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Quantitative performance of forward fill/flush differential flow modulation for comprehensive two-dimensional gas chromatography

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Abstract

GC×GC is an advanced separation technique allowing to achieve quantitative and qualitative characterization of complex samples. In order to perform two-dimensional separation, the system must provide suitable peak modulation which will direct short impulses of first column flow towards the second column. Forward fill/ flush differential flow modulation is a cost effective and no cryogen requiring approach which allows modulation over a wide range of analytes with very different boiling points. However, optimization of the flow modulation process can be difficult to understand and quantitation performance might be compromised if the parameters of the modulation process are not properly set. Modulated peak shape can be a good indication of the efficiency of the modulation process, however it is not sufficient to guarantee good quantitation. Different average velocities in the beginning and end of the thermally programmed GC run may cause different efficiency of the modulation process in various parts of the chromatogram. The purpose of this work is to investigate quantitative performance of the forward/fill flush modulation and delineate parameters that determine the effectiveness of the modulation process and its ability to properly reflect the quantitative composition of the investigated sample.

1. Introduction

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One-dimensional GC (1D GC) is a 'go-to' technique for the analysis of volatile and semi-volatile samples. Many regulatory and industrial methods rely heavily on the elaboration of this technique coupled with different detection solutions. However, for highly complex samples, 1D GC possesses shortcomings owing to many compounds coelutions. This favoured the emergence and the use of multidimensional and among them comprehensive gas chromatographic techniques. Comprehensive two-dimensional GC (GC×GC) involves a separation in the first column which is followed by the subsequent separation in the second column, resulting in the entire sample being subjected to a dual separation process. Introduction of the comprehensive separation methodology has brought a significant increase in peak capacity and signified a paradigm shift in the quality of chemical composition measurements for complex samples [1–5].

Modulator is the essential part of the GC×GC set-up. Its role is to successively sample the eluate from the first column and direct it towards the second column in a discontinuous but periodic manner [6]. Modulation is often designated as the bottleneck segment of the GC×GC system owing to the technical limitations of its performance. First practical realization of the modulation for GC×GC was effected by Liu and Phillips [7] who employed a two-stage thermal desorption modulator (TMD) in combination with two open-tubular GC columns (solutes were trapped at the entry of the second column in the thick film of stationary phase and periodically desorbed by electric pulses). Shortly after TMD was introduced, rotating thermal modulator known as 'sweeper' was developed by the same group [8]. This system brought improvement in terms of durability (in the previous system capillaries required frequent replacement), however it involved issues with moving parts and difficulties with modulation of higher boiling point compounds. Many limitations of described thermal modulators were overcome with the introduction of cryogenic systems. Marriott and coworkers [9] pioneered longitudinally modulated cryogenic system in which liquid CO_2 was used to trap analytes. This system however involved moving parts and was able to trap analytes at temperatures only down to approximately −50°C. Further improvements of modulation system involved dual jet systems [10] and substitution of CO_2 with liquid N_2 which allowed to trap high volatility species [11]. As alternatives to thermal systems, valve based and flow modulators were also developed. Diaphragm valve modulator

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was introduced by Bruckner et al. [12]. Such a system resulted in well resolved peaks, however it suffered from low mass transfer and thermal limitations. Flow modulation for GC×GC was first proposed by Seeley et al. [13] with so called differential or simple fluidic modulator and had concomitant developments such as modified valve system proposed by Wang [14] or the pulse flow modulator of Amirav's group [15–17]. Bueno and Seeley [18] further eliminated the diaphragm valve and devised a flow-switching system which allowed 100% mass transfer and eliminated thermal issues. This concept resulted in the commercial capillary flow technology (CFT) modulator proposed by Agilent and which is used in this work. Later on, reverse flow modulator [19], flow modulation low pressure GC×GC [20] and recently dynamic pressure gradient modulation [21] brought new developments.

In terms of use, cryogenic modulators dominate owing to their simple and flexible configuration, easy method development, small peak widths and no issues with coupling to the MS. They also allow the application of long modulation periods, which still for many applications could be desirable. Flow modulation is advantageous in terms of the lack of volatility limits and avoidance of a cryogen use, however it is characterized by laborious method development and issues with direct coupling to the MS detector. Summary of the basic advantages and drawbacks of the two modulation approaches is provided in Table 1.

Flow modulators are characterized by the simplicity of design, low cost and robustness, which makes them a very good choice for a robust analysis of a large variety of samples [22–30]. Operation of the differential flow modulator however must be carefully optimized in order to avoid quantification issues. Issues with peak shape and full peak return to the baseline have long been recognised and several approaches have been described in order to insure optimal performance of the modulator and investigate its quantitative performance. Harvey et al. [31] proposed a symmetric flow path modulator to minimize flow perturbations in the second dimension and using a pressure restrictor at the end of the first dimension column to stop the flow in the first column during the flush cycle for allowing more flexibility in terms of applicable range of modulation times. Tranchida et al. [32] proposed flexible loop-type flow modulator which ensured better peak shape however loss of sensitivity was

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incurred due to diverting part of the eluate to the waste. Griffith et al. [19], Duhamel et al. [25] and Krupcik et al. [23] investigated reverse fill/flush modulator for improved performance of flow modulation. Seeley et al. [33,34] have described quantitative aspects for flow modulation, including full transfer and low duty cycle modulation approaches.

Purpose of this work is to investigate in detail the qualitative and quantitative performance of forward fill/flush flow modulation, and define conditions and generic rules which ensure its satisfactory quantitative performance over the entire analysis time. Influence of the most important factors on the modulation process is investigated. Model of the fluid circuit representing the flow modulation is first devised similarly to the one used in Giardina et al. work [35]. Then, several operating conditions have been tested and calculations of the modulation fill and flush distances have been performed. An approach is devised to provide fine understanding of the forward fill/flush modulation process and generic rules to make a choice of modulation parameters that will allow to achieve efficient modulation process for the goal of quantitation. Most importantly, it was highlighted that modulation efficiency changes during the thermally programed GC run, owing to the change of linear velocities in the modulation channel, thus the possibility of programmable injection time could be proposed.

2. Description of the forward/fill flush modulation system

Operation of the modulation system with forward fill/flush dynamics comprises a series of consecutive sampling and injection steps which are driven by the periodic switching of the three way solenoid valve receiving a supply of the carrier gas [36]. In order to preserve the ^{1}D resolution but also avoid oversampling of the ^{1}D peaks, around three modulations over the ^{1}D peak area are recommended [37,38]. Number of modulations can be set by selecting appropriate modulation period, hence modulation period is one of the critical parameters influencing modulation process efficiency.

Additionally, it is important to preserve good peak shape after modulation. Tailing, fronting, double peaks or even not completely modulated peaks can occur and as such can influence quantitative reliability of the analysis. Critical parameters in the optimization of the modulation process and

elimination of adverse effects on the peak shape for this type of modulator are identified to be: ^{1}D column flow, ^{2}D column flow, modulation fill time and modulator flush time (modulation period = fill + flush time).

^{1}D column flow directs the fill stage of the modulation process. In order to avoid overfilling of the modulation channel this flow should not be too high. On the contrary, ^{2}D flow ought to be high enough in order to enable quick flushing of the modulation channel during the designated flush time. If the ^{2}D flow is too high however, the ^{1}D eluate might be carried over from the ^{1}D column and follow the main peak, causing double peaks. Modulation fill time, coupled with the ^{1}D flow determines the extent of the filling of the modulation channel. Hence, modulation period should not be too long as this might cause overfilling of the channel. On the other hand, if the modulation period is too short, oversampling of the ^{1}D peaks might occur as well as wrap-around in the second dimension separation. Thus, a compromise must be achieved in order to minimize these two adverse phenomena. The modulator flush time should be long enough to efficiently flush the entire volume of the modulation channel but not too long because in this case carryover of the ^{1}D eluate from the previous modulation cycle occurs and double peaks result, as mentioned before. Changing any or all of these parameters will bear influence on the modulation process, which can be perceived in the change of the peak shape. However, the question is how the quantitative performance of the modulation process is affected and how it can be optimized.

3. Theory

In order to better understand the flow modulation process, a fluid circuit representing the forward fill/flush modulation system was devised. Fluid circuit is illustrated in Figure 1, with fill and flush modes represented separately. During the modulator filling stage, eluate from the first column is slowly traveling into the modulator channel, in accordance with the low flows applied in the first dimension of the GC×GC separation. This stage is immediately followed by the flushing phase, where a high gas flow rate is applied at the head of the modulator channel and a fast pulse of the carrier gas

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flow is directed towards the second column. The sum of the fill and flush times of the modulator is equal to the modulation period. Described cycle is generally repeated over the entire GC×GC run.

In the represented fluid circuit, if involved inlet and outlet pressures in each segment of the circuit are known, classical Hagen-Poiseuille equation, allows to calculate appurtenant volumetric flows:

$$F = \left(\frac{\pi r^4}{16\eta L}\right)\left(\frac{P_{in}^2 - P_{out}^2}{P_{out}}\right)\left(\frac{T_{ref}}{T}\right)\left(\frac{P_{out}}{P_{ref}}\right) \quad (1)$$

Where F is the outlet volumetric flow (m^3/s) at reference conditions, r is the column radius (m), η is the carrier gas viscosity (Pa·s), L is the column length (m), P_{in} is the inlet pressure (Pa), P_{out} is the outlet pressure (Pa), T is the oven temperature (K), T_{ref} is the reference temperature, typically 25 °C (298 K), P_{ref} is the reference pressure, typically 14.696 psia (101 325 Pa).

In the fill mode of the depicted fluid circuit, inlet pressure of the first column is set to achieve a chosen ^{1}D flow, based on the column dimensions which are assumed to be known. Inlet pressure on the head of the second column is also applied in order to achieve a suitable ^{2}D flow, based on the known dimensions of the second column. Outlet pressure of the second column depends of the type of the detection system connected to the end of the column and, in the most simple case, is equal to atmospheric pressure; for example, when FID detector is used. Outlet pressure of the modulator is the same as the inlet pressure of the second column. Inlet pressure of the modulator is the same as outlet pressure of the first column and it is the only pressure which is unknown in this case. This pressure can be perceived as the midpoint pressure of the column set, involving first column and the modulator.

In the flush mode, first column outlet pressure is the same as the modulator inlet pressure; it is applied by the Pneumatic Control Module so as to achieve the desired flow in the second column. Modulator outlet pressure is the same as the second column inlet pressure and it is the only pressure which is unknown in the 'flush mode'. As in the first case, it can be regarded as the midpoint pressure of the column set consisting of the modulator and the second column.

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Required midpoint pressure can be calculated, by taking into the account that the volumetric flow at reference conditions in each segments of the column set has to be the same (mass conservation condition). Thus, for fill mode following equation applies:

$$\left(\frac{\pi {r_1}^4}{16\eta L_1}\right)\left(\frac{P_1^2-P_2^2}{P_2}\right)\left(\frac{T_{ref}}{T_1}\right)\left(\frac{P_2}{P_{ref}}\right)=\left(\frac{\pi {r_2}^4}{16\eta L_2}\right)\left(\frac{P_2^2-P_3^2}{P_3}\right)\left(\frac{T_{ref}}{T_2}\right)\left(\frac{P_3}{P_{ref}}\right) \quad (2)$$

Where r_1 is the ^{1}D column radius, η is the carrier gas viscosity, L_1 is the ^{1}D column length, P_1 is the ^{1}D column inlet pressure, P_2 is the ^{1}D column outlet pressure/modulator inlet pressure, T_1 is the column temperature, r_2 is the modulator channel radius, L_2 is the modulator channel length, P_3 is the modulator outlet pressure, T_2 is the modulator temperature, T_{ref} is the reference temperature, P_{ref} is the reference pressure, T is the column temperature.

If we assume that temperature is the same in column 1 and modulator, we can rewrite the equation 2 so as to calculate the ^{1}D column outlet pressure/modulator inlet pressure 'P_2':

$$\boldsymbol{P_2^2}=\frac{r_1^4L_2P_1^2+r_2^4L_1P_3^2}{r_2^4L_1+r_1^4L_2} \quad (3)$$

In the same way, the unknown modulator outlet pressure/^{2}D column inlet pressure 'P_3' in the fluid circuit representing the 'flush mode' of the modulator, can be calculated:

$$\boldsymbol{P_3^2}=\frac{r_2^4L_3P_2^2+r_3^4L_2P_4^2}{r_3^4L_2+r_2^4L_3} \quad (4)$$

Where r_2 is the modulator channel radius, L_2 is the modulator channel length, P_2 is the modulator inlet pressure, P_3 is the modulator outlet pressure/^{2}D column inlet pressure, r_3 is the ^{2}D column radius, L_3 is the ^{2}D column length, P_4 is the ^{2}D column outlet pressure.

When all pressures are known, volumetric flows at reference conditions in all fluid circuit segments can be calculated, which then in turn allow to calculate involved average velocities:

$$\boldsymbol{v}=\boldsymbol{F}\left(\frac{j}{\pi r^2}\right)\left(\frac{T}{T_{ref}}\right)\left(\frac{P_{ref}}{P_{out}}\right) \quad (5)$$

Where, $\left(\frac{T}{T_{ref}}\right)\left(\frac{P_{ref}}{P_{out}}\right)$ term is necessary to transform the volumetric flows at reference conditions 'F' into the corresponding volumetric flow. *j* is the compressibility factor:

$$j = \frac{3}{2}\left(\frac{\alpha^2-1}{\alpha^3-1}\right) \quad (6)$$

Where:

$$\alpha = \frac{P_{in}}{P_{out}} \quad (7)$$

Calculated average velocities in all fluid circuit segments, in fill and flush modes, allow to determine travelled distances by fill and flush volumes, based on the duration of the fill and flush periods of the modulation process. Examination of the calculated values of these quantities for the obtained empirical data, allows to understand how they influence the achieved efficiency of the modulation. Hence, parameters determining the success of the process can be identified, which allows for easier process optimization.

4. Materials and methods

For this study on the forward fill/flush modulation system, a standard mixture of n-paraffins (n-C8, n-C10, n-C12, n-C16, n-C18, n-C20, n-C22, n-C24, n-C26 and n-C28) diluted in toluene was used for optimization and quantitative performance tests. All chemicals used to prepare the n-paraffin mixture were of analytical grade quality (purchased from Sigma–Aldrich).

Agilent 7890A Gas Chromatograph with a G3486A CFT differential flow modulator and FID detection was used. Exact dimensions and shape of the modulator collection channel are not exactly known. However, according to literature, it was assumed that the volume of the collection channel is close to 35 µl and its length ~15 cm [35,36] with a circular shape. The selected column set consisted of a DB-1 ^{1}D column (100% dimethyl polysiloxane; 20 m, 0.1 mm ID, 0.4 µm; Agilent Technologies, Inc.) and a BPX-50 ^{2}D column (50% Phenyl Polysilphenylene-siloxane, 3.2 m, 0.25 mm ID, 0.25 µm; SGE). Carrier gas was hydrogen. 1 µL injections with a split ratio of 150:1 were performed on a

Multimode Inlet (MMI) from Agilent equipped with a single taper liner with glass wool. Injection port was heated to 300°C, then ramped to 330°C at 500°C/min, where it remained isothermal during 5 min. Temperature programing was 50-325 °C at 2.5°C/min. GC was run in a constant flow mode. FID conditions were as follows: 325 °C, air flow 400 ml/min, hydrogen 35 ml/min and make-up gas (nitrogen) 25 ml/min. Flow measurement were performed by using Alicat WHISPER MW Flow meter. All analysis were carried out in at least three replicates. Conditions that were varied for the analysis included ^{1}D Flow (0.10, 0.15 and 0.20 ml/min), ^{2}D Flow (6.3, 8.7, 11, 13.5, 18 and 20 ml/min), modulation period (3.0, 4.5, 5.0, 5.5, 6.0 and 8.0 s) and modulator flush time (0.16, 0.18, 0.20, 0.22 and 0.26 s). Overview of the selected conditions is provided in Table 2. Analysis have been repeated by using second modulator of the same kind with which similar results have been obtained.

Agilent ChemStation B.04.03-SP1 was used for GC data acquisition. GC Image 2.7 software was used for GC×GC chromatogram display and integration. For fluid circuit modelling, all calculations were performed in Excel®.

5. Results and discussion

5.1. Methodology

In order to investigate the influence of modulation parameters on both qualitative and quantitative results, a standard mixture of n-paraffins, covering a wide boiling point range: n-C8 to n-C28, was analysed by GC×GC-FID with different conditions of the modulation process.

Modulated peak shape was investigated through visual inspection for the different n-paraffins in the test mixture and the peak asymmetry was determined according to the following formula:

$$\boldsymbol{As} = \frac{\boldsymbol{W_{5.0}}}{\boldsymbol{t_w \cdot 2}} \quad (8)$$

Where *As* is the peak asymmetry factor, $W_{5.0}$ is the peak width at 5% peak height and t_w is the distance in minutes between the front of the peak and peak maximum (retention time) measured at 5% peak

height. However, as illustrated in Figure S1 in the supporting information, *As* could not be considered as the unique indicator for a good peak shape and visual inspection remains essential.

Relative response factors (RRFs) were calculated for all paraffins for each analysis. RRFs were deduced by taking into the account the FID determined blob volumes and known mass % of the analytes and calculated according to Equation 8. RRF of n-C18 is taken to be 1.

$$\boldsymbol{RRF_i} = \frac{\frac{\boldsymbol{m}}{\boldsymbol{m}}\%_i \cdot \boldsymbol{A_{ref}}}{\boldsymbol{A_i} \cdot \frac{\boldsymbol{m}}{\boldsymbol{m}}\%_{\boldsymbol{ref}}} \quad (9)$$

Where $m/m\%_i$ is the mass percent of investigated species in the prepared weighed standard mixture and A_i its blob volume, $m/m\%_{ref}$ is a mass percent of the reference species, in this case n-C18, and A_{ref} its blob volume. Theoretically, all the RRFs for n-paraffins should be similar with deviations less than +/- 10% compared to n-C18. Larger deviations may arrive from imperfect modulation or discrimination effects at the inlet but the latter were not observed in the used conditions.

For the different methods used, calculations were performed according to the fluid circuit described in the previous section. For 4 different oven temperatures (70°C – elution of n-C8, 100°C – elution of n-C10, 200°C– elution of n-C18 and 290°C– elution of n-C28) selected to span the entire n-paraffins elution range, average velocities were calculated in different segments of the system (^{1}D column, ^{2}D column and modulator). Even though temperature programing was in fact employed, assumption was made that the temperature changes very slowly, owing to the applied thermal rate and that system can be regarded as isothermal at chosen points of investigation. From these values, fill and flush distances that the carrier gas and the analytes travel during the fill and flush times in the modulator were calculated. They are obtained by multiplying average velocity in the modulation channel with the fill or flush time, respectively.

5.2. Experimental results

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Overview of the results obtained for 20 different methods applied are given in Table 3. Conditions of the modulation process, along with ^{1}D and ^{2}D flows for each run are provided in Table 2. For each of the four selected elution temperatures corresponding fill and flush distances were derived. Also determined was the time necessary to fully fill the modulation channel, which is a consequence of the choice of the ^{1}D flow, and the time necessary to flush one volume of the modulator channel which results from the chosen ^{2}D flow. Obtained quantities were closely looked at in conjunction with the appearance of the modulated peaks for four chosen elution temperatures.

Peak shape repeatability was investigated by performing repeated runs. Figure S2 in supporting information illustrates peak shape repeatability for six replicates of the same analysis. Chromatograms were highly repeatable, resulting in RRFs for all species whose RSD is less than 3%. Largest variability is obtained for n-C8, however this peak is very close to solvent peak (toluene) hence errors in blob volume estimations can be incurred.

5.3. Influence of modulation parameters on peak shape

In Table 3 highlighted in dark gray are all instances in which peak shape is close to ideal response perceived (minimal tailing or fronting). It is observed that in all cases, sum of fill and flush distance is close to 15 cm, which is the assumed length of the modulation channel. Additionally, for the efficient flushing of the channel, ratio of the two distances should be 2.5-3.5 times at least. Our assumption is that this could be owing to “non-ideal” flows with dead volumes or turbulences that require larger flushing volumes compared to filling volume to ensure the complete flush of the compounds in the collection channel. It is a purely empirical observation, supported also by our tests made on reverse fill/flush modulation system where similar behaviour was detected.

If the sum of the fill and flush distance is lower than the length of the channel, peak tailing occurs. On the contrary, if the sum of the two distances is higher than the length of the modulation channel, peak fronting is perceived. The fronting progressively increases with the sum increase and then double peaks start to occur. The way to understand this is to imagine that for efficient modulation the fill

volume needs to be pushed forward just as much so as to leave the channel in a tight band. If the total travelled distance is shorter than the length of the channel, a part of the band might still be in the channel when the next fill cycle begins, hence this part will follow the main band in a bit slower pace and cause the tailing effect. If the total travelled length is too high, this means that the flushing time is long which gives the time to the part of the next portion of the peak to enter the modulation channel. This part is in the modulation channel when the next fill cycle begins, hence after the flushing it elutes ahead of the main band and causes fronting. If the flush time is too long, then part of the next portion of the peak exits the channel and follows the main peak, hence double peaks are perceived.

The fact that the sum of the fill and flush distances is crucial and not only flush distance by itself is illustrated by comparing the results on the method 1 and 17 in Table 3. At 100°C around which n-C10 elutes, both analysis involve flush distance of 9.6 cm. In the former case, however, fill distance is 3.3 cm and in the later only 1.7 cm. Resultant modulated n-C10 peak is illustrated in Figure 2 for both analysis. It can be seen that in the first case acceptable peak shape is obtained with slight tailing indicating insufficient flushing (Figure 2A), however in the second case sum of fill and flush time is much lower than the length of the modulation channel hence significant portion of the modulated peak remains in the channel waiting for the next modulation cycle, hence double peaks are perceived (Figure 2B).

As it can be seen in Table 3 however, it is difficult to obtain optimal fill and flush distances at all temperatures spanning a wide elution range. During the thermally programed GC analysis run in the constant flow mode, with all other conditions constant, average velocities in the system segments are different at different oven temperatures. Namely, on the temperatures where first analyte elutes, i.e. n-C8: 70°C, average velocity will be lower than the one at heaviest analytes elution temperature, in our case n-C28: 290°C. To illustrate the effect on peak shape, Figure 3 shows generated GC×GC-FID chromatograms for analysed paraffin mixture, obtained at different conditions of ^{2}D flow. In 3A, ^{2}D Flow is 6.3 ml/min, which is too low for efficient flushing of especially lighter analytes, as indicated by their deformed peak shape. It can be seen in the figure how peak shape progressively becomes better from n-C16 to n-C28 as elution temperature increases. In 3B, ^{2}D flow is 13.5 ml/min, which

results in overall better peak shapes. With further increase of ^{2}D flow to 18 ml/min, in 3C it can be seen that peak doubling begins to be perceived. Peak doubling is progressively worse for heaviest paraffins. Calculated total modulation distance (fill + flush) is close to or higher than 20 cm for paraffins for which double peaks are observed (Table 3). In 3D, at 20ml/min ^{2}D flow, we can see how the double peak following the main peak becomes more intensive when compared to the previous figure.

In Table 3, few values are also highlighted in light gray. They correspond to conditions where good peak shape could be expected according to the total modulation distance (sum of fill and flush distance). However small deviations of the peak shape were still perceived. This is explained by the higher ^{1}D flow which was applied in all these instances. In the case of higher ^{1}D flow it can be assumed that during the flush cycle penetration of the column 1 flow into the channel will be more pronounced ('breakthrough') hence decrease of efficiency of the modulation process can be expected.

5.4. Influence of modulation parameters on quantitation

In Figure 4A, the influence of ^{1}D column flow on RRFs of the n-paraffins is depicted. In blue are the RRFs obtained in optimal conditions, i.e. 0.10 ml/min ^{1}D flow. It can be observed that with all other parameters constant, increasing the ^{1}D flow above the conditions ensuring optimal performance causes the perception of progressive loss of heavy analytes. Figure 3B shows the effect of changing the ^{2}D flow, with all other parameters constant. In that case, a ^{2}D flow of 11 ml/min ensures good quantitation and increasing the ^{2}D flow past the optimal flow causes decrease in the detected amount of heavier analytes, while decreasing the flow causes rather the loss of light analytes. The influence of the modulator flush time is illustrated in Figure 3C. Namely, it can be seen that the increase of the injection time from 0.18 s of only 20 ms will be detrimental for the quantitative determination of heavy analytes, while decreasing the injection time for 20 ms will cause the decrease in the detected amount of light analytes. In Figure 3D, the influence of the choice of the modulation fill time can be perceived. It can be observed that decreasing the fill time above optimal value causes deterioration of quantification of light analytes.

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From these experiments, we can conclude that the change of critical modulation parameters carries influence on light and heavy analytes that has different magnitudes, as already seen. Closer inspection of the modulated peaks in the regions of chromatogram where light and heavy n-paraffins elute, indeed demonstrates different levels of asymmetry of the obtained peaks, which also indicates varying efficiency of the modulation process. In order to evaluate the influence of the peak asymmetry on the quantitation performance, RRFs of analysed n-paraffins were compared for the different methods. As demonstrated previously, peak asymmetry factor cannot be regarded as a unique indicator of peak shape and visual inspection is also necessary. However, for peaks that completely return to the baseline between modulations, peak asymmetry factor values 0.85 from to 1.15 did not have a significant adverse effect on the quantification performance, as obtained quantitative results were in the range of acceptable values RRFs ~ 1± 0.1. It was found that acceptable deviations in peak shape are related to the deviation from the total modulation distance (fill + flush distance) of 15 cm for about 2 cm.

To illustrate the relation between the peak shape and quantitative performance, in Figure 5 and 6, chromatograms for methods no. 3 and 6 are compared. In the first case (Figure 5) peak shape is good for n-C10, but small fronting can be perceived for n-C28. In the second case (Figure 6), n-C10 demonstrates small tailing, while n-C28 has suitable asymmetry factor. In Figure 7 are the corresponding calculated RRFs. In red are the RRFs for method no. 3 and in blue are the RRFs for method no. 6. It can be observed that small deviation from perfect peak shape reflects a small loss of response for corresponding analytes, however the effect is not too important. More prominent tailing such as in the case of n-C10 in the second analysis will cause more significant loss of its response.

From these chromatograms and these quantitative results, it is noteworthy to observe that in the case of constant flow conditions and thermally programed GC runs that are classically used, travelling distances for same modulation parameters will always be different for analytes with different elution temperatures. Hence, if perfect modulation distance is achieved for light analytes, this distance is too high for heavy analytes, and vice versa. Thus, perfect peak shape and quantitation cannot be achieved in all areas if the chromatogram in the case of samples with a wide boiling point range and a

compromise has to be found so that the peak asymmetry and quantification for both light and heavy analytes remain satisfactory. Possible solutions to maintain proper peak shape all along the oven program would be for example flow programming in the second dimension which may be tedious, or the modification of the injection time depending on the oven temperature. To date, this last solution is however not available with commercial instruments and software sold with forward fill/flush modulation systems.

6. Conclusion

The most important parameters of the modulation process for forward fill/flush modulation systems were highlighted and their influence explained. Relationship between the fill and flush distances calculated through theoretical considerations and a fluid model of the modulation system and the length of the modulation channel was explored. It was demonstrated that the best results regarding peak shape and quantification are obtained when the sum of fill and flush distances are closed to the length of the modulation channel (15±2 cm). Additionally, even if the total distance is comparable to the length of the channel, but the ratio of flush and fill volume is too small (less than 2.5) or channel filling to fast, in these cases modulation is not efficient. Finally, it was observed that maintaining a proper modulation of light to heavy compounds is not straightforward with commercial instruments and some improvements have been suggested. To conclude, this study shows that for fine tuning of forward fill/flush flow modulation systems, it is always better to estimate quantitative performances by using a test mixture, as alone calculation and looking at peak shape are not enough to guarantee good results.

Credit author statement

Lelevic Aleksandra: Conceptualization, Investigation, Methodology, Writing - Original Draft. **Souchon Vincent**: Conceptualization, Methodology, Supervision, Writing - Review & Editing. **Geantet Christophe**: Conceptualization, Methodology, Supervision, Writing - Review & Editing. ***Lorentz Chantal****:* Supervision, Writing - Review & Editing. **Moreaud Maxime**: Supervision, Writing - Review & Editing.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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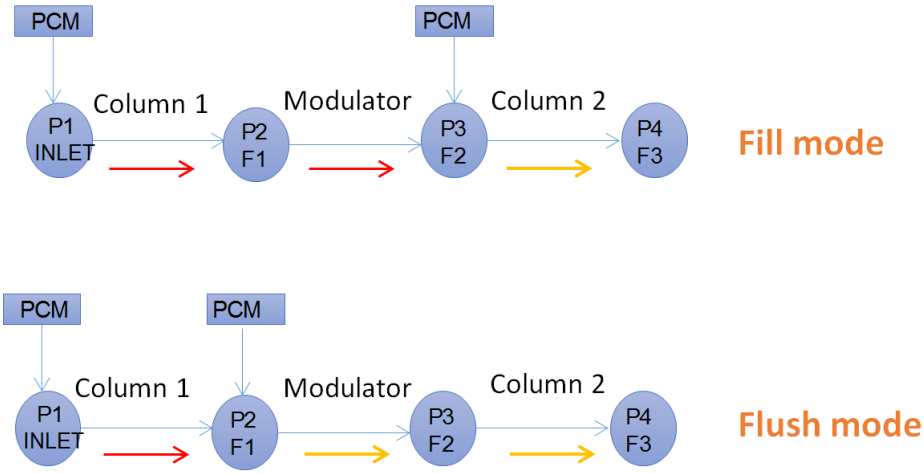
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Figure captions

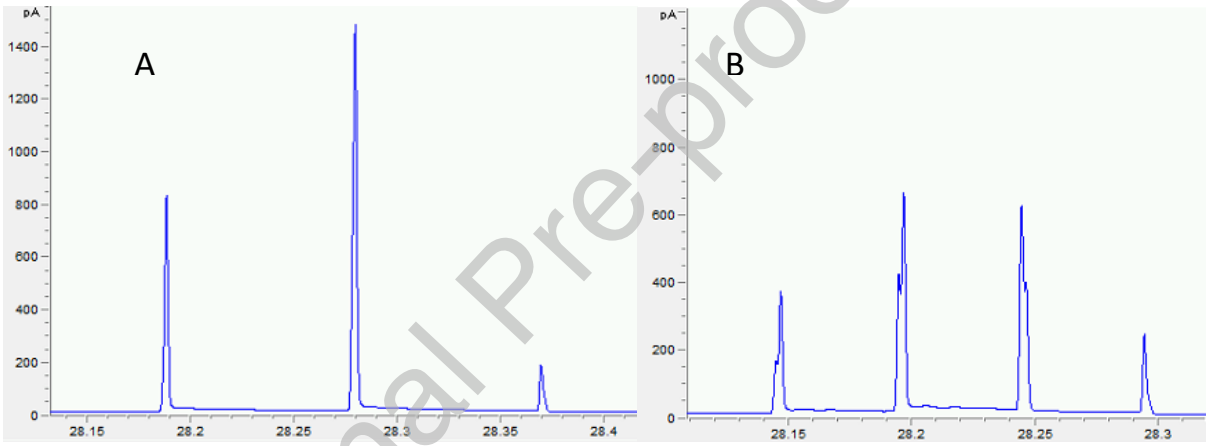
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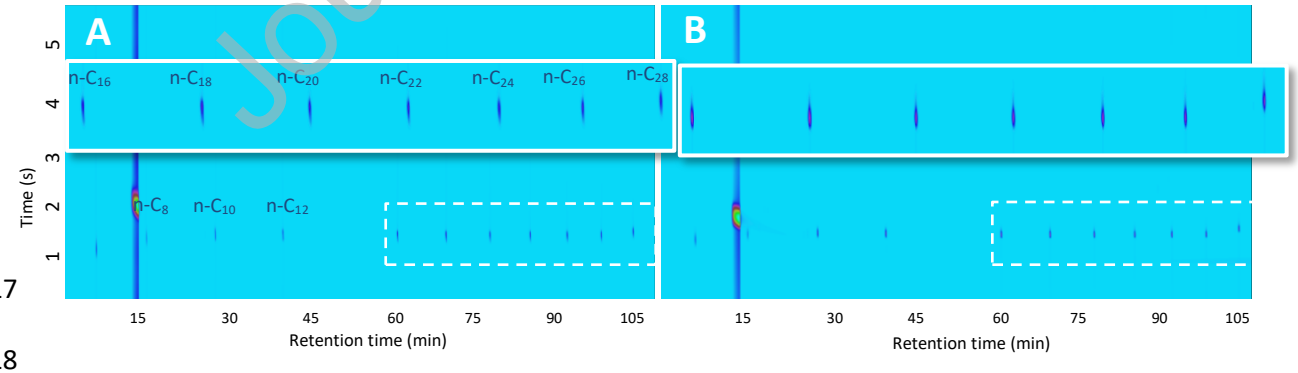
513 Figure 1 Fluid circuit representing the GC×GC system with differential flow modulation; P-pressure;

514 F-flow; PCM-pressure control module.



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516 Figure 2 Modulated peaks of n-C10: (A) Method no. 1; (B) Method no. 17.



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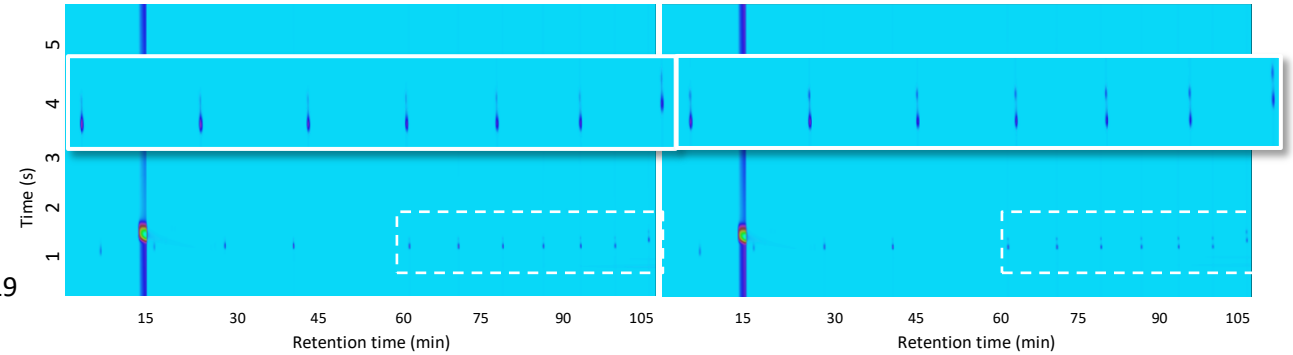


Figure 3 GC×GC-FID chromatograms for n-paraffins n-C8 to n-C28 in toluene at different conditions of 2D Flow : (A) Method no. 2; (B) Method no. 4; (C) Method no. 19; (D) Method no. 20.

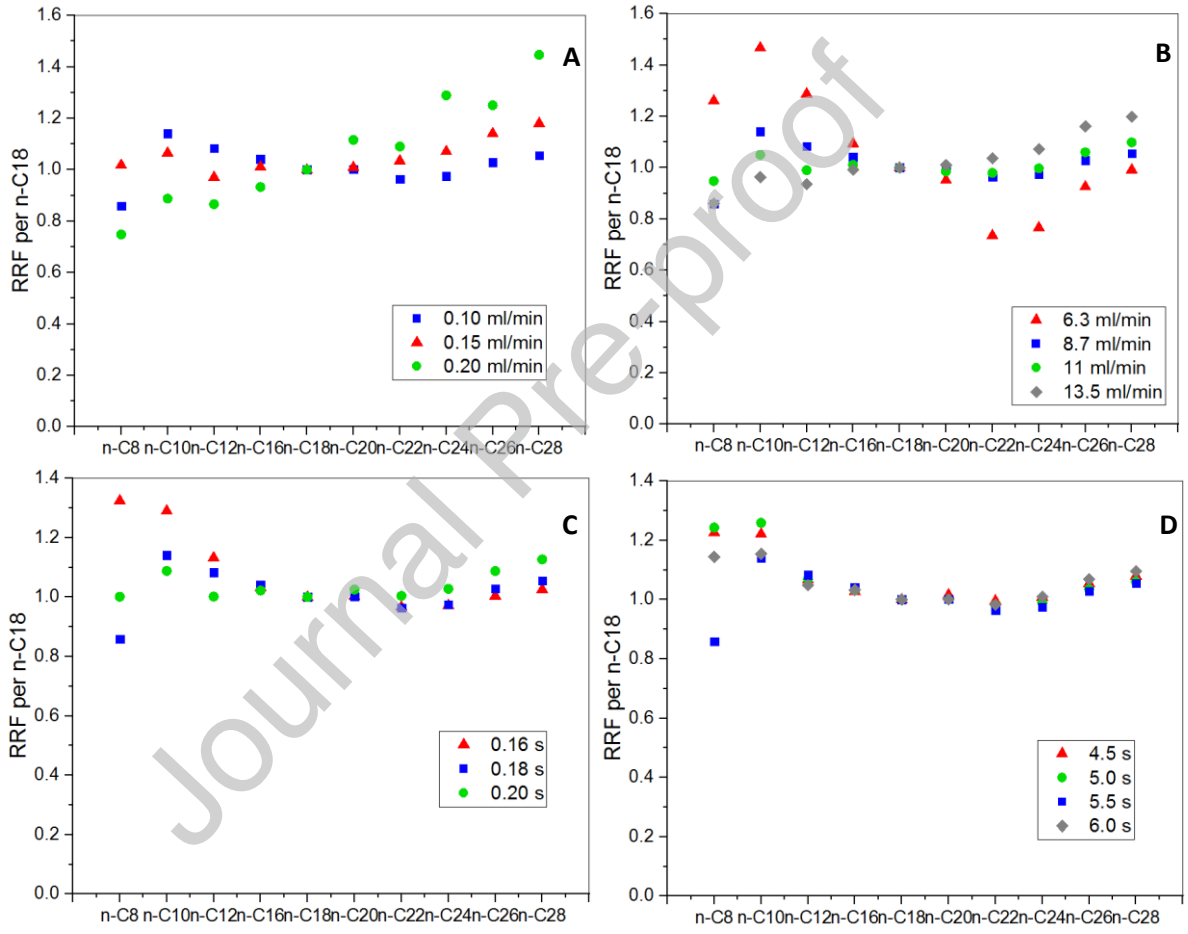


Figure 4 RRFs for n-C8 to n-C28 obtained in different modulation conditions; (A) 2D flow 8.7 ml/min, modulation period 5.5 s, flush time 0.18 s; (B) 1D flow 0.10 ml/min, modulation period 5.5 s, flush time 0.18 s; (C) 1D flow 0.1 ml/min, 2D flow 8.7 ml/min, modulation period 5.5 s; (D) 1D flow 0.1 ml/min, 2D flow 8.7 ml/min, flush time 0.18 s.

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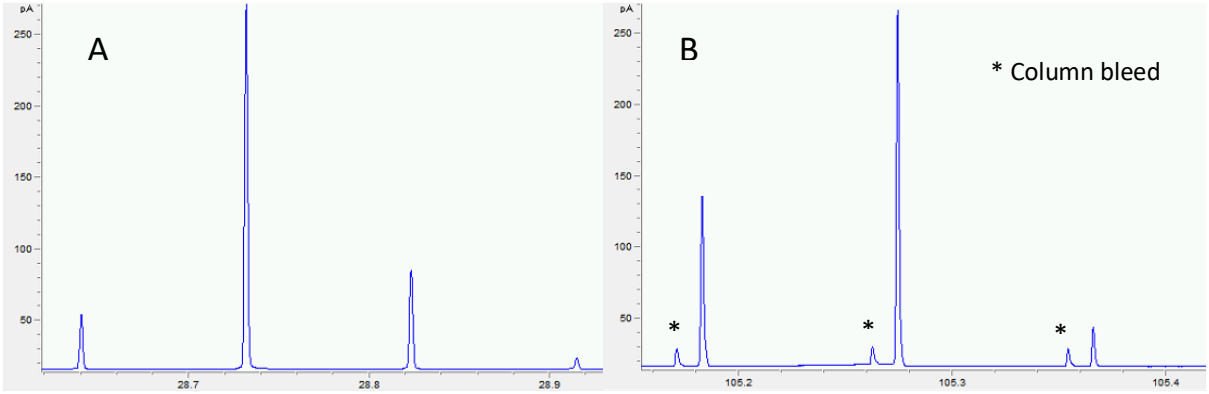


Figure 5 (A) Good peak shape for n-C-10. (B) Deviation from ideal peak shape for n-C28. Method n°3.

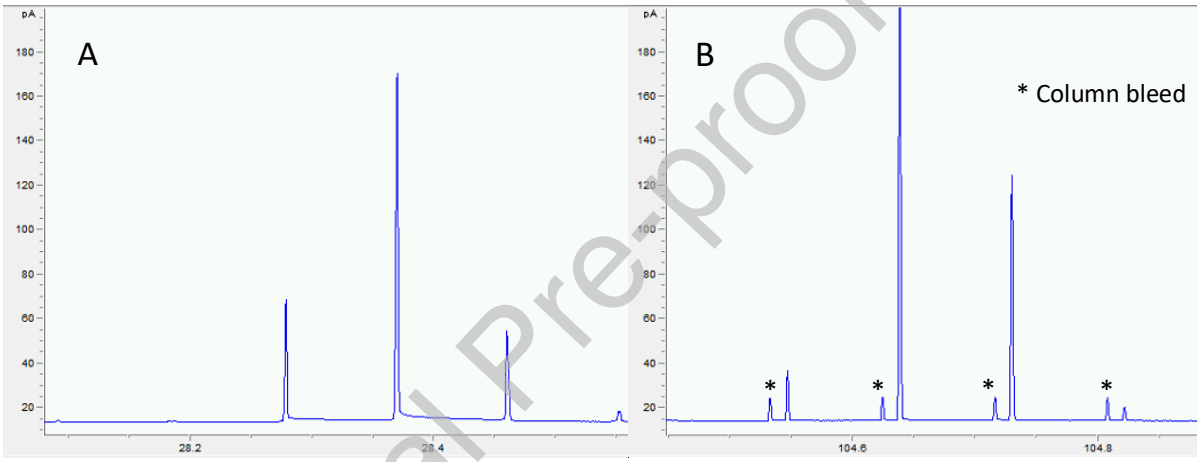
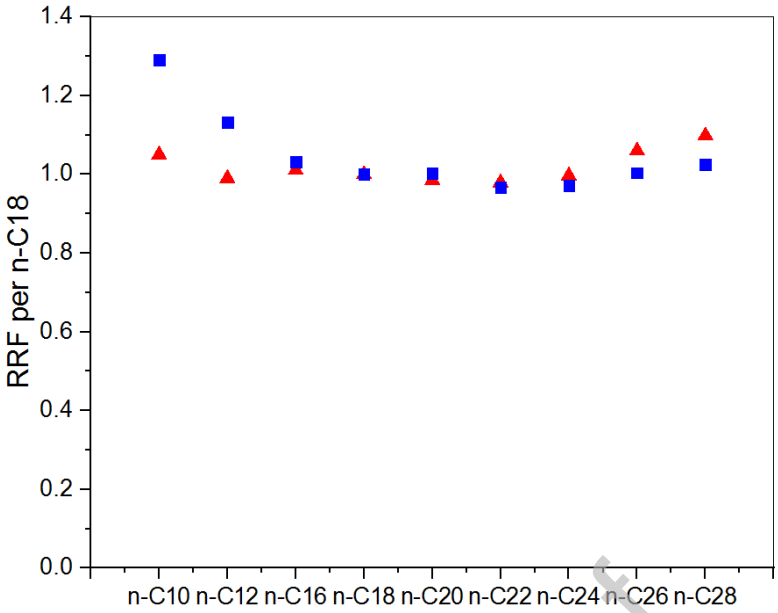


Figure 6 (A) Deviation from ideal peak shape for n-C10 causes quantitation issues. (B) Good peak shape for n-C28. Method n°6.

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535 Figure 7 RRFs, in red: Method n°3; in blue: Method n°6.

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538 **Table 1. Comparison of the advantages and disadvantages of the thermal and flow modulation systems.**

Thermal modulation	Flow modulation
Cold and hot gas jets are employed to efficiently trap and release analytes	**Auxiliary flow of gas is used to direct the flow of analytes into the second dimension**
✓ **No flow limitations (direct MS coupling)** ✓ **Simple and flexible configuration** ✓ **Easier method development** × **More expensive** × **Limited volatility range of analytes**	✓ **More cost effective** ✓ **No volatility limits** ✓ **Robust configuration** × **Laborious optimization** × **Flow limitations (limited coupling to MS)** × **Limited configuration flexibility**

539

540 **Table 2. Used modulation method data.**

Method No.	^{1}D Flow (ml/min)	^{2}D Flow (ml/min)	Modulation Period (s)	Flush time (s)
1	0.10	8.7	5.5	0.18
2	0.10	6.3	5.5	0.18
3	0.10	11.0	5.5	0.18
4	0.10	13.5	5.5	0.18
5	0.10	8.7	5.5	0.20

6	0.10	8.7	5.5	0.16
7	0.10	8.7	6.0	0.18
8	0.10	8.7	4.5	0.18
9	0.10	8.7	5.0	0.18
10	0.15	8.7	5.5	0.18
11	0.20	8.7	5.5	0.18
12	0.20	8.7	3.0	0.18
13	0.10	8.7	5.5	0.22
14	0.10	11.0	5.5	0.22
15	0.10	8.7	5.5	0.26
16	0.10	13.5	3.0	0.18
17	0.10	8.7	3.0	0.18
18	0.10	8.7	8.0	0.18
19	0.10	18	5.5	0.18
20	0.10	20	5.5	0.18

Table 3. Results for the modulation fill and flush distances at different temperatures and under different modulation conditions; also calculated are time to fill and time to flush one volume of the modulation channel. Dark grey shaded data correspond to the total modulation distances where good peak shape was observed (asymmetry factor 1±0.15).

Method No.	Ratio of flush and fill distances	Fill distance (cm)	Flush distance (cm)	Time to fill channel (s)	Time to flush channel (s)	**Total distance (cm)**	Fill distance (cm)	Flush distance (cm)	Time to fill channel (s)	Time to flush channel (s)	**Total distance (cm)**
		70 °C					**100 °C**				
1	2.9	3.2	9.2	25.3	0.23	**12.3**	3.3	9.6	24.2	0.20	**12.9**
2	2.1	3.4	7.1	23.4	0.29	**10.6**	3.6	7.5	22.3	0.27	**11.1**
3	3.7	3.0	10.9	26.9	0.20	**13.9**	3.1	11.3	25.9	0.19	**14.4**
4	4.5	2.8	12.6	28.7	0.17	**15.4**	2.9	13.1	27.7	0.17	**15.9**
5	3.2	3.1	10.2	25.3	0.23	**13.3**	3.3	10.6	24.2	0.22	**13.9**
6	2.6	3.2	8.2	25.3	0.23	**11.3**	3.3	8.5	24.2	0.22	**11.8**
7	2.7	3.5	9.2	25.3	0.23	**12.6**	3.6	9.6	24.2	0.21	**13.2**
8	3.6	2.6	9.2	25.3	0.24	**11.7**	2.7	9.6	24.2	0.23	**12.2**
9	3.2	2.9	9.2	25.3	0.24	**12.0**	3.0	9.6	24.2	0.23	**12.6**
10	1.9	4.7	9.1	16.8	0.20	**13.9**	4.9	9.5	16.1	0.19	**14.5**
11	1.4	6.3	9.1	12.6	0.17	**15.4**	6.6	9.5	12.1	0.16	**16.1**
12	2.7	3.3	9.1	12.6	0.23	**12.4**	3.5	9.5	12.1	0.22	**13.0**
13	3.6	3.1	11.2	25.3	0.23	**14.3**	3.3	11.7	24.2	0.22	**15.0**
14	4.5	2.9	13.3	26.9	0.20	**16.3**	3.1	13.8	25.9	0.19	**16.9**
15	4.3	3.1	13.3	25.3	0.23	**16.4**	3.2	13.8	24.2	0.22	**17.1**
16	8.5	1.5	12.6	28.7	0.19	**14.1**	1.5	13.1	27.7	0.19	**14.6**
17	5.5	1.7	9.2	25.3	0.26	**10.8**	1.7	9.6	24.2	0.25	**11.3**
18	2.0	4.6	9.2	25.3	0.20	**13.8**	4.8	9.6	24.2	0.19	**14.4**
19	6.0	2.5	15.3	31.5	0.15	**17.8**	2.6	15.8	30.6	0.14	**18.4**
20	6.7	2.4	16.4	32.7	0.14	**18.8**	2.5	16.9	31.7	0.13	**19.4**
Method No.	Ratio of flush and fill distances	Fill distance (cm)	Flush distance (cm)	Time to fill channel (s)	Time to flush channel (s)	**Total distance (cm)**	Fill distance (cm)	Flush distance (cm)	Time to fill channel (s)	Time to flush channel (s)	**Total distance (cm)**
		200 °C					**290 °C**				
1	2.9	3.7	10.7	21.6	0.20	**14.5**	4.0	11.6	20.0	0.17	**15.5**
2	2.1	4.1	8.6	19.5	0.23	**12.6**	4.4	9.3	18.0	0.20	**13.7**
3	3.7	3.4	12.6	23.3	0.17	**16.0**	3.7	13.4	21.9	0.15	**17.1**
4	4.5	3.2	14.4	25.1	0.15	**17.5**	3.4	15.3	23.7	0.14	**18.6**

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5	3.2	3.7	11.9	21.6	0.19	**15.6**	4.0	12.9	20.0	0.17	**16.8**
6	2.6	3.7	9.6	21.6	0.19	**13.3**	4.0	10.3	20.0	0.17	**14.3**
7	2.7	4.0	10.7	21.6	0.18	**14.8**	4.4	11.6	20.0	0.17	**15.9**
8	3.6	3.0	10.7	21.6	0.20	**13.8**	3.2	11.6	20.0	0.18	**14.8**
9	3.2	3.4	10.7	21.6	0.20	**14.1**	3.6	11.6	20.0	0.18	**15.2**
10	1.9	5.6	10.7	14.4	0.16	**16.2**	6.0	11.5	13.4	0.14	**17.5**
11	1.4	7.4	10.6	10.8	0.13	**18.0**	8.0	11.4	10.0	0.11	**19.4**
12	2.7	3.9	10.6	10.8	0.19	**14.5**	4.2	11.4	10.0	0.17	**15.7**
13	3.6	3.7	13.1	21.6	0.19	**16.8**	4.0	14.1	20.0	0.17	**18.1**
14	4.5	3.4	15.4	23.3	0.17	**18.8**	3.6	16.4	21.9	0.15	**20.1**
15	4.3	3.6	15.5	21.6	0.19	**19.2**	3.9	16.7	20.0	0.17	**20.6**
16	8.5	1.7	14.4	25.1	0.17	**16.1**	1.8	15.3	23.7	0.16	**17.1**
17	5.5	2.0	10.7	21.6	0.22	**12.7**	2.1	11.6	20.0	0.20	**13.7**
18	2.0	5.4	10.7	21.6	0.16	**16.2**	5.9	11.6	20.0	0.14	**17.4**
19	6.0	2.8	17.2	28.1	0.13	**20.0**	3.0	18.1	26.6	0.12	**21.1**
20	6.7	2.7	18.3	29.3	0.12	**21.0**	2.9	19.3	27.8	0.11	**22.1**

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