


# LIQUID Installation and Operation

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February 2019

Pacific Northwest National laboratory  
<https://github.com/PNNL-Comp-Mass-Spec/LIQUID>

1. Requires Microsoft .NET Framework 4.6.2 or newer:
  - a. <https://www.microsoft.com/en-us/download/details.aspx?id=53344>
2. LIQUID can read MS/MS data from Thermo Raw files or from .mzML files. A useful tool for creating .mzML files is MSConvert, which is part of ProteoWizardYou
  - a. <http://proteowizard.sourceforge.net/download.html>
  - b. Windows 64-bit installer (able to convert vendor files except T2D).
3. In the folder containing LIQUID program files, double click "LIQUID.exe"
4. You will see the following:

 LIQUID: Lipid Informed Quantitation and Identification

File ▾   Single Target   Global Analysis   Fragment Search

File loaded: none

Precursor Mass Error:

HCD Mass Error:

CID Mass Error:

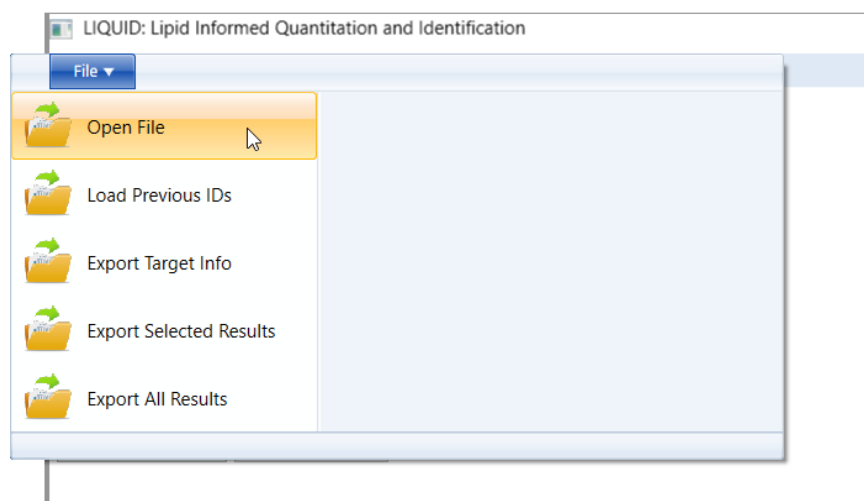
Ionization Mode:  ▾

☐ Average Spectra   **# of Targets: 0**

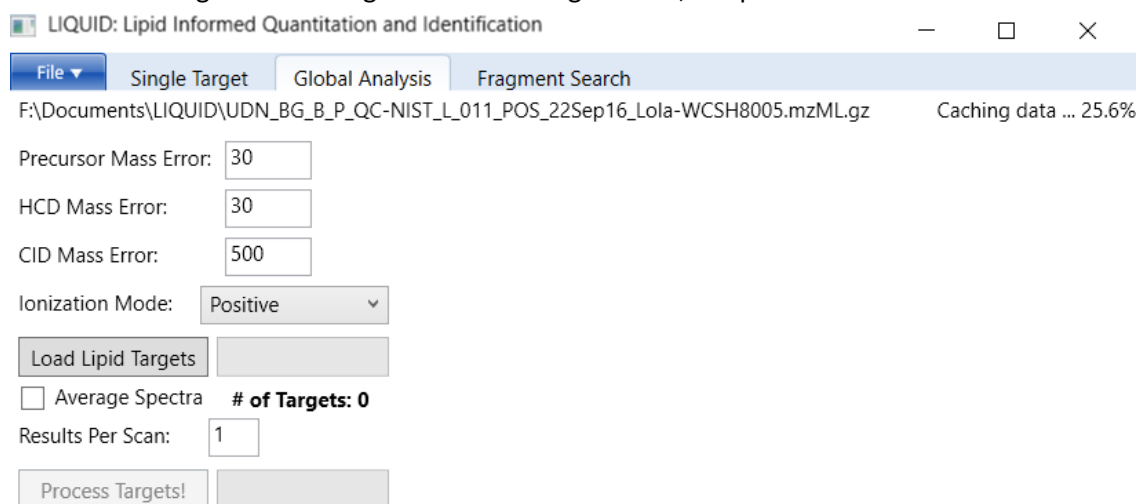
Results Per Scan:

5. Select the Global Analysis tab for untargeted lipidomics

6. Click on "File" and select your LC-MS/MS data file



7. The file will be indexed, creating a .pbf file in the same directory as the input file. Index progress is shown via the "Caching data" message. Once indexing finishes, the path to the file is visible.



8. If required, change the HCD and CID mass errors (in ppm)

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 that will be chosen)

LIQUID: Lipid Informed Quantitation and Identification

File ▾ Single Target Global Analysis Fragment Search

F:\Documents\LIQUID\UDN\_BG\_B\_P\_QC-NIST\_L\_011\_POS\_22Sep16\_Lola-WCSH8005.mzML.gz

Precursor Mass Error: 30

HCD Mass Error: 30

CID Mass Error: 500

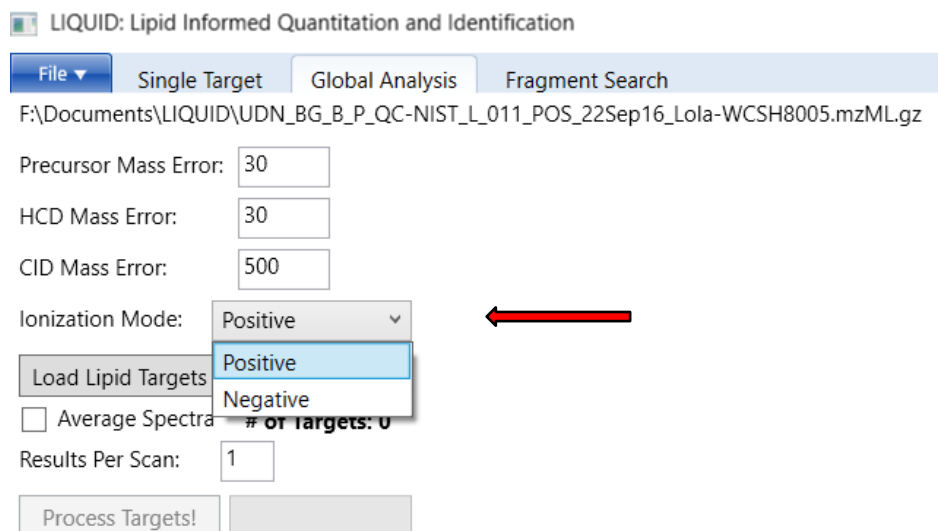
Ionization Mode: Positive ▾

Load Lipid Targets

☐ Average Spectra # of Targets: 0

Results Per Scan: 1

Process Targets!



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

LIQUID: Lipid Informed Quantitation and Identification

File ▾ Single Target Global Analysis Fragment Search

F:\Documents\LIQUID\UDN\_BG\_B\_P\_QC-NIST\_L\_011\_POS\_22Sep16\_Lola-WCSH8005.mzML.gz

Precursor Mass Error: 30

HCD Mass Error: 30

CID Mass Error: 500

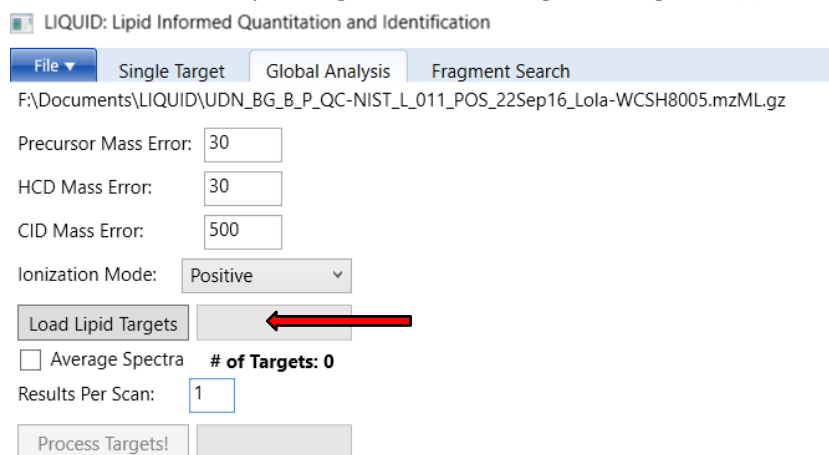
Ionization Mode: Positive ▾

Load Lipid Targets

☐ Average Spectra # 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 select the one appropriate to your data or select both if you need to analyze data from both ionization modes.
- Once uploaded, the number of targets will be shown.

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File ▾ Single Target Global Analysis Fragment Search

F:\Documents\LIQUID\UDN\_BG\_B\_P\_QC-NIST\_L\_011\_POS\_22Sep16\_Lola-WCSH8005.mzML.gz

Precursor Mass Error: 30

HCD Mass Error: 30

CID Mass Error: 500

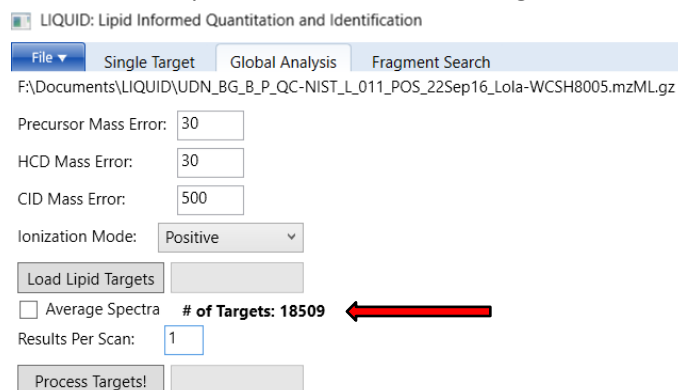
Ionization Mode: Positive ▾

Load Lipid Targets

☐ Average Spectra # of Targets: 18509

Results Per Scan: 1

Process Targets!



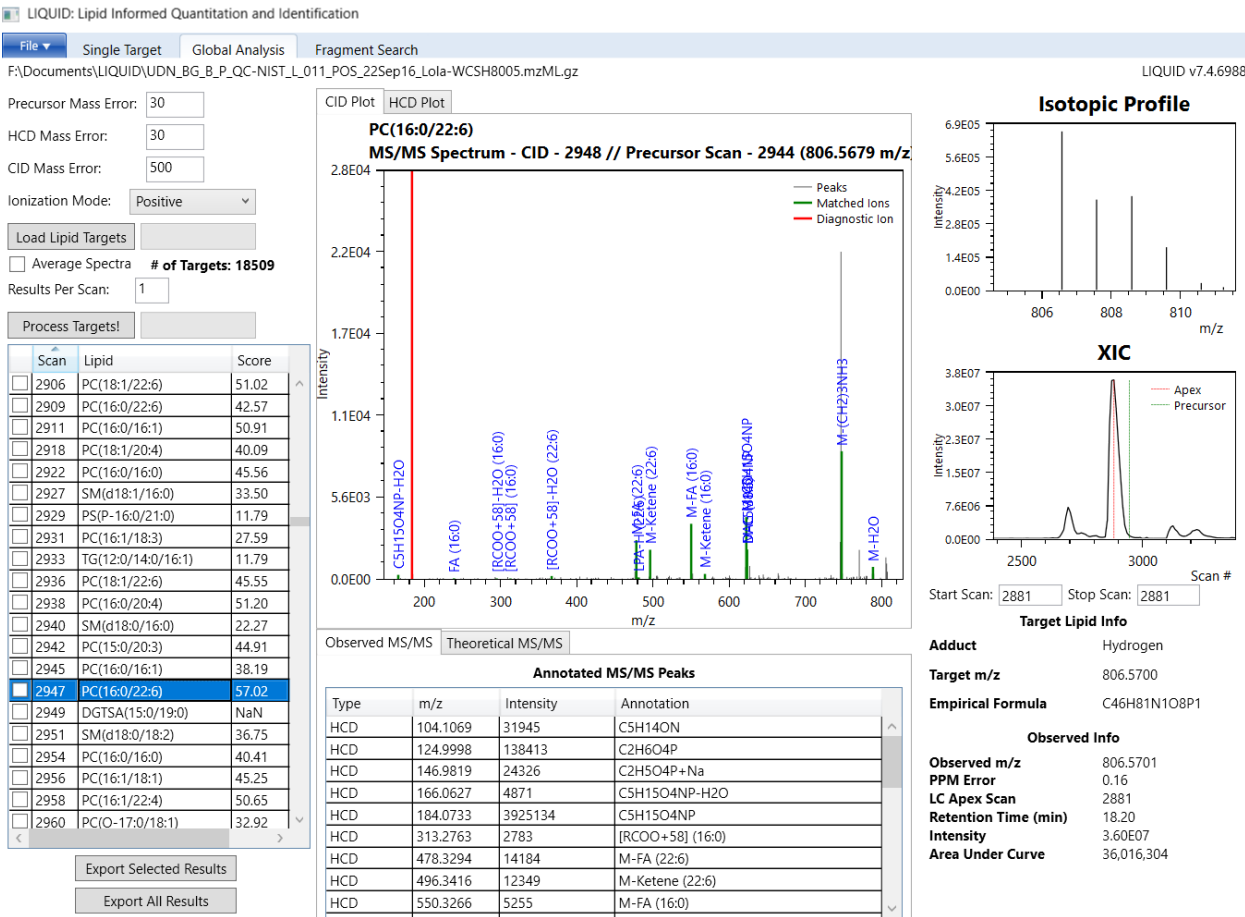
11. Select the number of results (lipid identifications) you want reported per MS/MS scan.  
If you select "1" the highest scored match will be shown.

12. Click "Process Targets"

a. A progress bar is shown to indicate processing progress.

13. After the search complete, search results are shown at the right, a mass spectrum is visible in the middle, and additional plots are shown at the right.

a. Higher scores mean a higher confidence result



14. To sort the results by lipid, and then by scan, left click "Lipid", then shift left click "Scan"

LIQUID: Lipid Informed Quantitation and Identification

File Single Target Global Analysis Fragment Search

F:\Documents\LIQUID\UDN\_BG\_B\_P\_QC-NIST\_L\_011\_POS\_22Sep16\_Lola-WCSH8005.mzML.gz LIQUID v7.4.6988

Precursor Mass Error: 30

HCD Mass Error: 30

CID Mass Error: 500

Ionization Mode: Positive

Load Lipid Targets

Average Spectra # of Targets: 18509

Results Per Scan: 1

Process Targets!

Scan	Lipid	Score
614	Cer(d14:1/0:0)	0.00
647	Cer(d14:1/0:0)	0.00
2112	Cer(d14:2/22:0(2OH))	18.32
4580	Cer(d16:0/25:0)	30.59
832	Cer(d16:1/0:0)	0.00
857	Cer(d16:1/0:0)	0.00
4504	Cer(d18:0/22:0)	33.14
4598	Cer(d18:0/24:0)	25.07
4637	Cer(d18:0/24:0)	34.10
4484	Cer(d18:0/24:1)	39.39
2220	Cer(d18:1/16:1)	24.99
4083	Cer(d18:1/22:0(2OH))	31.42
4443	Cer(d18:1/22:0)	23.61
4409	Cer(d18:1/24:0(2OH))	26.76
4583	Cer(d18:1/24:0)	23.41
4436	Cer(d18:2/24:0)	25.38
4468	Cer(d18:2/24:0)	16.96
4520	Cer(d19:1/22:0)	13.33
4508	Cer(d20:0/22:0(2OH))	31.84
4445	Cer(d20:1/22:3)	11.06
4859	CoQ10	0.00

Export Selected Results

Export All Results

CID Plot HCD Plot

**Cer(d18:0/22:0)**

MS/MS Spectrum - CID - 4505 // Precursor Scan - 4501 (624.6284 m/z)

Intensity

m/z

Observed MS/MS Theoretical MS/MS

**Annotated MS/MS Peaks**

Type	m/z	Intensity	Annotation
HCD	254.2847	12404	LCB-CH2 (d18:0)
HCD	266.2844	144710	LCB (d18:0)
HCD	284.2949	268658	LCB+H2O (d18:0)
HCD	340.3575	149171	FA short (22:0)
HCD	364.3534	1498	FA long (22:0)
HCD	588.6084	52307	M-2(H2O)
HCD	606.6181	700416	M-H2O
CID	254.3663	157	LCB-CH2 (d18:0)
CID	266.2625	3088	LCB (d18:0)

**Isotopic Profile**

Intensity

m/z

**XIC**

Intensity

Scan #

Start Scan: 4492 Stop Scan: 4492

**Target Lipid Info**

Adduct Hydrogen

Target m/z 624.6295

Empirical Formula C40H82N103

**Observed Info**

Observed m/z 624.6274

PPM Error -3.3

LC Apex Scan 4492

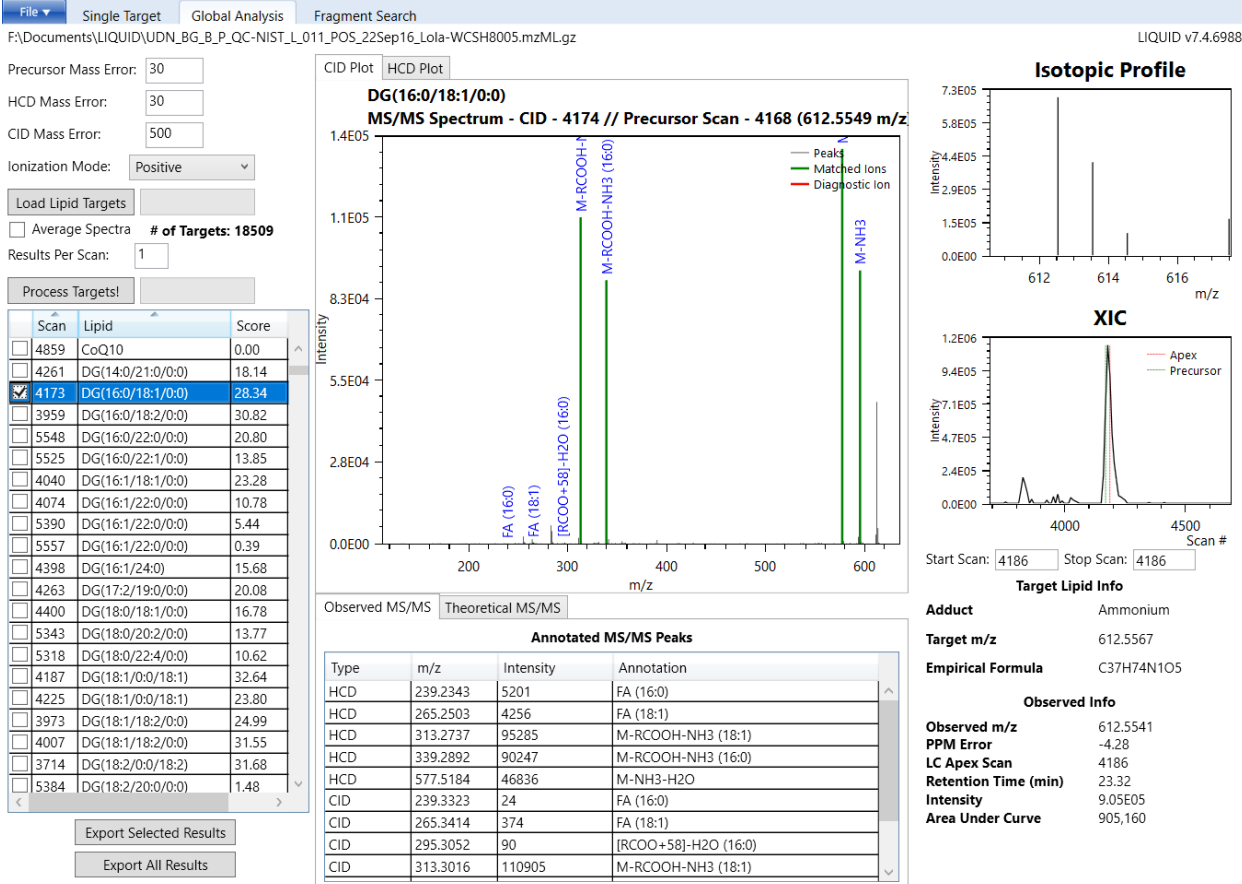
Retention Time (min) 24.75

Intensity 1.79E05

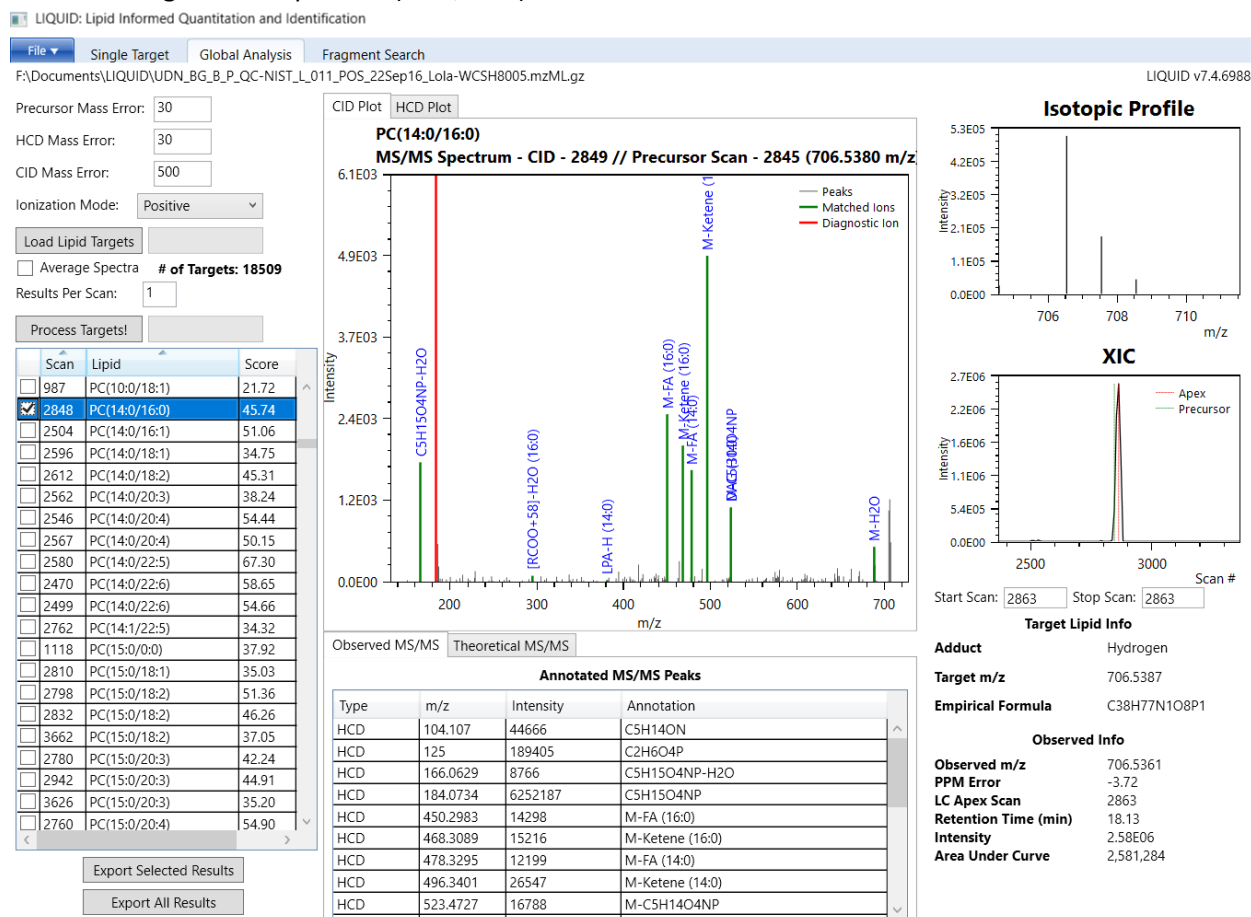
Area Under Curve 178,504

15. Start analyzing the results and validating the candidate identifications. Most of the results listed in the table are incorrect but what is correct and incorrect is usually easily deciphered. For example, DG(16:0/18:1):

LIQUID: Lipid Informed Quantitation and Identification



## 16. Another good example is PC(14:0/16:0)



## 17. Explanation of the window layout

- MS/MS fragments that match the identification are highlighted.
  - Red = diagnostic ion (if applicable)
  - Green = other matched fragments (e.g. fatty acids).
  - Gray = not matched.
- Plot Controls:
  - Zoom into the plot by dragging with your middle mouse button
    - If you don't have a middle mouse button (or you re-mapped it to double click), use Ctrl+Alt+Left Click to zoom in
  - Zoom out by double clicking the middle mouse button (or double Ctrl+Alt+Left Click)
  - Slide the chart left and right (pan) using the right mouse button
- You can look at your MS/MS data in both HCD and CID (if applicable)
- The "Theoretical MS/MS" tab shows which fragments the software is looking for
- The "Observed MS/MS" tab lists the annotations associated with observed ions
- The isotopic profile reflects the associated empirical formula for the candidate ID.
  - The red line in the XIC is where the software thinks the peak apex is located (the associated peak intensity value is based on this apex)
  - The green line in the XIC shows where the precursor scan is located.
- The PPM error and retention time (RT) are also provided to add another line of evidence that goes towards making a confident identification.

18. Once all of the confident identifications have been selected, click "Export Selected Results" below the results grid, or from the File menu

LIQUID: Lipid Informed Quantitation and Identification

File Single Target Global Analysis Fragment Search

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Precursor Mass Error: 30  
HCD Mass Error: 30  
CID Mass Error: 500  
Ionization Mode: Positive  
Load Lipid Targets  
Average Spectra # of Targets: 18509  
Results Per Scan: 1  
Process Targets!

Scan Lipid Score  
987 PC(10:0/18:1) 21.72  
2848 PC(14:0/16:0) 45.74  
2504 PC(14:0/16:1) 51.06  
2596 PC(14:0/18:1) 34.75  
2612 PC(14:0/18:2) 45.31  
2562 PC(14:0/20:3) 38.24  
2546 PC(14:0/20:4) 54.44  
2567 PC(14:0/20:4) 50.15  
2580 PC(14:0/22:5) 67.30  
2470 PC(14:0/22:6) 58.65  
2499 PC(14:0/22:6) 54.66  
2762 PC(14:1/22:5) 34.32  
1118 PC(15:0/0:0) 37.92  
2810 PC(15:0/18:1) 35.03  
2798 PC(15:0/18:2) 51.36  
2832 PC(15:0/18:2) 46.26  
3662 PC(15:0/18:2) 37.05  
2780 PC(15:0/20:3) 42.24  
2942 PC(15:0/20:3) 44.91  
3626 PC(15:0/20:3) 35.20  
2760 PC(15:0/20:4) 54.90

Export Selected Results  
Export All Results

CID Plot HCD Plot  
PC(14:0/16:0)  
MS/MS Spectrum - CID - 2849 // Precursor Scan - 2845 (706.5380 m/z)

Intensity  
6.1E03  
4.9E03  
3.7E03  
2.4E03  
1.2E03  
0.0E00

m/z  
200 300 400 500 600 700

Peaks  
Matched Ions  
Diagnostic Ion

Isotopic Profile

Intensity  
5.3E05  
4.2E05  
3.2E05  
2.1E05  
1.1E05  
0.0E00

m/z  
706 708 710

XIC

Intensity  
2.7E06  
2.2E06  
1.6E06  
1.1E06  
0.0E00

Scan #  
2500 3000

Start Scan: 2863 Stop Scan: 2863

Target Lipid Info

Adduct Hydrogen  
Target m/z 706.5387  
Empirical Formula C38H77N108P1

Observed Info

Observed m/z 706.5361  
PPM Error -3.72  
LC Apex Scan 2863  
Retention Time (min) 18.13  
Intensity 2.58E06  
Area Under Curve 2,581,284

Observed MS/MS Theoretical MS/MS

Annotated MS/MS Peaks

Type	m/z	Intensity	Annotation
HCD	104.107	44666	C5H14ON
HCD	125	189405	C2H6O4P
HCD	166.0629	8766	C5H15O4NP-H2O
HCD	184.0734	6252187	C5H15O4NP
HCD	450.2983	14298	M-FA (16:0)
HCD	468.3089	15216	M-Ketene (16:0)
HCD	478.3295	12199	M-FA (14:0)
HCD	496.3401	26547	M-Ketene (14:0)
HCD	523.4727	16788	M-C5H14O4NP

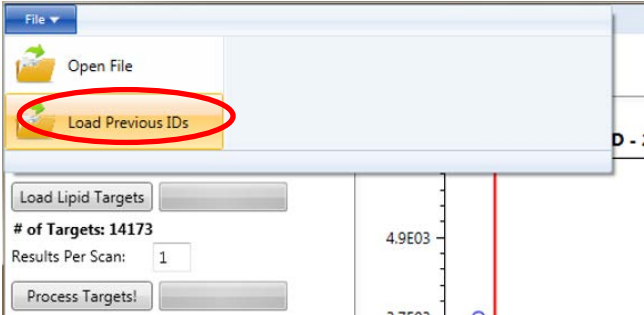
LIQUID: Lipid Informed Quantitation and Identification

File

Open File  
Load Previous IDs  
Export Target Info  
Export Selected Results  
Export All Results



19. To reload previously exported results, you must first re-process the raw or .mzML file then select "File",  
"Load Previous IDs"



20. Optionally re-sort the results by Lipid, then Scan

