FLUX™ GCxGC

The operation, use, and concepts behind a diverting flow technique

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Introduction

Comprehensive two-dimensional gas chromatography (GCxGC) offers dramatic improvements over traditional GC for the analysis of complex mixtures in various application fields—including, but not limited to—petrochemical, food, flavor, fragrance, environmental, metabolomics, and forensics. This is due to chromatographic resolution gains realized by adding an orthogonal second dimension of chromatographic separation. The modulator, considered the heart of the GCxGC system, is a device which injects the effluent from the primary column to the secondary column. There are two primary forms of modulators in GCxGC; thermal- and flow-based. We will describe these techniques here in general terms, but will then focus on flow modulation. Thermal-based modulators rely on a cooling mechanism to trap eluent from the first column, followed by heating to inject the eluent onto the second column for further separation. These modulators rely on the use of a cryogen such as liquid nitrogen for optimal cold-trapping performance. Flow-based modulation relies on either switching valves to inject on-column, or in some cases, combining valves with highly different flows (between the first column and the second) to flush a sample loop onto the second column. Both types provide a GCxGC alternative that is virtually consumable-free. The choice of which GCxGC approach is most appropriate depends on the application that will be performed on a particular system. While no system does everything, this technical brief describes the functionality and capabilities of LECO's *FLUX* Diverting Flow Modulator.

Before describing the operation principles of *FLUX,* we will discuss the general properties of flow-based modulation and how *FLUX* is different than other techniques in the field. First, the absence of cryogens makes flow modulation a cheaper alternative to thermal modulation. This is particularly important when dry nitrogen and/or liquid nitrogen are not readily available. If an application doesn't require ultimate GCxGC performance or sensitivity, flow-based modulation is a perfectly suitable technique to implement in a laboratory. Until the introduction of *FLUX,* flow modulation-based GCxGC operated by filling a sample loop with column 1 effluent before a high flushing flow of carrier gas (10-30 mL/min) was used to flush the sample loop onto column 2. This mode of modulation is often referred to as differential flow modulation due to the large difference in flow rates between column 1 and the flushing flow/column 2 flow rate. Several models of reverse-fill-flush differential flow modulators are commercially available and are usually coupled with an FID or dual detection (FID and MS).[1] The differential flow rates cause these types of GCxGC systems to have several drawbacks. First, these systems have lower chromatographic resolution due to less than optimum column flow rates, and second, the high flow rates through column 2 require a splitter prior to the introduction of a sample into a mass spectrometer, which sacrifices sensitivity. Alternatively, this high flow can be coupled to an FID detector which is responsive to higher flow rates, certainly a good pairing of techniques. The last significant drawback is the use of restrictors, which can be very cumbersome to users (that is, the use of various lengths of capillary columns). These restrictors are used at the vent line and for the split line (split to vent at the detector, but also split at the connection between the 2 columns) and are needed to determine flow rates through a set of non-intuitive calculations. Overall, the ease-of-use and performance of differential flow modulator systems are inferior to thermal modulators. One can optimize a system with thermal modulation without re-connecting and re-measuring restrictors.

Seeley et al. recently described a multimode flow modulator, which can be operated as either a differential flow modulator (full transfer) or a diverting flow modulator. When operated in the diverting mode, the modulator has several advantages over other flow modulators. The advantages of this type of modulation include lower column flow rates which are equivalent to thermal modulation, typical GC flow rates familiar to everyday users, and it eliminates the need for a splitter to couple to a mass spectrometer. These flow rates are at or near optimal for best chromatographic performance. Additionally, this diverting flow technique achieves narrower second dimension peak widths than differential flow modulation, even approaching those of thermal modulation. There are disadvantages to diverting flow modulation as well; they include a low duty cycle on the first GC eluent stream, leading to a loss in overall sensitivity compared to thermal modulation. This low duty cycle means the user must set the modulator to sample the first dimension GC peak at least three times (three or more slices) to avoid a loss in quantitative precision. However, this is easily avoided by following good practices—by first collecting a GC run to determine the peak widths produced under your specific method conditions, so the sampling period can be set to ensure three slices of the peaks of interest. Since the diverting flow modulator lends itself especially well to coupling with mass spectrometry, and can be set up in a very user-friendly configuration, LECO developed a commercialized version of this flow modulator known simply as *FLUX.*

FLUX Flow Modulator

LECO worked in collaboration with Dr. John Seeley, a key innovator in flow-based modulation, to commercialize the concept of the diverting flow modulator. The modulator, described previously[2], operates by using an auxiliary gas flow which opposes the effluent from column 1, sending it to waste during the divert state of the modulator. The auxiliary gas flow rate is slightly larger than the flow through column 1, and during the divert state it supplements the flow through column 2, as well as forcing the entirety of the column 1 flow to waste. When the switching valve is actuated, the modulator changes to the inject state, and the majority of the auxiliary gas flow is directed straight to waste, thus enabling the flow from column 1 to transfer directly into column 2; see Figure 1.

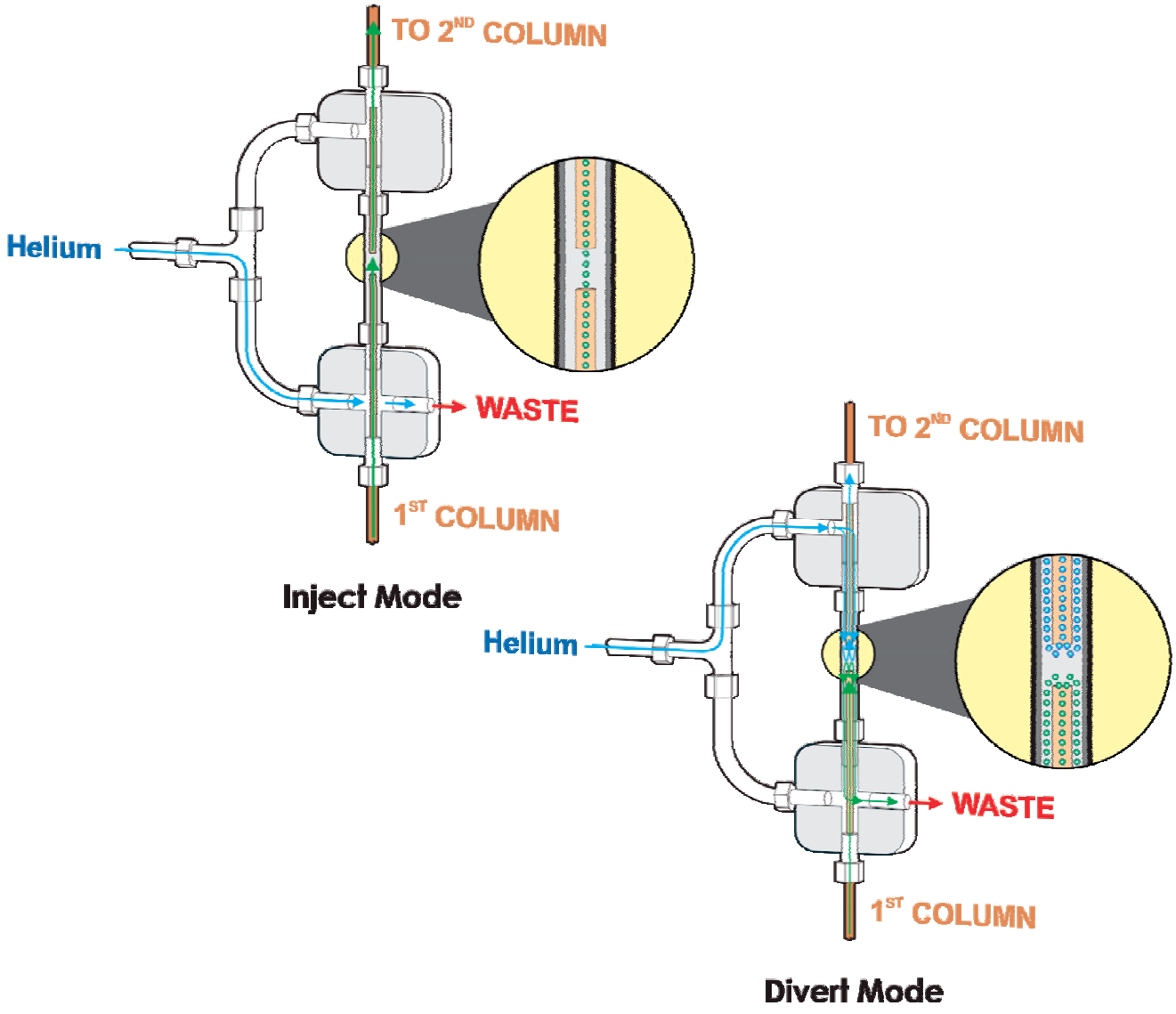

Figure 1. *Top:* The divert state of the diverting flow modulator. The auxiliary gas opposes the flow from column 1 forcing it to waste (exhaust). The auxiliary gas in this state makes up the flow of gas through column 2, and a portion is also sent to waste with the entire column 1 flow. *Bottom:* The inject state of the modulator. The auxiliary gas is sent straight to waste, while the effluent from column 1 is transferred onto column 2.

The *FLUX* Flow Modulator is comprised of a cross fitting which column 1 enters, and a tee fitting which column 2 exits. The cross and tee are connected by a length of tubing which is crimped in the center. This crimp positions the GC columns at an appropriate distance from each other to ensure optimal transfer of analyte from column 1 to column 2. All of this is mounted onto a bracket which is attached to the inner wall of the GC oven just below the secondary oven. This bracket can slide on the vertical axis, allowing users to move it up or down for access to fittings when making connections to the cross and tee. The exhaust and switching flow tubes are permanently connected to the cross and tee fittings. The switching flow tubes pass through the oven wall to the switching valve between the side panel and the outside oven wall. The switching valve is then connected to a

PCM module for auxillary flow control (3.5 mL/min) of the switching gas. This gas should be the same carrier gas as the main flow from column 1. The exhaust line from the cross runs inside the exterior panels to the upper rear of the GC, where it is plumbed into a split vent trap prior to terminating into the secondary channel of the PCM module. The secondary channel back pressure regulates the exhaust flow to maintain the desired flow through column 2.

Figure 2. The *FLUX* flow modulator is pictured right. The tee is mounted to the top portion of the modulator bracket; the cross is mounted to the bottom portion. The cross and tee are connected by a crimped tube which is used to position columns 1 and 2.

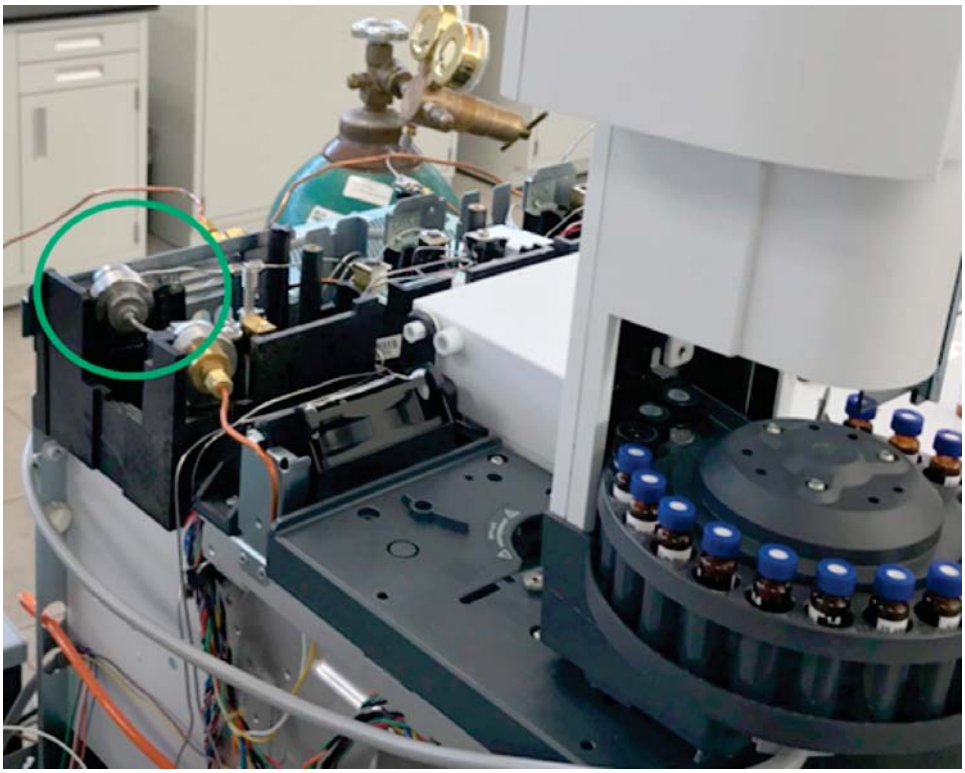

Figure 3. A split vent trap (circled in green) is used to clean the exhaust flow from the modulator prior to it being introduced into channel 2 on the PCM module for back pressure regulation.

Setting up the modulator is straightforward and relatively simple—essential attributes of any user-friendly system. Only two additional connections need to be made for the *FLUX* modulator compared to conventional GC. The primary column needs to be inserted into the cross until a hard stop is encountered, which corresponds to the crimp in the connecting tube. The 360 µm nut with captive ferrule is simply tightened using the provided hand tool to lock the column into place and provide a gas-tight seal. An open-ended wrench will provide too much torque and has the potential to break the 360 nut if overtightened. The same procedure is repeated for the secondary column, which is inserted down into the tee until it encounters a hard stop at the connecting tube crimp. Connecting the columns to the modulator and connecting the GC inlet and transfer line is all that is required to get up and running. There is no need for complicated spreadsheets to calculate and adjust flow restrictor lengths, costly cryogens, or excessive consumption of high purity gases.

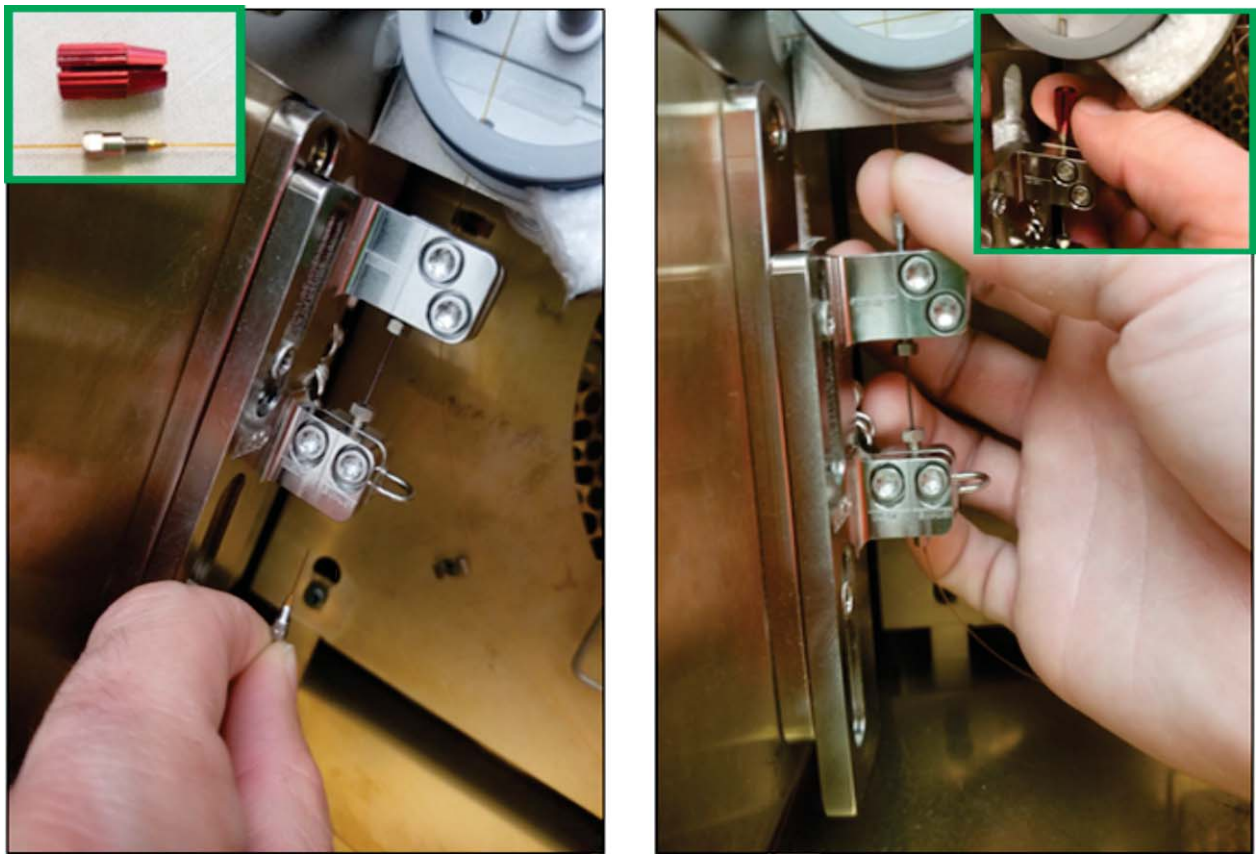

Figure 4. Setting up the modulator requires two simple connections. *Left:* Insert the primary column up through the cross and tighten the 360 ferrule and nut with the hand tool (upper left inset). *Right:* Insert the secondary column down through the tee, and then tighten the 360 nut in place with the hand tool.

Our focus on creating a very user-friendly system involved determining the optimal switching gas flow, as well as the best inject duration times for the modulator. Typical GC flow rates range from 0.5 mL/min up to 2 mL/min when coupled to an MS detector; note that higher flow rates lead to decreased ionization efficiency and can tax the pumping system of the MS. For column flow rates within this range, the optimal switching flow was determined, which produced narrow reinjections and well-shaped peaks. As one could expect, the modulator has a small inherent lag between the time the valve is actuated to switch the modulator from the divert state to the inject state, and the time the effluent from column 1 actually begins to enter into column 2. The design of the modulator was optimized to minimize this lag to produce fast, reproducible modulations. Essentially the software calculates the optimal flow based on the user's desired column flow rate—that is, small losses are accounted for by the software such that the user does not need to manage extra parameters.

To further simplify the operation of the *FLUX* modulator, the inject duration was optimized to provide narrow peaks while maintaining optimal sensitivity. The user is given three choices of inject durations within the optimal range of sensitivity. The short inject duration option (30 ms) provides very narrow peaks at the lower end of the optimal sensitivity range, while the long inject duration option (80 ms) provides significantly wider peaks, though still narrow by flow modulation standards, and better sensitivity. The mid-range option is the recommended default value of 50 ms, this value provides narrow peaks and near optimal sensitivity.

By determining optimal values for the auxiliary gas flow and inject durations, and then fixing those values in the software, the modulator operation becomes very user-friendly. There are less variables to understand and manage, making the learning curve to producing quality GCxGC data much faster. The theme of ease-of-use continues with the instrument software. When entering the column configuration into the GC Method the user makes sure to have the Flow Modulator option entered into the flow path, (see the red highlight in Figure 5 below). A simple click to select Collection Mode allows users to switch between GC and GCxGC without the need to change hardware (see blue highlight).

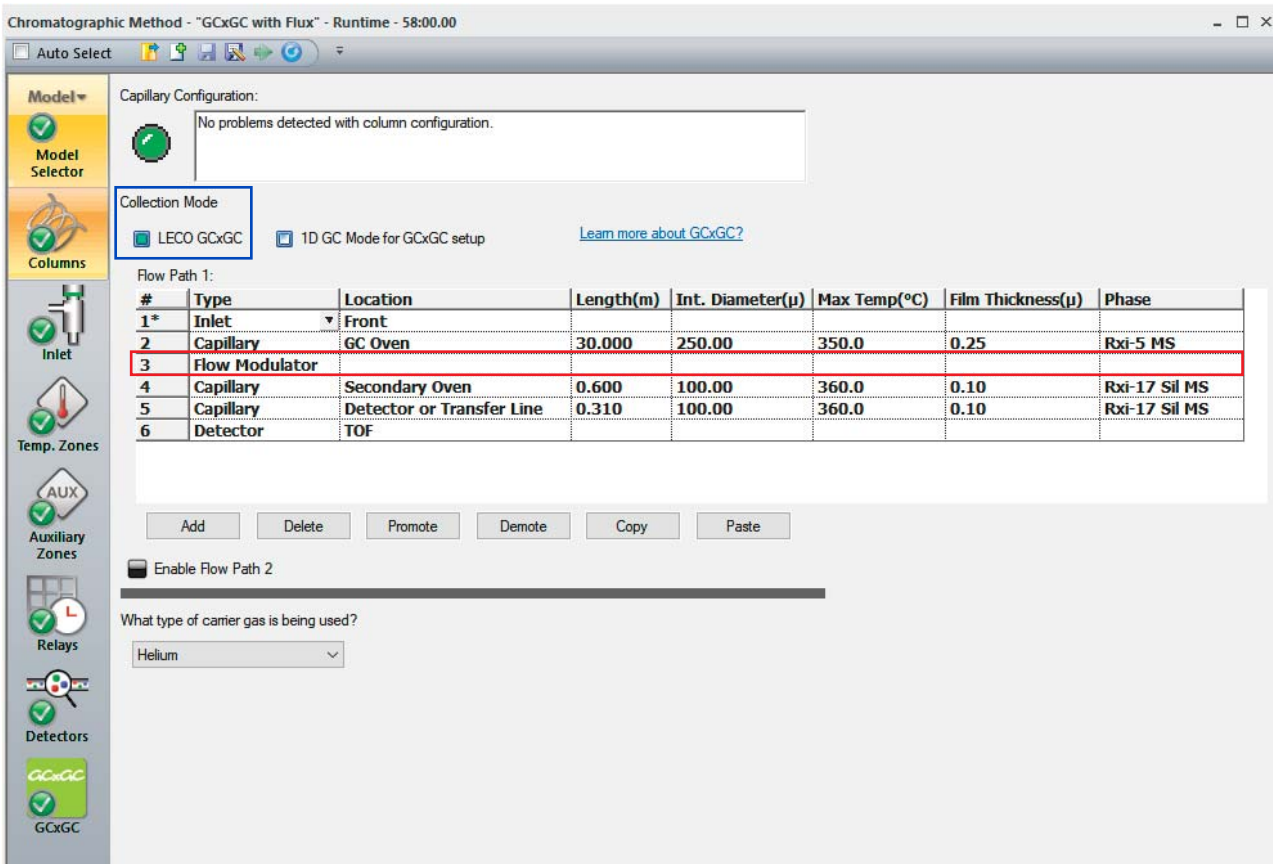

Figure 5. Shown above is the column configuration tab for a chromatographic method in LECO's ChromaTOF® brand software. The collection mode allows a user to switch between GC and GCxGC with a simple click. It can be seen that GC collection is selected for this example. Also, highlighted in red is the Flow Modulator line for the flow path.

The user sets up the GC Method the same way they would for conventional GC, selecting their desired flow rate, inlet split, temperature ramps, etc. The only additional requirement is to set the second dimension time and select an inject duration in the GCxGC tab. As mentioned previously, three optimal inject durations were pre-determined experimentally. These values appear in a drop down menu in the GCxGC tab. The user can read about the tradeoffs of each setting, and select the one which best suits their experiment. Properly setting the second dimension retention time is critical with this type of modulator to maintain quantitative precision. The second dimension time should be ~1/3 of the first dimension GC peak width, or three samples should be taken across the peak eluting from column 1.[3] It is recommended to follow good GCxGC method development practice by first running in GC mode to determine the peak widths produced under your specific method conditions, and then determine your second dimension time, see Figures 6 and 7.

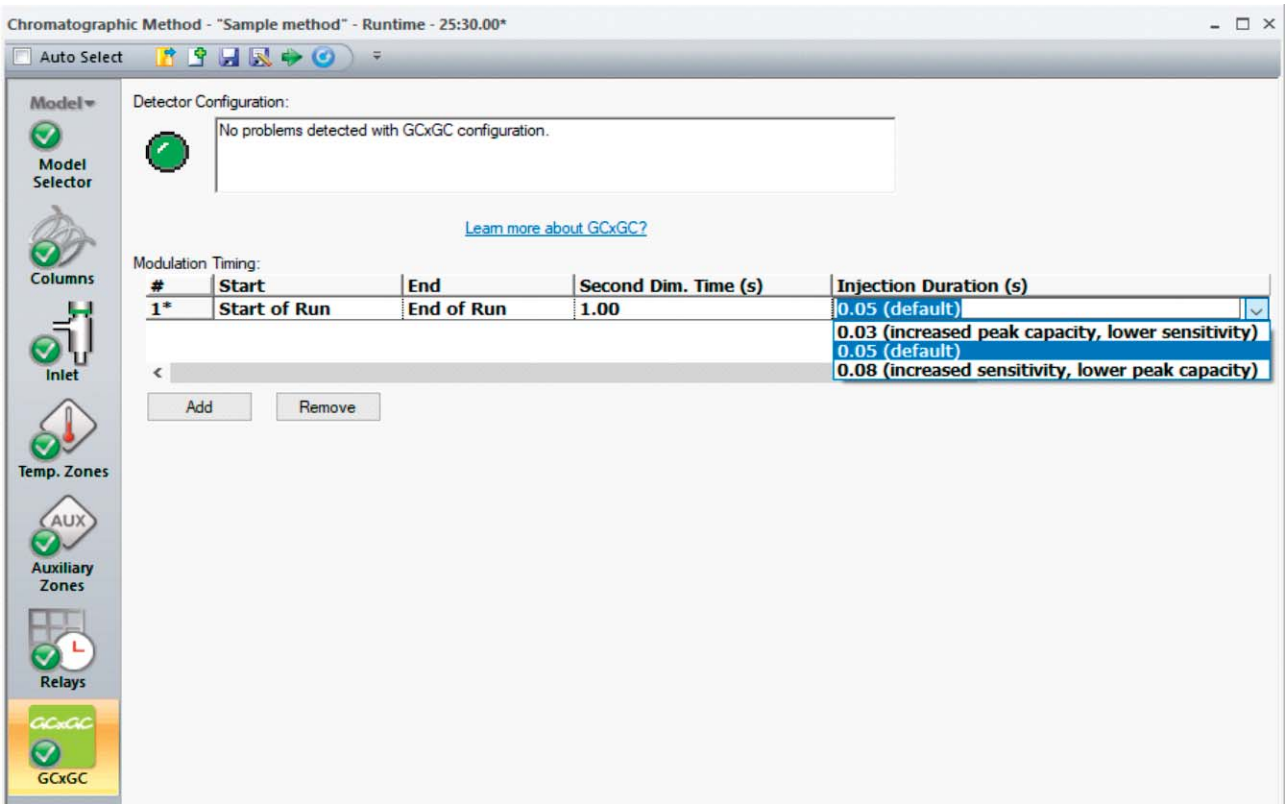

Figure 6. Shown above is the GCxGC tab for the Chromatographic method. This user-friendly tab provides three drop down options for setting the inject duration during a GCxGC run. The only other parameter to set is the second dimension time which should be 1/3 of the narrowest first dimension peak width.

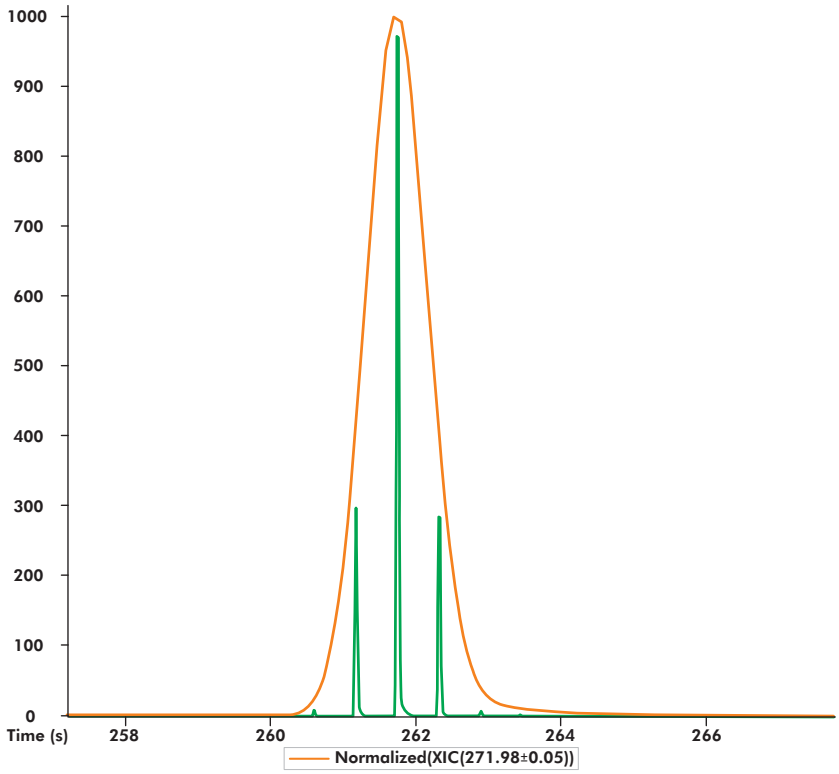

Figure 7. Shown above is an example of properly setting the second dimension separation time. The orange trace shows a GC peak width of ~1.8 s. The green trace which corresponds to the GCxGC run with a 0.6 s second dimension separation time provides at least three samples across the peak which is necessary for quantitative data precision with a low duty cycle modulator.

Performance

The flow modulator backs up its ease-of-use claim with excellent performance. While thermal modulation represents the best performance in terms of sensitivity and separation capacity, the diverting flow modulator outperforms differential flow modulators by producing narrower peaks on average while providing similar sensitivity, since differential flow modulation requires a flow splitter when coupled to an MS detector. Furthermore, the flow rate through the second dimension column is at or near optimal for efficient chromatography for diverting flow modulation, while the extremely high flows for differential flow modulation are not optimal chromatographically. Thus, diverting flow modulation provides a better second dimension separation, which is an important factor for two-dimensional chromatography in general. The *FLUX* Flow Modulator coupled to the Pegasus® BT GC-TOFMS yields an IDL below 1 pg, which is an excellent sensitivity level for many applications. Peak widths are narrow, typically <100 ms FWHH, while differential flow modulators yield average peak widths in the 100-300 ms range, see Table 1 and Figure 8.

Table 1. Shown above are the peak widths (FWHH) for four compounds from the Grob mixture collected using thermal modulation, and three prototype *FLUX* flow modulators. Thermal modulation gave the narrowest peaks, but the *FLUX* modulator yielded similar values, all below 50 ms

Instrument	FWHH (msec)
Undecane	
Thermal	39
Flux 1	41
Flux 2	41
Flux 3	37
Dimethylaniline	
Thermal	35
Flux 1	46
Flux 2	45
Flux 3	40
Methyl Undecanoate	
Thermal	27
Flux 1	43
Flux 2	42
Flux 3	38
Dicyclohexylamine	
Thermal	30
Flux 1	46
Flux 2	42
Flux 3	38

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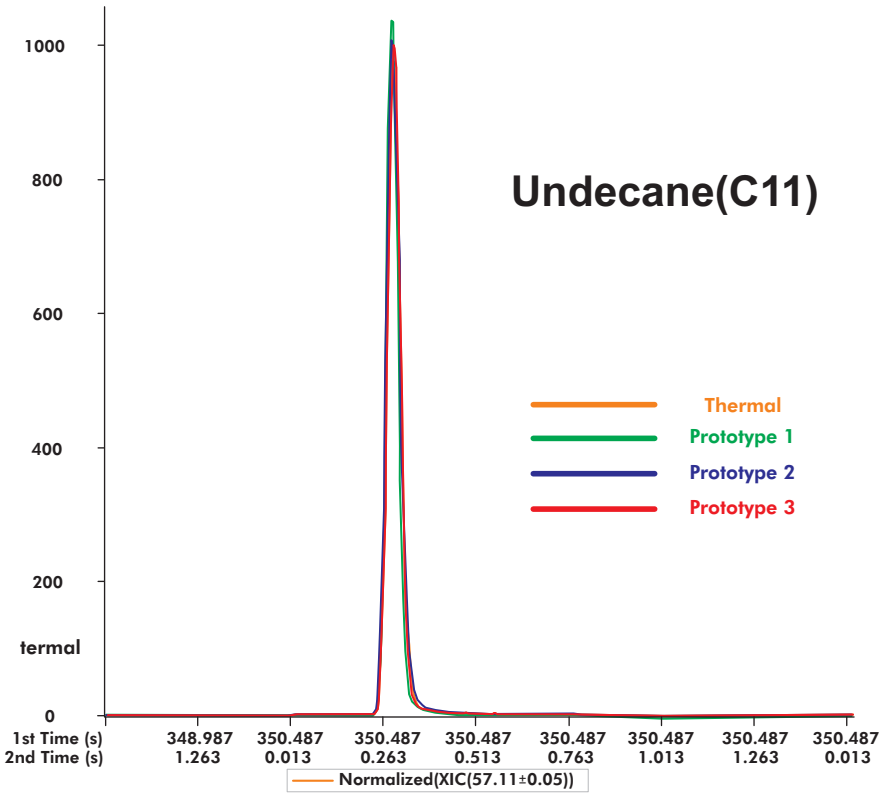

Figure 8. Shown above are overlays of a single modulation of the peak for Undecane, from Table 1. The orange trace corresponds to thermal modulator data, while the green, blue, and maroon traces were collected on *FLUX* Flow Modulator prototypes. Peak widths were between 37 ms and 41 ms FWHH.

The volatility range of the modulator is greater than that of a thermal modulator, since modulation occurs without cryogenically trapping analytes. Hence, even the most volatile analytes can be modulated. Figure 9 demonstrates this, showing a modulated peak for methane (C1 alkane), while liquid nitrogen-based thermal modulators are typically limited to compounds less volatile than C4 (butane), and recycling cooler-based Thermal modulators are typically limited to compounds less volatile than C8 (octane). The upper limit of the volatility range, like in thermal modulation, is actually limited by the column set being used and its maximum operational temperature limitations.

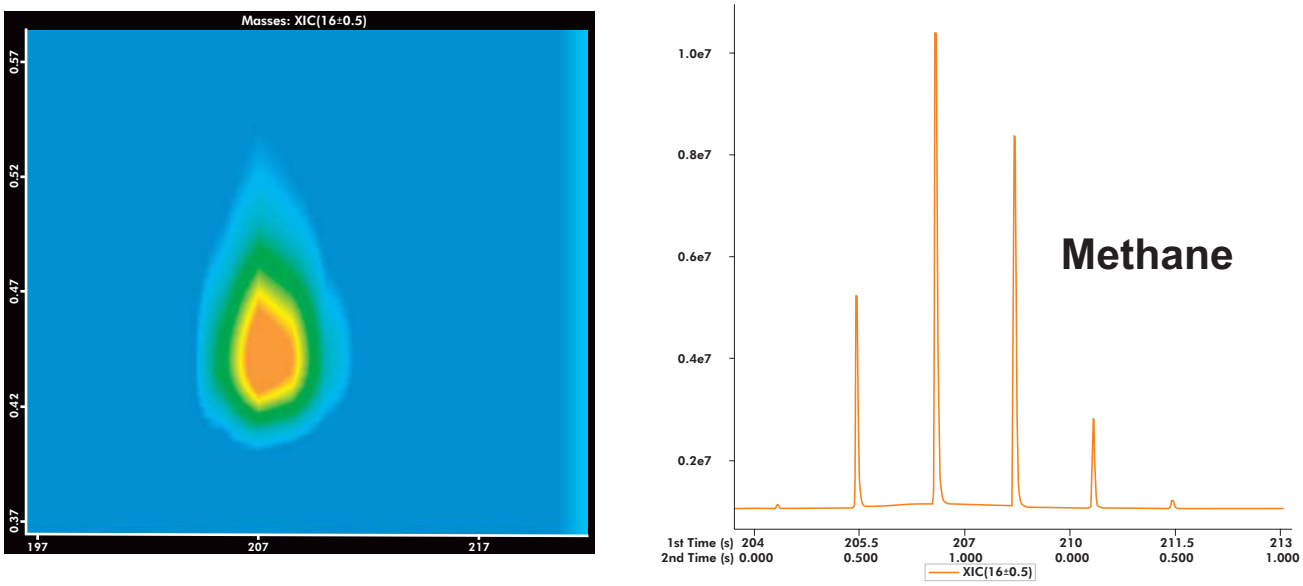

Figure 9. *Left* – the GCxGC contour plot for methane (C1); *Right* – its corresponding linear chromatogram.

Conclusions

The *FLUX* Flow Modulator is a unique flow modulator-well suited for use with MS detectors that provides a lower cost of ownership, and yet satisfactory analytical performance. The modulator's key features include:

- A truly user-friendly system, with no additional flows to manage, restrictors to cut, etc.
- Narrow peak widths that provide greater chromatographic resolution than the broader peaks produced by differential flow modulators. The peak widths are approaching those generated by thermal modulation.
- Sensitivity that is equivalent to, or better than, that obtained with the classic *Pegasus* 4D-C.
- A broader volatility range than thermal modulation, starting at C1.
- No need for splitter since carrier gas flow rates are comparable to Thermal modulation and one-dimensional GC. When compared to differential flow modulation this leads to a faster first-dimension separation (using splitter increases the pressure at the outlet to column 1) and flow rates are within the optimal range for good chromatography (efficiency and/or speed optimized flows).

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[1]Griffith, J. F., Winniford, W. L., Sun, K., Edam, R., Luong, J. C. A Reversed-Flow Differential Flow Modulator for Comprehensive Two-Dimensional Gas Chromatography. J. Chrom. A. 1226 (2012) 116-123
[2]Seeley, J. V., Schimmel, N. E., Seeley, S. K. The Multi-mode Modulator: A Versatile Fluidic Device for Two-dimensional Gas Chromatography. J. Chrom. A, 1536 (2018) 6-15
[3]Seeley, J. V. Theoretical Study of Incomplete Sampling of the First Dimension in Comprehensive Two-dimensional Chromatography. J. Chrom. A, 962 (2002) 21-27

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