Accelerating lipid profiling of human samples for biomarker discovery using UFLC-IT-TOF

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Introduction
Untargeted metabolite profiling has become an integral part of systems biology research and is helping to further our understanding of the physiological and pathophysiological processes related to diet, environmental factors, disease and the impact of pharmaceutical treatments. In practical terms global metabolite profiling needs to scale into account not only the inherent complexity and diversity of human metabolism but also analytical technologies that deliver high mass accuracy and sensitivity to allow a comprehensive measurement of metabolic changes.

In this paper, we describe the application of a LCMS IT-TOF to detect lipid classes covering a LC cycle time of 10 minutes (peak widths between 1.7 seconds) with high mass accuracy. Ion signal stability was assessed by a pooled QC sample analyzed throughout the batch. Despite the compressed nature of the LC cycle time, the peak area variance was less than 10% (≤±17) batch analysis time 20 hours) for a series of phospholipids. Endogenous lipid metabolites were identified using high security MS data and verified using external search engines (http://www.lipidmaps.org; http://www.hmdb.ca; http://www.genome.jp/kegg/).

Results
In metabolite profiling it is important to assess the system reproducibility throughout the batch analysis. In this study the pooled QC sample was used to characterize the reproducibility of the system; whilst the EDA suggests that variability of ≤15% of the nominal value represents an acceptable degree of reproducibility, in long term profiling studies the tolerance is often relaxed to between 20-40%. In this study, ion signals which resulted in a relative standard deviation (RSD) ≤20% and retention time RSD<1%, were considered in subsequent principal component analysis.

Table 1. The data above shows the reproducibility of the system to a number of phospholipids with different signal intensities for a pooled QC sample (n=17). Raw ion current data; ion current areas calculated using LabSolutions software.

<table>
<thead>
<tr>
<th>Lipid</th>
<th>m/z</th>
<th>IS</th>
<th>RT</th>
<th>Area</th>
<th>S/N</th>
<th>RT Max</th>
<th>S/N Max</th>
<th>RSD</th>
<th>RSD %</th>
<th>RSD % Max</th>
<th>Slope</th>
<th>Y-axis</th>
<th>Ion signal stability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPCho(16:0/0:0)</td>
<td>520.3398</td>
<td>1.00</td>
<td>4.65</td>
<td>100</td>
<td>1.00</td>
<td>4.65</td>
<td>100</td>
<td>2.48</td>
<td>0.002</td>
<td>2.48</td>
<td>0.002</td>
<td>2.77</td>
<td>0.003</td>
</tr>
<tr>
<td>GPCho(18:3/0:0)</td>
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<td>4.65</td>
<td>100</td>
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</tr>
<tr>
<td>GPCho(20:5/0:0)</td>
<td>520.3398</td>
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<td>4.65</td>
<td>100</td>
<td>1.00</td>
<td>4.65</td>
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</table>

Table 2. Following the alignment of the raw data files the data array was filtered to include ions above a set tolerance (%RSD area ≥20%, %RSD RT ≥2%, minimum number of pooled QC ions was >98%). Pooled samples prepared by protein precipitation with cold acetone typically resulted in a data array dominated by phospholipid ion signals. The table shows the average mass accuracy measured throughout the batch analysis; the mass accuracy was calculated for each sample (R) using the end value for the most intense ion in the spectrum (S).

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<td>4.65</td>
<td>1.15</td>
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<td>1.15</td>
</tr>
</tbody>
</table>

Conclusions
* The study reports the application of a LCMS-IT-TOF to human plasma metabolite profiling. Using a scan speed of 10 scans per second, the peak area variance for a number of phospholipid ion signals was less than 10%, with a retention time variance less or equal to 5% for peaks widths typically between 3-7 seconds.

References
Koulman et al, RCM 2009; 23; 1411-1418

Figure 1. Mass chromatogram for a series of phospholipids from a pooled QC human plasma sample. Typical chromatographic peak width; 15 seconds (isooi ion current); 100msec; m/z 100; external mass calibration (ion accumulation time 20msec; 120 mode 10 spectra per second were acquired). In polarity switching mode, the switching time was 100msec.

Figure 2. Principal Component Analysis. Pooled QC data are closely clustered together and show no ‘run order’ change in signal response over the analytical run. (the data presented above consider the 1uL injection volume data only).