5. Query Type 2: List of Molecular Masses or m/z values

There are two ways to input this Query Type (see Figure 5.1)

Option 1: By uploading a .txt file of molecular mass or *m/z* and no intensity values (See Figure 5.2)

Option 2: By uploading a .txt file of *m/z* and relative intensity values (see Figure 5.2)

Scheme 5.1 depicts flowchart describing the syntax to input query as list of molecular masses or m/z values.

Figure 5.2 shows the snapshot of the window to input Query Type 2. Different parts of the window are explained below:

(a) Title bar: This bar gives information about the query window and the selected lipid database in use.

(b) File Upload: As the list of mass values can be queried using two different options (Figure 5.1), the input window rearranges itself accordingly. Figure 5.2 shows the change in input window for both the options.

For Option 1:

1. **Back:** This button rearranges the main window of Query Type 2 (Figure 5.1)
2. **Upload .txt file:** On clicking this button a file window opens, by which a .txt file containing Molecular Mass or *m/z* & no intensity values can be selected and uploaded.
3. **Mass Type:** After uploading the text file, Monoisotopic or Average Mass can be chosen. However, monoisotopic mass is set as default.

For Option 2:

1. **Back:** This button rearranges the main window of Query Type 2 (Figure 5.1)
2. **Relative Intensity Threshold (%):** A scrollbar has been provided to select an intensity threshold value for the uploaded text file containing m/z and relative intensity values (see Figure 5.2).

3. **Upload .txt file:** Clicking this button opens a file window to select and upload a .txt file consisting of Molecular Mass or m/z & intensity values.

4. **Mass Type:** After uploading a text file Monoisotopic or Average Mass can be selected. Monoisotopic mass is however set as default.

(c) Choose Molecular Mass / Type of Ion: A choice between six negative and eight positive ions have been provided in addition to 'Molecular Mass', for making a query using MS-LAMP.

(d) Window range: Depending on the resolution of the instrument used and the level of accuracy required by the user, it is possible to define the search within a mass range of -1.00000 to +1.00000 about the queried *m/z* or mass value. However, the default value is set at 0.5.

(e) Lipid category(ies): MS-LAMP has multiple search ability. More than one lipid category can be chosen by activating the check-buttons corresponding to lipid category (ies). (Note: refer section 9 to know about permissible combinations of check-buttons)

(f) Submit: By clicking the 'Submit' button, the above selected parameters are entered as input to the programme.

The result of the input query can be seen in Figure 5.3 consisting of different parts as explained below:

(a) Title Bar: Summary of the database in use and the input parameters are shown here.

(b) Queried Parameters: It gives review of the queried parameters.

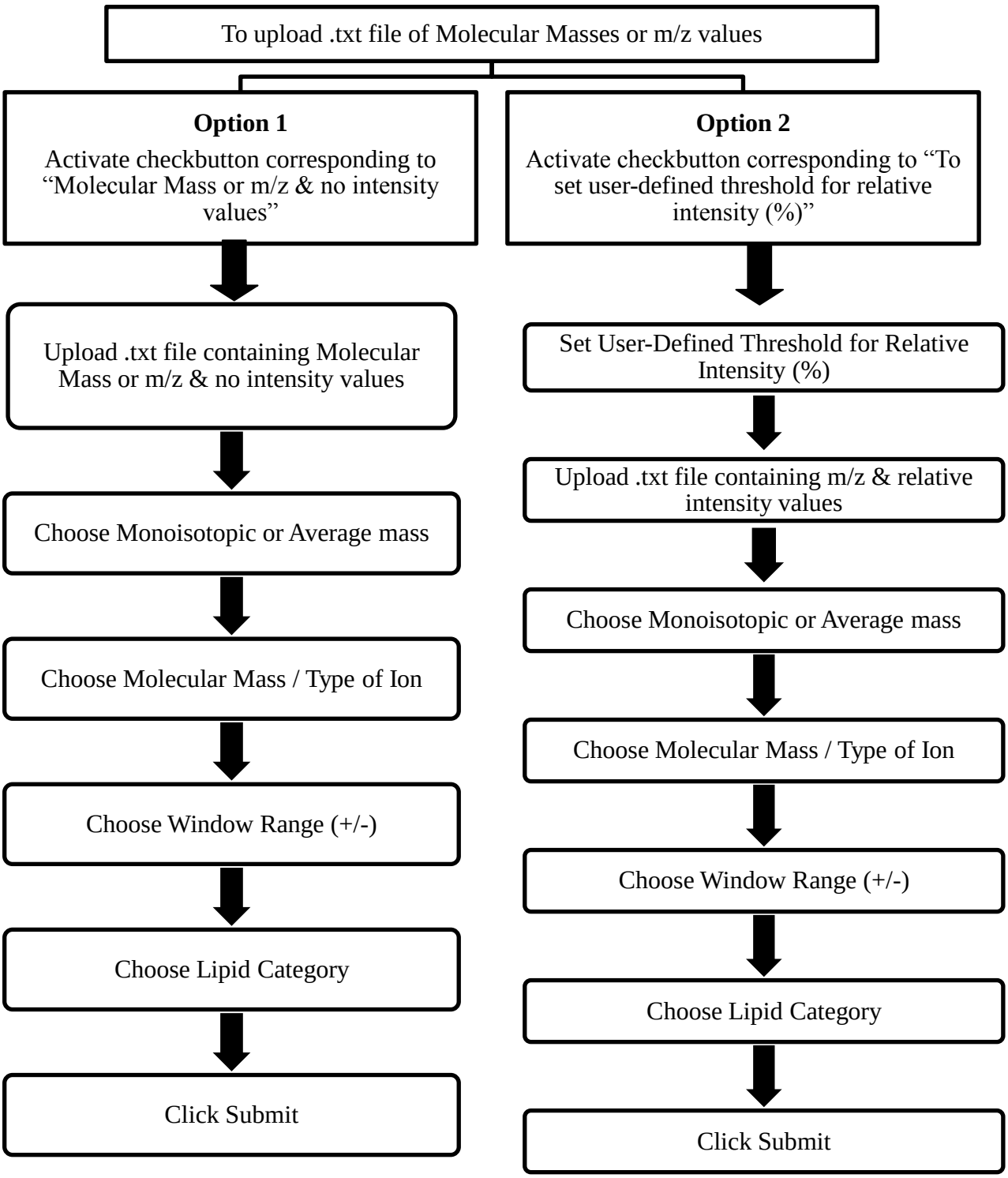
(c) Population distribution of lipids in different lipid categories chosen are shown here. These are clickable buttons that gives rise to a new window showing details (LM ID in case of General Lipidome MS-LAMP and Molecular Formulae in case of *Mycobacterium tuberculosis* (*M. tb*) Lipidome MS-LAMP) about lipid(s) obtained as result of the query (see Figure 5.4).

Upon clicking a button in this new window, molecular structure corresponding to that lipid can be viewed. In case of General Lipidome MS-LAMP, the structures are available as MD mol file, which can be opened using ChemSketch; while for *M. tb* Lipidome MS-LAMP, structures are available as .jpeg files.

(d) Output file types: Result of the query can be saved in 3 different formats:

1. Comma separated Value (.csv)
2. Text file (.txt)
3. Excel File (.xls)

(e) Search again: To start another search, this button can be clicked that would redirect to input window of Query Type 2 (Figure 5.1).



Scheme 5.1: Syntax to be followed to input **Query Type 2**: List of molecular masses or m/z values.

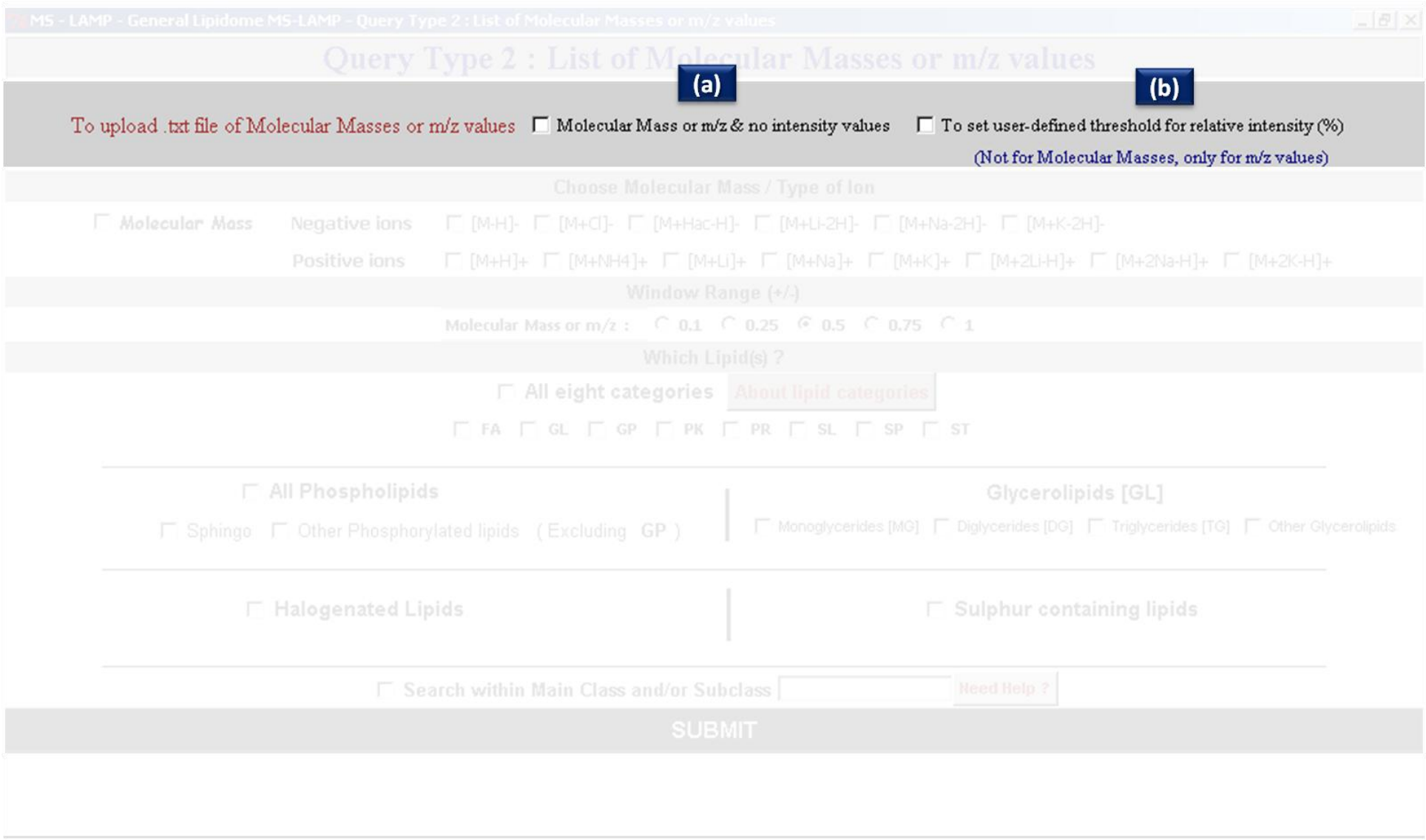


Figure 5.1: Snapshot of the main window of "**Query Type 2**: List of Molecular Masses or *m/z* values". There are two options to input: **(a) Option 1**: For uploading a text file of Molecular Masses or m/z & no intensity values, this check-button should be activated. Following this the window rearranges and buttons for uploading text file appear as shown in Figure 5.2. **(b) Option 2:** Activating this check-button allows a user to set a threshold for relative intensity values and then a text file containing *m/z* values can be uploaded (see Figure 5.2). Refer to Figure 5.5 for more details on the type of .txt files to be uploaded using these two options.

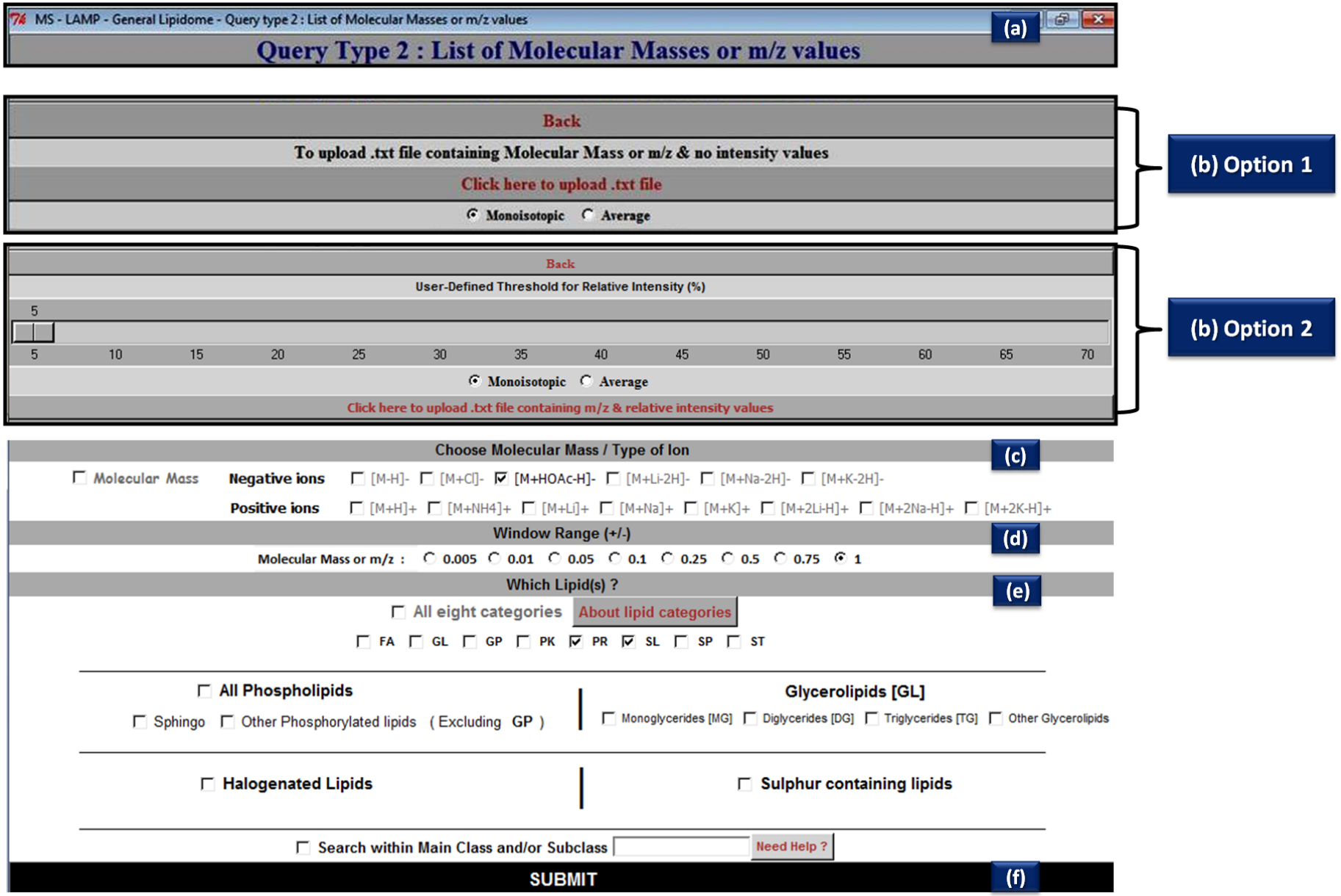


Figure 5.2: Snapshot of **Query Type 2** input window: **(a)** Title Bar **(b)** Two different features that would appear in this window depending on the choice of two different options, Option 1 & Option 2. In both the options, there is provision to choose monoisotopic or average mass. **(c)** To choose Molecular Mass/ Type of Ion **(d)** For selecting Window Range (+/-) **(e)** To choose lipid category(ies) **(f)** For submitting the chosen input parameters.

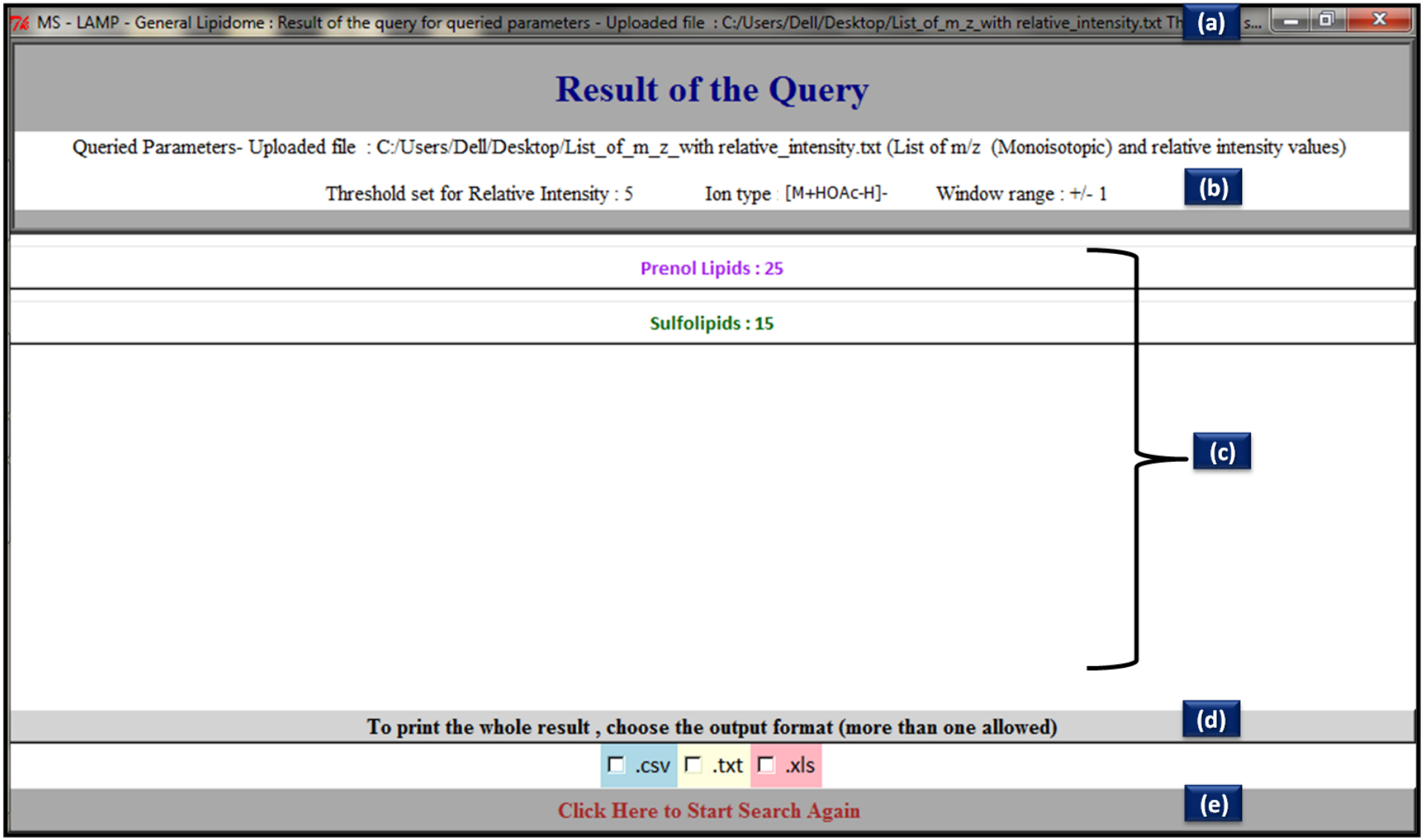


Figure 5.3: Snapshot of **Result of the Query** window displaying a glimpse of different lipid categories and number of lipids in each category that match to the queried criteria: A file containing list of *m/z* values and relative intensities was uploaded. As depicted in Figure 5.2, monoisotopic mass was selected; the threshold for relative intensity was set to 5; window range of 1 and type of ion, $[M+Hac-H]^-$ were chosen; and the search was directed to prenol and sulfur-containing lipids. It can be noted that corresponding to the input parameters, there are 25 prenol lipids and 15 sulfolipids. **(a)** Title Bar **(b)** Queried Parameters **(c)** Chosen lipid category(ies) **(d)** To choose different file types for saving the result **(e)** For beginning a new search, this button is to be clicked. The result obtained on querying General Lipidome MS-LAMP is shown here.

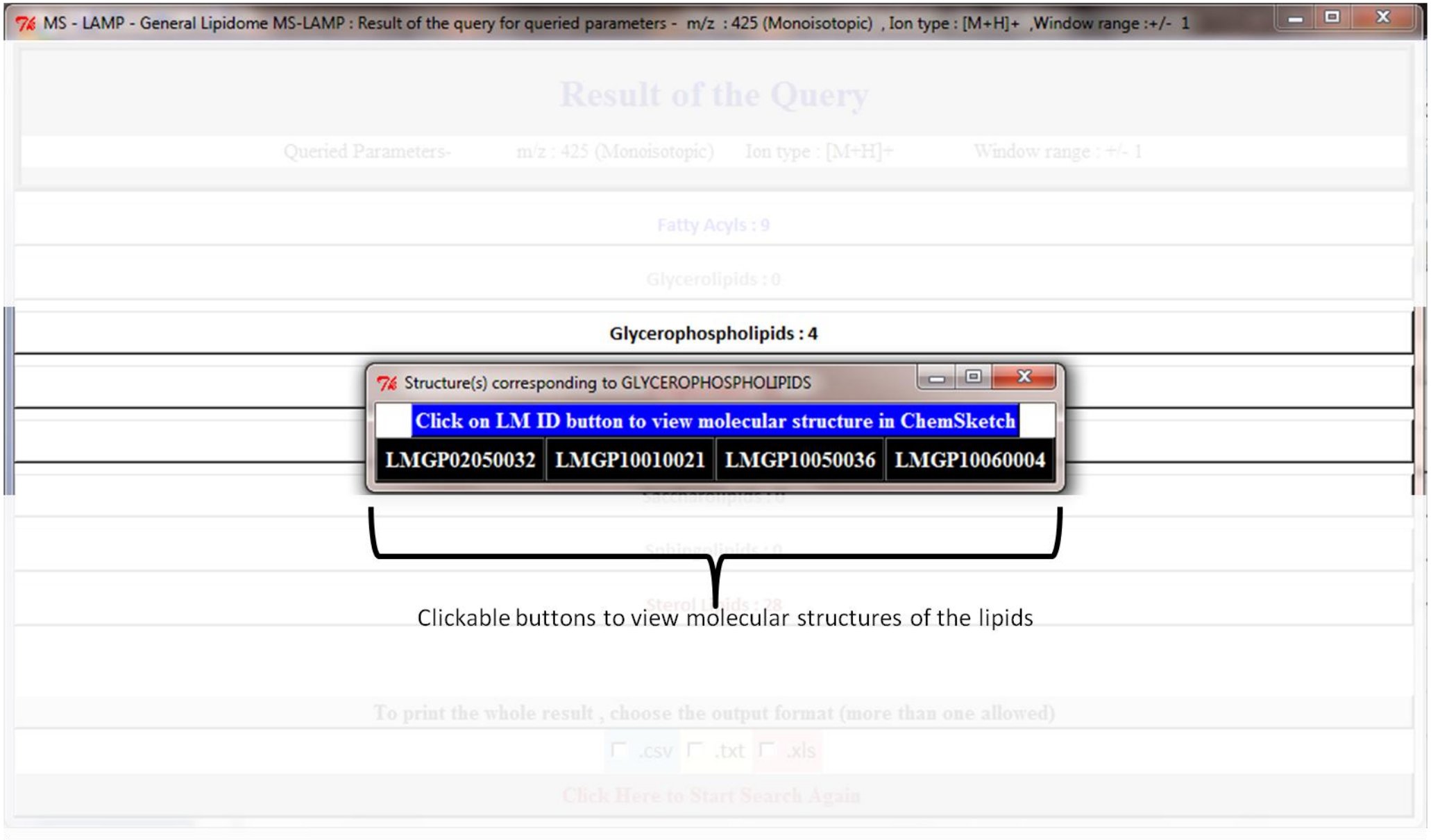


Figure 5.4: Figure showing snapshot of the window containing LM ID buttons, which can be clicked to view molecular structure of lipids that are obtained as result of the query , shown in Figure 5.3 (**Refer section 4 for more details on this**).

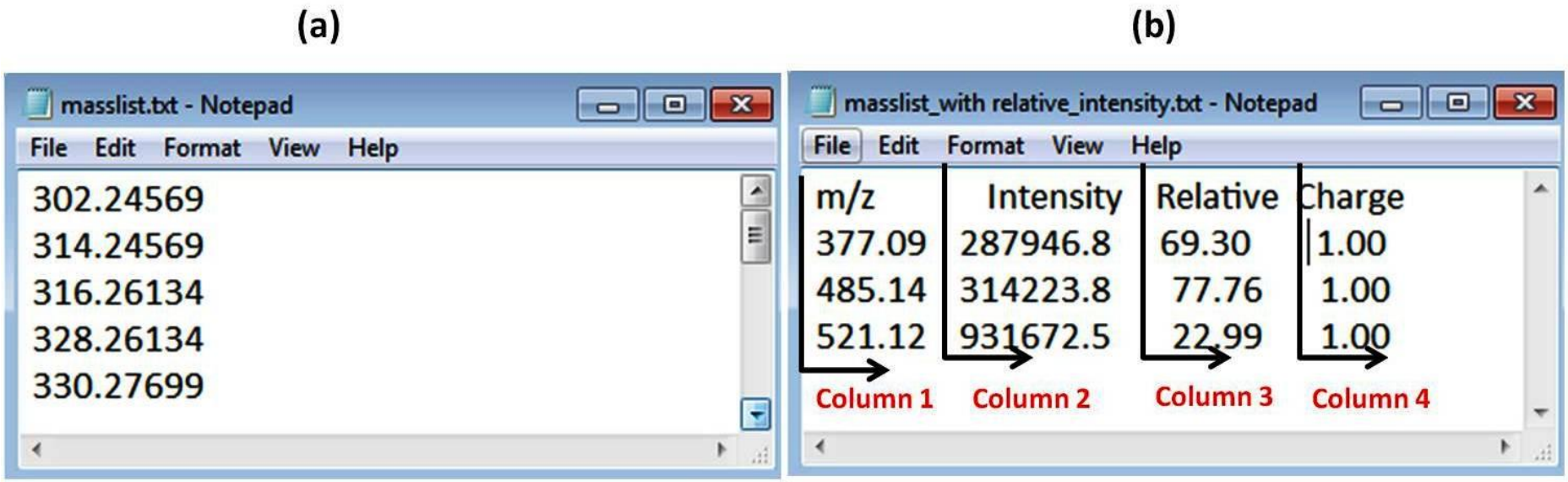


Figure 5.5: Files suitable to input **Query Type 2**: **(a) Option 1**: Example of a text file containing Molecular Mass or *m/z* and no intensity values. This file contains a single column and can be used for making a query corresponding to only "Molecular Mass or *m/z* & no intensity values" (Refer Figures 5.1 & 5.2) **(b) Option 2**: Example of a text file containing *m/z* and relative intensity values (To set user-defined threshold for relative intensity (%)) (Refer Figures 5.1 & 5.2). In this case, the .txt file to be submitted must have four columns comprising *m/z*, absolute intensity, relative intensity and charge, respectively in column numbers 1, 2, 3 and 4, where each column is separated by a tab (as shown here). This file is suitable for the entering query using **Option 1** as well, i.e. for making a query using "Molecular Mass or *m/z* & no intensity values". In that case, the software will consider column 1 only and ignore other three columns.

Note: Regarding submitting query by **Option 2**; where the user sets a threshold for relative intensity (%) (See Figure 5.2): When this option is chosen, the programme considers values in columns 1 (*m/z* values) and 3 (Relative Intensity values) only, while the entries in columns 2 and 4 are disregarded.