Introduction

Mass spectrometry has been an important tool of modern and clinical research for the discovery and quantification of metabolites and other small molecules. In bioanalytical applications, it is particularly useful for the analysis of low-molecular-weight compounds derived from, or transformed in cells of living organisms. While no single instrument is capable of fully profiling the metabolome, the unique capabilities of high resolution time-of-flight mass spectrometry (HRT) make it an essential tool for metabolomics research.

Sample Preparation

The rats were 7 to 9 weeks old, were fed a Western diet and preserved with EDTA.

Instrument Parameters

- **GC Parameters**
  - GC: Agilent 7890 and 7890A GC System
  - Column: 30 m x 0.32 mm x 0.25 µm silica bonded phase
  - Injector: 300 °C, split/splitless
  - Transfer Line: 300 °C
  - Detector: FID

- **Mass Spectrometer**
  - LECO Pegasus GC-HRT
  - Transfer Line: 300 °C
  - Mass Selectivity: 300000 (2 m, 0.25 µm, 0.25 µm cell)
  - Mass Range: 50 to 650 Da (0.00169 ppm, 9 amu)

- **Calibration**
  - FTIR (internal)
  - CI-Reflectron: 5 nm, quadrupole

- **Front End**
  - High Resolution (R = 25000, FWHM)

Experimental

Electron Impact and Chemical Ionization High Resolution Time-of-Flight Mass Spectrometry Analyses of Blood Plasma Samples

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Results and Discussion (EI-HRT)

Analytical ion Chromatograms (AIC) for fatty, and amino fatty acid derivatization (Figure 1). The plasma contained chemically diverse compounds including amino acids, fatty acids, nitric oxide, fatty alcohols, and sterols (Figure 2). Table 1 lists the retention times (Rt) and molecular ions (compounds represented). The 266 most intense ions in each AIC were used to compile a library database. The LEVO-384 GC-HRT software facilitated high sample throughput by providing excellent similarity matches for molecular ions. Figure 3 and 4, demonstrate using only four values for each analyte, resulted in formulas with accurate mass values ranging from 0.000 to 0.453 ppm.

Results and Discussion (CI-HRT)

CI-HRT can also be used to eliminate internal standard variability. For example, the number 1 and 2 library hits for 1-palmitoyl ethanolamine (16:0-PE-ethanolamine) were identical (Figure 5). The number 1 hit was assigned as cholesteryl palmitate (C30H50O2Si), whereas the number 2 hit was assigned as cholesteryl palmitate (C30H50O2Si, Figure 6). TMS derivatives of cholesteryl palmitate (C30H50O2Si) and cholesteryl palmitate (C30H50O2Si, Figure 7). Table 2: Representation Compounds in Zebrafish Plasma (Obese). The CI-HRT data revealed that the 3rd hit, cholestadiene (C32H50O2Si), was the actual compound.

Statistical Analysis

There were no significant mass spectral differences between TMS-16:0-PE and TMS-18:0-PE. These results are consistent with previous studies that have used negative CI-HRT to profile small molecules in biological samples.

Conclusion

The Pegasus GC-HRT is an ideal instrument for the analysis of plasma samples. The combination of EI and CI-HRT greatly facilitate confident identification of metabolites.

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**Table 1: Representation Compounds in Zebrafish Plasma (Obese)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rt (s)</th>
<th>Area</th>
<th>LM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-palmitoyl ethanolamine</td>
<td>16:0-PE-ethanolamine</td>
<td>50 to 650 Da (0.00169 ppm, 9 amu)</td>
<td>835/1000</td>
</tr>
<tr>
<td>Cholesteryl palmitate</td>
<td>C30H50O2Si</td>
<td>50 to 650 Da (0.00169 ppm, 9 amu)</td>
<td>211/1000</td>
</tr>
<tr>
<td>Cholestadiene</td>
<td>C32H50O2Si</td>
<td>50 to 650 Da (0.00169 ppm, 9 amu)</td>
<td>211/1000</td>
</tr>
</tbody>
</table>

**Figure 1: EI-HRT AIC: Fatty, Amino Fatty Acids, and Sterols**

**Figure 2: EI-HRT AIC and XIC Showing Cholesteryl Palmitate (C30H50O2Si)**

**Figure 3: EI-HRT Peak True Mass Spectral Data for TMS Derivatives of Cholesterol (A) and Gallotonin (B)**

**Figure 4: EI-HRT Peak True Mass Spectral Data for TMS Derivatives of Cholesterol (A) and Gallotonin (B)**

**Figure 5: EI-HRT Peak True Mass Spectral Data for TMS Derivatives of Cholesterol (A) and Gallotonin (B)**

**Figure 6: CI-HRT Peak True Mass Spectral Data for TMS Derivatives of Cholesterol (A) and Gallotonin (B)**

**Figure 7: CI-HRT Peak True Mass Spectral Data for TMS Derivatives of Cholesterol (A) and Gallotonin (B)**

**Figure 8: CI-HRT Peak True Mass Spectrum of Cholestadiene**

**Figure 9: Peaks in Zebrafish Plasma (Lean) (A), Fatty (B) and Obese (C)**