

# Utilizing GCxGC for Advanced Analytical Analysis of Volatile and Semi-Volatile Organic Compounds

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## Background

Volatile and semi-volatile organic compounds are some of the most widely studied samples. Traditional analytical methods rely on single dimension gas chromatography (GC) coupled to selective detectors or mass selective detectors (MS). More recently, the use of comprehensive two-dimensional gas chromatography (GCxGC) as an advanced analytical tool has gained significant popularity for complex sample analysis, target species in a heavy matrix, screening of difficult samples and quantification of compounds below traditionally reported L.O.D's. Recent advances to GCxGC instrumentation have made this technique more robust to routine analysis, screening of complex samples and the simultaneous quantitative detection of compounds found within. This paper will introduce GCxGC and explore the opportunities that make it a viable analytical tool for advanced analysis of food and flavors, metabolomics, petroleum, forensic and environmental samples.

## 1. Introduction

Since its invention just over a decade ago, the novel technique of comprehensive two-dimensional gas chromatography (GCxGC) has been developed to separate and analyze complex samples such as petroleum 1-5, flavors 6, environmental 7-9 and even human breath samples 10. The technique of GCxGC employs two coupled columns of different selectivity and subjects the entire sample to a two-dimensional separation. Effluent from the primary column is modulated to produce sharp chemical pulses, which are rapidly separated on the second column. A separation plane is produced by the two orthogonal retention time axes for the columns 11. Usually, the first column contains a non-polar stationary phase and the second column a polar stationary phase. This combination allows components to be independently separated, first according to their volatility and then according to their polarity. In comparison to conventional single column gas chromatography, GCxGC has much higher peak capacity because the available peak capacity is the theoretical product of the peak capacity of both dimensions. Other advantages

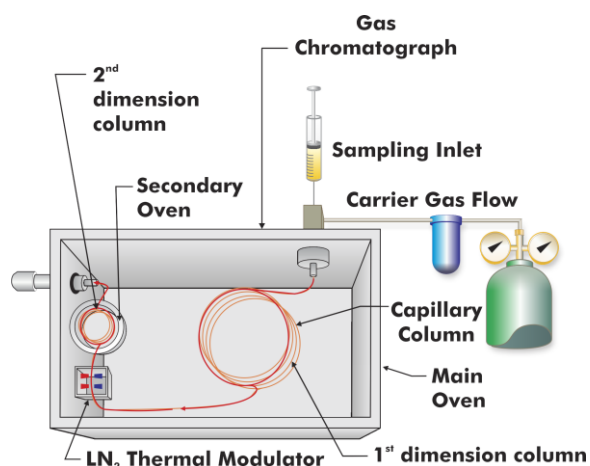
of GCxGC include enhanced detectability due to analyte refocusing, true background around resolved peaks, more reliable identification due to two retention times and well ordered bands of compound groups 12. The key element in a GCxGC system is the modulator, which focuses segments of the effluent from the primary column and re-injects them onto the secondary column. Different types of modulators have been designed and shown to be capable of producing GCxGC chromatograms 13-19.

The two most common types of modulators are thermal based and valve based. Thermal modulators are the most widely used in the commercial industry and deliver the best performance with respect to modulated peak widths across a wide volatility range. Thermal modulators rely on a cryogenic media (LCO<sub>2</sub> or LN<sub>2</sub>) to cool the cold-jet gas and as such have limited their use to laboratory based instruments. Valve based modulators are very simple in design and deliver the ultimate performance for highly volatile components. Unfortunately, due to the added carrier gas needed to sweep the components out of the modulator, valve based systems have limited application in quantitative MS work.

GCxGC should not be confused with 2DGC, a multi-dimensional system consisting of two-columns in series, or heart-cut 2DGC, a dual column system where compounds of interest are re-directed to a second analytical column. Only GCxGC delivers comprehensive analytical results where the entire primary column effluent is modulated and analyzed by a second orthogonal column. The results of GCxGC are unparallel and complex samples analyzed by

this technique often result in detection, identification and quantification of more than 10x the number of peaks identified by 1DGC alone.

## 1.1 Schematic of GCxGC



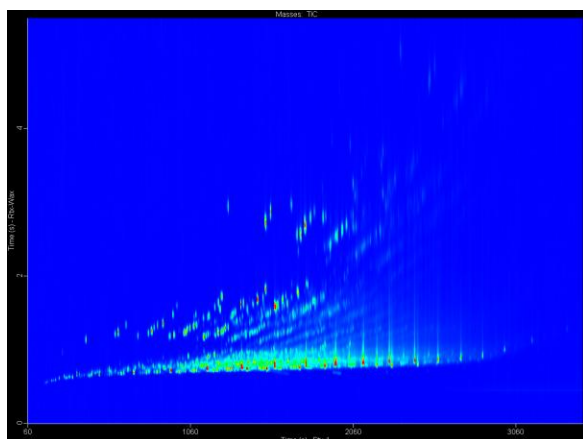
*Figure 1. Schematic of a GCxGC system showing 1<sup>st</sup> dimension column, thermal modulator and 2<sup>nd</sup> dimension column located inside a secondary oven.*

## 2. GCxGC Data Processing

GCxGC data is collected in a continuous stream from the detector and displayed on a two-dimensional retention plane (contour plot) as opposed to a single linear trace as is most common in one-dimensional GC. The contour plot peaks are represented by a color scale of intensity (blue is baseline and red is the most intense). User defined parameters such as modulation period (the time required between each modulation cycle and hence each rapid second column separation) and total run time are used to define retention time on each axis of the contour plot. The GCxGC contour plot is easily rotated on axis to produce a 3D image that will show the intensity of each peak along the z-axis.

All qualitative and quantitative aspects of data review are easily performed in commercially available software. Current platforms allow peak finding, retention time in both dimensions, peak area, report generation as well as more advanced statistical calculations. GCxGC data can easily be exported as net NetCDF, ASCII or mzXML files for comprehensive statistical analysis.

A common question is often asked about GCxGC data file sizes. Depending on your detector type and sampling rate, files usually range from low MB to low GB. A 60 minute GCxGC-TOFMS acquiring at 200 spectra/s will produce a 2 GB size file.



**Figure 2.** GCxGC contour plot for a petroleum sample. More than 3500 compounds were identified.

### 3. Advantages of GCxGC

The advantages that GCxGC provides over single dimension GC can be summarized in the following categories:

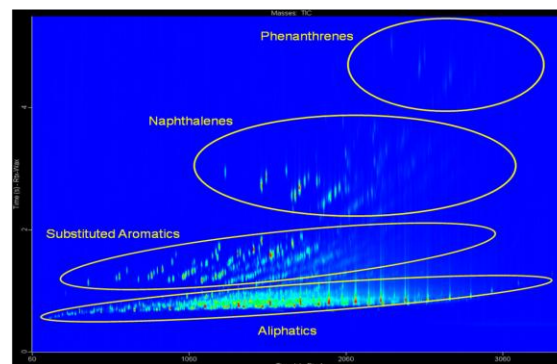
#### - Enhanced Chromatographic Resolution

Utilizing two columns with orthogonal phases results in greater separation power. Traditional

configuration uses a long non-polar column in the 1<sup>st</sup> dimension and short polar column in the 2<sup>nd</sup> dimension. Mixtures are separated by boiling point on the 1st column and then by polarity on the 2<sup>nd</sup> column. This orthogonal exposure yields to enhanced chromatographic resolution and theoretically decreases the opportunity for component co-elutions. Compounds that co-elute on the primary column (boiling point) are trapped, modulated and then sent to the second column where they are separated based on polarity. This continuous process greatly decreases the number of co-elutions in the overall analysis

#### - Structured Contour and 3D Plots

Processing the linear data stream into a mathematical matrix based on modulation period allows the analyst to view the data in a visual multi-dimensional fashion. The data can be viewed in a top-down contour plot as shown in figure 3 or as a 3-dimensional rotating plot as shown in figure 4.



**Figure 3.** A GCxGC contour plot displaying the structures of a petroleum sample. Aliphatic compounds, Substituted Aromatics, Naphthalenes and Phenanthrenes are all separated but grouped together for easy identification.

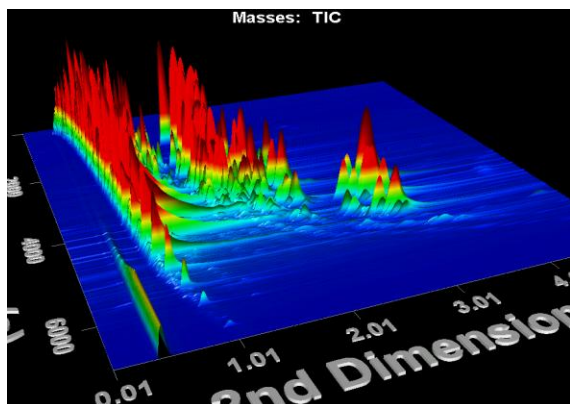


Figure 4. Three dimensional plot of a petroleum sample.

### - Enhanced Detectability

When the column effluent from the 1st dimension is focused and re-injected into the second column for a rapid analysis, the effects of peak broadening are greatly minimized. The resulting peaks, as recorded by the detector, are commonly 50-150 ms full width half-height. The focusing effect combined with minimal band broadening effects results in narrow peaks that deliver an enhanced S/N value. Common enhancements are on the order of 5-10x depending on concentration and detector acquisition rate. An experimental representation of peak detectability is shown in figure 5.

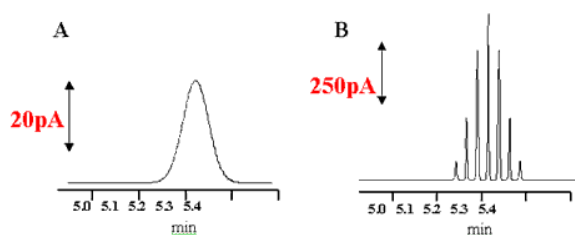


Figure 5. Enhancement of Peak Amplitude as a result of the modulation process in GCxGC. The overall Gaussian peak shape and peak area is maintained and the modulation process results in multiple sharp peaks with increased amplitude.

### - Extended Theoretical Peak Capacity

Peak capacity ( $C_p$ ) is defined as the number of peaks that can be separated by a user defined resolution in a single analysis. Traditional 1-dimensional GC analysis can deliver a theoretical peak capacity of 100+ peaks, depending on column length, internal diameter, stationary phase film thickness and temperature programming rates. The equation for peak capacity in a temperature programmed environment is defined below in equation (1).

$$C_p = \left( \frac{\Delta t_r}{V_{1/2} + V_{1/2}} \right) - 1 \quad (1)$$

When multi-dimensional techniques are employed, the peak capacity in the first dimension is relatively similar to a 1D GC analysis with the exception of peak broadening, which is a function of the pressure drop across the two columns. However, the rapid second dimension analysis is conducted under pseudo-isothermal conditions and as a result of this, the peak capacity is calculated based on an isothermal separation. The equation for isothermal peak capacity ( $C_{p_i}$ ) is defined below in equation (2).

$$C_{p_i} = 1 + \frac{\sqrt{N}}{4R_s} \ln \left[ \frac{t_{r(last)}}{t_m} \right] \quad (2)$$

In order to calculate the theoretical peak capacity of a GCxGC system ( $C_{p_i}$ ) one can derive that the peak capacity is a product of both dimensions. The resulting numbers are staggering, with typical analysis delivering a theoretical capacity of greater than 5000. The

derived equation for total peak capacity in GCxGC is shown in equation (3).

$$Cp_t = \left[ \left( \frac{\Delta t_r}{\sqrt{V_{1/2, \text{p}}} + \sqrt{V_{1/2, \text{s}}}} \right) - 1 \right] \times \left[ 1 + \frac{\sqrt{N}}{4R_s} \ln \left[ \frac{t_{r(\text{last})}}{t_m} \right] \right] \quad (3)$$

Experimental studies are shown in figure 10.

### 3. Sample Injection System

Traditional laboratory instruments utilize a variety of sample inlets such as liquid injection, Solid Phase Micro Extraction (SPME), Thermal Desorption, Pyrolysis as well as sorption traps. In order to make GCxGC widely applicable to a variety of applications and environments, efforts are under way to design application specific inlets and sample collection devices. One of the more versatile systems is a custom designed multi-bed sorption trap that consists of a molecular sieve, Carbo Pack X, Carbo Pack B and Carbo Pack Y. This design allows for air sampling, off gas analysis of solids as well as static and dynamic headspace analysis of various types of samples.

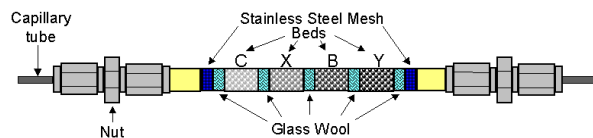


Figure 5. Schematic design of a multi-bed sorption trap system for GCxGC analysis.

### 4. Detectors for GCxGC

As stated previously, GCxGC produces very narrow bands at the detector. The resulting peaks are 50-150 ms and as such require a very fast data acquisition rate. Qualitative analysis

requires a minimum of 10 points across a fully resolved peak. In order to perform quantitative work and/or compound identification in heavy matrix, a recommendation of 18-20 data points across a peak is required for deconvolution algorithms to perform accurately. With these requirements a 100 ms GCxGC peak would need a detector acquisition rate of 200 Hz to properly define the peak. Typical detectors used with GCxGC include Flame Ionization (F.I.D.), Electron Capture (E.C.D.), fast acquisition Time-of-Flight Mass Spectrometry (TOFMS), and fast scanning quadrupoles that operate in Select Ion Monitoring (S.I.M. - target analysis only).

### 5. Applications for GCxGC

GCxGC is widely known as an advanced analytical tool for complex sample analysis. Over the last ten years multiple review papers have been published and the number of research groups and relevant applications is increasing steadily<sup>20</sup>. Of significant importance is the contribution of GCxGC to the fields of environmental analysis (trace level detection of P.O.P's), petroleum (sulfur speciation and fingerprinting), food and flavors (off-flavor compounds and batch processing) as well as metabolomics (human breath, biomarker discovery, plant and animal small molecule analysis). In the field of space science, the analysis of meteorite fragments, planetary soil and dust analysis as well as the study of primordial soups has been explored by GCxGC. The examples below highlight the features that GCxGC offers for complex sample analysis.

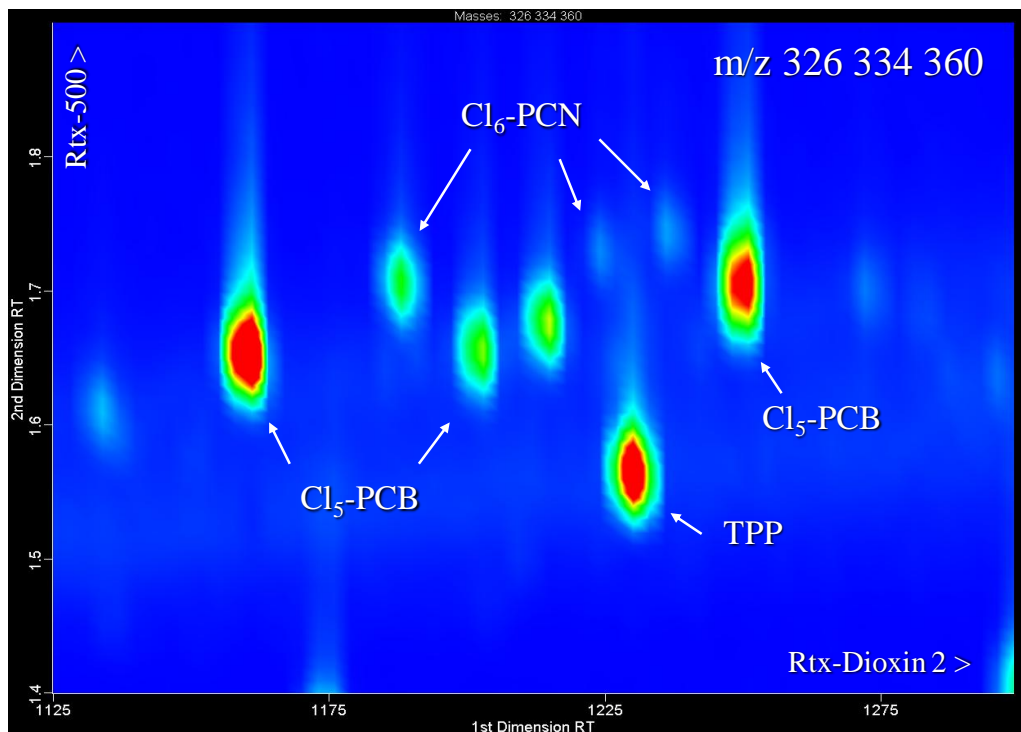


Figure 6. GCxGC contour plot of PCB's and PBN's in a fish extract.

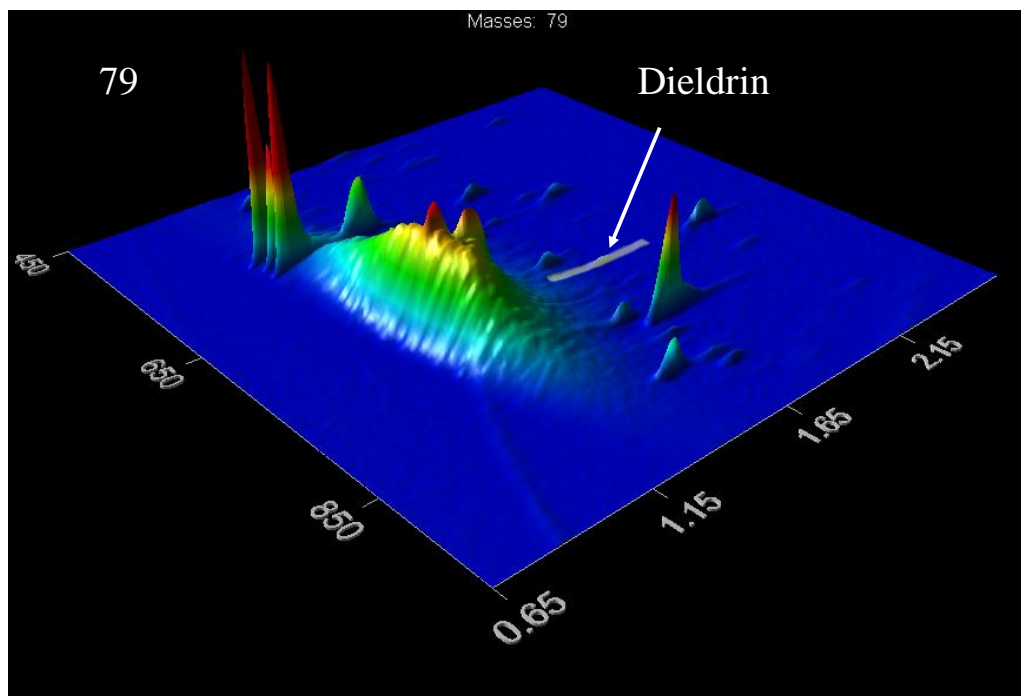


Figure 7. GCxGC analysis, identification and quantification of Dieldrin in a cucumber extract.

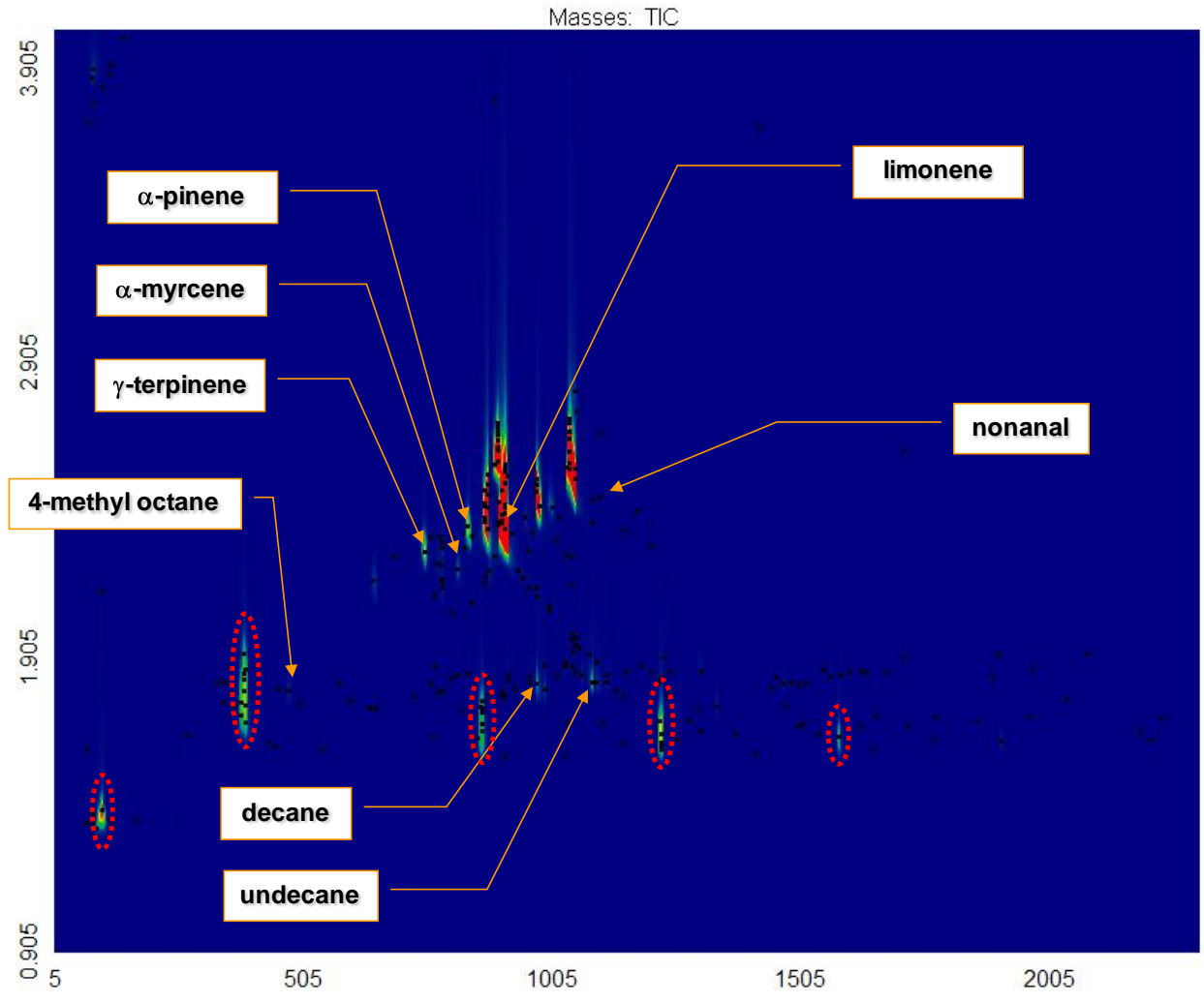
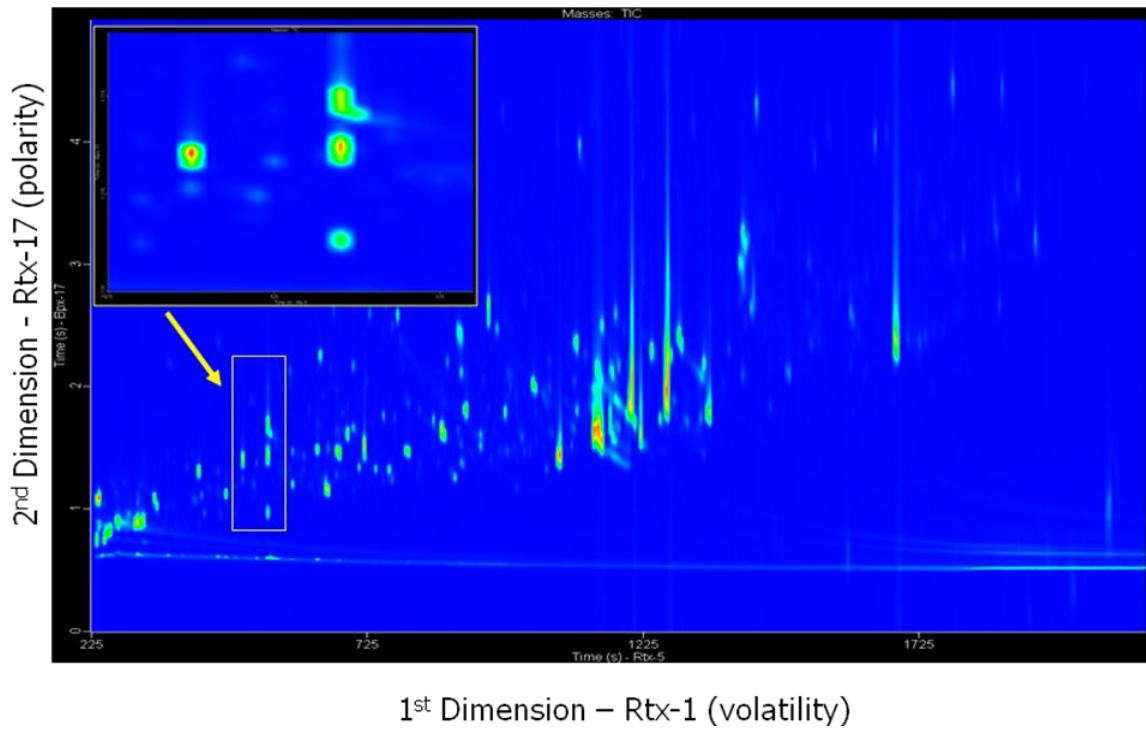
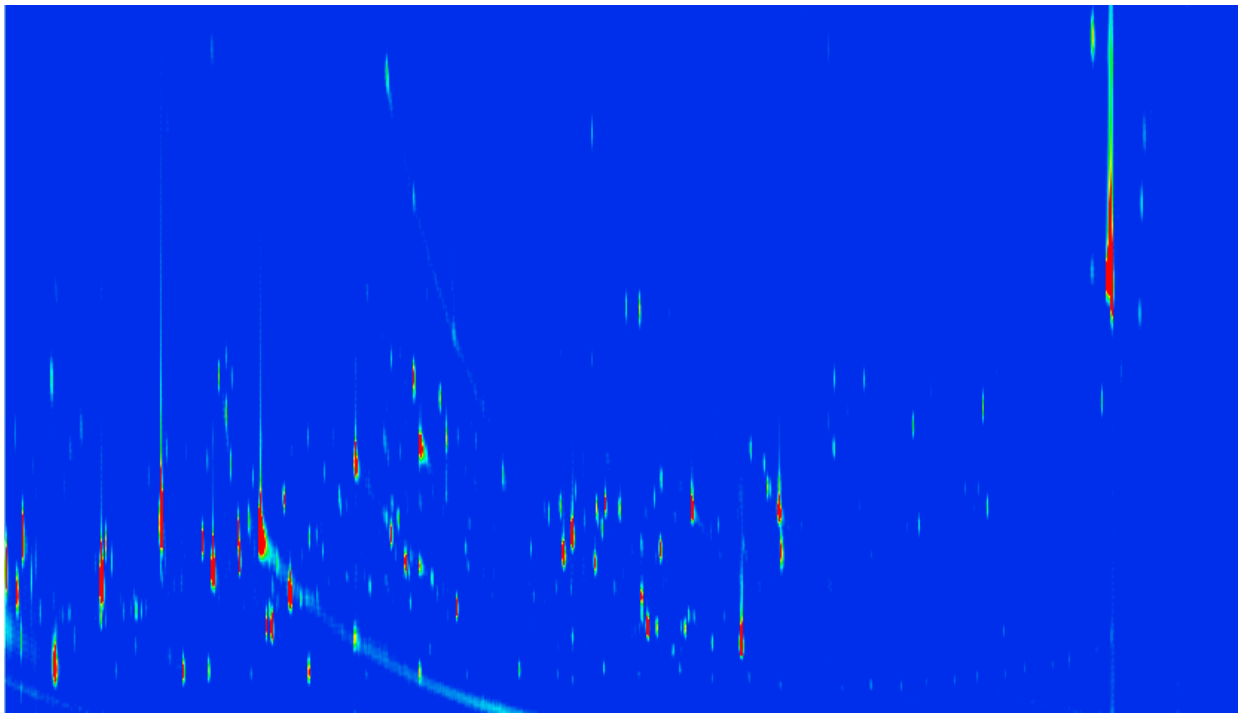
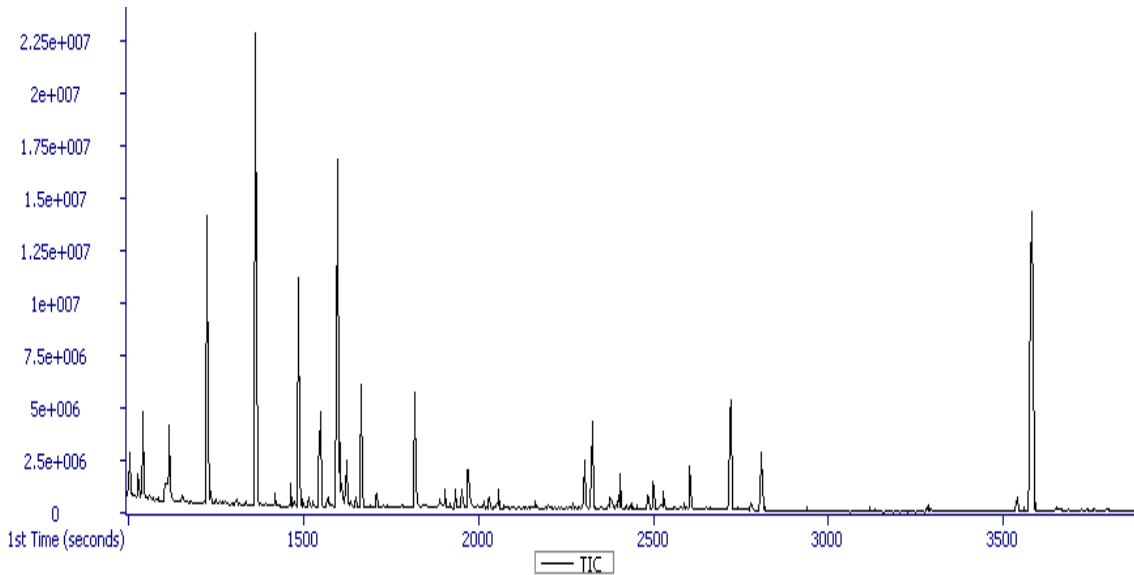


Figure 8. GCxGC analysis of Human Breath after drinking a flavored sports drink.



**Figure 9.** A derivatized metabolomic sample displaying an area where 3 compounds co-eluted on the primary column but were ultimately separated.



**Figure 10.** A derivatized "Metabolomic" sample that has been analyzed by both GCMS and GCxGC-MS. GCxGC allows the analyst to have a clearer and more structured view of the sample while maintaining its analytical integrity. Trace level compounds (not detected in the GCMS analysis) as well as saturated compounds are easily separated and identified on the GCxGC retention plane. 538 peaks with a S/N of >100 were detected in the GCMS analysis. 1227 peaks with a S/N >100 were detected in the GCxGC-MS analysis.

## Summary and Conclusion

The use of GCxGC for complex sample analysis, trace level detection of target compounds and advanced screening of biological samples has become a viable tool for analytical researchers. Key advantages such as increased peak capacity, enhanced resolution and increased detectability are necessary for simultaneous

trace level screening and identification in complex matrices. Advances in sample collection systems, modulators and high speed detectors has aided in the use of GCxGC as a more routine technique.

For a comprehensive analysis of your samples please contact Volatile Analysis or visit us on the web at [www.volatileanalysis.com](http://www.volatileanalysis.com)..

Samples used in this publication were analyzed on a LECO, Pegasus 4D GCxGC-TOFMS system operating with ChromaTOF software.

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