Untargeted Metabolomics: Tandem LC-MSMS
# Column and Flow Rate Selection

<table>
<thead>
<tr>
<th>Type</th>
<th>Column ID</th>
<th>Flow rate</th>
<th>Solvent consumed*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>1.0-4.6 mm</td>
<td>0.050-1.00 ml/min</td>
<td>72-1440 ml</td>
</tr>
<tr>
<td>Capillary</td>
<td>0.3-1.0 mm</td>
<td>0.005-0.050 ml/min</td>
<td>7.2-72 ml</td>
</tr>
<tr>
<td>Nano</td>
<td>0.05-0.20 mm</td>
<td>100-1000 nl/min</td>
<td>0.144-1.44 ml</td>
</tr>
</tbody>
</table>

Reverse Phase and Normal Phase Selections:

- Reverse Phase (C4, C8, C18, etc...)
- Normal Phase (Silica, Amide, Amido, etc...)
Predicted Increase in Sensitivity

- 20 μl out of 100 μl injected
  - Analyzed on a 2.1 mm ID column at 200 μl/min
  - LLOQ = 10 nM
  - Analyzed on a 0.7 mm ID column at 22 μl/min
  - LLOQ = 1.1 nM
  - Analyzed on a 0.3 mm ID column at 4.3 μl/min
  - LLOQ = 200 pM
  - Analyzed on a 75 μm ID column at 0.27 μl/min
  - LLOQ = 12.5 pM

- If 1 μl is injected, then LLOQ is 250 pM
The Time of Flight (ToF) chamber allows for rapid detection and high mass accuracy as compared to traditional triple quadrupole instrumentation. All fragments are detected simultaneously instead of a precursor (parent ion) > fragment (daughter ion) pair.
High Resolution and Acquisition Speed

Precursor Scans collected over 100 msec;
Fragmentation Spectra collected as low as 10 msec.
Typical Instrument Acquisition Method

- Precursor Ion Scan: 250 msec
- Product Ion Scan: 50 msec (X 20)

Total Cycle Time is 1.25 seconds

The combination of precursor plus fragmentation scans in one method allows the investigator to simultaneously quantitate and qualitate potential compounds between experimental groups.
Evaluation of Collected Data

- Chromatographic reproducibility
- Sample intensity comparisons
- Mass accuracies between samples
Evaluation of Collected Data continued

- Chromatography tracings overlap
- Mass accuracy window set to 0.005 Daltons
Exporting Data to Statistical Programs

Programs like MS Convert can transform instrument raw files (.wiff, .dta, etc) in usable formats such as mzXML. Keep in mind when transforming the raw data, the size of the files can increase significantly.
Data Analyses Software

There are a host of software platforms ranging from free online resources to company specific programs.
Data Analyses Software continued

XCMS Online aligns chromatography and outputs user friendly schematics from uploaded data including PCA graphs, t-test, etc.