# ReadMe file for LipidQuant 1.0

## Basic instructions how to use LipidQuant 1.0 for automated data processing in lipid class separation - mass spectrometry quantitative workflows (updated July 31, 2021)

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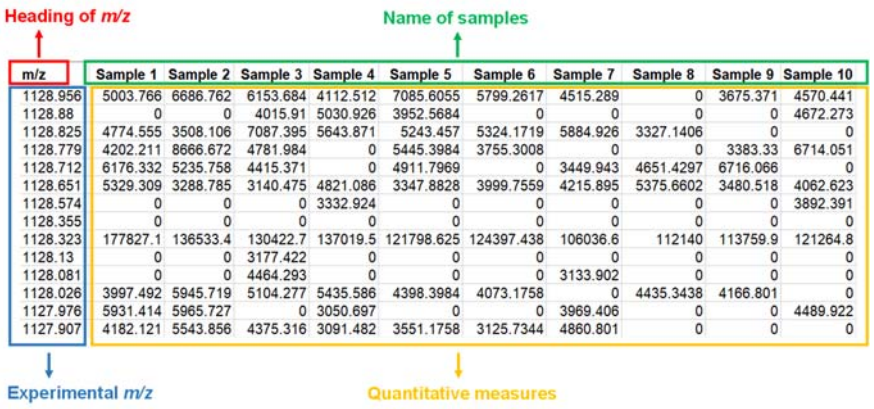
### Input data to LipidQuant 1.0

**1.** It has to be **txt format** or **Excel sheet** including all *m/z* features in the first column with the heading of *m/z* followed by individual samples containing the intensities or other quantitative measures for each *m/z* feature (**Figure 1**).

**Figure 1.** Example of an input table to the LipidQuant 1.0.




| 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 | 5931.414 | 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 |

**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 and Chol maybe 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, but not comma or dot.
- Decimal point (for*m/z* values and quantitative measures) has to be used, but not comma.

## LipidQuant 1.0

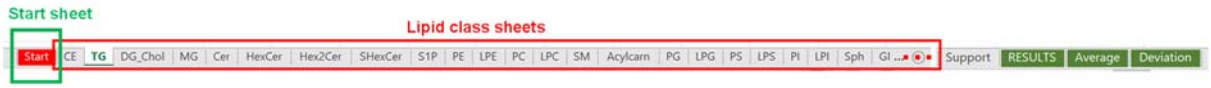
**1.** Open the LipidQuant 1.0.

**Attention!**

- Excel Macro has to be activated.

**2.** Go to the Start sheet (**Figure 2**), press the button "Clear all concentrations" to be sure that all data sheets are empty for starting a new processing.

**Figure 2.** Sheets of LipidQuant 1.0.




**3. Set the concentration of internal standards** and *m/z* **tolerance window** in all sheets of lipid classes, which you want to quantify (**Figure 3**).

**Attention!**

- You can set a maximum of 3 lipid standards within one lipid class.
- You have to define the order of the IS in the database (in cells C3, C4, or C5). Count the number of lines starting from line 10 until the IS is written (**Figure 4**).

**Figure 3.** Example of TG lipid class sheet with given IS information (annotation, order in the database, *m/z*, and concentration) and *m/z* tolerance window. The same structure is used for each lipid class sheet.

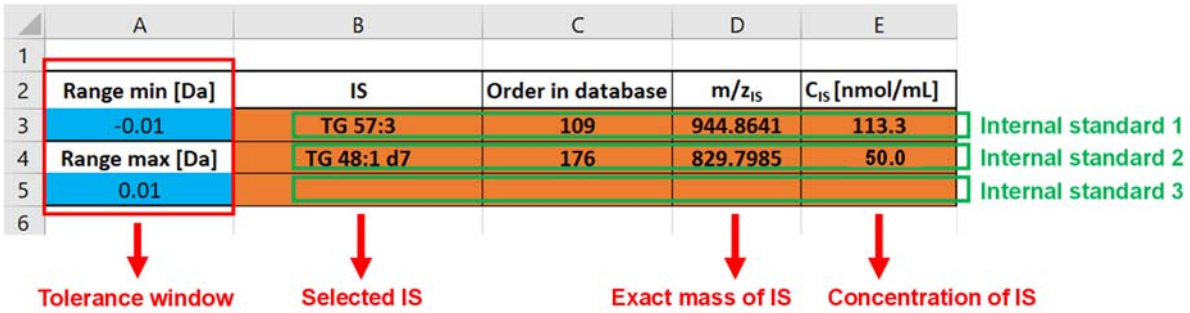
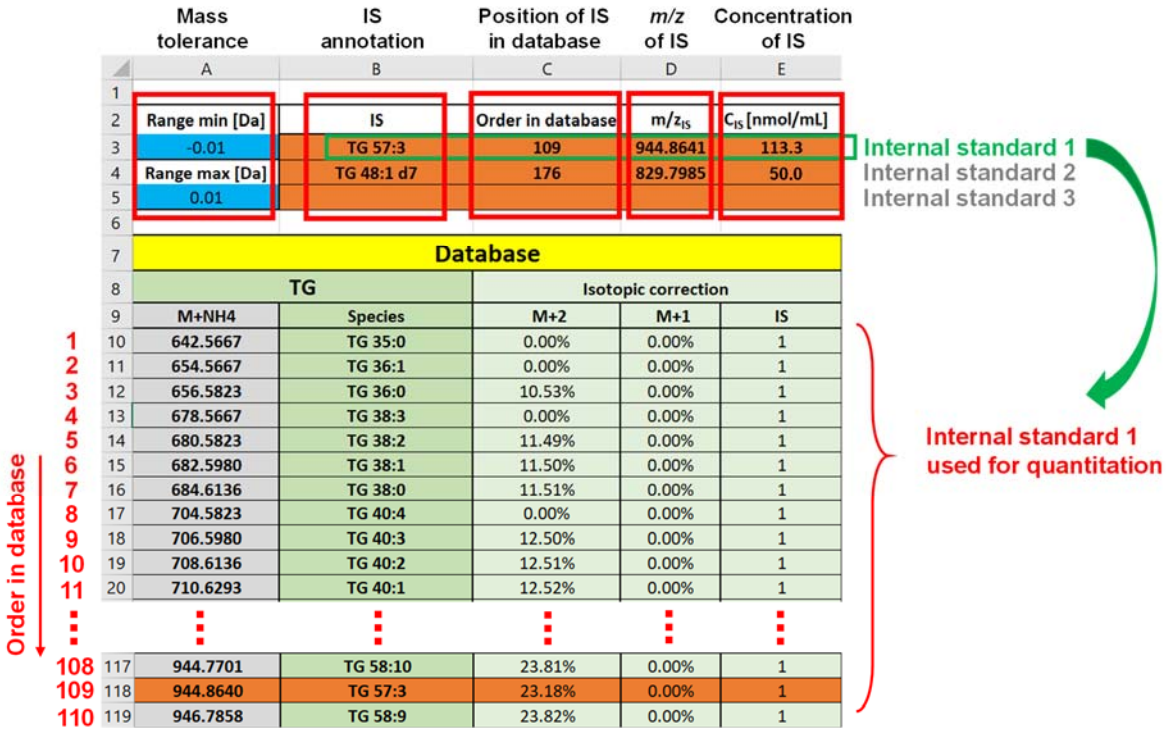


**Figure 4.** Definition of selected IS for quantitation. The same structure is used for each lipid class sheet.




**4. Define the internal standard (IS),** which should be applied for the quantitation of lipid species by setting the IS number 1, 2, or 3 to the lipid class database (column E) for all lipid classes, you want to quantify (**Figure 4**).

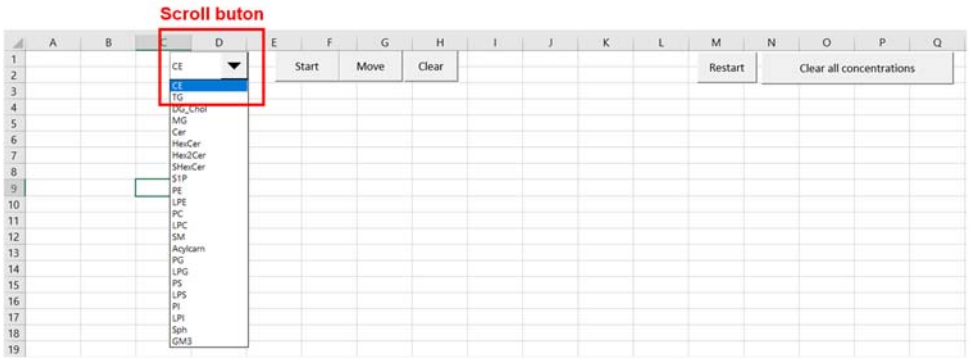
**Attention!**

- If you use the internal standard 2 or 3, you have to set number 2 or 3, respectively, to the database.

**5.** Go to the Start sheet of LipidQuant 1.0.

**6. Choose the lipid class**, which you want to quantify using the scroll button (**Figure 5**), open the input table of this class, select all, and copy the content into LipidQuant 1.0 by pasting in cell A1.

**Figure 5.** Start sheet of LipidQuant.




**7. Press Start button**. Now the LipidQuant 1.0 is comparing the exact *m/z* with the experimental *m/z* value according to the applied *m/z* tolerance.

**8.** When the processing is finished, a colored table appears. You can remove all lines, which are not green (**Figure 6**), as this lipid species are not within the tolerance, or follow subsequent instructions.

**Attention!**

- The number in the yellow column E illustrates only the position of the species in the database (class sheet).
- Light yellow highlighted lipid species (column C in **Figure 6**) are within two times the mass tolerance. When you decide to anyhow keep and quantify these lipid species, you have to put number 1 to the cell in column C and add the number of position of lipid species (cell in column E) in the database (**Figure 7**).
- Red marked lipid species (column C in **Figure 5**) show more detected lipids within the tolerance range. Remove all of them or choose the one you want to quantify, *i.e.*, the one closest to the exact mass and remove the second one (**Figure 8**).

**Figure 6.** Example of the colored table.

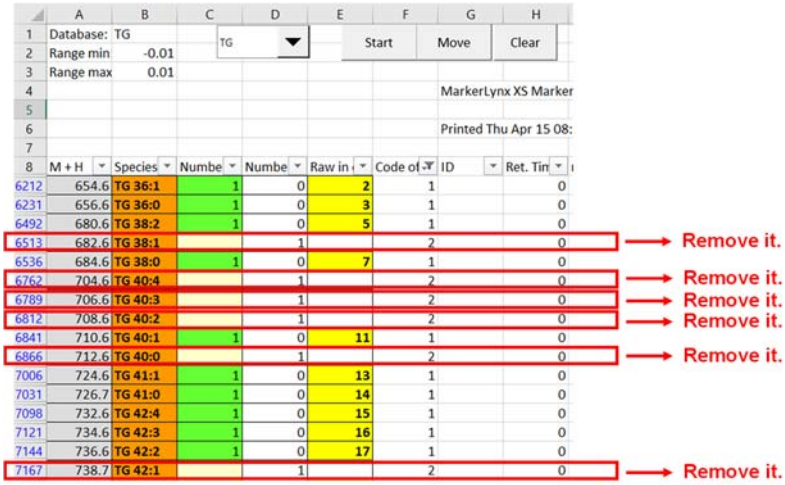


**Figure 7.** Example of changes in a colored table.

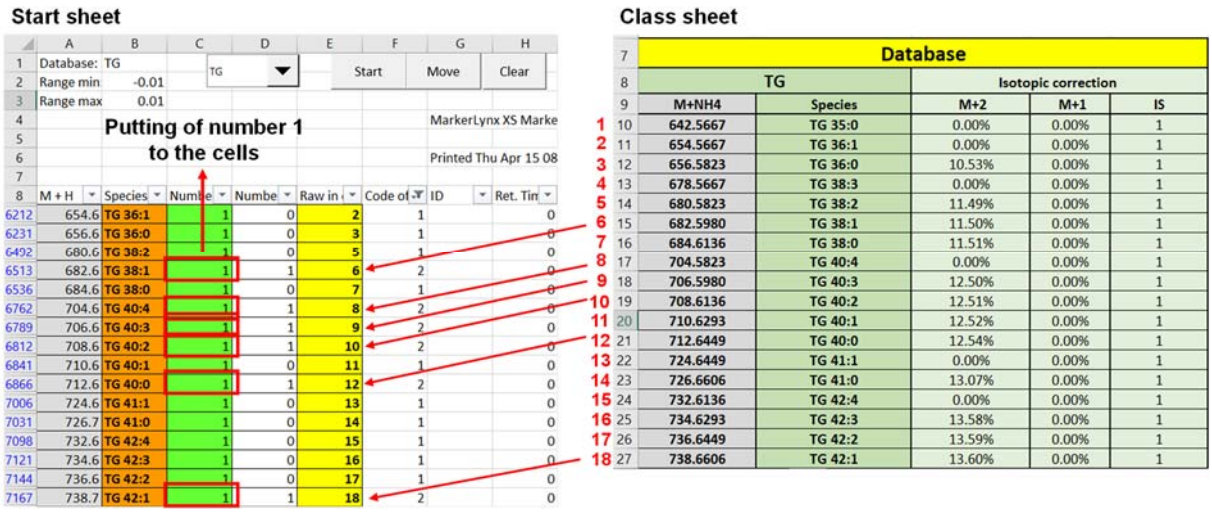
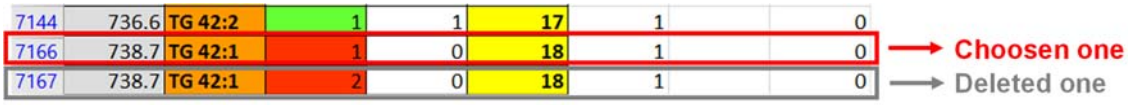



**Figure 8.** Example of red marked lipid species.




**9.** After removing or changing of some lines (cells in columns C and E), **press Move button**. Now the LipidQuant 1.0 performs the isotopic correction, quantitation and moves the results to the class sheet.

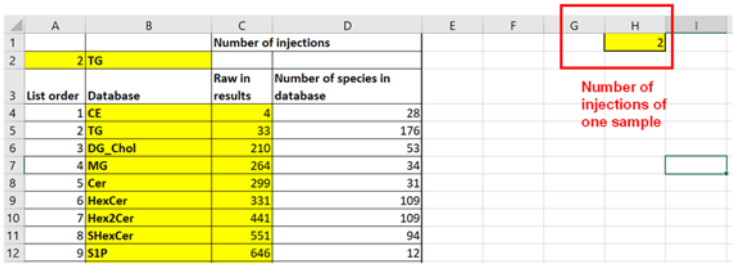
**10.** Once it is finished, a window appears with "Finish", **press OK.**

**11. Press Clear button** (in Start sheet) and continue with the next lipid class according to items 6 – 10 until you process all lipid classes for quantitation.

**12.** When you make multiple injections of one sample, set the number of injections (cell H1) in Support sheet **(Figure 9)**.

**13.** Go to the Result sheet and press Insert data. This may take longer time. You will get a summary table of your lipid species concentration in all samples.

**Figure 9.** Support sheet of LipidQuant.




**Attention:**

- The number of injections in Support sheet has to be set before you insert data to the Result sheet. Do not forget save changes.

**14.** Average and deviation of lipid species concentrations for multiple injections will be shown in the summary table in Average and Deviation sheets, respectively.

**Attention!**

- Multiple injections of one sample have to be in subsequent lines without any interruption.
- Average and deviation values in the corresponding sheets will be saved according to the name of the first injection of sample.

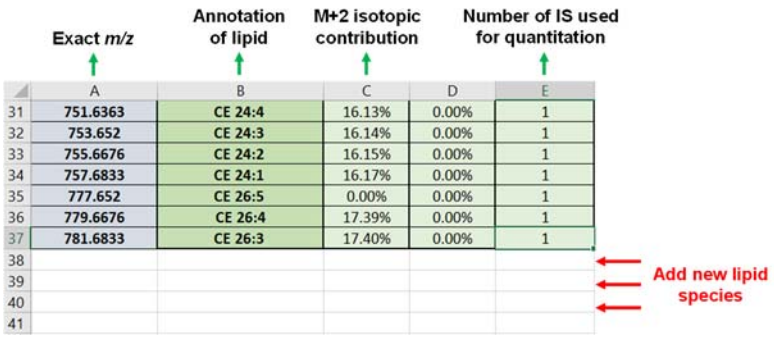
## Modification of LipidQuant 1.0

### Addition of more lipid species into the existing lipid class sheet

**1. Open the lipid class sheet**, which you want to modify.

**2. Add lipid species** including exact *m/z*, annotation of lipid, M+2 isotopic contribution of lipid, and the number of IS used for quantitation to the end of the lipid database (**Figure 11**).

**Figure 10.** Addition of new lipid species into the existing lipid class sheet.

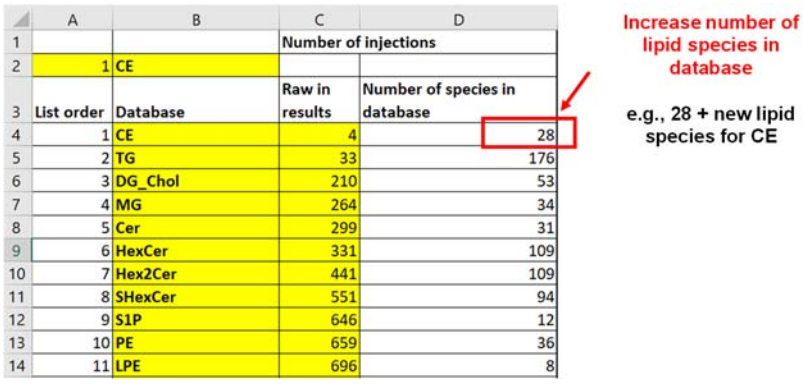



| | A | B | C | D | E |
|---|---|---|---|---|---|
| 31 | 751.6363 | CE 24:4 | 16.13% | 0.00% | 1 |
| 32 | 753.652 | CE 24:3 | 16.14% | 0.00% | 1 |
| 33 | 755.6676 | CE 24:2 | 16.15% | 0.00% | 1 |
| 34 | 757.6833 | CE 24:1 | 16.17% | 0.00% | 1 |
| 35 | 777.652 | CE 26:5 | 0.00% | 0.00% | 1 |
| 36 | 779.6676 | CE 26:4 | 17.39% | 0.00% | 1 |
| 37 | 781.6833 | CE 26:3 | 17.40% | 0.00% | 1 |
| 38 | | | | | |
| 39 | | | | | |
| 40 | | | | | |
| 41 | | | | | |

**3. Go to the Support sheet** to the LipidQuant 1.0.

**4.** Increase the number of lipid species in the database within the lipid class, which you want to modify (**Figure 12**).

**Figure 11.** Support sheet.




| | A | B | C | D |
|---|---|---|---|---|
| 1 | | | Number of injections | |
| 2 | 1 | CE | | |
| 3 | List order | Database | Raw in results | Number of species in database |
| 4 | 1 | CE | 4 | 28 |
| 5 | 2 | TG | 33 | 176 |
| 6 | 3 | DG_Chol | 210 | 53 |
| 7 | 4 | MG | 264 | 34 |
| 8 | 5 | Cer | 299 | 31 |
| 9 | 6 | HexCer | 331 | 109 |
| 10 | 7 | Hex2Cer | 441 | 109 |
| 11 | 8 | SHexCer | 551 | 94 |
| 12 | 9 | S1P | 646 | 12 |
| 13 | 10 | PE | 659 | 36 |
| 14 | 11 | LPE | 696 | 8 |

**5. Save** the changes.

**6.** Process data in the same way, as described above.

### Addition of new lipid class

**1.** Create a new lipid class sheet according to the existing one, which can be used as a template.

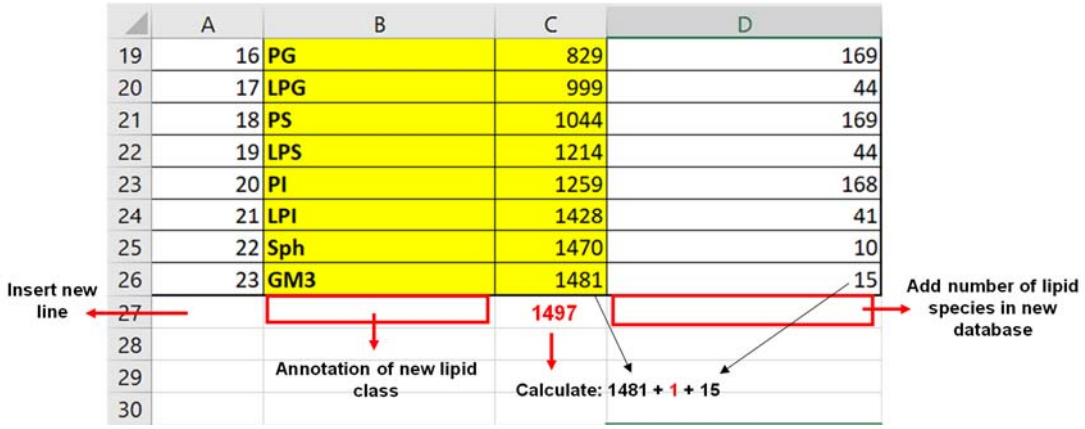
**2.** Add lipid species of the new created lipid class to the database including exact *m/z*, annotation, M+2 isotopic contribution, and number of IS used for their quantitation.

**3.** Add information about the used IS (annotation, *m/z*, the order in database, and concentration).

**4.** Go to the Support sheet.

**5.** Insert the new line, add the annotation of new lipid class (column B), the number of lipid species in the database (column D), and calculate the number of lines in the results (column C) (**Figure 13**).

**Figure 12.** Support sheet.



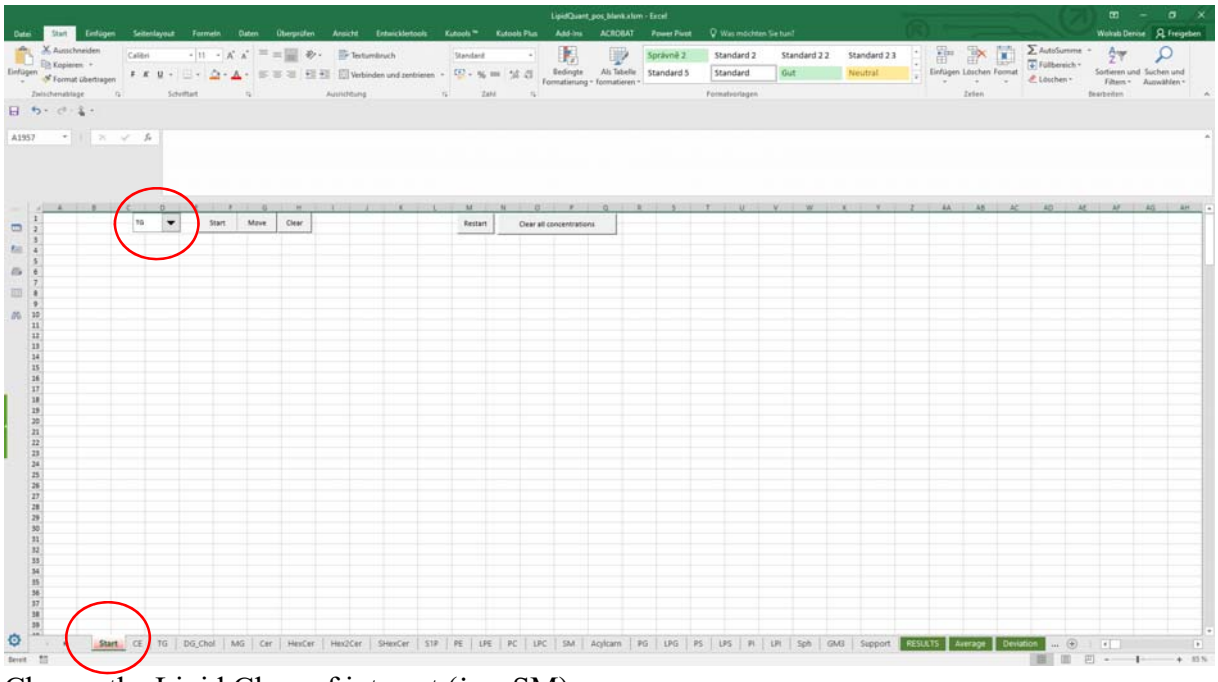

**6.** Save the changes. The new lipid class will appear in Start sheet (scroll button) automatically.

**7.** Process data in the same way, as described above.

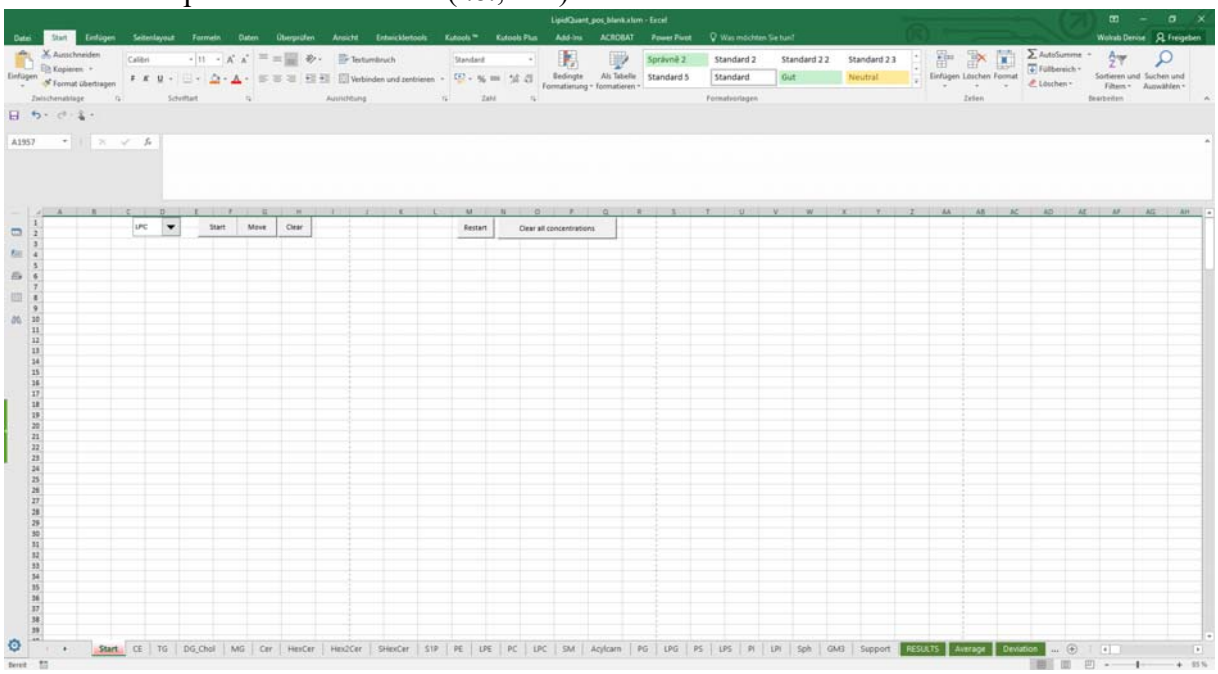
### Step-by-step walkthrough

**Examples:** SM+LPC

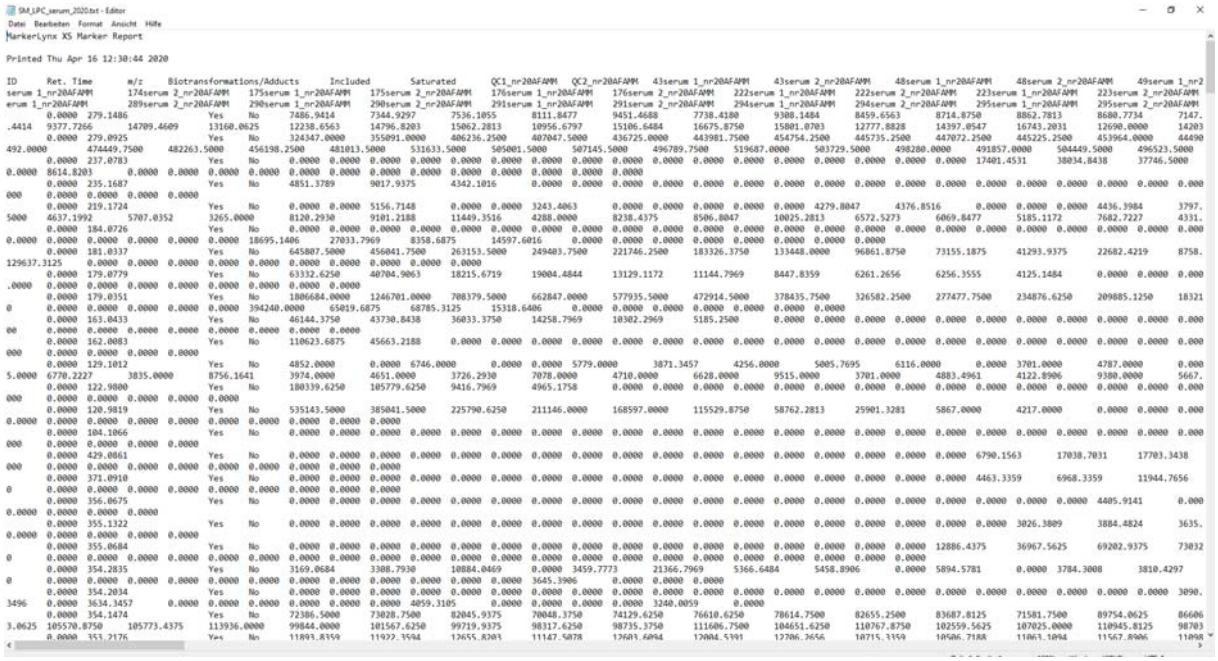
- Open LipidQuant 1.0.
- Go to the “Start” sheet.



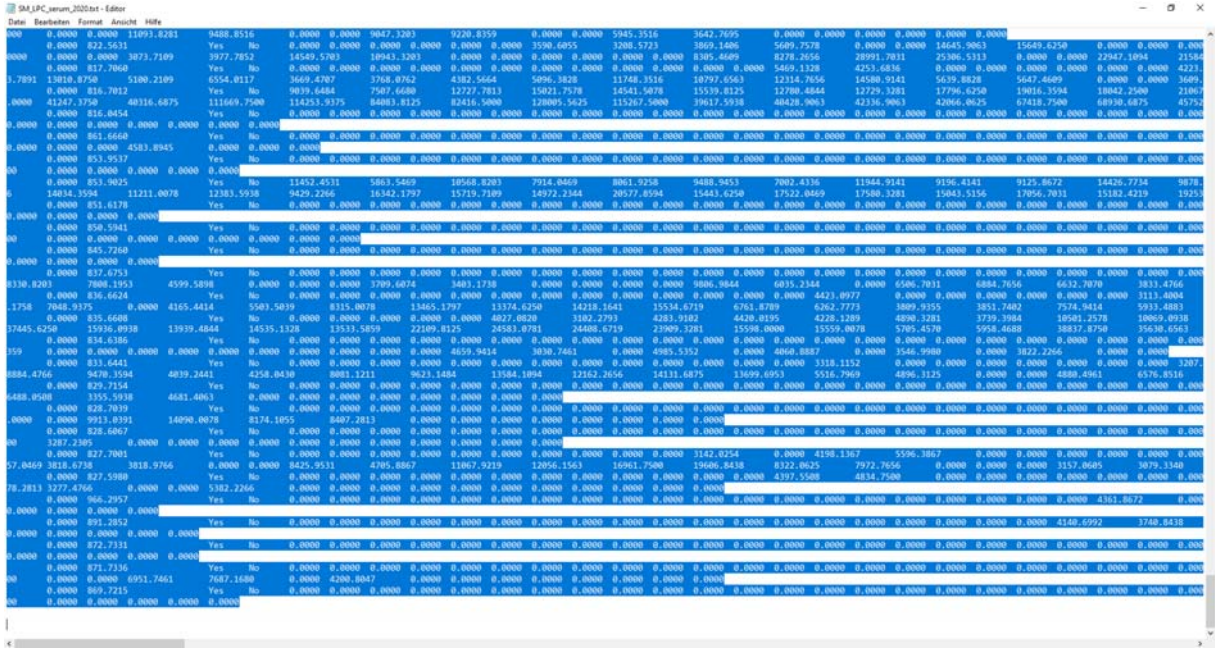
- Choose the Lipid Class of interest (*i.e.*, SM).



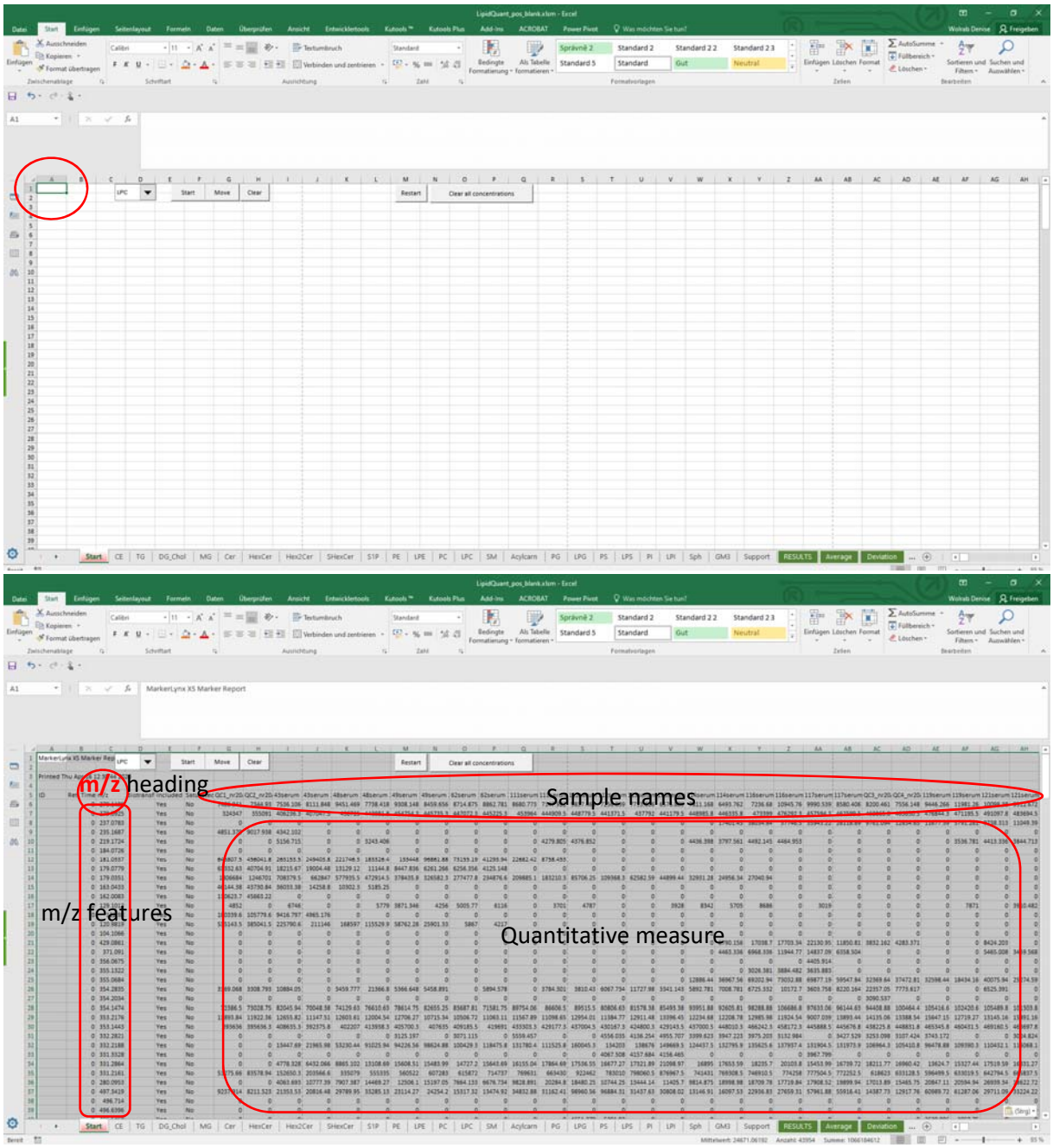
- Open the lipid class txt file generated in MarkerLynx or any other input file from a peak picking software (*i.e.*, SM_LPC_serum_2020 - classes elute close to each other, as no interferences are expected when they were processed together).



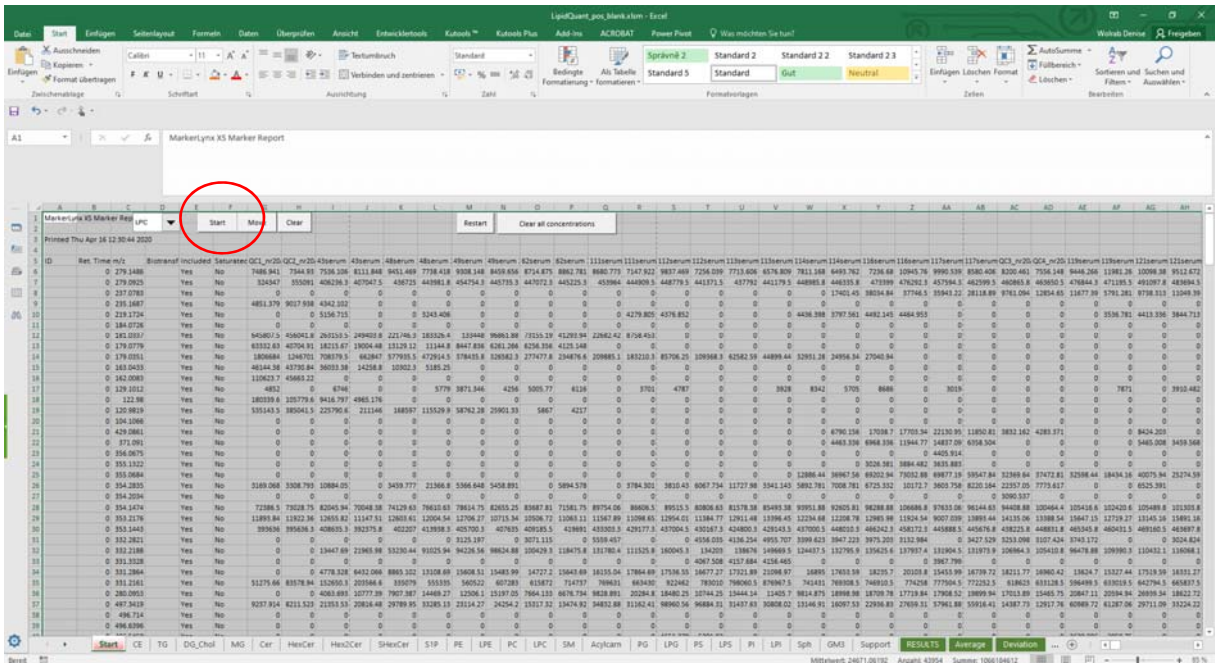
- Mark and copy the whole txt table (Ctrl+A and Ctrl+C).



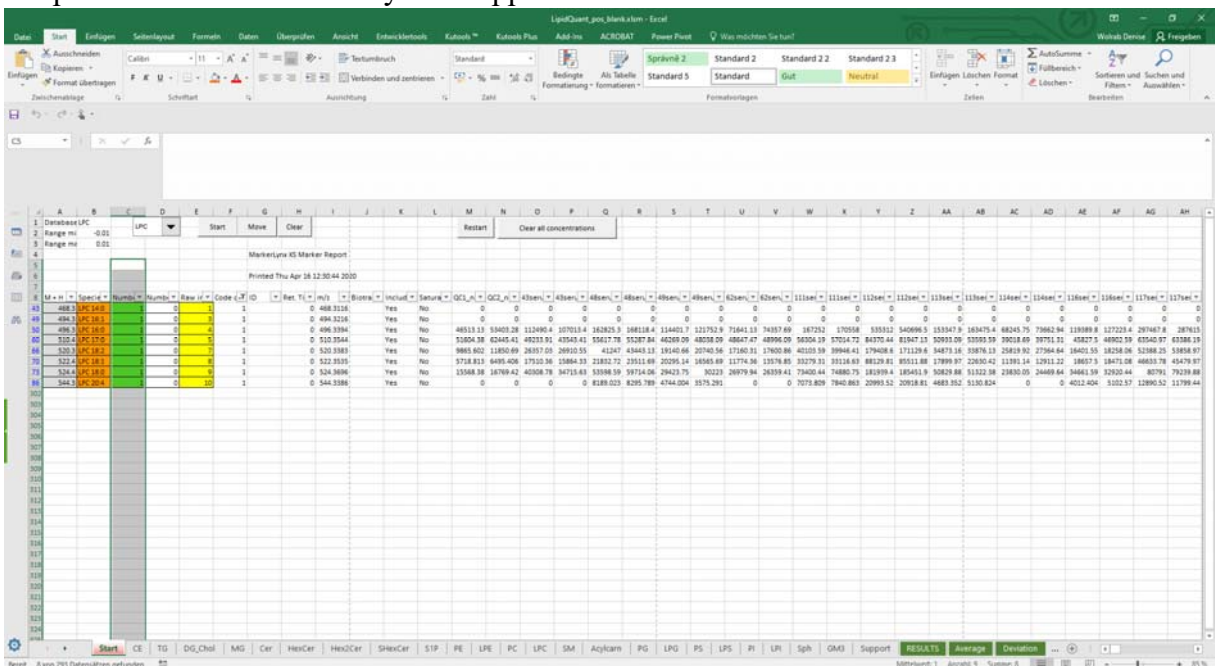
- Go to LipidQuant 1.0 and insert the table in A1.



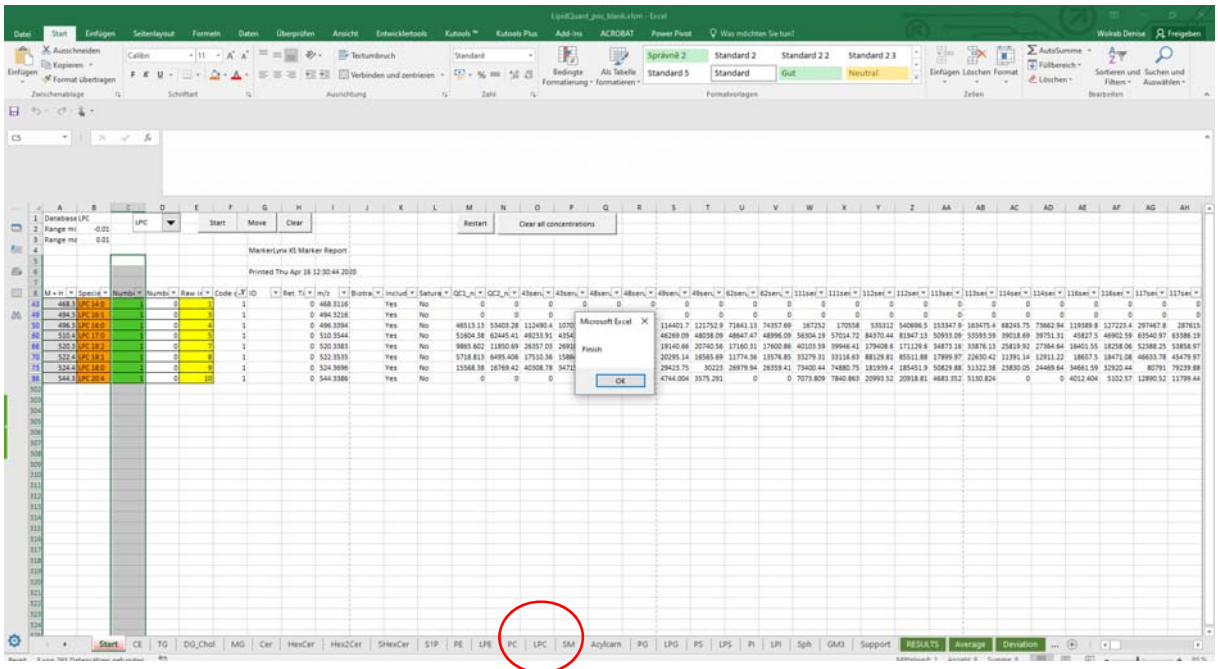

- Remark: If another peak picking software is used, then it is essential that you follow the general structure. You need a column with the heading “m/z” followed by the sample names and in the subsequent rows the*m/z* features and the quantitative measure, *i.e.*, signal response.
- Press “Start”.



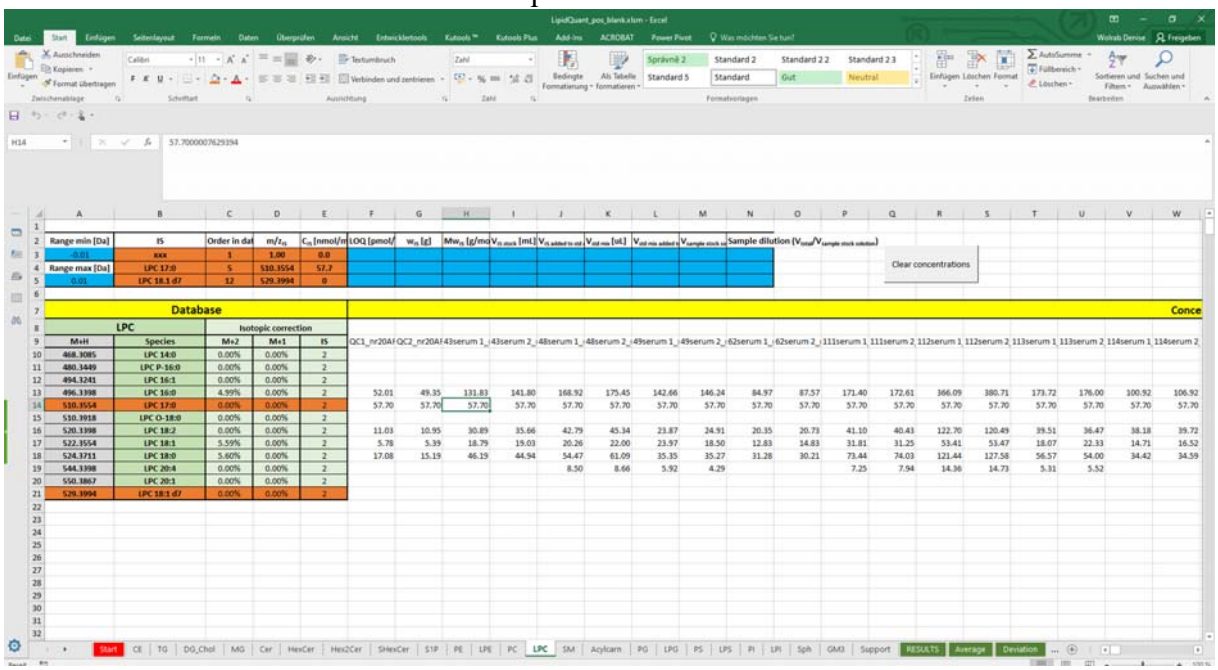
- A lipid identification summary table appears.



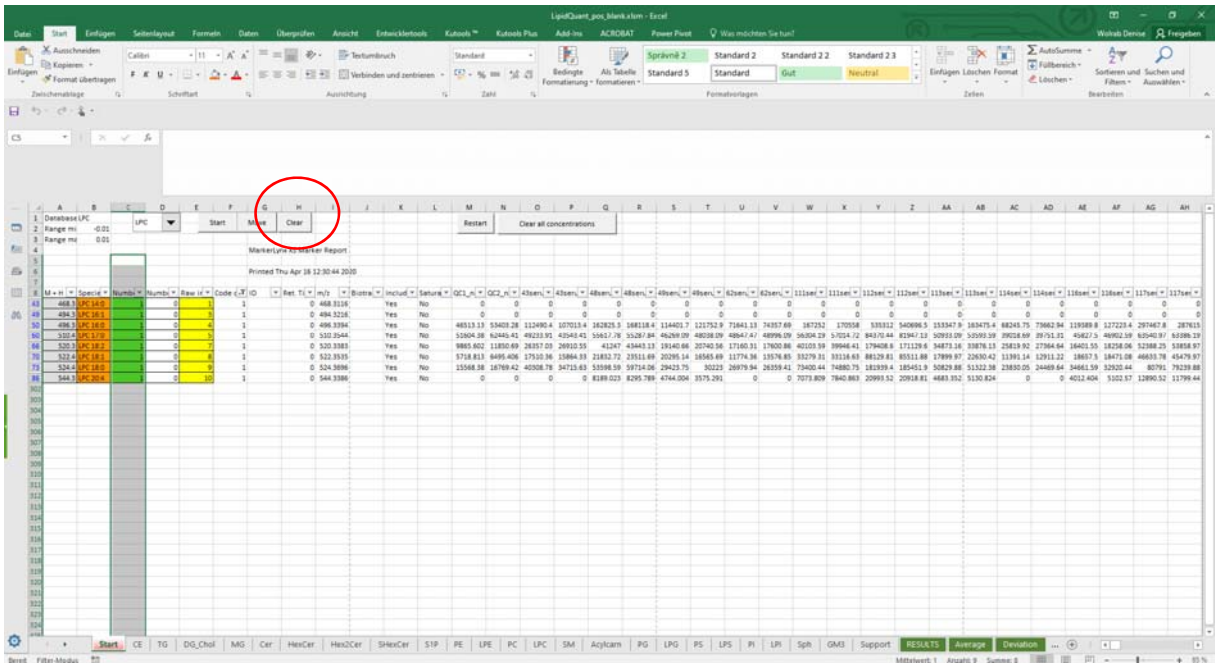
- Press “Move” and the identified lipid species will be quantified.
- When the quantitation is done, a window with “Finish” appears. Press “OK”.



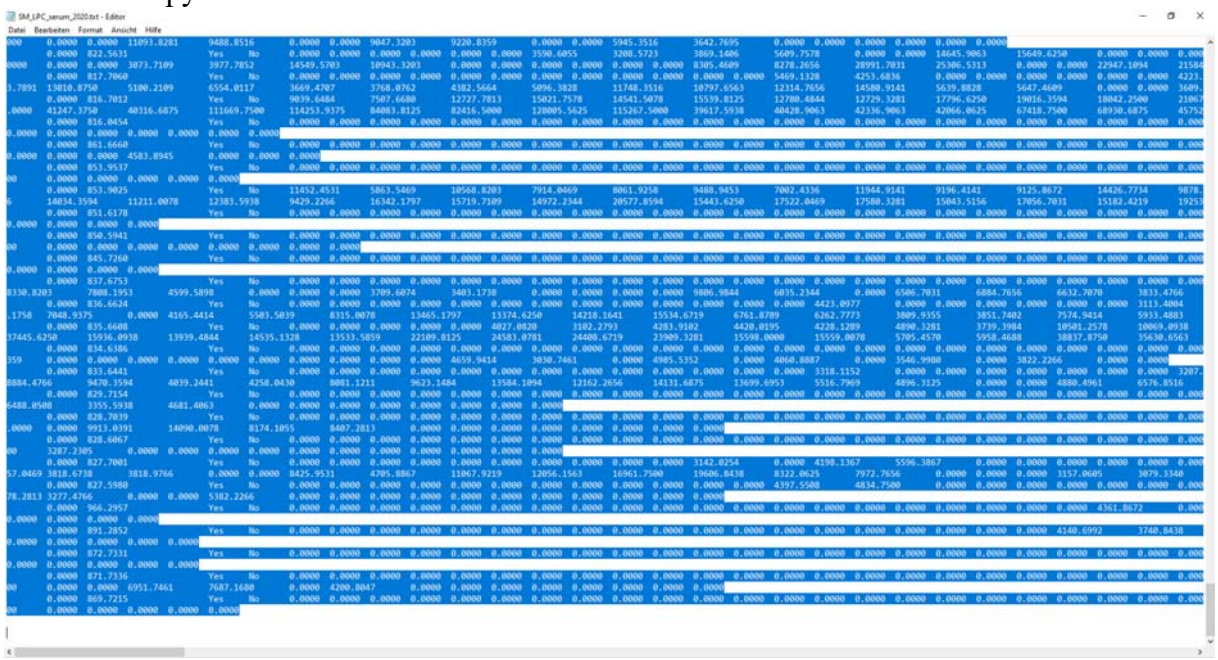
- Go to the LPC data sheet to monitor the quantitative results.



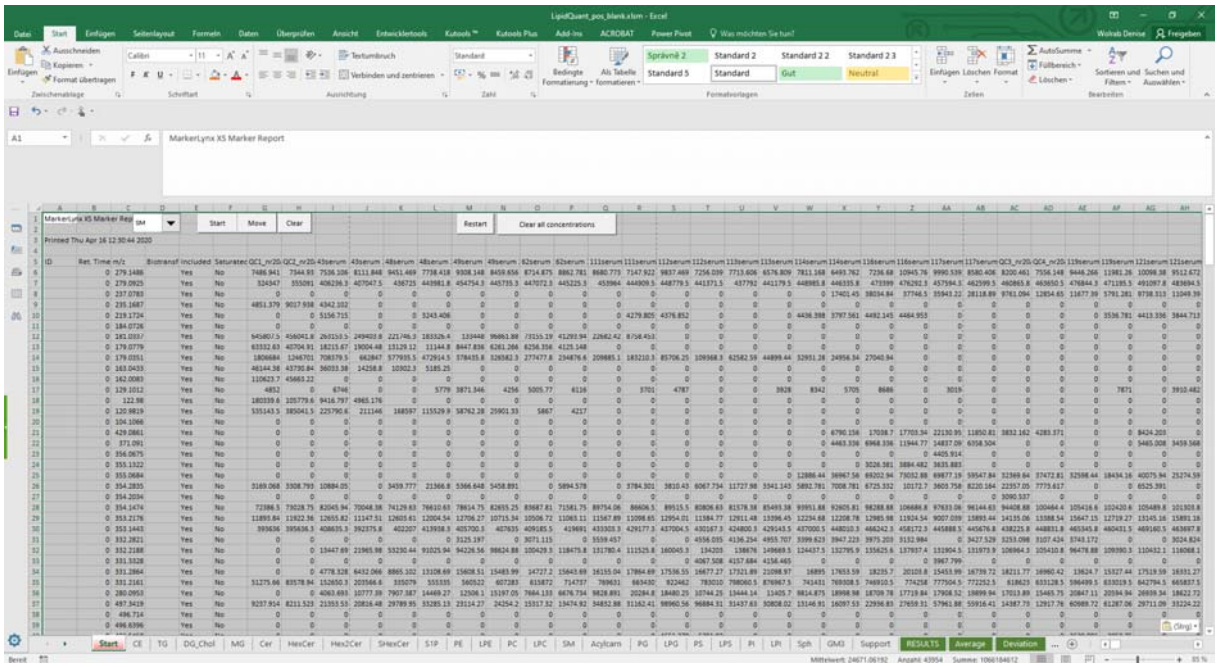
- Go back to the “Start” sheet and press “clear”.



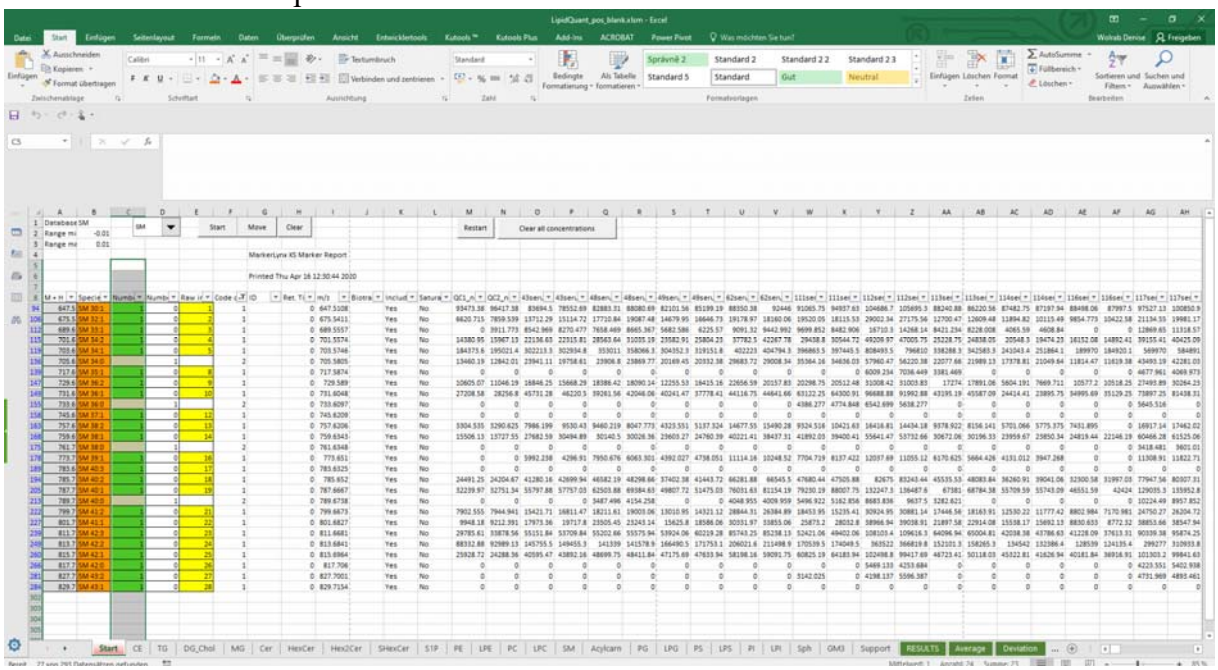
- Choose the next lipid class, *i.e.*, SM.
- Mark and copy txt table.



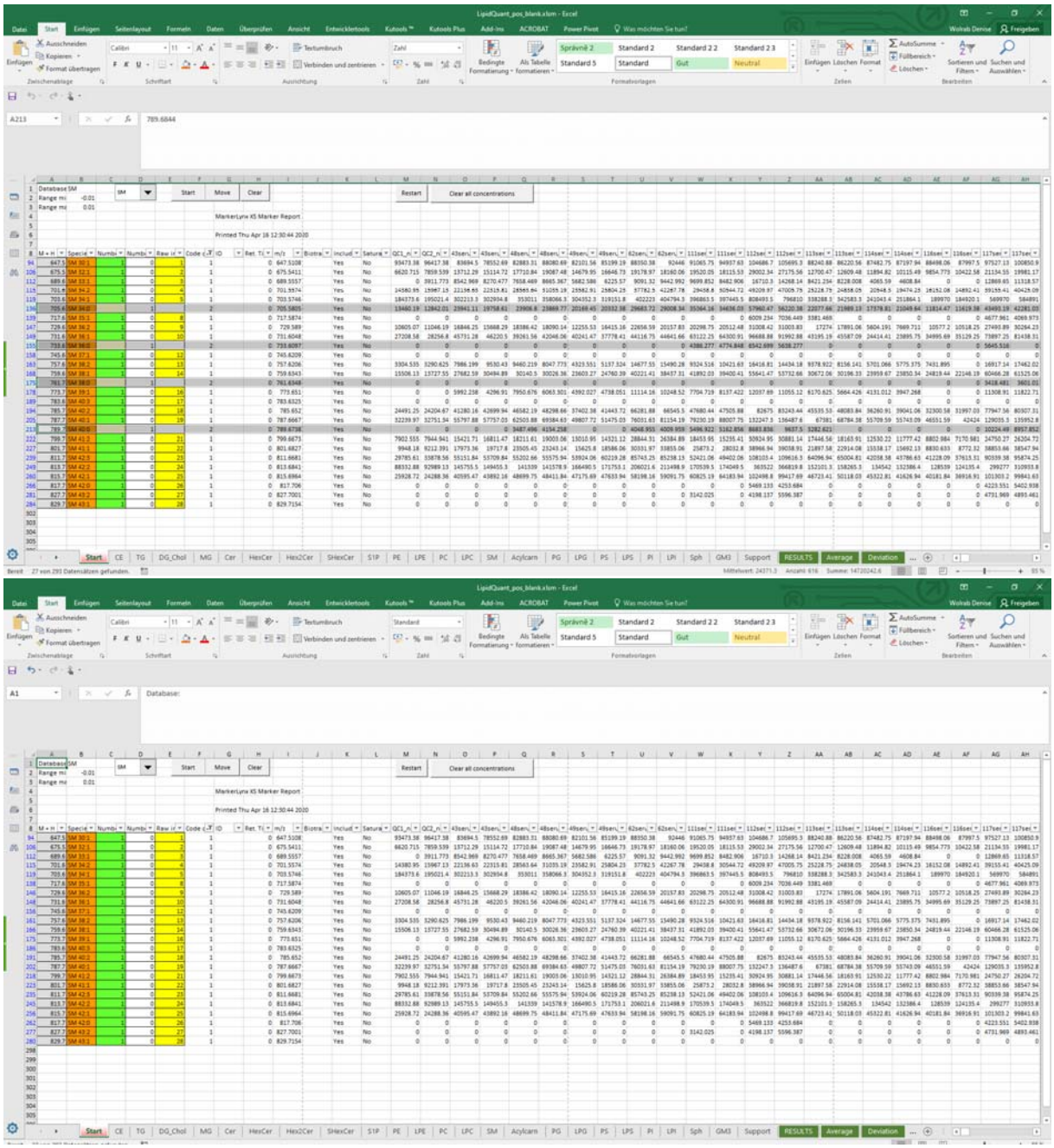
- Insert in A1 of the “start” sheet in LipidQuant 1.0.



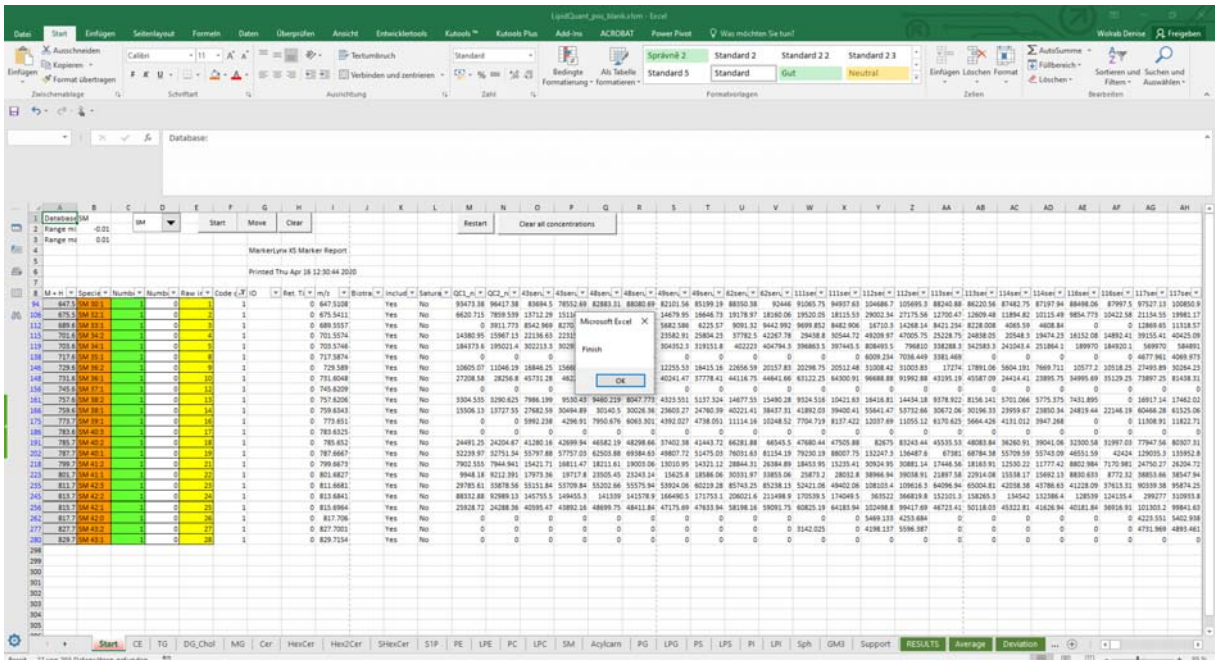
-
- Press “Start” for SM lipid identification.



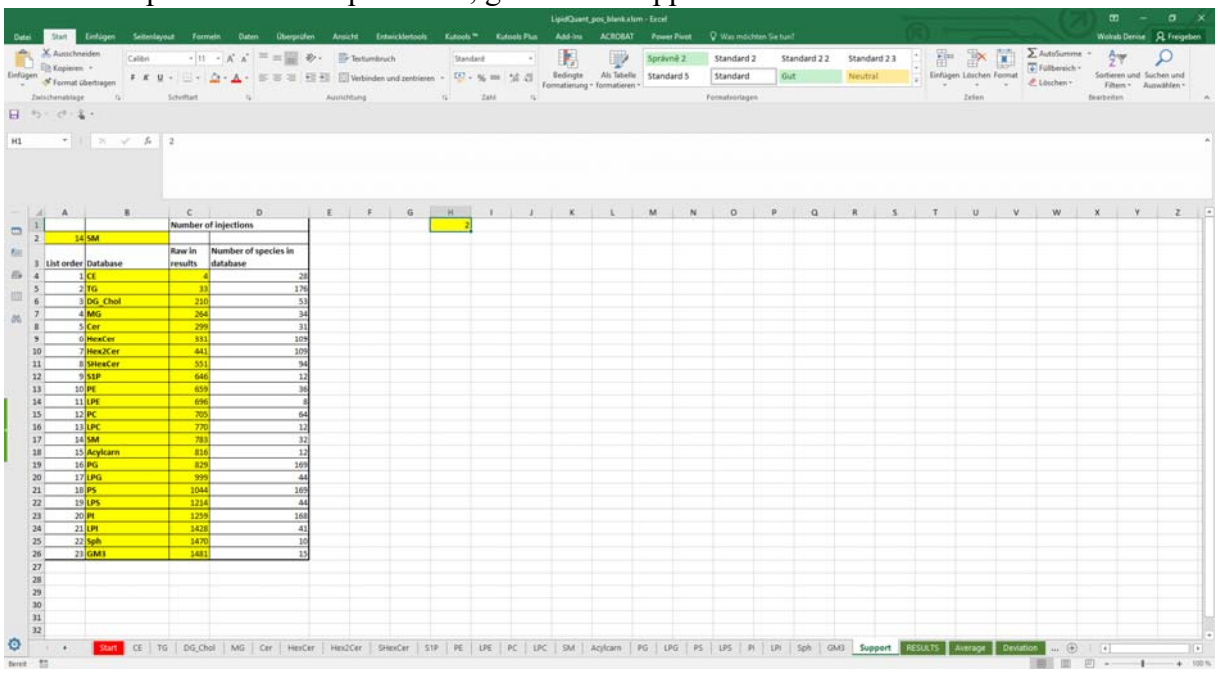
- *m/z* features in rows 136/155/175/213 are not within the defined mass tolerance range and should be deleted.



- Press “Move” for quantitation.

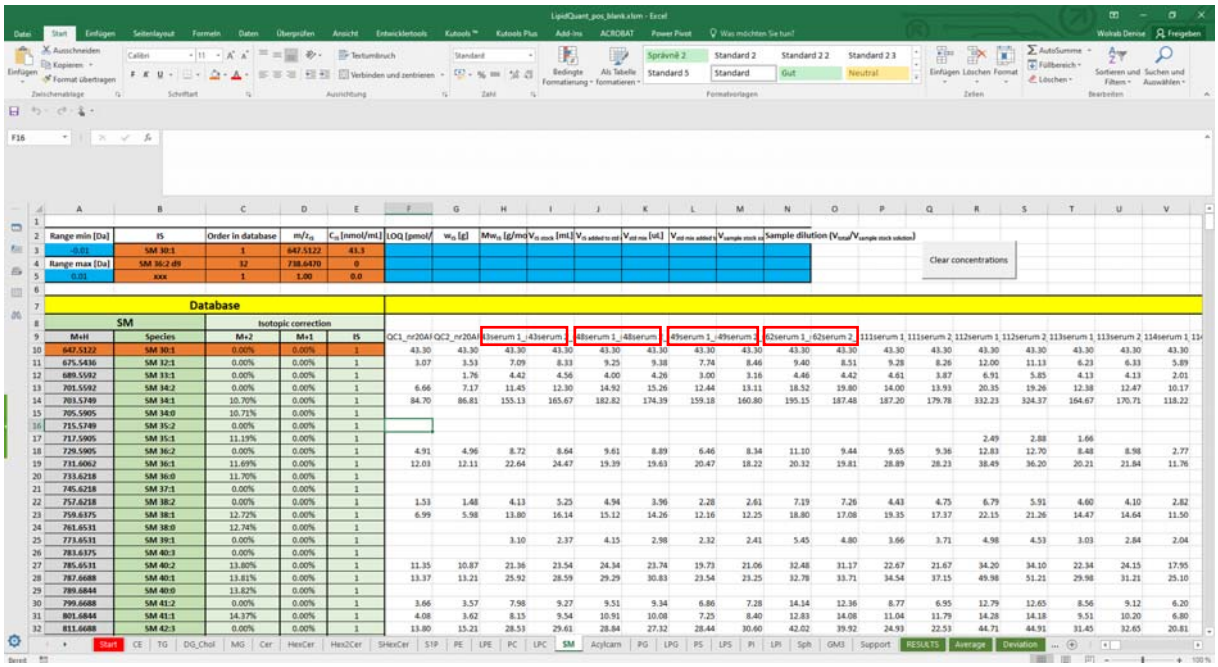


- Repeat steps for all lipid classes of interest.
- When all lipid classes are quantified, go to the “Support” sheet.

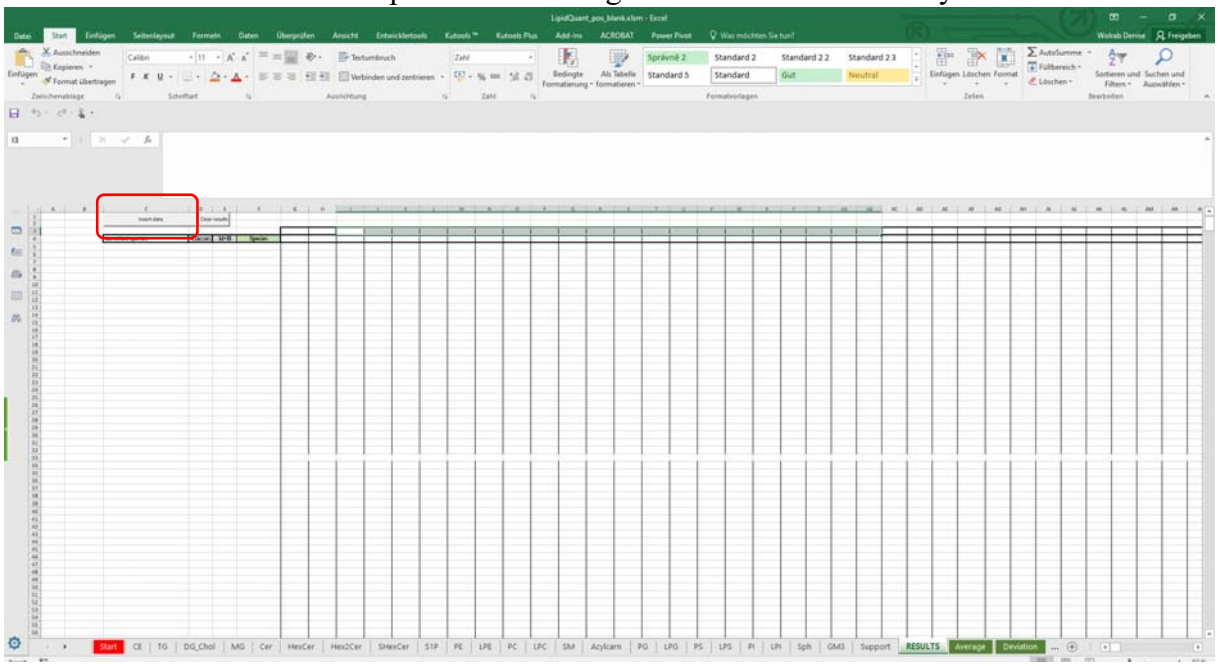


| | | Number of injections | | 2 |
|---|---|---|---|---|
| 14 | SM | | | |
| List order | Database | Raw in results | Number of species in database | |
| 1 | CE | 4 | 28 | |
| 2 | TG | 33 | 176 | |
| 3 | DG_Chol | 210 | 53 | |
| 4 | MG | 264 | 34 | |
| 5 | Cer | 299 | 31 | |
| 6 | HexCer | 331 | 109 | |
| 7 | Hex2Cer | 441 | 109 | |
| 8 | SHexCer | 551 | 94 | |
| 9 | S1P | 646 | 12 | |
| 10 | PE | 659 | 36 | |
| 11 | LPE | 696 | 8 | |
| 12 | PC | 705 | 64 | |
| 13 | LPC | 770 | 12 | |
| 14 | SM | 783 | 32 | |
| 15 | Acylcarn | 816 | 12 | |
| 16 | PG | 829 | 169 | |
| 17 | LPG | 999 | 44 | |
| 18 | PS | 1044 | 169 | |
| 19 | LPS | 1214 | 44 | |
| 20 | PI | 1259 | 168 | |
| 21 | LPI | 1428 | 41 | |
| 22 | Sph | 1470 | 10 | |
| 23 | GM3 | 1481 | 15 | |

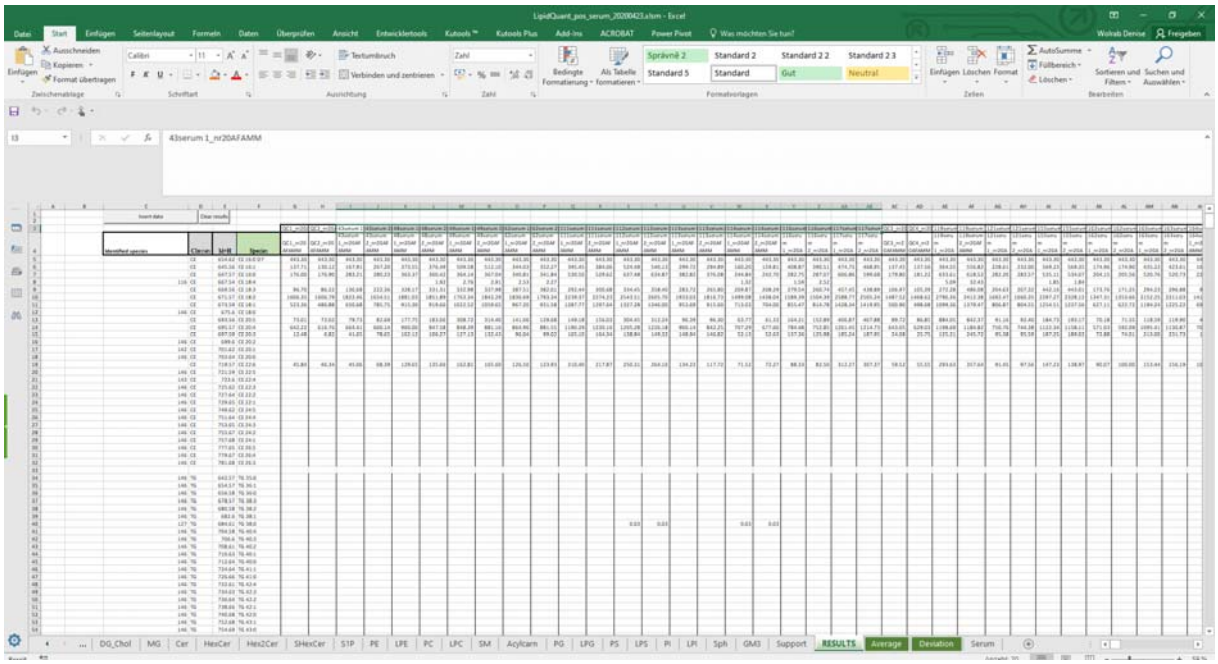
- Define multiple injections in H1 (in the presented study two injections, therefore put 2).



- Remark: Multiple injections need to be next to each other (*i.e.*, 43 serum 1 and 43 serum 2).
- Go to the “Results” sheet and press “Insert” to generate the final summary table.



- It may take some time.



- Remove empty lines and continue with statistical evaluation.