GCxGC: Theory, Practice and Optimization

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Presentation Outline

• An Introduction to GCxGC
  – What is GCxGC?
  – GCxGC Hardware
  – Interpreting GCxGC Chromatograms

• Optimization of a GCxGC Separation

• Applications
  – Advanced Data Processing
    • Classifications
    • Scripting
What is GCxGC?

Multi-Dimensional Gas Chromatography
– 2DGC vs. GCxGC
  • 2DGC
    – Heart-Cutting
      » Diverting a portion of effluent from a column onto a column of a different stationary phase
    – Multiple Columns
      » Splitting the effluent from a column onto multiple columns of differing stationary phases

• GCxGC (Comprehensive Two-dimensional Gas Chromatography)
  – Comprehensive
    » All material that enters the 1st dimension column passes through the 2nd dimension column to the same detector
    – Uses a “Modulator” to partition 1st column effluent as discrete plugs onto the 2nd dimension column
Heart-cutting (2DGC)
Heart-cutting (2DGC)

- Stationary Phase “A”
- Stationary Phase “B”
- Detector “A”
- Detector “B”

Inlet
Heart-cutting (2DGC)
Heart-cutting (2DGC)

Inlet

Stationary Phase “A”

Stationary Phase “B”

Detector “A”

Detector “B”
Heart-cutting (2DGC)

Detector

Detector “A”

Detector “B”
Heart-Cut 2DGC

- Each column requires an independent detector
- Each Heart-Cut must be targeted at a specific coelution
- Two data files
Capillary GC Schematic
Thermally Modulated GCxGC Schematic
Simplified GCxGC Flow

Inlet → Modulator → Detector

1st Dimension Column → 2nd Dimension Column
Modulation

• Modulator has two functions in GCxGC:

1) Collect and focus segments of effluent from the primary column

2) Act as the injector for the secondary column
Focusing in the Thermal Modulator

Cold Zone

Relatively Broad 1st Dimension Analyte Band

1st Dimension Column

2nd Dimension Column
Focusing in the Thermal Modulator

1st Dimension Column  2nd Dimension Column
Focusing in the Thermal Modulator
Focusing in the Thermal Modulator
Focusing in the Thermal Modulator
Focusing in the Thermal Modulator
Focusing in the Thermal Modulator
Focusing in the Thermal Modulator
Focusing in the Thermal Modulator
Focusing in the Thermal Modulator
Focusing in the Thermal Modulator

Hot Zone
Focusing in the Thermal Modulator
Focusing in the Thermal Modulator
Detector Requirements for GCxGC

Quantitation requires a minimum of 10 data points across a peak in order to define it.

- 50 ms peak width at base
- Need 10 data points / peak
- 5 ms between data points

Minimum Required Sampling Rate: **200 Hz** for a 50 ms wide peak
Volatility Range

Good Peak Shape and Width over Wide Volatility Range

- C10 n-alkane
- C20 n-alkane
- C30 n-alkane
- C40 n-alkane

Maximum column temperature too low for fast elution and a narrow peak

Column 1 - 10 m x 0.18 mm x 0.2 um Rtx-5, Column 2 - 1 m x 0.1 mm x 0.1 um DB-5, FID
Oven 40 deg (0.5 min.) 40 deg/min. to 350 deg, modulator +30 deg, secondary oven +10 deg (max. 350 deg)
GCxGC Hardware

Dual-Stage Quad-jet Thermal Modulator

• Utilizes LN₂ or a Closed-loop Chiller for Cooling

• Utilizes an Secondary Oven for Independent Temperature Control of the Individual Columns

• Modulation Occurs on the Beginning of the 2nd Dimension Column
LECO’s Dual-stage Quad Jet Thermal Modulator

Stage 1

COLD JETS

Stage 2

HOT JETS
LECO’s GCxGC

- Primary Column
- Secondary Oven
- Secondary Column
- Modulator
GC×GC: Dual-stage Quad-jet Thermal Modulation

1st Dimension Column

Hot Jets

1

2

Cold Jets

2nd Dimension Column
GC×GC: Dual-stage Quad-jet Thermal Modulation
GC×GC: Dual-stage Quad-jet Thermal Modulation

1\textsuperscript{st} Dimension Column  2\textsuperscript{nd} Dimension Column

Hot Jets

Cold Jets
GC×GC: Dual-stage Quad-jet Thermal Modulation

1st Dimension Column

Hot Jets

Cold Jets

2nd Dimension Column
GC×GC: Dual-stage Quad-jet Thermal Modulation

1st Dimension Column 2nd Dimension Column

Hot Jets

Cold Jets
GC×GC: Dual-stage Quad-jet Thermal Modulation

1st Dimension Column       2nd Dimension Column

Hot Jets

Cold Jets
GC×GC: Dual-stage Quad-jet Thermal Modulation

1st Dimension Column

Hot Jets

Cold Jets

2nd Dimension Column
GC×GC: Dual-stage Quad-jet Thermal Modulation

1\textsuperscript{st} Dimension Column

\begin{itemize}
  \item Hot Jets
  \begin{itemize}
    \item 1
    \item 2
  \end{itemize}
\end{itemize}

Cold Jets

\begin{itemize}
  \item 1
  \item 2
\end{itemize}

2\textsuperscript{nd} Dimension Column
GC×GC: Dual-stage Quad-jet Thermal Modulation

1st Dimension Column

2nd Dimension Column

Hot Jets

Cold Jets
GC×GC: Dual-stage Quad-jet Thermal Modulation

1\textsuperscript{st} Dimension Column  \hspace{2cm} 2\textsuperscript{nd} Dimension Column

Hot Jets

Cold Jets
GC×GC: Dual-stage Quad-jet Thermal Modulation

1st Dimension Column

Cold Jets

2nd Dimension Column

Hot Jets
GC×GC: Dual-stage Quad-jet Thermal Modulation

1st Dimension Column

Cold Jets

2nd Dimension Column

Hot Jets
GC×GC: Dual-stage Quad-jet Thermal Modulation

1st Dimension Column

Hot Jets

Cold Jets

2nd Dimension Column
GC×GC: Dual-stage Quad-jet Thermal Modulation

1st Dimension Column      2nd Dimension Column

Hot Jets

1 2

Cold Jets

1 2
GC×GC: Dual-stage Quad-jet Thermal Modulation

1st Dimension Column

Hot Jets

Cold Jets

2nd Dimension Column
GC×GC: Dual-stage Quad-jet Thermal Modulation

1\textsuperscript{st} Dimension Column  \hspace{1cm} 2\textsuperscript{nd} Dimension Column

Hot Jets

Cold Jets
GC×GC: Dual-stage Quad-jet Thermal Modulation

1st Dimension Column

2nd Dimension Column

Hot Jets

Cold Jets
GC×GC: Dual-stage Quad-jet Thermal Modulation

1st Dimension Column

Hot Jets

Cold Jets

2nd Dimension Column
GCxGC Overview

• GCxGC is accomplished through a series of rapid, independent 2nd dimension separations

• The modulator serves two functions: focusing sections of 1st dimension column effluent and acting as the injector for the 2nd dimension column

• GCxGC is comprehensive. All material that enters the 1st column passes through the modulator, the 2nd column and on to the detector
GCxGC Overview

- Primary column separates components based on volatility and also generates wide bands.

- The modulator focuses and re-injects time-fractions of the primary column effluent onto the second column for a second separation:
  - 5+ modulations per peak.

- The second column performs a rapid separation of each injected sample from the modulator based on polarity:
  - $t_m \sim 1.0$ s for a 1.0m column.
The GCxGC Process

The effluent from the primary column is focused and segmented by the modulator into a discrete “plug”. Each plug is then injected onto the secondary column by the modulator, where it is separated. The GCxGC process is a series of independent second column separations.
Contour Plot
Features of a GCxGC Contour Plot

- Alkyl-sub Phenanthrenes
- Alkyl-sub Napthalenes
- Alkyl-sub Benzenes
- n-Alkanes

GCxGC of Raw Diesel

- Alkyl-sub Phenanthrenes
- Alkyl-sub Napthalenes
- Alkyl-sub Benzenes
- n-Alkanes
Optimizing a GCxGC Separation
What is an “Orthogonal” Separation?

A separation scheme is orthogonal when two separations are performed by mechanisms that are independent from one another.

Example: SDS PAGE → LC
What is an “Orthogonal” Separation?

In GCxGC, an orthogonal separation occurs when the separation mechanisms of the two columns are independent from one another.
In GCxGC, the orthogonality of the column set determines how efficiently the available chromatographic “real estate” in the retention plane is used.
Retention Plane

ORTHOGONAL SEPARATION

2nd Dimension Retention Time

1st Dimension Retention Time

NON ORTHOGONAL SEPARATION LIM
Advantages of GCxGC

✓ Peak Capacity

$$ SN = \left( \frac{\Delta t_r}{(W_{1/2})_1 + (W_{1/2})_2} \right) - 1 $$

where

$$ SN_t = \sum_{i} (SN + 1) $$

= 85 peaks

$$ SN = \left( \frac{\Delta t_r}{(W_{1/2})_1 + (W_{1/2})_2} \right) - 1 \times CP = 1 + \frac{\sqrt{N}}{4R_s} \ln \left[ \frac{t_{r(last)}}{t_m} \right] $$

= 2550 peaks
Increased Peak Capacity in GC×GC

Theoretical Maximum Peak Capacity

Peak Capacity of 1st Dimension

×

Peak Capacity of 2nd Dimension

Actual Maximum Peak Capacity is Lower
Advantages of Thermally Modulated GCxGC

• Increased detectability resulting from focusing in the modulator

• Increased chromatographic resolution

• Increased peak capacity
Parameters Commonly Used In Optimization

• Temperature Program
  – *Rate of temperature increase for columns*

• Column Offset
  – *Temperature difference between 1st dimension column and 2nd dimension column*

• Modulator Offset
  – *Temperature difference between 2nd dimension column and modulator hot jets*

• Modulation Period
  – *Dwell time of modulation cycle*
Temperature Program

• Increasing the rate of the temperature program will cause the analytes retention time on the primary column to decrease.

• GCxGC uses lower temperature program rates so that each individual second dimension separation occurs under “isothermal” conditions. Having each second dimension separation occur under locally isothermal conditions helps maintain separation scheme orthogonality. This necessitates that the use of longer modulation periods requires lower temperature ramps.
Increased Temperature Program Rate

2nd Dimension Retention Time

1st Dimension Retention Time
Oven Temperature Offset

- Increasing the temperature offset between the first and second dimension columns will decrease retention times in the second dimension.

- Decreasing the temperature offset between the first and second dimension columns will increase retention times in the second dimension.
Increasing Temperature Offset

2nd Dimension Retention Time

1st Dimension Retention Time
Decreasing Temperature Offset

2nd Dimension Retention Time

1st Dimension Retention Time
GCxGC “Wrap-Around”
GCxGC “Wrap-Around”
Modulator Offset

- The modulator offset describes the difference in temperature between the modulator block (hot jets) and the 2nd dimension oven. Modulator offset relates to the injector function of the modulator. LECO recommends a modulator offset of at least +15 °C, relative to the secondary oven temperature.

- The modulator offset would be increased to assist in desorbing high boilers.
Temperature Offsets and Programming

- Modulator Offset
- Column Offset

- Col₂ Max Temp

Temperature (°C)

Time

- Mod
- Col₂
- Col₁

Δ15 °C
Modulation Period

• Increasing modulation period allows for the prevention of the wrap-around of compounds strongly retained on the 2nd dimension. It would also, however, decrease the number of slices taken of the 1st dimension peak and increase the likelihood of overloading the modulator.

• Decreasing the period would increase the number of 1st dimension slices, but would also increase the likelihood of wrap-around in the 2nd dimension and decrease the amount of separation obtained on the 2nd dimension column.
Increasing Modulation Period
Decreasing Modulation Period

2nd Dimension Retention Time

1st Dimension Retention Time
Resolution on the x-Axis

2nd Dimension Retention Time

1st Dimension Retention Time

6 sec 6 sec 6 sec 6 sec 6 sec

Leco®
Delivering the Right Results
Modulation Period

• The resolution on the x-axis is equal to the modulation period.

• Compounds that are separated by less than the modulation period are recombined in the modulator.

• A good rule of thumb is that the modulation period should be as short as possible while obtaining the necessary separation in the 2nd dimension.
GCxGC Applications

Environmental

Metabolomics

Petroleum
Environmental

GCxGC-TOFMS Analysis of PDBE’s and PCB’s in Fish
Environmental – PBDEs & PCBs in Fish
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Environmental – PBDEs & PCBs in Fish

- Tribromodiphenyl ether
- 5,8,11,14-Eicosatetraynoic acid
- Dehydroabietic acid methyl ester
- 3-(4-Methoxyphenyl)-2-propenoic acid 2-ethylhexyl ester
- 5-Heptyldihydro-2(3H)-furanone
Environmental – PBDEs & PCBs in Fish

- Tribromodiphenyl ether
- 5,8,11,14-eicosatetraynoic acid
- Dehydroabietic acid methyl ester
- 5-heptyldihydro-2(3H)-furanone
- 3-(4-Methoxyphenyl)-2-propenoic acid 2-ethylhexyl ester
Metabolomics

GCxGC-TOFMS Analysis of Sulfur-containing Metabolites of Asparagus in Urine
Pre-Asparagus Consumption
Post-Asparagus Consumption
Post-Asparagus Consumption
Petroleum

GCxGC-TOFMS Analysis of Diesel
Petroleum - Diesel
Petroleum - Diesel

~ 3280 Peaks w/ S/N ≥ 100
Petroleum - Diesel
Petroleum - Diesel
Petroleum - Diesel
Petroleum - Diesel
# Petroleum - Diesel

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Name</th>
<th>R.T. (s)</th>
<th>Classifications</th>
<th>UniqueMass</th>
<th>S/N</th>
<th>Height</th>
<th>Area</th>
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<tbody>
<tr>
<td>56</td>
<td>Cyclohexane, 1,1,3-trimethyl-</td>
<td>571.5 , 0.710</td>
<td>Alkanes</td>
<td>69</td>
<td>1301.2</td>
<td>5008.6</td>
<td>26512</td>
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<td>57</td>
<td>Cyclohexane, 1-ethyl-2-methyl-, cis-</td>
<td>582.5 , 0.700</td>
<td>Alkanes</td>
<td>55</td>
<td>370.8</td>
<td>2231</td>
<td>11696</td>
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<td>58</td>
<td>4-Nonene</td>
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<td>4643.7</td>
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<td>59</td>
<td>Cyclohexane, 1,1,2-trimethyl-</td>
<td>599 , 0.720</td>
<td>Alkanes; Alkenes</td>
<td>55</td>
<td>482.25</td>
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<td>599 , 1.210</td>
<td>C2 Benzenes</td>
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<td>285.33</td>
<td>912.7</td>
<td>7362.1</td>
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<td>Cyclohexane, 1,1,2-trimethyl-</td>
<td>610 , 0.720</td>
<td>Alkanes; Alkenes</td>
<td>69</td>
<td>194.63</td>
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<td>1,2,4,4-Tetramethylcyclopentene</td>
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<td>Alkenes</td>
<td>109</td>
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<td>Benzene, 1,3-dimethyl-</td>
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<td>C2 Benzenes</td>
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<td>215876</td>
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<td>C2 Benzenes</td>
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<td>153.51</td>
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<td>Heptane, 3,4-dimethyl-</td>
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<td>157.94</td>
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<tr>
<td>67</td>
<td>Cyclopentane, 1-methyl-3-(1-methylethyl)-</td>
<td>626.5 , 0.720</td>
<td>Alkanes; Alkenes</td>
<td>55</td>
<td>229.4</td>
<td>2143.6</td>
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<tr>
<td>68</td>
<td>Pentalene, octahydro-</td>
<td>626.5 , 0.810</td>
<td>Alkenes</td>
<td>67</td>
<td>407.51</td>
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<td>2,4-Heptadienal, (E,E)-</td>
<td>632 , 0.830</td>
<td></td>
<td>81</td>
<td>118.5</td>
<td>402.54</td>
<td>3241.6</td>
</tr>
</tbody>
</table>
Petroleum - Diesel
Petroleum - Diesel

Base Peak = m/z 91, 105 or 119

And

Rank(2) = m/z 134
Conclusion

• GCxGC is a versatile technique that is well-suited to the analysis of complex mixtures

• GCxGC provides benefits over a conventional 1DGC separation including:
  • Increased Detectability
  • Increased Chromatographic Resolution
  • Increased Peak Capacity

• LECO’s ChromaTOF software offers advanced data processing, such as Classifications and Scripting, which take advantage of the structured nature of GCxGC data
For More Information

Contact LECO at:

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