Parallel comprehensive two-dimensional gas chromatography

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Highlights:

- A parallel 2GC×2GC approach is introduced.
- Benefit of Independent flow control in each dimension is highlighted.
- Two entirely independent GC×GC separations for each injection are achieved.
- Four retention indices produced by using one injector, four columns and one detector.

ABSTRACT

We introduce an information rich analytical approach called parallel comprehensive two-dimensional gas chromatography (2GC×2GC). This parallel chromatography approach splits injected samples into two independent two-dimensional column ensembles and provides two
GC×GC separations by using contra-directional thermal modulation. The first-dimension (1D) and second-dimension (2D) columns are connected using planar three-port microchannel devices, which are supplied with supplementary flow via two pressure controller modules. Precise carrier gas flow control at the junction of the 1D and 2D columns permits independent control of flow conditions in each separation column. The 2GC×2GC approach provides two entirely independent GC×GC separations for each injection. Analysis of hop (Humulus lupulus L.) essential oils is used to demonstrate the capability of the approach. The analytical performance of each GC×GC separation in the 2GC×2GC experiment is comparable to individual GC×GC separation with matching column configurations. The peak capacity of 2GC×2GC is about 2 times than that of single GC×GC system, provided with the obtained modulation ratio (MR) of about 2 and 4 for the 2GC×2GC and GC×GC system, respectively. The dual 2D chromatograms produced by this single detector system provide complementary separations and additional identification information by harnessing different selectivity provided by the four separation columns.

Keywords: Gas chromatography; Comprehensive two-dimensional; 2GC×2GC; GC×GC; Contra-directional modulation ; Flow control

1. Introduction

With the aim of providing enhanced qualitative information compared to conventional comprehensive two-dimensional gas chromatography (GC×GC), Savareear et al. introduced two closely related multiplexed GC×GC approaches utilising contra-directional thermal modulation [1, 2]. Unlike other multi-column GC×GC methodologies [3-6], these multiplexed techniques employ a single detector to generate two two-dimensional (2D) separation windows for each injection within a single detector channel. Complementary separation and enhanced qualitative information were provided due to the selectivity differences between the different multiplexed stationary phase columns. By nature of the
multiplexed approaches, a fast second-dimension (\(2^D\)) separation is especially important to avoid overlap of the two separation windows in the single chromatogram [1]. Therefore the investigators configured their setups by using relatively short (e.g. 0.4 m) and narrow internal diameter (e.g. 100 \(\mu\text{m}\) i.d.) \(2^D\) columns coupled with conventional first-dimension (\(1^D\)) columns (i.e. 30 m \(\times\) 250 \(\mu\text{m}\) i.d.) [2]. However, these \(2^D\) column dimensions might not be the best choice for \(\text{GC} \times \text{GC}\) analysis [7]. These \(2^D\) columns lead to a considerably faster analysis, but at the expense of providing insufficient separation and band broadening due to overloading compared with longer and wider bore \(2^D\) columns (e.g. 1 m \(\times\) 250 \(\mu\text{m}\) i.d.).

Flow-mismatch between the two separation dimensions is a typical \(\text{GC} \times \text{GC}\) problem stemming from the coupling of columns with considerably different internal diameter \(1^D\) (regularly 250 \(\mu\text{m}\)) and \(2^D\) columns (often 100 \(\mu\text{m}\)). This flow-mismatch can be resolved by adopting a wider \(2^D\) column or by using \(1^D\) and \(2^D\) columns with the same internal diameter [5]. Flow conditions closer to optimal can be achieved in both dimensions, when columns with homologous internal diameter are used, leading to improved exploitation of the \(2^D\) stationary phase selectivity and increased \(2^D\) sample capacity [7-9]. A recent study has experimentally demonstrated that the use of homologous column diameter in both dimensions can substantially reduce the detrimental effect on underutilisation of the primary peak capacity (\(1^U\)) compared to that of comparatively narrow \(2^D\) column. Employing homologous column diameters in both separation dimensions is one of the key factors enabling a near-theoretical maximum in peak capacity gain (\(G_n\)) [10].

In the present investigation we employed a simple but effective method of independently controlling carrier-gas flow in the first- and second-dimensions. Recently, Luong \textit{et al.} utilised auxiliary flow control between the \(1^D\) and \(2^D\) columns for the purpose of \textit{Retention Time Locking} and \textit{Back-Flushing} in \(\text{GC} \times \text{GC}\) [11]. Similarly, changing the carrier-gas pressure at the junction of two series-coupled capillary GC columns has been reported as a versatile approach to achieve the best possible separation of multicomponent mixtures [12-14]. By changing the junction pressure, it is able to alter the relative retention position of components, and therefore adjust the selectivity of the column ensemble leading to optimal
separation. Independent control of flow conditions also greatly assists development of multi-column GC×GC approaches. To this end, we introduce a four-column multiplexed technique with two independent 1D columns each coupled to its own 2D column, and employ an additional gas supply and precise electronic pressure control at the midpoint between the two dimensions. Comparison of analyses with and without independent 2D flow control are made using otherwise matching column sets and the importance of applying flow control to adjust the separation speed in 2D is outlined. The capability of independent flow controlled 2GC×2GC is demonstrated by the analyses of hop (Humulus lupulus L.) essential oil with two column combinations comprising non-polar × polar and polar × non-polar column sets. Important features of the proposed independent flow controlled 2GC×2GC approach compared to multiplexed approaches introduced by Savareear et al. are discussed.

2. Experimental

2.1 Chemicals and reagents

Hop (Humulus lupulus L.) essential oil was prepared by hydro-distillation of dried hop cones (Hop Products Australia, North Hobart, Australia), and was diluted (1:20, v/v) in dichloromethane (Sigma-Aldrich, Castle Hill, Australia) prior to GC analyses.

2.2 Instrumentation and experimental conditions

All analyses were performed using a Leco GC×GC-FID instrument with an LN2 Cooled Thermal Modulator (LECO Australia, Castle Hill, Australia). The chromatograph was equipped with a split/splitless injector, operated with a 20:1 split ratio and inlet temperature of 200 °C. A 1 μL sample volume was injected. Hydrogen carrier-gas was supplied using a Parker Balston H2PEM-260 generator (Parker Hannifin, Castle Hill, Australia). Effluent from each secondary column was monitored by a single flame ionization detector (FID) operated at 100 Hz and 250 °C. Data were collected and processed using Leco ChromaTOF software.

2.3 GC×GC with independent flow control
Independently flow controlled GC×GC analyses were performed by using two different column combinations. A 60 m × 250 μm i.d. × 0.25 μm d_Γ SGE BPX5 column was used as the 1D column, and a 1.2 m × 250 μm i.d. × 0.25 μm d_Γ SGE SolGel-Wax column was used as the 2D column. The two columns were connected using a SilFlow 3-port micro channel device (Trajan Scientific and Medical, Ringwood, Australia). The 2D column was installed in the regular configuration through the GC×GC modulator and secondary oven. An auxiliary pressure controller module (PCM; Agilent Technologies, Mulgrave, Australia) was connected to the central port of the SilFlow device with 1.1 mm outside diameter SilFlow stainless steel capillary tubing sleeved to 1/16” at one end for connection to the PCM. A schematic diagram of the system is shown in Fig. 1. Carrier-gas flow rate in 1D was set at 2.5 mL/min; while 2D carrier-gas flow rate was set at 2.5 mL/min. The primary oven temperature program was 50 °C (1.1 min hold) to 245 °C (0.9 min hold) ramped at 3.5 °C/min. The 2D column offset was set at +15 °C from the primary oven, and modulator temperature offset was set at +25 °C relative to the secondary oven. The modulation period was of 2.0 s (hot pulse of 0.6 s) was used throughout. Another column combination comprising a 60 m × 250 μm i.d. × 0.25 μm d_Γ SGE SolGel-Wax column and 1.2 m × 250 μm i.d. × 0.25 μm d_Γ SGE BP10 was operated using the same conditions described above. All separation columns were from Trajan Scientific and Medical.

2.4 GC×GC without independent flow control

GC×GC analyses without independent flow control were performed using the column combinations and conditions described in Section 2.3, except that the 1D and 2D columns were connected directly using press-tight connectors (Restek Corporation, Bellefonte, PA). The carrier-gas flow rate was set at 2.5 mL/min for all experiments.

2.5 2GC×2GC with independent flow control

Independently flow controlled multiplexed 2GC×2GC analyses were achieved by using contra-directional modulation. Two parallel 2D columns were installed contra-directionally in the GC×GC modulator. All 2GC×2GC analyses were performed by splitting the flow from
the inlet into two 1D columns by means of a twin-hole graphite ferrule (Trajan). The two 1D columns used were: 1D1 (A) SGE BPX5 60 m \times 250 \mu m i.d. \times 0.25 \mu m d_i; 1D2 (B) SGE SolGel-Wax 60 m \times 250 \mu m i.d. \times 0.25 \mu m d_i. Each 1D column was connected to one 2D column using a SilFlow 3-port microchannel device, and two PCMs were connected to the central port of each SilFlow device. The two 2D Columns used were: 2D1 (C) SGE BP10 1.2 m \times 250 \mu m i.d. \times 0.25 \mu m d_i; 2D2 (D) SGE SolGel-Wax 1.2 m \times 250 \mu m i.d. \times 0.25 \mu m d_i. Flow from the two 2D columns was directly passed into a single FID by means of a twin hole graphite ferrule. For convenience of column installation and operation, the secondary oven was removed from the 2GC×2GC system. All four columns were heated using the main GC oven. An instrument schematic of the multiplexed independently flow controlled 2GC×2GC analytical system is illustrated in Fig. 2. The carrier-gas flow rate used in 1D was 1.2 mL/min; while the 2D carrier-gas flow rate used was 2.5 mL/min. To maintain appropriate separation space between the two separation windows in the chromatogram the total modulation period used was 4.0 s (hot pulse 1.6 s). The oven temperature program used was 50 °C (1.1 min hold) to 245 °C (0.9 min hold) ramped at 3.5 °C/min. The modulator temperature offset was set at +15 °C.

2.6 2GC×2GC without independent flow control

Multiplexed 2GC×2GC analyses without independent flow control were performed by using two sets of column combinations. The first column set used was the same as the independently flow controlled 2GC×2GC experiments. Total carrier-gas flow rate was set at 2.5 mL/min. Another column set used comprised a 60 m \times 250 \mu m i.d. \times 0.25 \mu m d_i SGE BPX5 1D column with a 0.45 m \times 100 \mu m i.d. \times 0.1 \mu m d_i Rtx-Wax 2D column along with a 60 m \times 250 \mu m i.d. \times 0.25 \mu m d_i SGE SolGel-Wax 1D column with a 0.45 m \times 100 \mu m i.d. \times 0.1 \mu m d_i SGE BPX35 2D column. Total carrier-gas flow rate was set at 1.0 mL/min. All separation columns were from Trajan Scientific and Medical, except the Rtx-Wax column ((Restek, Bellefonte, PA). All column connections were made using Restek press-tight connectors. All other configurations and conditions are identical to those described in Section.
3. Results and discussion

3.1 Performance and benefits of GC×GC-flow control system

The first part of this study was carried out by employing precise 2D flow control using an auxiliary PCM connected to a microchannel device at the union of the two separation columns. Close to optimal flow conditions in both dimensions can be achieved in a GC×GC column set having the same internal diameter in the first- and second-dimensions [7, 9].

Aligned with current thinking, we expected 1D head pressure to provide sufficient pneumatic control to achieve the best GC×GC results, since the optimal flow rate in each column is the same. However, it was evident that independently controlling 2D flow rate in GC×GC analysis of real-world samples provided a substantial improvement. Here, the flow rate was set at Speed Optimised Flow of 2.5 mL/min (hydrogen) [15]. Fig. 3 and Fig. 4 show the chromatograms obtained (A) without and (B) with independent flow control for the analyses of hop essential oil using BPX5×SolGel-Wax and SolGel-Wax×BP10 column combinations, respectively. The 2D separation space was inadequate in both column combinations without independent flow control (Figs. 3A and 4A) when a modulation period of 2 s was used. Some analytes were not eluted in their own modulation cycle from the 2D column, even when a 15 °C higher 2D oven temperature (which is the highest column offset commonly recommended) was applied. As a consequence, the ordered chromatographic structures are obscured and cannot be readily observed in the contour plots. In contrast, this problem was ameliorated by independently controlling flow in the two separation dimensions of the GC×GC system. All analytes were eluted from the 2D without wrap-around and displayed improved ordered structure (Figs. 3B and 4B). By applying a midpoint supplementary flow of carrier-gas, the head pressure of 2D column is adjusted to give an appropriate compression corrected pressure drop across the entire column set, and in turn have an appropriate correction of flow rate in both dimensions [16]. In the present study, the increased 2D head pressure leads to the
increased pressure drop in both 1D and 2D columns. This results in a decrease in carrier-gas velocity (increased hold-up time) for 1D and an increase in carrier-gas velocity (decreased hold-up time) for 2D. Hence, the analytes’ elution/retention time increased in the 1D column, and decreased in the 2D column. The observed small retention changes in 1D are due to the very long 1D column (60 m), which weakens the pressure variation. On the other hand, the elution time substantially reduced in the 2D corresponding to the great effect of decreased hold-up time for this short dimension, resulting in considerably reduced 2D peak widths. Consequently, improved peak shapes were obtained and more separation space can be provided in the 2D of the corresponding portions, as exemplified by the comparison between insert A2 and B2 (where a different shading was used for the colors indicating the peak intensity) derived from the marked out areas A1 and B1 in Fig 3. This practical advantage might be critical when analysing more complicated samples, in terms of separation efficiency. Moreover, the proposed approach also permits independent control of flow conditions in each dimension of the GC×GC system to maximise resolution by simultaneously achieving close to optimum flow rate in both columns. For instance, Efficiency Optimised Flow (i.e. 1.8 mL/min for present case) and Speed Optimised Flow (i.e. 2.5 mL/min for present case) can be readily applied to 1D and 2D, respectively.

Table 1 shows the effect of independent flow control on retention time, peak width at half-height and peak intensity for the selected peaks indicated in Fig 4. A wide range of variation for apparent 2D retention time between the two systems was presented, and the retention time differences are highly affected by wrap-around in the dependent flow-controlled experiment. A slightly extended retention for all peaks in 1D was displayed when flow control was applied, with 1.5% to 4.3% increases. Noticeably, with flow control the peak width at half-height in 2D was considerably reduced compared to without flow control, with the reduction percentage ranges from −12% to −43.6%, due to the provided supplementary flow for the 2D and higher elution temperature from 1D. Narrower peak widths in 2D can provide greater peak capacity and better resolution. Furthermore, peak compression and narrow peaks leads to concomitant increased response signal. Consequently, higher peak intensity was obtained
with independent flow control, with the percentage increases ranging from 3.5% to 38.4% compared to the system without independent flow control.

3.2 Performance and benefits of 2GC×2GC-flow control system

As a continuation of the previous multiplexed studies [1, 2], the current 2GC×2GC approach comprises two parallel and independent column combinations (non-polar×polar and polar×non-polar) with homologous 250 μm internal diameter. Correction of the pressure drop across each dimension was operated by applying flow control at the junction of each 1D and 2D column.

Two 2D columns were installed contra-directionally in the Leco dual-stage modulator. As shown in Fig. 2, the first column configuration comprising columns B and C was installed conventionally in the GC×GC system, namely from bottom to top via the modulator. The modulation period of this column set is identical to the set modulation time (i.e. 4.0 s). The second column configuration comprising columns A and D was installed contra-directionally (from top to bottom via the modulator). Hence the two modulation stages are inverted compared to the conventional setup. The modulation timing parameters (cool time till off/on, cool off/on time, heat time till on/off and heat on/off time) indicated that the cool time in the second-stage (top) of the modulator is always longer than that of the first-stage (bottom), which results in a defined time-interval of the whole modulation process between the two column sets. After calculating and validating the default modulator valve timing according to different set modulation period, it shows that this time-interval is exactly half of the set modulation period (results shown in Table S1). As a result, this multiplexed approach can be achieved without manual customisation of the modulation timing parameters [1, 2]. In this way, appropriate selection of the time-interval (or modulation period) can permit the peaks eluted from the alternate 2D separation to be separated completely in the two 2D separation windows within the single chromatogram.

Due to the nature of the multiplexed approach, the modulation period must be double to provide adequate separation space for the eluted peaks from the alternate 2D columns [1].
Under such conditions, in order to minimise modulation-induced loss of $^1$D resolution by using a minimum modulation period, one of the strategies is to obtain twice as fast $^2$D separations by using relatively short and narrow $^2$D columns. However, these $^2$D columns are not ideal for the analysis of complex real-world samples. The chromatogram of obtaining $0.45 \text{ m} \times 100 \text{ μm} \text{ i.d.}$ $^2$D columns for the analysis of hop essential oil is presented in Fig. S1(A). It shows that the desired fast analysis in the $^2$D was produced, since a 2 s separation window is sufficient to fit the peaks eluted from each of the $^2$D columns. However, this comes at the expense of providing insufficient separation when compared with wider bore $^2$D columns (e.g. $250 \text{ μm} \text{ i.d.}$), especially for the non-polar×polar column combination, presumably due to the extremely high carrier gas velocity. Substantially improved separation in the $^2$D was achieved when the diameter of the polar $^2$D column was changed from $100 \text{ μm}$ to $250 \text{ μm}$ (Fig. S1(B)). These results demonstrate that it is necessary to employ a $250 \text{ μm} \text{ i.d.}$ capillary as $^2$D column for the analysis of complex samples.

In order to maintain the same efficiency as the column dimension of $0.45 \text{ m} \times 100 \text{ μm} \text{ i.d.}$, 1.2 m length should be chosen when using the $250 \text{ μm} \text{ i.d.}$ column. However, it will contradict the requirement of a fast $^2$D separation if $1.2 \text{ m} \times 250 \text{ μm} \text{ i.d.}$ $^2$D columns were employed for the proposed 2GC×2GC approach. As shown in Fig. 5, the peaks eluted from the two column combinations were severely re-mixed with each other when a 4 s modulation period was applied. Despite of the careful adjustment of oven ramping ($3.5 \text{ °C/min}$, $5 \text{ °C/min}$, and $7 \text{ °C/min}$), flow rate ($2.5 \text{ mL/min}$, $3.5 \text{ mL/min}$), and modulation period (4 s, 5 s), insufficient separations of many overlapped peaks were still observed.

In this instance, adjustment of $^2$D column head pressure (i.e. alteration of $^2$D flow rate) can provide a more promising alternative on tuning the dual $^2$D separation in a 2GC×2GC approach while using $1.2 \text{ m} \times 250 \text{ μm} \text{ i.d.}$ $^2$D columns. The effectiveness of integrating flow control with this developed multiplexed system to adjust the separation speed in $^2$D is shown in Fig. 6. Two completely separated GC×GC chromatograms can be clearly viewed in a single window, and without peaks from either separation interfering with peaks from the
other (Fig. 6B). Moreover, satisfactory separations were obtained for both column combinations. The capability of implementing independent control of flow conditions in each dimension by this integrated setup allows easy fine-tuning for the parallel systems. In the present study, *Speed Optimised Flow* was applied for each of the 2D columns, while a slightly sub-optimal 1D flow rate was applied. Although the low 1D flow rate has a negative effect on 1D resolution, a higher modulation ratio resulted from the broadened 1D peaks can be obtained to compensate the doubled modulation period. Moreover, analytes will be eluted from the 1D at higher temperatures, increasing the separation speed in 2D. The average peak width at base in 1D ($\omega_b$) of about 9 s and 8 s has been obtained for the 2GC×2GC and GC×GC system, respectively (data calculated based on the 10 selected peaks in Fig. 6). By employing a modulation period of 4 s, the obtained modulation ratio ($M_R$) is at least 2 in the 2GC×2GC experiment, which is sufficient for semi-quantitative analysis purposes according to the recommendations of Khummueng *et al.* [17]. Using a longer 1D column (e.g. 73 m) at *Efficiency Optimised Flow Rate* would be a preferred approach.

Comparison of the chromatograms obtained from 2GC×2GC (Fig. 6B) and two individual GC×GC systems (Fig. 6A and 6C) with matching column configurations for the analyses of hop essential oil, shows that the integrity of the two separately performed GC×GC separations are well preserved in the 2GC×2GC experiment. The corresponding chromatograms present remarkable congruence and the number of peaks separated in each GC×GC analysis is similar to the corresponding portion of the 2GC×2GC analysis. Moreover, these complementary sets of information provided by the two different column combinations can be acquired simultaneously with a single injection. Consequently, there is a considerable gain of time and a two-fold increase of sample throughput without any loss of quality. Additionally, according to the GC×GC peak capacity equation $n_{c,2D}=\frac{1_t}{M_R^2\omega_2}$ [18], where $1_t$, $1M_R$ and $2\omega_2$ are the first dimension run time, modulation ratio, and the second dimension peak width at the base, respectively. The peak capacity production ratio for GC×GC and 2GC×2GC system for a similar $1_t$ and $2\omega_2$, is given by $n_{c,2GC×2GC}/n_{c,GCGC}=\frac{M_{R,GCGC}}{M_{R,2GC×2GC}}$. Hence, it is obvious that the peak capacity production of 2GC×2GC is about 2 times...
than that of single GC×GC system, provided with the obtained $M_R$ of about 2 and 4 for the 2GC×2GC and GC×GC system, respectively.

In order to demonstrate the potential and practical usefulness of the integrated 2GC×2GC with independent flow control in more detail, parameters such as repeatability of retention times, peak areas and peak widths at half-height were investigated. Repeatability in both column combinations was calculated as the relative standard deviations (RSD) of five consecutive injections of a hop essential oil sample. Results for selected peaks are summarised in Table 2. For all analytes, RSD were found to be below 0.5% and 2.5% ($n = 5$) for the $^1$D and $^2$D retention times, respectively. Acceptable RSD were attained for both peak areas (5.3% – 7.3%) and half-height peak width (2% – 4.2%). The results indicated that the multiplexed system could be used as an interesting approach for in depth characterisation of complex samples. Although the proposed approach provided two $^1$D and two $^2$D retention times for a single analyte, no attempt was made to assign identity to any of the components in hop essential oil. Based on the previous study [2], the approach described here is amenable to coupling with mass spectrometry without any further modification of the instrumental configuration. Coupling with mass spectrometry would facilitate further sample characterisation.

4. Conclusions

Appropriate flow control at the junction of $^1$D and $^2$D columns permits the possibility and simplicity of implementing GC×GC and 2GC×2GC experiments using 250 μm homologous i.d. column combinations. The proposed integrated system is robust and has the unique ability to design two entirely independent conventional GC×GC separations for each injection, yielding two independent GC×GC chromatograms viewed in a single window leading to an appreciable gain of productivity. The analytical performance and information content of each GC×GC separation in the 2GC×2GC experiment is comparable to individual GC×GC system with matching column configurations. The dual GC×GC chromatograms produced by this
single detector system provide complementary separation and additional identification information due to the different selectivity of the four separation columns, which is highly promising for qualitative analysis. Coupling 2GC×2GC to mass spectrometry: four independent retention times (or retention indices) with mass spectrometry should provide unequivocal assignment of individual peak identity in multicomponent samples.

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References


[17] W. Khummueng, J. Harynuk, P.J. Marriott, Modulation ratio in comprehensive two-

Figures and Tables

**Fig. 1.** A schematic diagram of the GC×GC-flow control analytical system.

**Fig. 2.** Instrument schematic of the multiplexed 2GC×2GC-flow control system.

**Fig. 3.** GC×GC-FID chromatograms obtained (A) without and (B) with independent flow control for analyses of hop essential oil using BPX5 × SolGel-Wax column combination. Inserts A2 and B2 are blow-ups of area A1 and B1, respectively.

**Fig. 4.** GC×GC-FID chromatograms obtained (A) without and (B) with independent flow control for analyses of hop essential oil using SolGel-Wax × BP10 column combination.

**Fig. 5.** Two-dimensional separation space for analyses of hop essential oil obtained using long (1.2 m) and wide-bore (250 μm i.d.) 2D columns using 2GC×2GC without independent flow control system.

**Fig. 6.** Comparison of GC×GC-flow control (A, C) and 2GC×2GC-flow control (B) chromatograms for the analyses of hop essential oil.
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2GC×2GC  

Re-submission Version
Yan et al.

2GC×2GC

Re-submission Version
Table 1 Comparison of first dimension retention time and apparent second dimension retention time ($t^1_{R}$ and $t^2_{R}$, respectively), half-height peak width ($\omega_h$) and peak intensity for selected peaks (indicated in Fig. 4) obtained with or without flow control (FC) using SolGel-Wax $\times$ BP10 column combination.

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Table 2 Repeatability of selected peaks (indicated in Fig. 6) in hop essential oil with 2GC×2GC-flow control system.

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