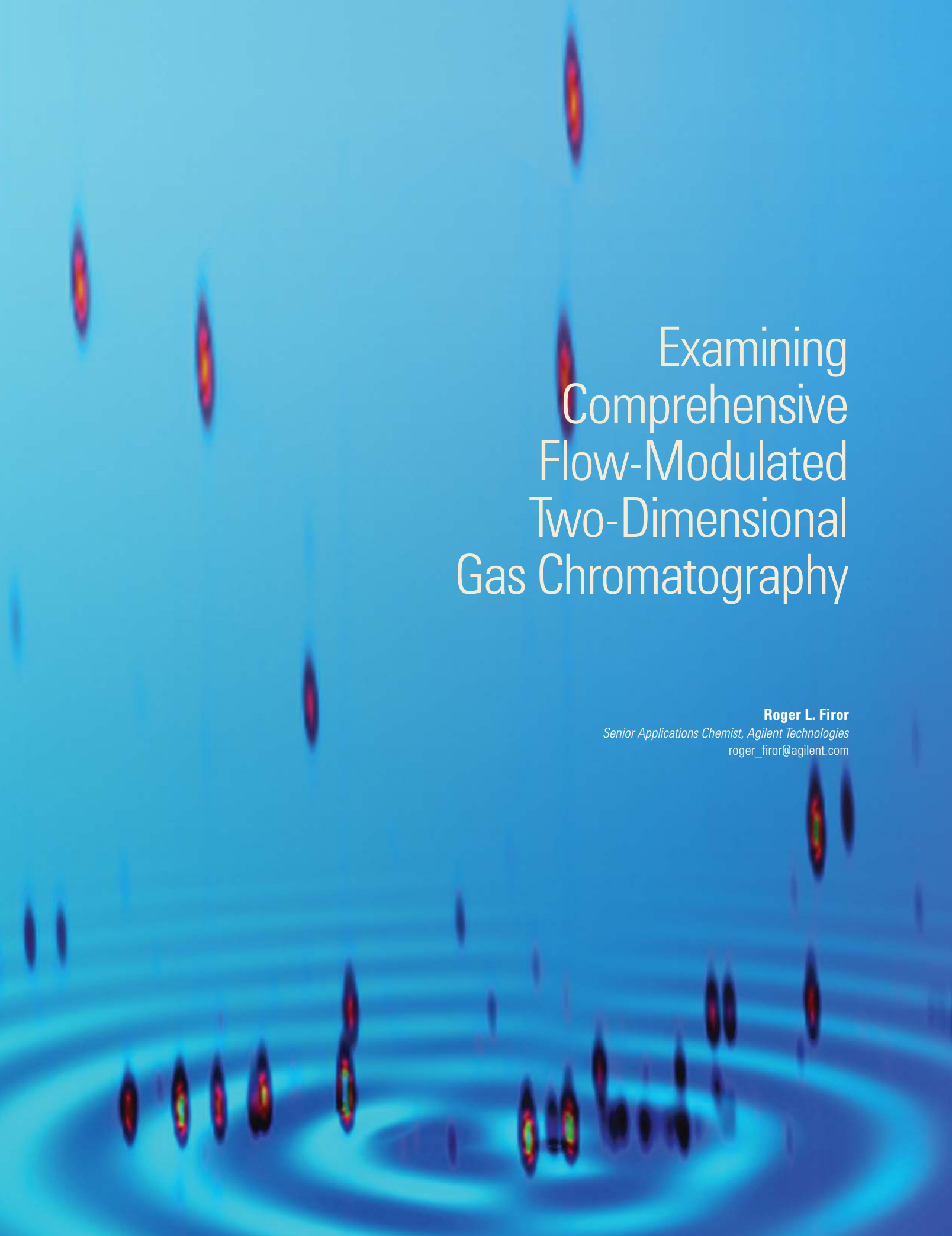

Examining Comprehensive Flow-Modulated Two-Dimensional Gas Chromatography

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Gas chromatography (GC) is the most widely used tool in separation science today. It so well established, however, many practitioners view it as a mature technology that offers little opportunity for innovation. Rather, its widespread use means any worthwhile innovation in hardware, software, or applications can have a significant impact. As proof, at least three significant developments have advanced the art of GC:

- Fast, high-resolution capillary columns have replaced packed columns of limited separation ability.
- "Hyphenated" techniques that couple gas chromatographs with mass spectrometers (GC/MS), for example.
- Electronic controls that provide very precise and repeatable control of flow and pressure in chromatographic systems.

One relatively recent development that has enhanced GC is two-dimensional GC, called "GCxGC" or "2D GC." This hyphenated technique is recognized for its powerful separation capabilities in the analysis of complex mixtures such as those found in the petrochemical and fragrance industries.

The 2D GC methodology utilizes two capillary columns of usually very different polarities installed in series. Between the two columns resides a device known simply as a flow modulator that is interfaced to an auxiliary programmable control module (PCM) on the GC through a three-way solenoid valve.[1] Within the flow modulator, analyte bands from the first column are collected in a fixed-volume channel and successively launched quickly into the short second column in very narrow bands (Figure 1). Any separation that occurs on the first column is preserved during transfer to the second column.

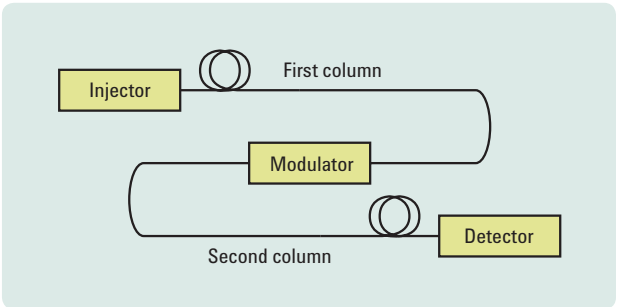


Figure 1. 2D GC utilizes a primary column (conventional separation), a flow modulator, a second column (very fast separation) and a fast detector.

Compared to one-dimensional separations, 2D GC can increase peak resolution and peak capacity, resulting in a greater number of individual compounds being separated. Per unit of time, its peak-generating ability is much greater than that of a single-column separation.

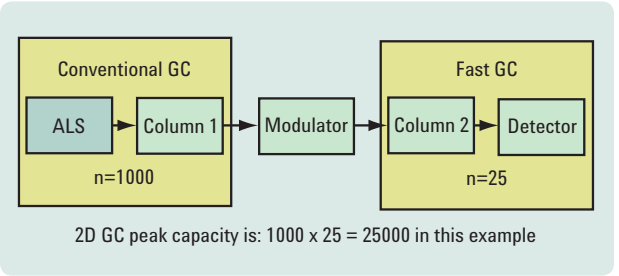


Figure 2. 2D GC provides a significant increase in separation power compared to 1D methods.

Creating a better modulator

The key component in a 2D GC system is the modulator. This device must transfer effluent from the first column to the second precisely and with high repeatability under a wide range of carrier flow rates and temperatures — without adding any extraneous chromatographic artifacts.

Most systems available today employ thermal modulation techniques in which rapid cooling (using a cryogenic jet) followed by rapid heating (using a hot-gas jet) immobilizes and then remobilizes the analytes. In such systems the cryogenic jet is always on and repetitive firing of the hot jet sends sharp bands of material to the second column for separation and detection.

The fabrication of conventional devices involves machining of stainless steel blocks. While this works well for simple devices such as unions, the complex structures required for controlled flow diversion (e.g., Deans switches or three-way splitters) are much more challenging. The need for low dead volume requires holes typically drilled at 0.25 mm inside diameter (ID) in size. This level of precision is possible only for short distances, making manifold construction very difficult and costly. The approach also results in significant thermal mass, which is undesirable in a chromatographic oven.

1. This concept was first demonstrated by John Seeley of Oakland University, Rochester, Michigan.

Agilent has developed a unique modulator that is simpler to operate and provides a lower cost of ownership compared to thermal modulation. Rather than using cryogenics, flow differentials trap or focus analytes as they exit the first column. This approach is based on Agilent's capillary flow technology hardware, which utilizes a new way of fabricating complex structures. Combined with several other improvements, this allows construction of in-oven devices that make difficult GC application problems easier to solve.

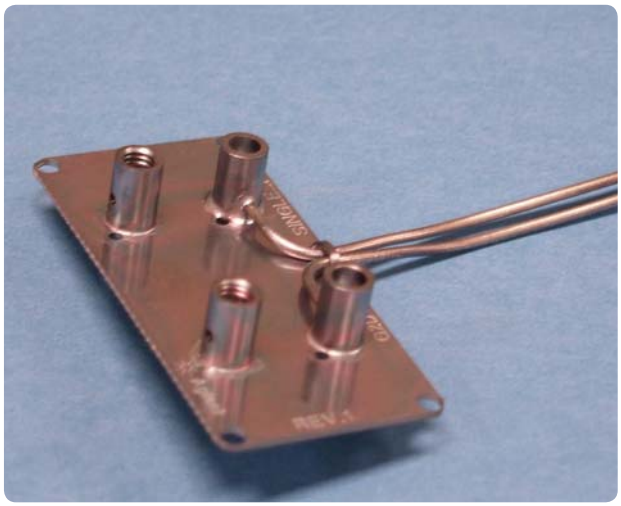


Figure 3. Agilent's modulator design includes specialized fittings attached by projection welding.

A planar structure incorporates flow tees and collector channels (Figure 3). All external connections are made through specialized fittings incorporated by projection welding onto the plate, providing near-zero unswept volumes and leak-tight seals. Agilent's design includes two major innovations: a diffusion-bonded manifold plate and a metal ferrule connector. The plates are the basis for low-mass, low-dead volume devices that enable diversion and splitting of gas flows to create a number of previously difficult capillary configurations.

Similar to integrated circuit manufacturing, the forming of this flow architecture uses photolithographic techniques to create the channels on one or both of two plates that are bonded together to form a metallic sandwich containing the desired internal flow channels. The ability to create these channels and provide external flow connections over very small distances within the GC oven dramatically reduces dead volume. What's more, the low thermal mass of the integrated plate provides for efficient heat transfer that might otherwise result in thermal lag and cold spots in the sample path. Figures 4 through 7 show examples of subassemblies, fittings and finished devices typical of capillary-flow technology. These structures are chemically deactivated to reduce interaction with active compounds.

Figure 4. Photolithographic milling ensures precise channel geometry and low dead volume in capillary-flow devices.

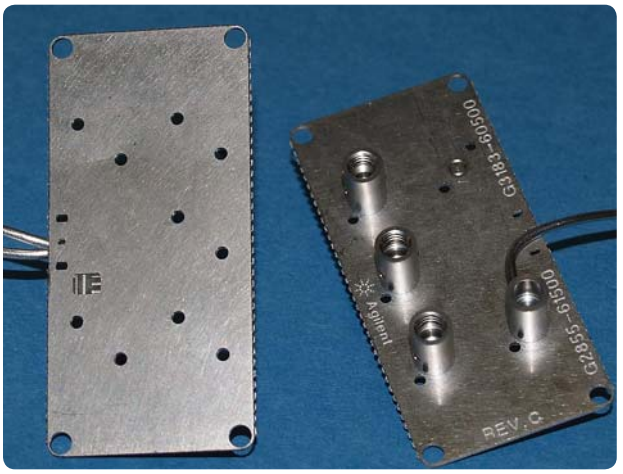


Figure 5. Top and inside views of a capillary-flow purged splitter.

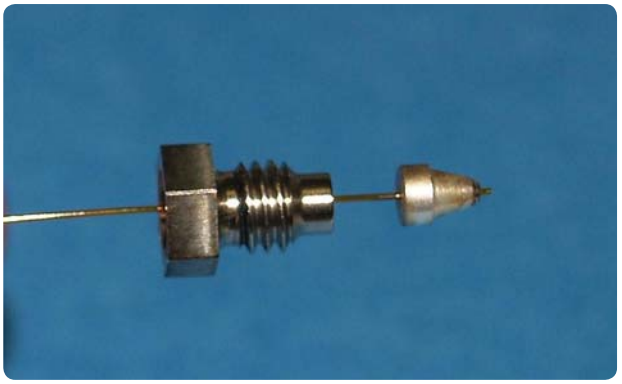


Figure 6. This subassembly provides a leak-tight connection to the modulator.

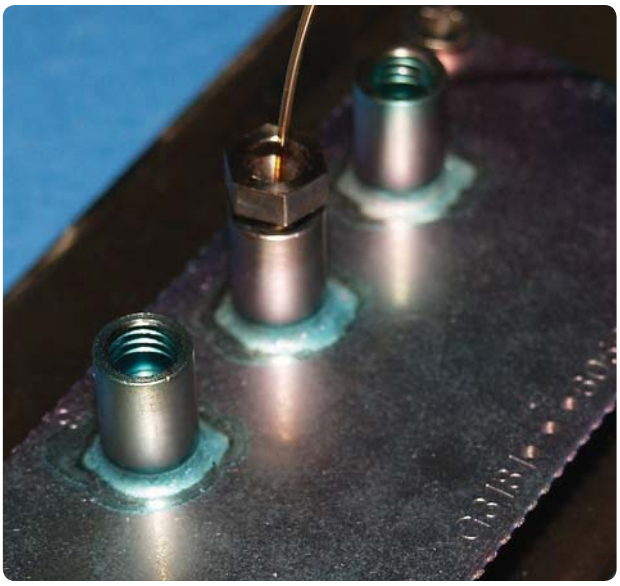


Figure 7. Close-up view of a connection to a capillary-flow device

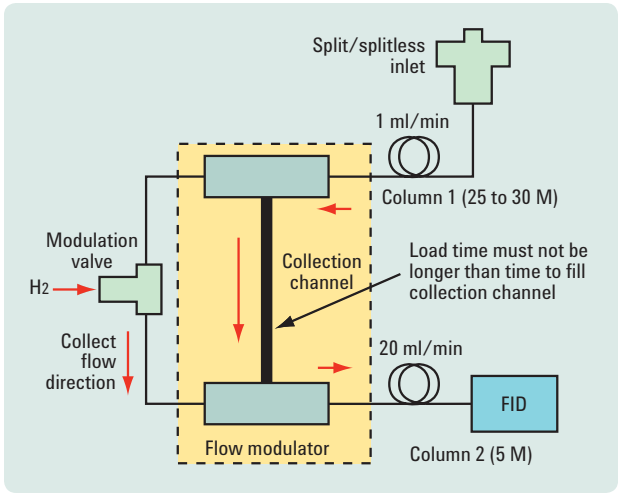


Figure 8. Flow rates and flow directions during the load or "collect" portion of the modulation cycle

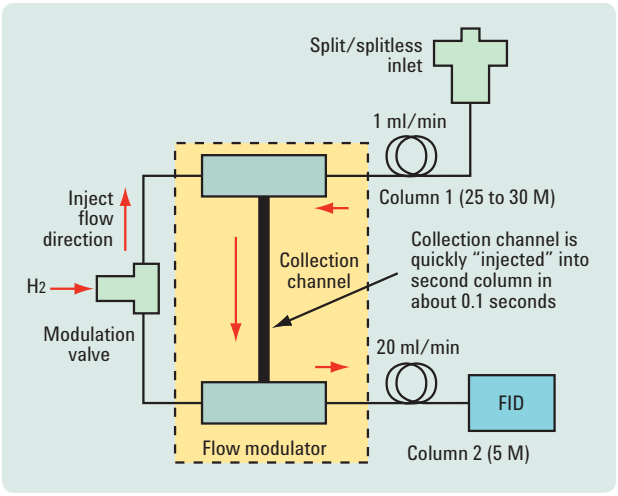


Figure 9. Flow rates and flow directions during the transfer or "inject" portion of the modulation cycle

Outlining system functionality

Figures 8 and 9 illustrate the modulator. A three-way solenoid valve installed on top of the GC oven interfaces to a PCM module; the periodic switching of this solenoid drives the modulator. The precisely timed and synchronized switching between the "collect" and "flush" states directs discrete sample pulses continuously to the second column for additional fast separation during the entire chromatographic run.

Typical experimental parameters

GC:	Agilent 7890A
Detector:	FID at 200-Hz data collection rate, split/splitless inlet
Carrier:	Hydrogen
Column 1:	30 m x 0.25 mm x 0.25 um HP-5 ms, 19091S-433
Column 1:	Pressure: 21.5 psig at 50° C, constant flow mode
Column 2:	5 m x 0.25 mm x 0.15 um INNOWAX
Column 2:	Flow: 20 ml/min, constant flow mode
Oven program:	50° C (1.0 min) to 260° C (4 min) at 8° C/min.
Modulator period:	1.4 seconds collect, 0.12 second flush
2D analysis software:	GC Image (www.gcimage.com)

Load or collect state (Figure 8)**:** The primary column effluent containing analytes exits the first column after undergoing initial separation at the top tee. The second column flow, sourced by the PCM through the solenoid, enters at the bottom tee. In this configuration, first-column analytes will fill the collection channel; some compression occurs in the channel due to elevated pressure at the first-column exit.

Inject or flush state (Figure 9)**:** Flow from the solenoid is directed to the top tee. A high flow of typically 20 ml/min rapidly flushes the collection channel, transferring material in a very narrow band onto the second column where any analytes in the channel undergo rapid separation.

Capillary columns and speed: Total analysis time in a typical 2D GC system is essentially the same as with a 1D setup. A 2D chromatographic method will use oven temperature programs and column dimensions (25 to 30 m x 0.25 mm ID) similar to those in a 1D system. The typical goal is to achieve maximum peak capacity and display the maximum number of well-separated

discrete compounds. Higher temperature program rates and shorter columns with smaller ID can be used; however, this may sacrifice separation for overall reduction of analysis time. A typical column set consists of a low-polarity column coupled to a very short (3 to 5 m) polar column (both with 0.25 mm ID). The second column must be very short to provide separation of all injected analytes during a typical 1.5-second modulation cycle or be driven at high-temperature program rates in a separate oven module.

Applying 2D GC

Figure 10 shows unmodulated and modulated peaks of a pure analyte (n-butylbenzene with approximately four modulations across the peak). Each modulated peak is very narrow due to the focusing effect of the modular and the speed of transfer to the second column. The peak height increases relative to the unmodulated peak since all mass is conserved. Ideally, the areas of the modulated peaks should equal the area of the unmodulated peak; the areas were within three percent for this test. Peak widths at half height for modulated butylbenzene are approximately 65 to 75 ms. Very narrow peaks are required for the technique to work properly and those shown in Figure 10 are similar to those obtained with thermal modulation systems.

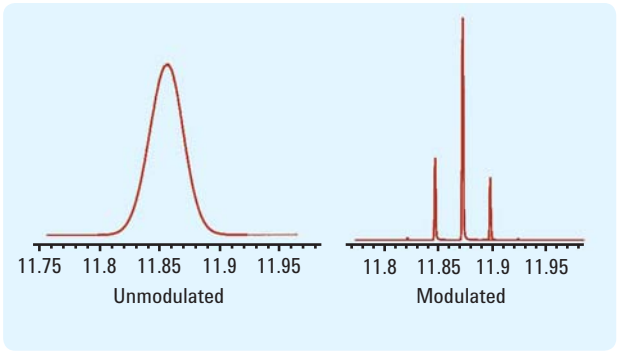
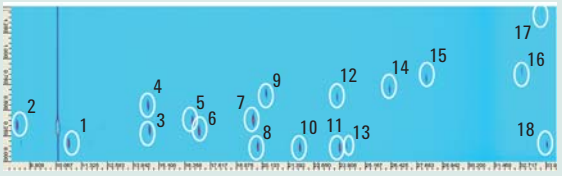


Figure 10. Unmodulated and modulated n-butylbenzene peak from a hydrocarbon test sample used to check modulator performance and timing

After checking for peak shapes and mass conservation, a performance test sample is run to verify the separation results and check for wrap-around effects (Figure 11). Wrap around is an undesirable consequence of compounds not completing elution from the second column during the modulation cycle in which they were injected.



1. Octane
2. Fluorobenzene
3. Propylbenzene
4. Bromo-2-fluorobenzene
5. Indane
6. Butylbenzene
7. Tetralin
8. Dodecane
9. Naphthalene
10. Tridecane
12. Fluorobiphenyl
13. 1, 3, 5-Tributylbenzene
14. Acenaphthalene
15. Fluorene
16. Terphenyl
17. 2-Methylanthracene
18. Elcosan

Figure 11. This 2D GC display verifies the separation results from the hydrocarbon test sample.

The ability to visualize hydrocarbon class separations is a major attraction of 2D GC. Using non-polar column followed by a polar column produces hydrocarbon type retention in the following order: 1) alkanes, 2) cyclic alkanes, 3) olefins, 4) single-ring aromatics and 5) multi-ring aromatics. Figure 12 shows a 2D image of kerosene: Chemical classes are clearly discernible for all of these petrochemical materials, especially the aromatics.

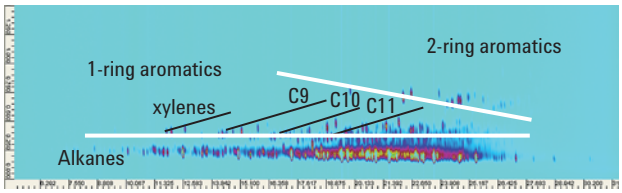


Figure 12: The one-ring and two-ring aromatics are clearly visible in this 2D measurement of kerosene.

Processing the acquired data

Specialized software is required to extract information from 2D GC data. Generally, the data are presented in a two-dimensional flat view where the retention time for the first dimension is along the "x" axis in minutes and the retention time of the second dimension is along the "y" axis in seconds. The software parses the chromatogram or raw data into this construct given knowledge of the modulation period (Figure 13). In visualization, the elution of chemical compounds from the two columns produces a cluster of pixels with values greater than background; colors are used to represent intensity or chemical amount. Figure 14 shows a 2D image based on the parsing of a raw modulated chromatogram.

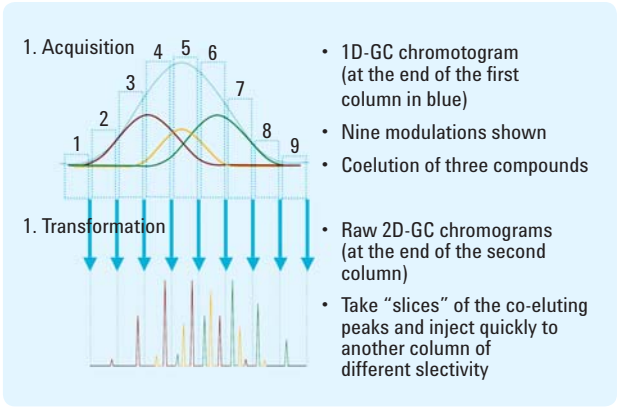


Figure 13. After GCxGC modulation, the red peak elutes last, the green peak elutes first and all three are completely separated.

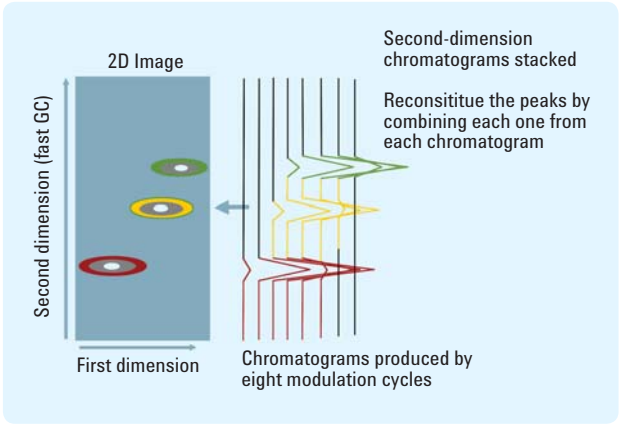


Figure 14. To visualize data in two dimensions, the modulated chromatograms are assembled as shown here

These 2D images lend themselves to pattern recognition techniques. Principal component analysis (PCA), a data reduction tool used to classify samples, can be a powerful quality assurance (QA) tool when applied to final characterization of other complex products such as coffee, flavors and perfumes (Figure 15). This shows the potential to apply common chemometric pattern-recognition tools for sample classification to discern product differences, quality, adulteration and authenticity.

Note that the applications described in this article use a 1D flame ionization detector; however, the technique lends itself to coupling with a mass spectrometer to provide another dimension and confirmatory compound identification. Time-of-flight (TOF) mass spectrometers are typically used due to the need for high data collection rates. Quadrupole-based mass spectrometers such as the Agilent 5975C may also be used in limited-scan or selected ion monitoring (SIM) data acquisition modes of operation.

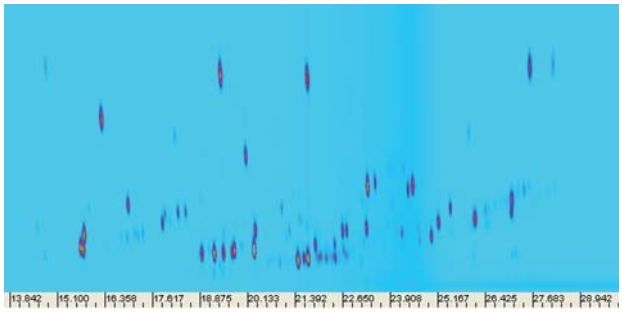


Figure 15. 2D GC can be used as a quality assurance tool for final characterization of complex products such as perfumes (shown).

Conclusion

For more than 10 years, 2D GC has been largely confined to the research laboratory, used only by skilled practitioners of gas chromatography. With reliable, easy-to-use hardware integrated into the gas chromatograph, the technique is now ready for more routine lab settings. Much of this is made possible by the advanced fabrication methods embodied in Agilent's capillary flow technology, and through firmware and software control of the resulting devices.

Extracting informative results depends on processing of 2D-GC data — and this remains a significant challenge. Specifically, quantitation requires more effort compared to traditional 1D GC and well-established hyphenated techniques such as GC/MS. In the next few years, however, we expect to see significant advances as 2D GC becomes better known and more widely used.