GC×GC-(HR)TOFMS: Why we Love it!

JF Focant et al.

Acknowledgements
✓ JEOL, LECO, Agilent, Thermo…
✓ Restek, Sigma-Aldrich/Supelco, SGE
✓ JSB, Gerstel, Markes…

GC×GC is Very Young
✓ GC×GC was invented just a few years ago
✓ This is why it is only used by experts
✓ It is much more expensive than regular GC
✓ It is much more complex than regular GC
✓ Runs in GC×GC last for ages
✓ GC×GC is only for petroleum sample analyses

The Early Days

First report on GC×GC

Modulation
✓ What is a modulator?
  • Interface between the two columns that samples narrow bands from the eluate of the 1D column,
  • For fast re-injection into the 2D column, producing fragments that are analyzed sequentially.
First report on GC×GC

The Idea

Classical

GC

GC×GC

Peak capacity \( (n_p) = 1D_{nc} \times 2D_{nc} \)

\[ t_{run\ 1DGC} = t_{run\ GC×GC} \]
The Principle

Instrumental Setup

SSL, PTV

(Cryogenic) Modulator

Detector

Loop Modulator

Carrier Gas

Cold Jet

Hot Jet

Zoex Corp.
**Modulation Process**

- Sampling rate 0.25 Hz
- Each 'slice' is a separate second dimension chromatogram

**Signal at the Detector**

<table>
<thead>
<tr>
<th>Time (sec)</th>
<th>Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1520</td>
<td>0</td>
</tr>
<tr>
<td>1524</td>
<td>4</td>
</tr>
<tr>
<td>1528</td>
<td>0</td>
</tr>
<tr>
<td>1532</td>
<td>4</td>
</tr>
<tr>
<td>1536</td>
<td>0</td>
</tr>
<tr>
<td>1540</td>
<td>4</td>
</tr>
<tr>
<td>1544</td>
<td>0</td>
</tr>
<tr>
<td>1548</td>
<td>4</td>
</tr>
<tr>
<td>1552</td>
<td>0</td>
</tr>
</tbody>
</table>

**Displaying the Data**

- Contour Plot
- 3D Plot

![Signal Plot](image1.png)

![Modulation Process Diagram](image2.png)

![Displaying the Data Diagram](image3.png)
The Added Value of Mass Spectrometry

State of the Art GC×GC

How to handle such multi-dimensional data sets??

Superstring theory, Hypercube, Tesseract, ...

Case Study #1: Cadaveric Decomposition
GC×GC-TOFMS gives us…

- Multi-dimensional date sets (sensitivity ++)
- Access to 'hidden' peaks
- $t_R^1$, $t_R^2$, deconvoluted MS signals, …
- > thousands of peaks (4-6 slices per peak)
- Several Gb file sizes

Making sense of such large data sets starts to be THE challenge…
TD-GC×GC-TOFMS

Trial carried out with pigs vs controls

e.g. Active Decay Trend
PCA (Ctrl vs HumInsIncl)

Correlation Loadings

70+ analytes

Pixel-Based Approach

First Dimension Retention Time Mean ($t_R^1$) [min]

Second Dimension Retention Time Mean ($t_R^2$) [s]

Fisher Ratio Plots

2D Fisher ratio bubble plot for percent responses of compounds detected in 24 chromatograms

Fisher Ratio Plot Cutoff

FR > 75

Identification

$^{1}t_R$, $^{2}t_R$, LRI, Lib Search, Molecular Formula, …
Compounds  | Formula  | Exact mass  | Real mass  | Diff (ppm)  
--- | --- | --- | --- | ---  
DMDS  | C₂H₆S₂  | 93,9915  | 93,9911  | -4  
DMTS  | C₂H₆S₃  | 125,9631  | 125,9632  | 1  
DMTeS  | C₂H₆S₄  | 157,9367  | 157,9352  | -9  
DMPeS  | C₂H₆S₅  | 189,8839  | 189,9073  | 123  

Mass Accuracy  

Barely any signal  

Case Study #2: Cancer Research  

Further reduction in cancer death rates can be accelerated by applying existing cancer control knowledge across all segments of the population, with an emphasis on those in the lowest socioeconomic bracket…  

Exhaled Breath Analysis (EBA)  

- Disease-related endogenous volatile biomarkers  
- Not that new…  

VOCs as Biomarkers…  

- Exhaled breath contains lots of VOCs  
- Health status fingerprint  
- Ease of accumulation  
- Ease of sampling  
- Fast and non-invasive
**VOC Measurements**

- PTR-MS
- SIFT-MS
- GC-MS
- E-nose

**GC×GC-(HR)TOFMS**

- **Sorbert Tubes:** Tenax® and carbopack®
- **TD:** Markes, desorption at 300°C for 3min
- **GC×GC-TOFMS:** LECO Peg 4D, JEOL 4G
- **Columns:** Rtx-5 (30m x 0.18mm x 0.2μm) as 'D and Rtx-17 (1m x 0.1mm x 0.1μm) as 'D
- **Oven T program:** 45°C (0.2min); 5°C/min until 245°C (1min); 30°C/min until 280°C (6min).
- **Modulation period:** 4 s
- **MS:** EI TOF at 70 eV, 25-100 Hz

**GC×GC-(HR)TOFMS**

- Patients
- Controls
- Alignments
- Creation of composite images
- Fisher ratio
- F Critical
- Various (un)supervised statistics (PCA, HCA, PLS, ...)

**GC×GC-(HR)TOFMS**

- Accessing patients and getting controls is somewhat complicated
- We need to gain orthogonal information's
- Another ‘source’ of VOCs can be considered
- What about VOCs produced by cancer cells?
- Would we see a specific signature???
Let’s Grow some Cells…

✓ Cell lines: MCF-7 breast cancer
  A-549 lung cancer
✓ Culture in DMEM @ 37°C under contr. CO₂
✓ T-75 boxes (20mL DMEM), triplicated

Sampling Based on Confluency

A-549 30% confl.
A-549 80% confl.
A-549 70% confl.

Measures on ‘Used’ DMEM

• PDMS 100µm SPME, 2h at 37°C, 250°C, split 10
• Rxn-5MS (30m, .25, .25) × Rxn-17Sil (1m, .15, .15)
• P<sub>1</sub>: <sup>13</sup>C, T<sub>2</sub>: 10°C
• 100 Hz, 35-450 amu, 70eV
• 2h cycle time

Apex of Composite Image

Apex of ‘VIP’ Analytes

PCA MCF-7 vs A-549 vs DMEM

Sampling at
Day-2 for MCF-7
Day-3 for A-549
PCA MCF-7 vs A-549 vs DMEM

Sampling at Day-2 & Day-5 for MCF-7
Day-3 and Day-5 for A-549

PCA MCF-7 Day-2 vs Day-5
PCA A-539 Day-3 vs Day-5
Time trend?

PCA MCF-7 Day-3 vs Day-5
PCA A-539 Day-3 vs Day-5
What Analytes?

✓ DMEM specific analytes...

What Analytes?

✓ Cancer cell specific analytes...

What Analytes ID?

✓ Exact mass identification of putative biomarkers...

✓ Duplication of selected samples on HRTOF

Take Home Message

✓ GC×GC-HRTOFMS is powerful tool (complex data)

✓ Supervised statistics needed (biological diversity)

✓ The cell culture approach reduces ‘flat tables’

✓ Next steps are:
  • Extract robust analyte identities
  • Compare analytes from cells to breath VOCs
  • Get primary cultures (biopsies) started on CRC