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Active modulation in neat carbon dioxide packed column comprehensive two-dimensional supercritical fluid chromatography

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Highlights:

- An active interface between the two SFC dimensions in SFCxSFC is presented.
- Peak compression is demonstrated in SFCxSFC for better modulation efficiency
- Effect of operating conditions are highlighted thanks to the use of design of experiments

Abstract:

After demonstrating in a first paper the feasibility of SFCxSFC without decompression of the mobile phase, a modified interface has been developed in order to perform active modulation between the two SFC dimensions. In this paper, it is shown that the new interface enabled independent control of modulation parameters in SFCxSFC and performed a band compression effect of solutes between the two SFC dimensions. The effectiveness of this new modulation process was studied using a Design of Experiments. The SFCxSFC prototype was applied to the analysis of a real oil sample to demonstrate the benefits of the active modulator; in comparison to our previous results obtained without active modulation, better separation was obtained with the new interface owing to the peak compression occurring in the modulator.

Keywords:

Supercritical Fluid Chromatography, comprehensive 2D SFC, modulation

1. INTRODUCTION

Development of commercial two dimensional chromatographic systems such as comprehensive two dimensional gas chromatography (GCxGC) and, more recently comprehensive two dimensional liquid chromatography (LCxLC) allowed the analysis of otherwise demanding matrixes [1]. However, as in gas chromatography (GC), elution of poorly volatile compounds in GCxGC requires high temperatures [2-3] that can facilitate the degradation of both expected and unexpected analytes present in complex matrixes and/or thermal degradation of stationary phases as well. The use of supercritical fluid chromatography (SFC) in two-dimensional chromatographic systems has been proposed and performed recently [4-8]. On one hand, SFC can overcome the limitations of GC for the analysis of low volatility compounds requiring a pre-separation step such as group type separation before GCxGC [4-6]. On another hand, it can be integrated in 2D, or more, separation scheme in dense phase such as SFCxLC reported in reference [7] or LCxSFC reported in reference [8]. In both cases, SFC has the definitive advantage to be performed in less extreme conditions [9] and to be milder towards both analytes [10] and stationary phases. Indeed, carbon dioxide used as eluent in SFC is reported to be mild towards stationary phases in the chemical aspects of interactions, as well as in mechanical aspects [11-13]. Moreover, SFC allows the use of both LC and GC oriented detectors, from Flame Ionisation detection (FID) to Mass Spectrometry (MS) and simultaneous multidetection systems [14]. These positive features point towards new developments of SFC, including the instrumentation.

The development of a comprehensive 2D SFC could be an effective answer to afore mentioned problems in GCxGC. As in other multidimensional chromatographic systems, one of the major key points is the transfer of analytes between the two separations. In the work presented by Hirata et al. [15], stop flow in the first dimension followed by total decompression of the first dimension mobile phase was proposed; the total analysis time was equal to the first dimension analysis time plus the time the flow was stopped. Despite it was not mentioned in the paper, the risk of cross contamination owing to the precipitation of analytes in the interface, and the loss of analytes during decompression, were possible drawbacks using such a non-continuous transfer between the dimensions. Another potential problem was the difficulty in the re-mobilisation, by the mobile phase, of compounds that could/should precipitate within the transfer module during the inter-dimensional transfer. These technical impediments do not present themselves as

problematic in comprehensive two-dimensional chromatographic systems where inter-dimensional transfer is performed in dense conditions, i.e without decompression, as in LCxLC.

Such a transfer mechanism was proposed recently by Guibal et al. [16] for SFCxSFC in an approach using a real on-line mode of analysis (like GCxGC or LCxLC). The use of an LCxLC like interface, with neat CO_2 in supercritical conditions, permitted the transfer of the first dimension flow into the second dimension, without decompression, thus evading the possible loss of analytes. Despite the good orthogonality shown on the chromatograms, this interface was not able to allow for a peak compression effect in the second dimension [16], unlike GCxGC: band-broadening occurring in the first dimension was not suppressed by the modulator and the peaks in the second dimension where quite broad; thus, the peak capacity remained low compared to GCxGC. In order to improve the efficiency of the technique in a way SFCxSFC would be closer to GCxGC rather than LCxLC from this point of view, a new interface that completely separated the two analysis dimensions and matter transfer between the two dimensions of separation is presented in this article. The transfer module has been designed so that it was possible to set distinctly and independently the mobile phase parameters (pressure and flow rate) in three regions of the system: first dimension, transfer region, and second dimension. This new set up could perform effective peak compression between the two dimensions without total decompression of the mobile phase by playing with the density of the fluid via the different pressure values applied in the modulator and in the second dimension. Design of experiments (DoE) was used both to determine which parameter had a significant influence on peak compression and to visualize this influence using a test mixture composed of hydrocarbons having various volatilities and molecular masses. The efficacy of the setup was demonstrated on a real sample.

2. EXPERIMENTAL

2.1.- Chemicals

CO_2 (99.995%) was supplied by Messer (Mitry-Mory, France).

Two test mixtures were prepared in heptane (Merck Millipore, Saint-Quentin en Yvelines France). First test mixture contained C9, C10, C11, C12, C14, C15, C16, C18, C19 and C20 alkanes, naphthalene and anthracene. Their concentration was in the range of 0.05-0.3 mg/mL. Second test

mixture contained C30 alkane and fluoranthene. Their concentration was 0.05 mg/mL. Solutes were provided by Sigma-Aldrich (Saint-Quentin Fallavier, France)

Real sample of coal tar vacuum distillate was provided by IFP Energies Nouvelles, Solaize, France.

2.2.- SFCxSFC Apparatus

The 2D SFC apparatus was constructed in the laboratory from the system presented in ref [16]. It consisted of two Berger SFC FCM-1200 (Mettler-Toledo Auto Chem, Viroflay, France) and a SFC Aurora A5 / HPLC Agilent 1100 (Agilent Technologies France SAS, Les Ulis, France) fluid delivery system, and an extensively modified HP 5890 gas chromatograph equipped with a Flame Ionisation Detector (FID). The Aurora/Agilent 1100 system was used as a CO_2 delivery system for the first dimension. One of the Berger SFC FCM-1200 pumps was used to supply CO_2 to the transfer module, while the other was the second dimension CO_2 pump. All of the pumps were equipped with computer-controlled backpressure regulators (BPR) in order to control the column outlet pressure and the flow of the CO_2 in both dimensions and in the transfer module. Sample introduction into the first dimension was obtained using an air-actuated high pressure injection valve (Valco, model 06T0107H, VICI AG International, Schenkon, Switzerland) equipped with an external 67µL loop. Two ten-port valves (Valco, model C72-1690ED) were used as hardware core of modulation for inter-dimensional analyte transfer. Prior to the modulation, the flow from the first dimension was split using a T-piece and a restrictor in order to reduce the volume of the mobile phase to be transferred in the second dimension (see figure 1). Doing so, a multiwavelength UV detector (UV MWD HP 1050) could be placed on the diverted flow before reaching the back pressure regulator of the first dimension; it could be used as an auxiliary in monitoring and setting of the first dimension conditions; it was unused for these experiments. The split ratio was adjusted to allow the adequate filling of the transfer loop during one modulation period as explained in [16].

After the second dimension, the flow of mobile phase was split using a small volume T-piece. This allowed the simultaneous use of the FID and, eventually Diode Array Detector (DAD HP 1050) detectors. The DAD was placed on the main stream before the BPR used to control of pressure in the second dimension; note that the DAD was only used for the setting of preliminary operating conditions. Desired split ratio between the BPR (DAD when used) and the FID was

99 % : 1 % FID): it was obtained using a laboratory-made integral restrictor (50µm I.D., deactivated fused silica capillary) placed upstream of the FID detector. Flow of CO_2 through the FID was monitored on daily basis to ensure a constant flow during the experiments.

The system and data acquisition were controlled using 3D-SFC Chemstation 3.4 (Agilent Technologies France SAS) for the Berger pumps, UV detectors (when used, as indicated above) and FID, and Chemstation 4.0 (Agilent Technologies France SAS) for the Aurora system. The FID was used for 2D separations monitoring: the FID signal was exported as a cvs file and processed on a Matlab routine developed in the laboratory to build the 2D color plots.

The transfer module between the two dimensions and control system were developed for this work within the research group (Figure 1). It consisted of two ten-port micro bore switching valves (Valco, model C72-1690ED) mounted between the first dimension column outlet and second dimension column inlet. Electronically timed actuators, one for each valve, controlled the actuation of valves. The first valve was used for both the comprehensive sampling of the first dimension column effluent into two sample loops, and the transfer to the second valve. The second valve was the interface towards the second dimension; it allowed a fast trapping and transfert of analytes using a different set of operating conditions in comparison to those used in both the first and second dimensions. Both valves, as well as all the columns, T-piece unions, and most of the tubings were placed inside the HP5890 oven that allowed the regulation of temperature at 50°C for all experiments presented in this paper. All parts of the system were interconnected using 0.25 ID 1/16" stainless steel tubing. The explanations of system operation and experimental conditions applied in the different parts of the system are described in the text or the figures legend.

2.3.- Columns

The same columns as in our first paper [16] were used because they were shown to provide suitable orthogonality (i.e. a separation according to carbon number in the first dimension combined to a separation according to polarity in the second dimension), as it is usually done in GCxGC of petroleum compounds [17] or as recently proposed in RPLCxSFC [8].

Stationary phase in the first dimension was:

- Capcell Pak C18 ACR, 4.6 mm I.D. x 250 mm (Shiseido, AIT, Houilles, France) packed with 5 µm particles

Stationary phase in the second dimension was:

- Syncronis bare silica 3 mm I.D. x 50 mm (ThermoFisher Scientific, Les Ulis, France) packed with 1.7 µm particles

2.4.- Experimental Design

Studied parameters were mobile phase flow rates and pressures in the three parts of the system: the flow in the first dimension (^1F), the flow in the second dimension (^2F), the flow in the transfer module (^tF), the pressure in the first dimension (^1P), the pressure in the second dimension (^2P), and the pressure in the transfer module (^tP). Thus, six factors were studied (3 zones x 2 parameters) and a 2^{6-2} fractional factorial design with 16 experiments was selected. The resolution of this design was IV, which meant factors and first order interactions (2 terms) could be estimated independently. Three center points (repetitions) were added to the sixteen factorial experiments. The chromatographic response monitored was peak width in the second dimension.

Calculation of the effects and their significance were carried out using JMP 10.0 (S.A.S Institute Inc, Cary, NC, USA) and Excel 2007 (Microsoft Corporation, Courtaboeuf, France)

3. RESULTS AND DISCUSION

3.1.- Transfer module

Ideally, an efficient comprehensive two dimensional chromatographic system should allow a fast transfer of analytes from the first dimension to the second dimension, without any loss of resolution and efficiency of separation in both dimensions. In SFC, it should permit a "fast injection" of the first column effluent into the second column without any decompression in the transfer module; transferred volumes and time required to transfer the fractions coming from the first dimension should be compatible with a fast analysis in the second dimension.

Moreover, the transfer module between the two dimensions (the modulator) should allow the total transfer into the second dimension of the first dimension mobile phase contained in the sampling loop used for modulation without any loss of material. As shown in our previous paper [16], this could be obtained using a split between the two dimensions: the volume transferred from the first to the second dimension was adjusted via the split ratio and the volume of the transfer loop. The split ratio was measured according to the method suggested by Guibal et al. [16], where a small

amount (0.5% of flow) of ethanol was continuously added to the mobile phase flow, the increase of detector response was monitored as a function of sampling time. As soon as the loop was totally filled, the response measured via the second dimension part of the 2D system was constant. The sampling time, for all the values of mobile phase flow investigated in the first dimension, was 30 s [16]. Using this design and the selected operating condition, the transfer module only allowed the direct transfer of the volume of the sample loop into the second dimension for the second dimension analysis. However, the chromatographic conditions (pressure and flow rate) used in the second dimension were found to influence the process of analyte transfer and efficiency in the second dimension as shown in the table 2 of reference [16]. In order to separate the transfer step from the second dimension analysis, the transfer module has been modified (figure 1) to distinctly set the mobile phase parameters (pressure, flow-rate and duration) in the three regions of the system: first dimension, transfer region (with a control of the parameters developed specifically for this work), and second dimension.

The modified module consisted of two ten-port valves equipped with an electric actuator and operated by an electronic timer. The first valve (sampling valve on the top of figure 1A), as said before, was used for continuous sampling of the mobile phase from the first dimension and was fitted with two 45 µL sampling loops; it allowed for one loop to be filled while the content of the other one was being transferred to and separated in the second dimension as presented in [16]. The second valve (the transfer valve, bottom of figure 1A) has been specifically added to enable two distinct sources of mobile phase to be used in the second dimension. One source, with its own set of pumping and pressure parameters, was used for transferring the analytes from the sample loops located on the first valve to the second dimension column inlet (Transfer valve in position A, figure 1A). The second source, having its own pump, pressure regulator and set of parameters, was used to deliver the mobile phase to the second dimension in order to perform the separation (transfer valve in position B, figure 1B). To balance the pressure and flow fluctuations that occurred when the position of the transfer valve was changed, a “dummy” column was mounted in parallel to the second dimension column. This column was identical to the column used for the separation in the second dimension: same stationary phase and dimensions were used in order to generate the same

pressure drop whatever the position of the transfer valve. This setup allowed for a continuous control of both mobile phase sources with minimal influence of the different mobile phase paths on the flow-pressure conditions in the system. Furthermore, it still permitted, eventually, to use multiple detectors after the second dimension column, in our case a FID and, for some preliminary experiments, a UV DAD, as in Guibal's original set up [16].
To test the effectiveness of this modification of the system and the impact of transfer conditions on the analysis, a series of tests was carried out after the determination of a suitable modulation sequence using the new interface.

3.2.- Modulation sequence

Based on results described in reference [14], the sampling modulation period was set to 30 seconds, permitting a comprehensive sampling of the first dimension column effluent (the flow rate in the first dimension was 1ml/min) while maintaining the possibility of a reasonably fast second dimension analysis (performed at 1.6 ml/min). This gave a frame for a series of tests aiming at determining the best ratio between - the time required for complete transfer (performed at a flow rate of 1ml/min) from the sampling loop to the second dimension column - and - the second dimension analysis itself -, the sum of both being 30 seconds. This was carried out by injecting dodecane, and determining the minimum time required for the complete flushing of the sampling loop followed by an equilibration step. The best results were obtained for a 12-second flushing time and an 18-second analysis time. Using the test mixtures, neither wrap-around nor cross contamination occurred while acceptable resolution was obtained using both light and heavy hydrocarbons test mixtures. Figure 2 shows the sequence of valves actuation and position.

3.3.- Study of transfer parameters using a design of experiments

To determine which experimental factors could affect the chromatographic responses and how, a design of experiments was carried out. The potentially influent factors were selected considering the instrumental design, and the influence of mobile phase flow rate and pressure on SFC

separations [11,18]. Studied parameters were mobile phase flow rates and pressures in the three parts of the system: the flow in the first dimension (^1F), the flow in the second dimension (^2F), the flow in the transfer module (^tF), the pressure in the first dimension (^1P), the pressure in the second dimension (^2P), and the pressure in the transfer module (^tP). They were selected because they could affect dramatically the separation in the second dimension (for a constant separation duration) as well as mass transfer between dimensions especially using of a compressible fluid such as neat supercritical carbon dioxide. As six factors had to be studied (3 zones x 2 parameters) and to reduce as much as possible the number of experiments, a 2^{6-2} fractional factorial design with 16 experiments was selected. The resolution of this design was IV, which meant factors and first order interactions (2 terms) could be estimated independently. Three center points (repetitions) were added to the sixteen factorial experiments.

3.3.1. Choice of the factors and responses

The chromatographic response monitored was peak width in the second dimension. As the purpose of the work was to monitor a "peak compression" effect, a variation in this response should allow an evaluation of the transfer mechanism, and its influence on the analysis.

As explained previously, the DoE chosen allowed not only the evaluation of the main effects of the six factors but also of their first order interactions (two-factor interaction). Therefore, it could be possible to establish if the effect of a factor depended on the level of another factor.

To make a comprehensive study of transfer influence as well as inter-dimensional pressure and flow interactions, maximum and minimum pressure and flow in each part of the system that could realistically be applied were defined taking into account both instrumental and chromatographic limitations. Thus, values of both pressure and flow of supercritical CO_2 were a compromise between separation requirements and instrumental limitations (the maximum pressure allowed by our system in the three studied zones of our set up being 400 bar). Pressure was monitored at the outlet of columns (inlet of BPR). The selected values of investigated factors, which corresponded to the low and high levels used for the DoE are reported in Table 1.

The use of high pressure in the transfer step, meaning an increase of the density of the CO_2 and of the solubility of analytes, should influence the introduction of solutes and their separation conditions into the second dimension. It was assumed that the best hypothetical transfer conditions should combine high flow and low pressure of the mobile phase: High flow should allow for a fast and total transfer and flushing of the sampling loops and simultaneous introduction of analytes into the second dimension column, while low pressure (low density) should enable efficient trapping of analytes by the stationary phase used in the second dimension column; under proper conditions of transfer, a peak compression effect should occur between the two dimensions without the need of total decompression of the mobile phase contrary to what was presented in the reference [15].

3.3.2. Matrix of experiments

The two test mixtures, respectively composed of light hydrocarbons (from C9 to C20, naphthalene, anthracene) and heavy hydrocarbons (C30 and fluoranthene), were analyzed using the conditions reported in the matrix of experiments in coded values (table 2). The sixteen experiments were randomized whereas the three center points were evenly distributed to check that no drift affected the system. Two series of analysis were executed, one using test mixture 1, and the other using test mixture 2.

3.3.3. Effects of parameters

Main effects and two-terms interactions for the six factors considered were computed by means of least square regression. The significance of the factors and interactions was evaluated using the residual error of the regression. A significance level of 1% was chosen to allow focusing on the most significant factors. Table 3 shows the results using the peak width of C12 alkane as the response.

It appeared that the most significant factor was ${}^{t}F$, the flow in the transfer module. Its effect had negative value meaning that to favor the peak compression it must be set at the high level. The second most significant factor was ${}^{2}F$, the flow in the second dimension. It had also an effect with a negative value. However, a significant interaction existed between these two parameters (${}^{t}F$ x ${}^{2}F$)

meaning that the effect of ${}^{2}F$ depended on the level of ${}^{t}F$. As this interaction was ranked second in the order of magnitude (stronger in absolute value than the effect of ${}^{2}F$) and presented a positive sign, ${}^{2}F$ level must be set at the low level. These results demonstrated unambiguously the importance of the transfer parameters and the peak compression phenomenon.

Thus, in agreement with one of our hypothesis, the peak compression effect increased when the flow in the transfer module was high. The reason of this effect is not trivial and can be related to a better sweeping of the sampling loop and/or a longer exposition to low density conditions in the inlet of the second dimension column. As an example of this observation, figure 3 shows the FID signal of the test mixture 1, with two different sets of chromatographic parameters.

Encircled are the instrumental responses of the same compounds. The chromatogram on the right was obtained using high flow in the transfer phase as well as the second dimension, while the one on the left was obtained using low flow in both phases. Figure 4 presents the 2D plots of the chromatograms reported in the figure 3. The peak compression effect on the second dimension is easily observable by a reduction of peak width in the second dimensions by a factor of 5 on the chromatogram of assay 10 (circled spots).

Figure 5 presents the side-by-side 2D plots of light hydrocarbons in the conditions of assays 4 and 13 of the DoE: Assay 4 had low values for flow in the transfer phase while maintaining high values of pressure. The second dimension parameters were kept at high levels. Assay 13 had high flow value in the transfer region while all the other parameters except ${}^{1}F$ were set at low values. Tailing and broadening of peaks were present in the chromatogram obtained in assay 4 conditions thus emphasizing the influence of transfer region flow and pressure on the peak shape in 2D SFC analysis.

Test mixture 2, containing heavy hydrocarbons, had also been injected using the conditions of the matrix of experiments. In figure 6, a similar behavior to the one of figure 5 was observed, using this batch of heavier solutes, in the operating conditions used for light hydrocarbons in figure 5. Compression effect was still present,- but not as pronounced in comparison to the experiments carried out using test mixture 1-, and was less influenced by the transfer conditions. It is assumed this was related to higher affinity of the heavier solutes of mixture 2 for the stationary phase used in the second dimension: for all compounds of test mixture 2, retention on the second dimension column should be much higher than that of light hydrocarbons of test mixture 1; however, the remobilization of heavy solutes of test mixture 2 for the second dimension separation could be more difficult owing to both higher trapping by the stationary phase and lower solubility in the mobile phase. This can be compared to the thermal modulation problems that can occur in GCxGC if the modulation temperature is too low because the time needed to remobilize the compounds after cryogenic focusing is related to their volatility [19].

Overall, the best conditions were obtained when the conditions used in assay 13 (first dimension ^{1}F=2ml/min, ^{1}P=120 bar, transfer, $^{t}\boldsymbol{F}$**=2.1ml/min**, $^{t}\boldsymbol{P}$**=70 bar**, second dimension ^{2}P=150bar, $^{2}\boldsymbol{F}$**=0.8ml/min**).

3.3.4 Application to a real sample

The system was tested using a real sample – a vacuum distillate of coal tar. The same sample was already investigated and discussed in our first demonstration of the feasibility of SFCxSFC [16]; it was used here for comparison purpose. Improvements obtained using the modified interface are clearly visible on the side-by-side comparison of 2D plots reported on the Figure 7. Left plot was obtained without the use of the new transfer module while the plot on the right was obtained in the same conditions using the active transfer module and applying the best conditions of transfer, *i.e.* at high flow and low pressure, in order to obtain the best peak compression effect. It is clear again that peaks were wider and less resolved when the transfer module was not used. The space occupancy expressed as the spreading angle of peaks was almost 90° for the analysis without compression during the transfer, as well as for the modified system, in the conditions of our

experiments (indeed, orthogonality was not supposed to be affected by the peak compression effect). The analysis carried out with the active transfer module looked quite similar to a GCxGC plot. However, compared to high temperature GCxGC of heavy oil fractions [2-3] it was obtained at much lower temperature, 50°C. As can be seen on figure 7, a better separation was achieved both in the first dimension (depending on the molecular weight, the effect is related to the better sensitivity obtained using the peak compression), and in the second dimension (as a function of polarity) where the peak compression is responsible of significant peak width reduction. In the second dimension, it is assumed that the alkanes eluted as the first large band of spots on the bottom on the 2D plot. Owing to their higher polarity, the aromatic hydrocarbon groups eluted above the alkanes; as they are supposed to bear 1 to 6 aromatic rings, it is likely to believe there is group type separation in the second dimension as some organization in bands can be evidenced from the 2D plot. Indeed, bare silica column has been shown to suit for group type separation in SFC [4-6]; this is the reason why it was chosen for this work [16].

4. - CONCLUSIONS

A new interface has been designed for SFCxSFC to enable independent control of transfer conditions between the two dimensions of separation. The impact of the independent control of supercritical fluid conditions during the transfer between dimensions was demonstrated to affect peak width and to enable the expected effect of peak compression.

Indeed, in the transfer step, using a high flow of CO_2 and a low density via low pressure of the mobile phase, peak width of light hydrocarbons was reduced regardless of the conditions used in the two dimensions of separation except the flow rate in the second dimension. A low flow rate applied for the separation in the second dimension gave the best results despite there was a quite good tolerance to higher flow rates. This can be an advantage to avoid wrapping around by reducing the analysis time: to reduce retention time, the flow rate can be increased in the second dimension and/or the pressure can be increase in order to increase the eluent strength of the mobile phase.

Heavy hydrocarbons also exhibited the same behavior that has been attributed to compression within the second column because they were much strongly retained. It is worth mentioning that the series of drastic changes in pressure applied to the second dimension did not affect the second

dimension column that could be used during months without any evidence of degradation of its performances!

Compared to the pioneering results presented by Hirata et al. in ref 15 where decompression occurred between the two dimensions, the new interface described in this paper should enable faster mobilization of compounds after trapping and limit the risk of loss of compounds during the transfer in the interface.

As a variety of detectors can be used in SFC, the potential of this set up would be amplified if mass spectrometry would be hyphenated too, together with on-line supercritical fluid extraction for real sample preparation prior to the analysis. Currently, the system is restricted to the use of pure carbon dioxide in the first dimension because the density and the eluent strength of the mobile phase are strongly affected by modifiers. It seems reasonable to assume that peak compression would be less effective or even ineffective if modifiers were used in the first dimension. Should FID be not mandatory for detection, modifiers could be implemented in the second dimension.

5. REFERENCES

1 M. Adahchour, J. Beens, and U. A. T. Brinkman, Recent developments in the application of comprehensive two-dimensional gas chromatography, J. Chromatogr. A, 1186 (2008) 67-108.

2 T. Dutriez, J. Borras, M. Courtiade, D. Thiébaut, H. Dulot, F. Bertoncini, M.-C. Hennion, Advances in Quantitative Analysis of Heavy Petroleum Fractions by Liquid Chromatography-High-Temperature Comprehensive Two-Dimensional Gas Chromatography: Breakthrough for Conversion Processes, Energy Fuels, 24 (2010) 4430-4438

3 T. Dutriez, M. Courtiade, D. Thiébaut, H. Dulot, F. Bertoncini, M.-C. Hennion, Extended characterization of a vacuum gas oil by offline LC-high-temperature comprehensive two-dimensional gas chromatography, J. Sep. Sci., 33 (2010) 1787-1796

4 F. Adam, F. Bertoncini, D. Thiébaut, D. Espinat, M.-C. Hennion, Supercritical fluid chromatography hyphenated with twin comprehensive two-dimensional gas chromatography for ultimate analysis of middle distillates, J. Chromatogr. A, 1217 (2010) 1386–1394.

5 B. Omais, T. Dutriez, M. Courtiade, N. Charon, J. Ponthus, H. Dulot, D. Thiébaut, SFC-GCxGC to Analyse Matrices from Petroleum and Coal, LC GC Europe, 24 (2011) 352-365

6 H. Potgieter, R. van der Westhuizen, E. Rohwer, D. Malan, Hyphenation of supercritical fluid chromatography and two-dimensional gas chromatography–mass spectrometry for group type separation, J. Chromatogr. A 1294 (2013) 137– 144.

7 I. François, P. Sandra, Comprehensive supercritical fluid chromatography×reversed phase liquid chromatography for the analysis of the fatty acids in fish oil, J. Chromatogr. A. 1216 (2009) 4005-4012.

8 M. Sarrut, A. Corgier, G. Crétier, A. Le Masle, S. Dubant,S. Heinisch, Potential and limitations of on-line comprehensive reversed phase liquid chromatography×supercritical fluid chromatography for the separation of neutral compounds: An approach to separate an aqueous extract of bio-oil, J. Chromatogr. A. 1402 (2015) 124-133

9 A. Dulaurent, L. Dahan, D. Thiébaut, F. Bertoncini, D. Espinat, Extended Simulated Distillation by Capillary Supercritical Fluid Chromatography, Oil Gas Sci. and Technol. 62 1 (2007) 33-42

10 M. M. Schwartz, H. E. Brownlee, R. G. Boduszynski, F. Su, Simulated distillation of high boiling petroleum fractions by capillary supercritical fluid chromatography and vacuum thermal gravimetric analysis, Anal Chem. 59 (1987) 1393-1401

11 E. Lesellier, C. West, The many faces of packed column supercritical fluid chromatography – A critical review. J. Chromatogr. A, 1382 (2015) 2-46

12 T. A. Berger and W. H. Wilson, Packed column supercritical fluid chromatography with 220000 plates, Anal Chem 65 (1993)1451-1455

13 C. Bouigeon, D. Thiébaut, M. Caude, Long packed column supercritical fluid chromatography: influence of pressure drop on efficiency. Anal. Chem. 68 (1996) 3622-3630

14 G. Lavison, F. Bertoncini, D. Thiébaut, J.-F. Beziau, B. Carrazé, P. Valette, X. Duteurtre, Hypernated Supercritical Fluid Chromatography (SFC/UV-FID-AED-FTIR-MS). Application to car lubricant analysis, J. Chromatogr. A, 1270 (2012) 318-323

15 Y. Hirata, T. Hashiguchi, E. Kawata, Development of comprehensive two-dimensional packed column supercritical fluid chromatography, J. Sep. Sci. 26 (2003) 531-535.

16 P. Guibal, D. Thiébaut, P. Sassiat, J. Vial, Feasability of neat carbon dioxide packed column comprehensive two dimensional supercritical fluid chromatography, J. Chromatogr. A 1255 (2012) 252-258

17 Gas chromatography and 2D-gas chromatography for petroleum industry. The race for selectivity. F. Bertoncini, M. Courtiade-Tholance, D. Thiébaut Editors, Editions TECHNIP, Paris, France, 2013

18 Practical supercritical fluid chromatography and extraction, M. Caude et D. Thiébaut Editors, Chromatography: a comprehensive treatise, Harwood Academic Publishers, Chur, Suisse, 1999

19 R.M. Kinghorn, P.J. Marriott, Enhancement of Signal-to-Noise Ratios in Capillary Gas Chromatography by Using a Longitudinally Modulated Cryogenic System, J. High Resol. Chromatogr. 21 (1998) 32-38

Figure 1. Transfer module: Simultaneous filling of sampling loop 1 by the mobile phase of the first dimension and transfer (12 seconds) of sampling loop 2 content to second dimension (both valves are in position A, figure 1 A). Figure 1B: Same position of the sampling loop, transfer valve in analysis position during 18 seconds (Position B)

Figure 2. Valves positions as a function of the modulation period. The sampling Valve (1) allows the alternate filling of sample loops, thus ensuring continuous collection of analytes from the 1st dimension. The transfer Valve (2) allows the transfer of each cut into the 2nd dimension and the second dimension analysis to be performed in different operating conditions (pressure and flow rate).

Figure 3. Raw FID chromatograms of light hydrocarbons analyzed using conditions of assays 10 and 12. The difference in peak shape (in circles) is obvious, with peaks in assay 12 showing broadening in respect to peaks in assay 10. Red circles: Left, Naphthalene; right : Anthracene. Alkanes elute first on the second dimension. Conditions: assay 10, first dimension ^1F=1.2ml/min, ^1P=120 bar, transfer, ^tF=2.1ml/min, ^tP=110 bar, second dimension ^2P=250bar, ^2F=1.2ml/min; assay 12, first dimension ^1F=1.2ml/min, ^1P=120 bar, transfer ^tF=1.3ml/min, ^tP=70 bar, second dimension ^2P=150bar, ^2F=0.8ml/min.

Figure 4. 2-D plots of assays 10 and 12, peak broadening and tailing are visible in the second dimension (same conditions and identification as in figure 3). Conditions: assay 10, first dimension ^1F=1.2ml/min, ^1P=120 bar, transfer, ^tF=2.1ml/min, ^tP=110 bar, second dimension ^2P=250bar, ^2F=1.2ml/min; assay 12, first dimension ^1F=1.2ml/min, ^1P=120 bar, transfer ^tF=1.3ml/min, ^tP=70 bar, second dimension ^2P=150bar, ^2F=0.8ml/min.

Figure 5. 2-D plots of assays 4 and 13 for light hydrocarbons mixture, with same conditions in the first dimension, but different conditions in transfer phase and second dimension. (same identification as in figure 3). Conditions: assay 4, first dimension ^{1}F=2ml/min, ^{1}P=120 bar, transfer, ^{t}F=1.3ml/min, ^{t}P=110 bar, second dimension ^{2}P=250bar, ^{2}F=1.2ml/min; assay 13, first dimension ^{1}F=2ml/min, ^{1}P=120 bar, transfer, ^{t}F=2.1ml/min, ^{t}P=70 bar, second dimension ^{2}P=150bar, ^{2}F=0.8ml/min.

Figure 6. 2-D plots of heavy hydrocarbons analysis in the same operating conditions as in figure 5. Parameters of assays 4 and 13 from table 2. Red circles: Bottom, Triacontane; top: Fluoranthene. Compression occurred, but was less influenced by transfer conditions. Conditions: Conditions: assay 4, first dimension ^{1}F=2ml/min, ^{1}P=120 bar, transfer, ^{t}F=1.3ml/min, ^{t}P=110 bar, second dimension ^{2}P=250bar, ^{2}F=1.2ml/min; assay 13, first dimension ^{1}F=2ml/min, ^{1}P=120 bar, transfer, ^{t}F=2.1ml/min, ^{t}P=70 bar, second dimension ^{2}P=150bar, ^{2}F=0.8ml/min.

Figure 7. An example of real sample analysis: vacuum distillate of coal tar. On the left the chromatogram obtained without compression, on the right the chromatogram obtained with compression using the new transfer interface. First dimension: flow ^{1}F=1,3 ml/min, pressure gradient from 100 bar to 250 bar; pressure ramp of 1.7 bar/min starting at 9 min; transfer: flow ^{t}F=2.1 ml/min, pressure ^{t}P=70 bar; second dimension: flow ^{2}F=1ml/min, pressure ^{2}P=200 bar; temperature 50 °C (all dimensions).

Table 1 Coded and original values of the studied factors.

Table 2 Matrix of experiments in coded values.

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CAPTIONS

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Figure 1

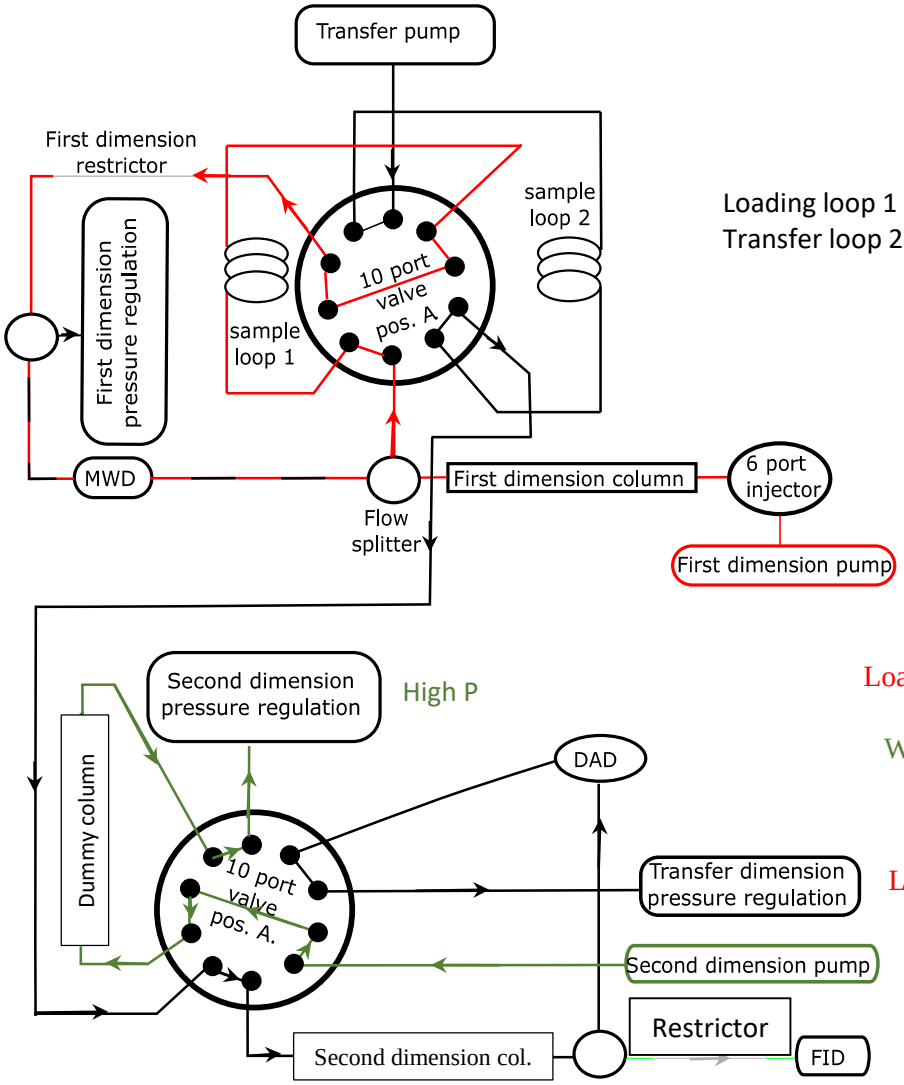

Fig 1A

Position A
Loading in dimension 2
(low P)
Waiting for analysis
(High Pressure)

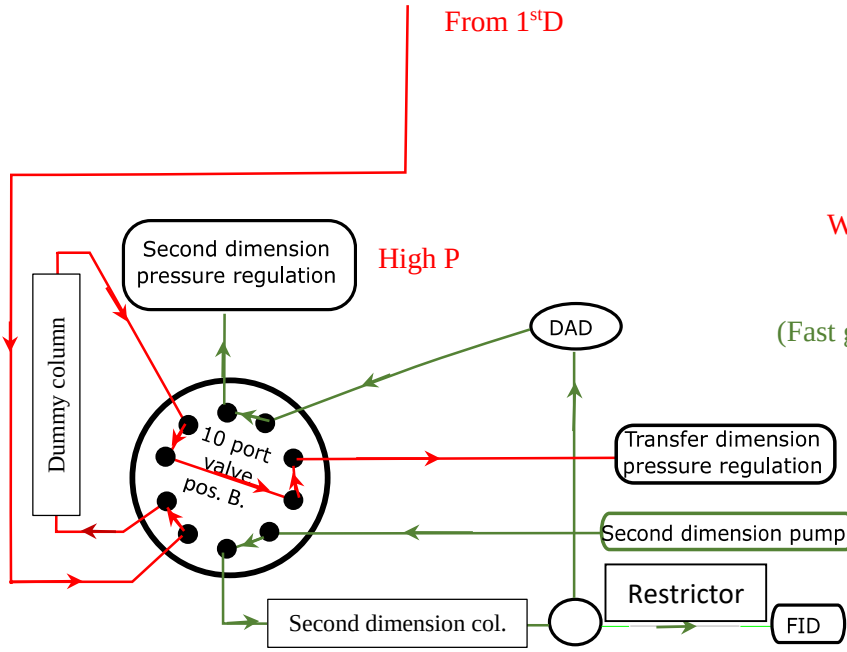

Fig 1B

Position B
Wait transfer from Dim 1
(low P)
Analysis Dim 2
(Fast gradient from low to high P)

Figure 2

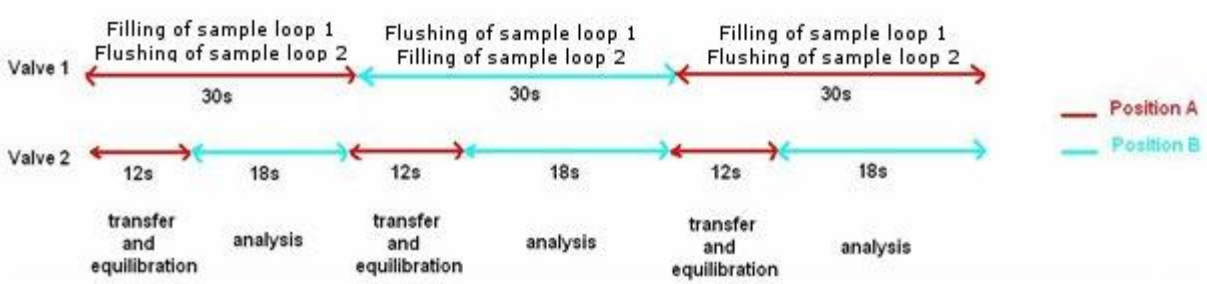

Figure 3

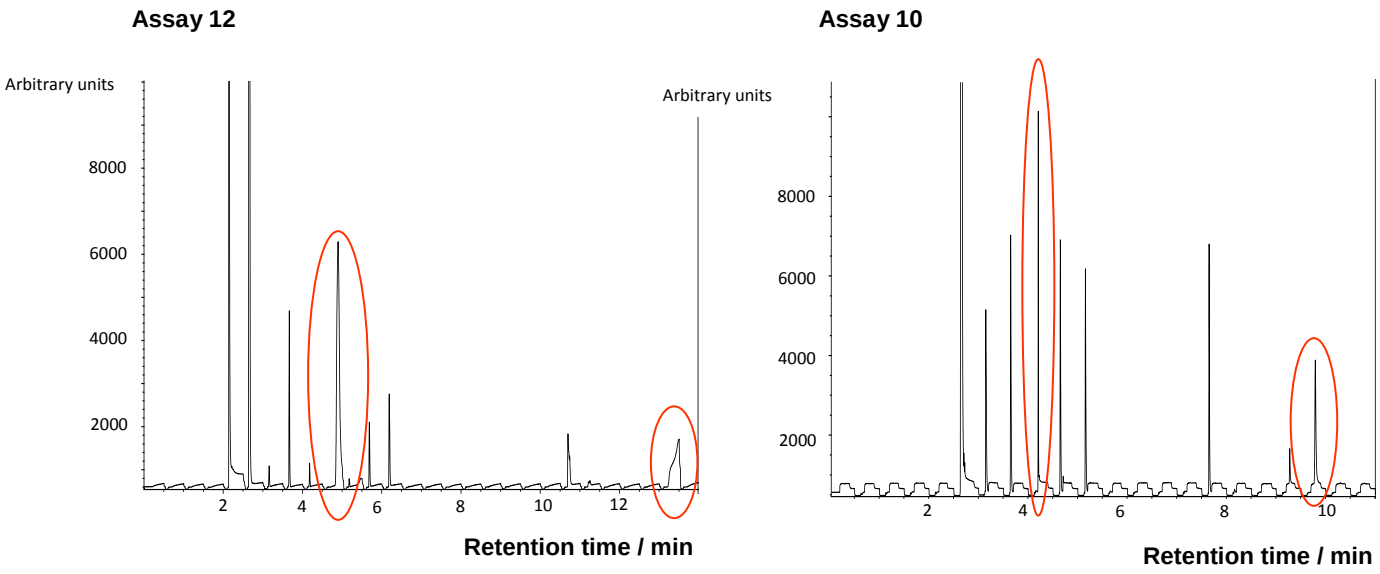

Figure 4

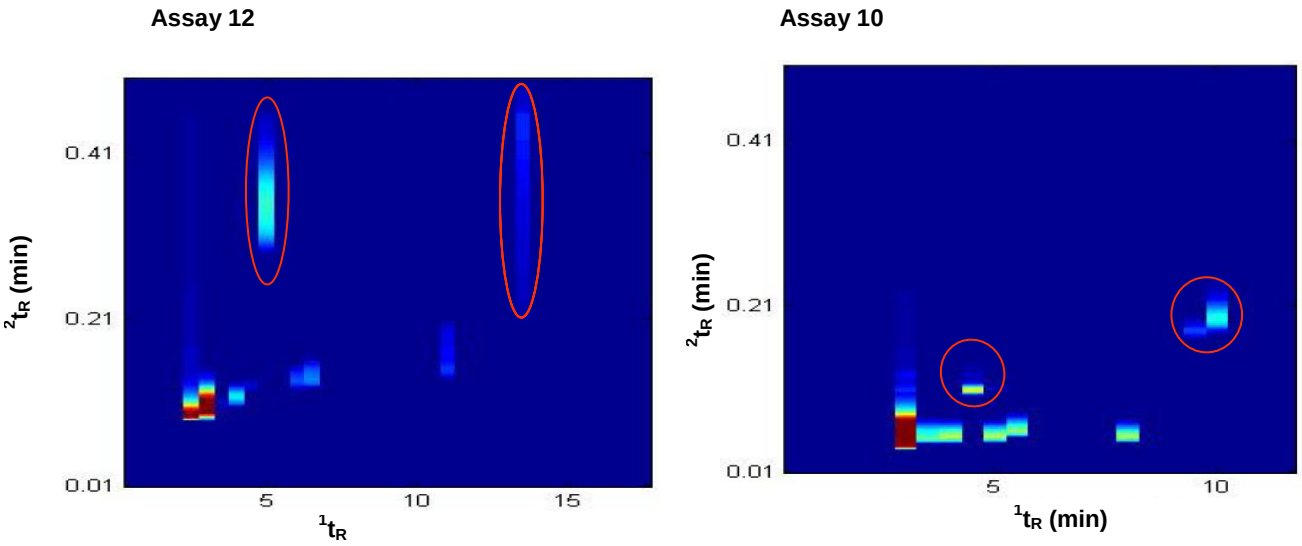

Figure 5

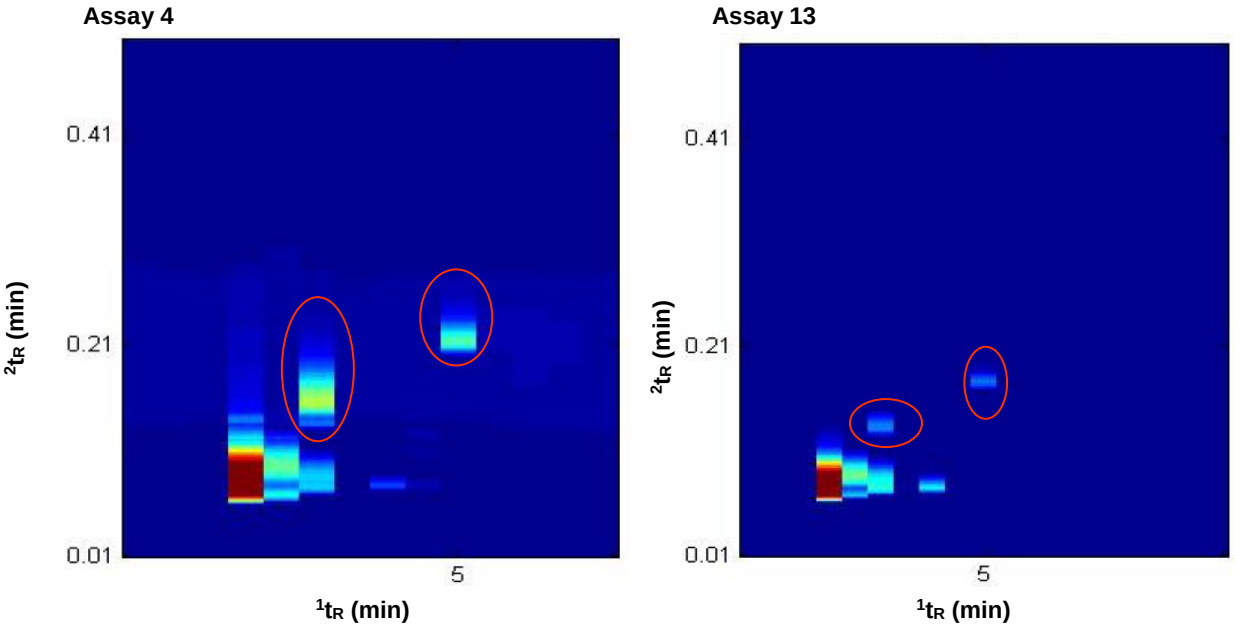

Figure 6

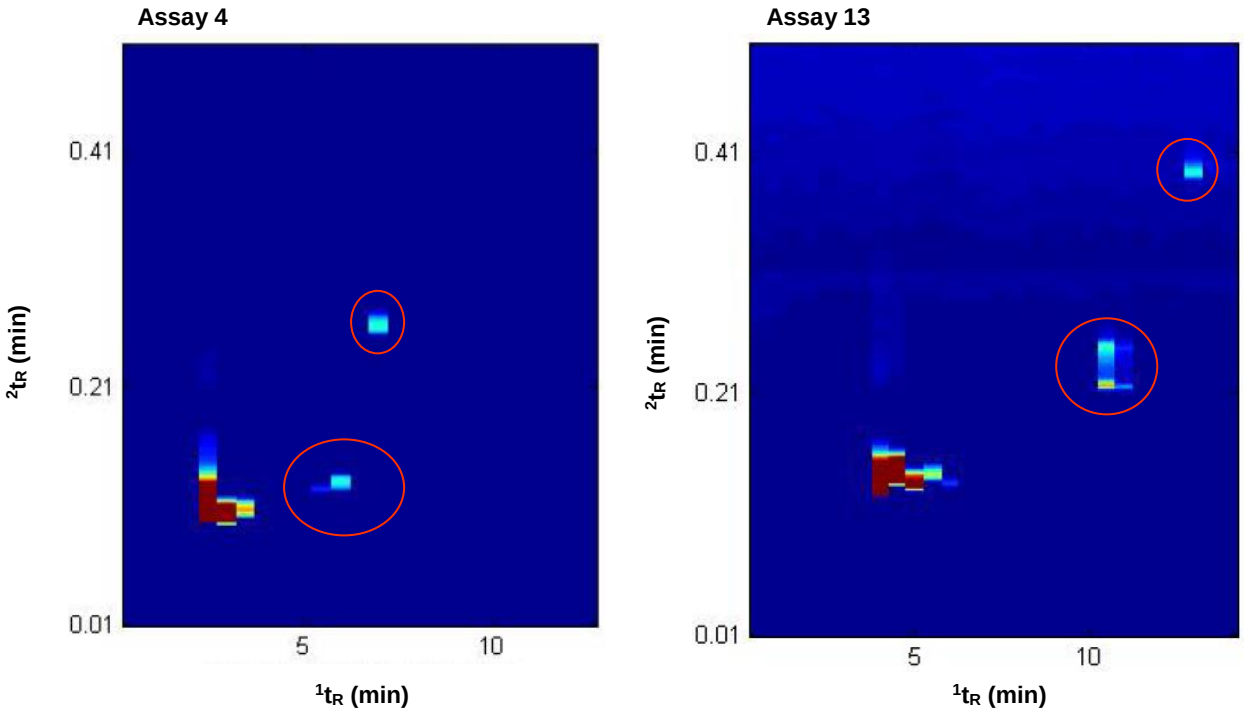

Figure 7

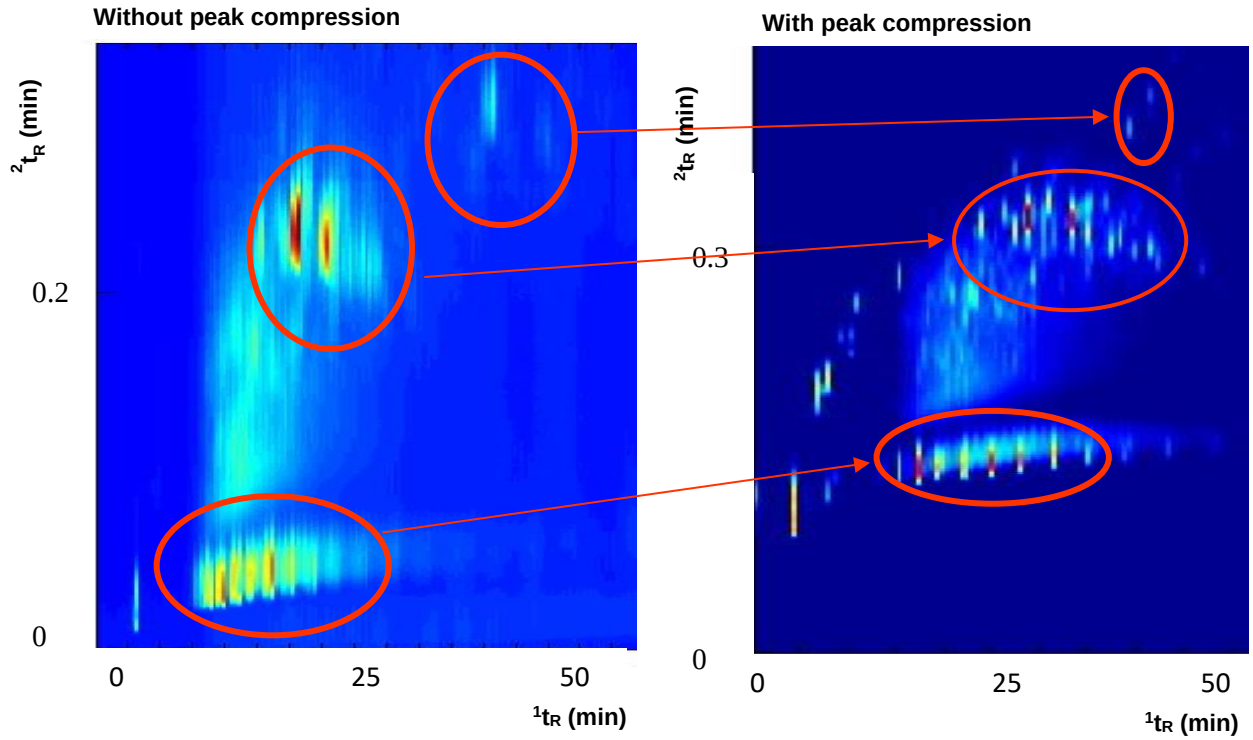

Table 1

Table 1 Coded and original values of the studied factors.

	First Dimension			Transfer			Second dimension		
	+	0	-	+	0	-	+	0	-
Flow **ml/min**	**2**	**1.6**	**1.2**	**2.1**	**1.7**	**1.3**	**1.2**	**1.0**	**0.8**
Pressure **bar**	**200**	**160**	**120**	**110**	**90**	**70**	**250**	**200**	**150**

Table 2

Table 2 Matrix of experiments in coded values.

Sample order	^{1}P	^{1}F	^{t}P	^{t}F	^{2}P	^{2}F
center point	**0**	**0**	**0**	**0**	**0**	**0**
1	+	+	+	-	+	-
2	+	+	-	-	+	+
3	+	-	+	+	+	-
4	-	+	+	-	+	+
5	-	-	+	-	-	+
6	-	-	-	+	+	-
7	+	-	+	-	-	-
8	+	+	-	+	-	+
center point	**0**	**0**	**0**	**0**	**0**	**0**
9	-	+	-	-	+	-
10	-	-	+	+	+	+
11	+	-	-	+	+	+
12	-	-	-	-	-	-
13	-	+	-	+	-	-
14	-	+	+	+	-	+
15	+	+	+	+	-	-
16	+	-	-	-	-	+
center point	**0**	**0**	**0**	**0**	**0**	**0**

Table 3

Table 3 Sorted effects and interactions determined using the peak width of dodecane. The estimate corresponds to the regression coefficient for the considered effect or interaction, the Standard Error corresponds to the standard deviation on this coefficient, the t-ratio is the ratio of the estimate to its standard deviation and Prob>|t| is the p-value corresponding to the t-ratio (bilateral test). The * indicates significant value with α = 1%.

Term	Estimate	Std Error	t Ratio	t Ratio	Prob>|t|
^{t}F	-0,010488	0,000562	-18,67		0,0003*
$^{t}F \times {}^{2}F$	0,007	0,000562	12,46		0,0011*
^{2}F	-0,00515	0,000562	-9,17		0,0027*
$^{t}P \times {}^{t}F$	0,0050125	0,000562	8,92		0,0030*
$^{1}P \times {}^{1}F$	0,003925	0,000562	6,99		0,0060*
$^{1}F \times {}^{2}F$	0,003125	0,000562	5,56		0,0115
$^{t}P \times {}^{2}P$	0,0028625	0,000562	5,10		0,0146
^{1}P	-0,002525	0,000562	-4,50		0,0205
$^{2}P \times {}^{2}F$	0,002525	0,000562	4,50		0,0205
^{1}F	-0,002237	0,000562	-3,98		0,028*
$^{1}P \times {}^{2}P$	0,0019	0,000562	3,38		0,0430
^{t}P	-0,001612	0,000562	-2,87		0,0640
$^{1}P \times {}^{t}F$	0,0015	0,000562	2,67		0,0756
^{2}P	-0,001312	0,000562	-2,34		0,1015
$^{t}P \times {}^{1}F$	0,0003125	0,000562	0,56		0,6167