


LIQUID Installation and Operation

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<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 ProteoWizard
 - 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:

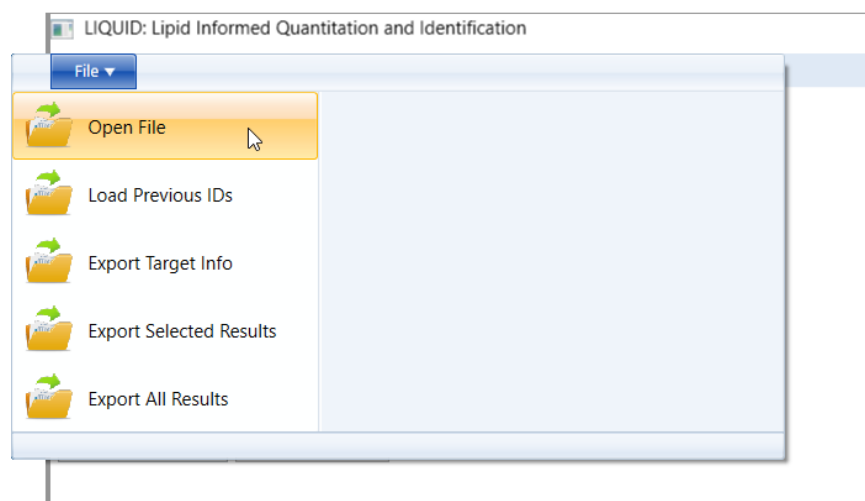
Ionization Mode: ▾

☐ Average Spectra **# of Targets: 0**

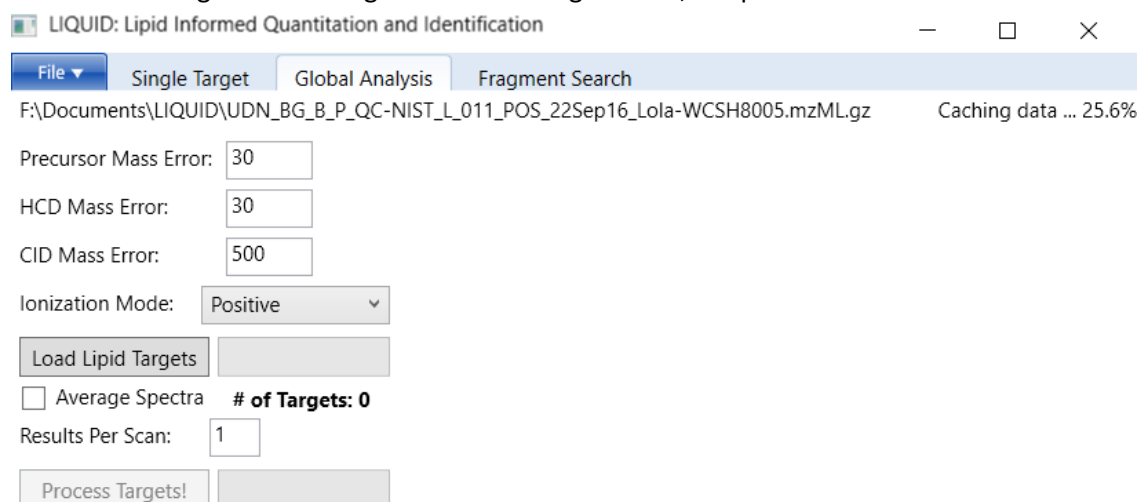
Results Per Scan:

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

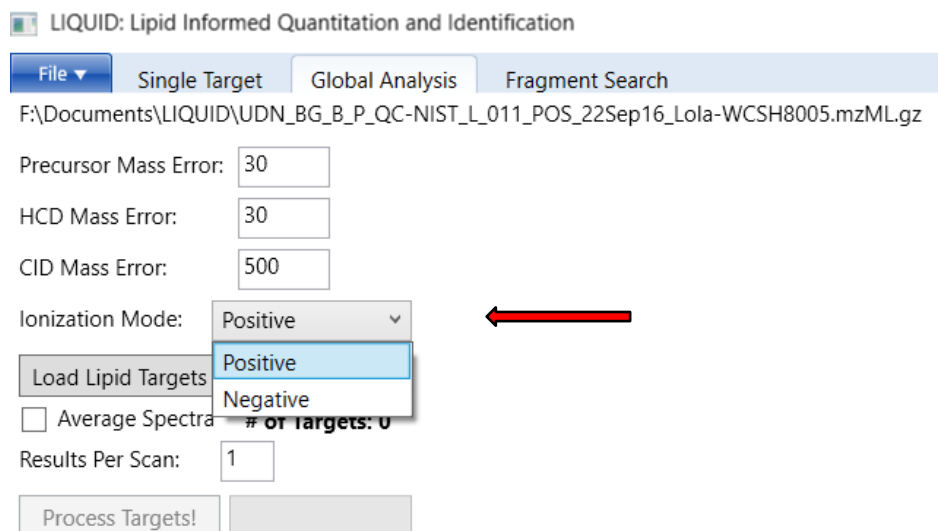
Ionization Mode: Positive ▾

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

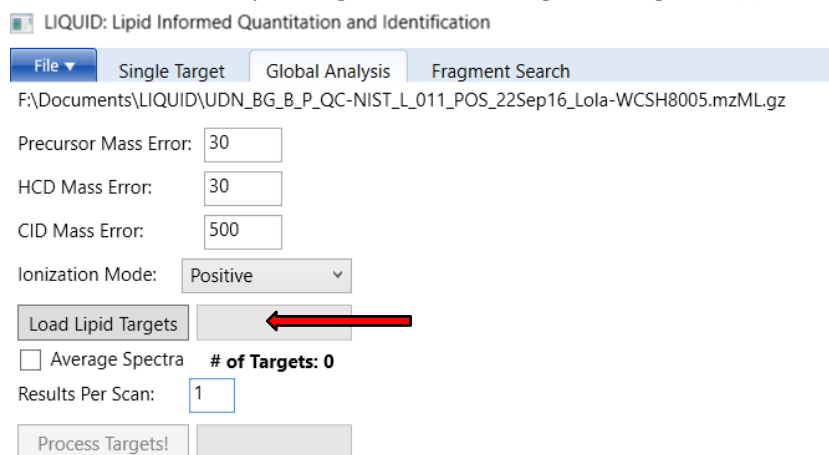
Ionization Mode: Positive ▾

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.

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

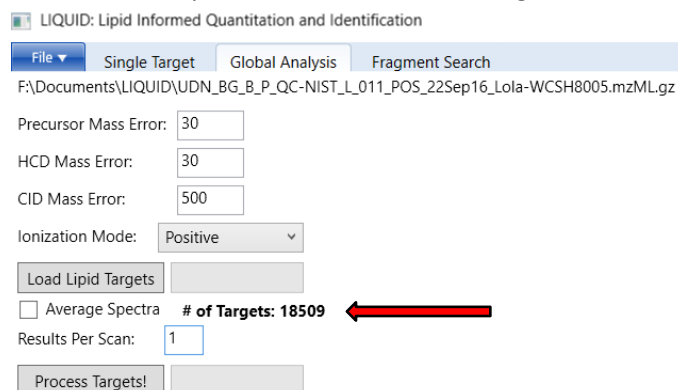
Ionization Mode: Positive ▾

Load Lipid Targets

☐ Average Spectra # of Targets: 18509

Results Per Scan: 1

Process Targets!



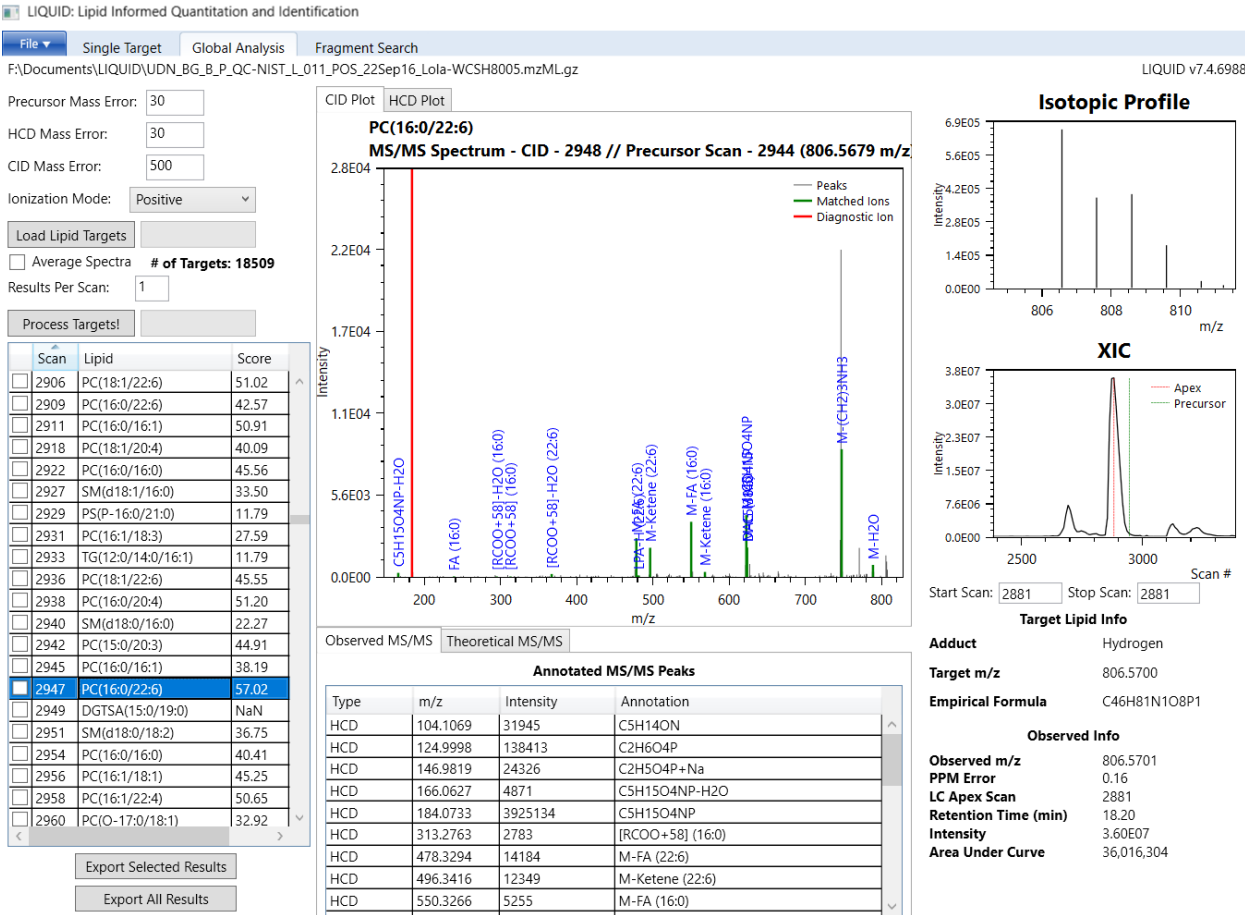
11. Select the number of results (lipid identifications) you want reported per MS/MS scan.
If you select "1" the highest scored match will be shown.

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

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

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

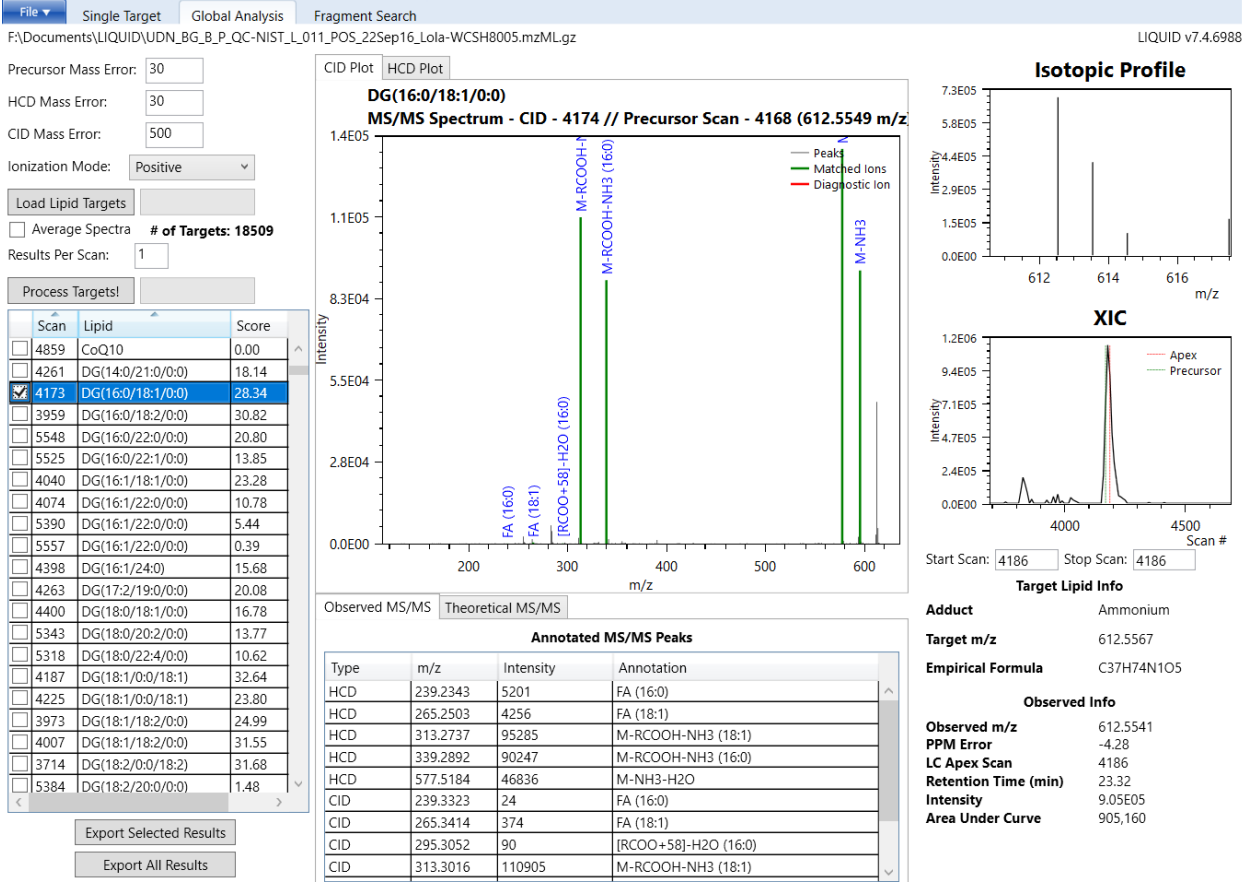
Observed MS/MS Theoretical MS/MS

Annotated MS/MS Peaks

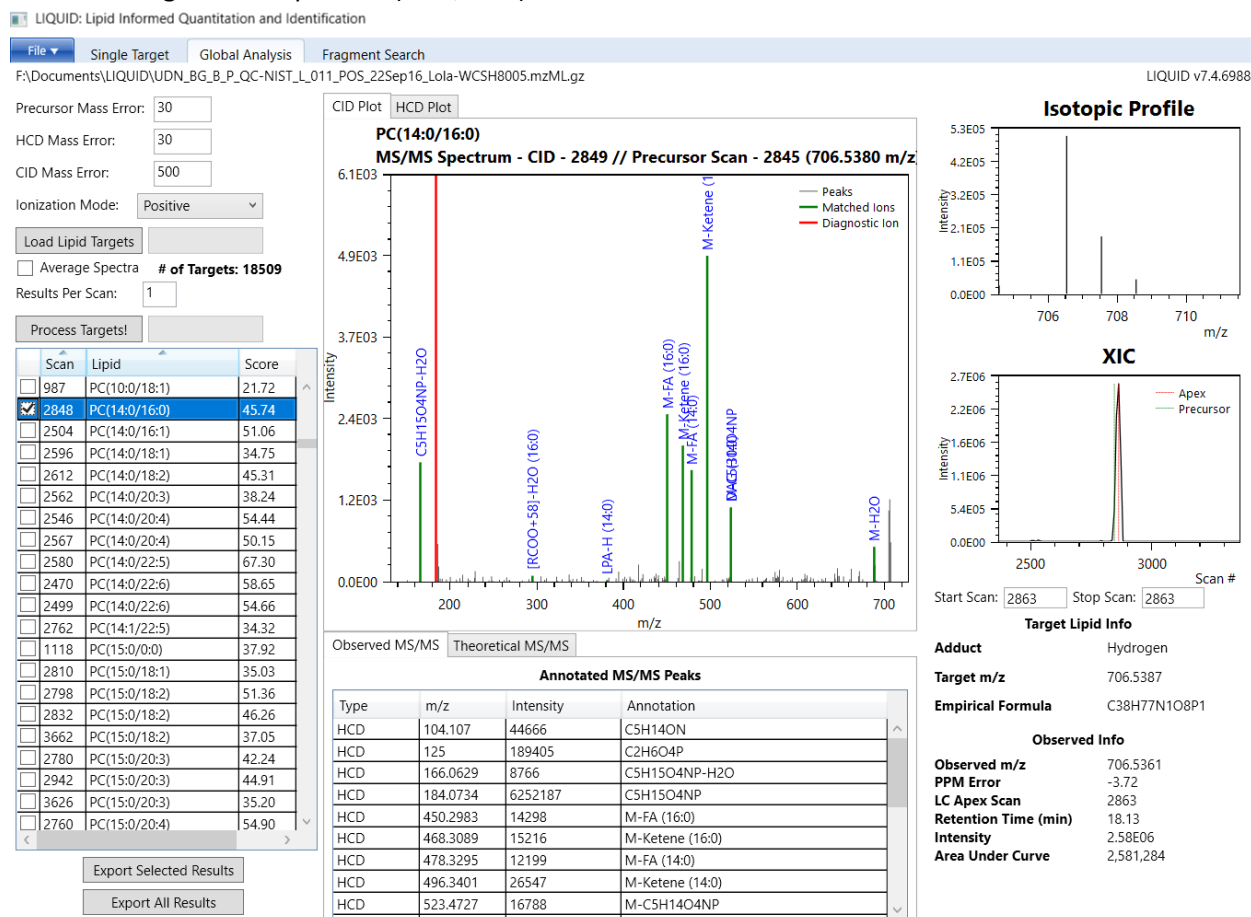
Type	m/z	Intensity	Annotation
HCD	254.2847	12404	LCB-CH2 (d18:0)
HCD	266.2844	144710	LCB (d18:0)
HCD	284.2949	268658	LCB+H2O (d18:0)
HCD	340.3575	149171	FA short (22:0)
HCD	364.3534	1498	FA long (22:0)
HCD	588.6084	52307	M-2(H2O)
HCD	606.6181	700416	M-H2O
CID	254.3663	157	LCB-CH2 (d18:0)
CID	266.2625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

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



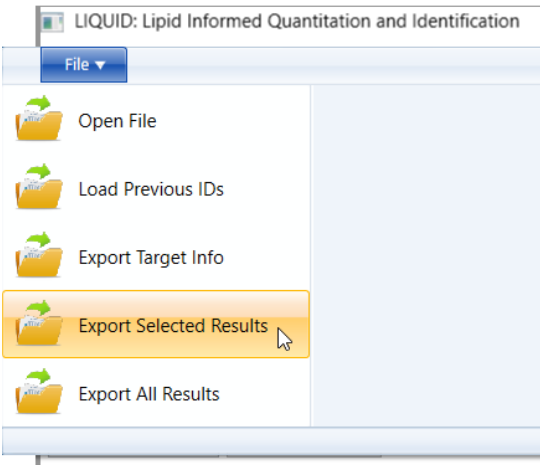
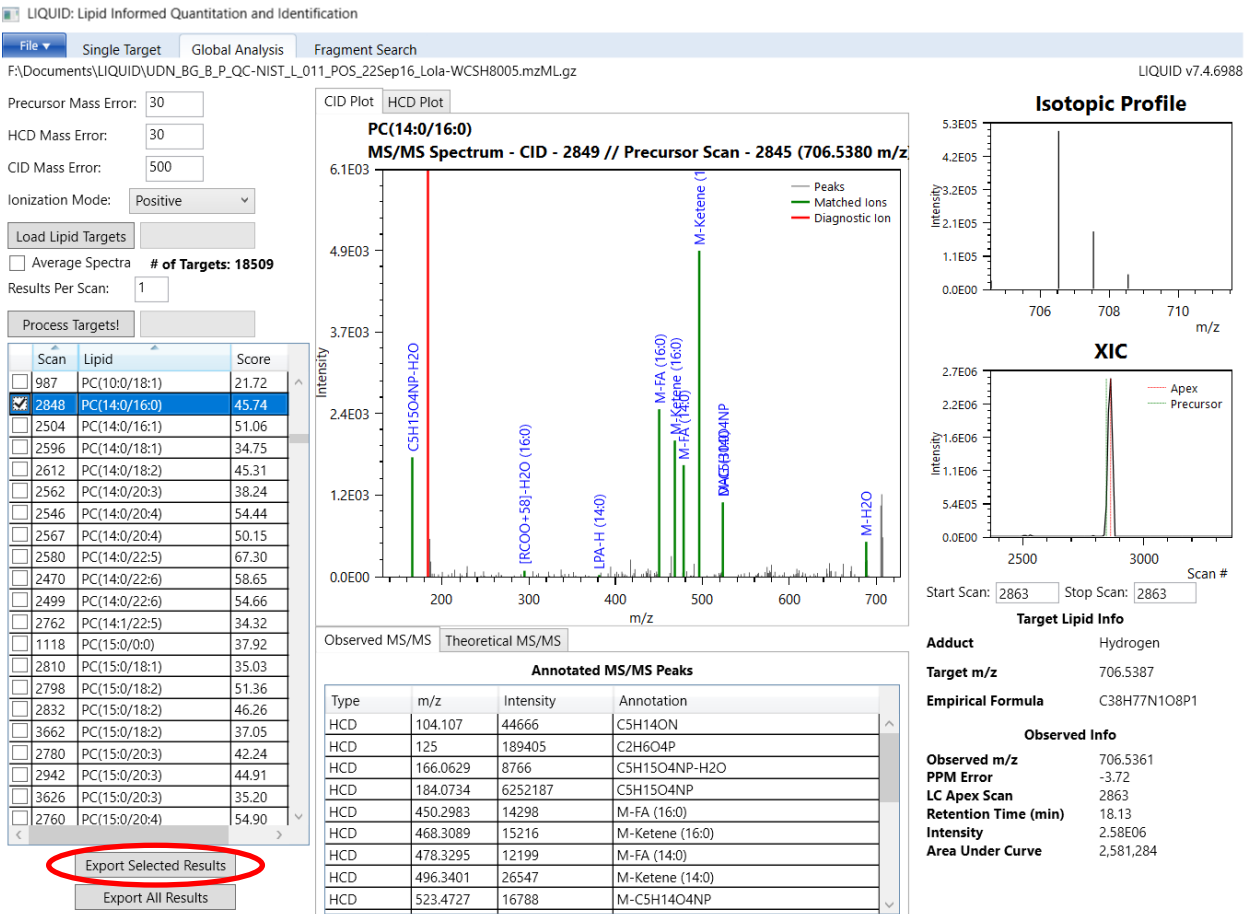
16. Another good example is PC(14:0/16:0)



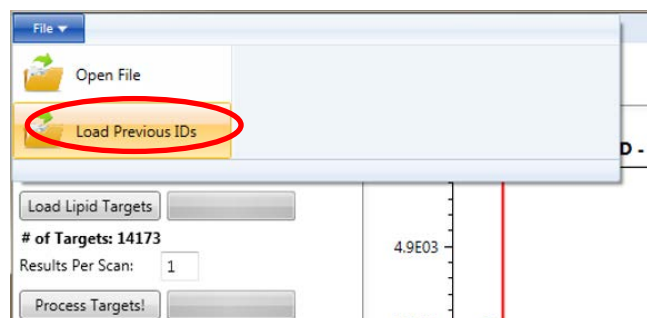
17. Explanation of the window layout

- 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



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

