

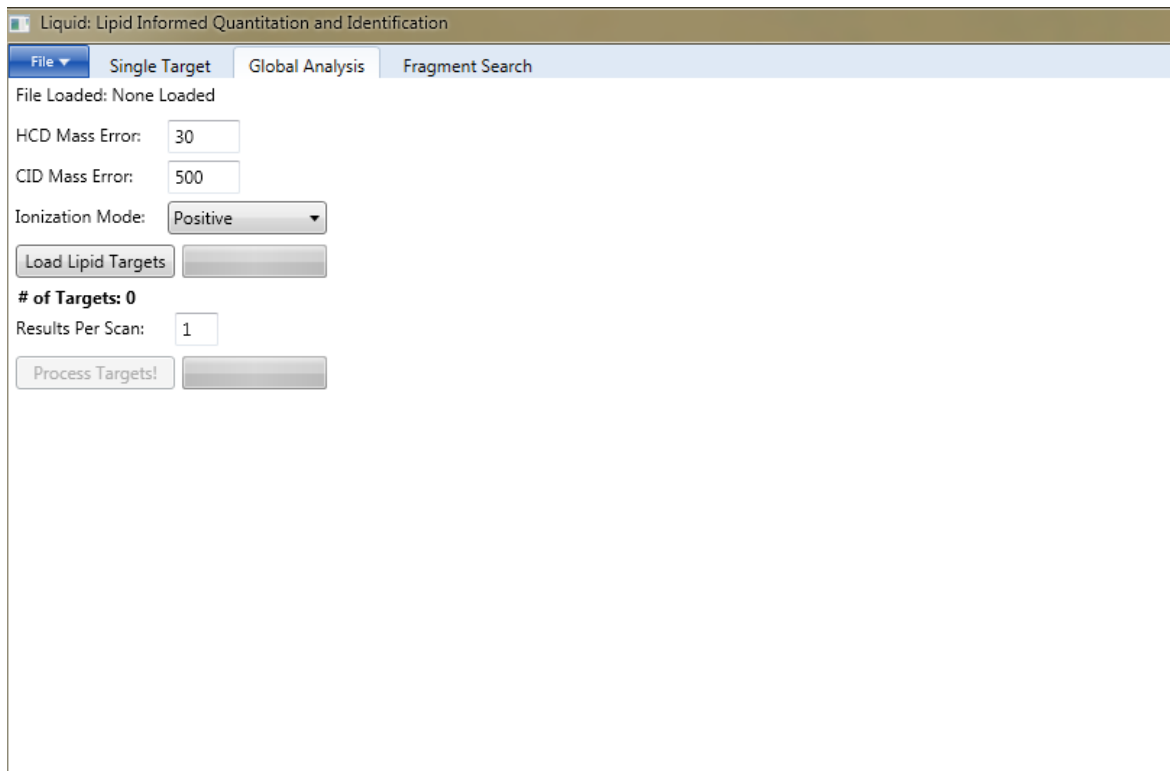
LIQUID Installation and Operation

By Jennifer E. Kyle
(Jennifer.Kyle@pnnl.gov)

November 2016

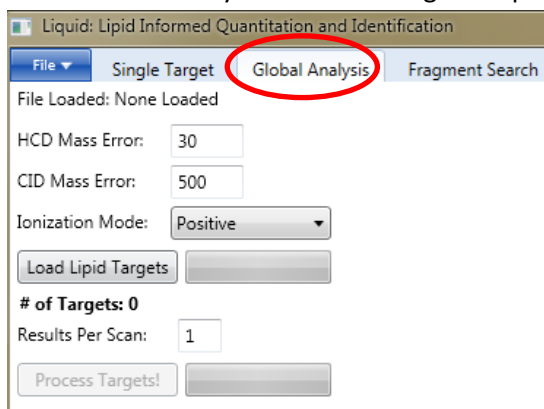
Pacific Northwest National laboratory

1. Click on the links below and download the associated files
 - a. NET 4.5.1: <http://www.microsoft.com/en-us/download/details.aspx?id=40773>
 - b. Thermo MS File Reader: download and install the MSFileReader by creating an account at <https://thermo.flexnetoperations.com/control/thmo/login>, then logging in and choosing "Utility Software". Select MS File Reader 3.1 SP2 , then download MSFileReader_x64.exe
2. You will need to have xcalibur installed on your computer or MSConvert to create mzML files (LIQUID accepts .raw or .mzML files) of your MS/MS data files
 - a. <http://proteowizard.sourceforge.net/downloads.shtml> (Windows 64-bit installer(no T2D support)). This contains the msconvert program.
3. In the folder containing LIQUID program files, open "LIQUID.exe"
4. You will see the following:

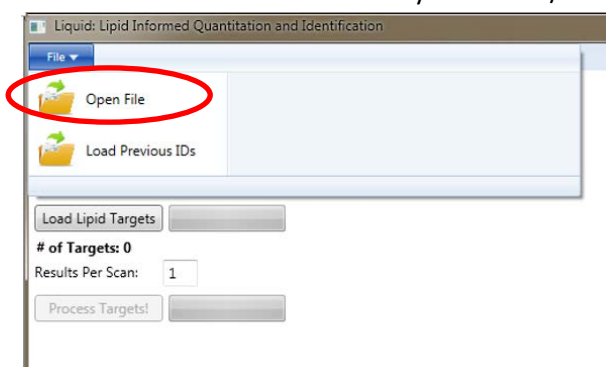


The screenshot displays the 'LIQUID: Lipid Informed Quantitation and Identification' application window. The 'Single Target' tab is selected, showing a 'File Loaded: None Loaded' status. Below this, there are input fields for 'HCD Mass Error' (set to 30) and 'CID Mass Error' (set to 500). The 'Ionization Mode' is set to 'Positive' via a dropdown menu. A 'Load Lipid Targets' button is present, followed by a status line indicating '# of Targets: 0'. The 'Results Per Scan' is set to 1. At the bottom, there is a 'Process Targets!' button. The interface also includes a 'File' menu and tabs for 'Global Analysis' and 'Fragment Search'.

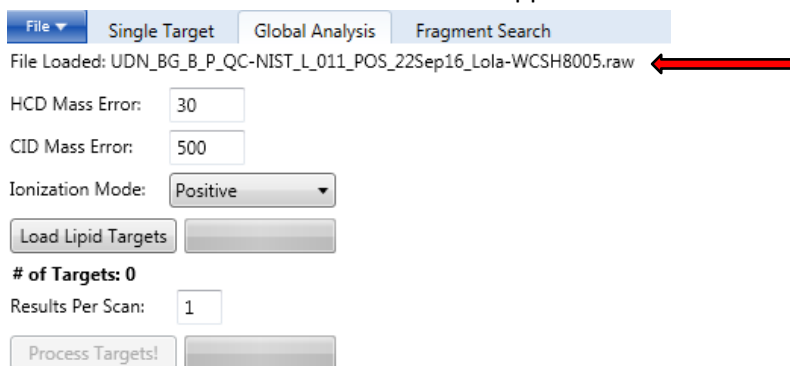
5. Select Global Analysis tab for untargeted lipidomics



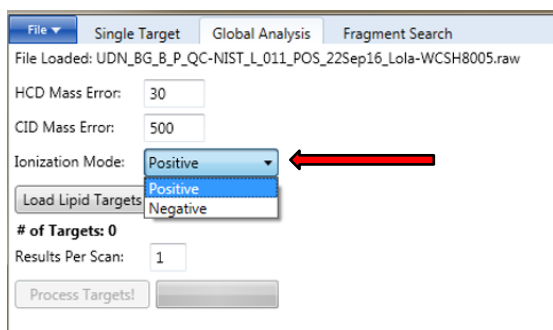
6. Click on 'File' and find and insert your LC-MS/MS data file



7. When the file is loaded the file name will appear



8. If required, change the HCD and CID mass error
9. Select the appropriate ionization mode from the drop down menu (this needs to match the LC-MS/MS data file and also the associated target list must be uploaded)



File ▾ Single Target Global Analysis Fragment Search

File Loaded: UDN_BG_B_P_QC-NIST_L_011_POS_22Sep16_Lola-WCSH8005.raw

HCD Mass Error: 30

CID Mass Error: 500

Ionization Mode: Positive ▾

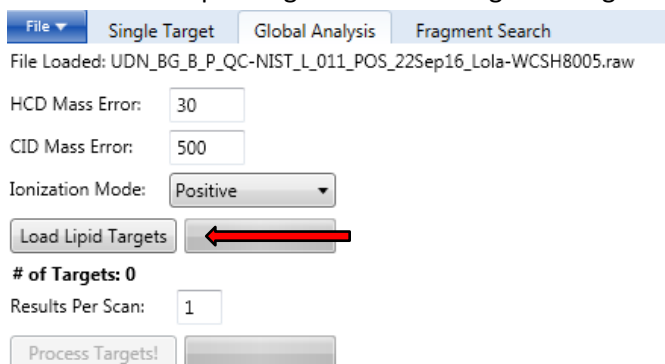
Load Lipid Targets Positive Negative

of Targets: 0

Results Per Scan: 1

Process Targets! []

10. Click on “Load Lipid Targets” to load the global target file(s)



File ▾ Single Target Global Analysis Fragment Search

File Loaded: UDN_BG_B_P_QC-NIST_L_011_POS_22Sep16_Lola-WCSH8005.raw

HCD Mass Error: 30

CID Mass Error: 500

Ionization Mode: Positive ▾

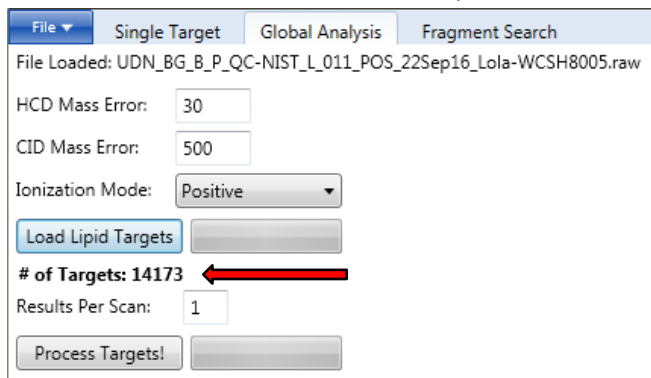
Load Lipid Targets []

of Targets: 0

Results Per Scan: 1

Process Targets! []

*There are separate global target files for samples analyzed in positive ionization and negative ionization. You can upload the one appropriate to your data or upload both if you will be analyzed data from both ionization modes. Once uploaded, the number of targets will be shown.



File ▾ Single Target Global Analysis Fragment Search

File Loaded: UDN_BG_B_P_QC-NIST_L_011_POS_22Sep16_Lola-WCSH8005.raw

HCD Mass Error: 30

CID Mass Error: 500

Ionization Mode: Positive ▾

Load Lipid Targets []

of Targets: 14173

Results Per Scan: 1

Process Targets! []

11. Select the number of results (lipid identifications) you want per ms/ms scan. If you select ‘1’ the highest scored match will be shown.

File ▾ Single Target Global Analysis Fragment Search

File Loaded: UDN_BG_B_P_QC-NIST_L_011_POS_22Sep16_Lola-WCSH8005.raw

HCD Mass Error: 30

CID Mass Error: 500

Ionization Mode: Positive ▾

Load Lipid Targets

of Targets: 14173

Results Per Scan: 1

Process Targets!

12. Click "Process Targets"

- Data will appear when the file has been processed (typically 60 seconds, about 10 sec after initial processing)

File ▾ Single Target Global Analysis Fragment Search

File Loaded: UDN_BG_B_P_QC-NIST_L_011_POS_22Sep16_Lola-WCSH8005.raw

HCD Mass Error: 30

CID Mass Error: 500

Ionization Mode: Positive ▾

Load Lipid Targets

of Targets: 14173

Results Per Scan: 1

Process Targets!

Scan	Lipid	Score
42	PE(16:3/0:0)	8.45
98	PE(16:3/0:0)	16.26
125	PE(16:3/0:0)	9.18
150	PE(16:3/0:0)	16.88
200	PE(16:3/0:0)	19.99
233	PE(16:3/0:0)	8.45
256	PE(16:3/0:0)	8.45
281	PE(16:2/2:0)	11.34
308	PC(16:3/0:0)	-2.62
310	PC(16:2/2:0)	11.25
314	PS(22:2/0:0)	12.45
326	PS(12:0/14:0)	32.66
328	PS(13:0/20:5)	26.11
330	PE(16:4/17:1)	22.50
332	PC(18:1/2:0)	20.15
335	PC(18:2/0:0)	9.66
348	PE(20:5/20:5)	31.30
470	carnitine(10:1)	0.00
512	PE(16:3/0:0)	15.11
519	carnitine(10:0)	0.00
548	PE(16:3/0:0)	15.63

Export Selected Results

Export All Results

CID Plot HCD Plot

carnitine(10:0)
MS/MS Spectrum - CID - 520 // Precursor Scan - 514 (316.2500 m/z)

Intensity

m/z

Observed MS/MS Theoretical MS/MS

Type	m/z	Intensity	Annotation
HCD	155.1432	23977	FA (10:0)
HCD	257.175	178997	M-C3H9N
CID	85.0211	3533	C4H5O2
CID	155.1578	1238	FA (10:0)
CID	257.1563	15499	M-C3H9N

Isotopic Profile

Intensity

m/z

XIC

Intensity

Scan #

Start Scan: 505 Stop Scan: 505

Target Lipid Info

Adduct Hydrogen

Target m/z 316.2488

Empirical Formula C17H34N1O4

Observed Info

Observed m/z 316.2481

PPM Error -2.09

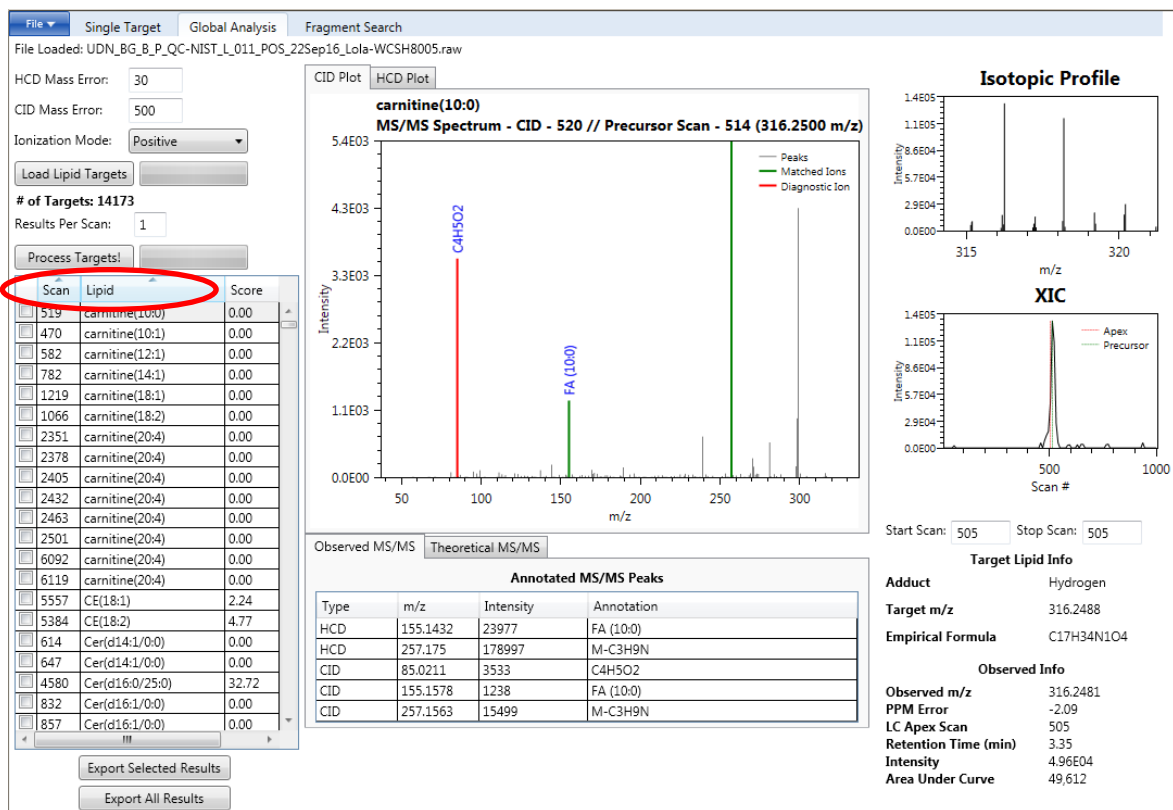
LC Apex Scan 505

Retention Time (min) 3.35

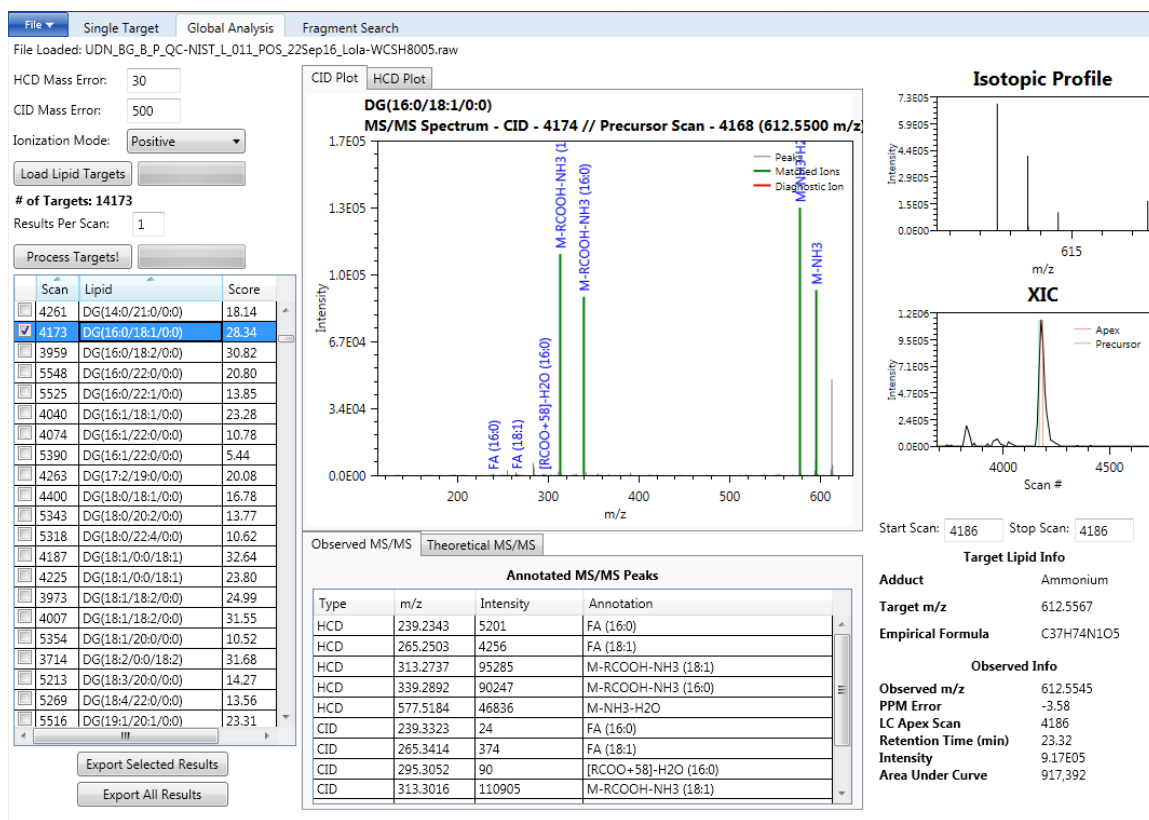
Intensity 4.96E04

Area Under Curve 49,612

13. To organize the results table, click on 'Lipid' then hold shift on your keyboard and click on 'Scan'

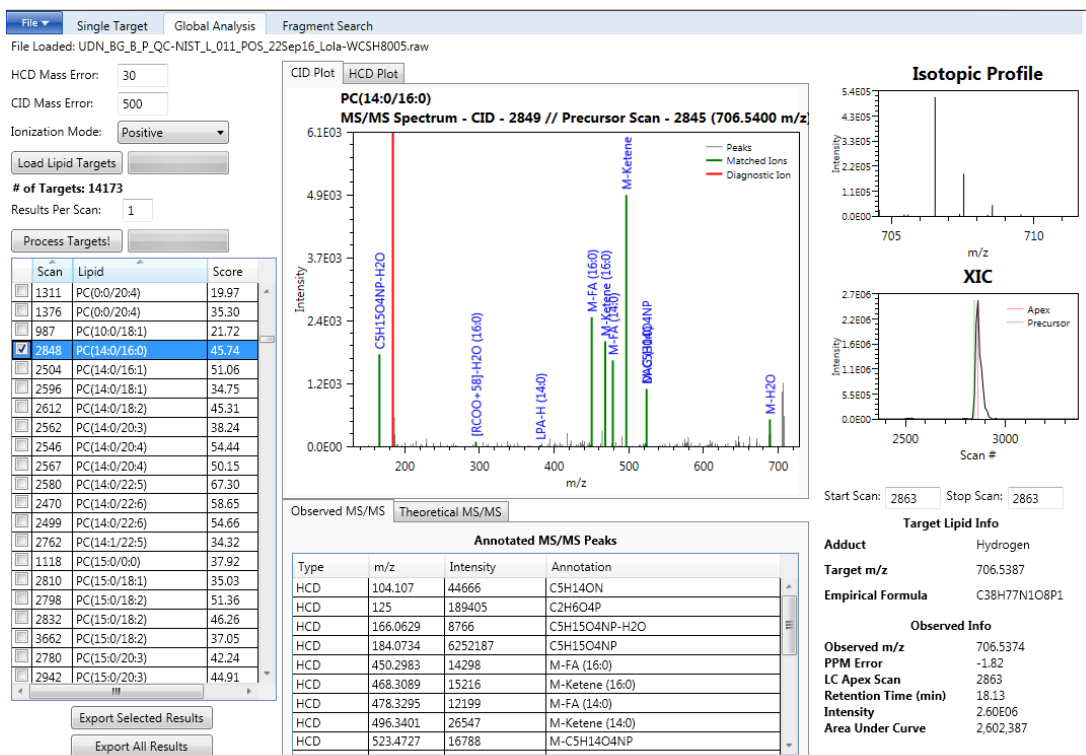


14. Start analyzing data and validating the candidate identifications. Most of the results listed in the table are incorrect (will be improved shortly) but what is correct and incorrect is usually easily deciphered. Example, DG(16:0/18:1):

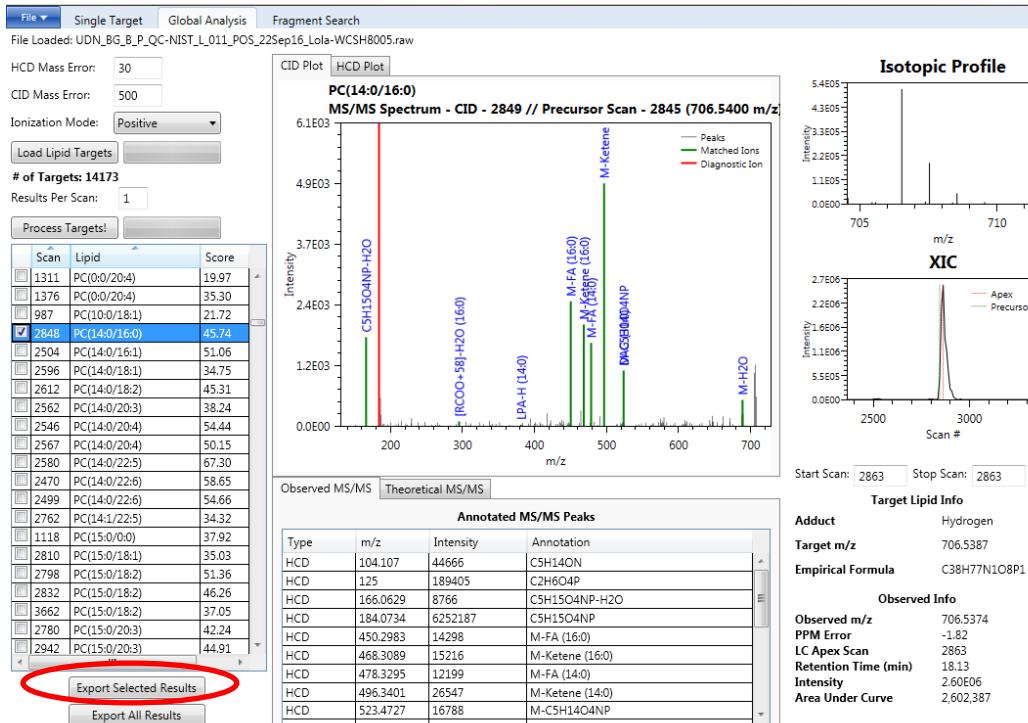


15. Another example (PC(14:0/16:0)) and quick preview on making an identification

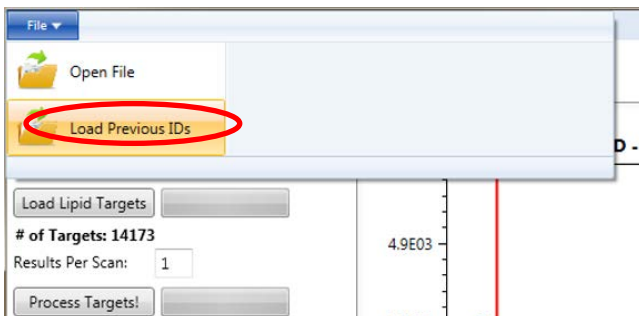
- MS/MS fragments that match the identification are highlighted. Red = diagnostic ion (if applicable) and green = other matched fragments (e.g. fatty acids). Gray = not matched. You can look at your MS/MS data in both HCD and CID (if applicable) and you can also see what fragments the software is looking for (Theoretical MS/MS tab) and a list of what was observed with associated annotation (Observed MS/MS). The isotopic profile for the associated empirical formula is shown. The MS level XIC is also shown. The red line in the XIC is where the software thinks the peak apex is located (this will give you the associated peak intensity value) and the green line in the XIC shows where the precursor scan is located. The PPM error and retention time (RT) is also provided to add another line of evidence that goes towards making a confident identification.



16. Once all of the confident identifications have been selected, click “Export Selected Results”



17. To reload previously exported results, reprocess the raw file then go to “File” and click “Load Previous IDs”



18. Once loaded organize the output chart (see Step 13) for the selected lipids to be checked

