Untargeted Metabolomics: Tandem LC-MSMS

Column and Flow Rate Selection

Reverse Phase and Normal Phase Selections:
• Reverse Phase (C4, C8, C18, etc...)
• Normal Phase (Silica, Amide, Amido, etc...)
Instrument Configuration

AB Sciex 5600 Triple ToF Mass Spectrometer (Q-Tof MS)

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 qualify and quantify 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 m2XML. Keep in mind when transforming the raw data, the size of the files can increase significantly.
There are a host of software platforms ranging from free online resources to company specific programs.

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