

Instrument: Pegasus[®] BT 4D

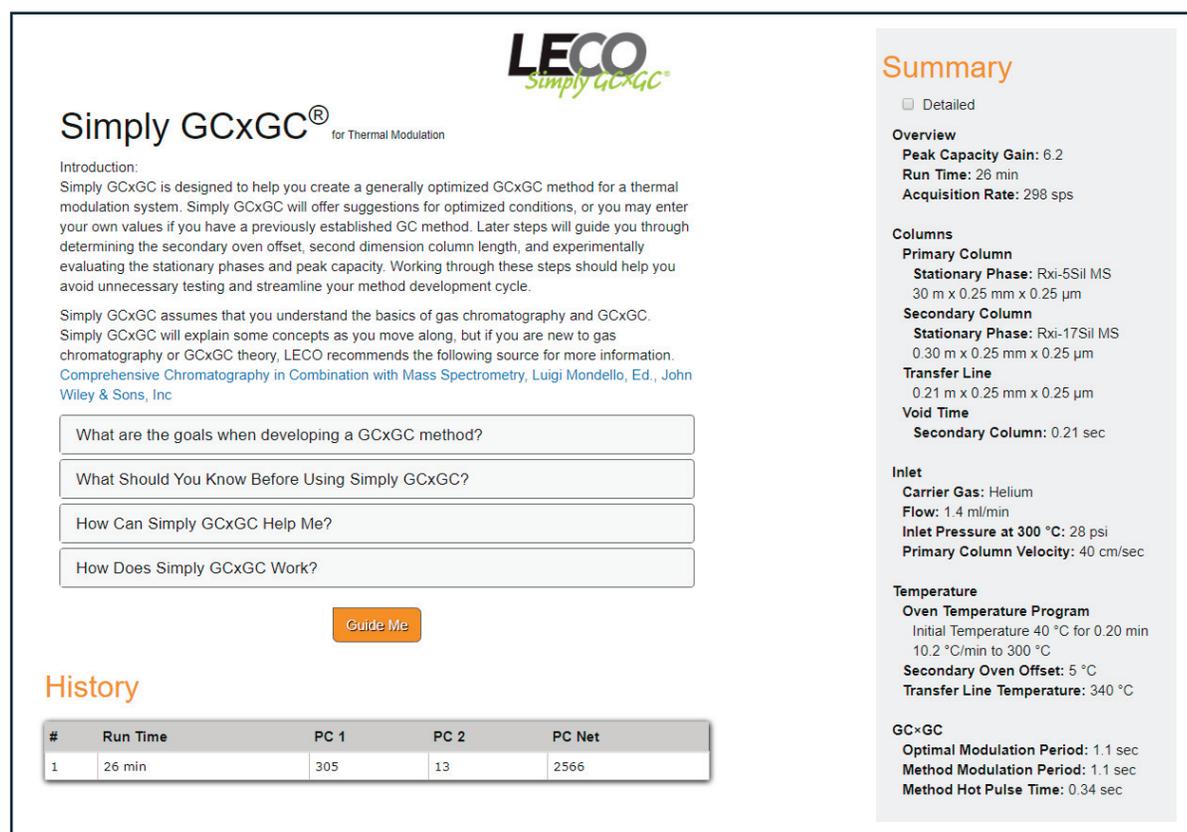
Introduction to Simply GCxGC[®]: An Online Guide for Optimizing Comprehensive Two Dimensional GC Separations

LECO Corporation; Saint Joseph, Michigan USA

Key Words: Simply GCxGC, 2D GC, Method Development, Diesel

Introduction

Comprehensive two-dimensional gas chromatography (GCxGC) combined with time-of-flight mass spectrometry (TOFMS) is a well-established technique for characterization of petroleum samples because of the ability to separate compound classes into distinct structured bands of analytes in a 3D contour plot that can then be identified with library-matched mass spectra. However, transferring traditional one-dimensional gas chromatography methods to optimized two-dimensional methods can be daunting because of the sheer number of options available for setting up commercial GCxGC systems—choosing orthogonal column phases, secondary oven temperature offsets, modulator temperatures, and second dimension separation period lengths. Using a new online tool, *Simply GCxGC*, the process of translating an existing analysis into an optimized GCxGC method is experimentally determined for a sample of diesel fuel. Calculations that maximize use of two-dimensional space and minimize analyte wrap-around are applied to user-controlled parameters to make the most of peak-capacity increases and further enhance the chromatographic resolution gains from the additional dimension of separation.



Simply GCxGC[®] for Thermal Modulation

Introduction:
Simply GCxGC is designed to help you create a generally optimized GCxGC method for a thermal modulation system. Simply GCxGC will offer suggestions for optimized conditions, or you may enter your own values if you have a previously established GC method. Later steps will guide you through determining the secondary oven offset, second dimension column length, and experimentally evaluating the stationary phases and peak capacity. Working through these steps should help you avoid unnecessary testing and streamline your method development cycle.

Simply GCxGC assumes that you understand the basics of gas chromatography and GCxGC. Simply GCxGC will explain some concepts as you move along, but if you are new to gas chromatography or GCxGC theory, LECO recommends the following source for more information. *Comprehensive Chromatography in Combination with Mass Spectrometry*, Luigi Mondello, Ed., John Wiley & Sons, Inc

History

#	Run Time	PC 1	PC 2	PC Net
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-5Sil MS
 30 m x 0.25 mm x 0.25 μm
Secondary Column
Stationary Phase: Rxi-17Sil MS
 0.30 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: 1.1 sec
Method Hot Pulse Time: 0.34 sec

Figure 1. Screenshot of the online tool, Simply GCxGC. The left sidebar shows the different steps for setting up an optimized analysis, while the right Summary area gives details on experimental parameters. An interactive, reviewable history of how changes in parameters affect calculated peak capacity is displayed at the bottom of the central column.

Experimental

A variety of experimental parameters were explored, using the *Simply GCxGC* web tool as a guide. Parameters that remained constant throughout the various steps for optimizing the separation on a sample of commercial diesel fuel are shown in Table 1 below.

Table 1. GCxGC-TOFMS (Pegasus® BT 4D) Conditions

Gas Chromatograph	LECO GCxGC Quad Jet Thermal Modulator & L-PAL 3 Autosampler
Injection	1 μ L liquid injection, split 100:1 @ 250 °C
Carrier Gas	He @ 1.4 mL/min, Corrected Constant Flow
Transfer Line	250 °C
Mass Spectrometer	LECO Pegasus BT 4D
Ion Source Temperature	250 °C
Mass Range	50-635 m/z
Acquisition Rate	Method Dependent

Results and Discussion

The experimental workflow begins with user input of parameters used in a one-dimensional GC-MS method, followed by a few experimental runs to evaluate sample loading and help determine optimum GCxGC parameters. Additional experiments are then run to increase either overall peak capacity or increase second dimension peak capacity after an initial set of conditions is proposed, with a goal to reach an optimal separation that matches the user's desired runtime or column availability.

Figure 2 shows the initial separation achieved for the diesel sample collected in conventional (1D) GC. After input of the 1D method parameters, with use of a 0.25 mm x 0.25 μ m Rxi-5MS column as the primary column, a 0.25 mm x 0.25 μ m Rxi-17MS column is recommended by the tool as the secondary column for a petroleum application. A 2 m length of secondary column is attached to the primary column, going through the modulator, with the initial amount specified in *Simply GCxGC* of 0.3 m coiled into the secondary oven.

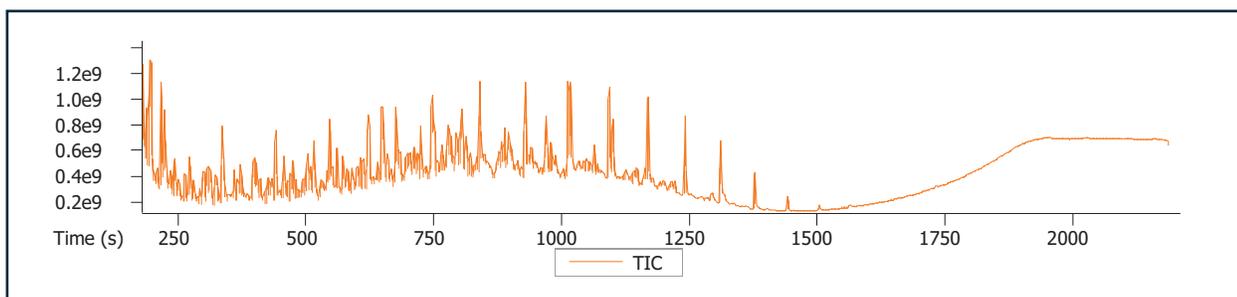


Figure 2. 1D Diesel Sample on Rxi-5MS 30 m x 0.25 mm x 0.25 μ m column chromatogram is pictured above.

In the first step to convert this analysis from a 1D separation to a GCxGC separation, sample loading is evaluated, because the cryofocusing process in the modulator could cause overloading of the secondary column. A zoomed-in view of the chromatogram of diesel sample collected with experimental GCxGC values for evaluation of sample loading is shown in Figure 3. The mass trace for m/z 57.07, a characteristic mass for alkanes expected to be present in diesel in high concentrations, is displayed. Peak shapes are shown to be symmetric and without plateaus, demonstrating acceptable sample loading when the inlet is set to split 100:1. When an overloaded peak is observed, one would expect an asymmetric or even a split peak.

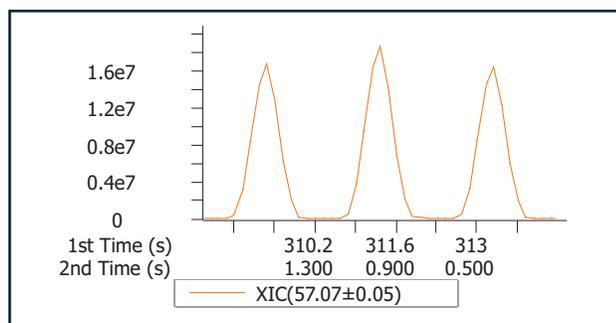


Figure 3. Zoomed view of linear chromatogram for evaluation of sample loading.

After sample loading is evaluated, two injections of the diesel sample (one with +5 °C and one with +40 °C secondary oven offset) are run to determine optimum values for secondary oven offset and 2nd dimension separation time, sometimes called modulation period. The goal is that all peaks of interest are maintained in the sections between the void time line (orange dotted line), and the void time + optimum 2nd dimension separation time line (yellow dotted line) shown in Figure 4.

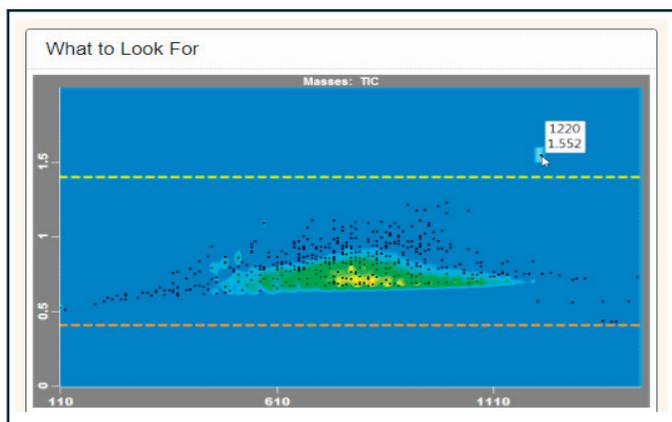


Figure 4. Screen capture of what to look for when determining void time and optimum 2nd dimension separation time, as shown in Simply GCxGC.

Elution times for both the first dimension and second dimensions of chromatography for the last eluting component are entered into the *Simply GCxGC* tool, which then returns optimum secondary oven temperature offset, 2nd dimension separation time, and hot pulse values for the current column configuration and primary oven temperature ramp. Additional suggestions for increasing or decreasing 2nd dimension column length may also be presented. This step can be repeated multiple times as other configurations are explored. Depending on user goals, the tool assists with developing methods for optimal chromatography within a shorter runtime or the maximum peak capacity given the available column sets. The contour plots from the first iteration of this experiment are shown in Figure 5 below.

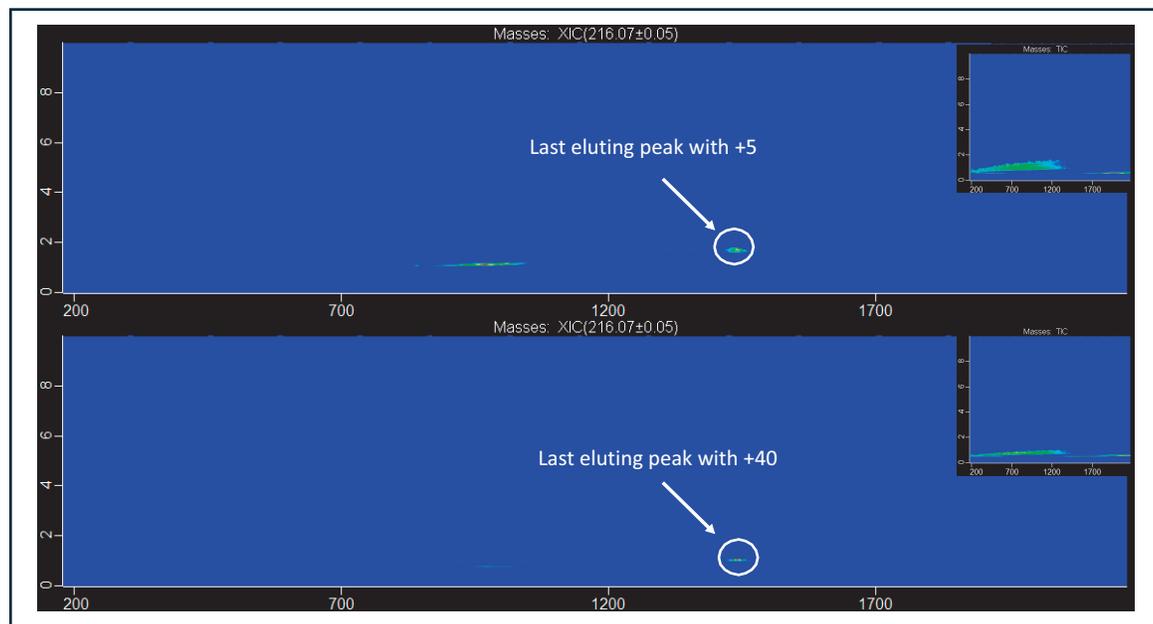


Figure 5. Zoomed view of contour plots of GCxGC acquisitions of diesel with secondary temperature offset set at +5 °C and +40 °C for finding the last eluting peak to set proper GCxGC parameters, with XIC of last eluting peak displayed. TIC overview is shown in the upper right corner of each plot.

Once the sample is run with the optimized parameters, peak capacity (PC) can be evaluated, and the user decides whether or not an increased peak capacity is needed. Figure 6 shows the first set of parameters calculated using Simply GCxGC, which returned a net peak capacity of 2523.

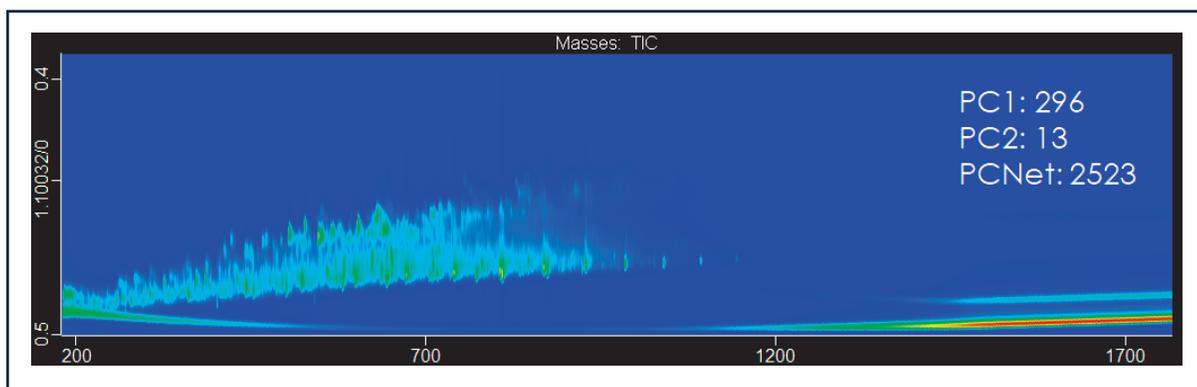


Figure 6. GCxGC Diesel Sample on Rxi-5MS 30 m x 0.25 mm x 0.25 μm coupled to Rxi-17SilMS 2 m x 0.25 mm x 0.25 μm column using calculated optimal GCxGC parameters of 0.3 m in secondary oven, 10.3 $^{\circ}\text{C}/\text{min}$ ramp rate from 50 $^{\circ}\text{C}$ to 300 $^{\circ}\text{C}$, carrier gas flow rate of 1.4 mL/min, +16 $^{\circ}\text{C}$ secondary oven offset, 2nd dimension separation time of 1.1 s with 0.33 s hot pulse, and spectral acquisition rate of 309 spectra/s.

A sequential optimization sequence guided by Simply GCxGC using the same first dimension column are shown in Figures 7-9 with the goal to increase peak capacity with every step. First, an increase in net peak capacity is achieved by slowing down the ramp rate, with results shown in Figure 7.

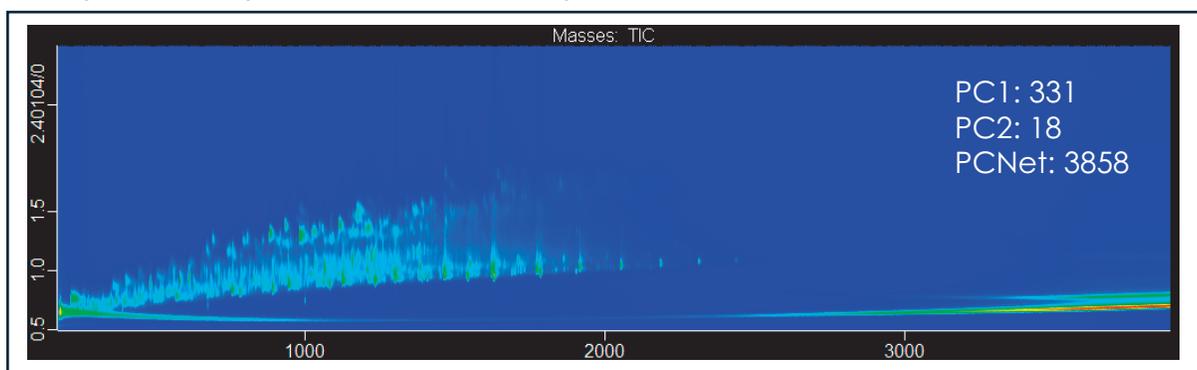


Figure 7. GCxGC Diesel Sample on Rxi-5MS 30 m x 0.25 mm x 0.25 μm coupled to Rxi-17SilMS 2 m x 0.25 mm x 0.25 μm column using calculated optimal GCxGC parameters of 0.3 m in secondary oven, 4.2 $^{\circ}\text{C}/\text{min}$ ramp rate from 50 $^{\circ}\text{C}$ to 300 $^{\circ}\text{C}$, carrier gas flow rate of 1.4 mL/min, +9 $^{\circ}\text{C}$ secondary oven offset, 2nd dimension separation time of 2.4 s with 0.73 s hot pulse, and spectral acquisition rate of 192 spectra/s. Total peak capacity calculated at 3858.

Then, to increase the peak capacity in the second dimension of separation, a column of smaller inner diameter was used, changing from the original 0.25 mm to 0.18 mm. Results for the change in secondary column inner diameter are shown in Figure 8.

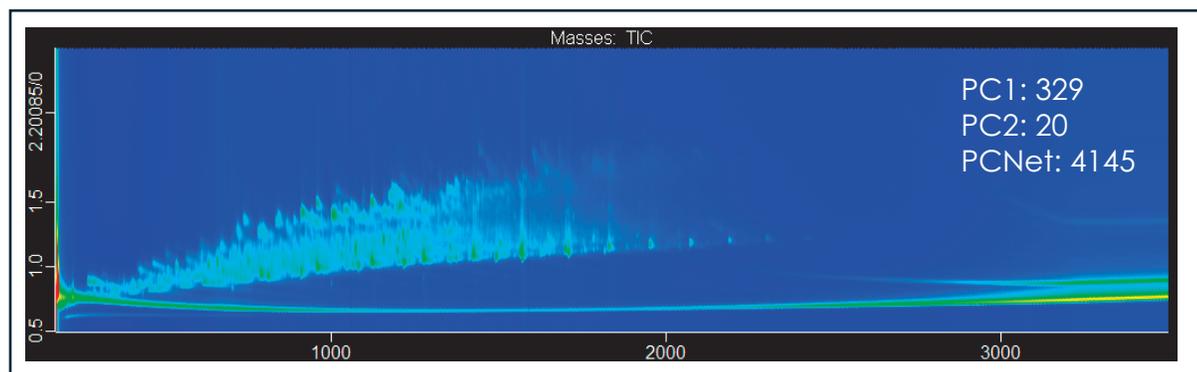


Figure 8. GCxGC Diesel Sample on Rxi-5MS 30 m x 0.25 mm x 0.25 μm coupled to Rxi-17SilMS 2 m x 0.18 mm x 0.18 μm column using calculated optimal GCxGC parameters of 0.3 m in secondary oven, 4.7 $^{\circ}\text{C}/\text{min}$ ramp rate from 50 $^{\circ}\text{C}$ to 300 $^{\circ}\text{C}$, carrier gas flow rate of 1.4 mL/min, +10 $^{\circ}\text{C}$ secondary oven offset, 2nd dimension separation time of 2.2 s with 0.60 s hot pulse, and spectral acquisition rate of 234 spectra/s. Total peak capacity calculated at 4145.

For further increase in peak capacity, an additional loop of secondary column is wound into the secondary oven and the ramp rate is reduced even further, for a final peak capacity of 5620, more than double the value returned from the first set of optimized parameters. The separation of more components in the diesel are evident comparing Figure 9 to Figure 6.

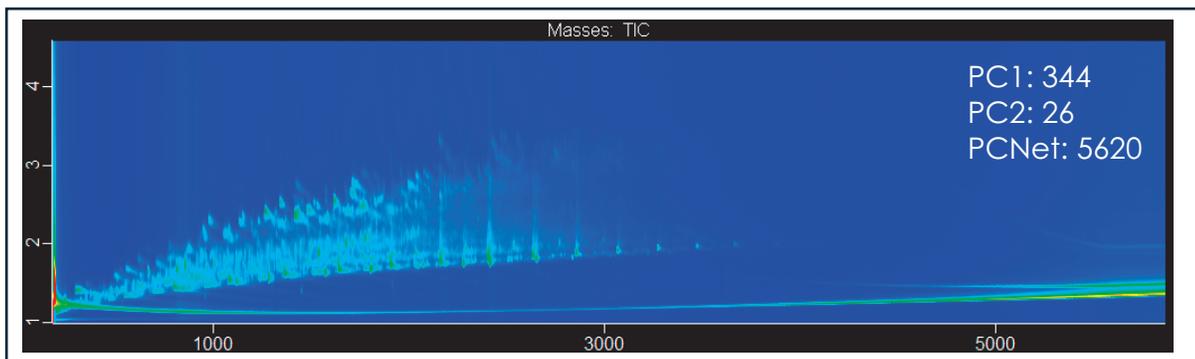


Figure 9. GCxGC Diesel Sample on Rxi-5MS 30 m x 0.25 mm x 0.25 μ m coupled to Rxi-17SilMS 2 m x 0.18 mm x 0.18 μ m column using calculated optimal GCxGC parameters of 0.45 m in secondary oven, 2.7 $^{\circ}$ C/min ramp rate from 50 $^{\circ}$ C to 300 $^{\circ}$ C, carrier gas flow rate of 1.4 mL/min, +15 $^{\circ}$ C secondary oven offset, 2nd dimension separation time of 3.6 s with 1.10 s hot pulse, and spectral acquisition rate of 178 spectra/s. Total peak capacity calculated at 5620.

Chromatographic separation with the column stationary phase paradigm reversed was then explored for the same diesel sample. A 30 m x 0.25 mm x 0.25 μ m Rxi-17SilMS column was coupled to 2 m x 0.25 mm x 0.25 μ m Rxi-5MS and the initial optimal parameters returned a net peak capacity of 2523, as shown in Figure 10.

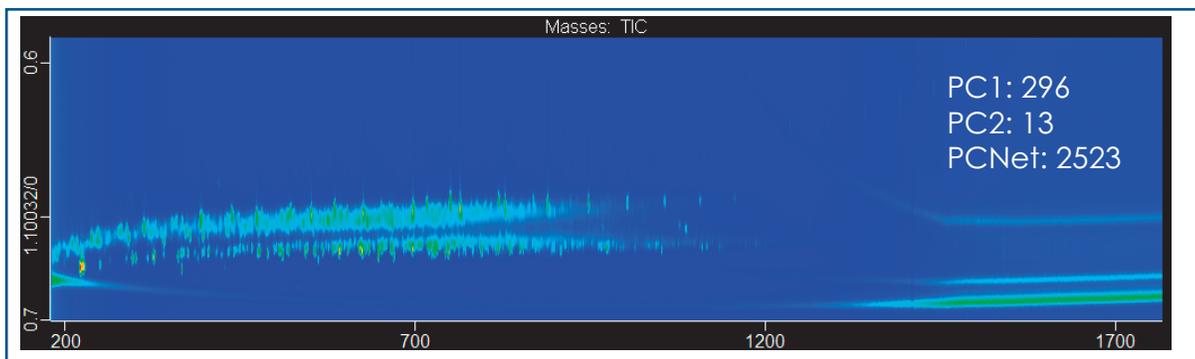


Figure 10. GCxGC Diesel Sample on Rxi-17SilMS 30 m x 0.25 mm x 0.25 μ m coupled to Rxi-5MS 2 m x 0.25 mm x 0.25 μ m column using calculated optimal GCxGC parameters of 0.3 m in secondary oven, 10.3 $^{\circ}$ C/min ramp rate from 50 $^{\circ}$ C to 300 $^{\circ}$ C, carrier gas flow rate of 1.4 mL/min, +32 $^{\circ}$ C secondary oven offset, 2nd dimension separation time of 1.1 s with 0.33 s hot pulse, and spectral acquisition rate of 309 spectra/s. Total peak capacity calculated at 2523.

Similar method developments steps were then taken with this setup to increase net peak capacity, as shown in Figures 11-13. First, additional net peak capacity was gained by slowing down the oven ramp rate, with results shown in Figure 11.

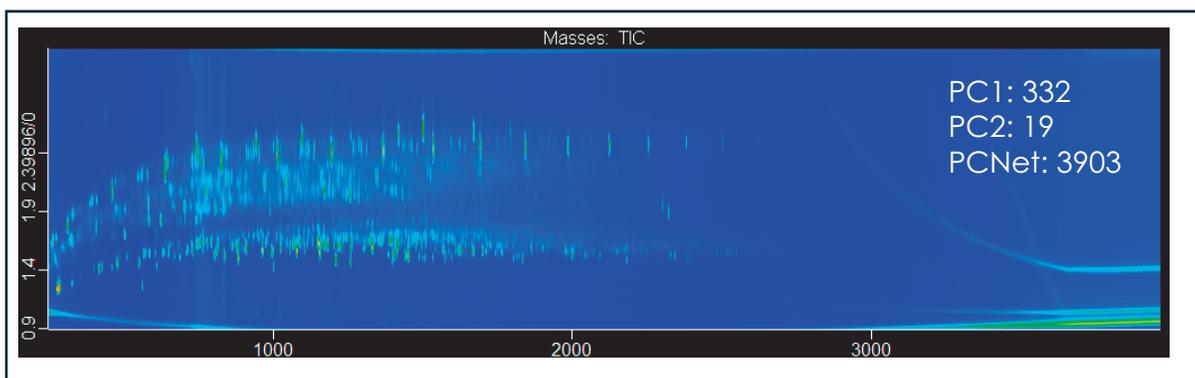


Figure 11. GCxGC Diesel Sample on Rxi-17SilMS 30 m x 0.25 mm x 0.25 μ m coupled to Rxi-5MS 2 m x 0.25 mm x 0.25 μ m column using calculated optimal GCxGC parameters of 0.3 m in secondary oven, 4.1 $^{\circ}$ C/min ramp rate from 50 $^{\circ}$ C to 300 $^{\circ}$ C, carrier gas flow rate of 1.4 mL/min, +9 $^{\circ}$ C secondary oven offset, 2nd dimension separation time of 2.4 s with 0.73 s hot pulse, and spectral acquisition rate of 193 spectra/s. Total peak capacity calculated at 3903.

Then, the inner diameter of the secondary column was changed from 0.25 mm to 0.18 mm and an additional loop was added to the secondary oven to increase second dimension peak capacity. This came with a loss in net peak capacity, but provided more 2nd dimension chromatographic resolution between components that elute in the same first dimension of separation, as can be seen in Figure 12.

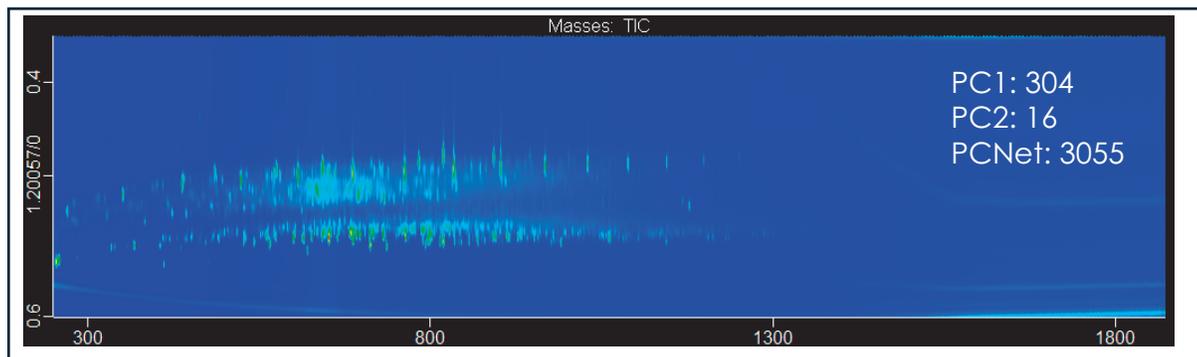


Figure 12. GCxGC Diesel Sample on Rxi-17SilMS 30 m x 0.25 mm x 0.25 μ m coupled to Rxi-5MS 2 m x 0.18 mm x 0.18 μ m column using calculated optimal GCxGC parameters of 0.45 m in secondary oven, 9.6 $^{\circ}$ C/min ramp rate from 50 $^{\circ}$ C to 300 $^{\circ}$ C, carrier gas flow rate of 1.4 mL/min, +9 $^{\circ}$ C secondary oven offset, 2nd dimension separation time of 1.2 s with 0.30 s hot pulse, and spectral acquisition rate of 349 spectra/s. Total peak capacity calculated at 3055.

Finally, the ramp rate was reduced again to increase the net peak capacity and yield a final optimized net peak capacity of 4485, as shown in Figure 13.

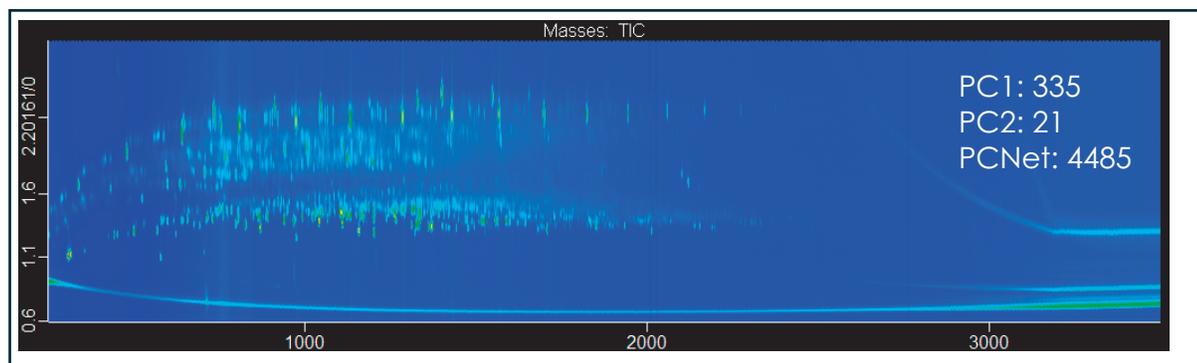


Figure 13. GCxGC Diesel Sample on Rxi-17SilMS 30 m x 0.25 mm x 0.25 μ m coupled to Rxi-5MS 2 m x 0.18 mm x 0.18 μ m column using calculated optimal GCxGC parameters of 0.45 m in secondary oven, 4.7 $^{\circ}$ C/min ramp rate from 50 $^{\circ}$ C to 300 $^{\circ}$ C, carrier gas flow rate of 1.4 mL/min, +9 $^{\circ}$ C secondary oven offset, 2nd dimension separation time of 2.2 s with 0.65 s hot pulse, and spectral acquisition rate of 248 spectra/s. Total peak capacity calculated at 4485.

While the net peak capacity from experiments run with the nonpolar stationary phase as the primary column was larger, if a greater separation of compound classes into a structured chromatogram were desired, the reversed column phase paradigm could offer better results. Different compound-to-column-phase affinities affect the utility of the GCxGC separation space in ways that can be tailored for the most effective desired chromatographic resolution for a given application.

Conclusion

Using the *Simply GCxGC* tool to set up and optimize GCxGC experiments yields excellent two-dimensional separations. With initial parameters providing a helpful starting point for analysis, *Simply GCxGC* provides a guide for step by step optimization that leads to greater peak capacity results and better chromatographic resolution for a given sample, regardless of the initial column phase used. Although a classic petroleum application was demonstrated here, *Simply GCxGC* can be effectively employed to optimize your GCxGC separations for other application fields as well, whether food, flavor and fragrance, metabolomics, or environmental analyses.

