

# **Instructions on how to use LipidQuant 2.1 for automated data processing in lipid class separation - mass spectrometry quantitative workflows**

**(updated August 2, 2023)**

## **Installation of LipidQuant 2.1**

1. Download and install “MyAppInstaller\_mcr.exe”.
2. Open “LipidQuant.exe”.

### **Attention!**

- The software can be found at <http://holcapek.upce.cz>
- Downloading the “MyAppInstaller\_mcr.exe” will automatically install the MCR component and extract LipidQuant.exe.
- Once the MCR component has been installed on a certain computer, it does not need to be installed again. This means that more advanced versions of this software can be installed simply by updating LipidQuant.exe file, without the need to reinstall “MyAppInstaller\_mcr.exe”.

## Data input to LipidQuant 2.1

1. It must be in **.txt format** including all  $m/z$  features in the first column aligned among all measured samples with the heading  $m/z$  followed by individual samples containing the intensities or other quantitative measures for each  $m/z$  feature (**Fig. 1**).

$m/z$	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
1128.956	5003.766	6686.762	6153.684	4112.512	7085.6055	5799.2617	4515.289	0	3675.371	4570.441
1128.88	0	0	4015.91	5030.926	3952.5684	0	0	0	0	4672.273
1128.825	4774.555	3508.106	7087.395	5643.871	5243.457	5324.1719	5884.926	3327.1406	0	0
1128.779	4202.211	8666.672	4781.984	0	5445.3984	3755.3008	0	0	3383.33	6714.051
1128.712	6176.332	5235.758	4415.371	0	4911.7969	0	3449.943	4651.4297	6716.066	0
1128.651	5329.309	3288.785	3140.475	4821.086	3347.8828	3999.7559	4215.895	5375.6602	3480.518	4062.623
1128.574	0	0	0	3332.924	0	0	0	0	0	3892.391
1128.355	0	0	0	0	0	0	0	0	0	0
1128.323	177827.1	136533.4	130422.7	137019.5	121798.625	124397.438	106036.6	112140	113759.9	121264.8
1128.13	0	0	3177.422	0	0	0	0	0	0	0
1128.081	0	0	4464.293	0	0	0	3133.902	0	0	0
1128.026	3997.492	5945.719	5104.277	5435.586	4398.3984	4073.1758	0	4435.3438	4166.801	0
1127.976	5831.414	5965.727	0	3050.697	0	0	3969.406	0	0	4489.922
1127.907	4182.121	5543.856	4375.316	3091.482	3551.1758	3125.7344	4860.801	0	0	0

**Fig. 1.** Example of an input table in LipidQuant 2.1.

2. Lipid class separation: one .txt file = one lipid class.

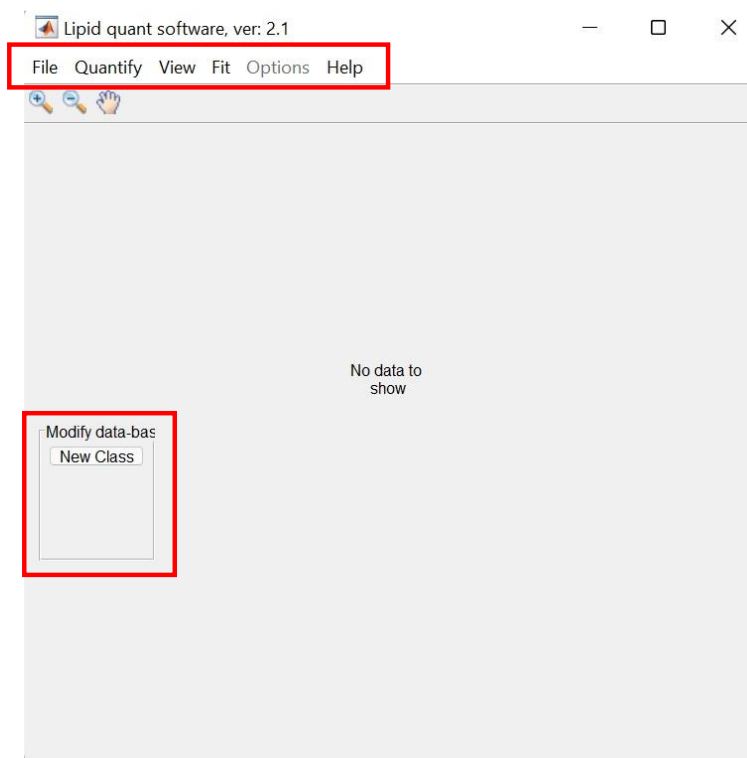
### Attention!

- One .txt file can be used for more lipid classes due to the same or almost the same elution window, *e.g.*, SM + LPC or DG + Chol may be included in one file. Make sure that there are no mass interferences between two lipid classes in one .txt file.
- Individual columns in .txt format have to be separated by a tabulator, not by a comma or a dot.
- The decimal point (for  $m/z$  values and quantitative measures) must be used, not the comma.

## LipidQuant 2.1

#1. Open LipidQuant 2.1.

#2. The main panel will appear (**Fig. 2**).

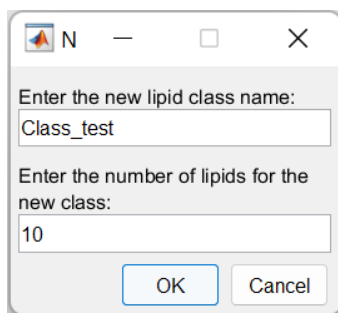


**Fig. 2.** The main panel in LipidQuant 2.1.

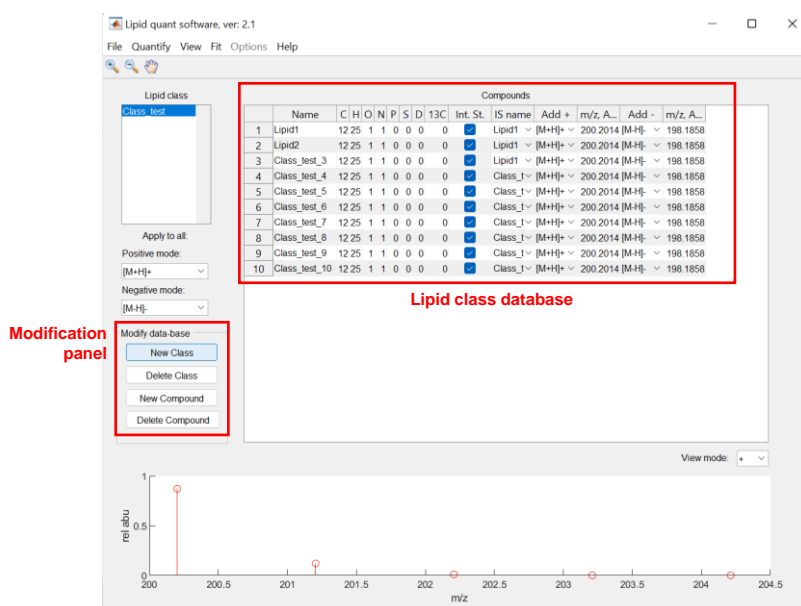
#3. Prepare a new project by creating a lipid database. There are two ways to do this:

### A/ Create a new lipid database from scratch.

Click on “New class”. The window shown in **Fig. 3** will appear. Enter the required information and press “OK” button. A Lipid database window (**Fig. 4**) will appear. Fill in the required information: name of the lipid species, elemental composition of the lipid species, internal standards, and adducts. Use “Modify database panel” to add/delete other lipid classes or compounds.



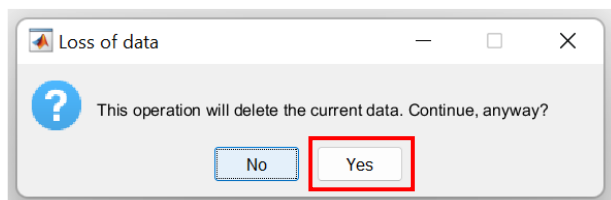
**Fig. 3.** New class window.



**Fig. 4.** Lipid class database window.

## B/ Create a new lipid database from an Excel template.

Click on “File” → “Import Excel”. This message will appear in the window shown in **Fig. 5**. Click on “Yes” button.



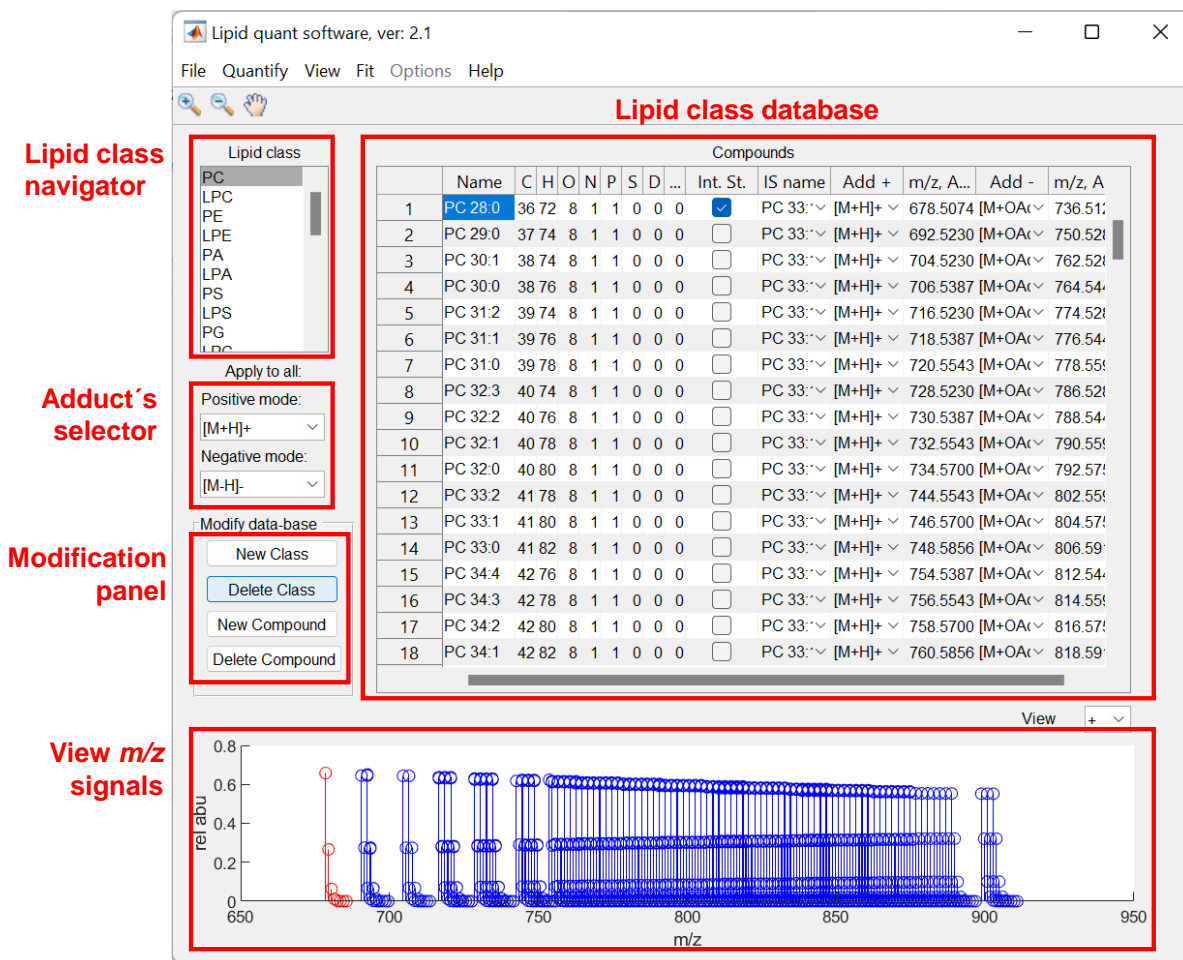
**Fig. 5.** Warning message.

## Attention!

- An example lipid database (Excel template) is provided and can be downloaded at: <http://holcapek.upce.cz>
- The example lipid database includes 27 lipid classes: PC, LPC, PE, LPE, PA, LPA, PS, LPS, PG, LPG, PI, LPI, SM, Cer, HexCer, Hex2Cer, SHexCer, GM3, S1P, Sph, Acylcarnitine, CE, Chol, TG, DG, MG, and FA.
- The example lipid database can be freely modified by adding new lipid classes or lipid species. Keep the Excel structure!

**#4.** The calculation of isotopic profiles will be in progress. It will take several minutes.

**#5.** Once done, the main window of LipidQuant 2.1 will change as shown in **Fig. 6**.



**Fig. 6.** The main panel with the loaded lipid database.

### Attention!

- You can click between lipid classes using “Lipid class navigator” (**Fig. 6**).
- You can create or delete class/compound using “Modification panel” (**Fig. 6**).
- “View *m/z* signals” panel displays the collection of compounds for a given class with all isotope patterns. The lipid species selected in the lipid class database (**Fig. 6**) are displayed in red. Click on “View” “+” or “-” to observe the isotopic distribution of the whole lipid’s set for the selected positive or negative adduct.

**#6.** Select the adduct of interest using “Adduct’s selector” (**Fig. 6**).

### Attention!

- LipidQuant 2.1 supports following adducts so far:  $[M+H]^+$ ,  $[M+H-H_2O]^+$ ,  $[M+2H]^{2+}$ ,  $[M+K]^+$ ,  $[M+Na]^+$ ,  $[M+Li]^+$ ,  $[M+NH_4]^+$ ,  $[M-H]^-$ ,  $[M-2H]^{2-}$ ,  $[M+Cl]^-$ ,  $[M+OAc]^-$ ,  $[M+HCOO]^-$ .

**#7.** Specify the lipid species used as an internal standard for each lipid class to be quantified (“Int. St.” column, **Fig. 7**).

**#8.** Assign the specified internal standard to each lipid species within the lipid class to be quantified (“IS name” column, **Fig. 7**).

		Lipid species composition								Compounds		Selected adducts and their theoretical m/z in positive and negative mode			
	Name	C	H	O	N	P	S	D	13C	Int. St.	IS name	Add +	m/z, Ad...	Add -	m/z, A...
172	PC O-42:3	50	96	7	1	1	0	0	0	<input type="checkbox"/>	PC 33:1 D7	[M+H] <sup>+</sup>	854.7003	[M+OAc] <sup>-</sup>	912.7057
173	PC O-42:2	50	98	7	1	1	0	0	0	<input type="checkbox"/>	PC 33:1 D7	[M+H] <sup>+</sup>	856.7159	[M+OAc] <sup>-</sup>	914.7214
174	PC O-42:1	50	...	7	1	1	0	0	0	<input type="checkbox"/>	PC 33:1 D7	[M+H] <sup>+</sup>	858.7316	[M+OAc] <sup>-</sup>	916.7370
175	PC O-42:0	50	...	7	1	1	0	0	0	<input type="checkbox"/>	PC 33:1 D7	[M+H] <sup>+</sup>	860.7472	[M+OAc] <sup>-</sup>	918.7527
176	PC O-43:6	51	92	7	1	1	0	0	0	<input type="checkbox"/>	PC 33:1 D7	[M+H] <sup>+</sup>	862.6690	[M+OAc] <sup>-</sup>	920.6744
177	PC O-43:5	51	94	7	1	1	0	0	0	<input type="checkbox"/>	PC 33:1 D7	[M+H] <sup>+</sup>	864.6846	[M+OAc] <sup>-</sup>	922.6901
178	PC O-43:4	51	96	7	1	1	0	0	0	<input type="checkbox"/>	PC 33:1 D7	[M+H] <sup>+</sup>	866.7003	[M+OAc] <sup>-</sup>	924.7057
179	PC O-43:3	51	98	7	1	1	0	0	0	<input type="checkbox"/>	PC 33:1 D7	[M+H] <sup>+</sup>	868.7159	[M+OAc] <sup>-</sup>	926.7214
180	PC O-43:2	51	...	7	1	1	0	0	0	<input type="checkbox"/>	PC 33:1 D7	[M+H] <sup>+</sup>	870.7316	[M+OAc] <sup>-</sup>	928.7370
181	PC O-43:1	51	...	7	1	1	0	0	0	<input type="checkbox"/>	PC 33:1 D7	[M+H] <sup>+</sup>	872.7472	[M+OAc] <sup>-</sup>	930.7527
182	PC O-43:0	51	...	7	1	1	0	0	0	<input type="checkbox"/>	PC 33:1 D7	[M+H] <sup>+</sup>	874.7629	[M+OAc] <sup>-</sup>	932.7683
183	PC O-44:6	52	94	7	1	1	0	0	0	<input type="checkbox"/>	PC 33:1 D7	[M+H] <sup>+</sup>	876.6846	[M+OAc] <sup>-</sup>	934.6901
184	PC O-44:5	52	96	7	1	1	0	0	0	<input type="checkbox"/>	PC 33:1 D7	[M+H] <sup>+</sup>	878.7003	[M+OAc] <sup>-</sup>	936.7057
185	PC O-44:4	52	98	7	1	1	0	0	0	<input type="checkbox"/>	PC 33:1 D7	[M+H] <sup>+</sup>	880.7159	[M+OAc] <sup>-</sup>	938.7214
186	PC O-44:3	52	...	7	1	1	0	0	0	<input type="checkbox"/>	PC 33:1 D7	[M+H] <sup>+</sup>	882.7316	[M+OAc] <sup>-</sup>	940.7370
187	PC O-44:2	52	...	7	1	1	0	0	0	<input type="checkbox"/>	PC 33:1 D7	[M+H] <sup>+</sup>	884.7472	[M+OAc] <sup>-</sup>	942.7527
188	PC O-44:1	52	...	7	1	1	0	0	0	<input type="checkbox"/>	PC 33:1 D7	[M+H] <sup>+</sup>	886.7629	[M+OAc] <sup>-</sup>	944.7683
189	PC O-44:0	52	...	7	1	1	0	0	0	<input type="checkbox"/>	PC 33:1 D7	[M+H] <sup>+</sup>	888.7785	[M+OAc] <sup>-</sup>	946.7840
190	PC 33:1 D7	41	73	8	1	1	0	7	0	<input checked="" type="checkbox"/>	PC 33:1 D7	[M+H] <sup>+</sup>	753.6120	[M+OAc] <sup>-</sup>	811.6175

Lipid species

Internal standard selection

**Fig. 7.** Lipid class database.

**#9.** Save created project: “File” → “Save project”.

### Attention!

- The project can be saved whenever you make changes.

**#10.** Click on “Quantify” → “Method”. The window shown in **Fig. 8** will appear.

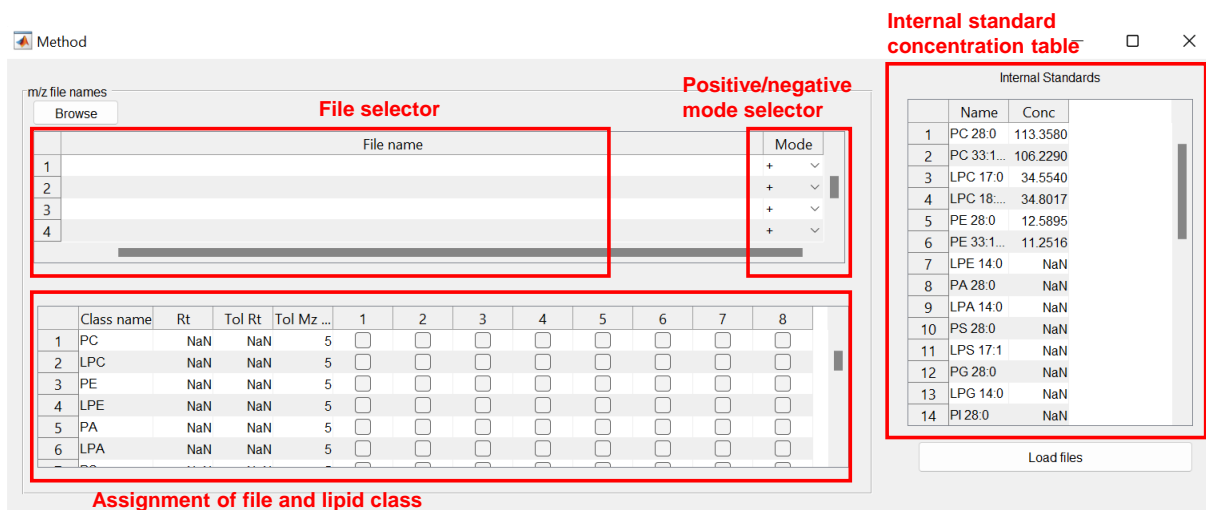
**#11.** Load all input.txt files using the “Browse” button (see Input data to LipidQuant 2.1 note).

**#12.** Select the positive or negative mode using “Positive/negative mode selector”.

**#13.** Fill in the internal standard concentration table.

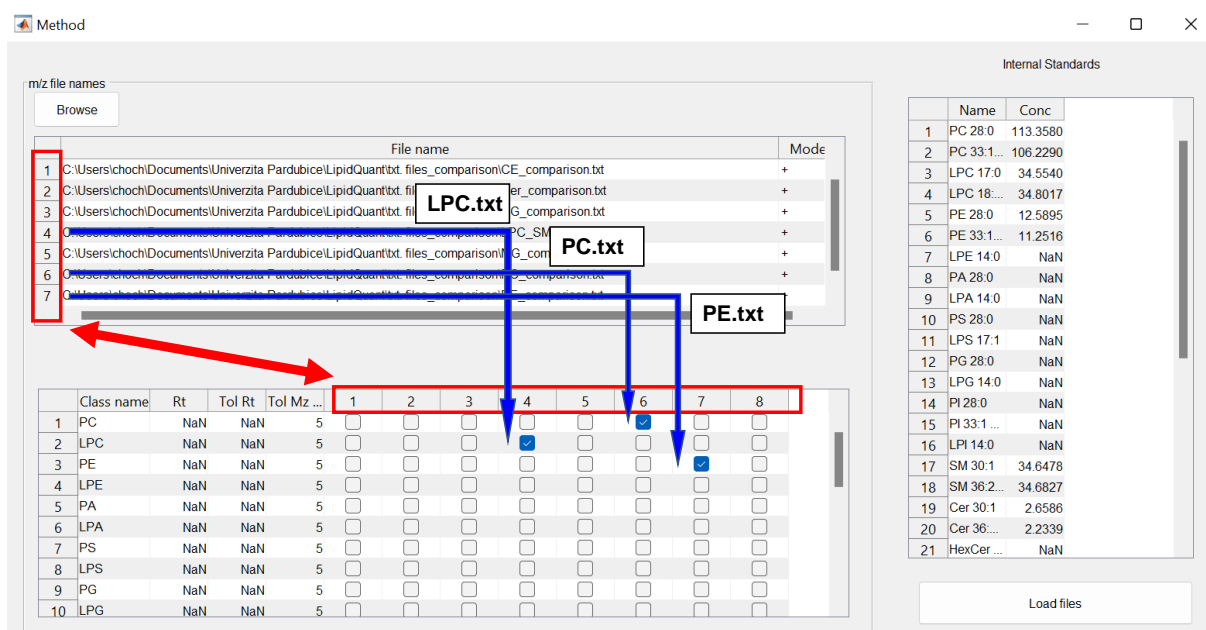
### Attention!

- The internal standard concentration table includes the internal standards selected in step #7.



**Fig. 8.** Method window.

**#14.** Assign .txt files to lipid classes as shown in **Fig. 9**.



**Fig. 9.** Assignment of files and lipid classes.

### Attention!

- One .txt file can include more lipid classes. Do not forget to tag all lipid classes that appear in one.txt file.

**#15.** Apply  $m/z$  tolerance (ppm).

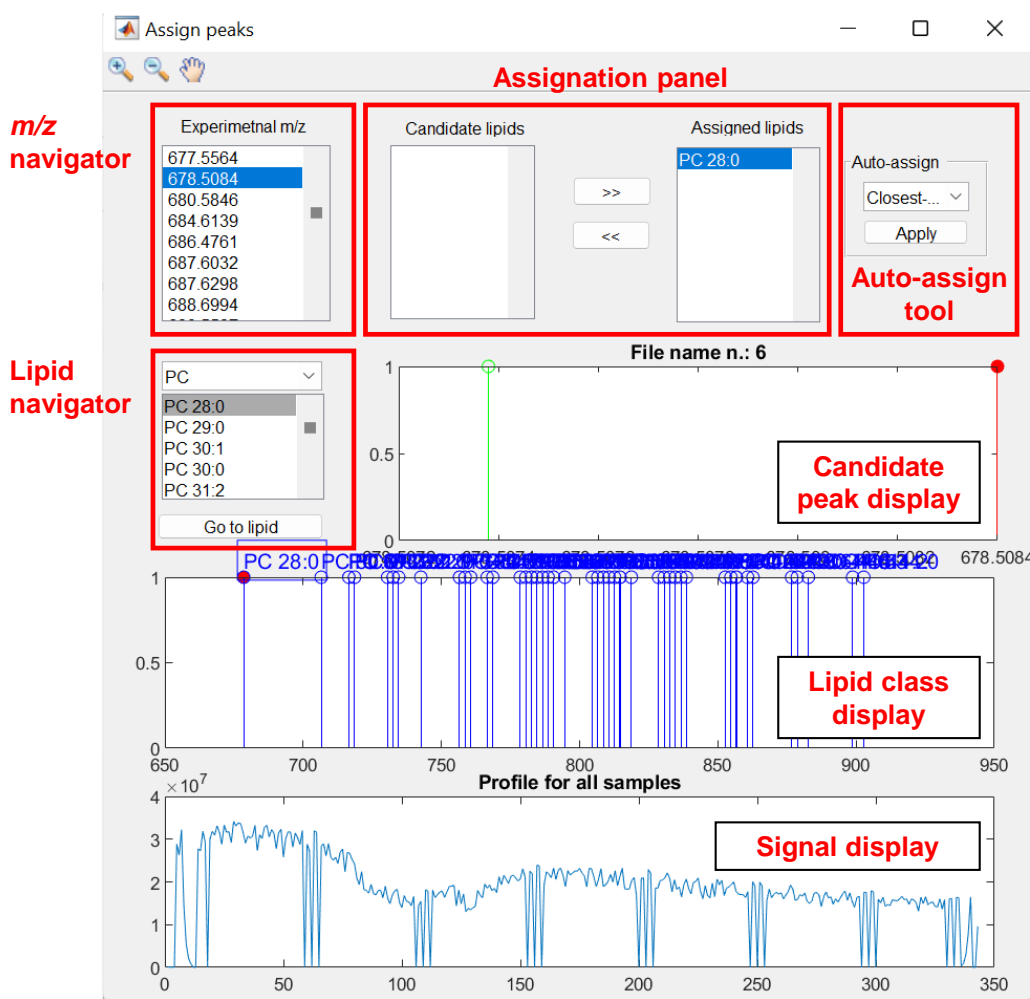
### Attention!

- This tolerance controls the error margin for each compound (within a class) to be assigned to an experimental  $m/z$  value. In other words, the difference between the

theoretical  $m/z$  value for a compound and the experimental values found in the .txt files, cannot be larger than “ $Tol\ m/z$ ” value.

**#16.** Click on “Load Files” button. Wait a few minutes. Once completed, the message (“Reading files...”) will disappear.

**#17.** In the main window of LipidQuant 2.1, click on “Quantify” → “Peak assignment”. The window shown in **Fig. 10** will appear.



**Fig. 10.** Peak assignment window.

**#18.** Select the preferred automatic method for peak assignment (Closest-unique, Closest-sum or Highest) from the “Auto-assign tool” panel to automatically assign experimental signals to lipids.

#### Attention!

- **Closest-unique** assigns the closest experimental  $m/z$  to the theoretical  $m/z$  value of the lipid species with applied  $\Delta m/z$  tolerance, but it allows to assign only one experimental

$m/z$  to the theoretical  $m/z$ . The assignment of experimental  $m/z$  values to a theoretical  $m/z$  value outside of the  $m/z$  tolerance interval (defined in step #15) is not allowed.

- **Closest-sum** enables the assignment of more experimental  $m/z$  values to one theoretical  $m/z$ , if the theoretical value is within the  $m/z$  tolerance interval for two experimental  $m/z$  (the resolution of mass spectrometry does not allow the separation of these molecules). Finally, the sum concentration (of all possible candidates) is reported. As previously, the assignment of experimental  $m/z$  values to the theoretical  $m/z$  value outside of the  $m/z$  tolerance interval (defined in step #15) is not allowed.
- **Highest** assigns the theoretical  $m/z$  value to the highest experimental value located within the  $m/z$  tolerance interval. The potential disadvantage of closest-sum or closest-unique algorithms is that the artificial noise very close to the theoretical value can be misassigned to theoretical compounds, whereas the abundant peak is not assigned. The algorithm uses the average across all samples used for the data processing.
- The selection among three methods for peak assignment must be done by the operator after the careful inspection of data for the particular method and the type of mass spectrometer. Our preference for HILIC/MS and UHPSFC/MS from QTOF instruments from Waters is the use of Closest-unique option.

**#19.** Once you have selected your preferred assignment method, click on “Apply” button. The assignment can take several minutes. When finished, the window “Assigning peaks...” will disappear.

#### Attention!

- Use the “Lipid navigator” panel to select the lipid species to visualize (**Fig. 10**).
- The “ $m/z$  navigator” panel shows a list of all experimental  $m/z$  of all determined lipid species sorted according to their masses.
- Candidate lipids will appear when more than one lipid meets the criteria ( $m/z$  tolerance). The closeness of theoretical  $m/z$  (green) and experimental  $m/z$  of assigned (red) and possible candidate lipids (blue) is visualized in “Candidate peak display” panel (**Fig. 10**).
- The “Lipid class display” (**Fig. 10**) visualizes  $m/z$  values of the other lipids belonging to the same class.
- The “Signal display” (**Fig. 10**) shows the signals of selected lipid species in all samples.

**#20.** Save project: “File” → “Save project”.

**#21.** Click on “Quantify” → “Apply quantification”. The concentration calculation will be in progress. It can take several minutes. Once it is done, the window “Fitting data...” will disappear.

**#22.** Click on “View” → “Quantification results”. The window shown in **Fig. 11** will appear.

#### Attention!

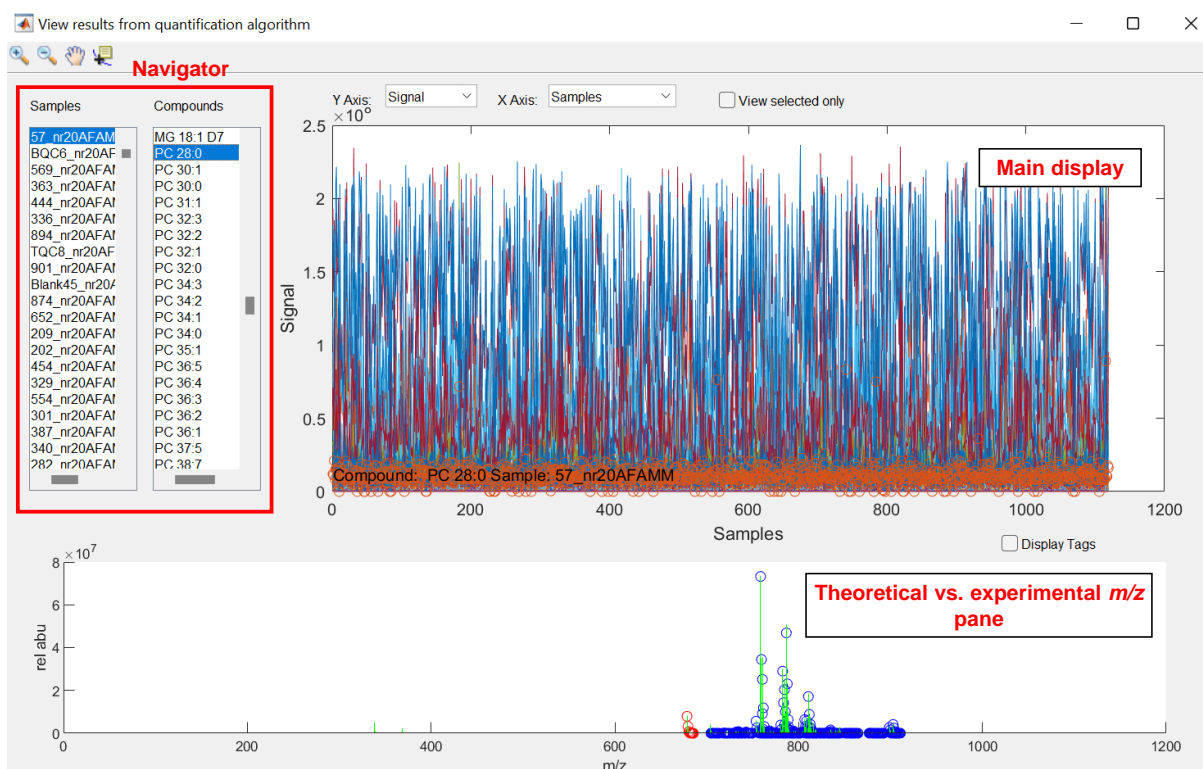
- The “Navigator” panel shows a list of all samples and all determined lipid species in these samples.
- The “Main display” visualizes the relationship between signal/concentration (Y axis) and samples/compounds (X axis).

- Click on “View selected only” (**Fig. 11**) to see only the selected trace in the navigator.
- The “Theoretical vs. experimental pane” displays a specific compound/sample case. Green traces correspond to experimental signals (for a given lipid class), blue stem plots correspond to the (fitted) values of theoretical compounds, while the red stem plot corresponds to the selected compound/sample in “navigator” panel.
- Click on “Display Tags” in the top panel to show the names of the compounds on top of the fitted isotope (exact mass).

**#23.** Click on “File” → “Export Excel” to get a summary table of signals and concentrations.

### Attention!

- The exported signals are not the raw measured signals, but those after isotopic correction are used for the calculation of concentrations.



**Fig. 11.** Quantification results window.