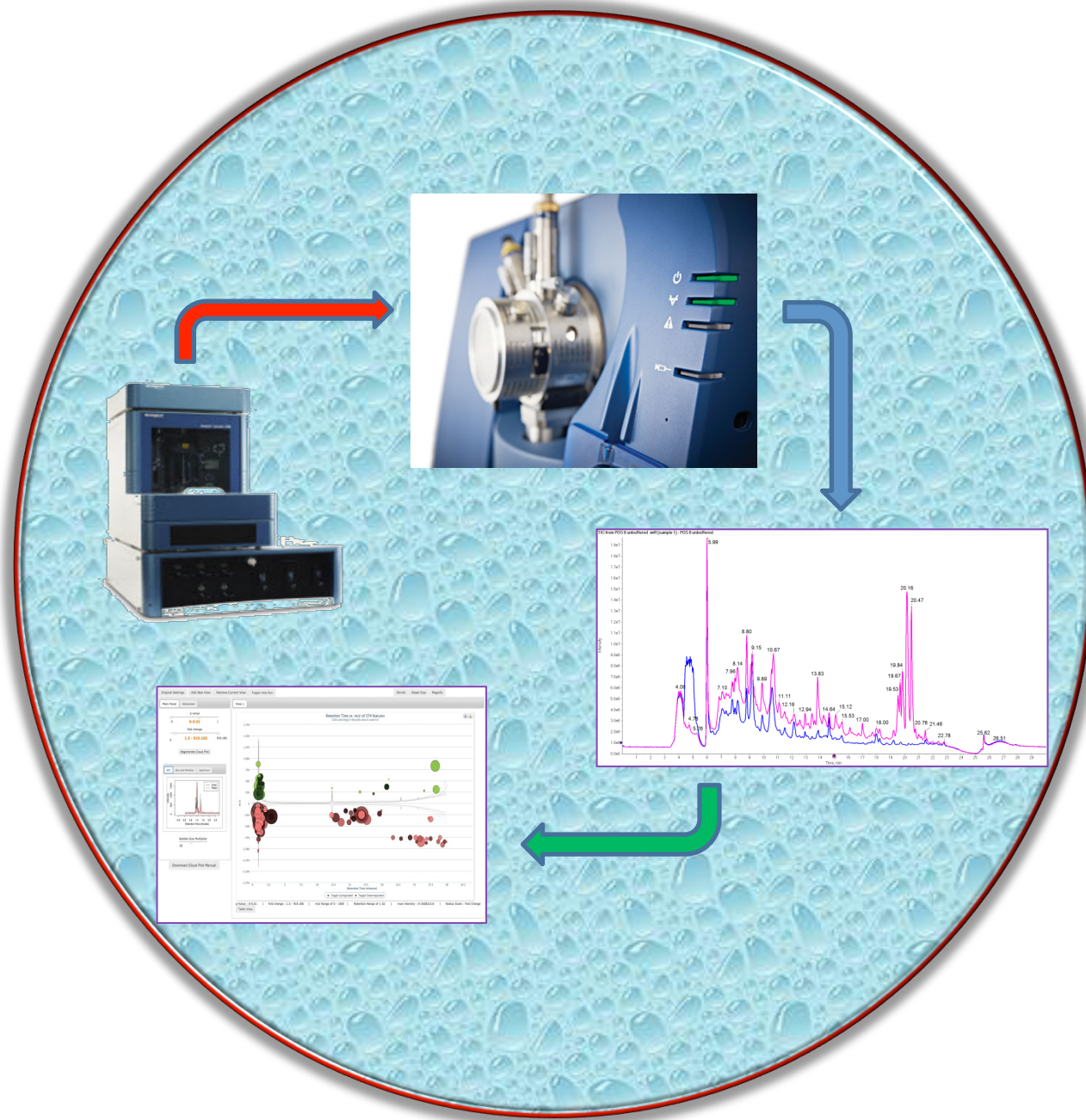# Untargeted Metabolomics: Tandem LC-MSMS
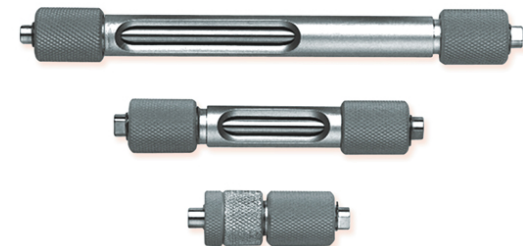
Column and Flow Rate Selection

Insert Barnes table for flow rates and sensitivity gain.
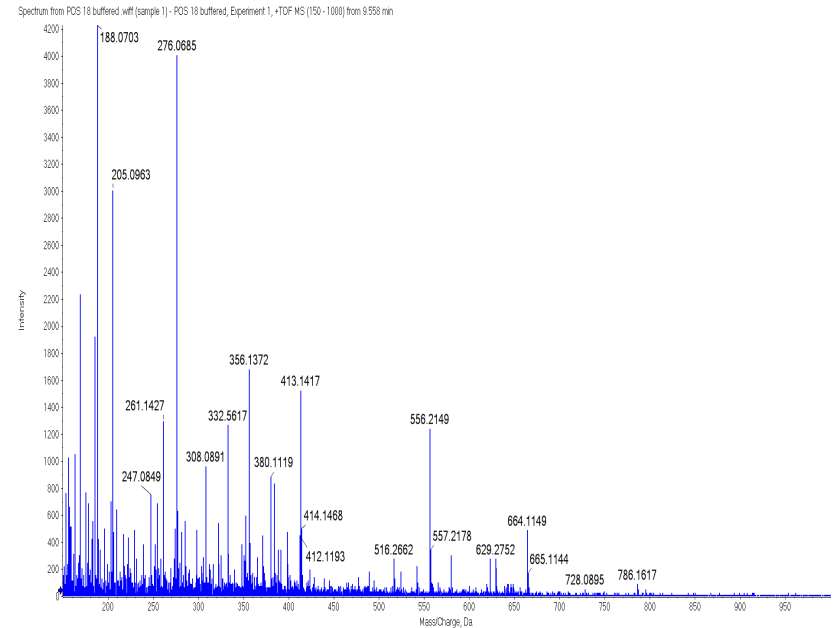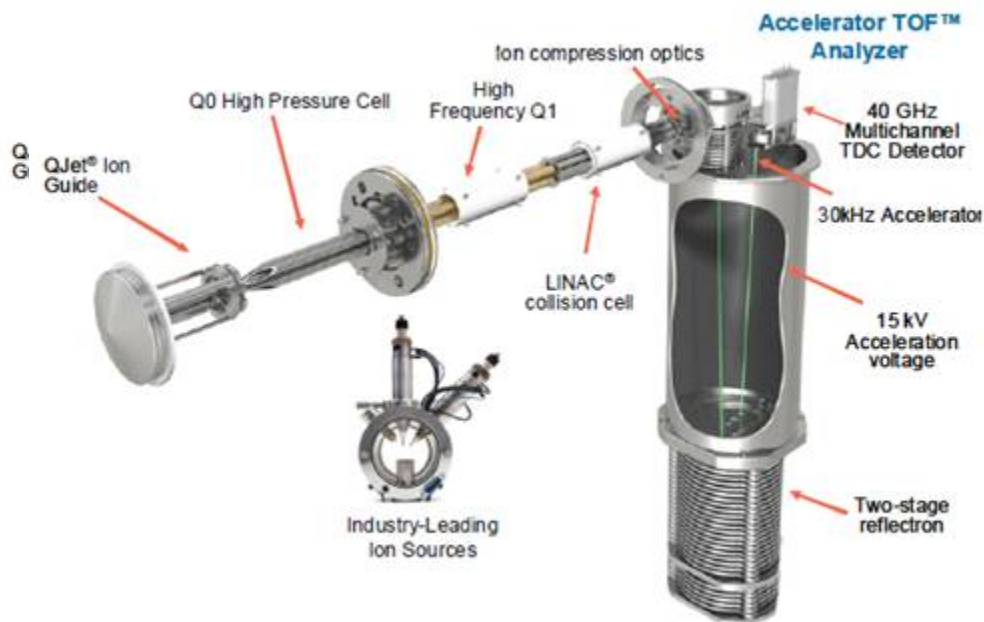
Reverse Phase and Normal Phase Selections:

• Reverse Phase (C4, C8, C18, etc…)
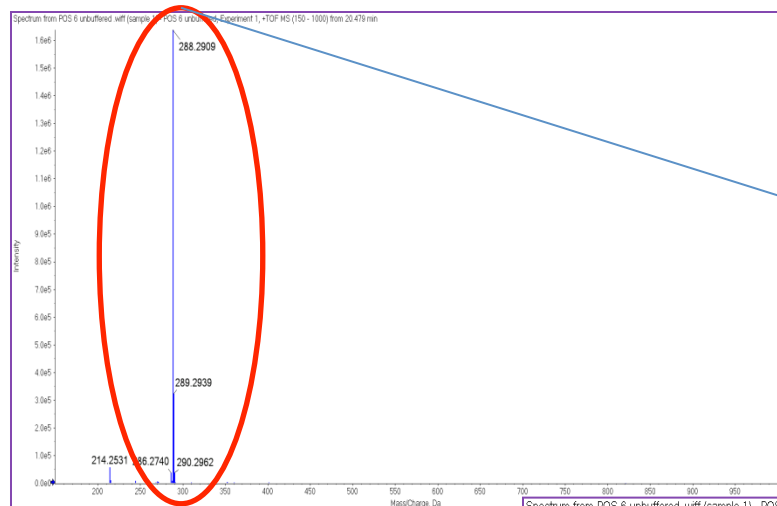• Normal Phase (Silica, Amide, Amido, etc…)

Instrument Configuration

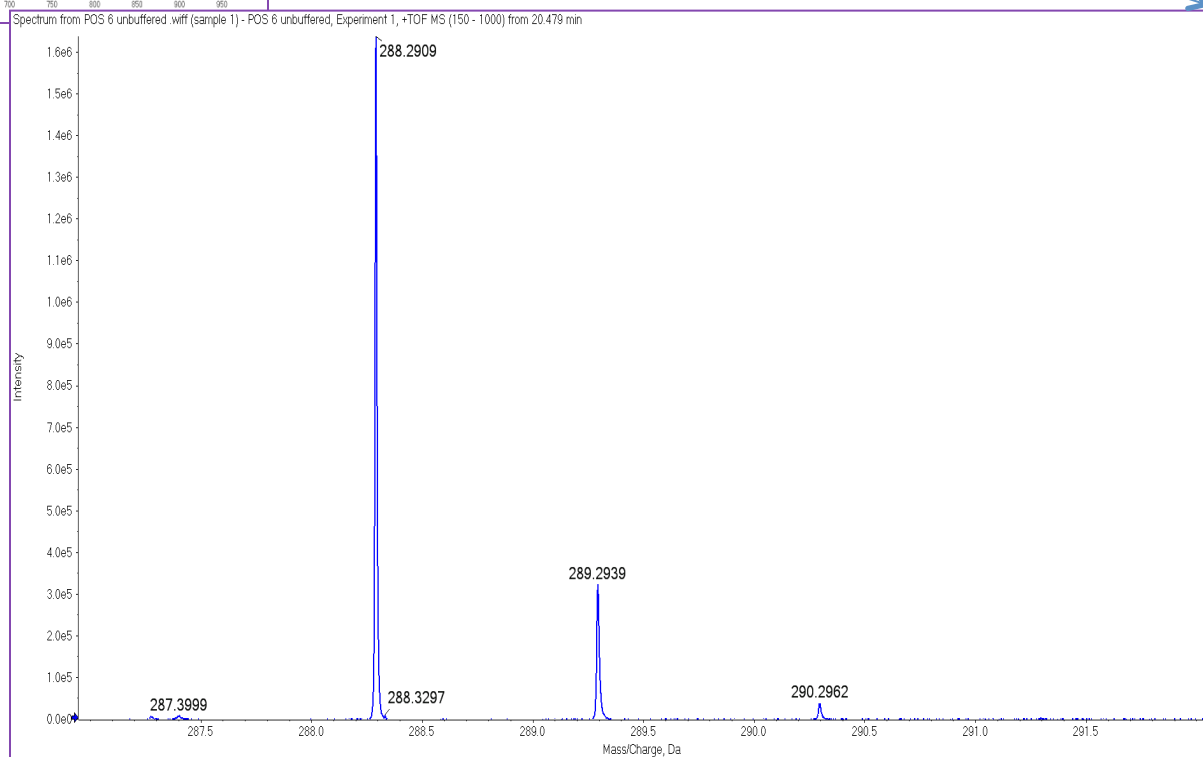AB Sciex 5600 Triple Tof Mass Spectrometer (Q-Tof MS)

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;
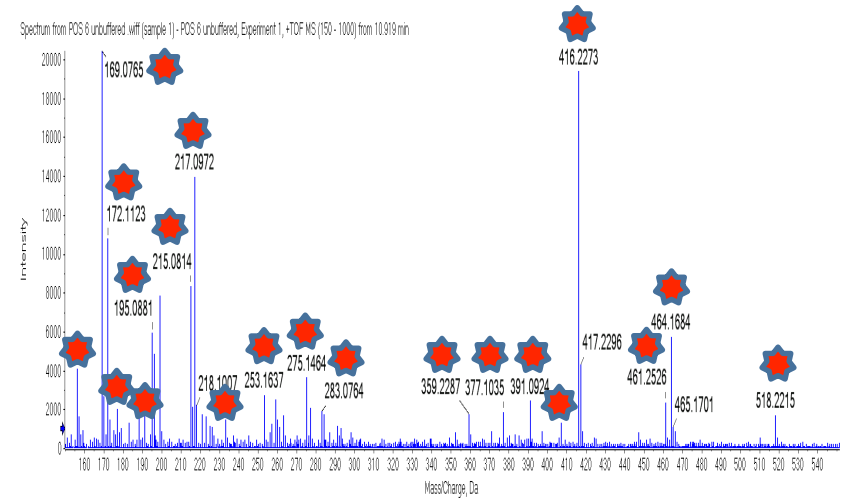Fragmentation Spectra collected as low as 10 msec.

Typical Instrument Acquisition Method

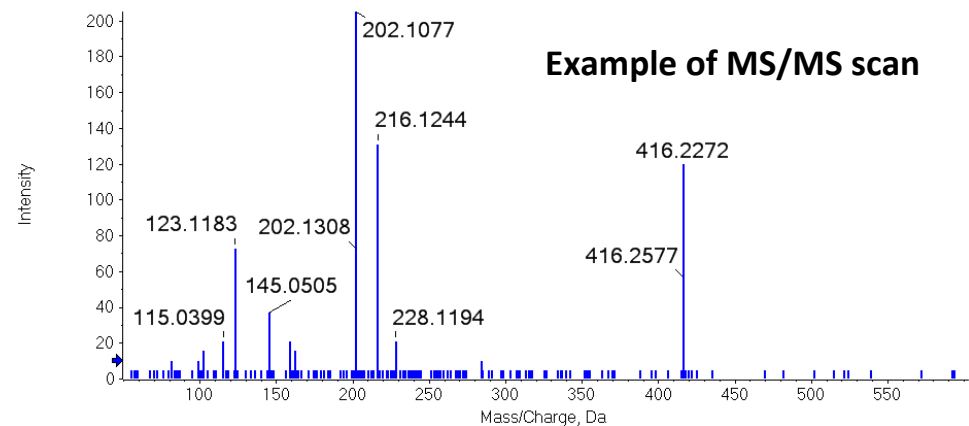• Precursor Ion Scan: 250 msec

↓

• Product Ion Scan: 50 msec (X 20)

Total Cycle Time is 1.25 seconds

Spectrum from POS 6 unbuffered .wiff (sample 1) - POS 6 unbuffered, Experiment 1, +TOF MS (150 - 1000) from 10.919 min
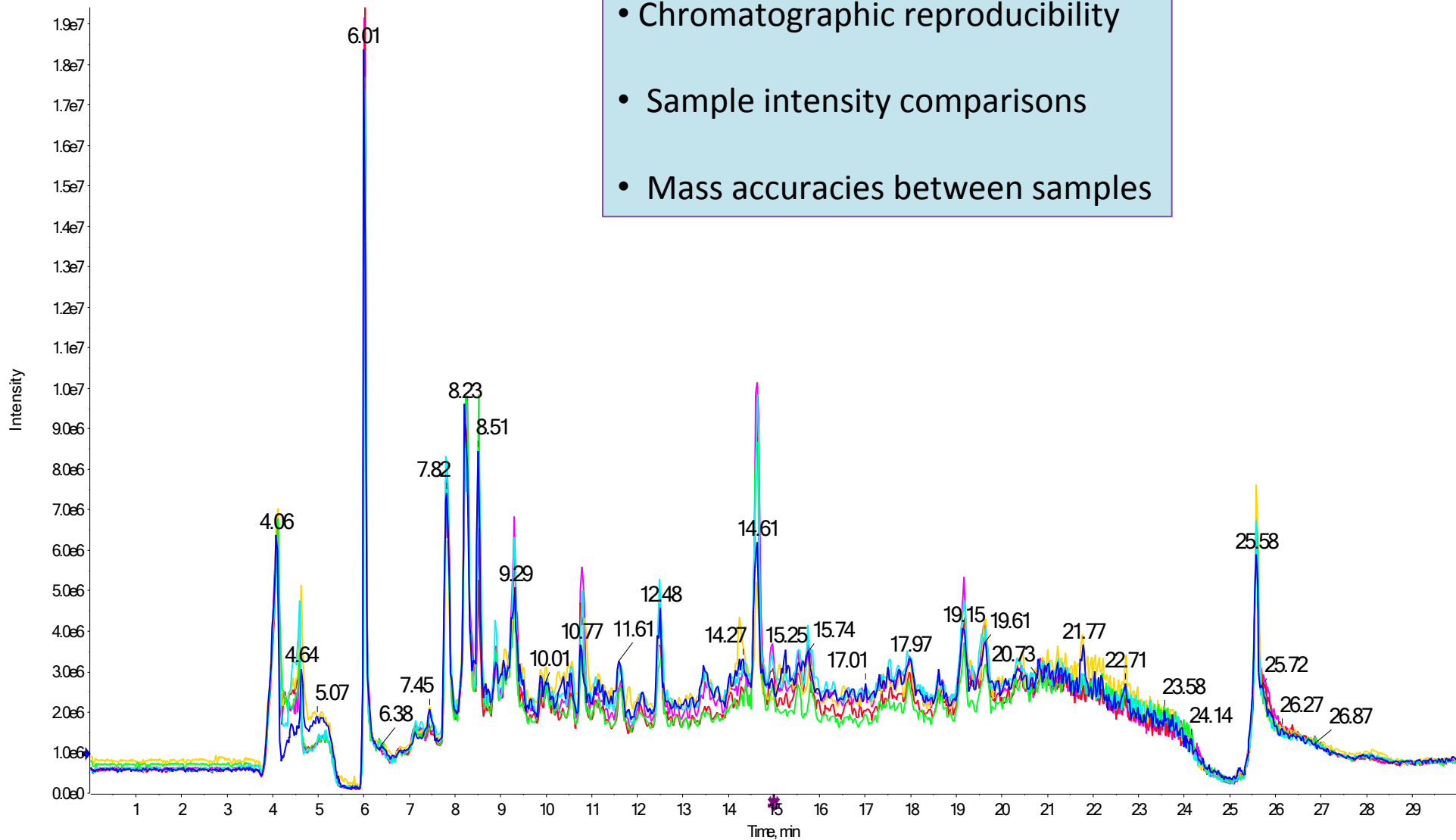
⬟ *Selected ion for fragmentation*

↓

The combination of precursor plus fragmentation scans in one method allows the investigator to simultaneously qualify and quantify potential compounds between experimental groups.
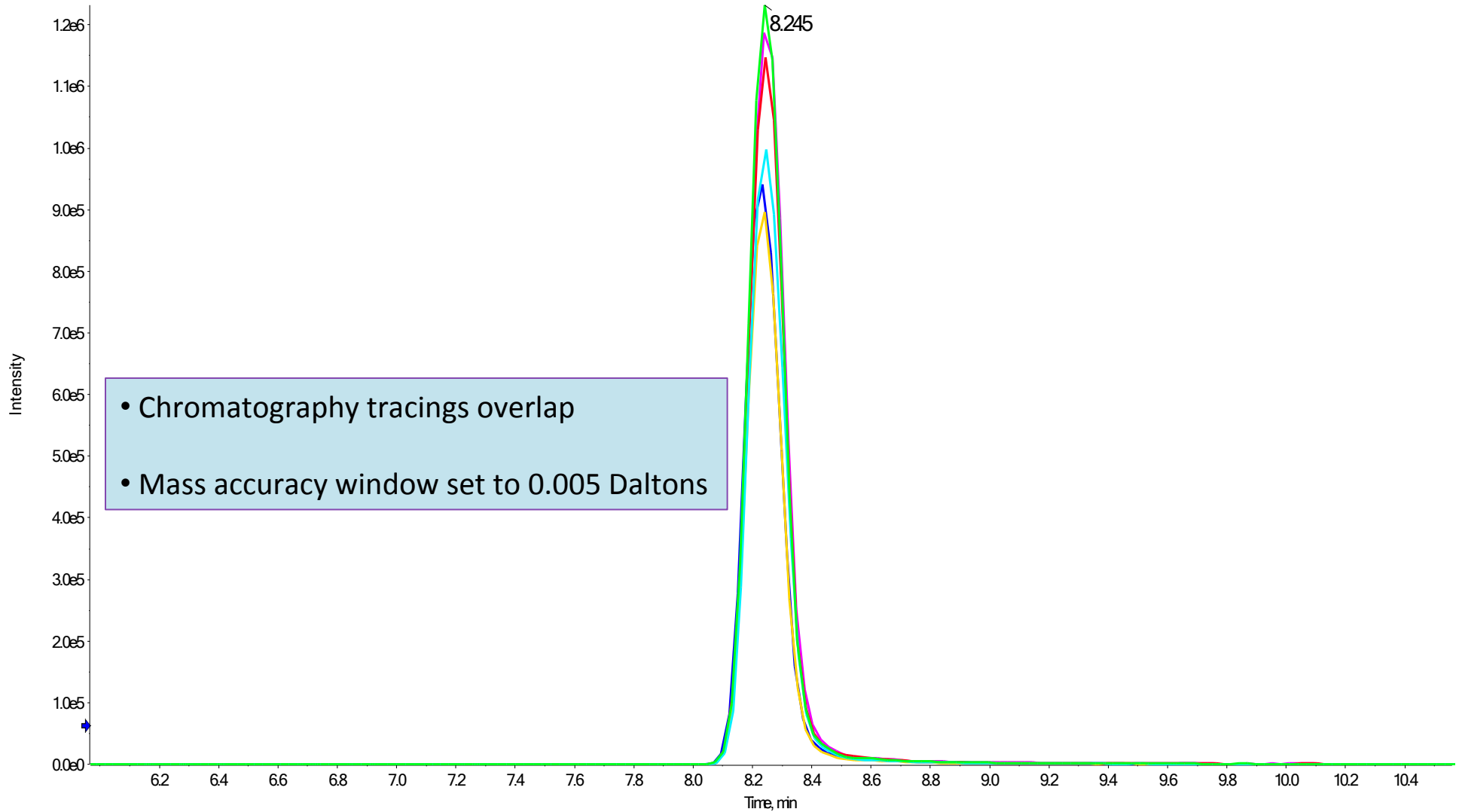

Spectrum from POS 6 unbuffered .wiff (sample 1) - POS 6 unbuffered, Experiment 6, +TOF MS^2 (50 - 1000) from 10.997 min
Precursor: 416.2 Da

Example of MS/MS scan

Evaluation of Collected Data

- Chromatographic reproducibility

- Sample intensity comparisons

- Mass accuracies between samples

Evaluation of Collected Data continued

XIC from PosNFc216-1.wiff (sample 1) - Pos NFc216-1, Experiment 1, +TOF MS (150 - 1000): 220.1174 +/- 0.0025 Da, Gaussian smoothed
XIC from PosNFc216-2.wiff (sample 1) - Pos NFc216-2, Experiment 1, +TOF MS (150 - 1000): 220.1174 +/- 0.0025 Da, Gaussian smoothed
XIC from PosNFc216-4.wiff (sample 1) - Pos NFc216-4, Experiment 1, +TOF MS (150 - 1000): 220.1174 +/- 0.0025 Da, Gaussian smoothed
XIC from PosVMP-1.wiff (sample 1) - Pos VMP-1, Experiment 1, +TOF MS (150 - 1000): 220.1174 +/- 0.0025 Da, Gaussian smoothed
XIC from PosVMP-2.wiff (sample 1) - Pos VMP-2, Experiment 1, +TOF MS (150 - 1000): 220.1174 +/- 0.0025 Da, Gaussian smoothed
XIC from PosVMP-4.wiff (sample 1) - PosVMP-4, Experiment 1, +TOF MS (150 - 1000): 220.1174 +/- 0.0025 Da, Gaussian smoothed

8.245

- Chromatography tracings overlap

- Mass accuracy window set to 0.005 Daltons
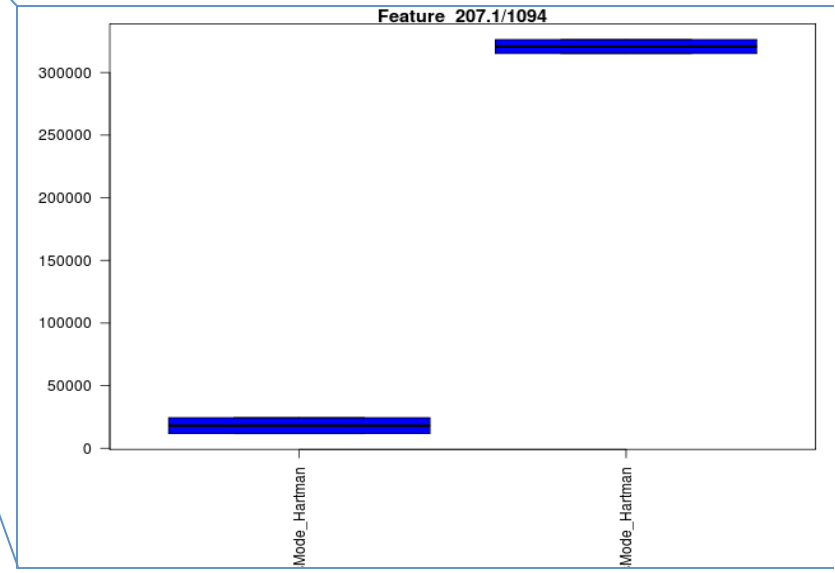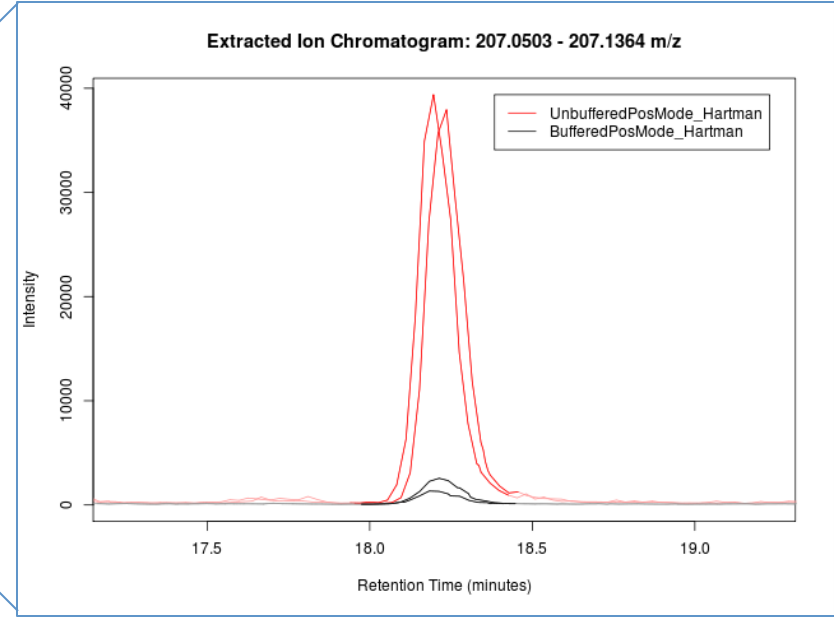
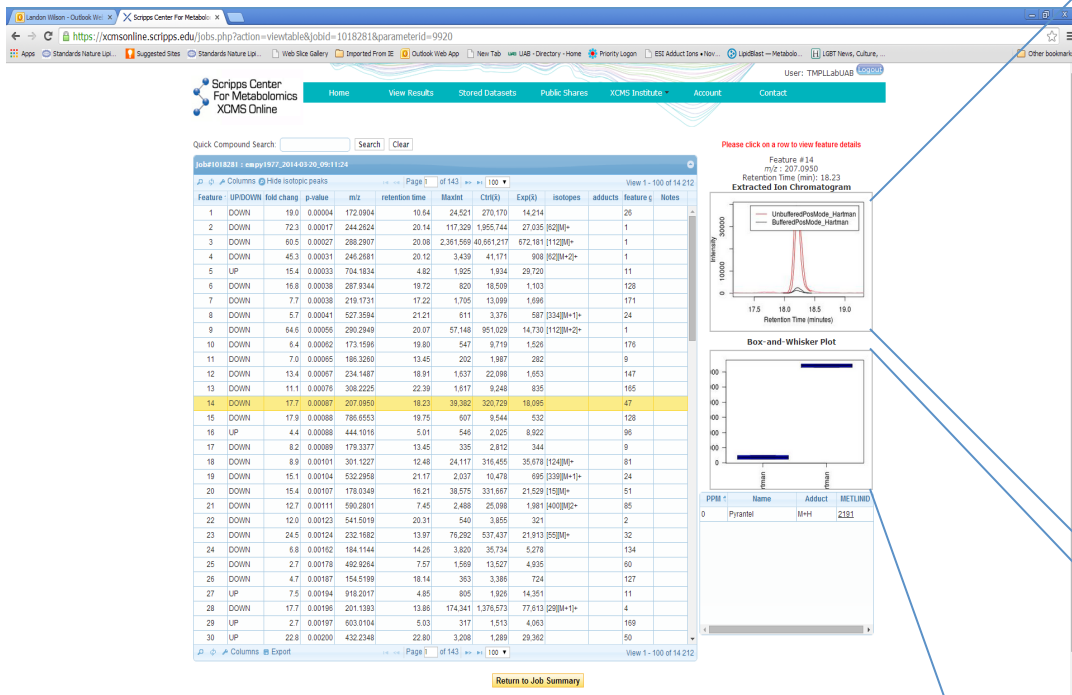# Exporting Data to Statistical Programs

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.