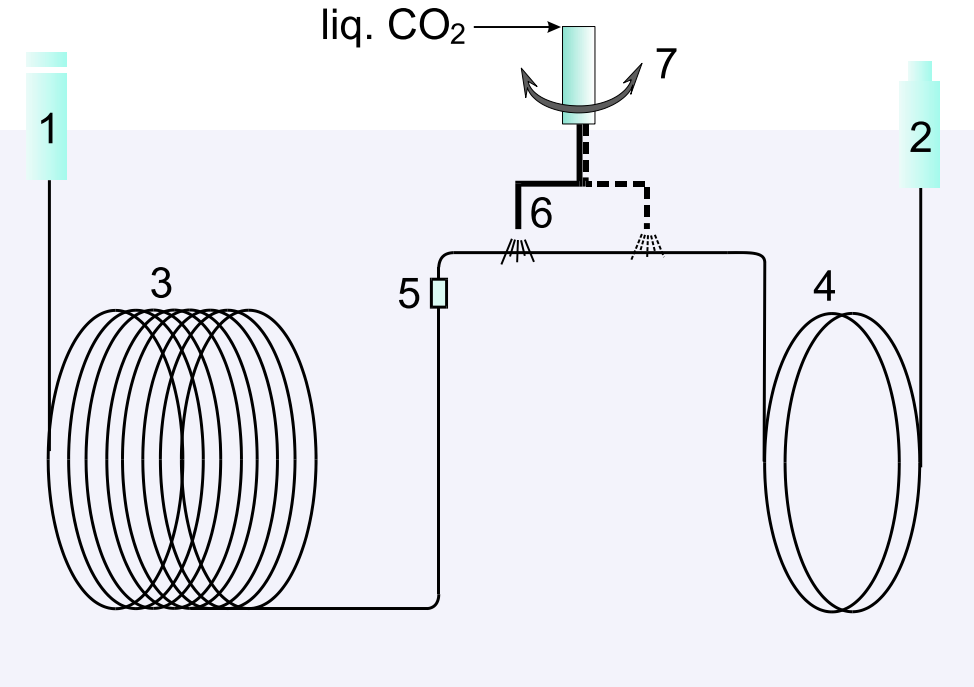


Figure 4.17. Schematic diagram of a GC×GC with a revolving, single-jet, dual-stage modulator.

1. injector, 2. detector, 3. first dimension column, 4. second dimension column, 5. column connection, 6. cooling jet, 7. revolving bar.

A dual-jet, dual-stage cryogenic modulator using liquid nitrogen that is sprayed directly onto the secondary column has been presented by Pursch *et al*. [50]. The spots on the head of the secondary column that hold the trapped fractions by cooling with liquid nitrogen are surrounded by small sleeves in which the nitrogen is sprayed. After the cooling has been terminated, these spots are heated for remobilisation of the trapped fraction by hot air. The bandwidths of the focussed fractions are comparable with the aforementioned cryogenic jet modulators. In view of the fact that liquid nitrogen is used as the coolant, the temperature at which the compounds are trapped is lower than that of liquid CO_2-cooled modulators and it therefore enables the modulation of compounds boiling as low as propane.

A single cryogenic jet, yet two-stage modulator has been presented by Ledford [50]. The schematic diagram is depicted in Fig. 4.17. It incorporates a vacuum-insulated chamber

in which either liquid CO_2 expands or into (with liq. N_2)-cooled nitrogen gas is fed. This chamber ends with a single nozzle continuously spraying a jet of cooled gas onto two overlapping spots of different windings of a loop of a non-coated, deactivated modulation capillary. Each of these spots is alternately heated by deflecting the cold gas jet from the capillary with a hot pulse of air to remobilize the trapped and focused fraction. The heating (or the deflecting) capacity of the air jet is such that it does not work properly at oven temperatures around 300°C or higher, so that high boiling compounds tend to exhibit band broadening. When using CO_2 as the cryogen, only compounds boiling above n-C_{12} (boiling point = 216ºC) are properly modulated [51]. When using (with liq. N_2)-cooled gas as the cryogen, even compounds boiling as low as butane (boiling point = 0°C) can be modulated. Bandwidths of the focused fractions are far narrower than 10 ms.

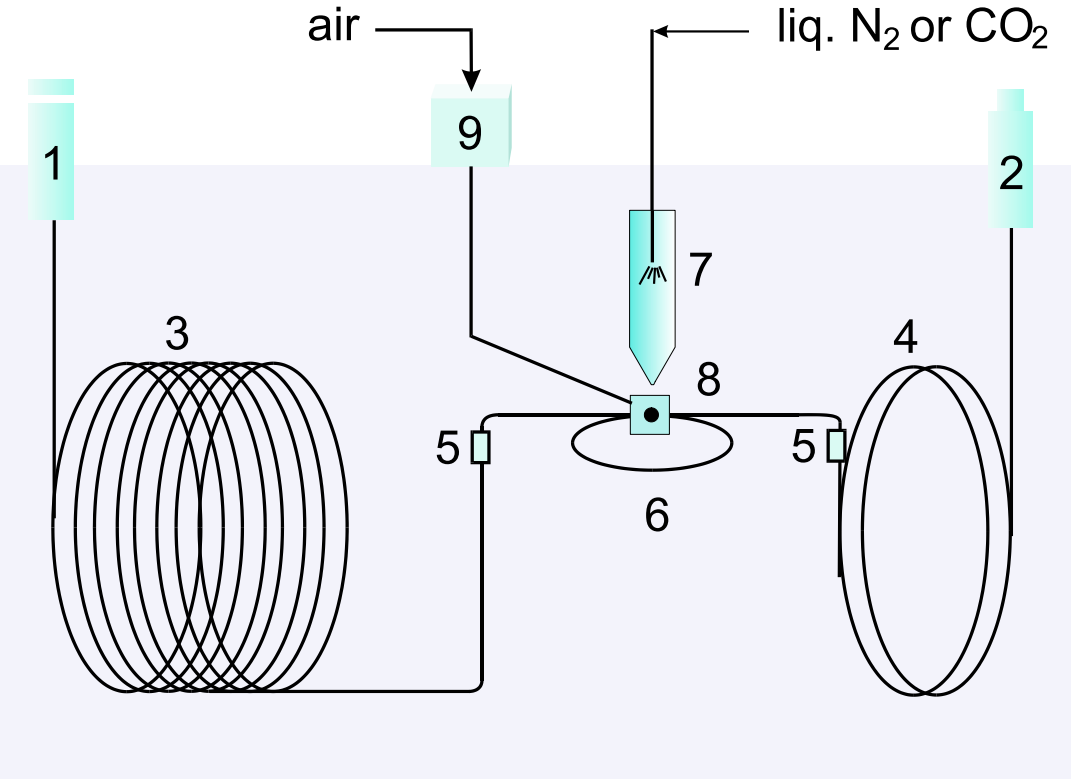


Figure 4.18. Schematic diagram of a GC×GC with a single cryogenic jet, dual-stage modulator.

1. injector, 2. detector, 3. first dimension column, 4. second dimension column, 5. column connections, 6. double loop of the modulation capillary, 7. vacuum-insulated cryogenic chamber with nozzle, 8. (modulating) hot jet, 9. valve

A compilation of the properties of the different modulators is presented in Table 4.1, including their robustness and ease of use. A comparison of the working properties of a number of thermal modulators has been reported in [46 and 63].

A modulator contained in GC×GC system in a 32"×36" box is presented by Libardoni *et al.* [53] and is schematically depicted in Fig. 4.19. The system is portable and self containing for on-site applications. It contains an electrically heated and liquid-cooled single-stage thermal modulator that requires no cryogenic materials. The stainless-steel modulator tube is coated with PDMS, which can be heated to 350°C for sample injection into the second-dimension column. The modulator tube, coated with PDMS, is cooled to –30°C by a 100 mL/min flow of PEG by means of a commercial liquid cooling system and a small recirculating pump. Resistive heating of the modulator tube is provided by a programmable power supply, which uses a voltage program that results in increasing modulator temperature during analysis. The modulator is able to focus and reinject compounds from n-pentane on to n-heptadecane and provides peaks with a peak width at half height of about 40 ms.

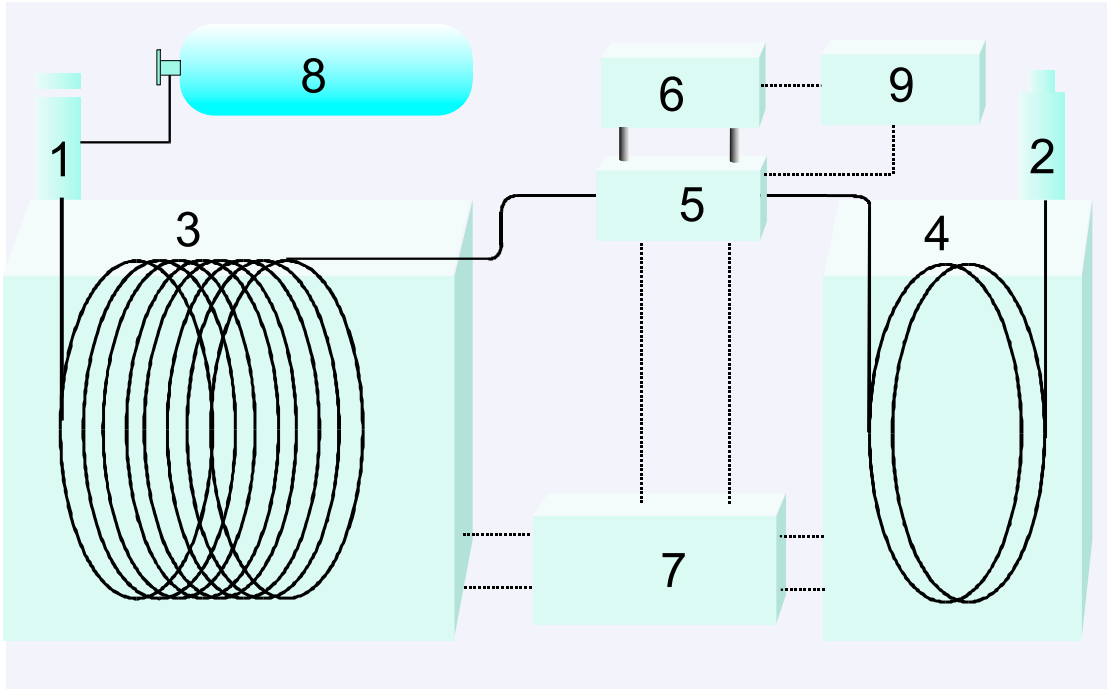


Figure 4.19. Set-up of a portable GC×GC system. 1. injector, 2. FID detector, 3. first column with at-column heating, 4. second column with at-column heating, 5. modulator, 6. cooling unit, 7. power supply, 8. hydrogen supply, 9. electronic controls

4.1.2.1 The flexibility of cryogenic modulators as switching devices.

Cryogenic modulators differ from heated modulators in more respects than only the way they perform the accumulation and focusing effect. Apart from acting as a modulation system for GC×GC, cryogenic modulators can in principle serve more applications in

(multidimensional) GC analyses. Provided its electronic control is sufficiently flexible and can be set in multiple ways, there are three possible functions for a cryogenic modulator:

A. Focusing of injection bands for (ultra) fast analysis
B. Targeted and non-targeted modulation for GC×GC
C. Peak amplitude enhancement in front of the detector

These three different functions are depicted in Fig. 4.20 and will be explained in the following subchapters.

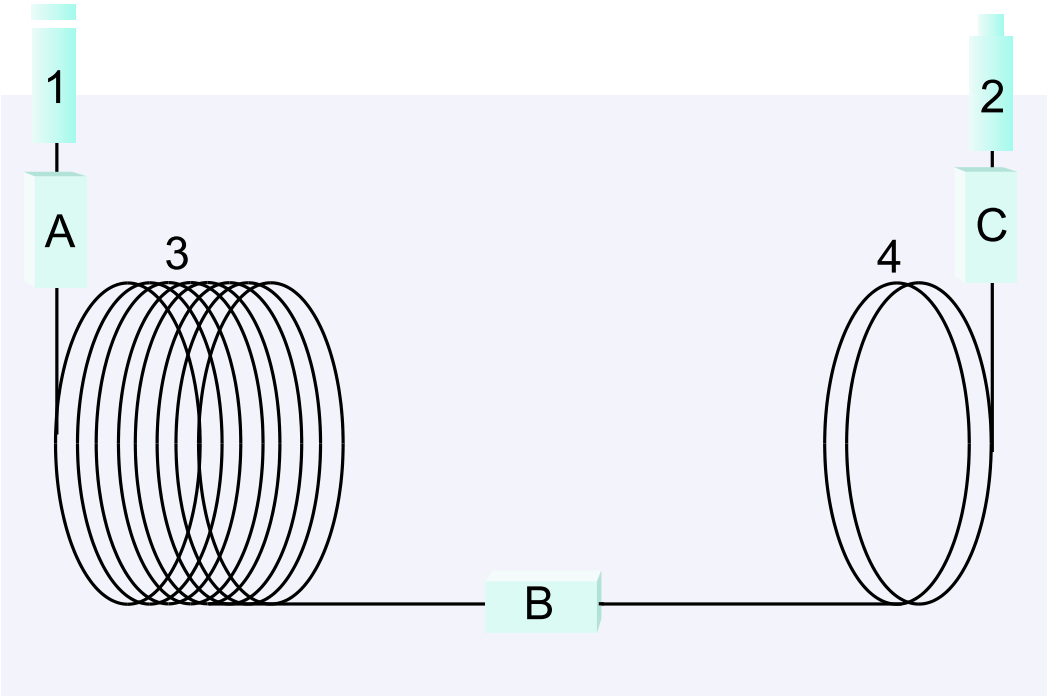


Figure 4.20. Three different functions of a cryogenic modulator on three different positions.

1. injector, 2. detector, 3. first column, 4. second column.

The three different functions of the cryogenic modulator are indicated by their positions:

A. focusing of injection bands for (ultra) fast analysis, B. GC×GC modulation or targeted MDGC, C. peak intensity enhancement

4.1.2.1. A Focusing of injection bands

Already from the beginning of GC, minimal-time operation has been envisaged by researchers in the field and subject of research work. Desty demonstrated that fast separations in GC could be achieved *e.g.* by employing narrow-bore columns [52]. However, the lack of the necessary column technology, the unavailability of detectors with sufficient small rise

time and fast data acquisition systems to acquire the data at the necessary high speed, prevented this fast chromatography to become a feasible technique.

Recently, the interest in fast chromatography has revived, now that both the column technology and the instrumentation have been improved accordingly. Fast GC is being implemented in process control, high-throughput analysis or simply for increasing the speed of routine analysis. However, one important part of the instrumentation has not been adapted for (ultra) fast GC sufficiently. Using standard injection systems the necessary narrow pulses that are needed as injection bands for fast GC in order not to significantly contribute to band broadening can hardly be reached. Here a cryogenic modulator can improve the situation and compress the bands of injected samples considerably. By simply assembling the modulator just behind the injector at the head of the analysis column, trapping and focusing of the total injected sample and releasing it very rapidly for fast analysis, enables that standard injection systems can be used for (ultra) fast GC. Now the modulator does not use the rapid pulsing modulation function, but merely traps and accumulates the complete sample in a single narrow band and subsequently launches it into the column.

4.1.2.1.B GC×GC and targeted MDGC.

When the modulator is assembled in between the two columns and produces rapid pulsed fractions it can act as a GC×GC modulator as is extensively explained in this and previous chapters. This way of using the modulator, *i.e.* trapping and focusing adjacent narrow bands at regular time intervals throughout the entire 1D-chromatogram, is the normal way of employing the modulator. There is, however, a second way in which the modulator can be employed in the same position, which is designated as *targeted MDGC*. This technique has been advocated by Marriott *et al*. [39] using their LMCS, but other cryogenic modulators can also execute it as well. In this mode only a limited number of (target) peaks have to be analysed and are trapped and focused as complete peaks or even as peak bundles. After their release from the trap (by the normal heating process), they are separated as complete, but very narrow peaks in the second column with high speeds and consequently enhanced peak amplitudes. This process is depicted in Fig. 4.21. A second example is shown in Fig. 4.22, in which all the – low concentration – contaminants of a main compound are accumulated in one very narrow fraction, released and separated in the second column with a high peak amplitude enhancement in the detector.

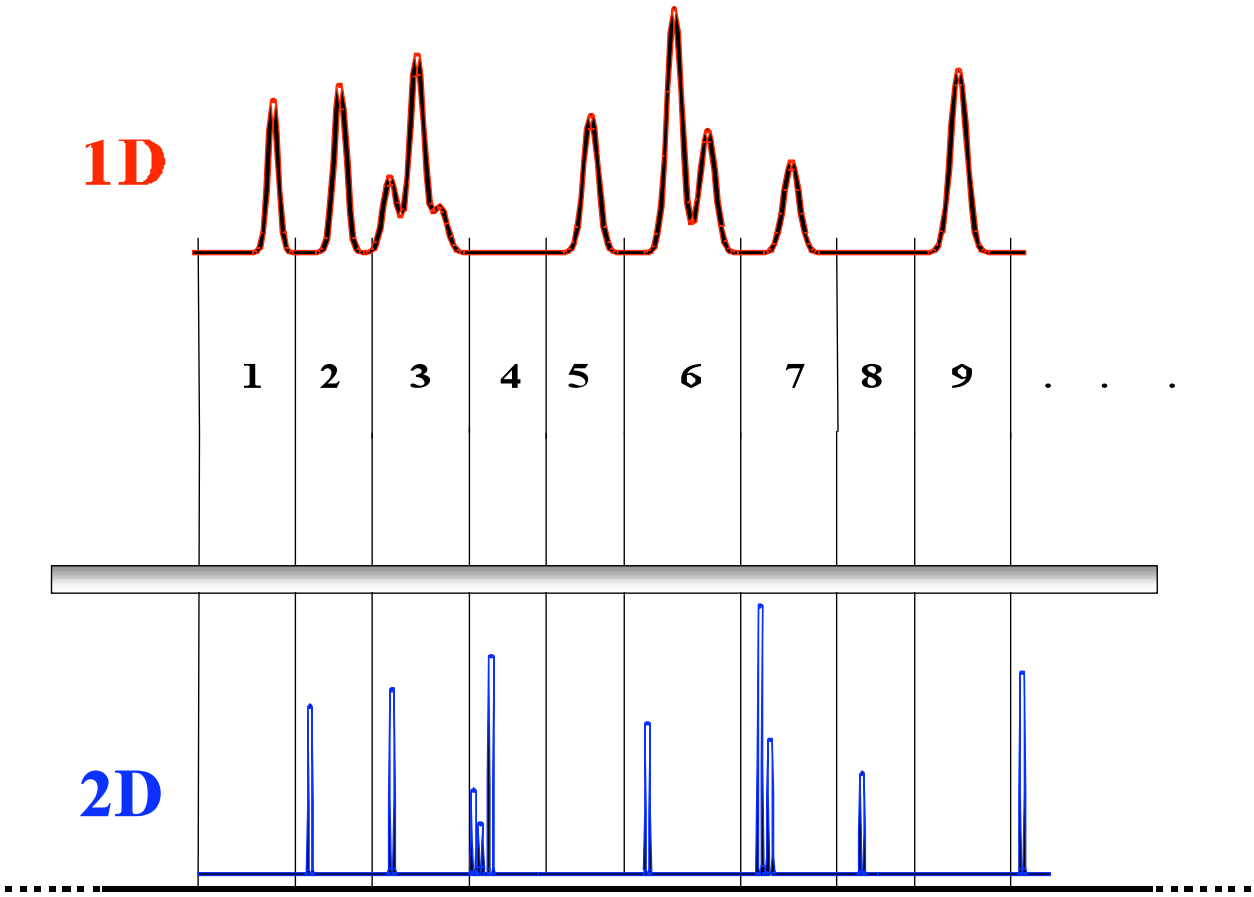


Figure 4.21. Targeted MDGC process with a cryogenic modulator. (Courtesy of Philip Marriott).

As a result of the compression in space of complete peaks, peak amplitude increase in the detector may be between 20 and 100.

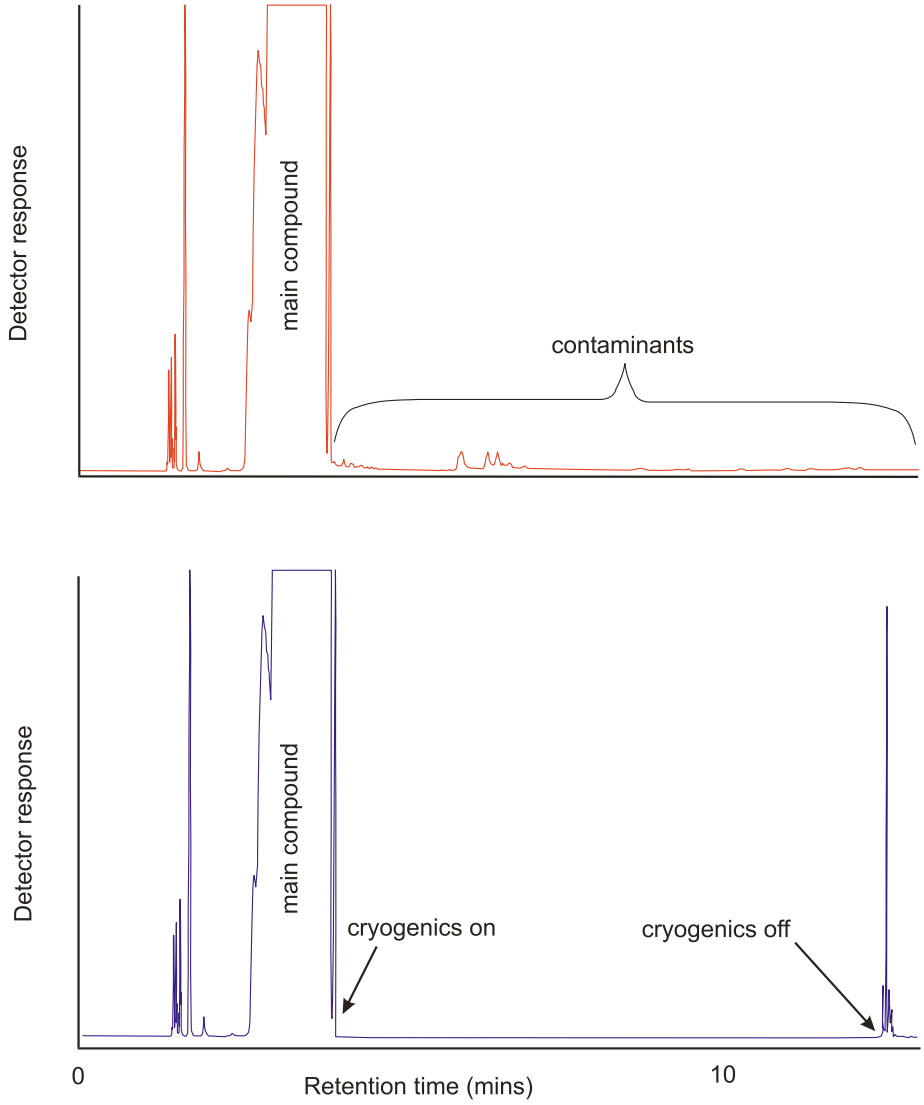


Figure 4.22. Peak sensitivity enhancement by targeted MDGC analysis.

The upper (red) trace is the normal 1D-GC analysis. The lower (blue) trace depicts the increase in peak amplitudes as a result of the accumulation and zone compression of all the contaminants eluting after the main peak.

4.1.2.1.C. Peak amplitude enhancement

When the cryogenic modulator is assembled at the end of the analysis column, just in front of the detector it can be used to enhance peak amplitudes. It should then be programmed such that it traps and compresses all individual peaks one by one and releases them at enhanced peak amplitudes. It is obvious that this process does not add any additional separation and can only be performed on relatively simple samples, containing only a limited number of components. The final sensitivity enhancement as a result of the peak amplitude enhancement is further discussed in Chapter 8.1.

4.2. Valve-based modulators.

As is the case with thermal modulators, there are also different types of valve-based systems. Bruckner *et al*. describe a diaphragm valve-based system that samples only very narrow timed fractions from the effluent of the first dimension [54] (schematically shown in Figure 4.21). Here the "focusing" is effectuated by very rapidly sampling only very narrow fractions (50 ms wide, twice a second) from the first column effluent, so that they do not need further focusing in order to serve as narrow input fractions for the second column. Eluting material from the first column in between two consecutive sampled fractions – being about 90% of the effluent – is vented off. Moreover, since the first column has an internal diameter of 0.53 mm and consequently a relative high carrier flow rate as compared to the 0.18 mm ID second column, a flow splitter is used to decrease the flow of carrier plus analyte after the first column. So, in effect, far less then 10% of the original injected amount of analytes is transferred to the second column. According to the definition of the GC×GC technique, this modulator does not provide a comprehensive separation (see also Fig. 4.23). But it is claimed that the chemometrics described in this paper enable a proper reconstruction of the original first dimension peaks provided that they have been sampled at least five times.

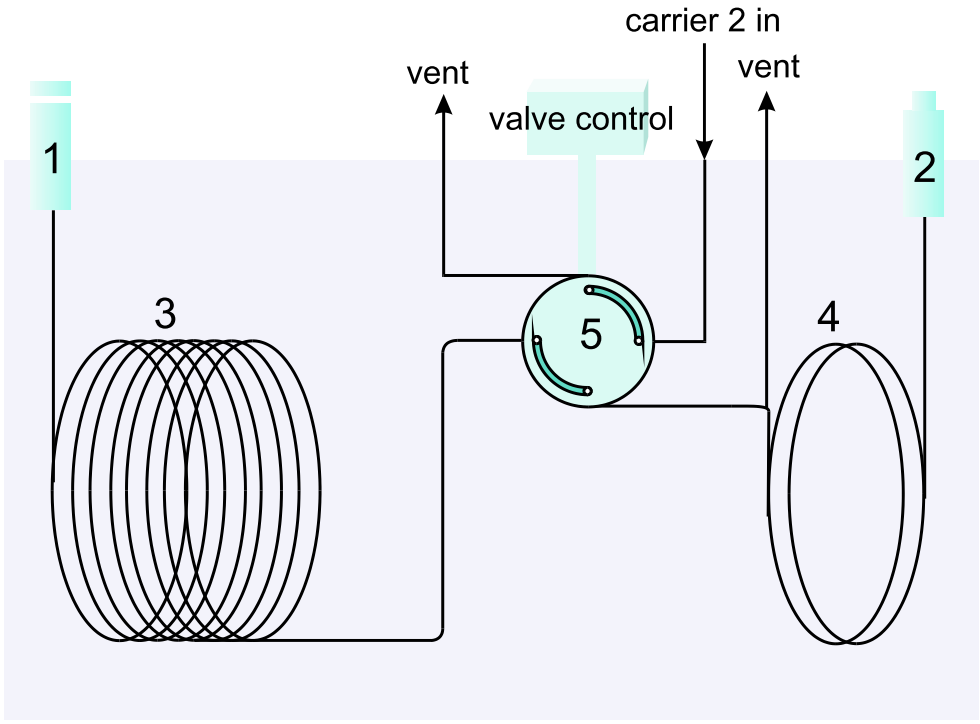


Figure 4.23. Schematic diagram of a GC×GC with diaphragm valve as a modulator.
1. injector, 2. detector, 3. first dimension column, 4. second dimension column, 5. four-port diaphragm valve

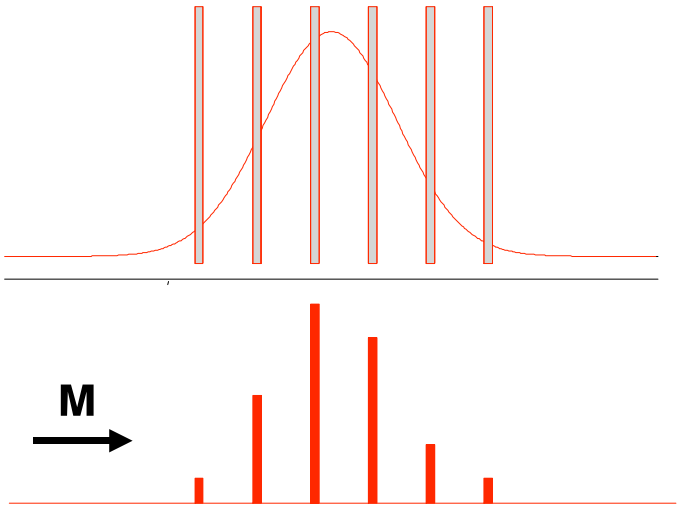


Figure 4.24. Sub-sampling with little conservation of mass. (Courtesy of Philip Marriott)

A different valve-based system has been described by Seeley *et al*. [55] (schematically shown in Fig. 4.25) in their flow diversion system. Here the focusing of the first column effluent fractions is achieved by focusing in time. The contents of the sample loop, being 80% of the time in series with the first column, is focused by a flow of carrier gas to the second column, of which the linear velocity is about 20 times higher than that of the first column. So, the duration of the injected plug onto the second column is about 5% of its original first dimension duration. It thus provides a compression in time of the fractions from the first column. Although this system also does not fully comply with the term "comprehensive" since it only conserves 80% of the original mass, it is almost comprehensive (see also Fig. 4.26).

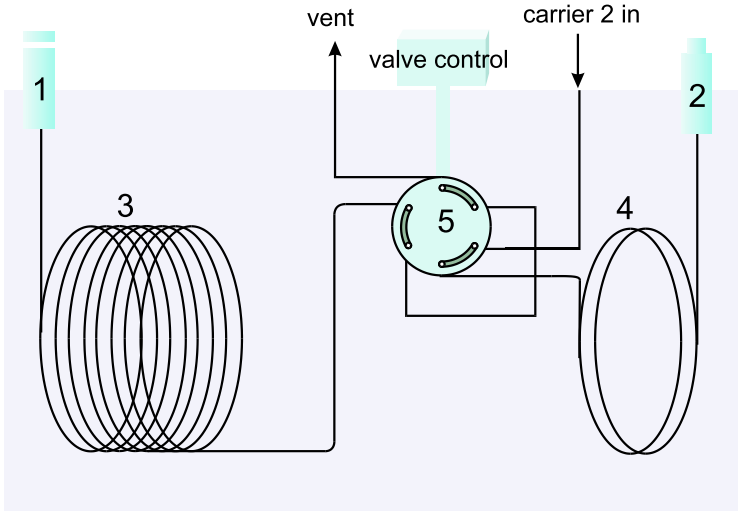
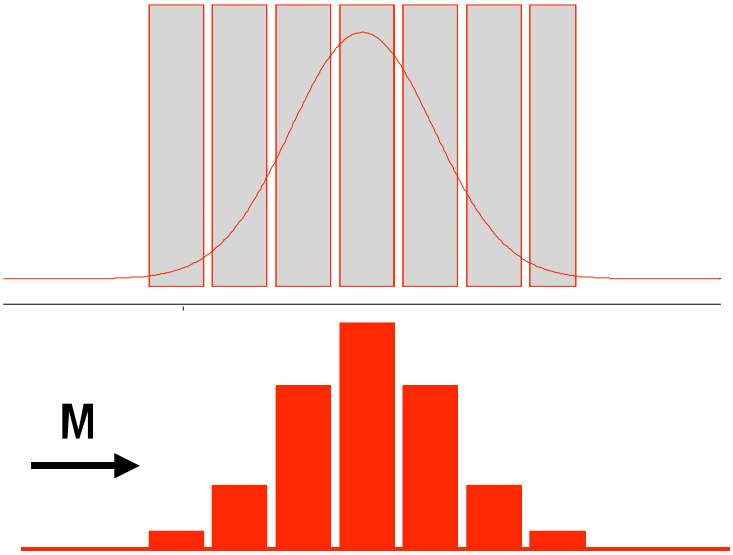


Figure 4.25. Schematic diagram of a GC×GC with flow divertor modulator.

1. *injector, 2. detector, 3. first dimension column, 4. second dimension column, 5. six-port diaphragm valve*

In two next papers [56, 57] the same authors describe a system with the same modulator and two secondary columns in parallel. In this paper they also describe different modulation times: sample loop filling time 0.9 s, modulation frequency 1 s. So here the sample loop is in series with the primary column during 90% of the time. Accordingly, as an average, the amount of transferred material from the first to the second column is 90%. The advantage of utilising a dual secondary column system and two different retention times in the second dimension for every separated component is commented in Chapter 6.



F*igure 4.26. Sub-sampling with a high degree of mass conservation. (Courtesy of Philip Marriott)*

Apart from the more or less non-comprehensiveness of valve-based modulators, they share another general and major disadvantage. Analytes in valve-based systems have to pass through the switching valve itself. Next to temperature limit restrictions – maximum allowable temperatures of the valves are typically <175°C – small amounts of (polar) compounds tend to be adsorbed onto the surface of these valves. As a consequence, valve-based modulators can only be used in the analyses of low boiling, low-polarity compounds.

Meanwhile, the set-up of valve-based modulators has been adapted such that they can be used at higher operating temperatures [59]. Diaphragm valve-based modulators adapted in this way have successfully been used for the analysis of compounds with boiling points up to n-pentadecane (270ºC). And although this is a major step forward in the applicability of valve-based modulators, it can not be considered to be "high-temperature" applications as is stated in the referenced publication. High temperature in GC is restricted to methods and methodologies that can handle compounds with boiling points well above 500ºC. For high-temperature applications of GC×GC, see also in Chapter 13, *Applications*, the example of the separation of steroids.

Since Harynuk and Górecki have demonstrated that stop-flow mode in GC×GC can be applied without a too serious loss in resolution, or a too serious increase in band broadening [60], this stop-flow mode has been employed to produce a valve-based modulator with a total transfer of the effluent from the first to the second column [61]. A similar approach is followed by Mohler et al. [62], by periodically blocking one of the two appropriate ports of the high-speed six-port diaphragm valve that is used as the modulator between the first and the second column. The modulation period and difference in head pressure between the two columns are found to be the two primary variables that are controlled to provide good detection sensitivity and 100% mass transfer from the first to the second column. The detection sensitivity is better with a longer modulation period. A limit of detection of 0.03 ng/L was obtained.

An interesting new approach in the field of valve-based modulators is the flow-switching device developed by Bueno et al.[61]. This device is related to the flow switching system, already developed by Deans in 1968 [19]. By utilising this system, the valve can be located outside the oven and is not in the sample path.

This valve merely serves to direct the various flows in different directions (see Fig. 4.27).

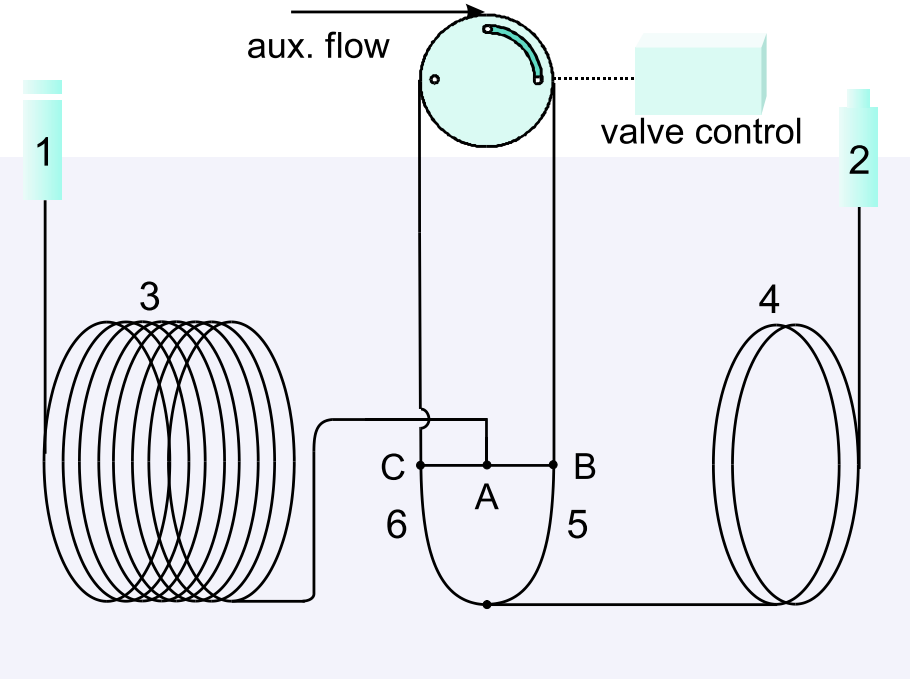


Figure 4.27. Schematic of the Deans flow-switching device used for differential flow modulation GC×GC.

1.injector, 2. detector, 3. first dimension column, 4. second dimension column, 5. fill loop, 6. flush loop

With the valve in the drawn position, the auxiliary flow will build up a pressure in point A such, that the flow from the primary column will flow to the left (flush) loop. After a second and switching the tree-way valve in the other position, the primary column flow will be directed to the right (fill) loop and vice versa. By setting the first- and second-column flow such that a ratio of F_1:F_2 = 1:20, the first-column effluent pulse will be compressed (in time) to form a 20-fold narrower second-column pulse (1.0 s pulse can be injected as a 50 ms pulse) and a differential flow modulator is created. This modulation technique has no inherent temperature restriction and 100% of the column effluent of the first column passes the modulator and is transferred to the second column. In other words, this is a fully comprehensive valve-based modulator. The secondary peaks that are produced with this system have peak widths at base of about 250 ms.

	Ref	Focusing effect			Bandwidth injection pulse (ms)	Application range (B.P. °C)	Sensitivity enhancement	Robust-ness	Simplicity in use	Compre-hensive
		Phase-ratio	Cryo	Valve						
Dual-stage heated	[14]	yes			16 - 20		10 - 20	– – –	+++	yes
Multi-stage heated	[37]	yes			300		1 - 2	+++	++	yes
Sweeper (standard)	[38]	yes			60	125 - 450	5 - 15	– –	–	yes
LMCS	[39]		CO_2		20 - 50	125 -	5 - 15	–	–	yes
Four-jets cryo	[41]		N_2		<10	30 - 500	10 - 40	++	0	yes
Two-jets cryo	[43]		CO_2		<10	125 - 550	10 - 40	+++	++	yes
Single-jet, single-stage cryo	[44]		CO_2		<10	125 - 550	10 - 40	+++	+++	yes
Rotating cryo	[46]		CO_2		?	125 - 550	10 - 40	+	0	yes
single-jet, dual-stage	[47]		CO_2		<10	215 - 550	10 - 40	+++	++	yes
			N_2		<10	0 – 550	10 – 40	+++	++	yes
Diaphragm valve	[50]			yes	50	0 - 300	1	+	+++	no
Differential flow	[51]			yes	50	0 – 300	20	+	+++	almost

Table 4.1. Compilation of the properties of GC×GC-modulators