Research Hype to Practical Analysis: Benefits of Comprehensive Two-Dimensional Gas Chromatography (GCxGC) for a Routine Laboratory

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Comprehensive two-dimensional gas chromatography (GCxGC) is a technique that has been around for over 25 years. Some may argue that the technique has been over-hyped, and that it is a complicated research tool that doesn't fit into routine analytical laboratories. However, several routine validated GCxGC methods have demonstrated that the technique is accurate, precise, and robust. Research has shown the benefits of GCxGC in a wide variety of applications (e.g. environmental, metabolomics, petroleum, food safety, fragrance). The benefits include increased peak capacity (i.e. resolution), structured two-dimensional chromatograms (i.e. contour plots), and sensitivity enhancement. The merits of GCxGC as a separation science are clear, but how will it benefit a routine laboratory? Multiple analyte classes can be combined into a single analysis to save instrument and sample preparation time. Manual review time for non-target screening methods can be reduced with the increased peak resolution of a GCxGC analysis by creating a better library match leading to faster, more confident peak identification. Sample characterization is improved with a GCxGC analysis which increases confidence in decisions based on analytical results. The extra resolution afforded by the GCxGC analysis allows the use of more economical detectors, like the flame ionization detector (FID) or electron capture detector (ECD).

Introduction

One-dimensional gas chromatographic (1D-GC) analyses of complex environmental, biological, or petrochemical samples often result in a chromatogram with a large portion of unresolved components. Mass spectrometry can be used to resolve some of the complexity but structural isomers and large concentration differences can complicate the data analysis and spectral interpretation. Some chromatographic resolution can be enhanced with an efficient, long, narrow bore, thin film capillary column, but increased analysis time and reduced sample loading capacity may be unattractive for high throughput laboratories with complex samples. Multidimensional gas chromatography (MDGC or 2D-GC) increases resolution by using two separate columns with two different stationary phases. One form of MDGC is heart-cutting. After an initial evaluation of the sample, a portion of the unresolved GC effluent can be diverted to a different column prior to detection. Heart-cutting can be a simple way to achieve a better separation of a complex mixture, however only a portion of the one-dimensional separation can be enhanced with the second-dimension column. Comprehensive two-dimensional gas chromatography (GCxGC) utilizes a high frequency modulator to divert the entire one-dimensional effluent onto a second-dimension column. Previous knowledge of the separation (or lack thereof) is not necessary to optimize the analysis since every peak is transferred to the second-dimension column. The detector records both the first and second dimension retention times, and the response is plotted to create a chromatographic plane (i.e. contour plot) (**Figure 1**). The chromatographic separation space is dramatically increased compared to a conventional 1D-GC analysis, yielding a big leap in potential peak capacity. In addition to increased resolution, the resulting chromatogram is also structured so that similar compounds elute in clustered bands across the chromatogram plane. The clustering of chemically and structurally similar compounds provide a helpful clue that can aid in peak identification. Another positive side-effect of a GCxGC analysis is increased compound detectability. When the modulator rapidly traps and “injects” the effluent of the primary column it focuses the peaks only a few seconds before being detected. This process enhances the signal-to-noise ratio (S/N).

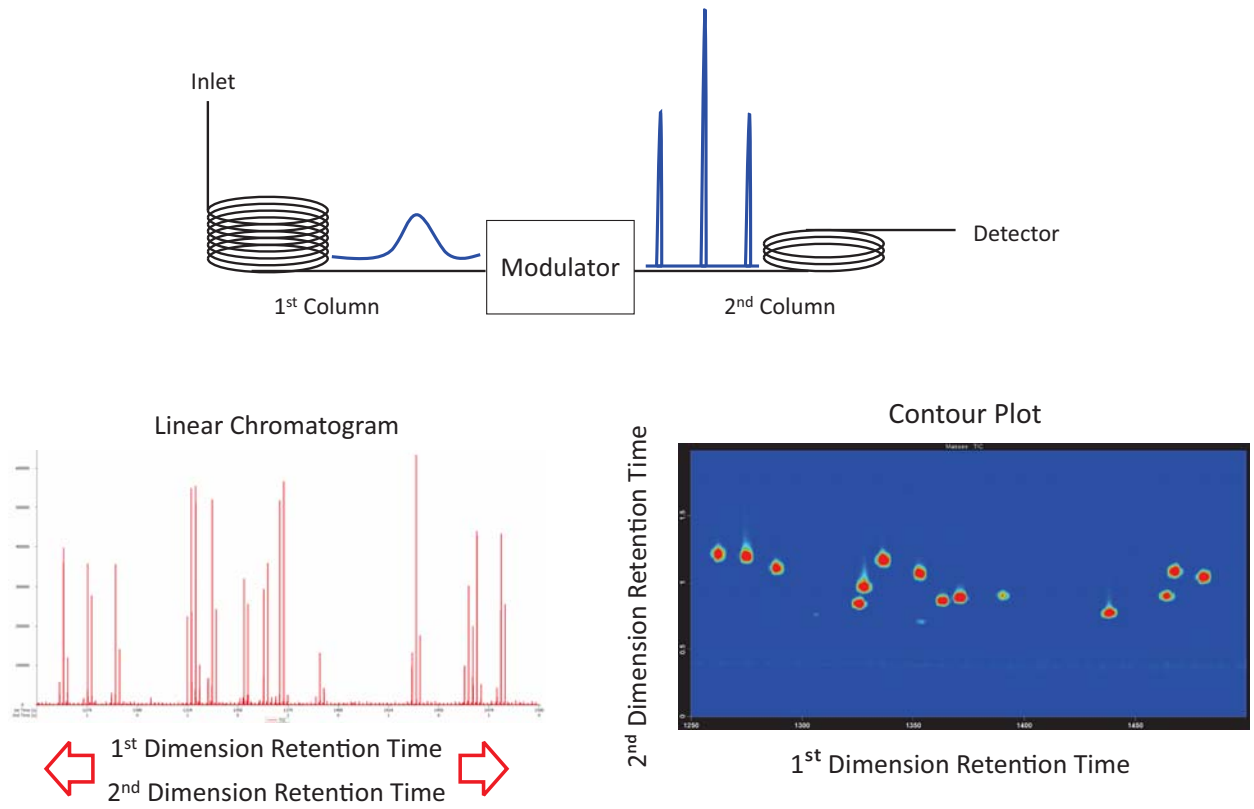

Figure 1: Overview of GCxGC: Peaks eluting from the 1st dimension column enter the 2nd dimension column through the modulator. The modulator rapidly traps the effluent from the primary column and "injects" this effluent to the secondary column. A fast detector simultaneously records the 1st dimension retention time and 2nd dimension retention time. A linear chromatogram is used for quantitation (e.g. integration, peak area, peak height, S/N), and the contour plot is a topographic type surface that is useful for data review and display purposes.

Innovative leaps in technology like GCxGC can take time before widespread adoption occurs. For example, the transfer of routine methods from packed GC to capillary GC took decades (you could argue the transfer is still in progress!). The enhanced resolution of a capillary GC analysis was first demonstrated in 1957 with the separation of m- and p-xylene[1]. Petroleum chemists were early adopters of capillary GC, and they benefited from the increased peak capacity (i.e. resolution) of complex samples. Although the technique was superior to packed columns, widespread adoption was slow due to ease of use and ruggedness of the capillary column (glass). The ruggedness issue was finally solved in 1979 with the introduction of fused silica capillary columns[2], but it still has taken decades to transfer routine accredited methods from packed to capillary column technology. The benefits of capillary columns are undeniable and have advanced our knowledge of the chemical composition of countless samples.

Much like the transfer of methodology from packed columns to capillary columns, the transfer of 1D-GC to GCxGC in regulated industries has been slow. The technique was first demonstrated in 1991[3]. Early adopters found the increased peak capacity was useful for complex environmental and petrochemical samples. The commercialization of a GCxGC instrument equipped with a thermal modulator in 2002[4] spurred research into a large cross-section of application areas (e.g. petrochemical, metabolomics, environmental, food safety, fragrance). Similar hurdles of ease of use and ruggedness have limited the adoption of the GCxGC platform. Method development and optimization have been expressed as difficult and tedious and only for an experienced analyst. In recent years these hurdles have been minimized, making it easier for implementation into a routine laboratory. Helpful guidelines[5] and tools[6] have been developed to aid users in GCxGC method development. Flow modulators have been commercialized for users interested in trying GCxGC at a lower initial cost. Method ruggedness has been demonstrated with several published validated methods[7-11]. GCxGC is currently in the process of moving from academic hype to everyday practical analysis. In a routine laboratory a comprehensive two-dimensional gas chromatographic analysis can save time by combining several compound classes into a single analysis, and short-cut sample preparation and cleanup by chromatographically resolving matrix interferences. When complete characterization of a sample is needed, a GCxGC analysis will accelerate discovery and provide insight into a sample that no other chromatographic technique can match. More economical detectors (e.g. FID, ECD) can be used for routine analyses instead of relying on mass spectrometers to resolve complex mixtures.

Save Time with Multi-Class Analyses

In routine high throughput laboratories; time is money. Laboratories tasked with monitoring environmental pollutants, for example, are facing reduced operating budgets, dirty samples (e.g. soils, sediments, sludge), and a growing cocktail of chemical contaminants. Contaminated samples often contain multiple classes of components. Contaminants such as polychlorinated biphenyls (PCBs) and brominated flame retardants (BFRs) are both halogenated compounds that are commonly analyzed using an ECD. The ECD is a non-specific detector; therefore, sample extracts must be carefully fractionated in order to avoid interferences. Fractionation steps can be reduced or eliminated completely when implementing a GCxGC method. By taking advantage of the second dimension separation space multiple classes of components can be analyzed in a single analysis **(Figure 2).**

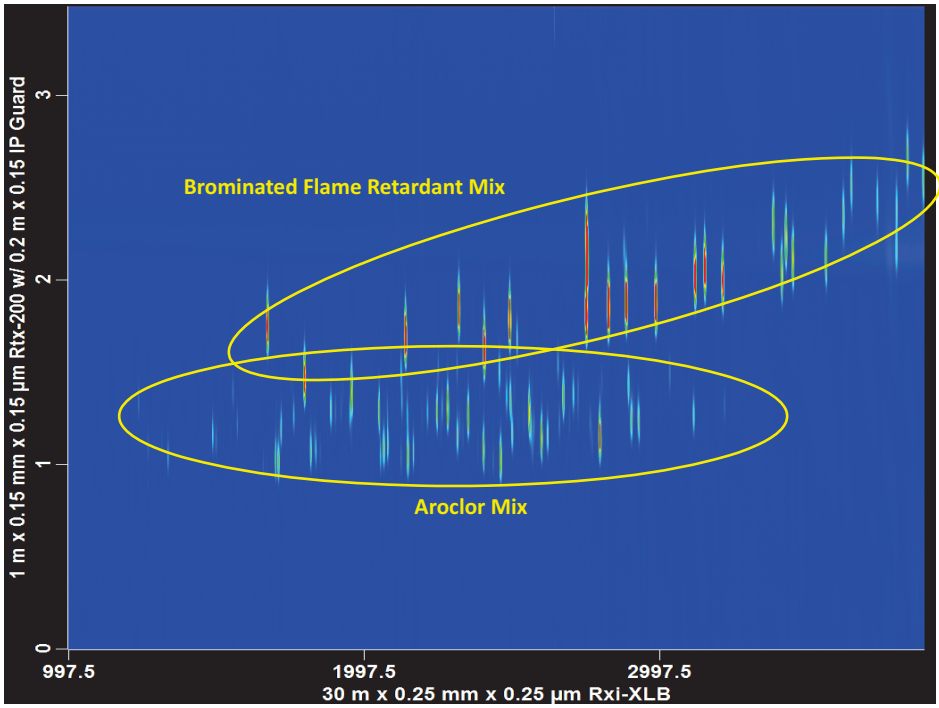

Figure 2: Multiple halogenated contaminants can be analyzed in a single injection using GCxGC-µECD. Brominated flame retardants (BFRs) and polychlorinated biphenyls (PCBs) are often found in the same sample extract. GCxGC reduces the need for fractionation steps.

The Canadian Ministry of the Environment and Climate Change (MOECC) Laboratory Services Branch (Toronto, Ontario) routinely uses three validated GCxGC-µECD methods[12-14]. The GCxGC methods are not only validated, but also participate in proficiency testing programs provided by an external organization to satisfy accreditation requirements. Two of these methods have been successfully accredited. The first regulatory GCxGC method was developed at MOECC in 2011 for a single analysis of PCBs, organochlorine pesticides (OCPs) and chlorobenzenes (CBz) in soils, sediments and sludge by GCxGC-µECD[8]. The micro-electron capture detector (µECD) is well suited for an environmental monitoring laboratory due to its low dead volume, superior sensitivity for halogenated compounds and low operating cost. With GCxGC-µECD, up to six injections were replaced with a single analysis targeting 118 compounds. In addition to reducing analysis time, sample preparation time was also reduced as fractionation of the extracts was no longer necessary. The method performance was evaluated using certified reference materials and demonstrated excellent agreement with certified values. Aroclor calculations were still possible using the targeted 82 PCB congeners allowing for historical comparisons to be made. Another important benefit of the GCxGC-µECD analysis is the ability to detect non-targeted halogenated contaminants that may have been obscured in a one-dimensional analysis. The locations of the unknown compounds in the GCxGC chromatogram can aid in tentative identification or compound specific classification (e.g. dioxins, PBDEs). Monitoring programs can benefit from the ability to screen for non-targeted analytes that could identify emerging contaminants, or catch illegal activity that would otherwise go unnoticed.

Reduce Solvent Usage with Simplified Sample Preparation

Sample preparation time can be lengthy for pesticide residue analysis in agricultural and food samples. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) sample preparation method is a multi-residue general extraction and cleanup method that has saved time-per-sample in high throughput food safety laboratories. The broad based extraction approach is advantageous for a simultaneous multi-class residue analysis; however, co-extractive matrix components are also present in the extract and can interfere with targeted analytes. The second dimension separation space in a GCxGC analysis can be used to chromatographically remove interferences from pesticides of interest and reduce the burden of extensive cleanup procedures **(Figure 3).**

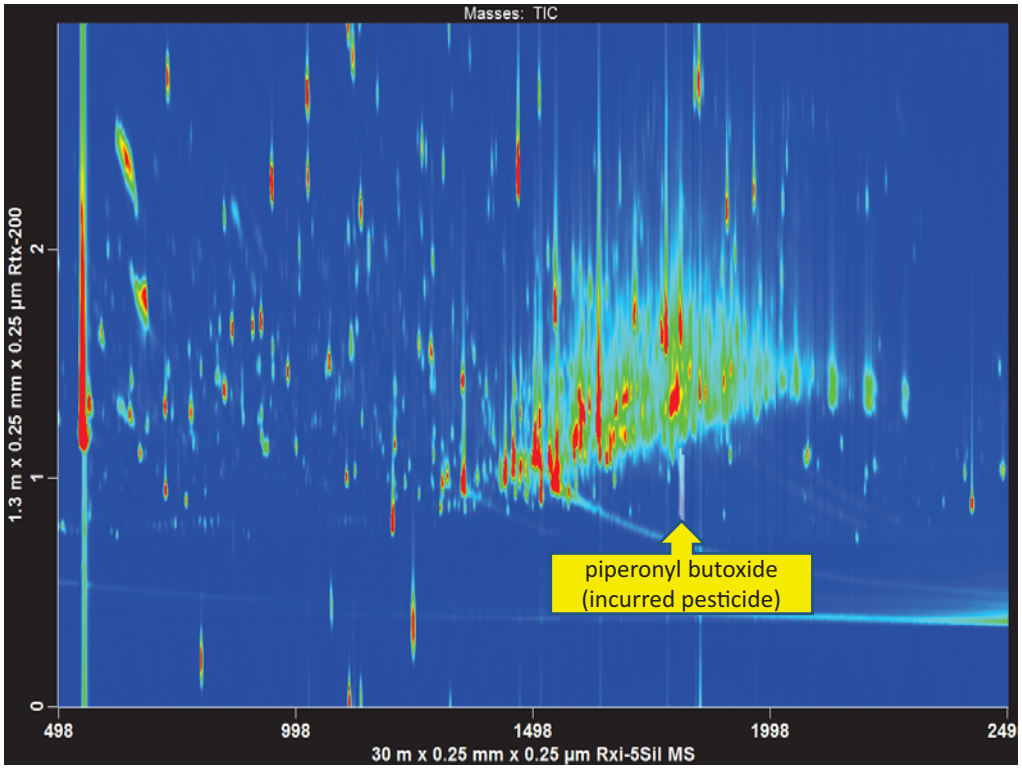

Figure 3: GCxGC-TOFMS was used to analyze a QuEChERS extract of finished tobacco. In a 1D-GC analysis matrix interference would have obscured the detection of a widely used pesticide ingredient, piperonyl butoxide. The automated peak find in the LECO ChromaTOF software identified piperonyl butoxide, with a library similarity of 876.

The QuEChERS approach to sample preparation has been modified for applications outside the scope of pesticide residues in fruit and vegetables. With a modification in extraction solvent, the QuEChERS approach can be used to determine PCBs, BFRs, and polycyclic aromatic hydrocarbons (PAHs) in high fat animal feed ingredients (e.g. palm oil, fish oil, rapeseed oil)[11]. A cleanup step is necessary prior to injection on the gas chromatograph to remove the non-volatile lipids present in the extract. Gel permeation chromatography (GPC) is often used to remove the co-extractive lipids; however, it is both solvent and time intensive. A simple cleanup using silica solid phase extraction (SPE) columns can also be used to remove the majority of fat prior to analysis. Although the SPE cleanup may not produce as clean extracts as GPC; a GCxGC analysis can chromatographically resolve co-extractives that would interfere with a 1D-GC analysis. This simplified sample preparation approach (QuEChERS extraction with silica SPE cleanup) was used in a validated screening method for PCBs, PBDEs, emerging BFRs, and PAHs in animal feed and ingredients using GCxGC with a time-of-flight mass spectrometer (GCxGC-TOFMS) for analysis[11]. Several marker compounds were selected for each compound class, and they were detected at or below the established maximum regulatory limit (MRL). The validation procedure determined with high confidence (95%) that the screening method would properly flag non-compliant samples for further confirmation. To further expand the utility of the screening method, data processing was automated to quickly identify other potential brominated or chlorinated contaminants. The comprehensive analysis of GCxGC-TOFMS also provides a complete record of a sample that can be used for retrospective data mining.

Decrease Manual Labor with Automated Peak Identification

As the number of potential chemical contaminants increase (in the environment, food, people, animals, etc), the need for routine non-target screening methods increase. One of the benefits of using GCxGC is the structured elution of components in a two-dimensional contour plot. With a non-specific detector like the FID or ECD, the contour plot can be used to group compounds based on their chemical class. When using a mass spectrometer (MS) as a detector, unknown peaks can be further identified by searching a mass spectral library (e.g. NIST). Library matching in a 1D-GC analysis can be complicated when numerous coelutions corrupt the mass spectrum, and it results in poor quality search results. The quality of the search relies on the similarity of the selected spectrum to the reference mass spectrum. A GCxGC-TOFMS can aid in unknown peak identification in a few ways. First, the extra peak capacity in GCxGC means more resolved peaks. If a peak is better resolved from surrounding interferences it is more likely to have a high library match (provided that the analyte in question is in the library). Second, a TOFMS can perform spectral deconvolution that mathematically pulls apart spectral overlap for co-eluting analytes. This also acts to "purify" a mass spectrum to obtain a better library match **(Figure 4)**. Finally, because of these factors, integrated software can automate the selection, search, and identification of unknown peaks and create a peak table. Although manual review is still necessary to verify the results of the automated peak find, the overall time to generate a list of identified peaks is significantly reduced.

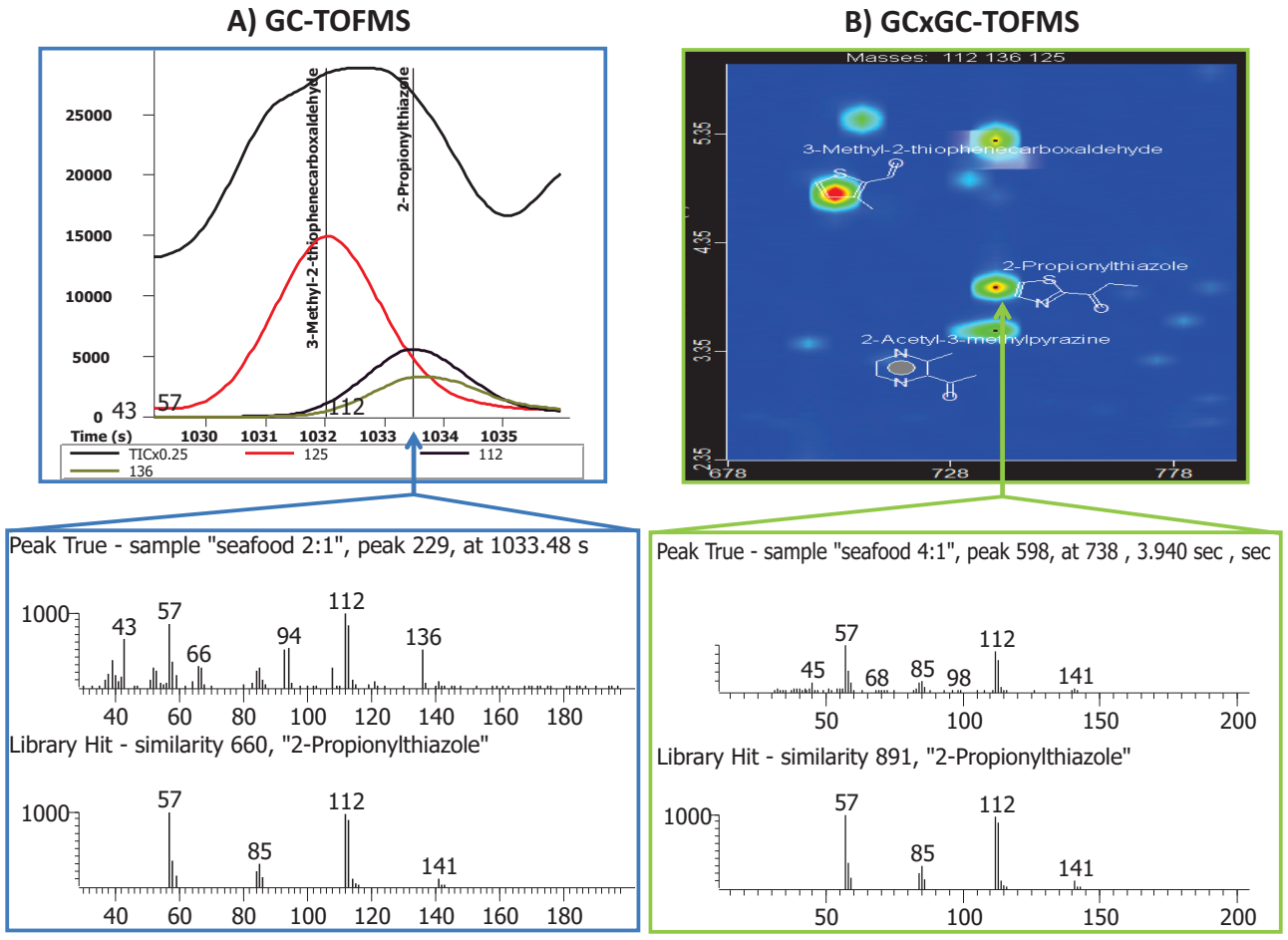

Figure 4: Side-by-side comparison of a GC-TOFMS and GCxGC-TOFMS analysis of the same pet food sample. A) In the GC-TOFMS mass spectrum the flavoring compound 2-propionylthiazole had a library similarity of 660. Spectral deconvolution was not able to resolve (or identify) a co-eluting compound with m/z 136 in the peak true mass spectrum. B) In the GCxGC-TOFMS contour plot the two co-eluting compounds were chromatographically resolved in the second dimension. The library similarity for 2-propionylthiazole was improved to 891 and the peak with m/z 136 was identified as 2-acetyl-3-methylpyrazine with a library similarity of 860 (not shown). Image courtesy of Joe Binkley, LECO Corporation

Accelerate Discovery with Increased Peak Capacity

One of the best (and most hyped) usages of GCxGC is to separate and identify all of the individual components that make up a sample. Characterization of a sample can be fundamental to forensic investigations, drug discovery, and early disease diagnosis. When a GCxGC method is developed to maximize chromatographic resolution (i.e. True Peak Capacity Increase)[5, 15] a fingerprint of the sample can be obtained that is useful for source apportionment in environmental forensics. The indicator molecules (i.e. biomarkers) are often small peaks in a GC chromatogram that can easily be obscured in a 1D-GC analysis (Figure 5).

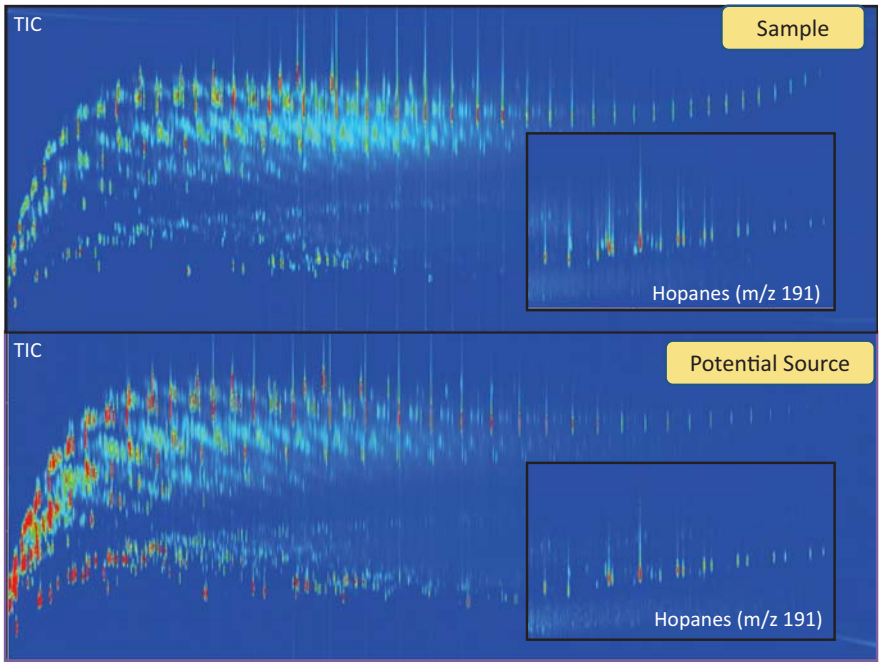

Figure 5: GCxGC-TOFMS contour plot of a tar ball sample and suspected crude oil source. Extracted ion chromatograms (XIC) are magnified to show the similarity between the two samples. The hopanes are molecular fossils that are resistant to weathering and are important biomarkers that aid in source apportionment. In a 1D-GC analysis the hopanes are more difficult to identify due to interferences with late eluting hydrocarbons.

In metabolomics, small molecules have been found to be sensitive indicators of disease. These biomarkers are essential to the understanding of disease with the goal of treatment and prevention. A validated GCxGC-TOFMS method discovered 11 more statistically significant biomarkers in human serum samples compared to a GC-MS method[10]. The enhanced resolution of the GCxGC analysis was paramount to the discovery of additional biomarkers. In addition, the extra sensitivity afforded by the modulation process of the GCxGC system allowed for a 30:1 split injection to be used instead of a splitless injection with the GC-MS method. A split injection is advantageous for reproducibility and increased column lifetime (less non-volatile material deposited onto column).

Save Money with Economical Detectors

In order to quantify the minor components of a complex sample, increased resolution is necessary through either the detector or gas chromatograph. Mass spectrometry is a powerful detector that can spectrally resolve and identify many co-eluting peaks. In a one dimensional GC-MS analysis, large concentration differences between co-eluting compounds, structural isomers, or multiple analytes eluting at the same time can decrease the utility of the MS. One approach to increase the specificity of the MS is to use high resolution mass spectrometry (HRMS) or tandem mass spectrometry (MS/MS). Both of these detectors have their place in an analytical laboratory, but they are expensive to purchase and operate. Increasing the chromatographic resolution with GCxGC, decreases the need for expensive mass spectrometer detectors. The FID is an economical, robust, and simple to operate detector.

Twenty laboratories were involved in an inter-calibration experiment using certified reference materials of Gulf of Mexico Crude Oil (SRM 2779). The laboratories were tasked with reporting the levels of various crude oil constituents (e.g. saturated hydrocarbons, aromatics, sterane and hopane biomarkers) in the NIST certified reference material[16]. Most of the laboratories used GC-MS for analysis, three laboratories used GC-MS/MS, and two laboratories used GCxGC-FID. Overall, the reported values using GCxGC-FID were in good agreement with the NIST certified reference values. The GCxGC-FID showed equivalent performance to GC-MS in the measurement of n-alkanes[17]. Only 8 of the 20 laboratories reported values for a list of several sterane and hopane biomarkers, one of which was the lab equipped with a GCxGC-FID. An additional benefit to using an FID in place of mass spectrometry is the ease of quantification, especially for hydrocarbons. The reliable detector response of an FID reduces the need for multiple internal standards to achieve accurate quantitation.

Conclusion

Any leap in technology requires an initial investment of resources. Capital equipment purchases, staff training and instrument operation costs are all factors when considering investing in GCxGC. Cautious buyers may question if the technique is viable in a routine environment, or how long will it take to realize a return on the investment. The examples in this article have offered a glimpse into the viability of GCxGC in a routine laboratory. Saving time in sample preparation, instrumental analysis and non-target data review can be an immediate payoff when implementing a GCxGC system. The ability to analyze a wide range of complex samples with simultaneous target and non-target detection is an attractive prospect that can be leveraged in a competitive landscape. Comprehensive two-dimensional gas chromatography is no longer just research hype; it is the separation science of the future. Are you ready?

If you are interested in learning more about GCxGC, please stay tuned for Part II in this series. Part II will focus on implementing a GCxGC system into a routine laboratory. Method development guidelines will be covered, including column selection and GCxGC operating parameters. The validated GCxGC method examples in this article will showcase data processing and review strategies for quantitative and semi-quantitative results.

Note: The author was compensated by LECO Corporation for her work in this piece. All thoughts and opinions expressed herein are those of the authors, and not influenced by the company and/or its affiliates in any way.

Author Biography

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Michelle Misselwitz is an experienced analytical chemist with expertise in gas chromatography (GC), comprehensive two-dimensional gas chromatography (GCxGC), mass spectrometry (MS) and sample preparation. As an applications chemist, she developed hot topic applications for environmental, food safety, environmental forensics, and botanical markets. Misselwitz has successfully combined her scientific and communication skills to present and write technical papers and training seminars for customers worldwide. With a decade of experience at Restek, a chromatography consumables company, and a B.S. in Chemistry from The Pennsylvania State University, Michelle is currently an independent consultant specializing in technical writing, presentations and GC method development.

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