

# A Web-Based Software Tool to Assist with GC×GC Method Development

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Comprehensive two-dimensional gas chromatography (GC×GC) has shown significant advantages over one-dimensional gas chromatography (GC) including increased peak capacity, signal enhancement and structured chromatograms. However, since its invention over 25 years ago, the technique has not been adopted as widely as expected. Two reasons that have been suggested for this slow adoption are difficulty in creating and optimising a GC×GC method, as well as understanding what constitutes a 'good' GC×GC method. This article describes a web-based software tool which guides the user through a step-by-step process of developing an optimised GC×GC method.

## Introduction

Gas chromatography (GC) has been a well-established analytical technique for over half a century, and it has gained wide acceptance for routine analyses. However, for complex samples the separation power of GC is insufficient in many cases. In 1991 Liu and Phillips introduced comprehensive two-dimensional gas chromatography (GC×GC) as it is now practiced [1]. Many believe this was a major step in GC development. Since then, the technique has developed into one of the most powerful separation techniques available [2, 3]. Over the years the technique and instrumentation have developed significantly and commercial instruments have become available [4]. The demonstrated performance has met the promised potential including near-theoretical, order-of-magnitude peak capacity gain [5], signal enhancement [6], and structured chromatograms [3]. However, given these significant benefits, the technique has not been widely adopted even though several validated methods have been published [7-9]. One of the reasons suggested for this slow adoption is difficult method development and users focused on their applications may not possess the necessary chromatographic expertise or interest in the chromatography for such method development.

In GC there are many established methods (regulatory, literature, column suppliers, instrument suppliers) from which an analyst may choose to start. From these established methods the analyst can then make basic method changes (heating rate, stationary phase) to adapt the method to their particular sample. In GC×GC, although

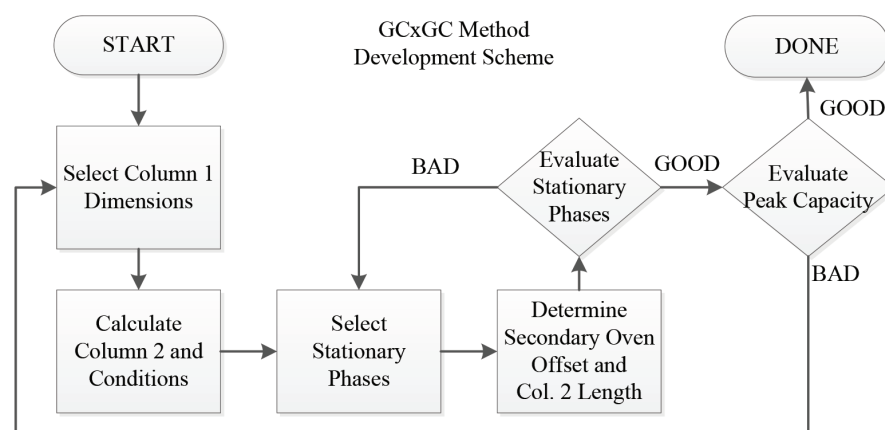


Figure 1. Flow chart outlining the basic steps that Simply GC×GC utilises to guide analysts through the method development process.

standard methods and procedures are available, there is not the same broad-based knowledge that exists with GC methods today. To develop a GC×GC method one must either start from the beginning or look for examples in the literature. Unfortunately, the literature contains some methods that are less than optimal. In some cases the peak capacity is reduced because the first dimension resolution is reduced by excessively long modulation periods and in other cases peak capacity may be similar to optimal, but the analysis time is significantly longer because of method conditions and/or instrumentation. To then modify the many parameters in a method to meet your purposes, the challenges are new and different from those of GC. There are descriptions in the literature of how varying certain parameters influence GC×GC separations starting with an early paper by Dallüge et al. in 2002 [10] and more recently by Mostafa et al. in 2012 [11], but these descriptions though good, do not provide an analyst with a straightforward, logical

path to an optimised GC×GC method. In fact, Mostafa et al. stated, "Therefore, it is quite obvious that GC×GC method optimisation is not straightforward, which is probably one of the reasons for the still somewhat limited usage of the technique."

This article describes a free, web-based software tool called Simply GC×GC™ (<https://www.leco.com/simple-gcxcgc>) that guides an analyst in a step-by-step manner through the process of developing a GC×GC method optimised for peak capacity and the utilisation of the two-dimensional separation space. The software tool (referred to simply as 'tool' from this point on) utilises theoretical calculations to compute optimum column dimensions and conditions, provides guidance in stationary phase selection and experimental steps to optimise the secondary column length and secondary oven temperature offset, and provides suggestions for adjusting the peak capacity as appropriate for the complexity of the sample.

**Guide Me**

- Convert 1D to GCxGC
- Stationary Phase Combinations
- Evaluate Sample Loading
- Determine Secondary Oven Offset
- Evaluate Stationary Phase
- Evaluate Peak Capacity
- Increase Peak Capacity
- Increase Secondary Column Peak Capacity
- Decrease Run Time
- Units / Settings
- Glossary
- Contact Us

**Determine Secondary Oven Offset**

Retention time on primary column (seconds): 710

Retention time on secondary column with offset of 5 °C (seconds): 1.075

Retention time on secondary column with offset of 40 °C (seconds): 0.620

Previous Next

Step 14 of 19

**History**

#	Run Time	PC 1	PC 2	PC Net
6	26 min	305	13	2566
5	26 min	305	13	2566
4	26 min	305	13	2566
3	26 min	305	13	2566
2	26 min	305	13	2566
1	26 min	305	13	2566

**Summary**

Detailed

**Overview**

Peak Capacity Gain: 6.2  
Run Time: 26 min  
Acquisition Rate: 298 sps

**Columns**

**Primary Column**  
Stationary Phase: Rxi-17Sil MS  
30 m x 0.25 mm x 0.25 µm

**Secondary Column**  
Stationary Phase: Rxi-5 MS  
0.45 m x 0.25 mm x 0.25 µm

**Transfer Line**  
0.21 m x 0.25 mm x 0.25 µm

**Void Time**  
Secondary Column: 0.21 sec

**Inlet**

Carrier Gas: Helium  
Flow: 1.4 ml/min  
Inlet Pressure at 300 °C: 28 psi  
Primary Column Velocity: 40 cm/sec

**Temperature**

**Oven Temperature Program**  
Initial Temperature 40 °C for 0.20 min  
10.2 °C/min to 300 °C  
Secondary Oven Offset: 5 °C  
Transfer Line Temperature: 340 °C

**GCxGC**

Optimal Modulation Period: 1.1 sec  
Method Modulation Period: 10 sec  
Method Hot Pulse Time: 3.0 sec

Figure 2. Example view of Simply GCxGC. Standard view consists of four parts: steps of software tool (left side), information section (top centre), history (bottom centre), and summary of method parameters and performance (right side).

## Experimental

**Sample:** A typical diesel fuel sample diluted 4:1 in dichloromethane (DCM) was used for all experiments. Neat DCM (Sigma Aldrich, <99.9%) was used as a blank.

**Methods:** For the following experiments, a Pegasus® 4D GCxGC-TOFMS system (Leco Corporation) equipped with an Agilent 7890 gas chromatograph (Agilent Technologies) and a Leco quad-jet, liquid nitrogen cooled thermal modulator was used. The Simply GCxGC web-based method development tool version 1.00 (Leco Corporation) was used to optimise GCxGC methods to maximise the peak capacity and utilisation of separation space. The column sets used were comprised of a 30 m x 0.25 mm x 0.25 µm Restek Rxi-17Sil MS primary column coupled via a SGE micro union to either a 1.5 m x 0.25 mm x 0.25 µm (or 0.5µm) Restek Rxi-5 MS or a 1.5 m x 0.10 mm x 0.10 µm Restek Rxi-5 MS second-dimension column (lengths are total lengths). Secondary column total lengths are the sum of the transfer line (0.2m), secondary oven (length as noted in the results), modulator (0.1m) and GC oven lengths. For all experiments helium was used for the carrier gas. A 200:1 split was used with the GC inlet held at 280°C and the transfer line held at 340°C. The temperature program consisted of a 0.5 min hold at 40°C, before ramping to 340°C. The oven heating rates, column flow rates, and modulation periods (the time duration of the second-dimension separation) were determined by the Simply GCxGC tool based on the dimensions of the column set

and as noted in the discussion. For each set of conditions two replicate injections of a DCM blank were performed prior to triplicate injections of the diesel sample. Note that the maximum temperature of 340°C was not necessary for the diesel sample, but only used as a generic temperature range for petroleum.

## Results and Discussion

In order to have a step-by-step process to optimise a GCxGC method there must be some logic behind it. For Simply GCxGC, the optimisation starts with an optimised first-dimension separation which is typically readily available. This method can easily be entered into Simply GCxGC, or alternatively an optimum flow (depends on inside diameters of primary and secondary columns) and an optimum heating rate (depends on the void time of the first-dimension) are calculated and suggested in the tool. From this optimised first-dimension, the optimum modulation period (approximately 3 modulations per base peak width for GCxGC-MS [12]) is then calculated based on the first-dimension peak width which the tool calculates. The optimum secondary column length is then calculated based on this optimum modulation period (second-dimension separation time). This computational engine, based on GCxGC theory [12, 13], was validated against experimental data and the results were presented at The Pittsburgh Conference & Exposition (PittCon) 2016 [14] and the 13th GCxGC Symposium in 2016 [15]. All

predicted peak capacity results were within +/- 20% of the experimental, while most were within +/- 10%.

Simply GCxGC follows the basic flow chart shown in Figure 1. In addition to guiding the analyst, the tool also provides thorough explanatory information regarding each step and the rationale behind it. The first step is the input of initial information (Leco-based GCxGC-TOFMS, carrier gas type, primary column dimensions, temperature range of temperature program, transfer line temperature). The default primary column is the common 30 m x 0.25 mm x 0.25 µm column. The secondary column is given the same ID as the primary column, unless it is changed in a later step for increased peak capacity in the second-dimension. Next the tool calculates the optimum flow [13] and heating rate which are recommended, but the analyst can change these values. Figure 2 provides an example view of the tool showing four different informational areas. The above described input information would appear in the information section in the centre at the top.

After the calculation of the remaining optimum method parameters (modulation period and secondary column length), the next step is the selection of stationary phases. The tool provides some general guidance and the phases to be tested are input as documented information shown in the Summary. The following step then begins the experimental part of method development as guided by the tool. The columns are installed and a GC method is created with the recommended conditions. An injection of a representative sample or standard is made to check for overloading. The amount of sample injected can then be adjusted as needed.

The next experimental step is to 'Determine Secondary Oven Temperature Offset'. In this step, the analyst is guided in the adjustment of the secondary oven temperature offset relative to the GC oven temperature and the secondary column length in order to utilise as much of the two-dimensional separation space as possible given a particular stationary phase combination and sample. The objective is to adjust the retention such that the last eluting peak in the second-dimension elutes at a second-dimension retention time of one modulation period plus one second-dimension void time. The void time of the next modulation can be used because peaks of the next modulation elute after the void time. The tool directs the analyst to analyse the representative sample at secondary oven temperature offsets of +5°C and +40°C relative to the GC oven with a very long modulation period

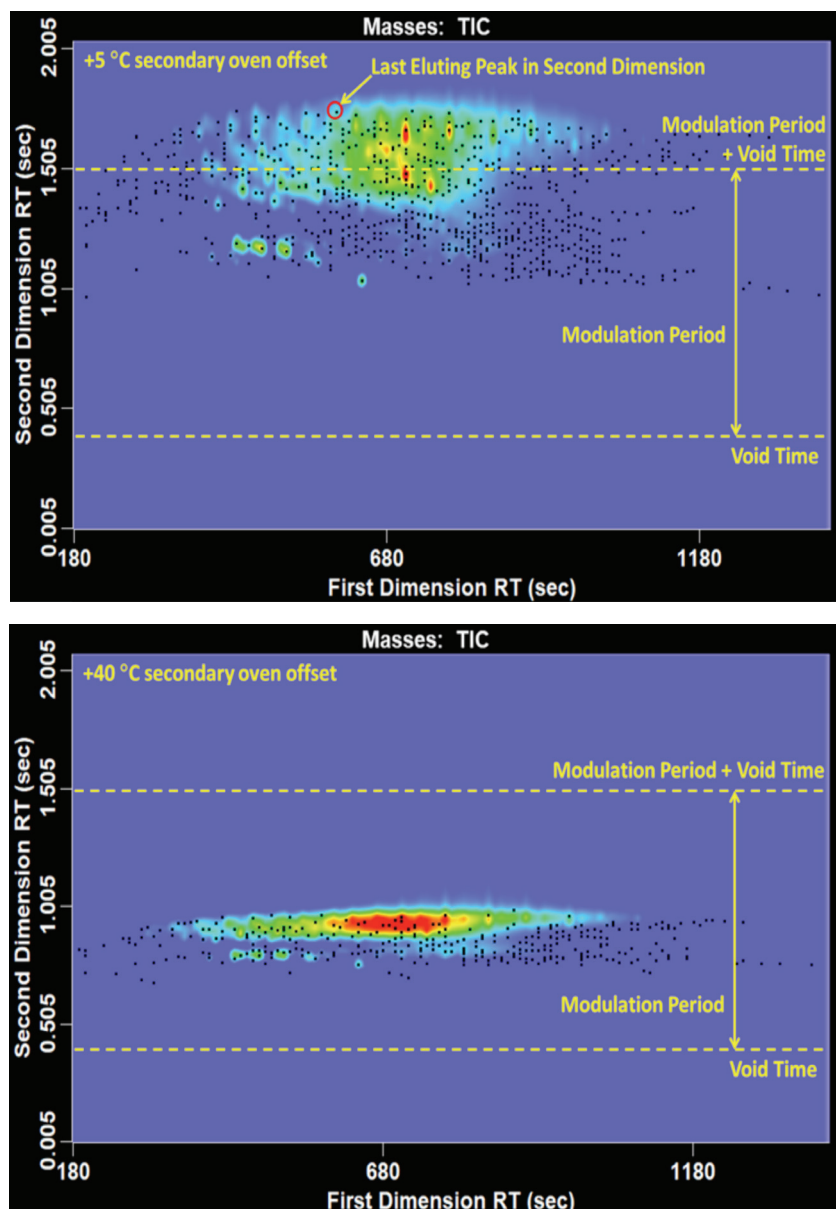


Figure 3. Example in which desired retention is between +5°C and +40°C secondary oven temperature offsets. Column set, 30x0.25x0.25/0.6x0.25x0.25; column flow, 1.36 mL/min; heating rate, 10.2°C/min; modulation period, 10 sec; secondary oven temperature offset, +5°C (top), +40°C (bottom).

(10 sec) to avoid any possible wrap around. (Wrap around is when peaks in the second-dimension elute in the next modulation period.) The user inputs the second-dimension retention times of the last eluting analyte in the second-dimension along with the corresponding first-dimension retention time. Figure 2 shows the view in which the retention time data is entered. There are three cases which the tool handles: 1) too much retention, 2) too little retention, and 3) retention with an offset between +5°C and +40°C. In the first case when the retention is too much, a recommendation is made to shorten the secondary column within the secondary oven and the +5°C and +40°C analyses run again. When the retention is too little, a recommendation is made to lengthen the secondary column within the secondary oven and the +5°C and +40°C analyses repeated. The tool will instruct

the analyst as needed in these cases. In the case in which the retention falls between the retention obtained for the +5°C and +40°C analyses, the tool calculates the necessary offset. The analyst then runs this predicted case with the optimum modulation period to confirm the result. If/when either a minimum or maximum length of secondary column is reached, the tool will instruct the analyst that a different stationary phase combination is needed.

Figure 3 is an example of the +5°C and +40°C analyses after lengthening the secondary column from 0.3 m to 0.6 m as instructed by the tool for too little retention at 0.3 m and 0.45 m lengths. The figure shows lines at the void time of the second-dimension separation and the modulation period plus void time. The tool calculates the offset so that the peaks in the second-dimension fit within the optimum

modulation period from void time to void time. Figure 4 is the result at the calculated offset of +17°C for the case shown in Figure 3. Note that the second-dimension axis is shifted so that the void time is at time zero and the last eluting second-dimension peaks are near the top of the contour plot at a time of one modulation period plus one void time.

In the next step, the evaluation of the stationary phase combination, the tool provides some guidance on whether the peaks are spread sufficiently throughout the two-dimensional separation space. The analyst must then decide if the general spread of the peaks is acceptable. If not, the tool returns to the step of selecting stationary phases. If the stationary phase combination is accepted, the tool moves on to evaluating the peak capacity. In this step, the tool guides the analyst in examining the separation more closely in both dimensions. The analyst can then decide to accept the separation or not. If the separation is not accepted the tool will suggest three ways to increase peak capacity and two ways to decrease peak capacity (in order to decrease analysis time). If an overall increase in peak capacity is needed, the primary column can be lengthened which will also lengthen the secondary column and increase peak capacity in both dimensions. Alternatively, if the analyst would rather not take the time to change the column set, but is willing to have a longer run time, a 'slow-down' option is provided. A slow-down is simply a decrease in oven heating rate which can increase the peak capacity in the first-dimension while also widening the first-dimension peak and increasing the optimum modulation period which will provide greater peak capacity in the second-dimension. Figure 5 shows the result of a slow-down of the optimum case in Figure 4. The separation time has doubled (due to the reduction of the heating rate from 10.2 to 5.1°C/min), but one can see that the peak markers (black dots in the figures) are more separated and groups of peaks are more apparent. If a method is to be routinely run, lengthening the columns is recommended because it provides a faster separation than a slow-down for the same peak capacity.

The tool also gives a third choice in increasing peak capacity which is for increasing the peak capacity mainly in the second-dimension. This is accomplished by having a smaller ID secondary column (mixed ID). The greater the difference in ID's between the primary and secondary columns the greater the peak capacity increase in the second-dimension. Typically, a 0.25/0.15 mm or 0.25/0.1 mm column combination (1D/2D) is recommended.

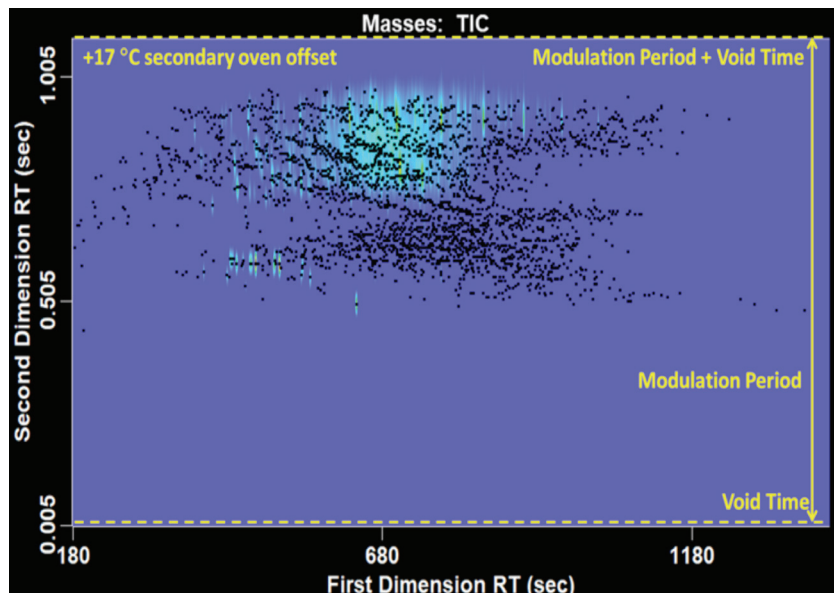


Figure 4. Result for calculated secondary oven temperature offset for retention to modulation period plus void time. Second-dimension axis is shifted 0.41 sec (calculated void time). Column set, 30x0.25x0.25/0.6x0.25x0.25; column flow, 1.36 mL/min; heating rate, 10.2°C/min; modulation period, 1.1 sec; secondary oven temperature offset, +17°C.

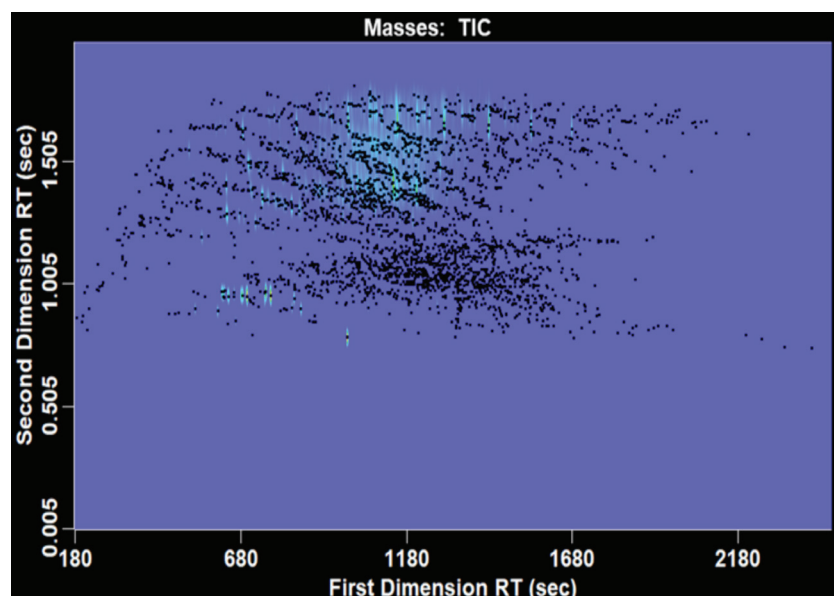


Figure 5. Contour plot of slow-down of conditions as shown in Figure 4. Heating rate cut in half from 10.2 to 5.1°C/min. Column set, 30x0.25x0.25/0.6x0.25x0.25; column flow, 1.36 mL/min; heating rate, 5.1°C/min; modulation period, 2.0 sec; secondary oven temperature offset, +17°C. Second-dimension axis shifted by 0.41 sec (calculated void time).

However, the analyst must keep in mind that the sample loading capacity decreases with ID, so the secondary column sample loading capacity is decreased significantly relative to the primary column.

Figure 6 is an example of the mixed ID case (0.25/0.1 mm) for increased second-dimension peak capacity. The optimum heating rate is only 3.4°C/min. In the case of mixed ID, the secondary column acts as a restrictor and decreases the flow velocity in the primary column. Additionally, the optimum flow is decreased to better accommodate the narrower bore secondary column. For a 0.25/0.25 mm ID

combination, the optimum flow is 1.36 mL/min, while for a 0.25/0.1 mm ID combination the optimum flow is 0.79 mL/min. The optimum modulation period is 2.9 seconds now since the flow and heating rate are lower. For the mixed ID case in Figure 6, the separation of compound classes and separation within classes are improved relative to the same ID case in Figures 4 and 5. This separation could be improved even further by a slow-down.

The tool is structured in a way to help novices not only learn how to do GC×GC method development, but also to develop effective GC×GC separations. For the more

expert analyst Figure 7 shows a couple of other method adjustments to be considered. Currently these adjustments are not part of the tool.

Adjustments of the secondary column length and the secondary oven temperature offset have limitations. If the stationary phase combination provides reasonably good spread of the peaks, then more (or less) retention in the second-dimension can also be achieved by increasing (or decreasing) the film thickness of the secondary column. The limitation of this approach is primarily the film thicknesses commercially available. The top contour plot in Figure 7 shows a 30 m x 0.25 mm x 0.25 μm primary column and a 0.3 m x 0.25 mm x 0.5 μm secondary column. In the earlier example shown in Figure 4 with a film thickness of 0.25 μm for the secondary column, the secondary column length was increased to 0.6 m to increase the retention time of the last eluting second-dimension peak to the limit of the modulation period plus the void time. With a 0.5 μm film thickness the optimum secondary column length is 0.3 m. Compared to the result in Figure 4, the result in Figure 7 (top contour plot) is slightly more compressed in the second-dimension.

The other adjustment we suggest is for more complete utilisation of the two-dimensional space, in particular the available space in the lower portion of the contour plot. In this initial version of the tool we kept it simple by only wrapping peaks into the void space (no peaks) of the next modulation period. However, as is apparent in all of the contour plots before Figure 7, there is more available space in the lower portion of the two-dimensional separation space, but that space may be filled with solvent, bleed, or other background peaks. In the contour plots in this article these peak markers were removed for best visualisation. With a mass spectrometer, the solvent and bleed peaks can generally be identified quite readily (banana-like shapes in the contour plots) and removed. In this case the maximum use of the second-dimension space can be achieved by wrapping the last eluting peaks around to the first eluting peaks. This is shown in Figure 7 in the bottom contour plot. All of the conditions are the same as in the upper contour plot except for the secondary oven temperature offset, which was lowered to increase retention in the second-dimension and achieve the desired wrap around. Note that the second-dimension axis is shifted such that the first eluting peak is at the bottom of the contour plot. The result is a very good separation in less than 30 minutes.

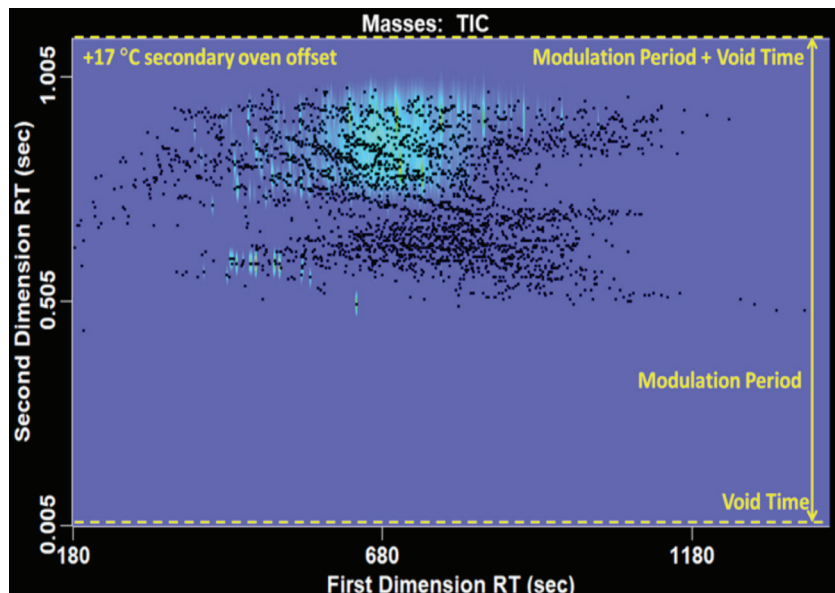


Figure 6. Contour plot of mixed ID (0.25/0.1) column set. Column set, 30x0.25x0.25/1.05x0.1x0.1; column flow, 0.79 mL/min; heating rate, 3.4°C/min; modulation period, 2.9 sec; secondary oven temperature offset, +14°C. Second-dimension axis is shifted up by 1.0 sec (calculated void time).

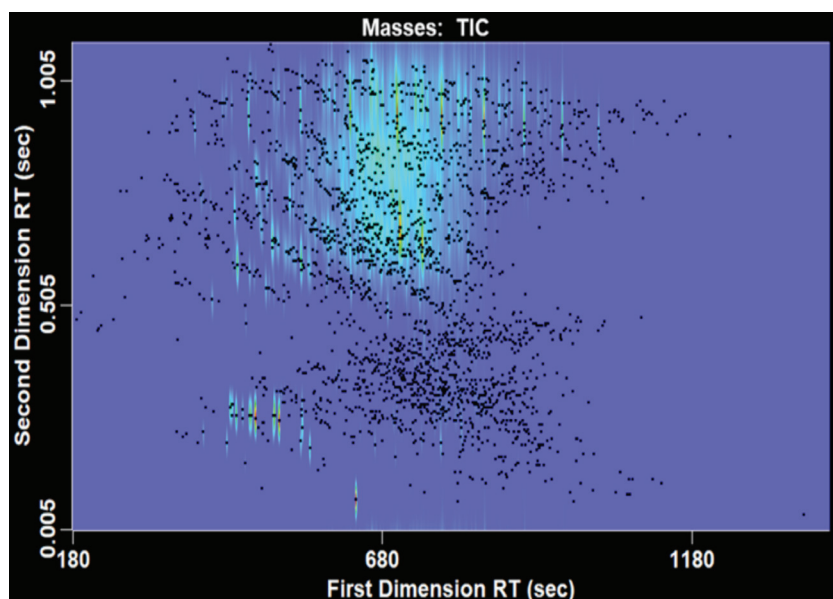
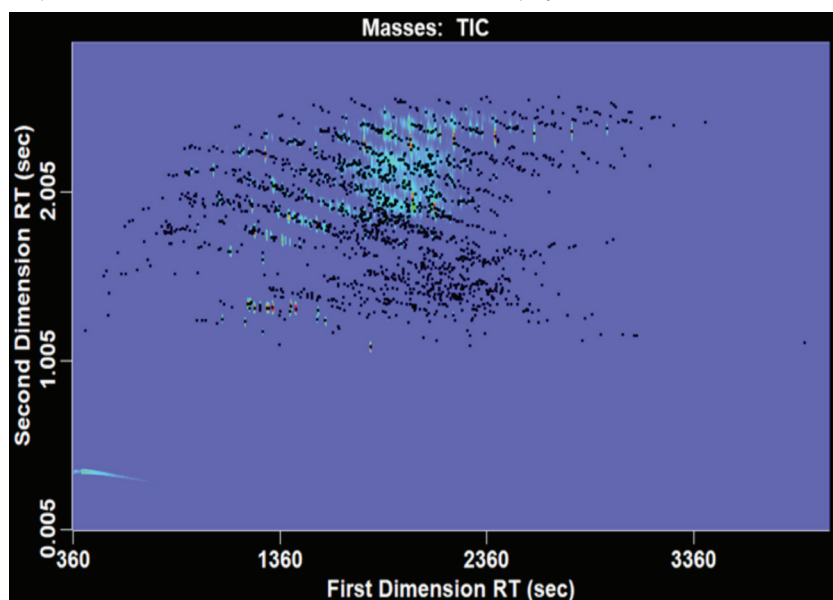


Figure 7. Contour plots for thicker film in second-dimension with second-dimension time axis shifted 0.21 sec (top), 1.05 sec (bottom). Column set, 30x0.25x0.25/0.3x0.25x0.5; column flow, 1.36 mL/min; heating rate, 10.2°C/min; modulation period, 1.1 sec; secondary oven temperature offset, +32°C (top), +8°C (bottom).

## Conclusion

A web-based software tool has been developed which assists an analyst in the development of an effective GC×GC method. Using diesel as an example of a complex sample, the steps in developing a method were shown along with the resulting separations. In addition to assisting in the development of a method, the software tool also provides tutorial information to help the analyst develop the skills for GC×GC method development similar to those of one-dimensional chromatographers.

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