Delivering the Right Results

Comparative Analysis of Lipophilic Wood Extractives by GCxGC-TOFMS for Enhanced Chromatographic Resolution and Compound Identification

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OVERVIEW

- Demonstrate the enhanced detection and increased peak capacity available with GCxGC-TOFMS for complex lipophilic wood extractives.
- Show how comprehensive two-dimensional chromatography resolves analytes that would otherwise totally coelute and be buried by high concentration analytes in one-dimensional chromatography.
- Illustrate deconvolution of fast acquisition non-skewed time-of-flight mass spectrometry (TOFMS) spectra for closely eluting analytes of similar chemical structure.
- Compare Soxhlet extractions using different solvents to maximize and isolate the detection of possible volatile components in wood by GCxGC-TOFMS.

INTRODUCTION

Metabolomic and lipidomic analyses present challenges that historically have relied upon conventional quadrupole GC/MS targeted methods of selected ion monitoring and tandem GC/MS/MS techniques. The complex nature of these sample types demand analytical solutions and instrumental methods that will identify the metabolic profile completely, as well as discover significant key components of interest. Comprehensive two-dimensional gas chromatography (GCxGC) expands the peak capacity of the chromatographic separation, thereby increasing resolution and analyte characterization necessary for complicated samples. Time-of-flight mass spectrometry (TOFMS) offers continuous full range non-skewed mass spectral information and fast acquisition rates ideal for metabolite identifications. Wood components are cellulose, hemicelluloses, extractives, and lignin. No single solvent can isolate all of the extractive material from wood, so more than one solvent is required. The chemical classes of extractives are resins, fatty materials, alcohols, and phenolic compounds.

This poster shows GCxGC-TOFMS data from the comparative analysis of lipophilic soxhlet extractions in wood utilizing various solvents and fractionation. Two-dimensional chromatographic data will show key component classes of interest (fatty acids, sterols, sterol esters, waxes) that highlight the contrast between ethanol and acetone extractions. Sample data will illustrate how comprehensive two-dimensional chromatography can effectively separate and resolve significant trace level components in the second dimension. The combination of GCxGC-TOFMS data and Deconvolution algorithms facilitate detection of trace level analytes that would otherwise be buried under high concentration compounds. Data presented in this poster illustrates the advantages and benefits of GCxGC-TOFMS to accurately characterize and identify differences in these complex lipophilic wood extractions.

This proof-of-concept research was conducted to demonstrate the capabilities of GCxGC-TOFMS to maximize separation power and component identification not possible by conventional single-dimension separations. The study was facilitated by and in cooperation with Roderquita K. Moore, Ph.D., research chemist for fiber and chemical research at the USDA Forest Service, Forest Products Laboratory, Madison, Wisconsin.

EXPERIMENTAL METHODS

Sample Preparation

The USDA Forest Service submitted 6 wood extractive samples for evaluation using GCxGC and TOFMS.

- Samples were prepared at the USDA Forest Products Laboratory by conventional soxhlet extraction.
- Various types of wood samples were extracted using acetone and ethanol to maximize the total number of analytes found from different chemical classes.
- Two samples were fractionated using hexane or chloroform to collect the sterols, sterol esters, triglycerides, and fatty acids.
- The chemical classes evaluated were resins, fatty materials, alcohols, and phenolic substances.

GCxGC-TOFMS Analysis Parameters

- Gas Chromatograph: Agilent 7890 equipped with a LECO dual stage quad jet thermal modulator
- Primary Column: 30 m x 0.25 mm id. x 0.25 µm film thickness Rxi-5SilMS (Restek Corp., Bellefonte, PA)
- Secondary Column: 1.5 m x 0.1 mm id. x 0.1 µm film thickness BPX-50 (SGE Analytical Science, Austin, TX)
- Carrier Gas: Helium set at 1.5 mL/min
- Injection Mode: Splitless or split 25:1
- Injection Volume: 1 µL
- Inlet Temperature: 250°C
- Primary Column Temperature Program: Initial temperature 140°C (0.5 min), ramped 7°C/min to 325°C, hold 7 min
- Secondary Column Temperature Program: Parallel ramp offset by +5°C (Total run time: 39.5 min)

Mass Spectrometer: LECO Pegasus® 4D

- Mass Range: 45 to 650 m/z
- Acquisition rate: 150 spectra/s
- Ion source temperature: 230°C
- Detector Voltage: 1650 V
- Electron Energy: -70 eV

GCxGC-TOFMS RESULTS

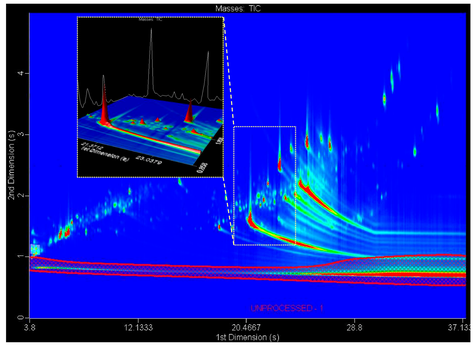

Figure 1. Wood sample type I (vial 1) Soxhlet extraction with acetone. The inset in the 2D contour plot above shows a 3D surface plot illustrating the value of GCxGC. This illustration shows that numerous minor components can be separated and resolved in the second dimension that would be buried in tailing high concentration analytes using one-dimensional chromatography.

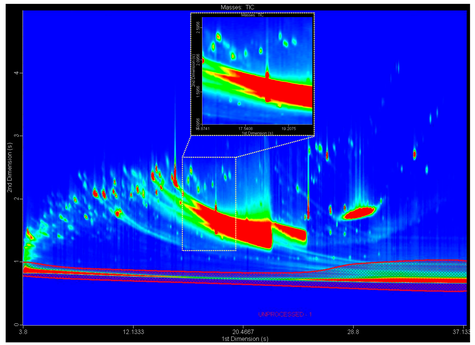

Figure 3. Wood sample type I (vial 3) Soxhlet extraction with ethanol. The inset shows the enhanced peak capacity and separation of minor components that would be buried in the heavy matrix of high concentration analytes.

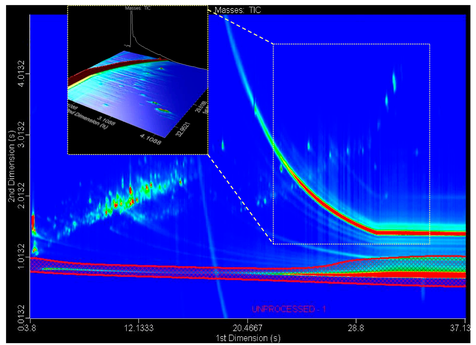

Figure 2. Wood sample type II (vial 2) Soxhlet extraction with acetone. The high concentration tailing peak in the figure above is abietic acid. The inset shown in the 3D surface plot shows an excellent example of how numerous trace components can be separated and identified by GCxGC that would otherwise be buried and lost by conventional GC.

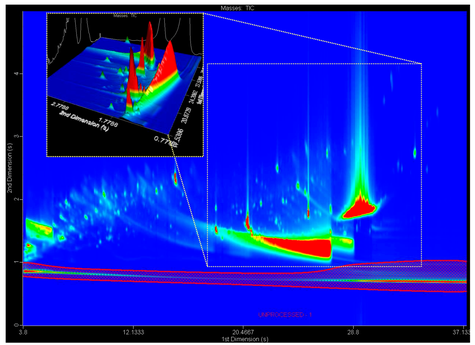

Figure 4. Wood sample type I (vial 4) Soxhlet extraction with ethanol. The GCxGC-TOFMS analysis shows the 3D inset rotated 90 degrees to illustrate separation in the second dimension. The second dimension view illustrates the enhanced separation and resolution power of GCxGC.

GCxGC ENHANCED PEAK CAPACITY

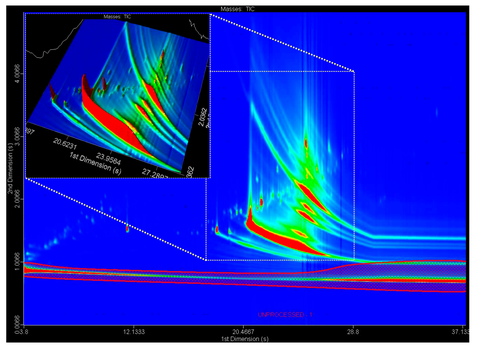

Figure 5. Wood sample (vial 6) from an acetone Soxhlet extraction fractionated to collect fatty acids in chloroform solvent. The inset in Figure 6 (below) shows the separation and resolution of many components, including a variety of fatty acids, in the second dimension that would otherwise coelute by single-dimension chromatography.

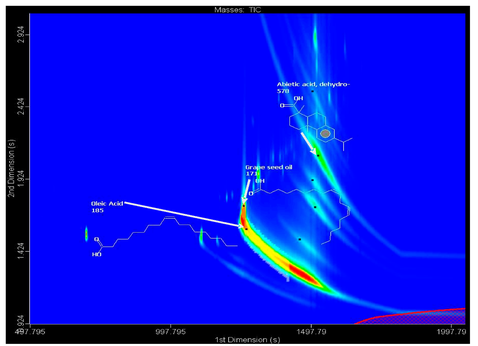

Figure 6. This figure shows the zoomed-in portion from the GCxGC-TOFMS analysis shown above in Figure 5. This illustration demonstrates the enhanced peak capacity achieved using GCxGC that is not possible using conventional one-dimensional chromatography. Notice the fatty acids grape seed oil and oleic acid, which would be totally coeluted if analyzed by one-dimensional chromatography.

TOFMS TRUE SIGNAL DECONVOLUTION

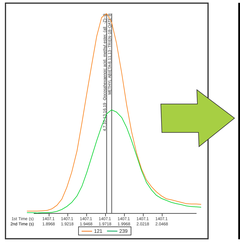

Figure 7. Shown above is the linear first dimension chromatogram of the unique mass ions for two compounds totally coeluted in the first dimension at 1407.1 seconds.

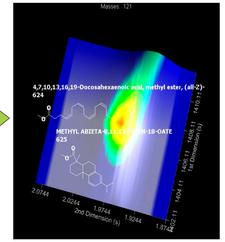

Figure 8. The 3D surface plot above shows the structures for 4,7,10,13,16,19-Docosahexaenoic acid, methyl ester and methyl Abieta-8,11,13-trien-18-oate which are separated by only 7 milliseconds in the second dimension separation.

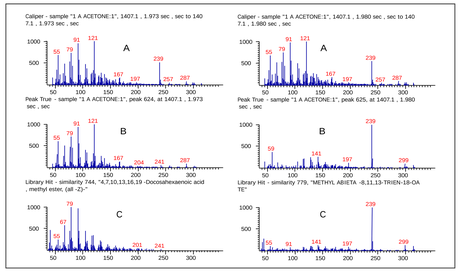

Figure 9. The figure above illustrates the Peak Find algorithm and True Signal Deconvolution accomplished using ChromaTOF® software. The mass spectral data show the deconvolution of the peaks found in Figures 7 and 8. The mass spectra labeled (A) are the Caliper total ion spectra before deconvolution. Notice that they are both very similar. The mass spectra labeled (B) are the deconvoluted Peak True mass spectra. The mass spectra labeled (C) are the library search matches with similarities of 74% and 78% respectively.

CONCLUSIONS

Excellent results were shown for the goals established for this collaborative study. The analysis of complex lipophilic wood extractives clearly demonstrates the enhanced detection and increased peak capacity available with GCxGC-TOFMS using the LECO Pegasus 4D instrument. The GCxGC-TOFMS results for the different wood extractive samples show how GCxGC separates and resolves components that would otherwise totally coelute and be buried by high concentration analytes in one-dimensional chromatography. A deconvolution example was given that confirms the value of fast acquisition non-skewed TOF mass spectra to identify closely eluting compounds with only 7 milliseconds of separation between the peak apexes. This proof-of-concept research shows that GCxGC-TOFMS is a valuable instrumental option for the comparative analysis of wood Soxhlet extractions that can maximize the detection and characterization of possible volatile components found in these complex samples.

LECO would like to thank Roderquita K. Moore, Ph.D., and the USDA Forest Service, Forest Products Laboratory, Madison, Wisconsin, for making this research collaboration possible.

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