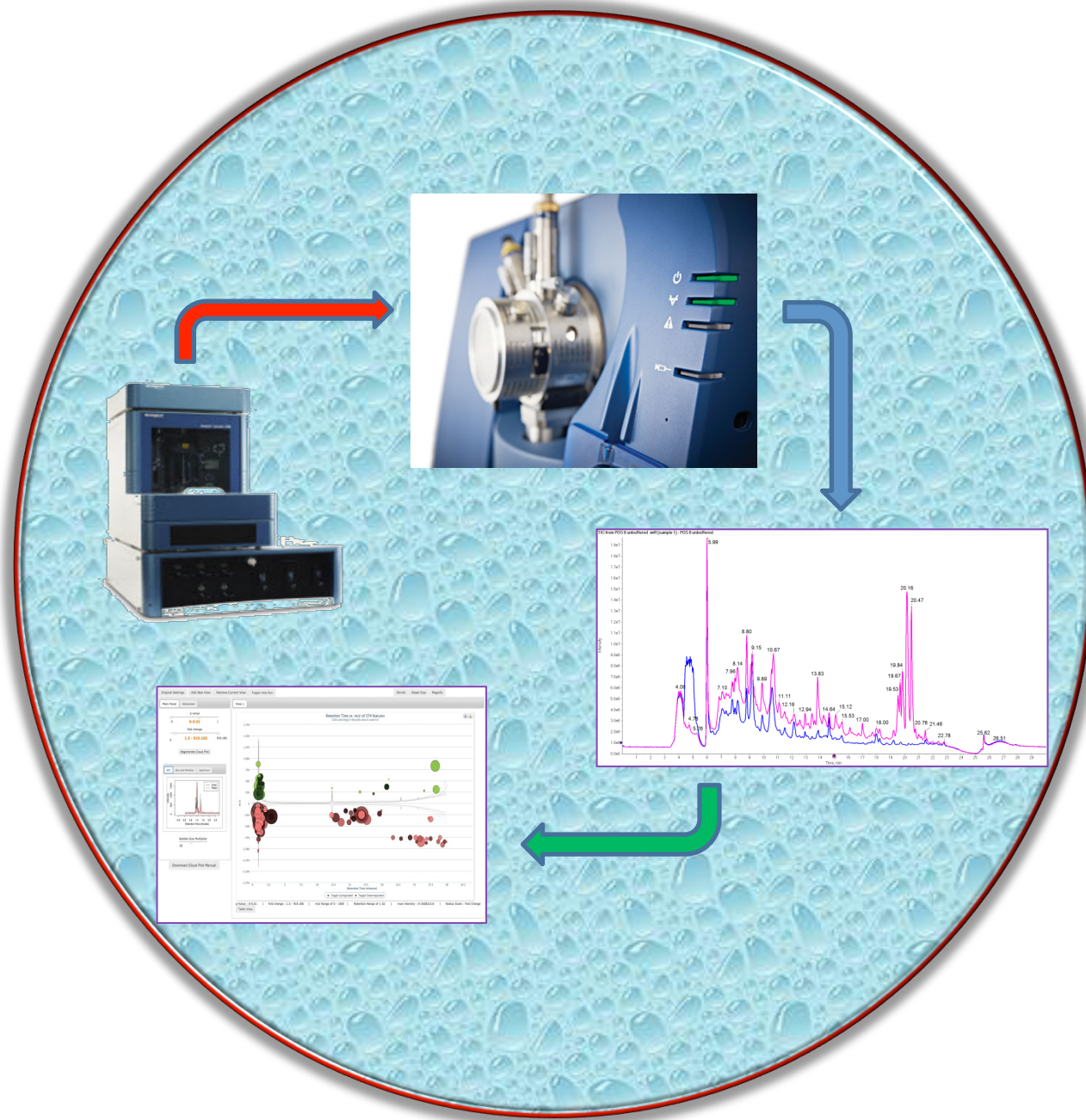


Untargeted Metabolomics: Tandem LC-MSMS

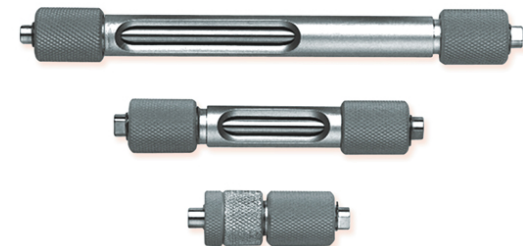


Column and Flow Rate Selection

Insert Barnes table for flow rates and sensitivity gain.

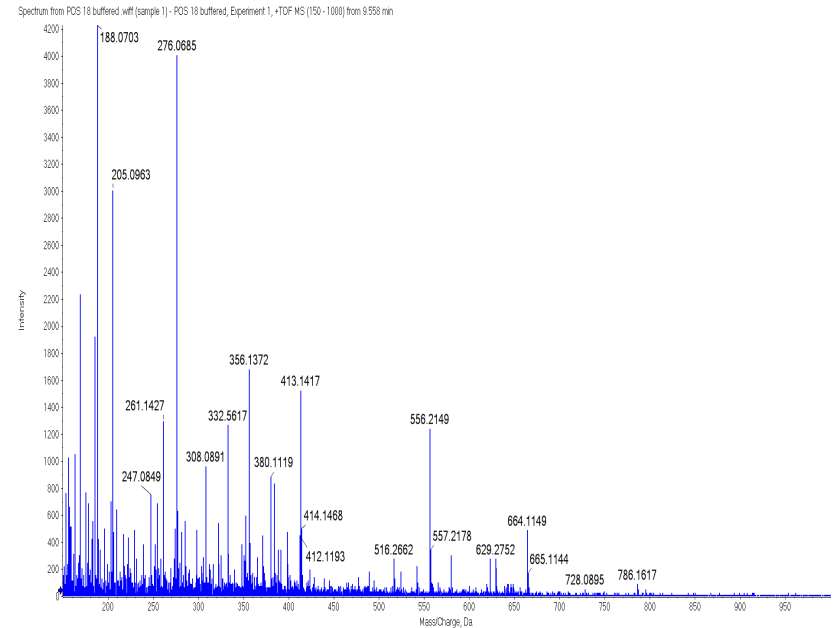
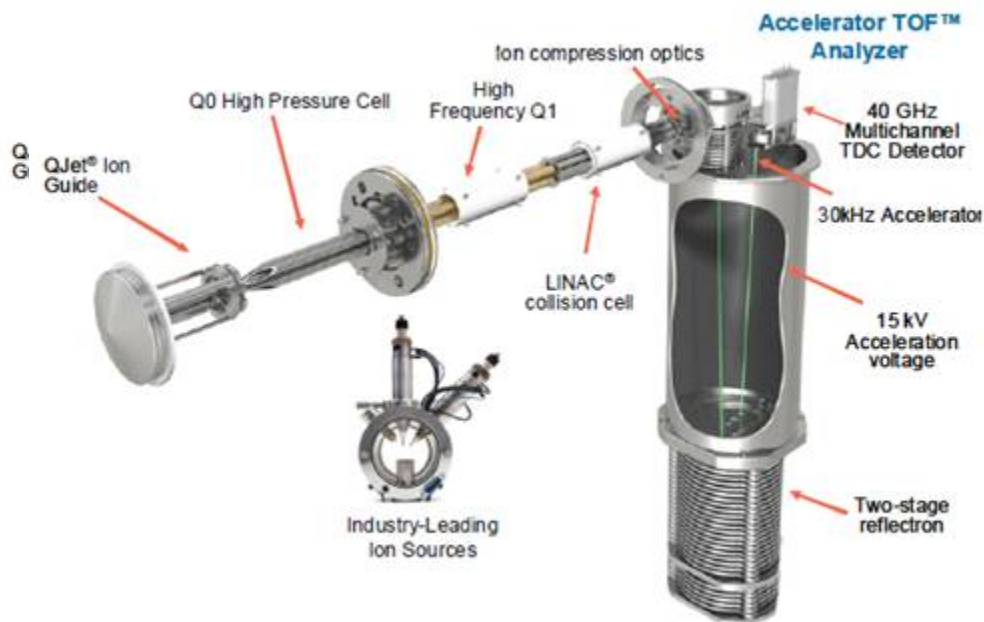
Reverse Phase and Normal Phase Selections:

- Reverse Phase (C4, C8, C18, etc...)
- Normal Phase (Silica, Amide, Amido, etc...)



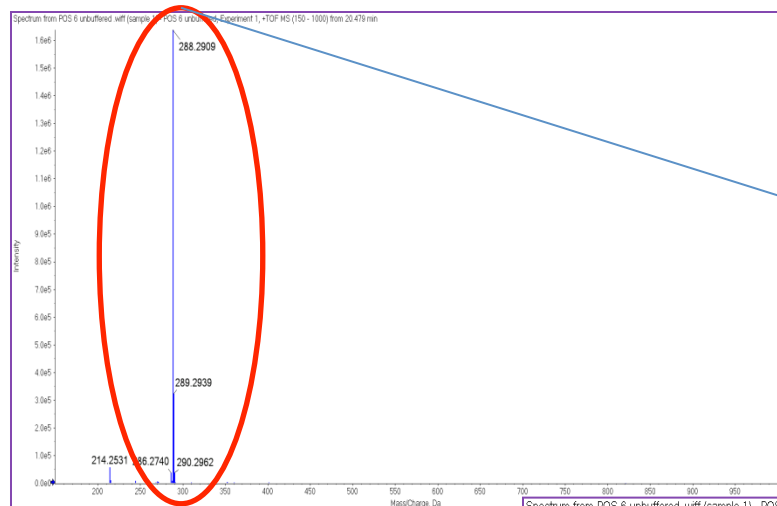
Instrument Configuration

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

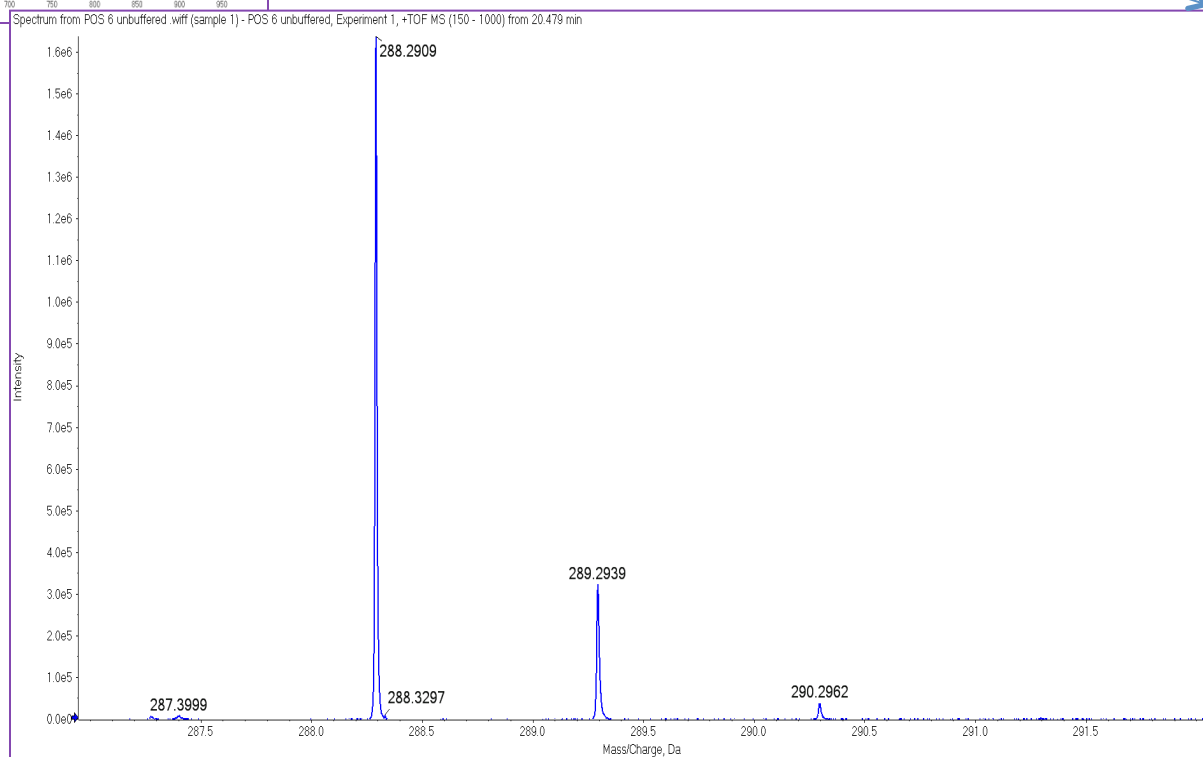


The Time of Flight (ToF) chamber allows for rapid detection and high mass accuracy as compared to traditional triple quadrupole instrumentation. All fragments are detected simultaneously instead of a precursor (parent ion) > fragment (daughter ion) pair.

High Resolution and Acquisition Speed



Precursor Scans collected over 100 msec;
Fragmentation Spectra collected as low as 10 msec.



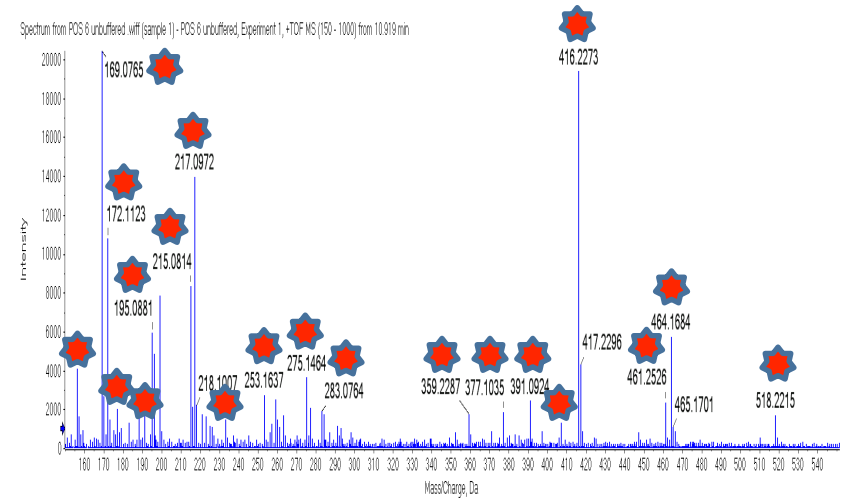
Typical Instrument Acquisition Method

•Precursor Ion Scan: 250 msec



•Product Ion Scan: 50 msec (X 20)

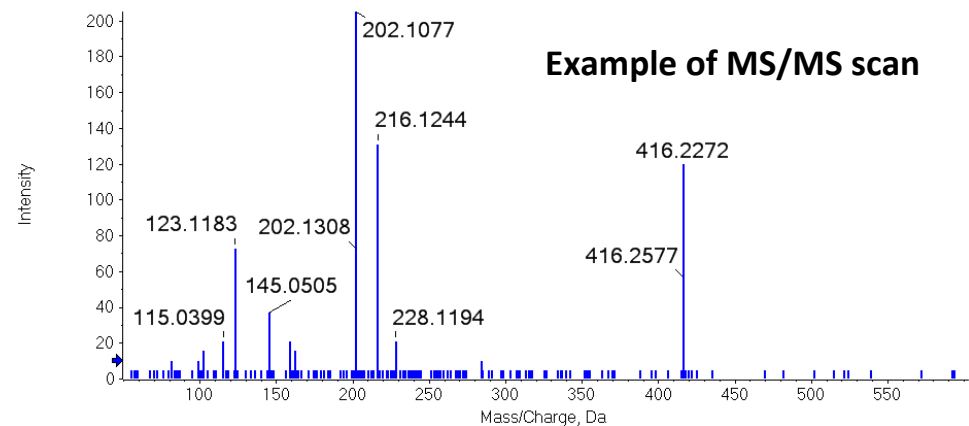
Total Cycle Time is 1.25 seconds



 **Selected ion for fragmentation**



Spectrum from POS 6 unbuffered.wiff (sample 1) - POS 6 unbuffered, Experiment 6, +TOF MS² (50 - 1000) from 10.997 min
Precursor: 416.2 Da

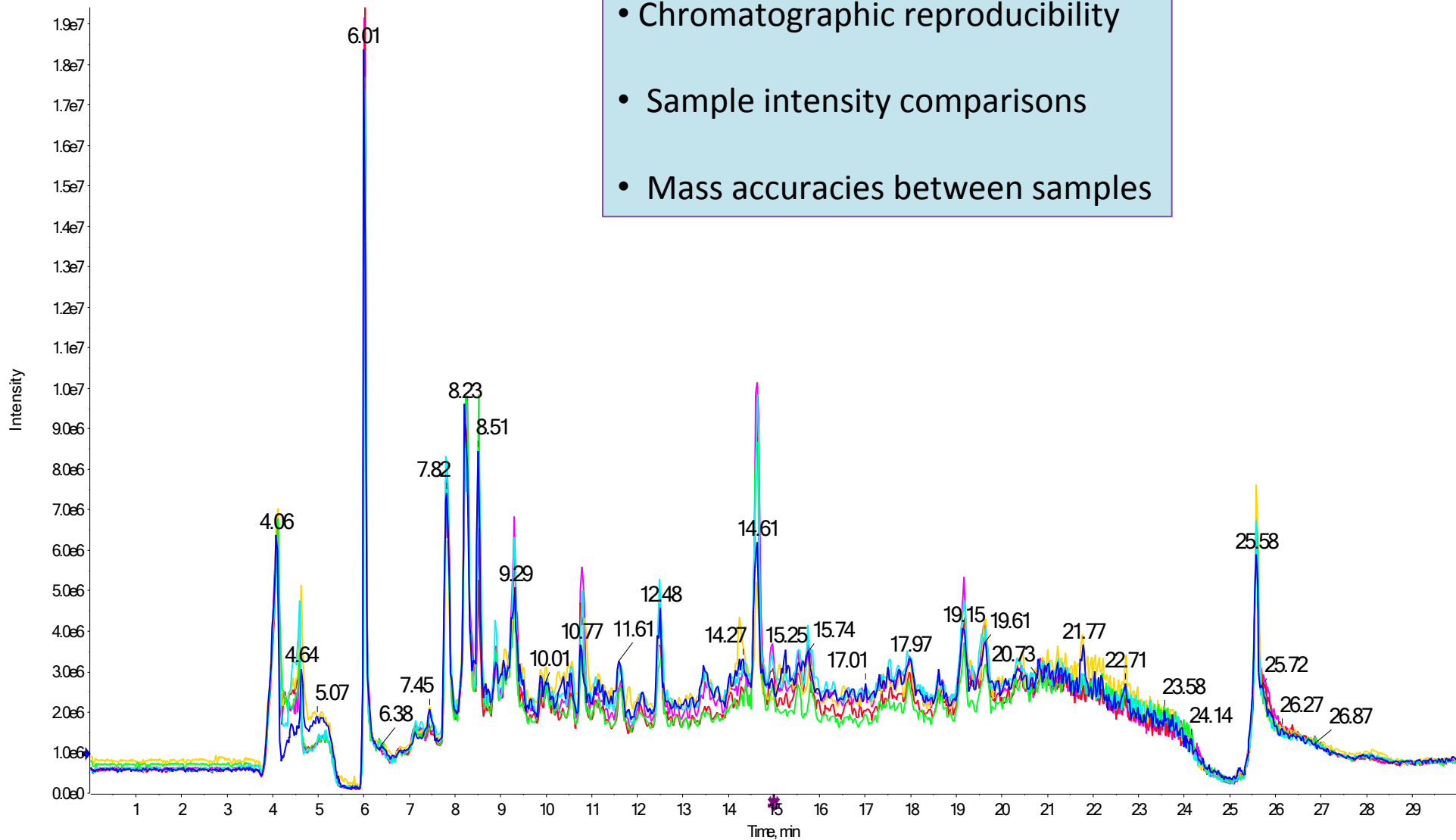


The combination of precursor plus fragmentation scans in one method allows the investigator to simultaneously qualify and quantify potential compounds between experimental groups.

Evaluation of Collected Data

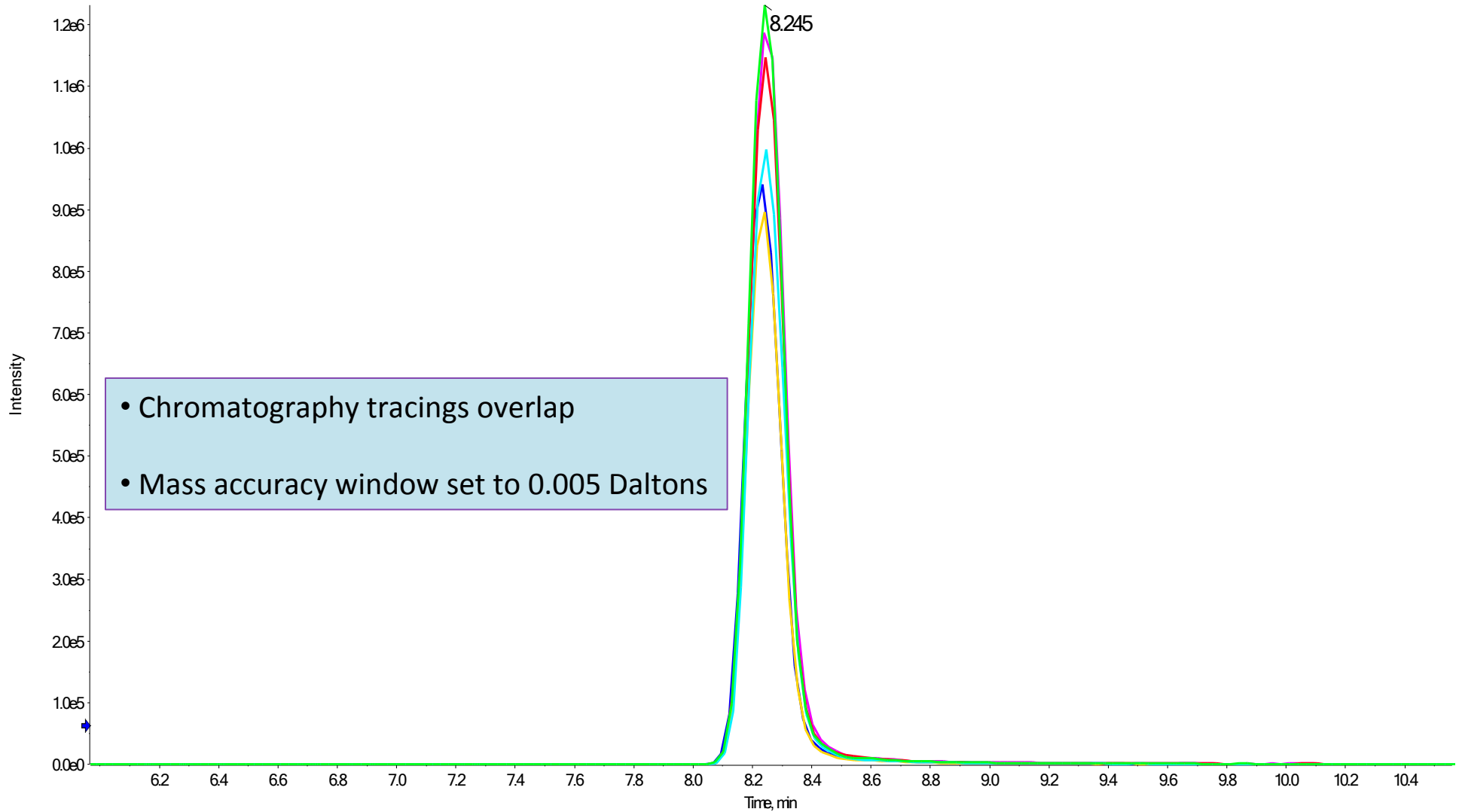
- TICfromPosNfc216-1.wiff (sample 1) - Pos Nfc216-1
- TICfromPosNfc216-2.wiff (sample 1) - Pos Nfc216-2
- TICfromPosNfc216-4.wiff (sample 1) - Pos Nfc216-4
- TICfromPosVMP-1.wiff (sample 1) - Pos VMP-1
- TICfromPosVMP-2.wiff (sample 1) - Pos VMP-2
- TICfromPosVMP-4.wiff (sample 1) - Pos VMP-4

- 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



Exporting Data to Statistical Programs

The image shows two windows from the MSConvert software. The left window is the main MSConvert interface, and the right window is the 'Open Data Source' dialog.

MSConvert (64-bit) Interface:

- File selection: List of Files (selected), File of file names.
- File: [] Browse
- Buttons: Add, Remove
- Filters: MS Level (dropdown), Levels: 1 - []
- Buttons: Add, Remove
- Filter table:

Filter	Parameters
titleMaker	<RunId> <ScanNumber> <ScanNumber>....
- Output Directory: [] Browse
- Options:
 - Output format: mzXML (dropdown), Extension: []
 - Binary encoding precision: 64-bit (selected), 32-bit
 - Write index: Use zlib compression:
 - TPP compatibility: Package in gzip:
 - Use numpress linear compression:
 - Use numpress short logged float compression:
 - Use numpress short positive integer compression:
- Buttons: Use these settings next time I start MSConvert, Start

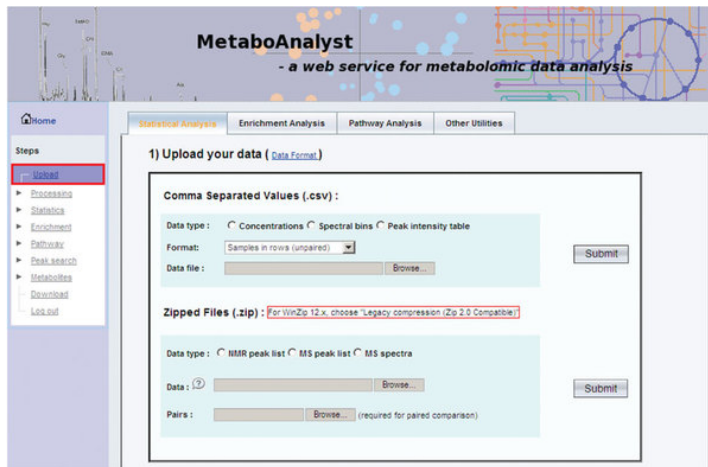
Open Data Source Dialog:

- Path: masspec2 > Desktop > Lalita Samant 051214 Files > 051114
- Table of files:

Name	Size	Type	Date Modified
Neg NF2c16-1.mzXML	1,244 MB	mzXML	5/12/2014 10:26:57.
Neg NF2c16-1.wiff	4,132 KB	ABSciex WI...	5/11/2014 4:50:51 P
Neg NF2c16-2.mzXML	1,145 MB	mzXML	5/12/2014 10:26:48.
Neg NF2c16-2.wiff	4,083 KB	ABSciex WI...	5/11/2014 6:08:27 P
Neg NF2c16-4.mzXML	1,168 MB	mzXML	5/12/2014 10:29:22.
Neg NF2c16-4.wiff	4,128 KB	ABSciex WI...	5/11/2014 7:25:59 P
Neg VMP-1.mzXML	1,236 MB	mzXML	5/12/2014 10:29:43.
Neg VMP-1.wiff	4,182 KB	ABSciex WI...	5/11/2014 12:58:08
Neg VMP-2.mzXML	1,095 MB	mzXML	5/12/2014 10:31:15.
Neg VMP-2.wiff	3,874 KB	ABSciex WI...	5/11/2014 2:15:43 P
Neg VMP-4.mzXML	1,114 MB	mzXML	5/12/2014 10:31:38.
Neg VMP-4.wiff	3,952 KB	ABSciex WI...	5/11/2014 3:33:18 P
- Source name: Neg NF2c16-1.wiff; Neg NF2c16-2.wiff; Neg NF2c16-4.wiff; Neg VMP-1.wiff; Neg VMP-2.wiff; Neg VMP-4.wiff
- Buttons: Open, Cancel
- Sources of type: Any spectra format

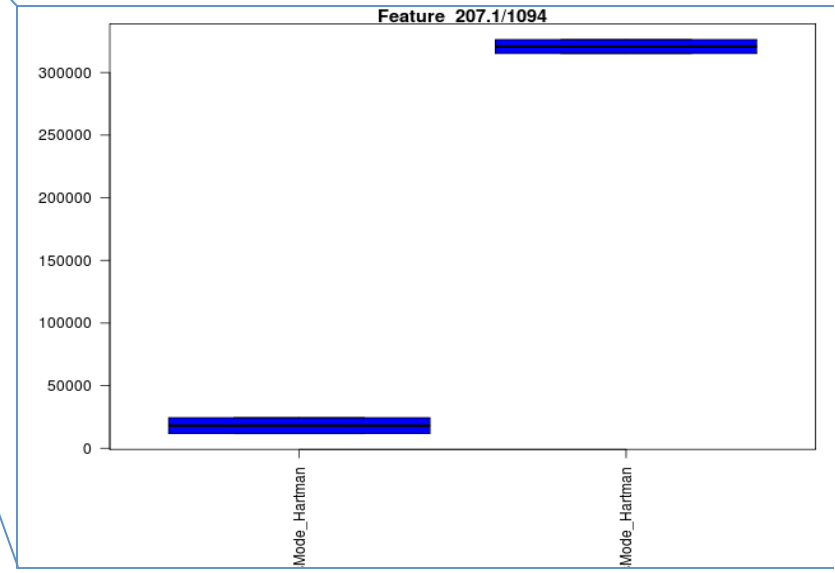
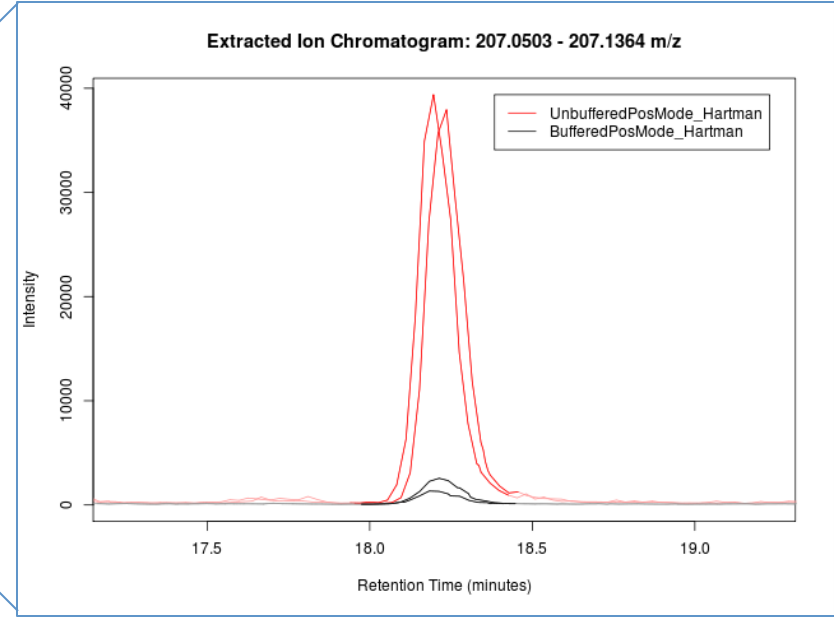
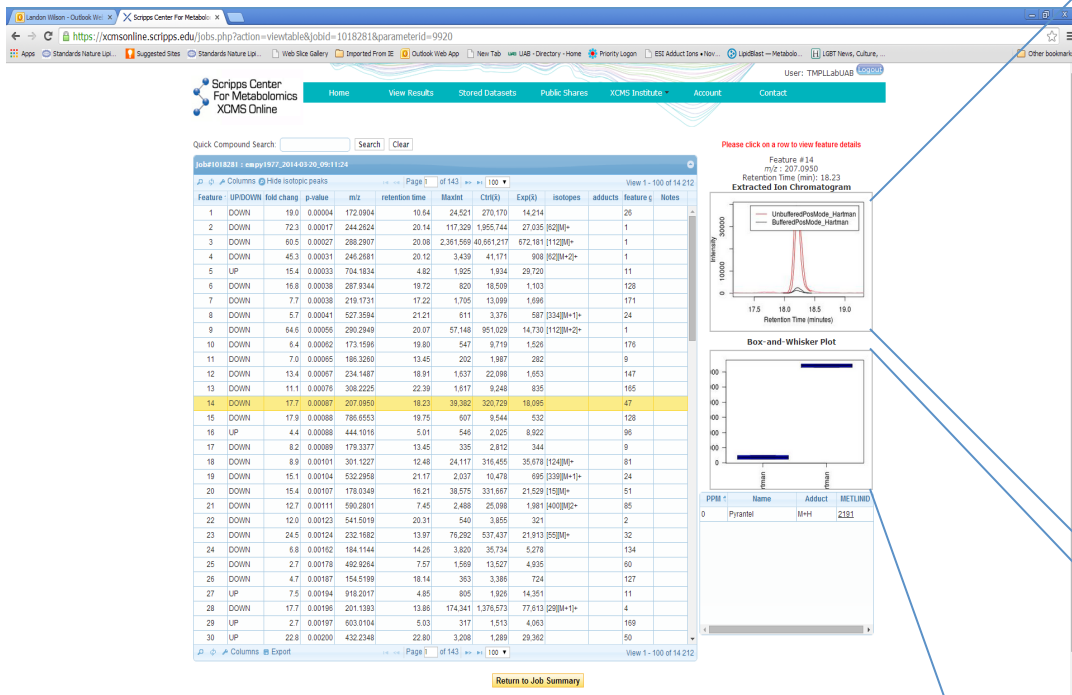
Programs like MS Convert can transform instrument raw files (.wiff, .dta, etc) in usable formats such as mzXML. Keep in mind when transforming the raw data, the size of the files can increase significantly.

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.