Advancing GC×GC through integrated sample preparation methods and optimized column formats
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Chapter 5

Comprehensive two-dimensional gas chromatography with a multi-capillary second dimension

Summary

Comprehensive two-dimensional gas chromatography (GC×GC) suffers from the impossibility to operate both dimensions at their optimum carrier gas velocity at the same time due to the different inner diameters of the columns typically employed. The use of multiple parallel capillary columns in the second dimension (GC×multi-GC) is studied as a means to achieve simultaneous optimum-velocity operation. A programme written in Microsoft Excel® was developed to calculate the efficiency of the two dimensions in GC×multi-GC for different numbers of columns in the second dimension. With the aid of this programme the appropriate number of columns was selected. Columns with maximum repeatability were specifically manufactured to grant suitable performance, i.e. to avoid band broadening effect caused by inter-column variations. 1D-GC experiments were carried out on the columns separately and combined in parallel. The performance of the parallel column set was consistent with that of the individual columns, with over 9,100 plates generated (approximately 10,000 plates/m). A GC×multi-GC set-up was successfully installed. Model experiments proved the possibility to operate both dimensions at their optimum linear velocity simultaneously. The suitability of the novel second dimension column format to perform multidimensional separations was also shown for a number of selected applications.

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Chapter 5

5.1. Introduction

Since its introduction back in 1991 [1], comprehensive two-dimensional gas chromatography (GC×GC) has become widely accepted for the analysis of very complex samples. The method offers a remarkable resolving power and unmatched peak capacity. Moreover, the structure of GC×GC chromatograms is a useful tool for class identification and determination of unknowns [2-5].

The column sets generally employed in GC×GC consist of a long normal-bore first dimension (1D) column and a short narrow-bore second dimension (2D) column. Thanks to the use of the short narrow-bore columns in the 2D, very fast second dimension separations can be obtained while providing very high efficiency, e.g. 10,000 plates. However, due to this combination of columns GC×GC is not used at its full potential. Due to the large difference in column diameters a flow mismatch occurs between the two columns making it impossible to operate both columns at their respective optimum linear velocity at the same time [6]. When high inlet pressures are used to obtain optimum performance in the 1D, the linear velocity in the 2D is far above optimum and its efficiency is sacrificed. At lower inlet pressures the separation capacity of the 2D is fully exploited, but the carrier gas velocity in the 1D is now below optimum. Therefore, a conscious choice needs to be made as one separation dimension can only be optimized at the expense of the other. A second limitation resulting from the use of narrow-bore 2D columns is that these columns have very low sample capacities. Hence, they are easily overloaded resulting in poor peak shapes and a loss of resolution [7, 8].

Stop-flow GC×GC [9-11] was introduced as an elegant solution to regulate the carrier gas flows in the two columns independently. In this methodology the first dimension flow is periodically stopped for a short time. During these pauses, the second separation is performed by delivering the carrier gas directly into the 2D via an auxiliary inlet. This way, the separation time of the second column is fully independent from the modulation time. 2D columns with 0.15-0.25 mm i.d. can be used and both separations can be performed at their optimum. The main limitations of this approach are the need for additional hardware and the complexity of the instrumental set-up. Tranchida et al. [12-14] presented another
interesting method to balance the carrier gas linear velocities in the two dimensions. These authors installed a splitter at the end of the primary column to adjust the flow rate entering the 2D. This way, high inlet pressures can be used to operate the 1D at its optimum while maintaining an appropriate velocity in the 2D. This approach is effective for solving the velocity mismatch, but poorer detection limits are obtained as only part of the analytes reaches the detector, typically only 20-50% of the flow goes to the FID [12, 14]. A column format which allowed achieving the same result with no loss in sensitivity would be preferable. Fitz et al. [15] introduced a single i.d. column set, i.e. 0.10 mm, with the long 1D and the short 2D having a thick and a thin film, respectively. By using two narrow-bore columns no flow mismatch occurred and it possible to achieve an extremely high peak capacity per unit of time. On the other hand, special hardware is required and the chromatographic space, although perfectly exploited, is limited because of the very low modulation times used (250-500 ms). In chapter four of this thesis we described the use of monolithic columns as 2D in GC×GC [16]. The benefit of using monoliths is that efficiency and column diameter are decoupled and the column diameter can be adjusted independently to provide high plate numbers at any flow rate. This way, simultaneous operation of the two dimensions at their optimum was successfully achieved. However, the preparation of suitable monoliths is rather challenging and the efficiency obtained is not yet comparable to that of standard GC×GC set-ups.

Multi-capillary columns (MCC) have been investigated for their potential to perform fast GC separations with increased loadability almost two decades ago [17-20]. These columns were made by combining over 900 capillaries of 40 µm internal diameter in parallel. When compared to standard (i.e. single) short narrow-bore columns, the MCC showed greatly enhanced sample capacity and generated a much higher volumetric flow. However, due to the difficulty to prepare exactly identical capillaries, the performance of the MCC was rather poor [19]. Slight variations in the length, diameter or film thickness of the individual columns have a detrimental effect on the efficiency of the MCC. To obtain the maximum performance possible, very strict requirements need to be fulfilled for the uniformity of the columns, especially in terms of i.d. and stationary phase film thickness [21]. With hundreds of capillaries this turned out to be impossible, which
explains why the MCC column was removed from the market only a few years after its introduction.

MCCs could provide an interesting solution to the flow mismatch encountered in GC×GC. A multi-capillary ²D column (multi-²D) would allow both dimensions to be operated at their optimum velocity: the rather high flow of carrier gas exiting the ¹D column is in fact split over multiple ²D capillaries. By carefully selecting the inner diameters of the two dimensions and the number of ²D columns, ¹D and ²D optimum linear velocity can be obtained simultaneously. An additional advantage is that the reduced pressure at the ¹D/²D junction will have a positive effect on the speed of the ¹D. If the number of columns used to construct the multi-capillary ²D is limited, the favorable effects on the linear velocities will be exploited whereas the uniformity problems encountered when using too many capillaries are greatly reduced.

In this work we will report the theoretical evaluation and practical use of a GC×GC column set-up with a multi-²D “column” (GC×multi-GC) consisting of two or three narrow-bore ²D capillaries. A calculation model is developed and applied to predict the optimum number of ²D columns. These capillaries were initially evaluated in terms of efficiency individually and then combined to assess their uniformity. The multi-²D columns were connected to the ¹D via chip-based or press-fit type splitters. The GC×multi-GC set-up was evaluated through model experiments aimed at operating the two dimensions at their optimum simultaneously. The analysis of real-life samples will also be shown to confirm the feasibility of this methodology for practical use.

5.2. Theory

Beens et al. [6] described a programme written in Microsoft Excel® to calculate the efficiency for the two dimensions in GC×GC. This software provides a very useful means to optimize the column set and the GC×GC conditions. Here this programme was modified to allow the use of multiple parallel second dimension columns. The programme provides estimates for the linear velocities and efficiencies in the two dimensions of the GC×multi-GC set-up operated under isothermal conditions. In order to do so, the assumption is made that all capillaries in the ²D are exactly the
same. The effects of possible variations will be discussed later. The programme by Beens starts with calculating the volumetric flows through the \(1D\) and the multi-\(2D\), \(1F\) and \(2F\). \(1F\) is given by:

\[
1F = \left( \frac{\pi \cdot 1d_1^2}{256 \cdot \eta \cdot 1L} \right) \cdot \left( \frac{1p_{in}^2 - 1p_{out}^2}{1p_{out}} \right) \cdot \left( \frac{T_{mod}}{1T} \right)
\]  

(1)

Similarly, \(2F\) can be expressed as:

\[
2F = m \cdot \left( \frac{\pi \cdot 2d_2^4}{256 \cdot \eta \cdot 2L} \right) \cdot \left( \frac{2p_{in}^2 - 2p_{out}^2}{2p_{out}} \right) \cdot \left( \frac{T_{mod}}{2T} \right) \cdot \left( \frac{2p_{out}}{1p_{out}} \right)
\]  

(2)

Here \(\eta\) is the dynamic viscosity of the carrier gas. \(1p_{in}\) and \(2p_{out}\) are the inlet pressure and the outlet pressure at the detector, and \(1p_{out}\) or \(2p_{in}\) is the midpoint pressure between the two dimensions. \(1L\) and \(1d_1\) are the length and the internal diameter of the \(1D\). \(2L\) and \(2d_2\) are their equivalents for the \(2D\). \(1T\), \(2T\) and \(T_{mod}\) are the temperature in the \(1D\), the \(2D\) and the modulator, respectively. The additional term \(m\) represents the number of parallel capillaries in the multi-\(2D\). If all temperatures are the same and realizing that, in case of non-splitting between the dimensions, \(1F\) is equal to \(2F\) (if at least expressed at the same temperature and pressure), Eq. (1) and Eq. (2) can be combined and rearranged to give:

\[
1p_{out}^2 = \left( \frac{1d_1^2 \cdot 2L \cdot 1p_{in}^2 + m \cdot 2d_2^4 \cdot 1L \cdot 2p_{out}^2}{m \cdot 2d_2^4 \cdot 1L + 1d_1^2 \cdot 2L} \right)
\]  

(3)

The average linear velocities in the columns can now be calculated as can the respective plate heights according to the procedures described in [6]. A detailed explanation of the equations used and of the algorithm developed is given in the Appendix. Moreover, in the article this chapter is based on information is provided on how free access to this software can be obtained.

Assuming that the separation achieved in the \(1D\) is not lost during the modulation and that the two mechanisms are orthogonal, the peak capacity of a two-dimensional system, \(n_{c,2D}\), is given by [22]:

111
where $n_{c,1D}$ and $n_{c,2D}$ are the peak capacities for the 1D and the 2D columns, respectively. As the theoretical model used in our software is valid only for isothermal conditions, the peak capacities were calculated using the well-known formula [23]:

$$n_{c,iso} = \frac{\sqrt{N}}{4R_s} \cdot \ln \left( \frac{1+k_\omega}{1+k_\alpha} \right) + 1$$

where $N$ is the plate number, $R_s$ is the desired resolution, and $k_\alpha$ and $k_\omega$ are the retention factors of the first- and last-eluting peaks, respectively. Assuming isothermal conditions is correct for the 2D, due to its fast elution and the very slow temperature programming ramps employed. On the other hand, using Eq. (5) $n_{c,1D}$ will be somewhat lower than in practice, where the 1D temperature is normally programmed. Nevertheless, this will give us an accurate representation of the systems at different flow conditions and thus of the potential benefit arising from simultaneous at-optimum operation.

A second programme was written in Microsoft Excel to predict the shape of the peaks eluting from two or three columns in parallel. This programme allows introducing $L$, $d_c$ and $d_f$, independently for up to three columns. This way, the effect of a poor column reproducibility on the peak shape of the columns’ ensemble could be calculated.

5.3. Experimental

5.3.1. Chromatographic instrument, materials and methods

All 1D-GC experiments and the initial GC×multi-GC experiments were performed on a Agilent Technologies (Santa Clara, CA, USA) 7890 Series GC System equipped with a split/splitless injector, a Zoex (Houston, TX, USA) loop modulation system and a flame ionization detector (FID) all provided by JSB (Eindhoven, the Netherlands). The hydrogen flow for the FID was produced by a hydrogen generator PG-H$_2$ Series
3 (Schmidlin-DBS AG, Neuheim, Switzerland). All subsequent GC\times multi-GC experiments were performed on an Agilent Technologies 6890 Series GC System equipped with a CombiPal Autosampler (ATAS GL, Veldhoven, The Netherlands), a split/splitless injector, a LECO (Mönchengladbach, Germany) dual-stage, quad-jet thermal modulator and a FID.

The columns assembled to form the multi-2D were obtained by cutting exactly 1.1 m-long pieces from a Rxi-5ms column (length 10 m, i.d. 0.075 mm, film thickness 0.075 µm) specifically manufactured by Restek (Bellefonte, PA, USA) to grant very high uniformity in terms of inner diameter and film thickness. The 1D-GC evaluation of the MCC was performed by installing three columns in parallel in the GC. Connection to the injector and the detector was achieved by fitting the three columns together through a single hole graphite ferrule for wide-bore columns. In the GC\times multi-GC set-ups, 0.9 m-long pieces of these columns were coupled to the 1D via 1-in-2-out or 1-in-3-out splitters. These were miniaturized chip-based devices (Fig. 1A) provided by NLISIS (Veldhoven, The Netherlands), or customized press-fit connectors (Fig. 1B) manufactured by Techrom (Purmerend, The Netherlands) with a silicon-based glue (Bison International B.V., Goes, the Netherlands) resistant to temperatures up to 300°C applied on the 2D side to provide additional sealing. The modulator was slightly modified by enlarging its holes to allow installing all 2D columns together. Modulation was performed on the multi-2D, resulting in an actual 2D length of 0.75 m. A Restek Stabilwax column (length 30 m, i.d. 0.25 mm, stationary phase 0.25 µm) was used as first dimension. A schematic representation of the GC\times multi-GC instrumental set-up is shown in Figure 1C. For the GC\times GC system used as reference, one single 2D column was connected directly to the 1D via a Meltfit connection (NLISIS).

In all experiments an injector temperature of 250°C was chosen and helium was used as the carrier gas. All GC\times GC and GC\times multi-GC chromatograms were recorded with a modulation time of 3 s. For the construction of the Van Deemter curves injections of 1 µL of the headspace of single test compounds or of a test mixture were performed and isothermal analysis was carried out at different inlet pressures. For the 1D-GC evaluation of the three narrow-bore capillaries and the MCC, a split ratio of 1:900 and an oven temperature of 70°C were used. For the evaluation of the two-dimensional set-ups the split ratio was 1:200 and the oven
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temperature selected was 40°C or 80°C. The efficiency of the $^1$D and $^2$D was assessed by performing the analysis with the modulator off and on, respectively. The two-dimensional applications performed to assess the GC×multi-GC set-ups were performed by injecting 0.1 µL of the sample with a split ratio of 1:200. The detailed oven temperature conditions employed in each case will be described below.

![Image](image.png)

Figure 1 – Photos of the chip-based (A) and the press-fit type (B) splitters with 3 outlets and schematic representation of the GC×multi-GC set-up with $m=3$ (C). M: Meltfit connection. S: flow splitter.

5.3.2. Chemicals

P.A. grade n-octane, n-nonane, n-decane and ethylbenzene were purchased from Aldrich (Zwijndrecht, The Netherlands). n-Hexane and n-heptane were purchased from Biosolve (Valkenswaard, The Netherlands). The test mixture of n-alkanes used to evaluate the narrow-bore columns used as multi-$^2$D was prepared by mixing 20 µL of each compound. The petrol-derived household cleaning product and the perfume were obtained from a local supermarket. The diesel oil sample was obtained from a local petrol station.
5.4. Results and discussion

5.4.1. Theoretical evaluation

The software developed (Section 5.2) was used to evaluate the effect of multiple ²D columns on the efficiency of the two dimensions. Figure 2 shows an example of Van Deemter curves calculated for standard GC×GC and GC×multi-GC (m=2 and m=3), respectively. As expected, when one short narrow-bore column is used as ²D, very different inlet pressures are necessary to obtain the minimum plate heights for the two dimensions (Fig. 2A). The ²D has its optimum at an inlet pressure of 160 kPa at 106 cm/s, but at that pressure the ¹D is far below optimum. This dimension reaches its optimum only at 333 kPa, i.e. 13 cm/s, but at this pressure the ²D velocity exceeds its optimum by far. Fig. 2B shows that with m=2 the ¹D and the multi-²D optima are much closer: at 182 and 240 kPa. The much lower pressure now required to operate the primary column at its optimum velocity is the result of the lower flow resistance of the ²D, which leads to lower \(^{1}p_{\text{out}}\). When three capillaries are used, the ¹D optimum is reached at an inlet pressure of only 205 kPa and at this pressure also the multi-²D gives its best performance. Clearly, in GC×multi-GC the optima of the two dimensions are much closer than in GC×GC. Optima in terms of inlet pressure can even be identical, making simultaneous optimum operation possible. According to this theoretical model, with a ²D column i.d. of 75 µm two columns provide a significant enhancement and three columns are already sufficient to achieve the desired flow conditions. Therefore, the use of even more columns in parallel, which would obviously even be more challenging from the practical prospective, is not necessary. On the basis of these results, the choice was made to investigate multi-²D columns made of two and three individual capillaries.

As expected, the maximum peak capacity achievable with the multidimensional system improves when the two dimensions are operated at their respective optimum (Fig. 2). With a single ²D column the maximum \(n_{c,2D}\), i.e. 8,300 peaks, is obtained when the ¹D is at the optimum. At these conditions \(2n_{c}\) is 30. With \(m=3\), the ²D is now also at its optimum at this inlet pressure with \(2n_{c}=38\), and the total peak capacity is now over 9,500 peaks. Hence, as expected, the GC×multi-
GC set-up provides improved resolving power, with beneficial effects for the separation of very complex samples. However, the extent of this improvement is rather small. This is due to the fact that, as can be seen in Eq. (5), $N$ is under a square root making its influence on $n_c$ limited.

![Calculated Van Deemter plots and peak capacity for the GC×GC (A) and the GC×multi-GC systems with $m=2$ (B) and $m=3$ (C), respectively. 1D: 30 m×0.25 mm i.d., 0.25 µm df. 2D: m×(1 m×0.075 mm i.d., 0.075 µm df). The thin solid and dotted lines indicate the plate height (H) for the 1D and the 2D calculated with programme developed. $k_1=5$, $k_2=2$. $T=70°C$. The bold solid line represents $n_{c,2D}$ calculated using Eq. (5) and Eq. (4). $k_ω=0.5$, $k_{ω}=60, k_ω=0.1, k_{ω}=5$.](image-url)
So far we assumed that all capillaries in the multi-2D were identical. However, given the difficulty of making equal columns, slight differences are hard to avoid in practice. Figure 3 shows the results obtained when simulating the peak shapes resulting from differences in the individual columns in a multi-2D with \( m = 3 \).

As can be seen, variations in length of only ±0.5% already cause severe band broadening while an error of 1% in length even leads to clear peak splitting (Fig. 3B). Similar (relative) variations in i.d. are even more detrimental and can lead to complete separation into distinct peaks (Fig. 3C). Inhomogeneities in film thickness, shown in Fig. 3D, also lead to severe band broadening. If these results are compared with those described in [21], it is clear that with low numbers of columns the impact of possible variations is even more significant. Therefore, very strict manufacturing requirements need to be fulfilled to assure good performance. On the other hand, preparing only three identical columns rather than several hundred is a much more realistic goal.

![Figure 3](image-url)

**Figure 3** – Effect of column variations on the peak shape on a multi-2D with \( m = 3 \). The figure shows the peak profiles calculated for identical (1 m×0.075 mm i.d., 0.075 µm \( df \)) capillaries (A) and for capillaries with differences in length (B), i.d. (C) and stationary phase film thickness (D). The dotted lines describe the case in which one column has the nominal dimension whereas the others have an error of +0.5% and -0.5%, respectively. The continuous lines illustrate a variance of ±1%.

\( p_m = 172 \) kPa. \( T = 70°C \). \( k = 2 \).
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5.4.2. Multi-capillary column

To obtain the high repeatability required, the columns used for the multi-2D were prepared by cutting adjacent pieces with equal length from the same capillary column specifically prepared to provide a high consistency in terms of i.d. and film thickness. As in practice coating is more challenging for polar stationary phases, the choice was made to use a non-polar 2D column, i.e. to work with a reversed-polarity column set.

In a first series of experiments three columns were evaluated independently. In order to simulate the highly critical situation encountered in the 2D, i.e. fast elution and very narrow peaks, low-boiling alkanes ranging from n-hexane to n-decane were selected as test compounds. A very high split ratio, i.e. 1:900, was used to minimize injection-related band broadening. The Van Deemter curves obtained for the three columns are the same, indicating a good uniformity. The results obtained for n-nonane are shown in Figure 4 as an example. It can be seen that the efficiency is close to that expected, i.e. 9,500 plates for a length of 1.1 m. The peaks obtained on the three columns were consistent both in terms of retention time (RSD≤0.8%) and peak width (RSD≤3.0%).

In a second series of experiments the columns were combined in parallel and evaluated as MCC. Good peak shapes were obtained with no signs of band broadening or split peaks. The Van Deemter curves were comparable with those obtained for the columns individually (Fig. 4). Table 1 shows some examples of the plate heights obtained. As can be seen the efficiency of the MCC, with over 9,100 generated plates, is very close to that of the individual columns. The repeatability in terms of retention times (RSD≤0.6%, n=3) and \( \omega_{0.5} \) (RSD≤2.7%, n=3) was satisfactory.

These promising results suggest that the strict column-uniformity requirements were fulfilled and the MCC was suitable for practical use. Therefore, it was decided to proceed to further evaluation of the column assembled as multi-2D.
Figure 4 – Comparison of the Van Deemter curves obtained for n-nonane at 70°C for the three Rxi-5ms (1.1 m×0.075 mm i.d., 0.075 µm df) columns individually (white, grey and black symbols) and combined as MCC (+). Every point is the average of 3 repeated injections.

Table 1 – Plate height at different carrier gas inlet pressure measured for n-nonane on the three Rxi-5ms (1.1 m×0.075 mm i.d., 0.075 µm df) columns individually and combined in parallel. T= 70°C.

<table>
<thead>
<tr>
<th>Inlet pressure (kPa)</th>
<th>Column A</th>
<th>Column B</th>
<th>Column C</th>
<th>MCCa</th>
<th>RSD% (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>0.268</td>
<td>0.283</td>
<td>0.248</td>
<td>0.254</td>
<td>5.9</td>
</tr>
<tr>
<td>48</td>
<td>0.166</td>
<td>0.179</td>
<td>0.171</td>
<td>0.182</td>
<td>3.0</td>
</tr>
<tr>
<td>62</td>
<td>0.136</td>
<td>0.138</td>
<td>0.143</td>
<td>0.143</td>
<td>2.1</td>
</tr>
<tr>
<td>69</td>
<td>0.126</td>
<td>0.128</td>
<td>0.129</td>
<td>0.131</td>
<td>1.3</td>
</tr>
<tr>
<td>76</td>
<td>0.125</td>
<td>0.126</td>
<td>0.130</td>
<td>0.122</td>
<td>2.8</td>
</tr>
<tr>
<td>83</td>
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<td>0.117</td>
<td>0.120</td>
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</tr>
<tr>
<td>90</td>
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<td>0.117</td>
<td>0.121</td>
<td>0.119</td>
<td>1.7</td>
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<tr>
<td>97</td>
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<td>0.116</td>
<td>0.117</td>
<td>0.5</td>
</tr>
<tr>
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<td>0.116</td>
<td>0.115</td>
<td>0.118</td>
<td>1.5</td>
</tr>
<tr>
<td>121</td>
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<td>0.119</td>
<td>0.121</td>
<td>0.119</td>
<td>0.8</td>
</tr>
<tr>
<td>138</td>
<td>0.124</td>
<td>0.129</td>
<td>0.132</td>
<td>0.127</td>
<td>2.1</td>
</tr>
<tr>
<td>155</td>
<td>0.132</td>
<td>0.133</td>
<td>0.132</td>
<td>0.130</td>
<td>1.1</td>
</tr>
<tr>
<td>172</td>
<td>0.140</td>
<td>0.147</td>
<td>0.144</td>
<td>0.137</td>
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<tr>
<td>190</td>
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<td>0.156</td>
<td>0.151</td>
<td>0.144</td>
<td>3.7</td>
</tr>
<tr>
<td>207</td>
<td>0.159</td>
<td>0.156</td>
<td>0.162</td>
<td>0.148</td>
<td>4.5</td>
</tr>
</tbody>
</table>

*a Multi-capillary: column A + column B + column C (in parallel)
5.4.3. GC×multi-GC

Optimization of the instrumental set-up

The splitter used to distribute the carrier gas flow exiting from the \(^1\)D column over the multiple \(^2\)D columns is crucial for obtaining a good performance in the GC×multi-GC experiments. Initially, the choice was made to use the chip-based splitters. Due to their very small internal volume and surface area they were expected to provide better performance in terms of band broadening and inertness. In the first experiments modulation was performed at the end of the primary column in order to facilitate installation and keep the instrumental set-up as simple as possible. The \(^2\)D peaks so obtained were broader than expected, with significant loss in efficiency. This was clearly caused by band broadening in the splitter device and could easily be solved by modulating on the multi-\(^2\)D, i.e. after the splitter. This way the peaks were refocused and all possible dead-volume effects were eliminated.

Initially the loop modulator was preferred as it allowed to install the multi-\(^2\)D without need for modifications. The parallel columns were simply coiled in the loop holder as normally done when using a single \(^2\)D column. Right away after a careful installation narrow, symmetrical \(^2\)D peaks were obtained, as shown in Fig. 5A. This results shows that modulating on three columns at once, even if coiled to give actually six columns, is fundamentally feasible. However, after a few injections clear peak splitting became visible (Fig. 5B). This was due to the fact the parallel columns were no longer aligned properly, causing differences between the actual modulator-detector for the individual columns. This was most likely caused by the intense blows of nitrogen periodically delivered by the hot jet, which was observed to induce movements in the loop. An additional reason for the limited stability could be the tension generated by having six columns, i.e. three columns coiled twice, in the loop.
The linear dual-stage quad-jet modulator was thus evaluated. The position of the multi-2D in front of the cold jets of the modulator was found to be critical. Poor installation caused breakthrough and peak splitting as the result of inhomogeneous cooling of the three columns and different lengths between modulator and detector for the individual columns. On the other hand, after careful adjustment it was possible to ensure effective trapping and identical 2D conditions. Although optimization was somewhat tedious and time-consuming, the set-up showed excellent robustness. Once appropriate performance was achieved, no further corrections were required during several weeks of operation. Figure 6 shows some examples of 2D peaks obtained by modulating on three parallel columns.
Figure 6 – Example of \(^2\)D peaks obtained with the GC×multi-GC set-up \((m=3)\) with the linear dual-stage, quasi-jet modulator. The modulation performed on the multi-\(^2\)D. Test compounds: n-hexane (A), n-heptane (B), n-nonane (C) and ethylbenzene (D). Inlet pressure=241 kPa. \(T=40^\circ\text{C} \) (A-C) and 80°C (D).

As can be seen the peaks are symmetrical and narrow, further suggesting that the strict requirements for the column repeatability in the MCC were fulfilled and that the chip-based column connectors indeed provide artifact-free, i.e. equal, flow splitting. Based on these results the dual-stage modulator was selected and used in all the following experiments.

**Efficiencies vs. inlet pressure**

The efficiency of a standard GC×GC column-set with one \(^2\)D column as a function of the inlet pressure was assessed as a reference. The efficiencies of the \(^1\)D and of the \(^2\)D were measured by performing isothermal analyses with the modulator off and on, respectively. As expected, the optimum velocities for the two dimensions were obtained at very different inlet pressures (Figure 7). For instance, for ethylbenzene the best performance is obtained at 172 kPa for the \(^1\)D and at 414 kPa for the \(^2\)D, respectively.
The efficiency of the two dimensions of our GC×multi-GC column set-up with two and three 2D columns in parallel was also investigated. The results obtained are also shown in Fig. 7. As can be seen the plate heights of the 1D are somewhat higher than expected for the column in use. This is probably caused by extra-column broadening occurring in the connections between the columns. However, it is not expected to have a significant influence on the position of the optimum and was therefore neglected for now. The performance of the multi-2D, on the other hand, is very good. In fact, the efficiency achieved here is even better than obtained during the 1D evaluation of the MCC (Section 4.2.1). An explanation for this is that even with the very high split ratio used the injection contribution to band broadening in the 1D experiments was not completely negligible for the very short retention times and narrow peaks generated, whereas in the multi-2D experiments this artifact is not present due to the efficient refocusing in the modulator. It can be observed that the minimum plate heights obtained for both \( m=2 \) and \( m=3 \) are slightly higher than with a single 2D column. This is most likely caused by very small variations in the multiple columns, i.e. to the presence of a “multipath” effect. In order to quantify this contribution, the experimental results obtained were compared to those calculated with our model for similar retention factors under the same operational conditions. With \( m=3 \) the experimental peak width at the 2D optimum for n-nonane is 160 ms at a retention time of 5.9 s, against the predicted 131 ms at 5.7 s. Clearly in this case some band broadening occurs in the multi-2D. On the other hand for ethylbenzene the results obtained, i.e. 138 ms at 5.8 s of retention, are consistent with the theoretical 147 ms at 6.15 s. This suggests that minor inhomogeneities in the ensemble are indeed present which results in a slight loss of performance only for the narrowest peaks. Nevertheless, in all cases the plate heights obtained, i.e. about 80-100 \( \mu \)m, are still more than suitable to fulfill the efficiency requirements asked of a 2D.

The most interesting aspect of Fig. 7 is that the experimental results obtained are in excellent agreement with the prediction generated by our computer programme (Section 5.2), confirming the correctness of our model. As expected, the 1D optima are shifted towards much lower inlet pressures. With \( m=2 \) the optima are significantly closer, the gap is in fact reduced from 250 to 80 kPa. With \( m=3 \) the maximum efficiency of both dimensions is obtained at exactly the same inlet
pressure, i.e. 200 kPa. As a result, in spite of the slightly lower plate numbers obtained in the two dimensions individually, the two-dimensional efficiency is improved. For example, the maximum $n_{c,2D}$ calculated as described in Section 6.2 with the experimental $N$ measured for ethylbenzene with $m=3$ (Fig.6) is increased from less than 6,500 peaks to 7,200 peaks, for an increment of 11%.

![Figure 7 – Van Deemter curves of n-nonane and ethylbenzene obtained with standard GC×GC (A) and GC×multi-GC with $m=2$ (B) and $m=3$ (C), respectively. Circles: $^1$D; triangles: $^2$D. Every point is the average of 3 repeated injections.](image)

The results described above confirm that the use of the multi-$^2$D is indeed a relatively simple yet effective approach to solve the $^1$D - $^2$D linear velocity mismatch issue. It is no longer required to choose which separation to exploit at the expense
of the other, it is now possible to exploit the maximum efficiency of the two dimensions simultaneously.

**Applications**

Selected samples were analyzed to assess the suitability of the GC×multi-GC column set-up and the two splitter devices for real-life applications. Figure 8 (chip-based connector) and 9 (press-fit type connector) show the chromatograms obtained with a multi-2D made of three columns for a petrol-derived household cleaning product, i.e. white spirit, and a diesel oil sample, respectively. The chromatograms demonstrate that very narrow and symmetrical peaks are obtained on the multi-2D with three columns in parallel. Efficient two-dimensional separations can be performed. Moreover, both connectors were observed to provide good inertness for this kind of samples. Figure 9 shows that the highly retained compounds give very wide 2D peaks as a result of the slow speed obtained in the multi-2D. This could be improved easily by using a set-up provided with a secondary oven. The second half of the chromatogram shows a very nice separation of the different groups, confirming the good exploitation of the 2D. This results, as expected, in a very good resolving power. On the other hand, the resolution along the primary column is rather poor for the early eluting species. This is due to an insufficient number of modulations. Because the linear velocity in the 1D is now higher as a result of the low flow resistance in the multi-2D, the compounds with low retention on the primary column are eluted as very narrow bands. Therefore, achieving a suitable number of modulations across a peak is more difficult. In order to verify this hypothesis, decreasing modulation times were tested (Fig. 10). This way a significant improvement in resolution was achieved: passing from a modulation time of 4 seconds to 2 seconds several overlapping peaks were fully resolved. This suggests that to maintain a good resolution in the first minutes of the GC×multi-GC analysis a careful choice of the modulation time and slow temperature programs are necessary, especially when this area of the chromatogram is complex.
Figure 8 – GC×multi-GC ($m=3$) chromatograms of a petrol-derived household cleaning product. Conditions: inlet pressure=207 kPa, oven: 40°C (2 min) to 80°C at 2.5°C/min; The dimensions were coupled with the chip-based flow connector.

Figure 9 – GC×multi-GC chromatogram ($m=3$) obtained for a diesel oil sample. The dimensions were coupled with press-fit connector. Conditions: inlet pressure=207 kPa, oven: 40°C (2 min) to 80°C at 2°C/min. Modulation time: 4 s.

Figure 10 – Effect of the modulation time on the resolution of the early eluting compounds for the diesel sample shown in Fig. 9. Modulation time: 4 s (A), 3 s (B) and 2 s (C). The dimensions were coupled with press-fit connector. Conditions: inlet pressure=207 kPa, oven: 40°C (2 min) to 80°C at 2°C/min.
Figure 11 – GC×multi-GC chromatograms of a perfume sample with $m=3$ (A) and $m=2$ (B), respectively. Conditions: A) press-fit connector, inlet pressure=275 kPa, oven: 60°C (0.2 min) to 250°C at 3°C/min; B) chip-based connector, inlet pressure=275 kPa, oven: 100°C (0.2 min) to 250°C at 5°C/min.

Figure 10 shows the two-dimensional chromatograms recorded for the same perfume sample by using 3 and 2 columns in parallel, respectively. The three-column experiment was performed using the press-fit connector while the chip connector was used for the two-column set. Some 1D peaks in Fig. 11A exhibit slight broadening/tailing, suggesting the presence of dead-volume and/or residual activity in the press-fit type connector. This artifact was not noticeable for the chip-based connectors, indicating a better inertness. Due to the lower speed obtained with decreasing $m$, a higher initial oven temperature and a faster temperature ramp were chosen for the set-up with $m=2$ than with $m=3$. As a result the separation obtained with $m=2$ is somewhat faster. This way, a comparable use of
the two-dimensional space was achieved. As can be seen, the results obtained in the two cases are quite consistent.

5.5. Conclusions

MCCs are an interesting 2D column format to solve the flow mismatch typically encountered in GC×GC. A computer programme written in Microsoft Excel® was developed to calculate the column efficiency for both dimensions in GC×GC with a single or multiple 2D columns in parallel. With the aid of this programme it was possible to assess that with multiple 2D columns simultaneous optimum operation in the two dimensions is fundamentally possible. The appropriate number of columns was selected based on this theoretical model. A second programme based on Microsoft Excel® was developed and used to predict the influence of the inter-column variability on the performance of the multi-2D.

The capillaries used to form the MCC were obtained from a column manufactured to provide high consistency in terms of i.d. and film thickness of the stationary phase. 1D-GC experiments performed on three columns individually and combined as MCC gave very good results, both in terms of absolute efficiency and column repeatability. The plate height obtained for n-nonane with the MCC was 0.13 mm, very close to the value of 0.12 mm obtained with the individual columns. Multi-2D columns made of two or three parallel capillary columns were successfully installed in GC×multi-GC set-ups. The multi-2D gave good results in terms of peak shape and efficiency. Symmetrical narrow peaks with peak widths at half-height down to 37 ms and plate heights as low as 80 µm were obtained. Model experiments showed that in GC×multi-GC it is possible to operate both dimensions at their optimum at the same time. The large inlet pressure gap between the optima of the two dimensions with a single 2D column, i.e. over 150 kPa, was completely eliminated when using three parallel 2D. A compromise is no longer required, both dimensions can be fully exploited. The two-dimensional chromatograms obtained for a few selected samples proved the suitability of the novel column combination for use with real-life samples. Future work should be focused on the hardware with the aim to reduce the instrumental complexity. However, this multi-2D approach clearly
is of great value and interest in application fields where a maximum resolving power is required.
Chapter 5

List of references