

MRMQuant

User Guide

Version 2.0
2024-10-02

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Part A. System Requirement and Installation

Minimum System Requirement:

Windows 10 or higher

CPU: Intel® Core™ i5 CPU

Memory: 8 GB

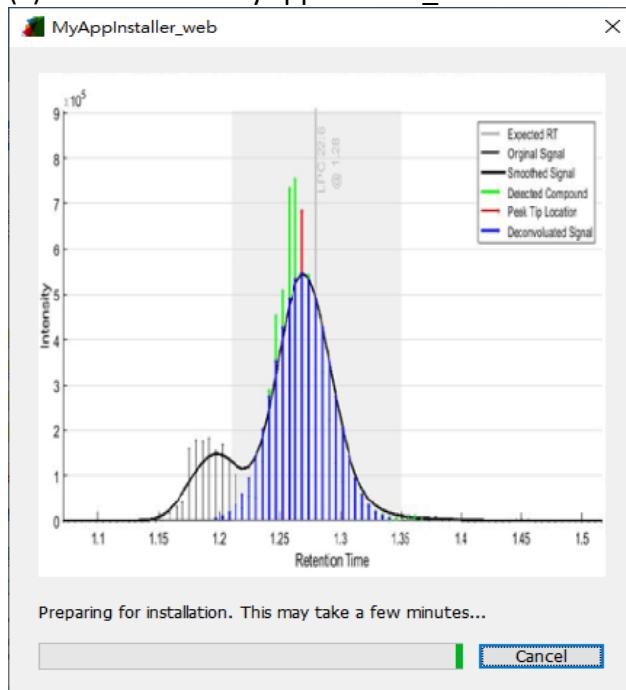
System type: 64-bit operation system

Recommended screen resolution: 1920×1080

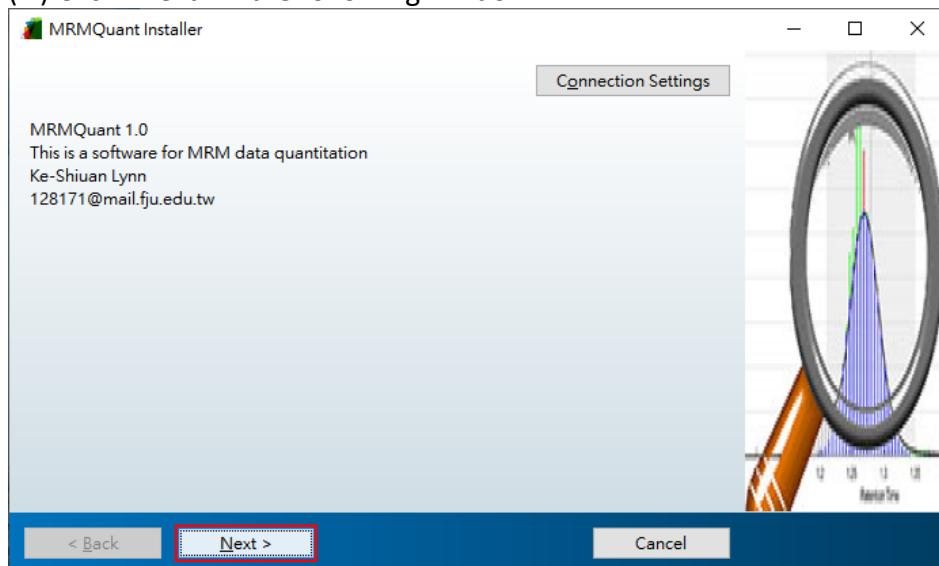
Installation Steps

(i) Make sure the computer is internet assessable (for downloading and installing MATLAB Runtime).

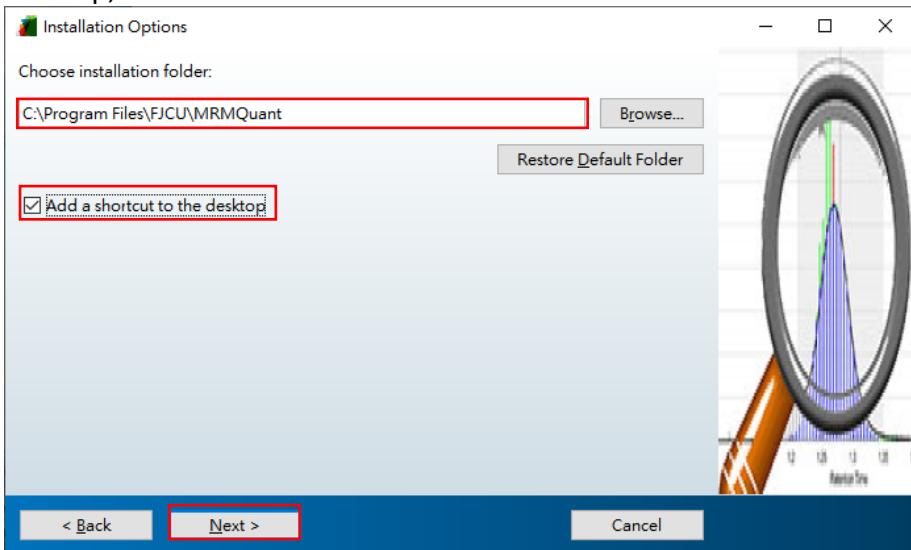
(ii) Execute the “MyAppInstaller_web.exe”.



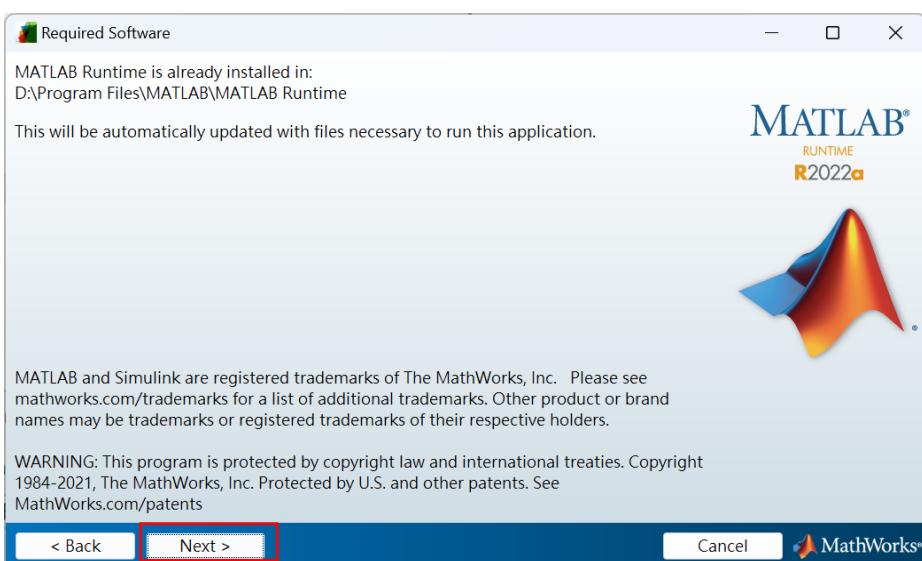
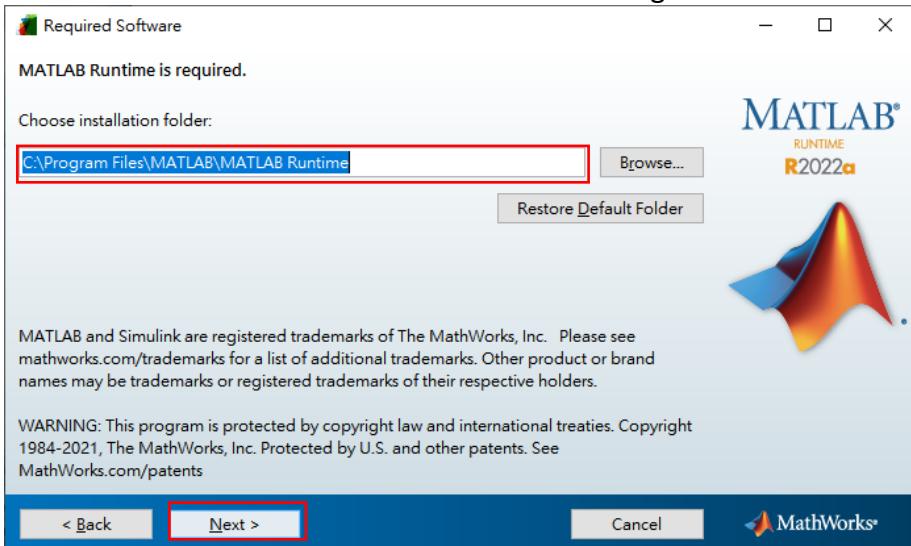
(iii) Click "Next" in the following window.



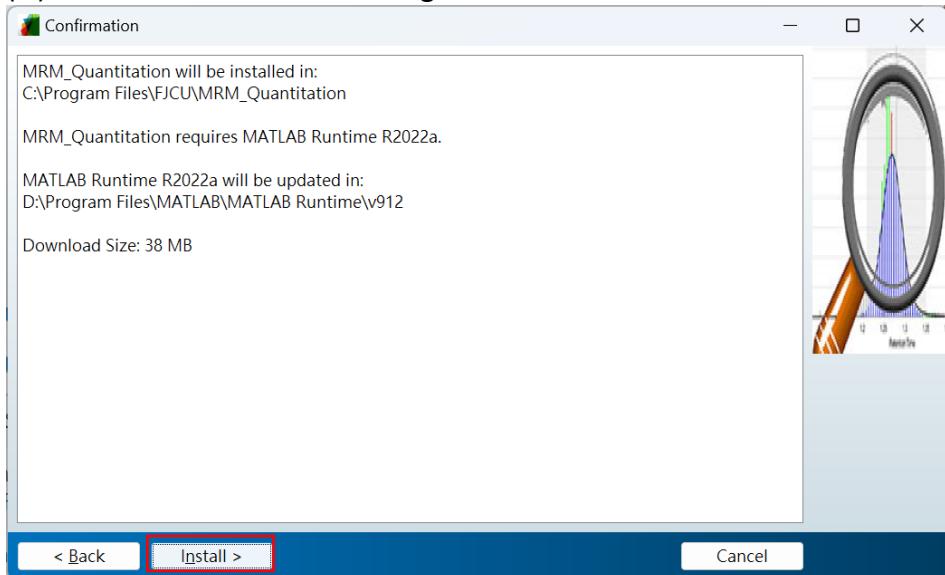
(iv) Select the folder in which the MRMQuant is to be installed, choose whether to create a shortcut to the desktop, and then click "Next".



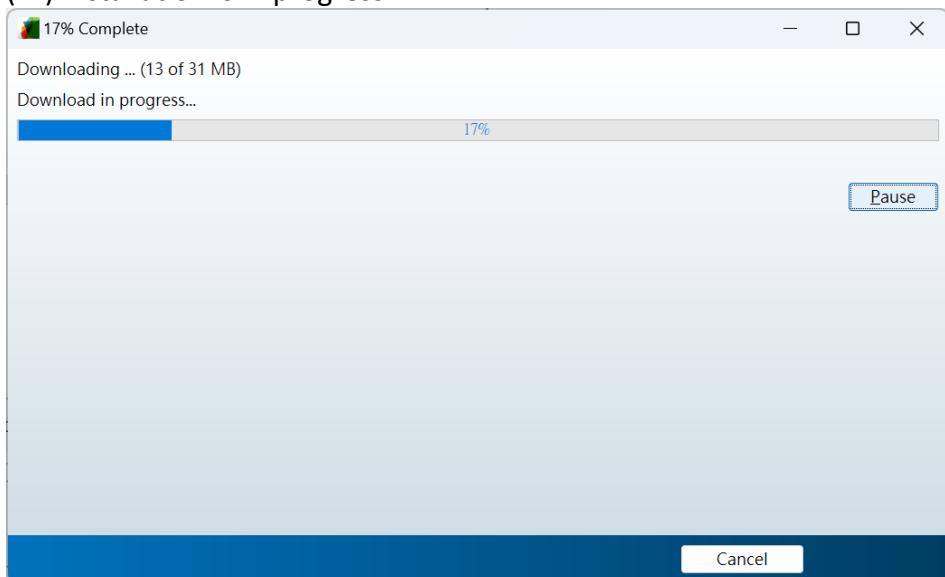
(v) If no MATLAB Runtime is ever installed, select the folder in which the MATLAB Runtime is to be installed and click "Next". Otherwise, if a different version of MATLAB Runtime has been installed, the program will install the MATLAB Runtime R2022a to the existing folder.



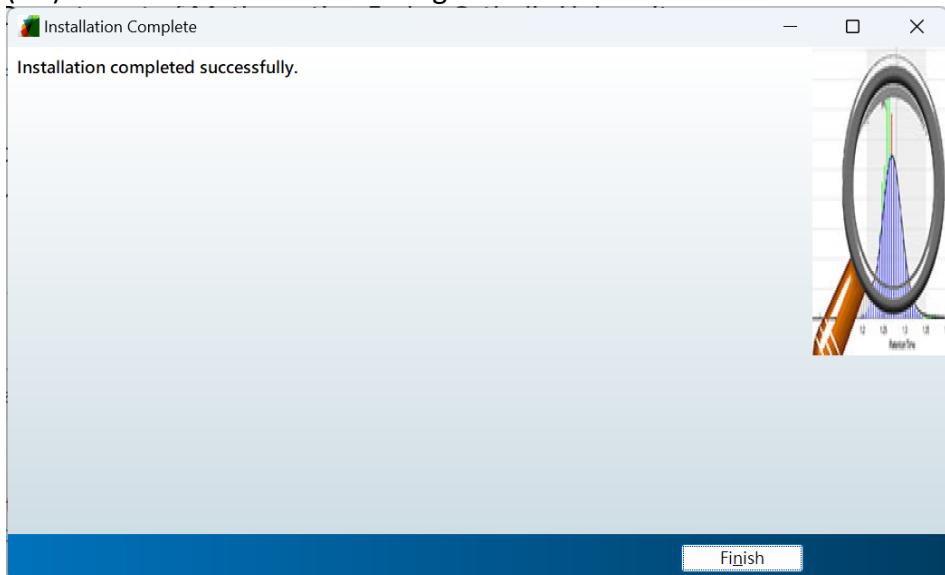
(vi) Click "Install" in the following window



(vii) Installation is in progress

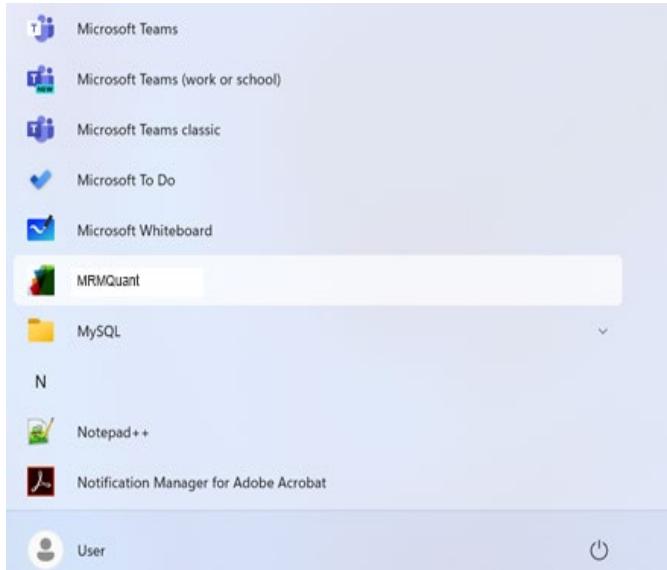


(viii) Click "Finish" in the following window to close the window.

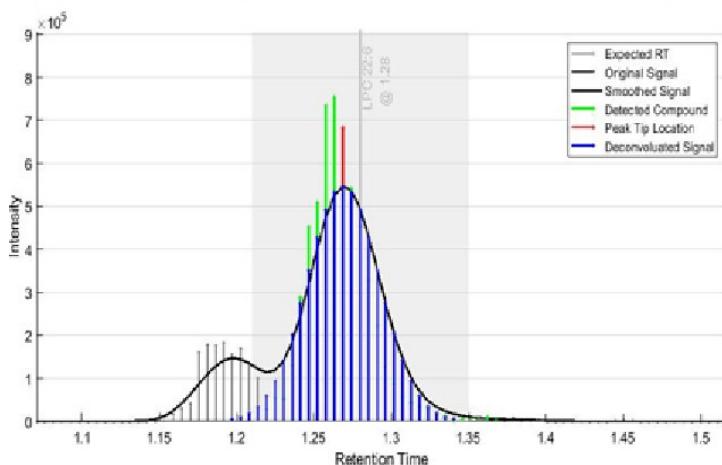


(ix) Copy the “data folder” and the “MRM_Method.csv” to your test data folder (optional).

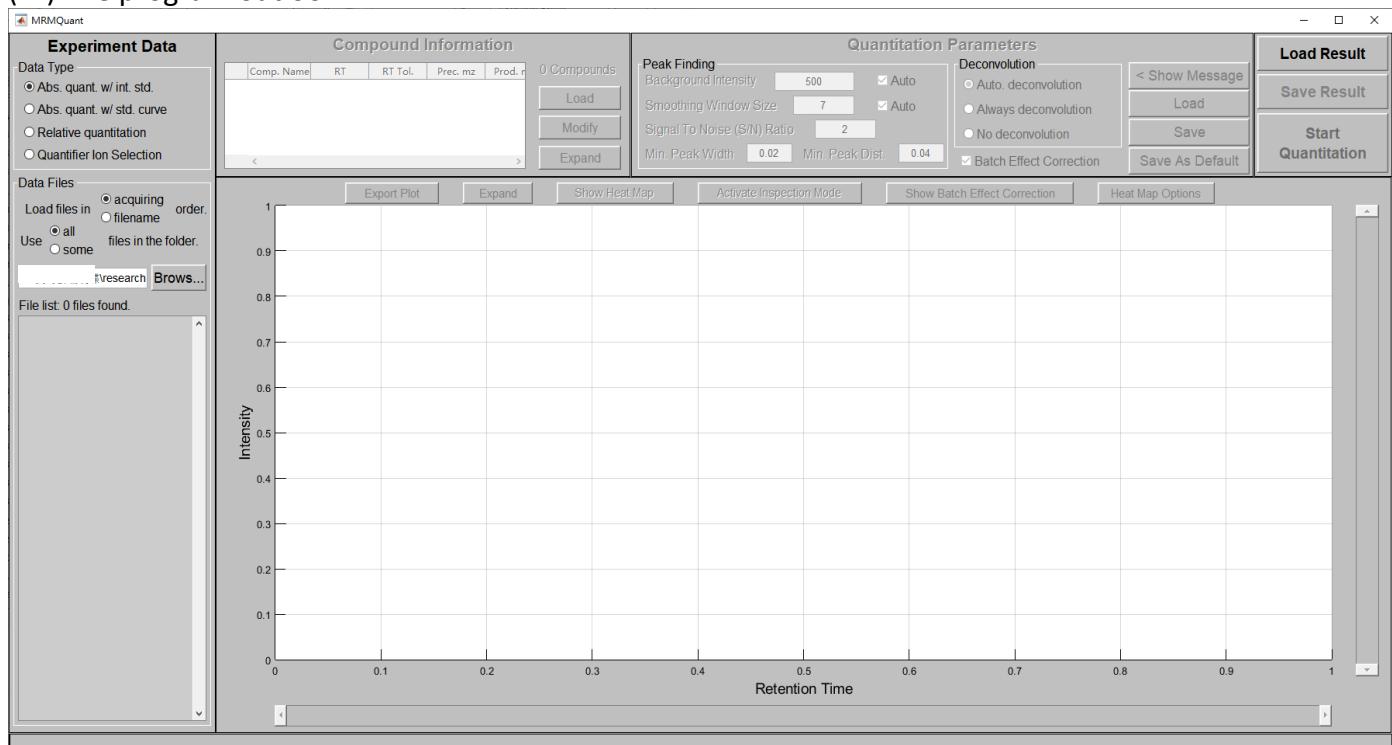
(x) Execute MRMQuant from the application list.



(xi) The starting logo



(xii) The program outlook



Contact Information

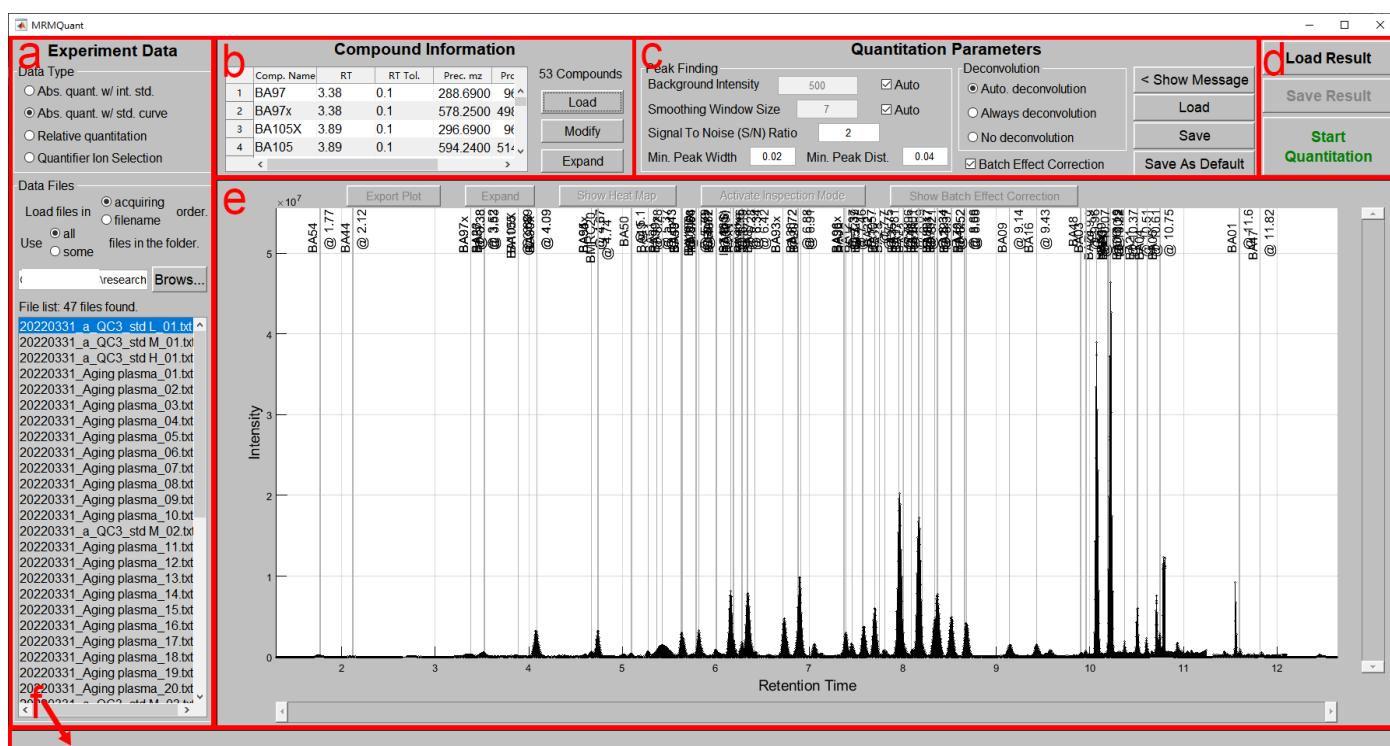
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System Overview

Figure 1 shows the key panels in the MRMQuant GUI. To perform quantitation to a set of sample files, the user needs to provide data and information in panels (a) through (c) in sequence. At the beginning, only the “Experiment Data” panel (panel (a)) is activated and the user selects an experiment type and loads the sample files in the panel. The files are loaded in the acquiring order for the subsequent batch effect correction. After all the sample files being loaded, the total ion chromatogram (TIC) of the first file will be shown in panel (e). Users can view the TIC of another file by clicking on its file name in panel (a). At this stage, the “Compound Information” panel (panel (b)) will be activated allowing the method file, which providing the compound-related information, to be loaded herein. Upon loaded, the compound names and their corresponding locations (RT) will be noted in the TIC. Then, the “Quantitation Parameters” panel (panel (c)) will be activated and the parameters including peak finding, peak deconvolution, and batch effect correction are to be input herein. Finally, the user can press the “Start Quantitation” button to start the quantitation process. During the quantitation process, the quantitation result in the heatmap form will be displayed in panel (e) and the complete percentage will be shown in panel (f).



if required. For each quantitated compound, absolute concentration can be converted from the computed abundance via its corresponding IS of known concentration and/or a standard curve generated via a series of standard compounds of known concentrations. Similar quantitation procedure will be performed on the SC MRM files and absolute concentrations are to be converted from the computed abundances via the SCs. Finally, the quantitation result, including calculated abundances, concentrations, quantitation status, and retention times, and SC functions for all the targeted compounds will be exported to a Microsoft Excel file. In addition, a MATLAB mat file will also be generated, which can be loaded into MRMQuant in the future to inspect and correct the existing quantitation result.

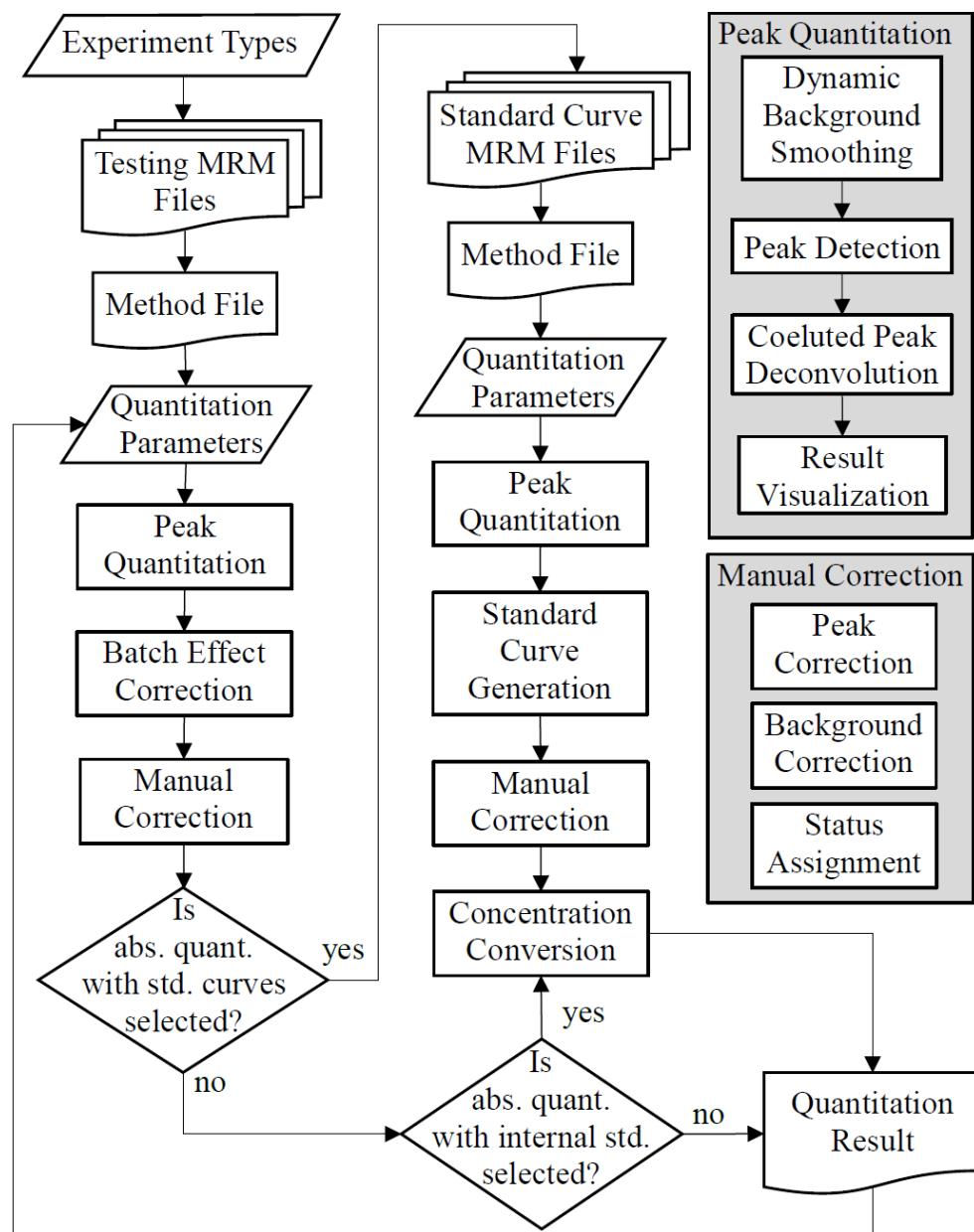


Figure 2. System Flowchart. The gray boxes in the right-hand side list the detailed functions in the peak quantitation and in the manual correction modules, respectively.

Part B. Step-by-Step illustration

Start MRMQuant

MRMQuant can be invoked by two methods, depending whether MATLAB has been installed in your computer. All the required files can be downloaded from <https://github/MRMQuant>. If MATLAB is not installed in your computer, download the files from the executable files from <https://github/MRMQuant/program/executable>. Double click on the "MRMQuant.exe" file in the folder to start MRMQuant, as shown in Figure 1(a). On the other hand, if MATLAB has already in your computer, download the MATLAB files from https://github/MRMQuant/program/MATLAB_source_code and then start MATLAB and run MRMQuant.m (as shown in Figure 1(b)) in the MATLAB editor. A graphical user interface should appear as shown below. At this stage, the **Experiment Data** panel will be activated for users to input MRM data-related information, as indicated by the red block in Figure 2.

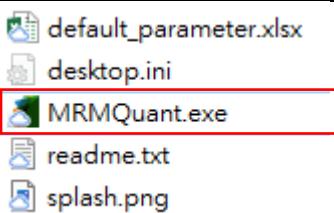
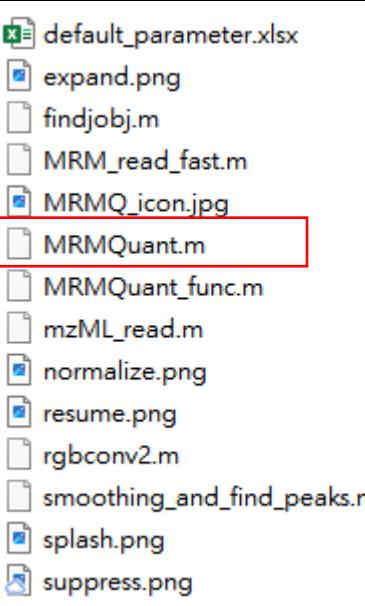
Execute MRMQuant via the executable file	Execute MRMQuant via MATLAB .m file
 (a)	 (b)

Figure 1. Two methods to start MRMQuant. (a) Users with MATLAB (b) Uses without MATLAB.

Select an experiment type

In the "Experiment Data" panel, users need to first select a data type, as shown by the red block in Figure 3. In MRMQuant, there are 3 options: absolute quant. w/ int. std, absolute quant. w/ std. curve, and relative quantitation. The first type was designed to compute concentrations (e.g., mol./mL.) of targeted compounds in the MRM sample files via internal standards of known concentrations intentionally put in the samples. Similar to the first type, the second type was designed to compute concentrations via standard curves constructed using another set of sample files with targeted compounds of known concentrations. The third type, on the other hand, was designed to compute relative abundances of the targeted compounds. The abundance means the integrated peak signals in an ion chromatogram in an MRM sample file.

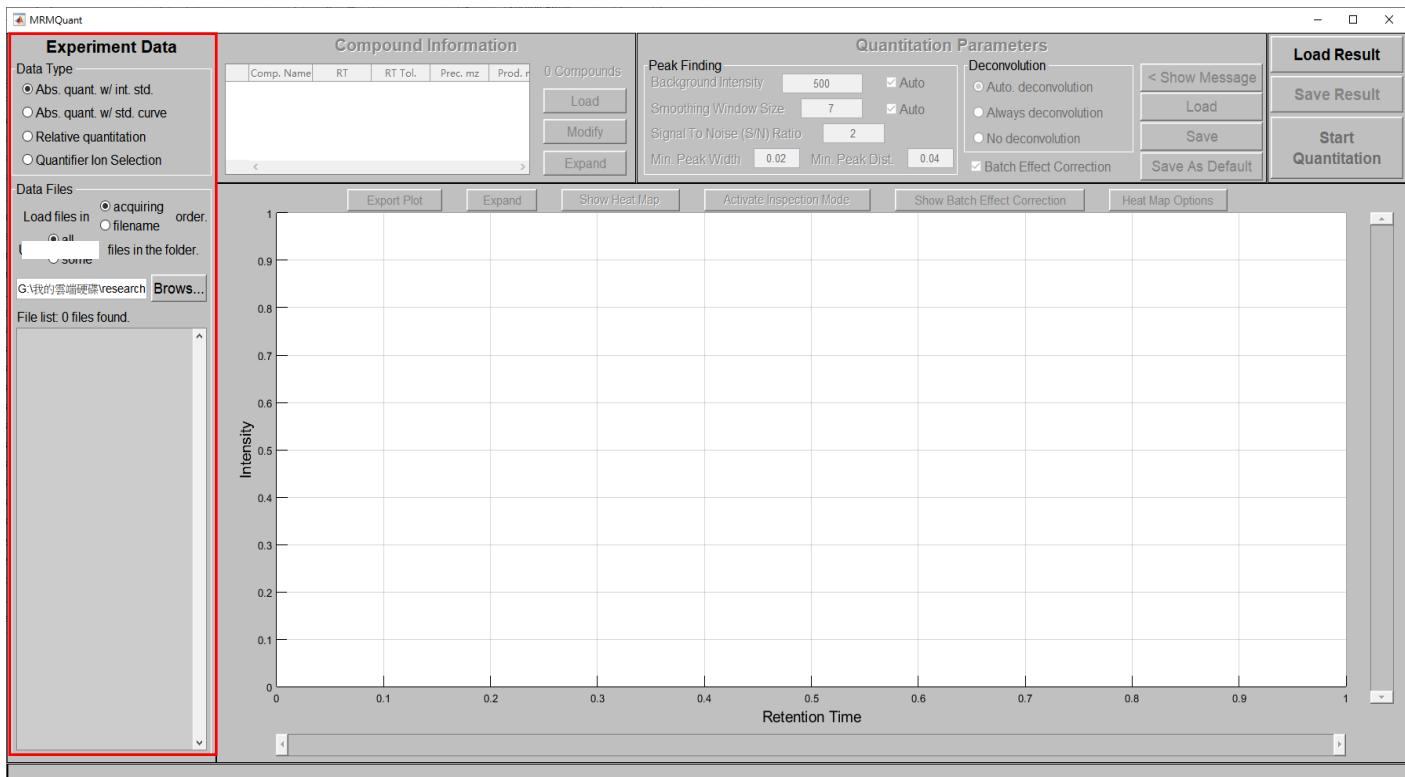


Figure 2. The graphical user interface of MRMQuant and the Experiment Data panel.

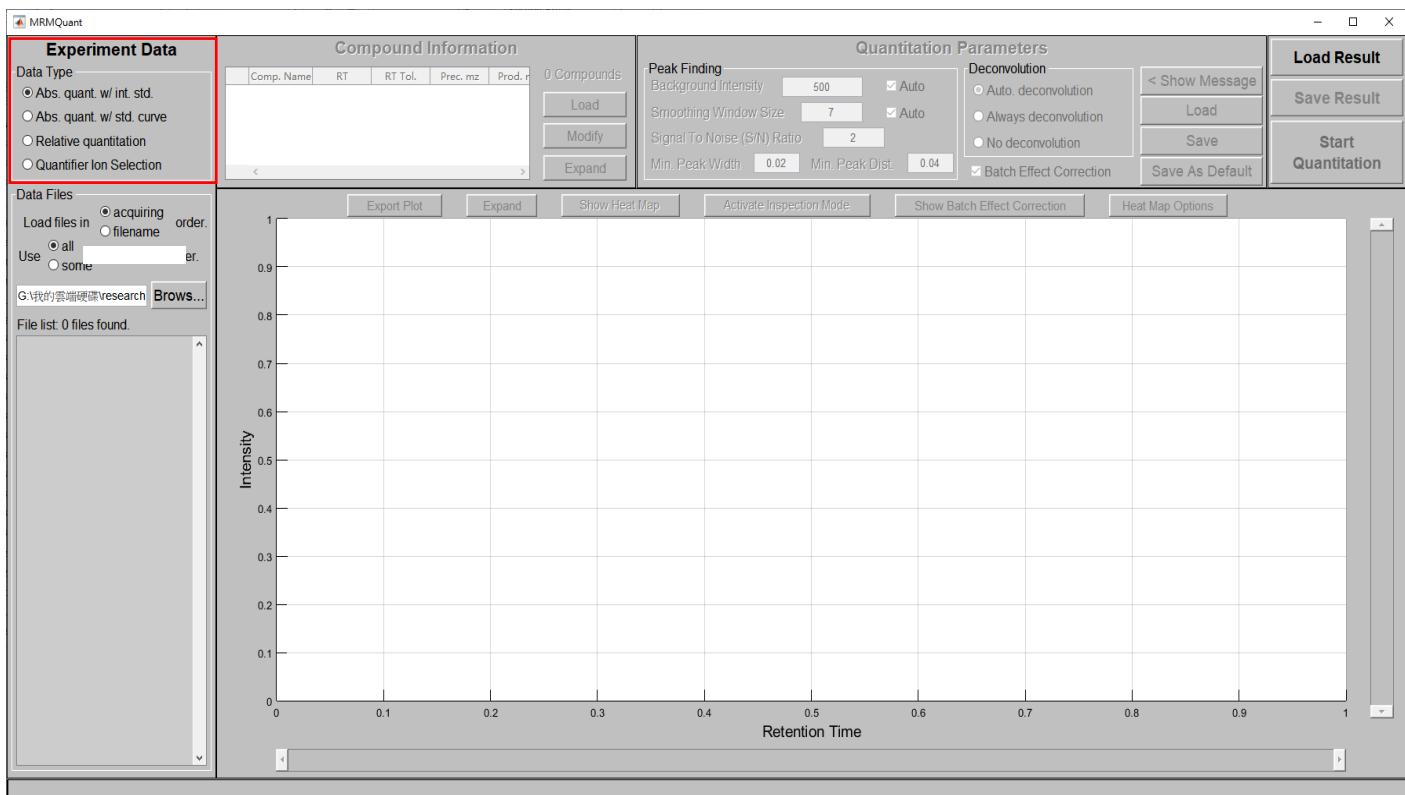


Figure 3. The data type panel in MRMQuant.

Load MRM sample files

Before the sample data is loaded into the system, users should select the order of how files are to be loaded, in acquiring or in file name order. The file order is important if batch effect correction is to be performed during the quantitation (refer to the batch effect connection section). In addition, users can also determine how many files in the specified folder are to be loaded, full or partial files in the folder. Once the file loading order and amount are determined, click the "Browse..." button in the left panel to select the folder/files to

be quantitated, as shown in Figure 4. The program should start to load all the sample files in the folder. A progress bar at the bottom of the interface provides the progress of the file-reading process (Figure 5). After all the data files are loaded, users can click on a file name in the left panel to inspect its corresponding total ion chromatogram (TIC).

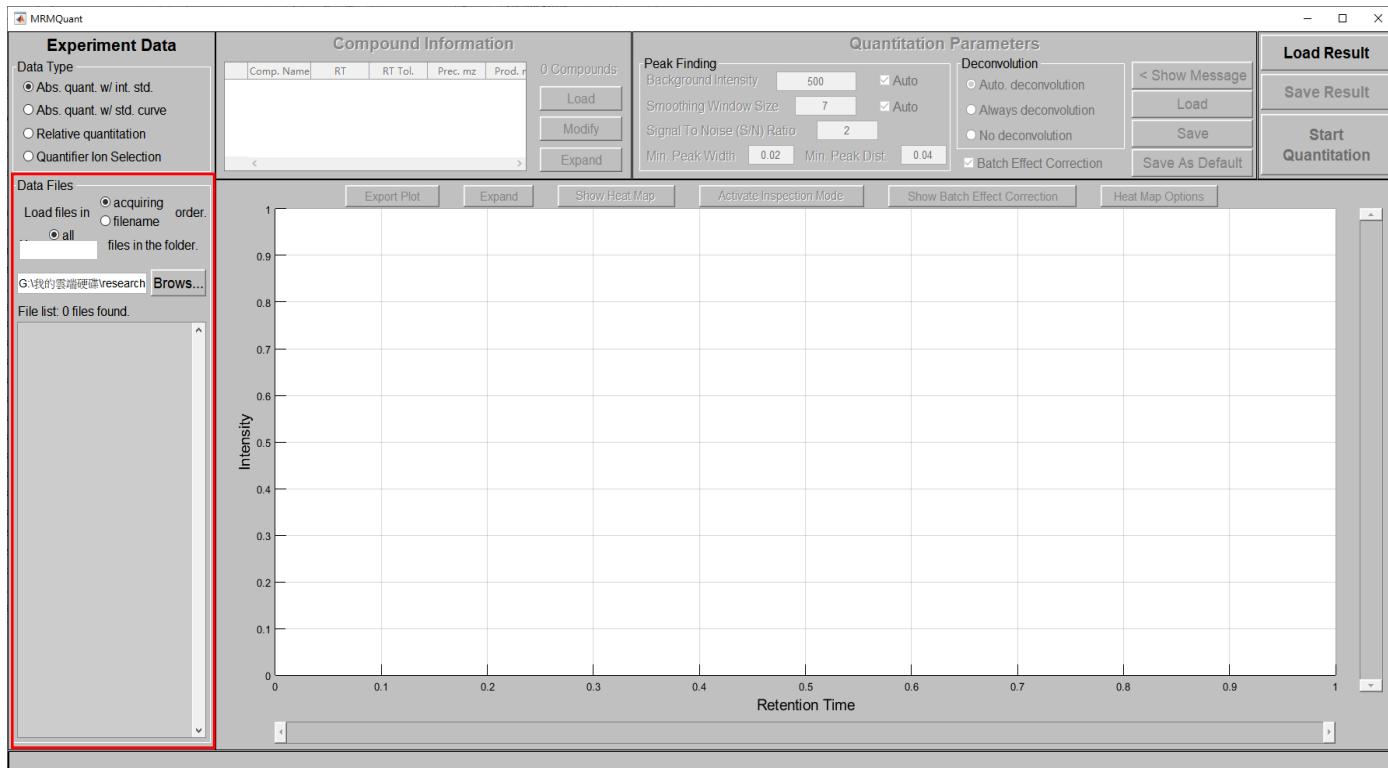


Figure 4. The “Data Files” panel for the import of MRM sample files

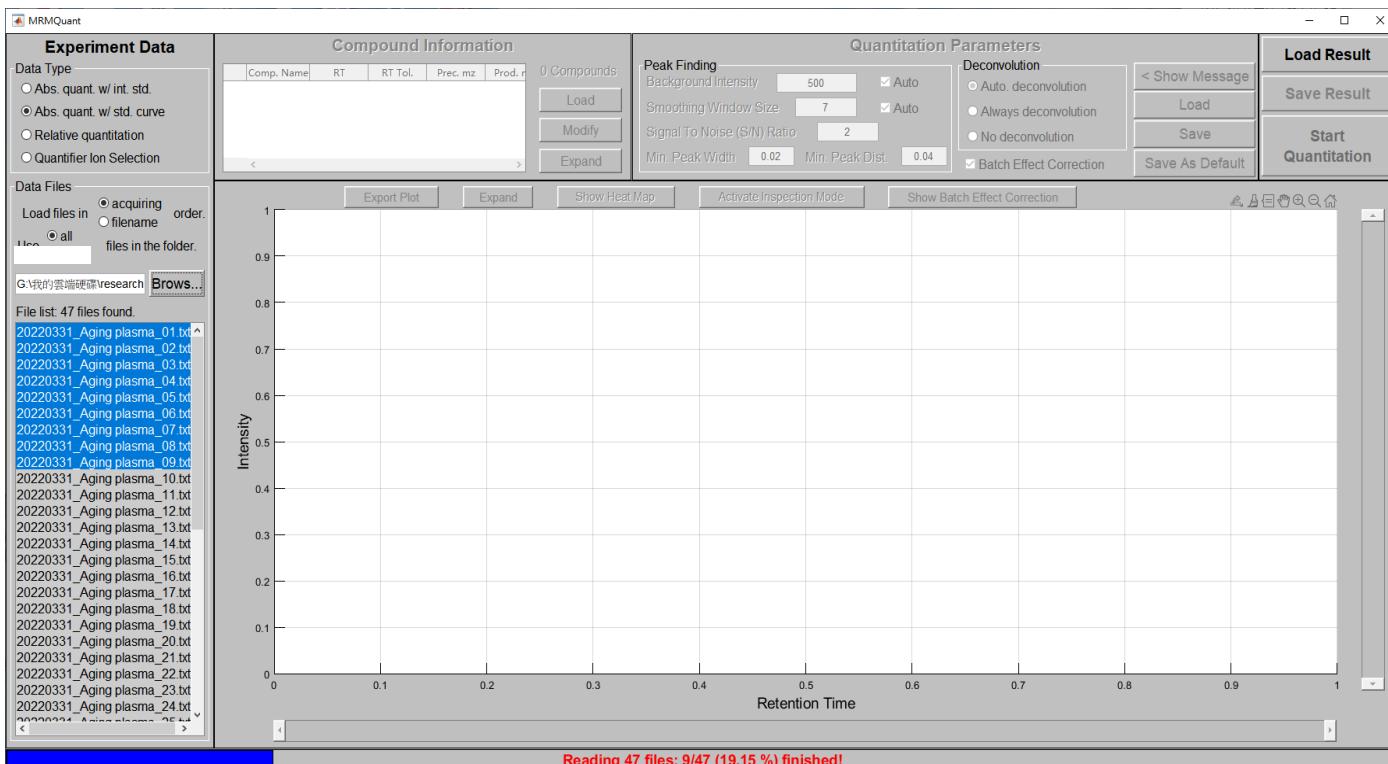


Figure 5. The progress bar at the window bottom reveals the file loading progress

Load/Modify compound information

Once the sample files are completely loaded into the system, the "Compound Information" panel will be activated. Press in "Load" button (as shown in Figure 6) to import the method files (in .csv file format) containing the targeted compound information. The required information for the two types of absolute quantitation are shown in Figure 7(a) and 7(b). For relative quantitation, the required columns are the first five columns in Figure 7(a). Noted that the MRMQuant allows multiple compounds in an ion chromatogram. However, compound names should be concatenated with "_" and RTs should be concatenated with ";" in the method file, as shown in Figure 7(b). The example method files can also be found in Appendix A1-A4 or https://github/MRMQuant/test_datasets. Note that, the column names in the method file do not need to be the same as those in the example, however, the data order has to be the same for the MRMQuant to read the correct information. In addition, MRMQuant will check the "Prec. mz" (precursor ion m/z) and "Prod. mz" (product ion m/z) for each ion chromatogram with those in the sample files. If the number of ion chromatograms or the m/z values are not matched in the two files, an error message will be shown and the method file will not be loaded. Furthermore, the internal standard of an "Abs. quant. w/ int std" experiment can be specified in an addition column as shown in Figure 7(a) or can be noted by adding "(IS)" after the compound name in the "Comp. Names" column.

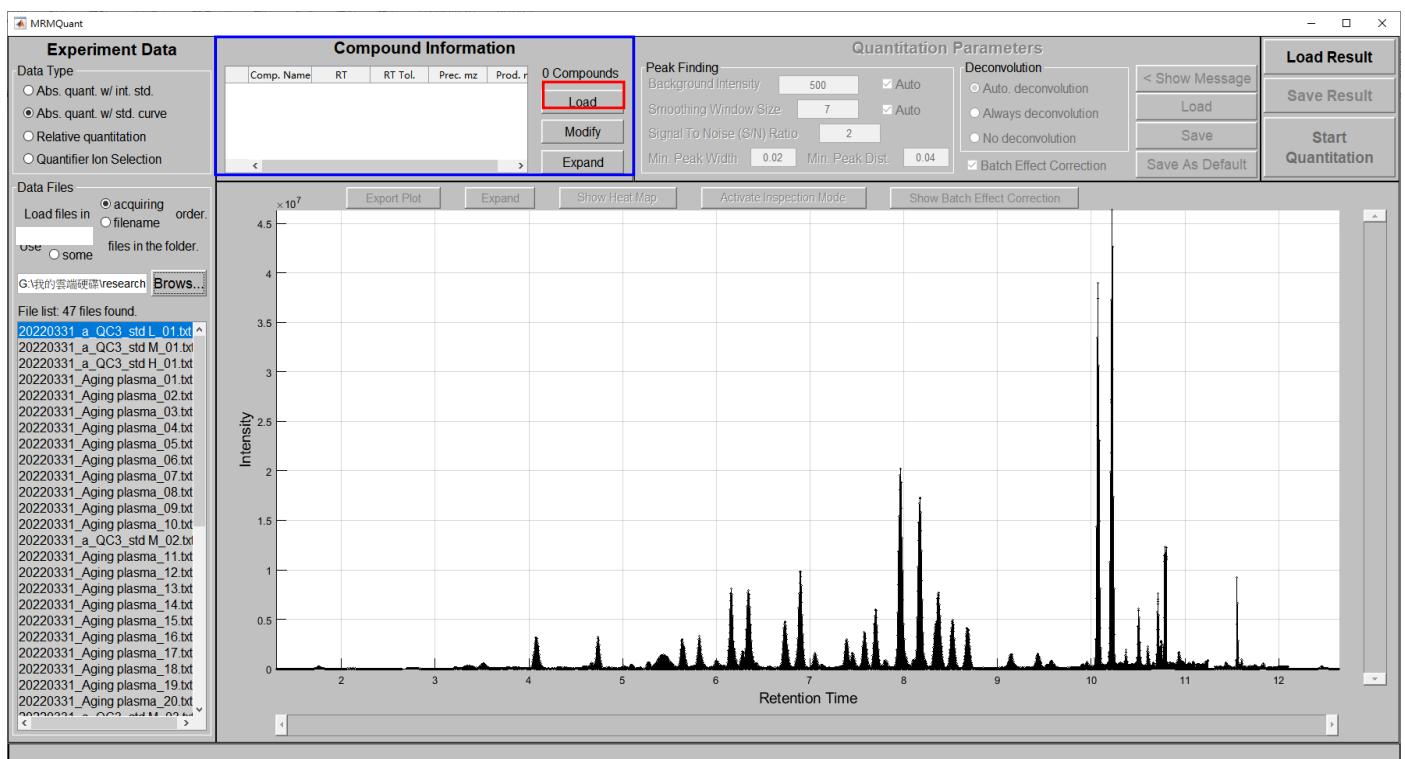


Figure 6. The "Compound Information" panel for MRM method import and modification

CompNams	RT	RT Tol.	Prec. mz	Prod. mz	IS	Conc	Comp. Names	RT	RT Tol.	Prec. mz	Prod. mz	IS
LPE 16:0	1.6	0.2	454.2928	313.2928	LPE 18:1 (d7)	1	BA97	3.38	0.1	288.69	96.954	IS71(IS)
LPE 18:2	1.34	0.2	478.3085	337.3085	LPE 18:1 (d7)	1	BA97x	3.38	0.1	578.25	498.29	IS71(IS)
LPE 18:1	1.7	0.2	480.3085	339.3085	LPE 18:1 (d7)	1	BA105X	3.89	0.1	296.69	96.954	IS71(IS)
LPE 18:0	2.2	0.2	482.3241	341.3241	LPE 18:1 (d7)	1	BA105	3.89	0.1	594.24	514.28	IS71(IS)
LPE 18:1 (d7)	1.68	0.2	487.3085	346.3085	LPE 18:1 (d7)	1	BA99_BA100	5.43;5.79	0.1	288.69	96.954	IS71(IS)
LPE 20:4	1.29	0.2	502.2928	361.2928	LPE 18:1 (d7)	1	BA99x_BA100x	5.43;5.79	0.1	578.25	498.29	IS71(IS)
LPE 22:6	1.23	0.2	526.2928	385.2928	LPE 18:1 (d7)	1	BA96	7.37	0.1	280.69	96.954	IS71(IS)
LPC 14:0	1.08	0.2	468.3085	184.07	LPC 18:1 (d7)	45	BA96x	7.37	0.1	562.25	482.29	IS71(IS)
LPC(P-16:0/0:0)	1.72	0.2	480.3449	104.08	LPC 18:1 (d7)	45	BA01	11.6	0.02	359.29	359.28	IS71(IS)
LPC(O-16:0/0:0)	1.78	0.2	482.3605	104.08	LPC 18:1 (d7)	45	BA03_BA04	9.96;10.61	0.05	373.27	373.27	IS71(IS)

(a)

(b)

Figure 7. Required columns in the method file. (a) Method file columns for absolute quantitation with internal standards. (b) Method file columns for absolute quantitation with standard curves.

Upon loaded, the compound names and their corresponding RT will be noted in the TIC, as shown in Figure 8. Users can modify the content of the MRM method file by clicking on the "Modify" button beneath the "Load" button. A new window consisting of an editable table will appear (Figure 9(a)). Each cell in the table can be modified. If a fixed RT difference is to be used, input the number in the top entry, press enter, and all the RT tolerances in the table will be changed simultaneously. When all the modifications are done, click on the "Ok" button at the bottom of the window to apply all modifications in the subsequent computations. Users can also click on the "Expand" button to enlarge the compound information panel to display more information in the window, as shown in Figure 9(b). After inspection, users can click on the "Shrink" button to resume to the original window.

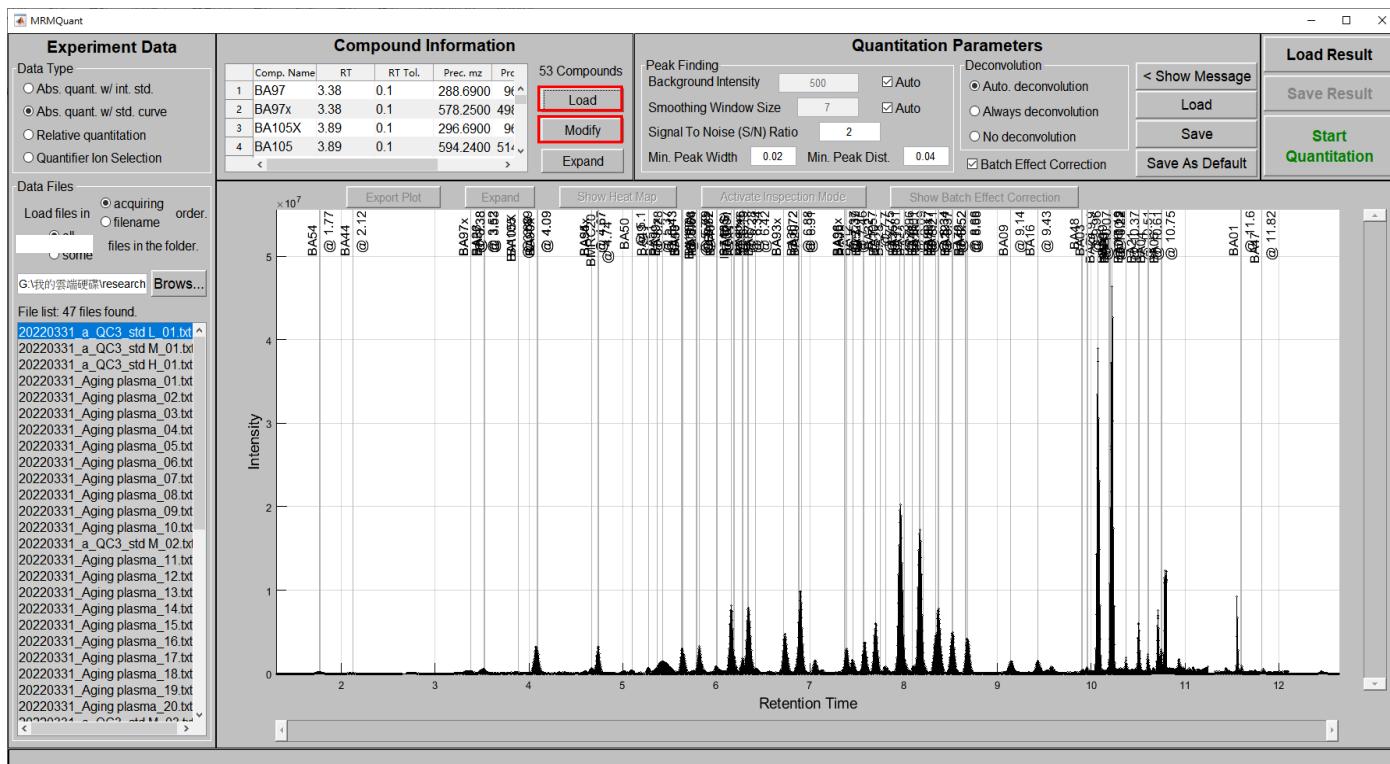


Figure 8. The TIC plot is noted with compound names and their corresponding retention times after the method file is loaded.

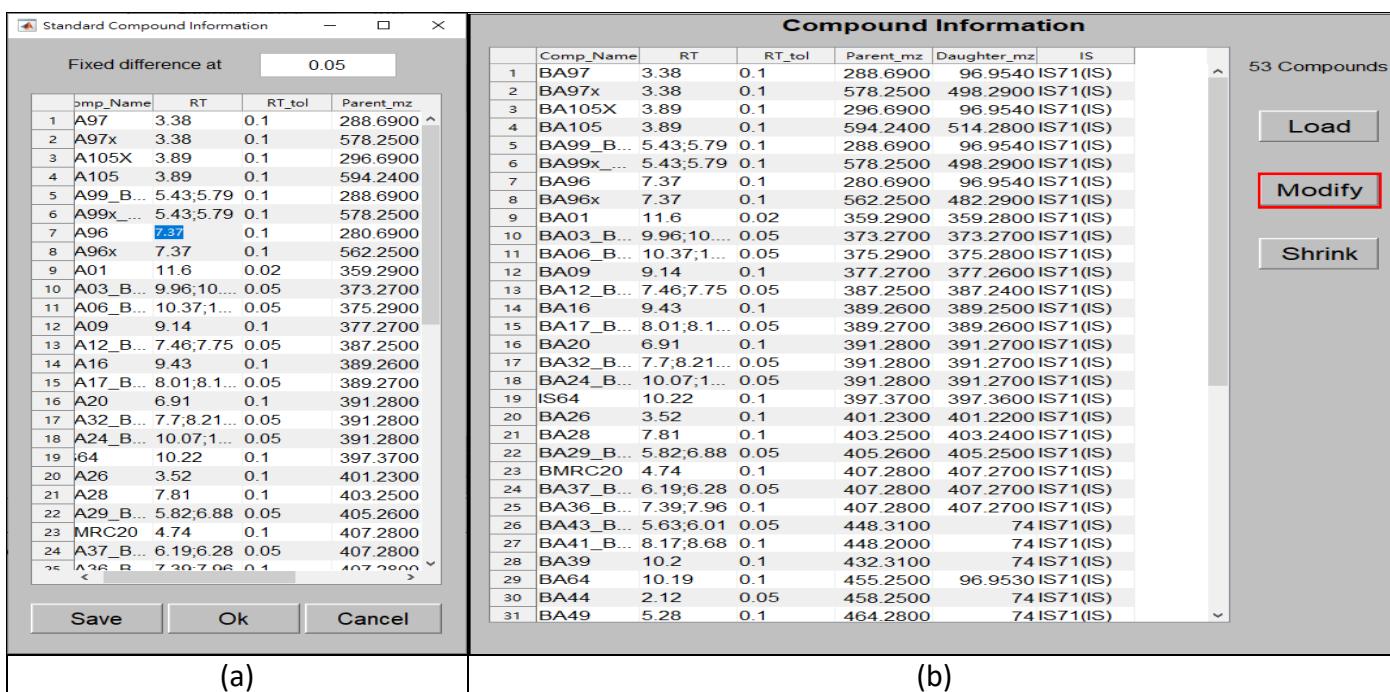
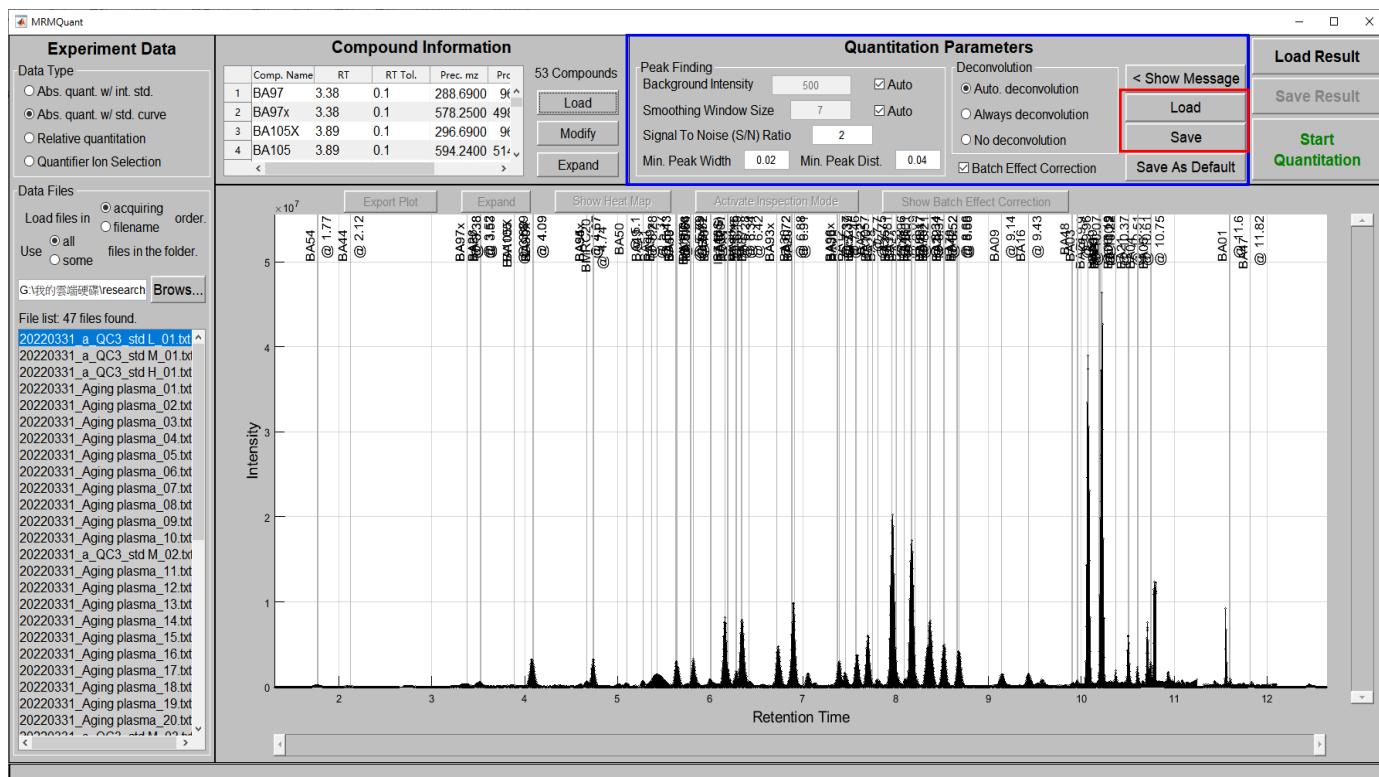


Figure 9. Pop-up windows for the compound information. (a) The editable window when the "Modify" button is pressed. (b) The window when the "Expand" button is pressed.

Adjust quantitation parameters

After the method file is loaded, the "Quantitation Parameters" panel will be activated, as shown in Figure 10. Parameters related to three major tasks in quantitation, including peak finding, deconvolution, and batch effect correction, are to be determined. For peak finding, users can adjust parameters such as background intensity (click on "auto" if it is to be determined by the system's build-in method), signal-to-noise (S/N) ratio, and the minimum peak width and minimum peak distance for coelution determination. For deconvolution, users can select automatic deconvolution, always deconvolution, or no deconvolution when a coeluted peak related to the targeted compound is found. Finally, users should determine whether to perform batch effect correction or not based on whether multiple batches of samples are used and whether QC samples are included in each batch. After modification of the parameters, users can choose to save the modifications as a file for future use by clicking on the "Save" button at the bottom. The modifications can also be saved as default settings by clicking on the "Save As Default" button so they will be used when the system starts. Users can also click on the "Load" button to use previously saved parameters.



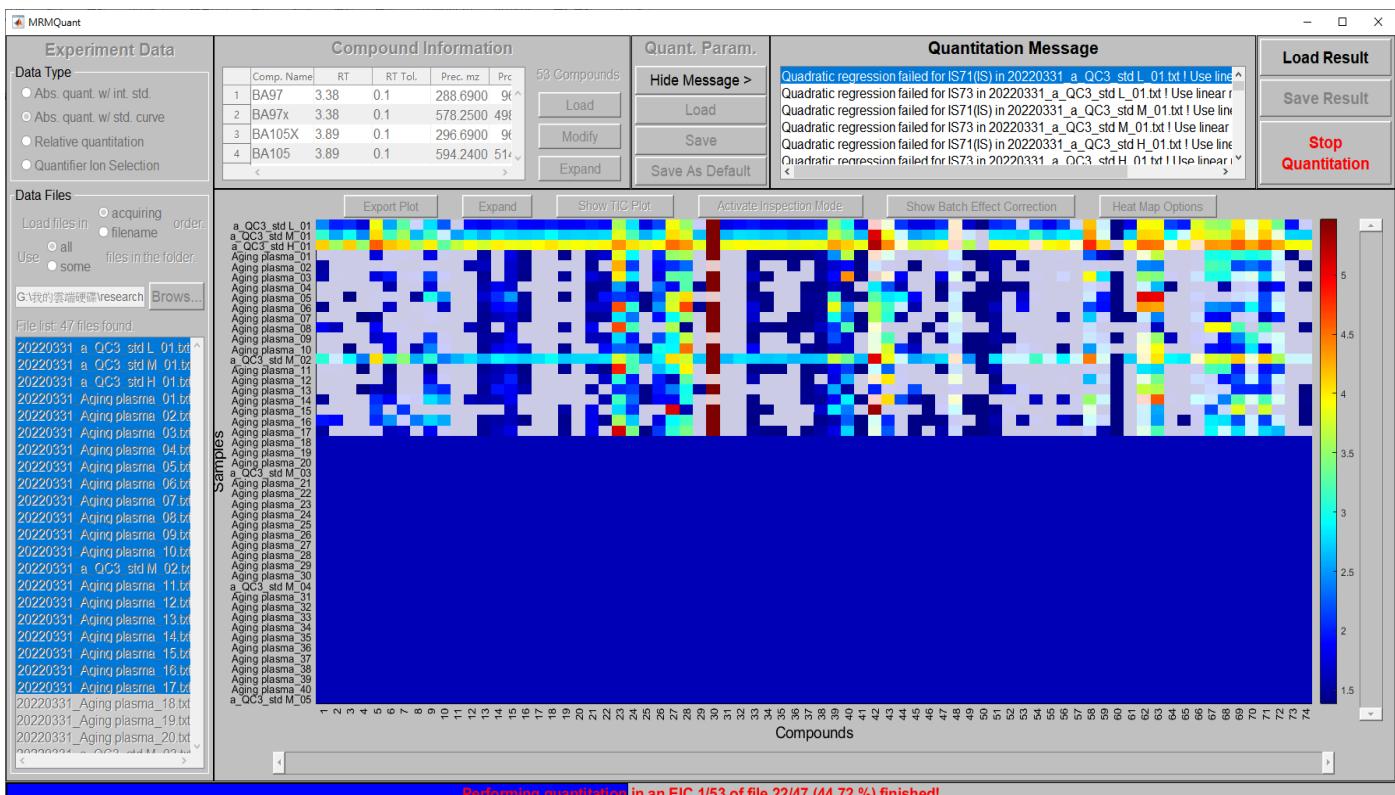


Figure 11. The heatmap and the progress bar during the quantitation process.

Once the entire quantitation process is finished, the heatmap will be rearranged to a pre-specified range. The default range is 10 samples (in vertical direction) and 50 compounds (in the horizontal direction) specified in the default_parameter.xlsx. The user can change the display range by clicking on the “Heatmap Option” button (refer to the Heatmap Option section). The user can use the vertical and horizontal scroll bars to change the viewing range. The user can also check the quantitation result of a cell in the heatmap including abundance, concentration (conc_org), normalized concentration(conc_norm), and quantitation status by moving the mouse cursor over the cell, as shown in the bottom middle of Figure 12. The color of the cells in the heatmap represents the concentration of the corresponding compound for the data type of absolute quantitation and the abundance of the compound for the data type of relative quantitation. The abundance is obtained by the trapezoidal integration of the peak area, where as the concentration is obtained by a further conversion of the abundance via an internal standard in the sample or via a standard curve. Moreover, the color is displayed in the logarithm scale by default. It can be change to its original scale via the “Heatmap Option” button. The magnitude of the computed value can be referred to the color bar showing at the right of the heatmap. Furthermore, for each inspection, according to the quantitation status, the color of a cell is masked with 20% transparent grey if its quantitation status is “no qualified peak” or “multiple possible peaks” to indicate minor problems and is painted in white if the status is “saturated signals”, “defect quantitation”, or “concentration unconvertable” to denote problems that deserve more attention. Such a mask can be removed by changing the setting in the “Heatmap Option”. Other than the above settings, the color scheme and the grid line of the heatmap can also be changed vis the “Heatmap Option” button.

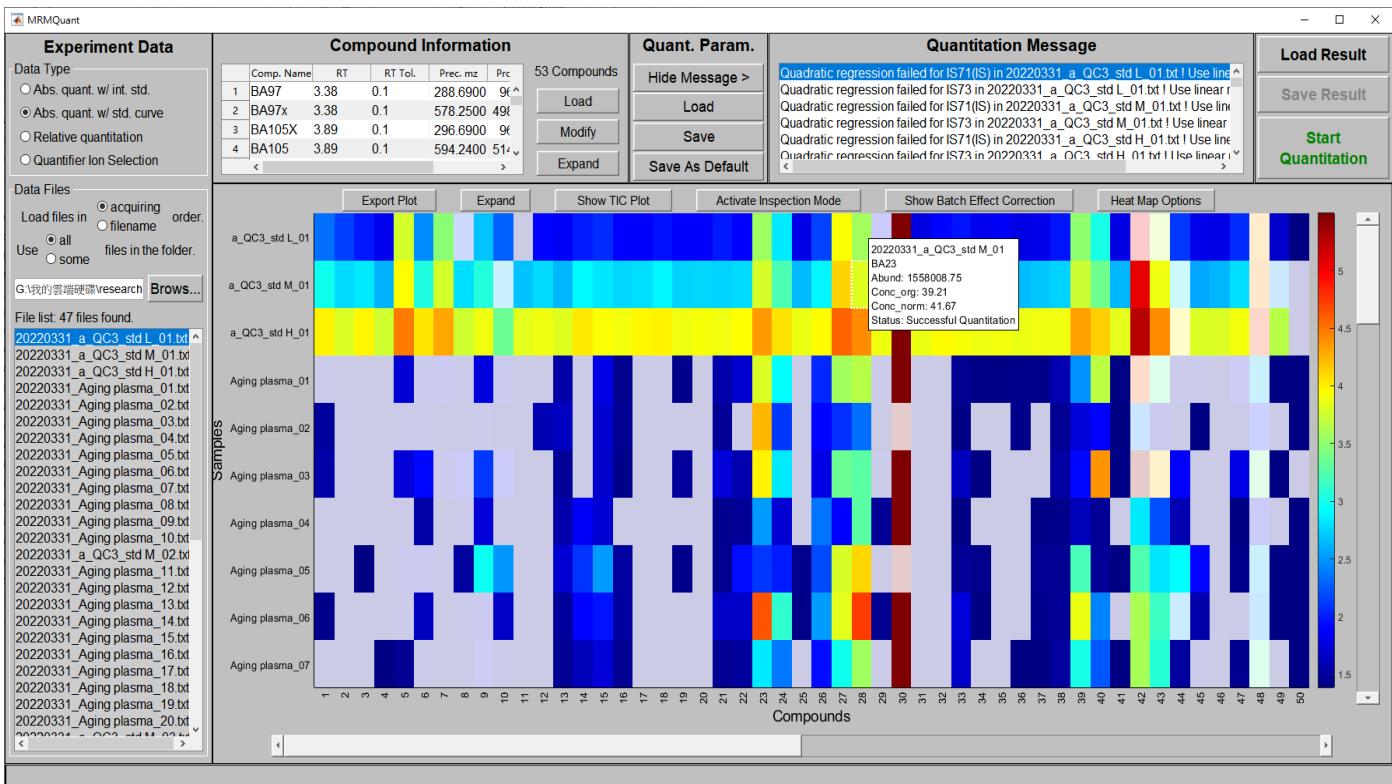


Figure 12. The 10-sample-50-compound heatmap after the quantitation is finished.

On the top of the heatmap, there are five buttons named “Export Plot”, “Expand”, “Show TIC Plot”, “Activate Inspection Mode”, “Show Batch Effect Correction”, and “Heatmap Options”. The first two buttons are used to ex-port and to expand the plot to full window, respectively. The third button is used to switch between TIC and heatmap in the panel. The fourth button activates/deactivates the inspection mode to enable/disable the Multiple Reaction Monitoring window to popup once a heatmap cell is clicked. The fifth button allows users to check the effectiveness of the batch effect correction and the last button is used to change the appearance of the heatmap. The details of the five buttons will be introduced in the following sections.

Export the heatmap plot

The quantitation heatmap can be exported to an image file by pressing the “Export Plot” button on the top of the heatmap. A window will pop up to let the user to adjust the size of the image, as shown in Figure 13(a). By clicking on the Rending in the left panel, the user can also select the color space, background color, renderer, and resolution, as shown in Figure 13(b). By clicking on the Fonts in the left panel, the user can change the font properties in the plot, as shown in Figure 13(c). By clicking on the Lines in the left panel, the user can also change the font properties in the plot, as shown in Figure 13(d). After the all the changes are made, the user can click on the “Export” button in the righthand side, a new window will appear to select the folder and file name of the output image as well as the image format, as shown in Figure 13(e). The supported image formats are listed in Figure 13(f).

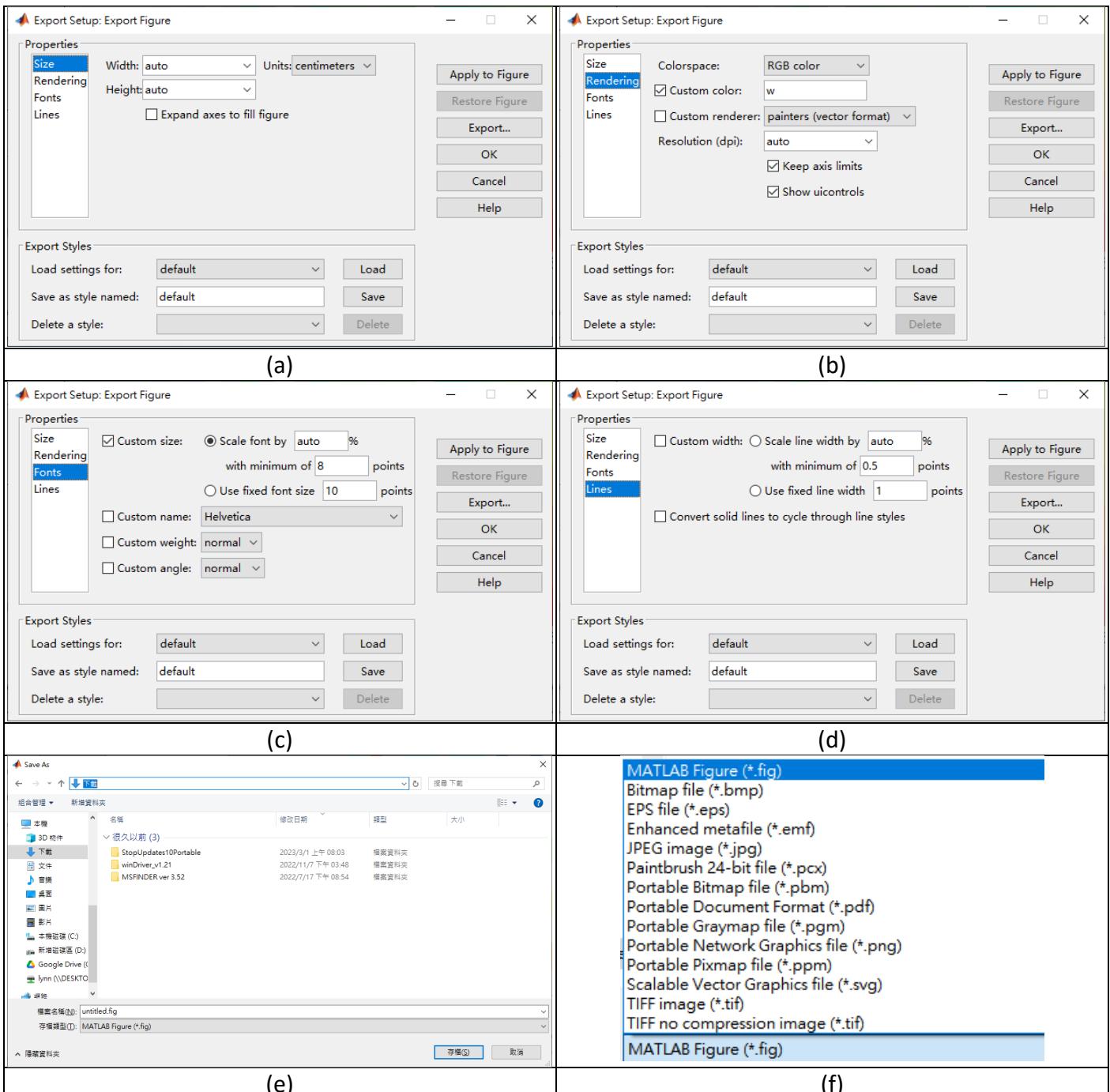


Figure 13. The windows to export the heatmap image.

Expand/shrink and show the heatmap/TIC plot

To better reveal the contents in the heatmap, the user can press the “Expand” button on the top of the heatmap, as shown in Figure 14. Similarly, a TIC plot can be also enlarged using this function. Alternatively, an enlarged TIC plot can be obtained using the “Show TIC plot” button on the top of the heatmap, as shown in Figure 15. The “Show TIC plot” button on the top of the heatmap will be renamed to “Show Heatmap” once the TIC plot is in display, and vice versa. On the other hand, the expanded plot can be resumed to its original size by pressing the “Shrink” button on the top of the plot.

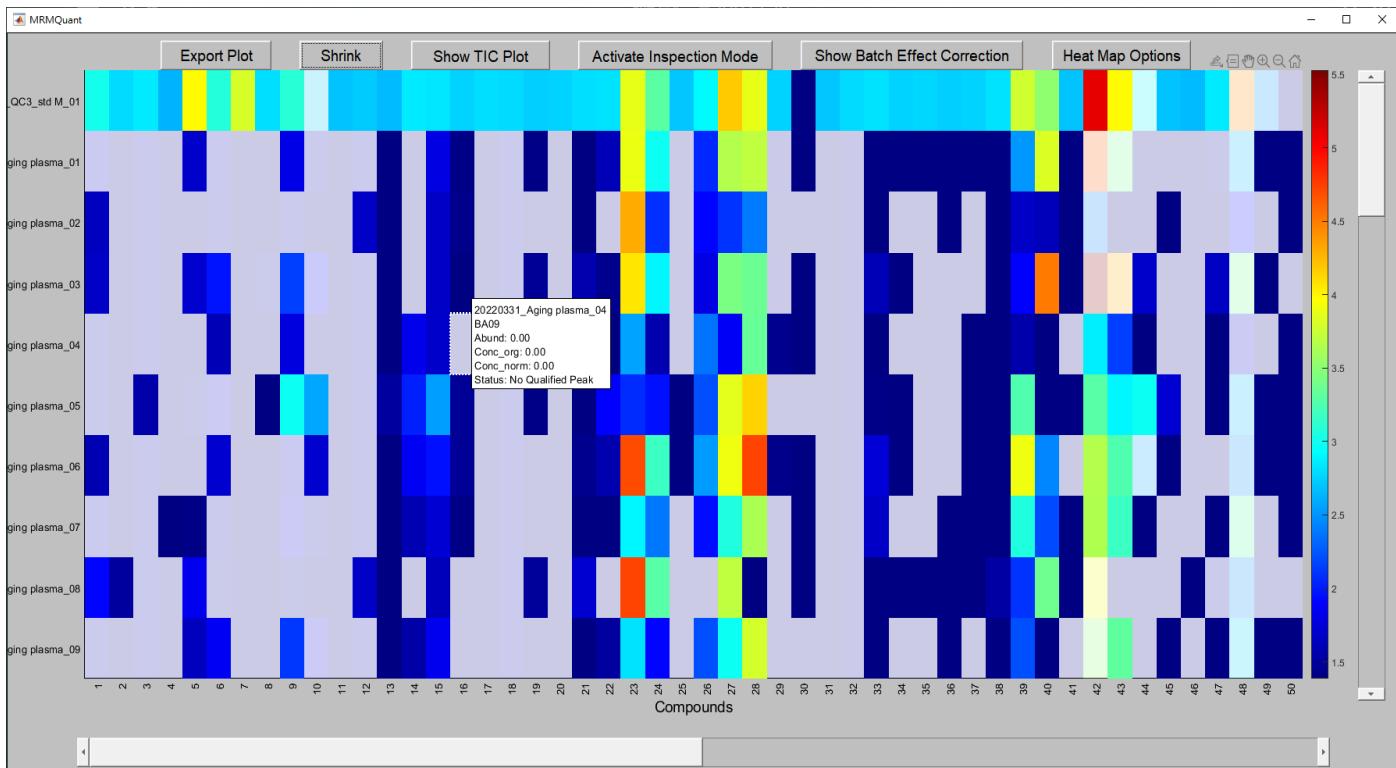


Figure 14. The expanded heatmap after the user presses the “Expand” button on the top of the heatmap. The “Expand” button will be renamed to “Shrink” once the expansion is done. The user can use the “Shrink” button to resume the plot to its original size.

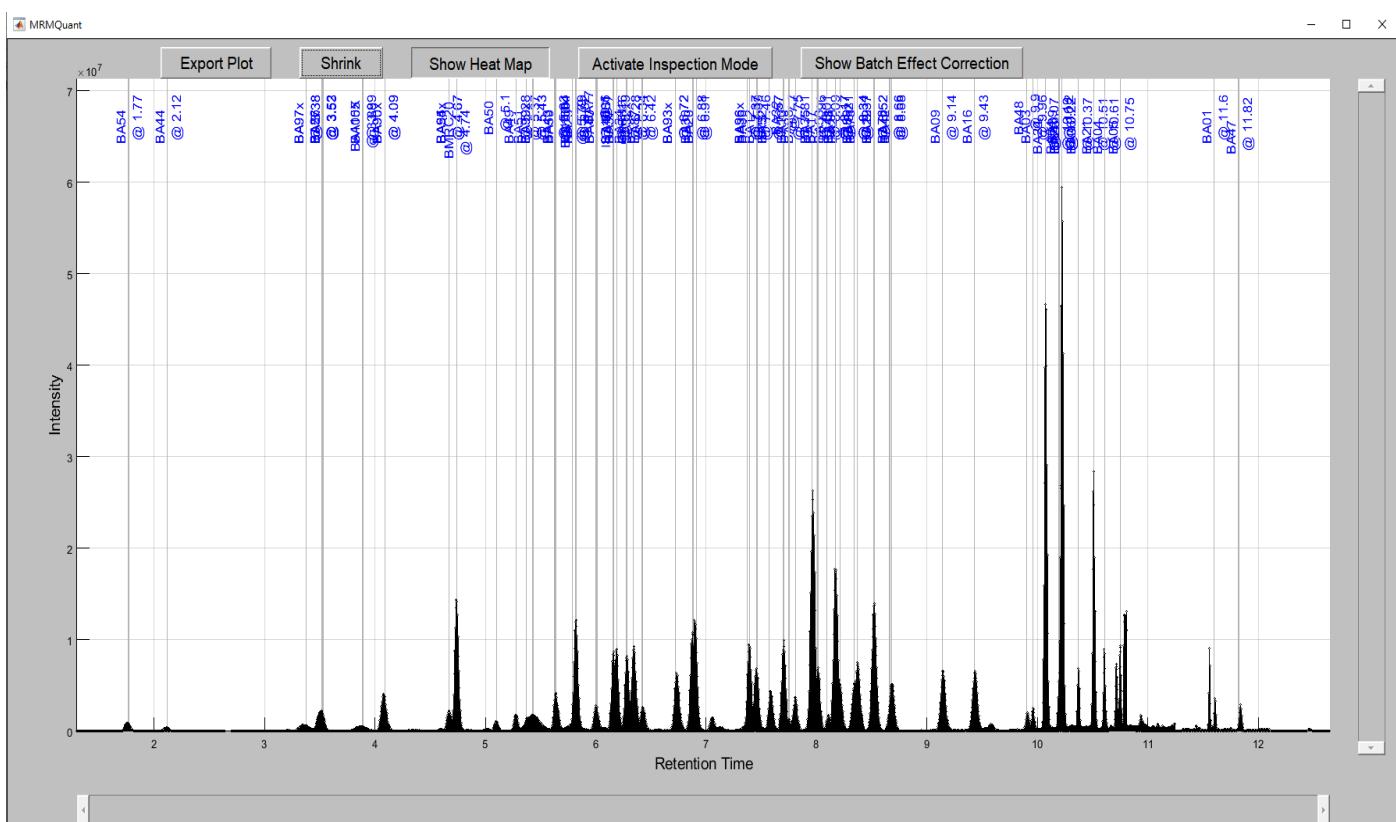


Figure 15. The expanded TIC plot of the current sample file (the highlighted file in the ‘Data File’ list). The user can use the “Shrink” button to resume to its original size or use the “Show Heatmap” button to change the display to heatmap.

Activate/deactivate inspection mode

In addition to checking the computed abundance/concentration and quantitation status by moving the mouse cursor over a heap map cell, the user can further inspect the detailed quantitation result of an ion chromatogram by activating the inspection mode using the “Activate Inspection Mode” button. Once the button is pressed, the text of the button becomes “Deactivate Inspection Mode” indicating the inspection mode is activated and the user can click on a heap map cell to inspect the quantitation details of the corresponding compound. As shown in Figure 16, when the inspection mode is activated and the cell in heat map or the compound name in TIC corresponding to the compound “BA42” in the sample “20220331_Aging_plasma_04” is clicked, the Multiple Reaction Monitoring window will appear showing the corresponding ion chromatogram.

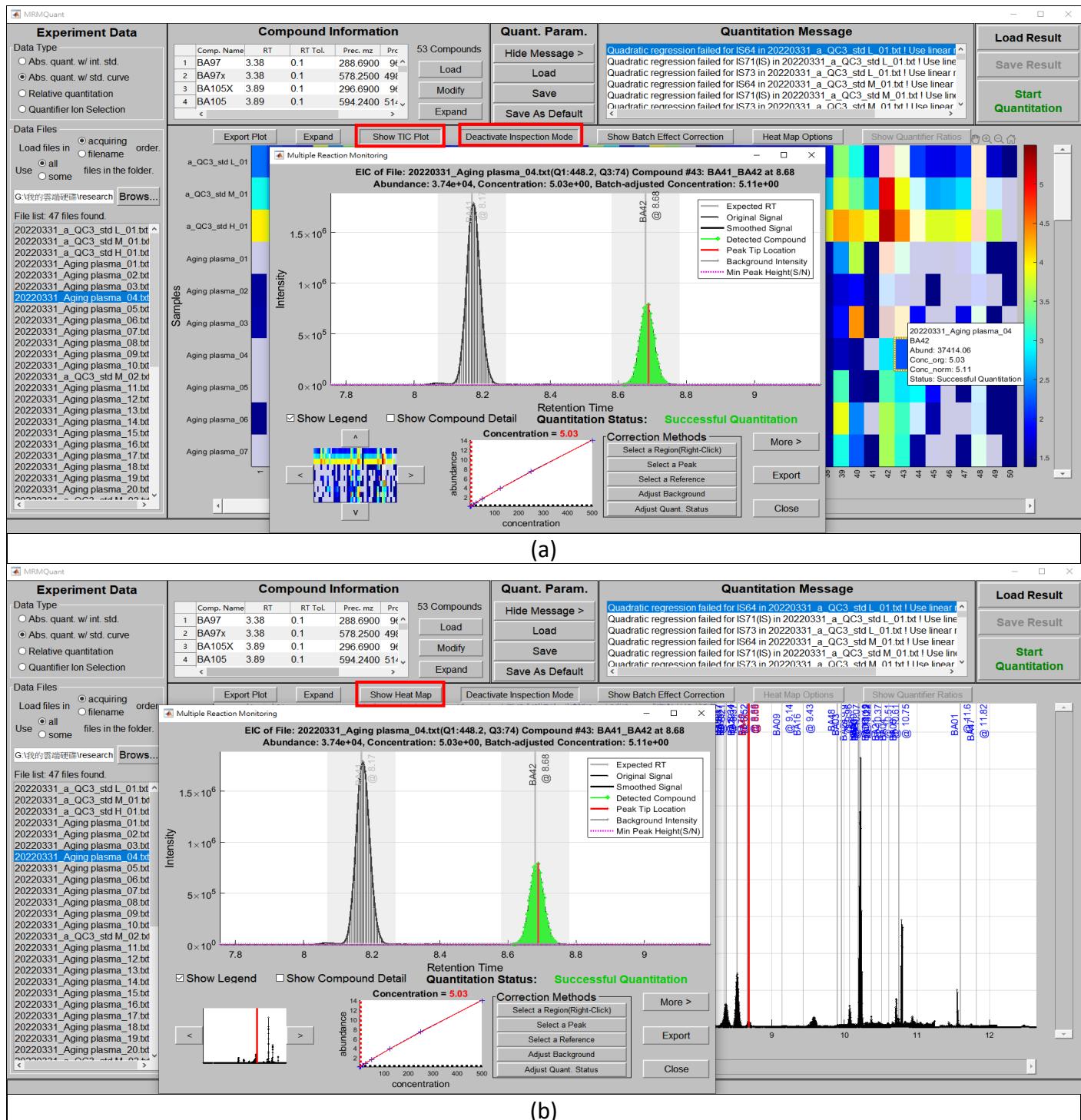


Figure 16. As the inspection model is activated, clicking on (a) a cell in the heap map or (b) a compound name in the TIC will bring up a window showing the quantitation details in the corresponding ion chromatogram.

The user can utilize the “Multiple Reaction Monitoring” window to navigate, inspect, correct, and to export the detailed quantitation result of a single ion chromatogram. There are three ways to bring up the window: (1) by clicking on a cell in the heatmap, (2) by clicking a compound name in a TIC plot, and (3) by clicking on one of the ion chromatograms in the standard curve window (refer to the Standard curve generation section). An example of the Multiple Reaction Monitoring window is demonstrated in Figure 17. Panel (a) in the figure shows the ion chromatogram of the selected MRM and compound. Colored lines and regions represent different information, for instance, black curve for the profile of the smoothed signals, gray blocks for the RT tolerances of the targeted compounds, vertical black, red, green, blue, and magenta lines for original signals, detected peak tip, detected compound peak, deconvoluted peak, and minimal peak height (according the given S/N ratio), respectively. As shown in the panel, MRMQuant can deal with multiple compounds in a chromatogram by automatically decomposing the coeluted peaks for better abundance estimation.

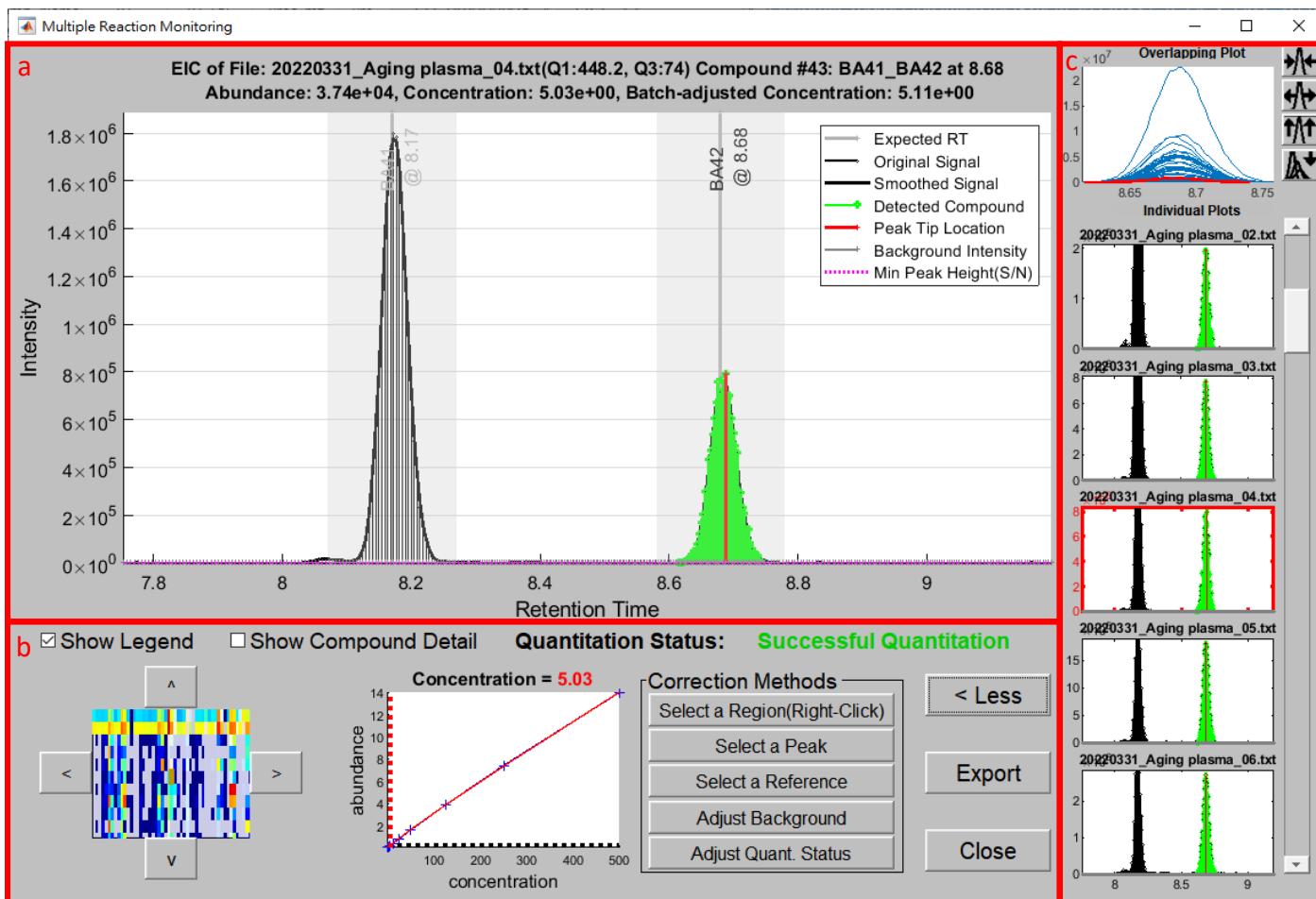


Figure 17. The Multiple Reaction Monitoring window. Panel (a) shows the original signals and the detected peak for the targeted compound in the selected MRM file. Panel (b) contains buttons corresponding to navigation, inspection, correction, and export functions. Panel (c) shows overlapped and individual detected peaks of the targeted compound in different sample files.

Panel (b) in the figure exhibits buttons to navigate, inspect, correct, and export the peaks in the ion chromatogram. On the top, there are two checkboxes to show/hide the legend in the panel (a) (as showed in Figure 18) and to show compound detail by displaying the zoom-in plot of the quantitated peak in the ion chromatogram (as showed in Figure 19) and a text message to show the quantitation status of the compound. On the bottom left, a mini-heatmap with four arrow buttons allows users to browse nearby compounds. On the right to the mini-heatmap, a plot shows the standard curve (the red solid line) of the current compound for the conversion of concentration (the red dotted line) from abundance (the black dotted line) of the current compound and its associated internal standard for the absolute quantitation experiment.

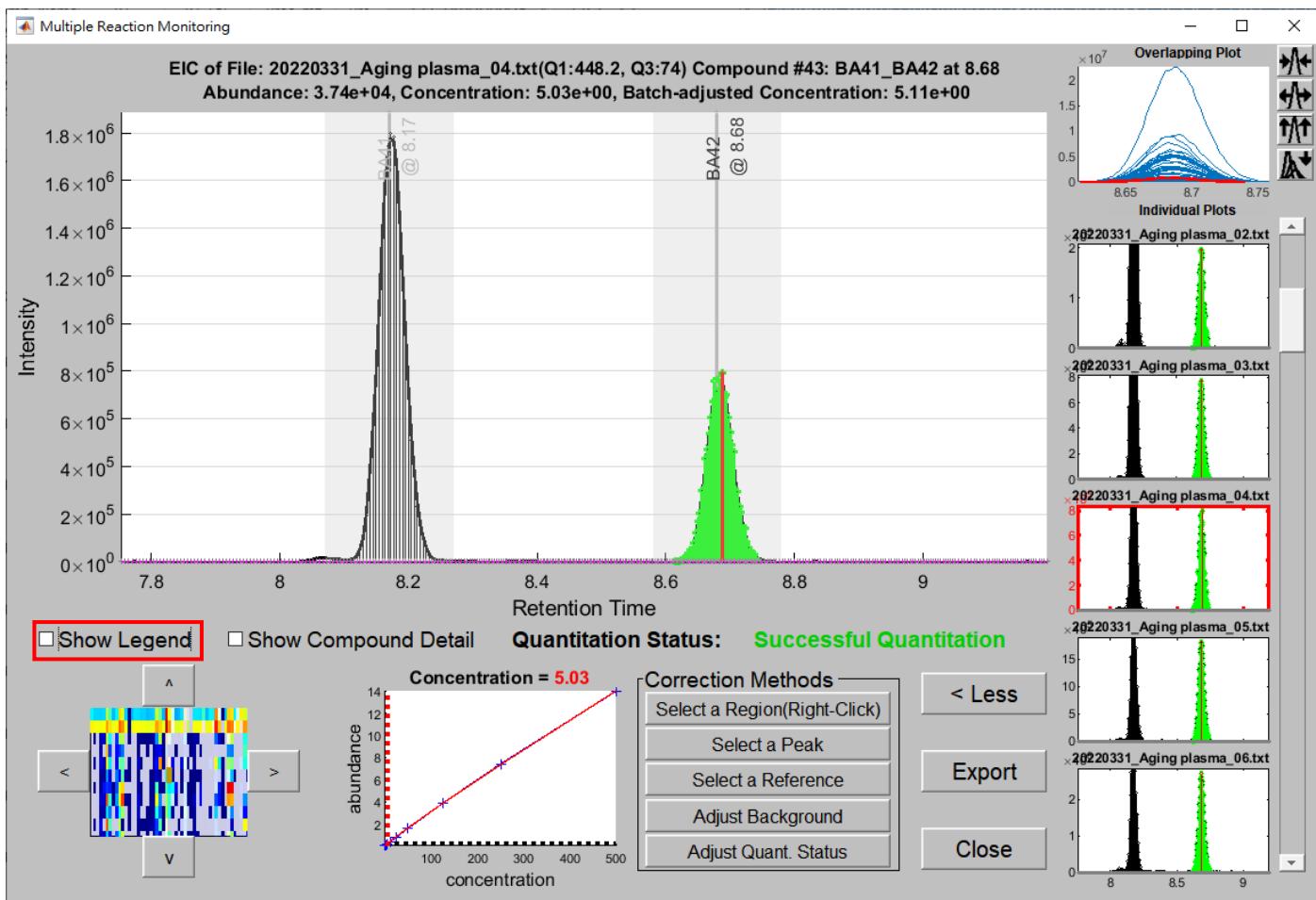


Figure 18. The legend in the ion chromatogram is hidden.

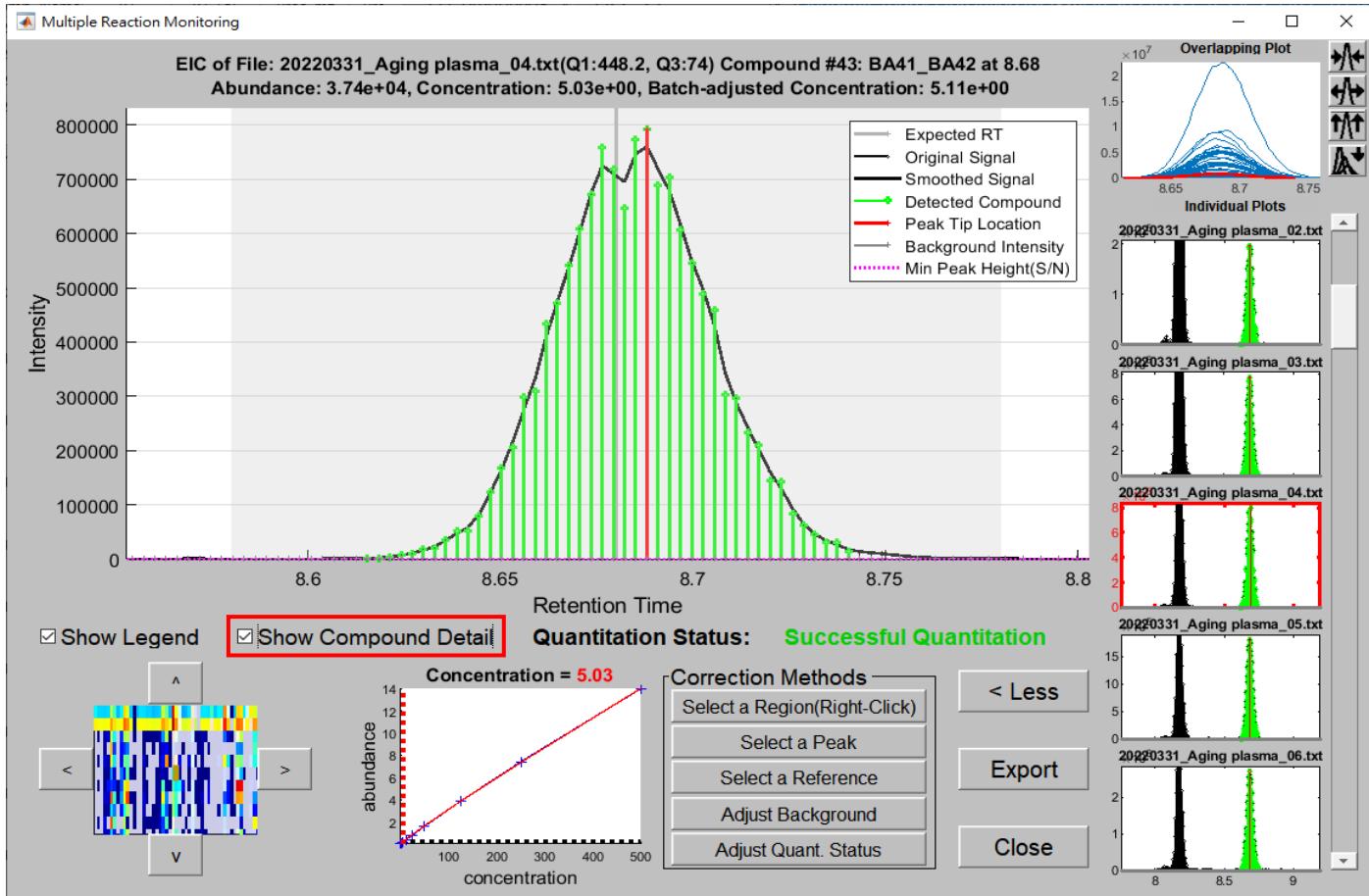


Figure 19. The zoom-in plot of the quantitated compound in the ion chromatogram.

On the right to the standard curve plot, there are five buttons designed to correct the quantitation by specifying (1) a peak region(Right-Click), (2) a peak tip, (3) a reference sample, (4) the background intensities, and (5) the quantitation status. Clicking on the “Select a Region(Right-Click)” using the mouse’s right button, a submenu will show up with two options “by mouse” and “by keyboard”, as shown in Figure 20. Choosing the “by mouse” option, the mouse cursor will turn into a “+” sign and the user can then click, hold, and drag a rectangle using the mouse containing the expected peak, as shown in Figure 21.

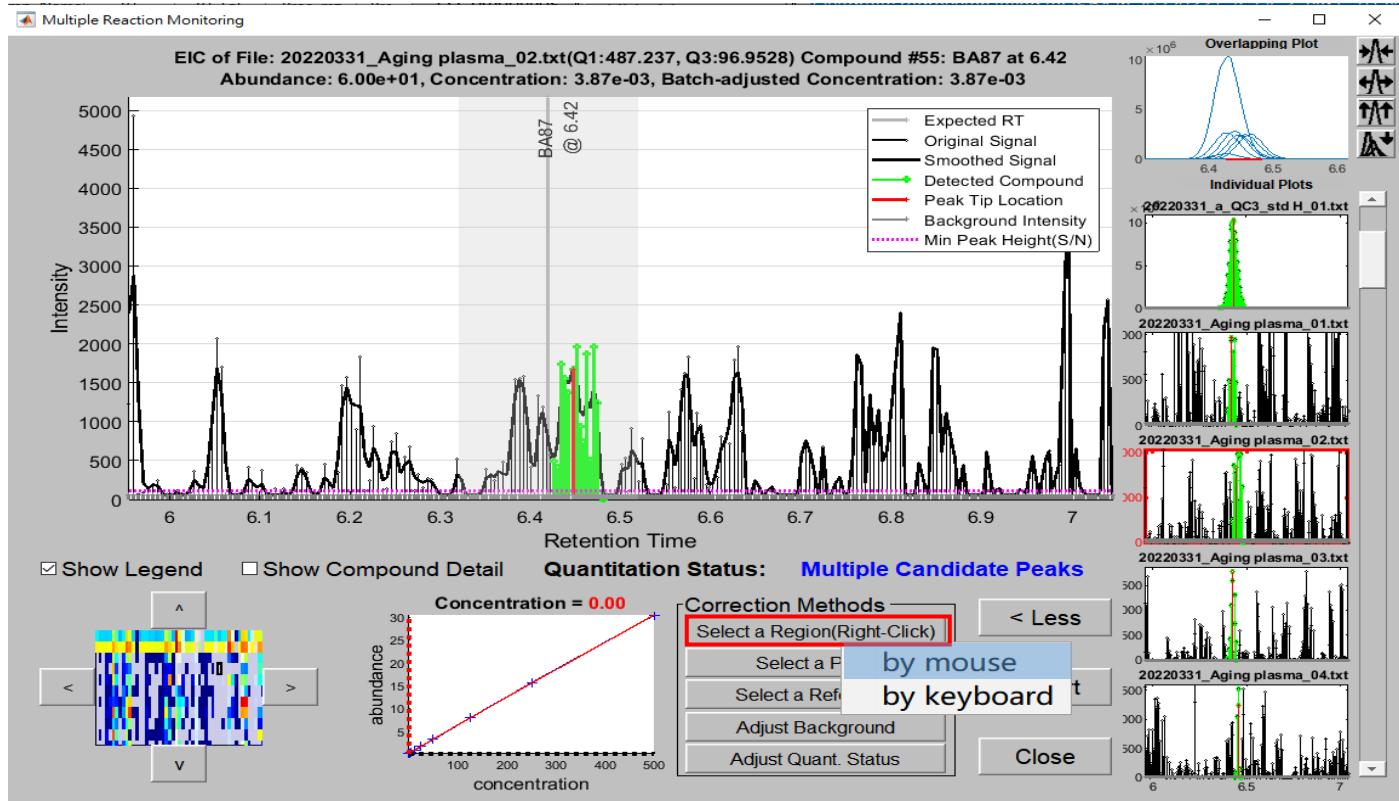


Figure 20. Two methods (“by mouse” and “by keyboard”) to modify a peak by selection the peak region.

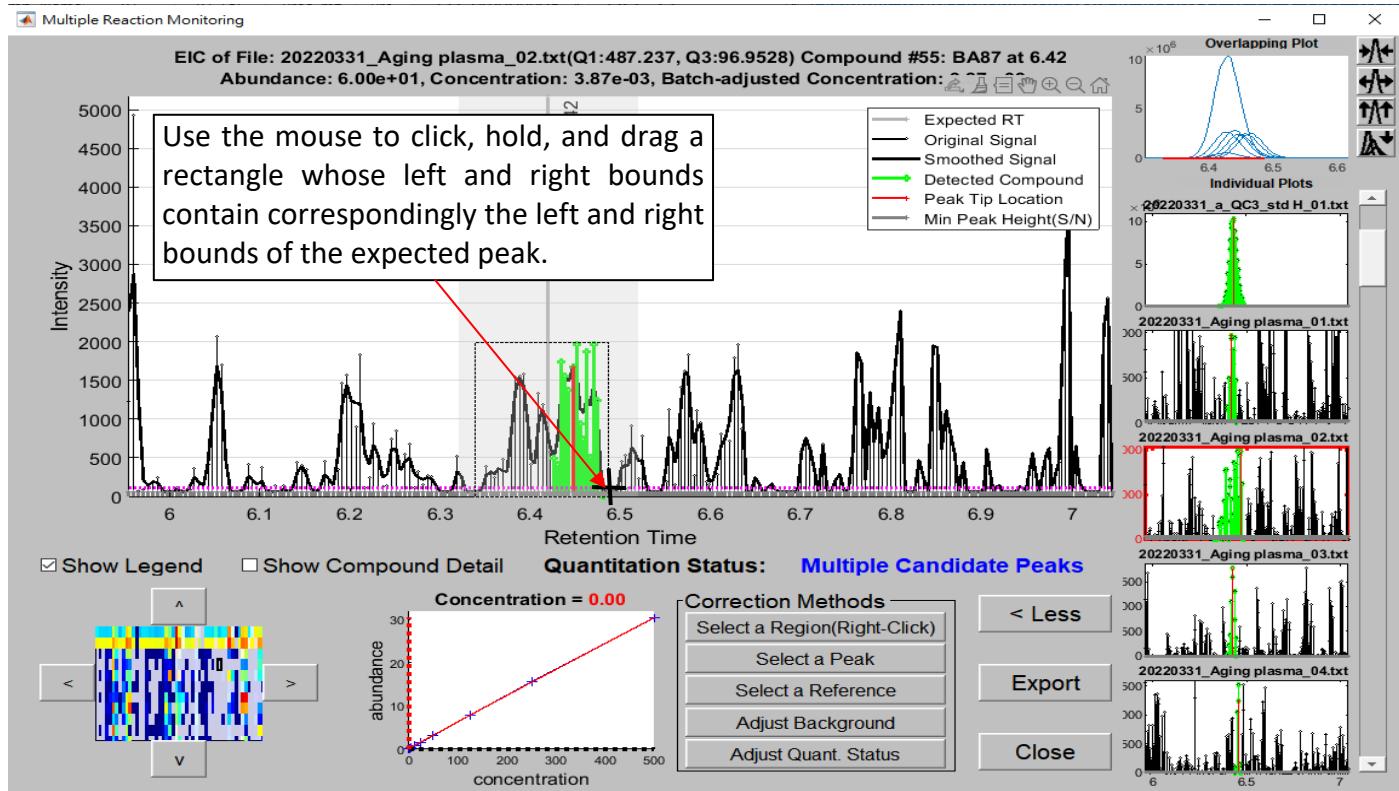


Figure 20. The expected peak region specified by the rectangle created by clicking and dragging the mouse cursor after choosing the “by mouse” option.

On the other hand, if the user chooses the “by keyboard” option (Figure 22), a window to input the left- and right-boundary will be displayed, as shown in Figure 23. Afterward, the user can also select whether to use the given value as the peak boundaries or as initial points to search for the nearby valleys as the peak boundaries. Once clicking on the Ok button, another window will appear for the user to select whether the similar modification is applied to only the current peak (as shown in Figure 24(a) and the resultant peak region is shown in Figure 25(a)) or to the same peak in multiple samples (as shown in Figure 24(b) and the resultant peak regions are shown Figure 25(b)). In addition, when applied to multiple samples, the user can select whether the peak left- and right- boundaries are defined by the two specified values or by the relative distances of the two values to the peak tip.

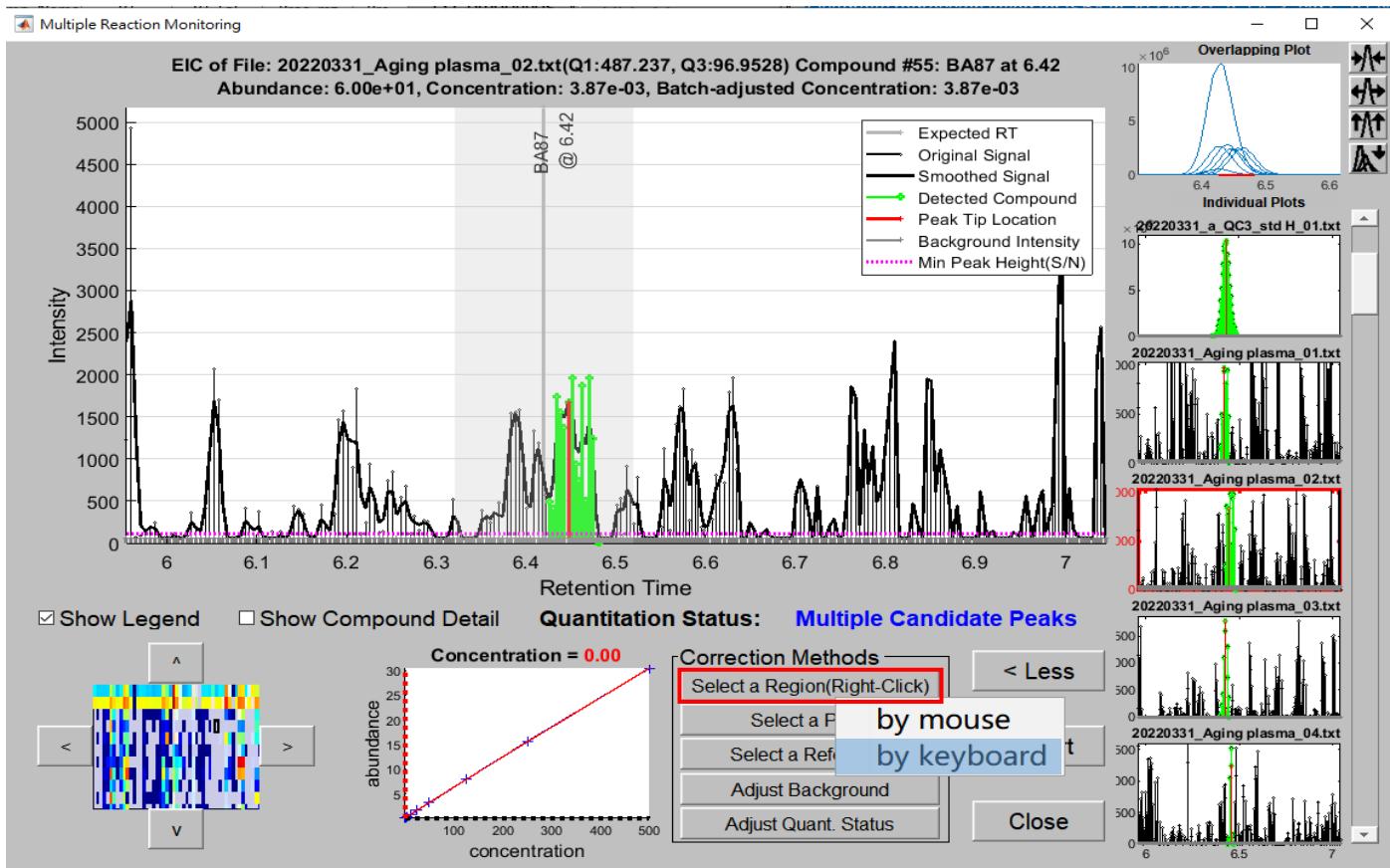


Figure 22. Choosing the “by keyboard” option to modify a peak region by manually keying in the left and right boundaries (in retention time).

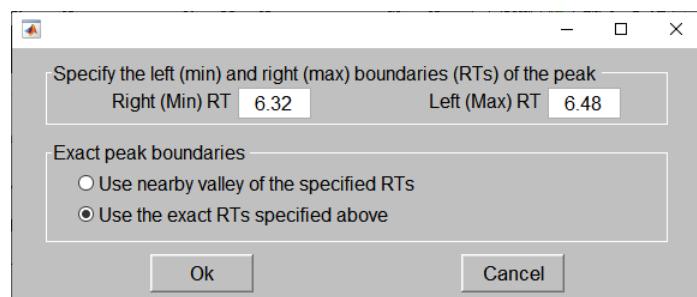


Figure 23. Input the left and right boundaries (in retention time) and determine whether to use the exact retention times for the peak boundaries or to allow the program to find the nearby valley for the boundaries.

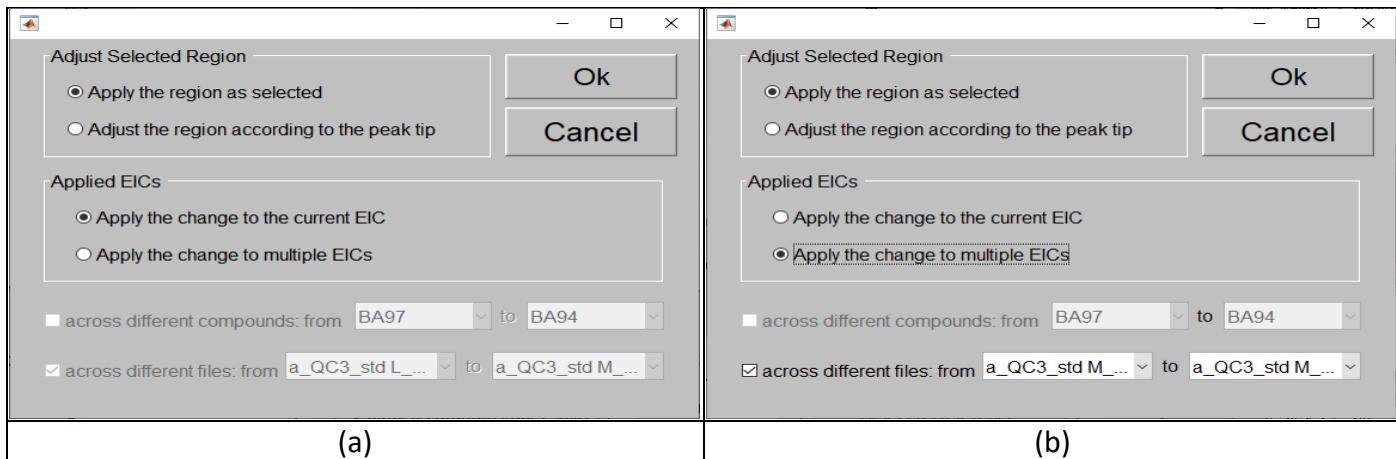


Figure 24. Modify the peak area using the given boundaries and (a) only apply to the current compound or (b) apply to the same compound in multiple compounds.

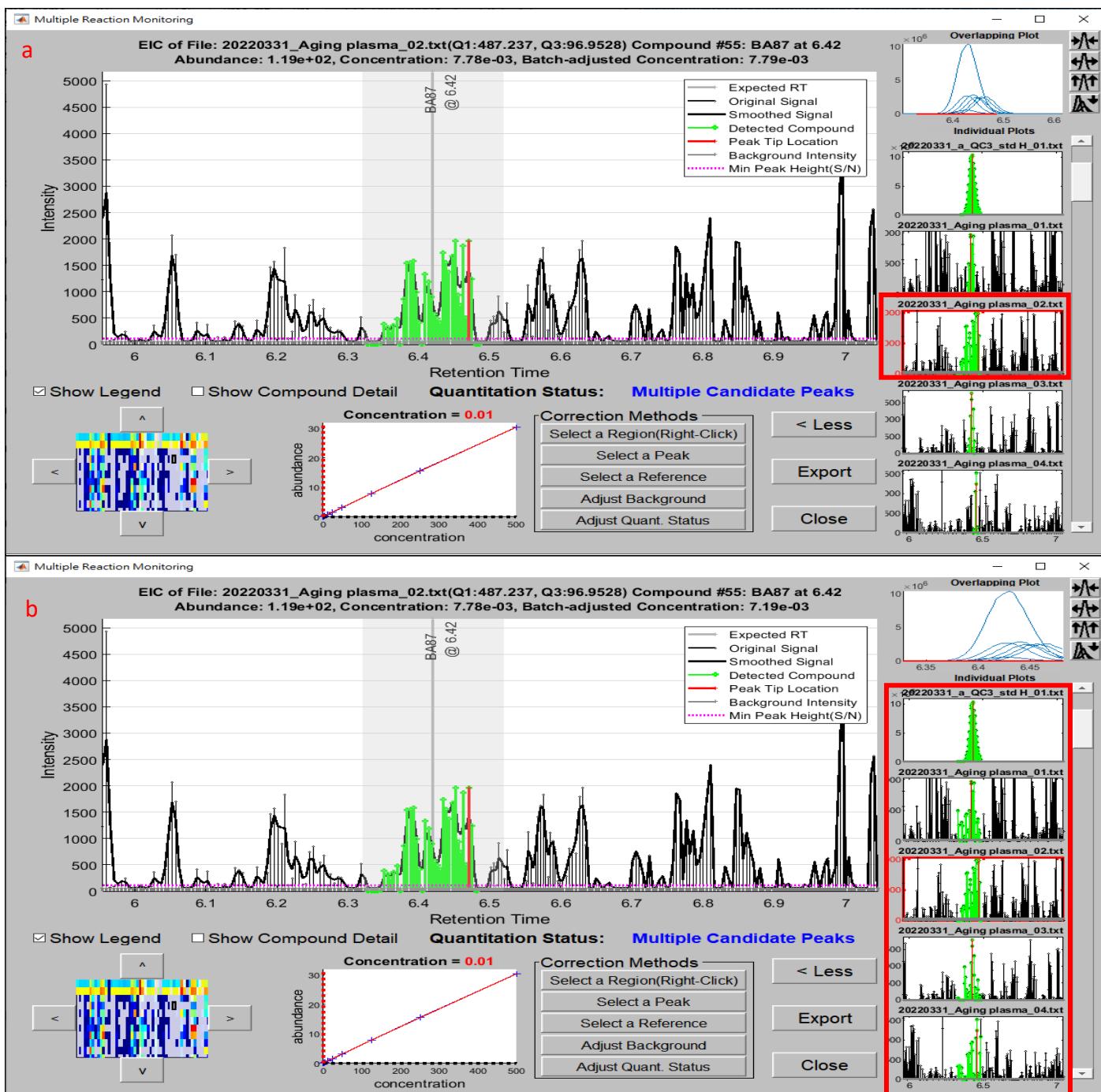


Figure 25. The resultant peak region and quantitated peak regions in the neighboring samples when modification is applied to (a) the current peak (b) the same peak in multiple samples.

If the peak shape of the targeted compound is smooth and clean but was misidentified by the program, the user can use the “Select a Peak” button. Once the button is pressed, the mouse cursor will turn into a long cross and the user simply needs to click on the neighborhood of the peak tip to change the peak to the correct one, as shown in Figure 26. Once the new peak is successfully recognized, the peak is annotated by the colors specified in the legend and a window will appear to allow the user to determine whether the same modification should be applied to multiple samples, as shown in Figure 27.

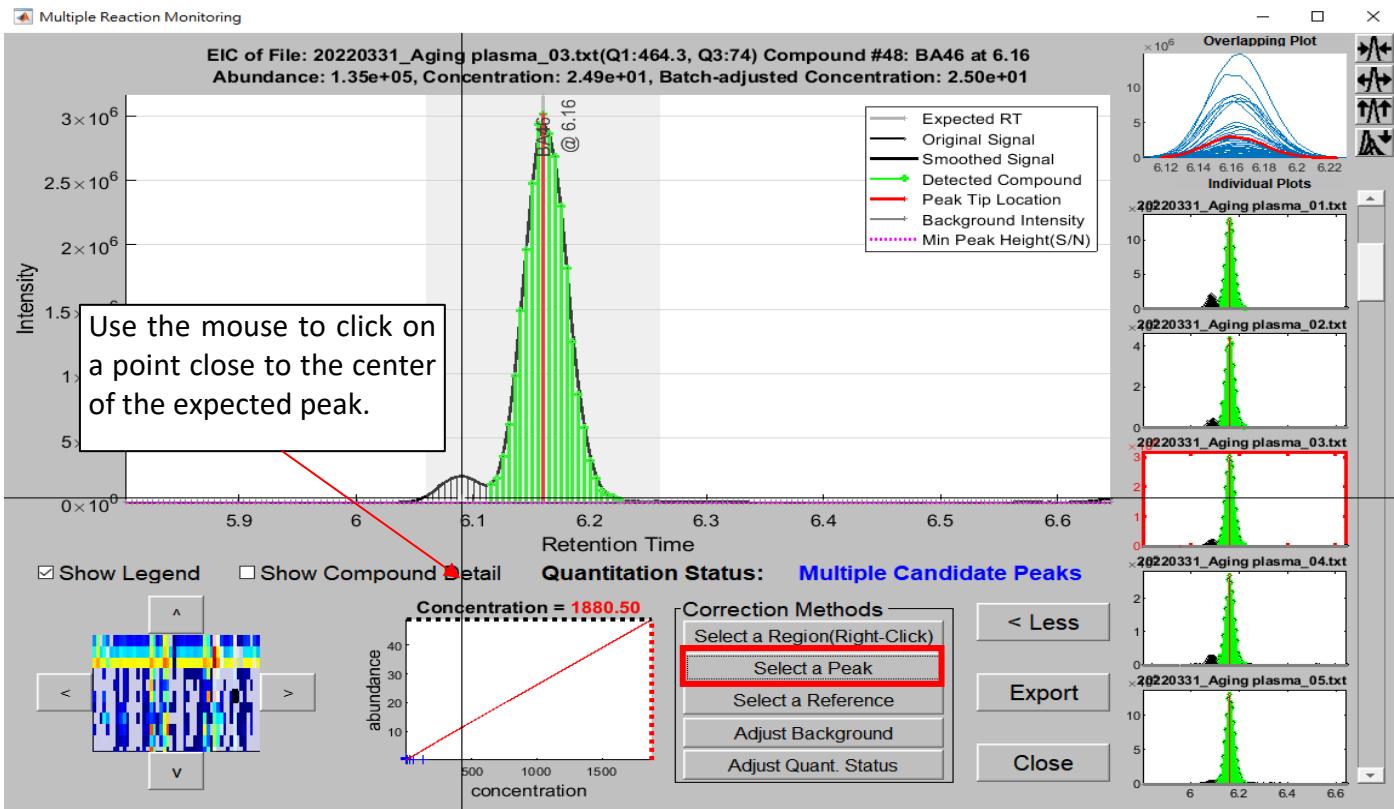


Figure 26. Change of peak corresponding to the targeted compound using the “Select a Peak” function.

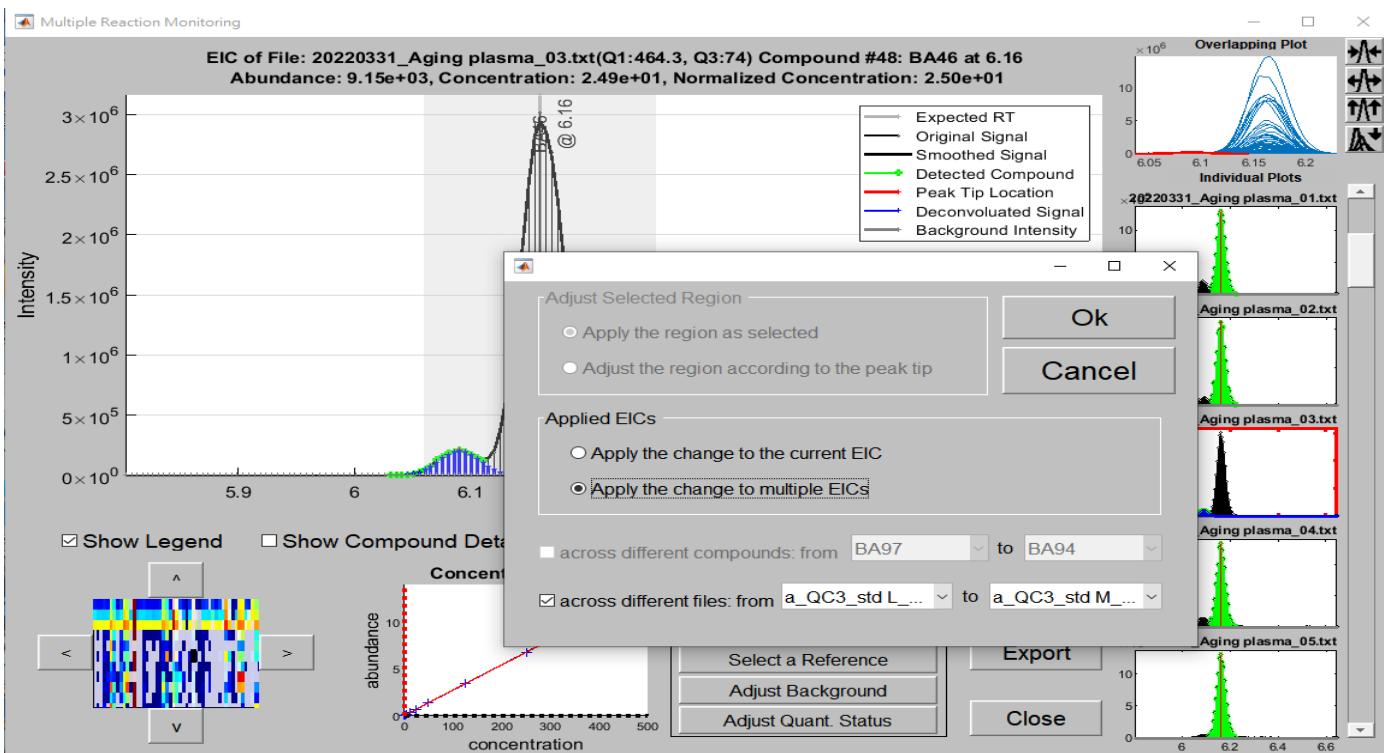


Figure 27. The new peak determined by the “Select a Peak” method and the window for the user to choose whether to apply the similar change to the same compound in other samples and which samples to be applied.

To further simplify the modification process, MRMQuant provides a “Select by Reference” button for simultaneously modify multiple compounds and multiple samples. This function requires a reference sample for each batch, as shown in Figure 28. The reference is recommended to be selected from QC samples or samples whose compounds are mostly abundant and inspected so that its compound boundaries can be used to find the possible peaks in the other sample.

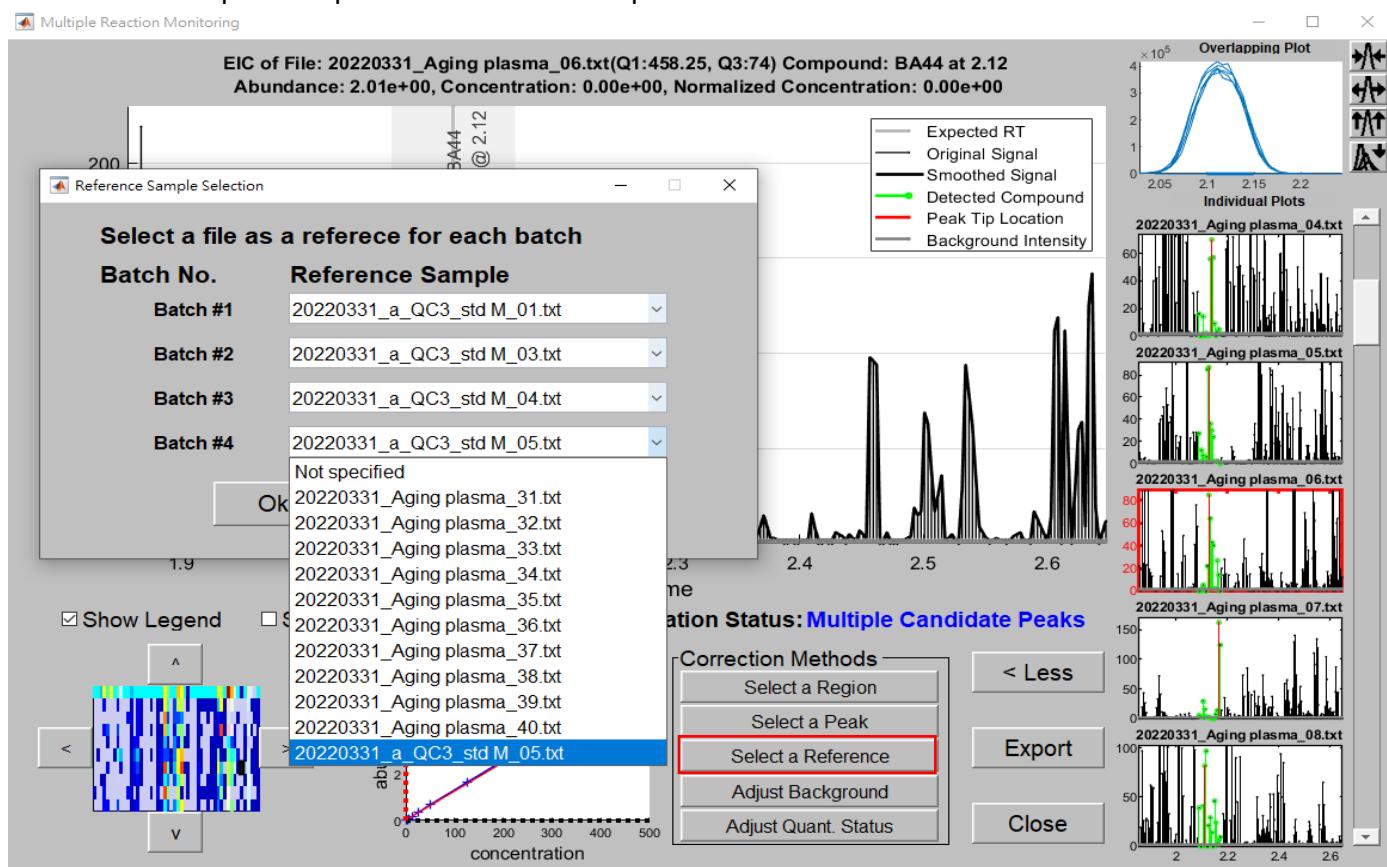


Figure 28. The “Select a Reference” function allow the user to assign a reference for each batch so that the peaks for the targeted compounds in other samples can be quantitated based on the conditions in the reference.

For an ion chromatogram whose background intensities exhibit considerable fluctuations, the constant intensity assumption in MRMQuant may not accurately reflect the profile of the background intensities under the peak area causing errors in the subsequent peak quantitation. To deal with such a situation, the user can utilize the “Adjust Background” button to indicate the correct intensities. Like the “Select a Peak” method, once the user presses the “Adjust Background” button, the cursor will turn into a long cross and the user needs to use a series of left click on the correct background intensities under the peak area and a right click to terminate the process, as shown in Figure 29. The peak abundance and concentration will be recalculated based on the new background intensities, as shown in Figure 30. In addition, the changes in the background intensity can be applied to multiple compounds and/or multiple samples, as shown in Figure 31.

Finally, the user can assign the quantitation status to “Successful Quantitation” using the “Adjust Quant. Status” button. Once the button is pressed, a window will appear to let the user determine whether the similar change should be applied to other samples, as shown in Figure 32.

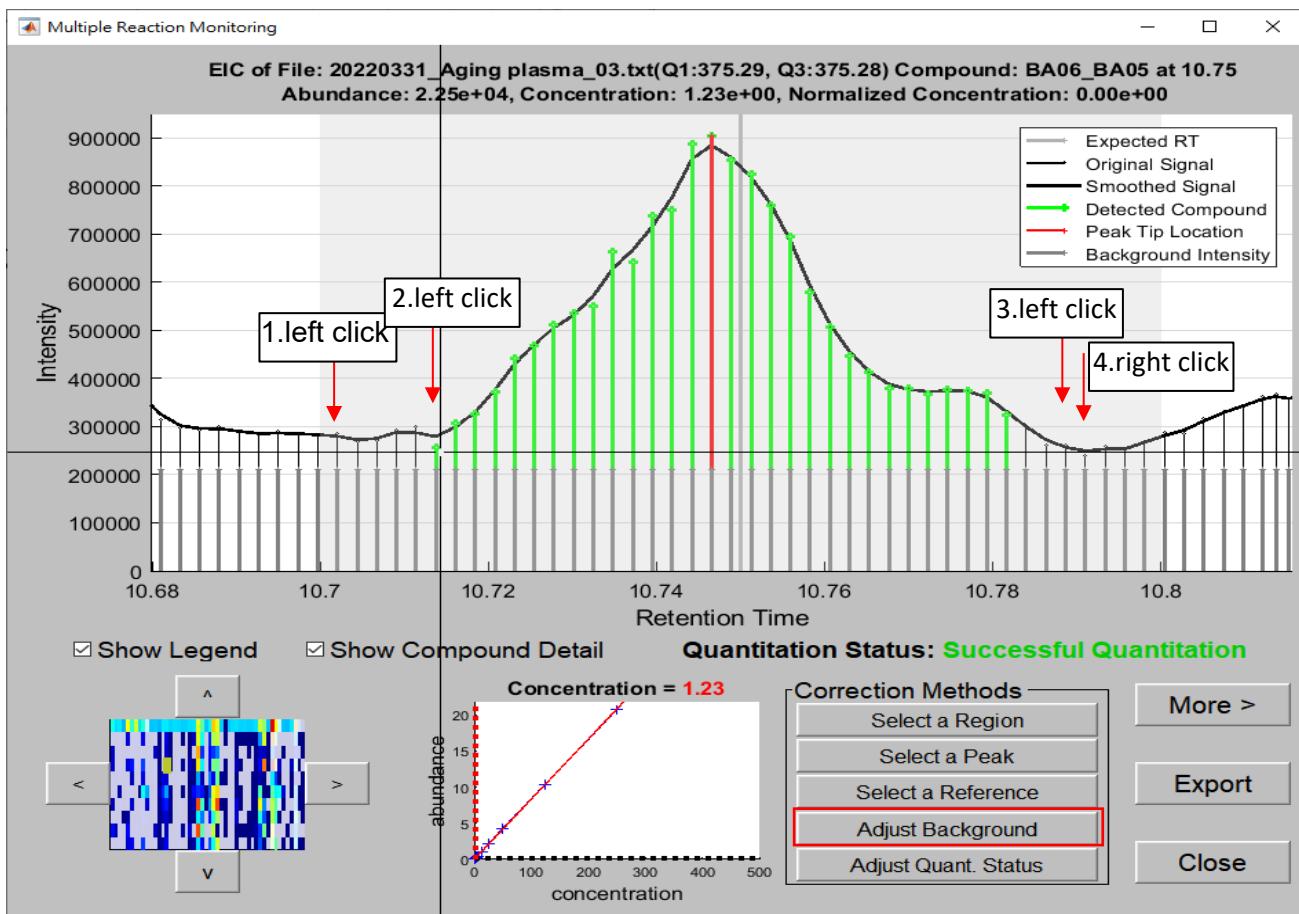


Figure 29. User specified background intensities using the “Adjust Background” method. After activate the “Adjust Background” method, the user specifies 4 points via 3 left clicks and one right clicks (indicating the termination of the process) for the new background intensities. The MRMQuant will use the new background intensities for the peak quantitation.

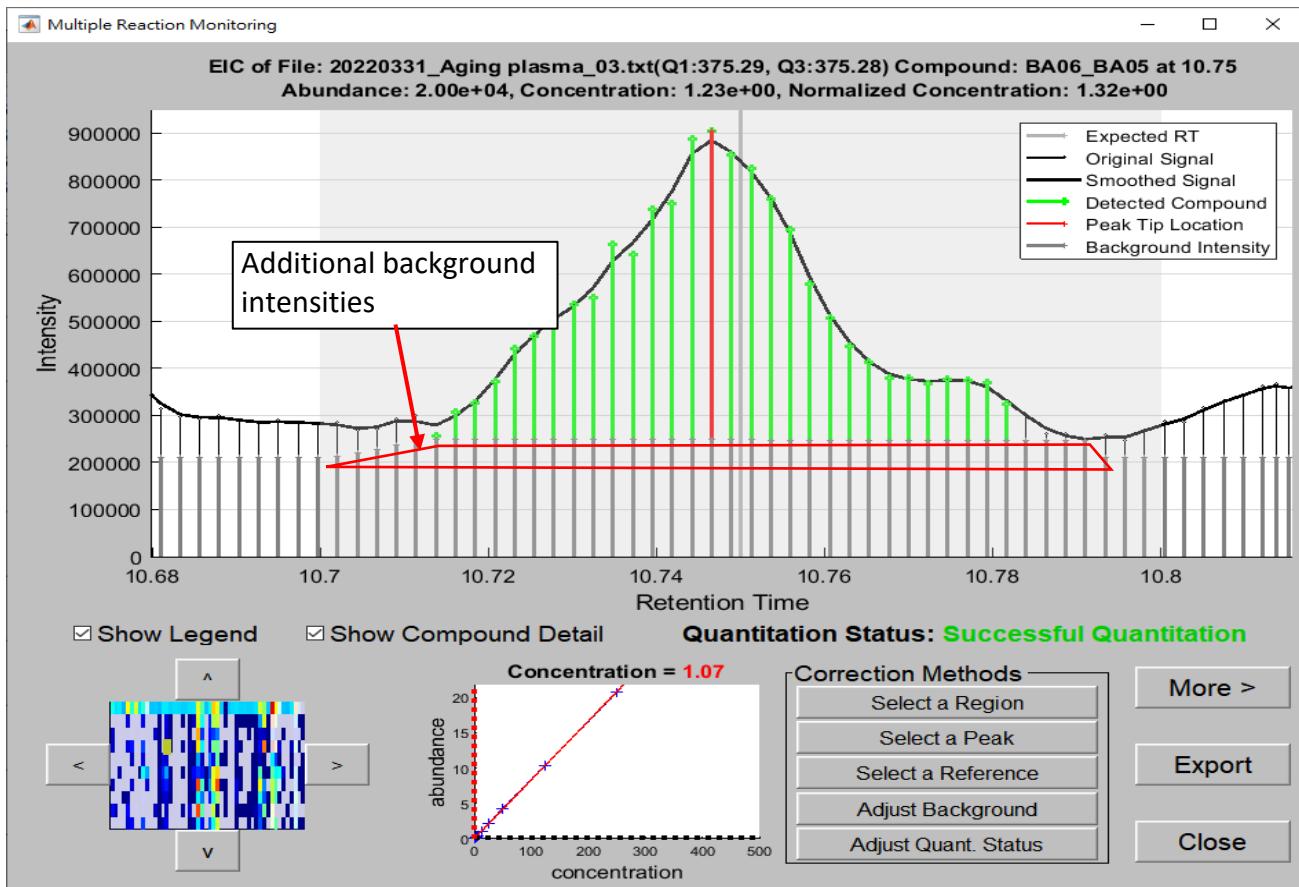


Figure 30. The smaller peak area due to higher background intensities specified by the user.

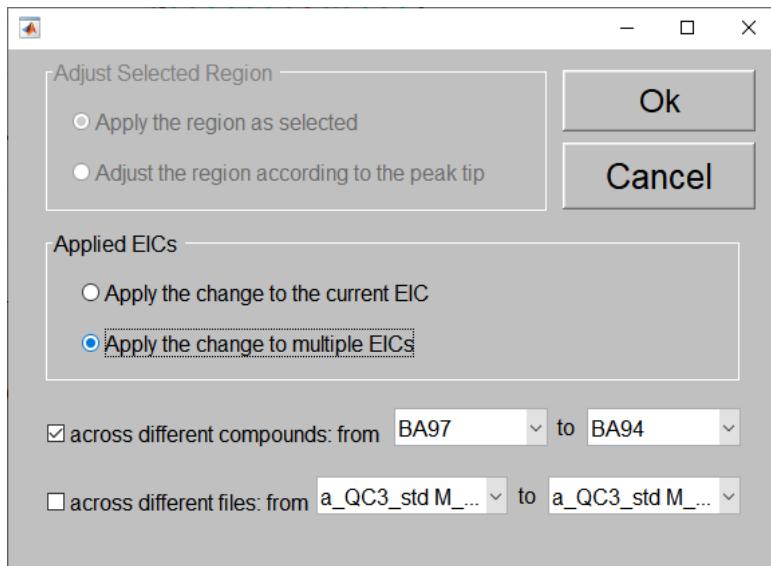


Figure 31. The changes in the background intensity can be applied to multiple compounds and/or multiple samples

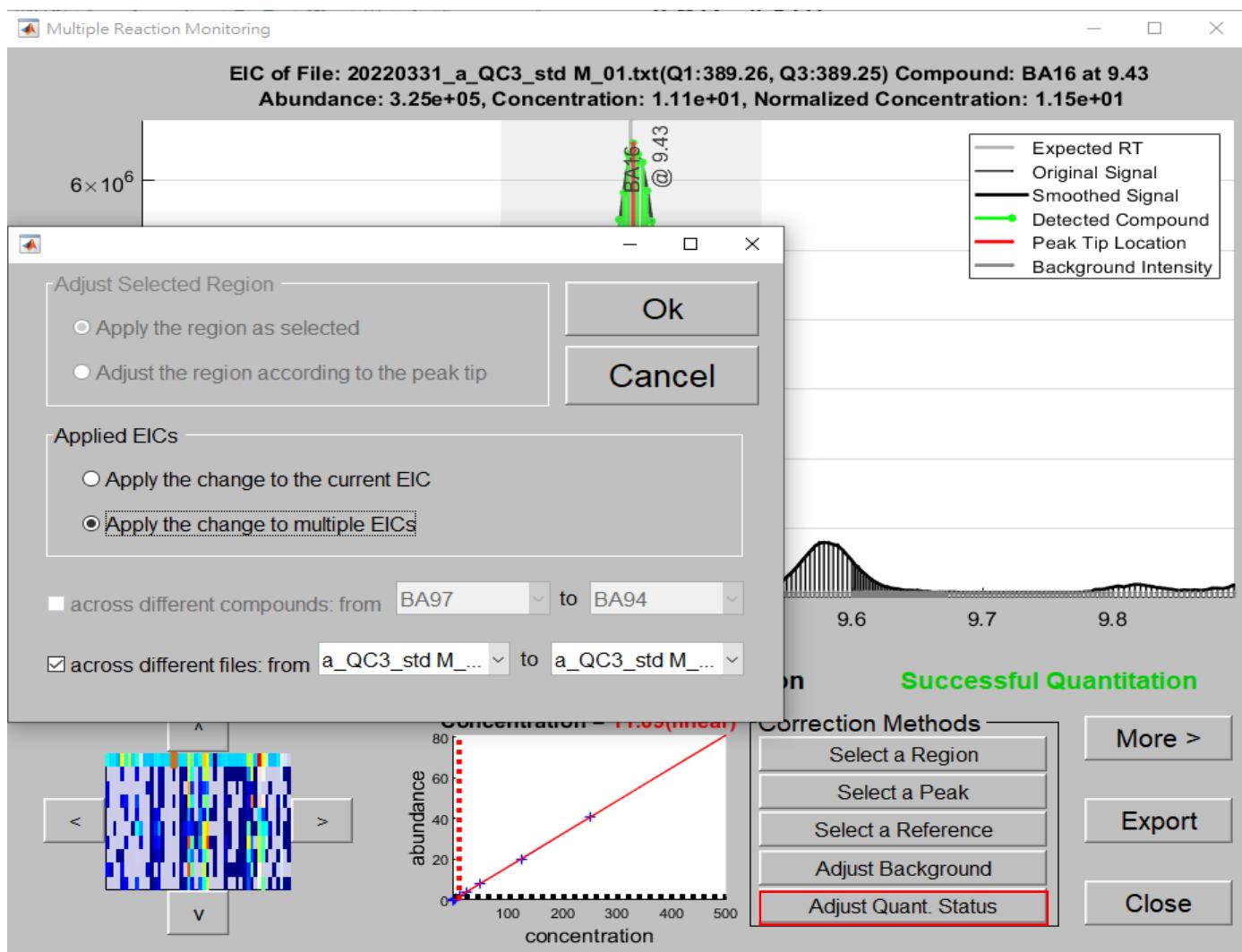


Figure 32. The “Adjust Quant. Status” changes the quantitation status of the current ion chromatogram to “Successful Quantitation”. Such a change can be applied to the same compound of multiple samples using options in the pup-up window.

There are three more buttons at the right-hand side of the correction methods. The “More >” button is used to show the same compound found in the other ion chromatograms. Once pressed, the text of the button will be changed to “Less <” and an additional panel (panel (c) in Figure 17) will appear to show such plots, as shown in Figures 33(a and b). This panel shows the overlapped peaks (zoomed-in horizontally) of all sample files at the top and the detected peak in individual file below for easy inspection of the same compound across sample files. The plot of overlapped peaks is useful in checking the consistency of the compound across all the sample files. To remove the panel, the user can press the “Less <” button. The user can further use the four buttons next to the overlapped plot to zoom out the peaks horizontally to the original RT range, to zoom in the peaks horizontally to the detected peaks only, to normalize the peak heights, and to resume the original heights, as shown in Figure 33(c-f). The “Export” button is used to export the ion chromatogram to an image file. Similar to the export of heatmap, once the button is pressed, a window will appear to let the user to choose rendering methods, line width, font type and size, resolution, image format, and file name of the image (refer to Figure 13 for details). Finally, the “Close” button is used to close the “Multiple Reaction Monitoring” window.

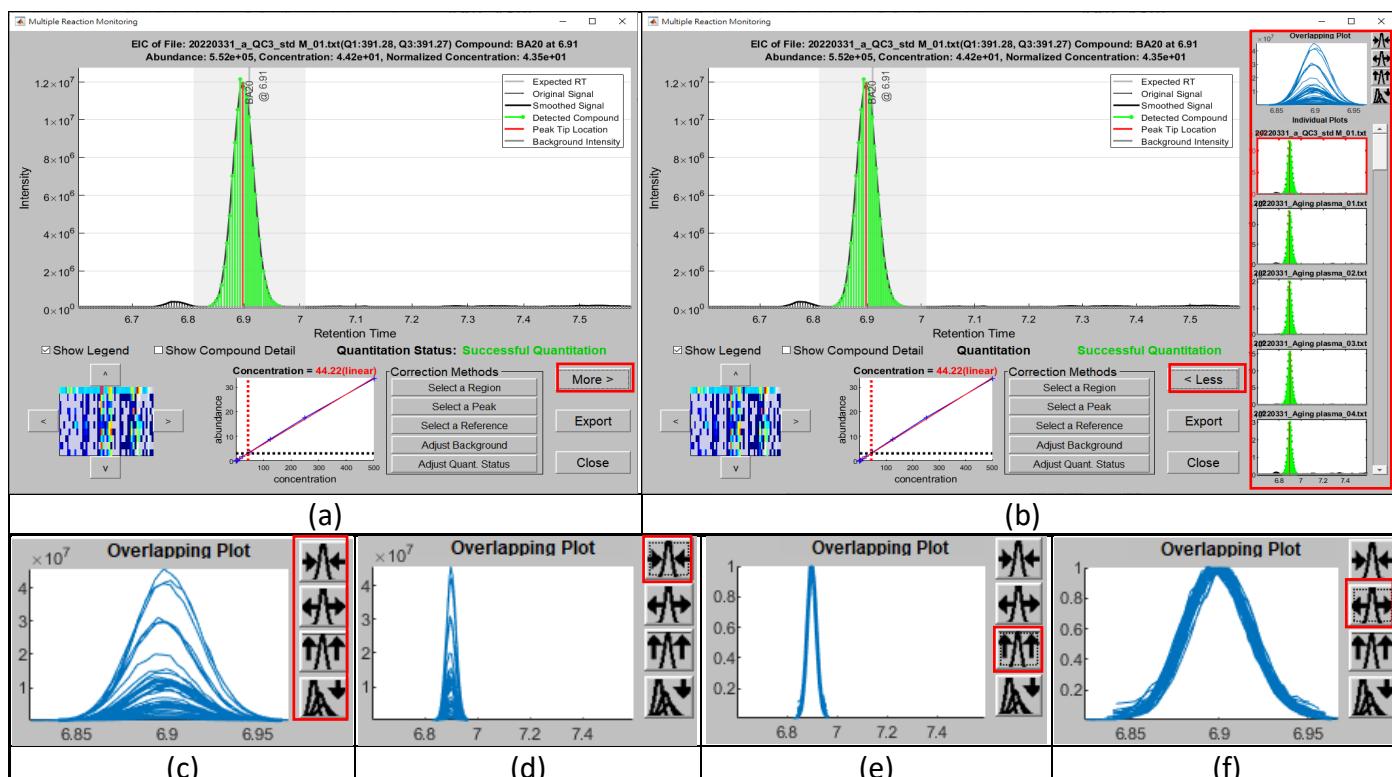
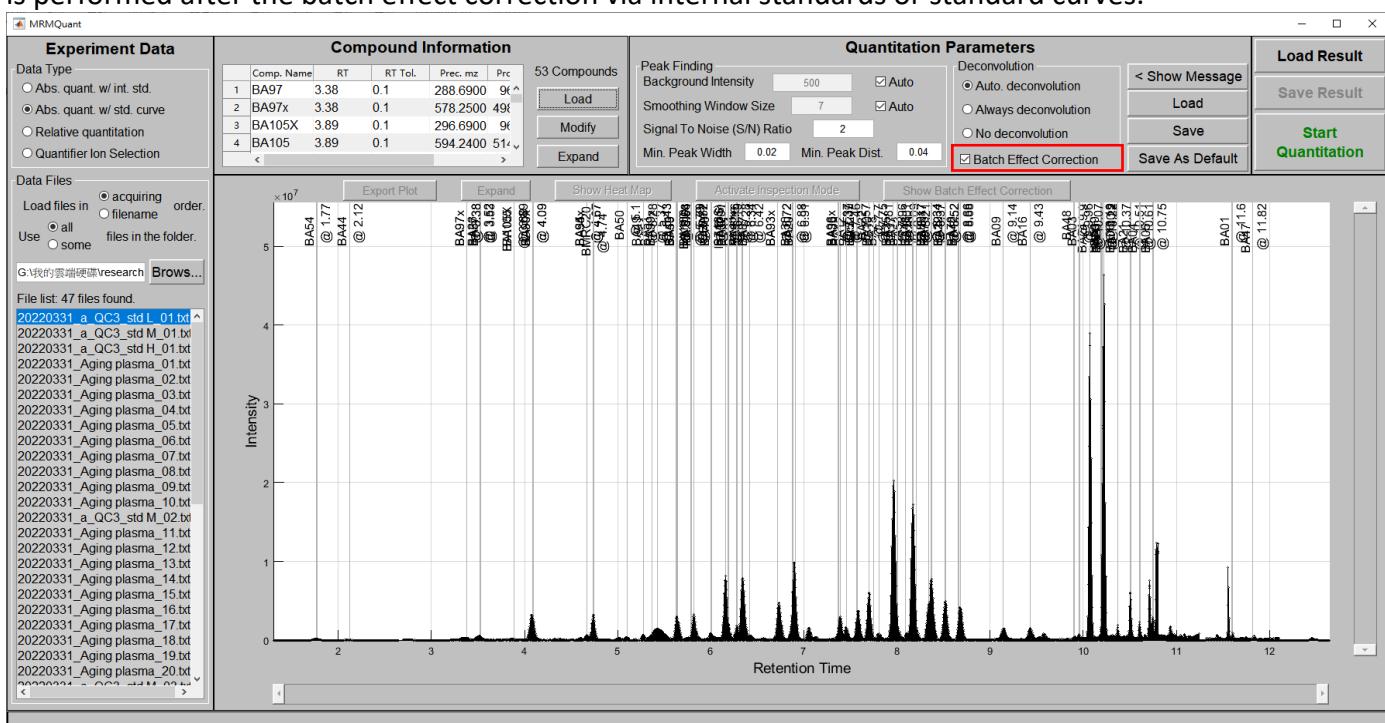


Figure 33. The additional panel that display the detected peak of a compound in different samples and an overlapped plot of the detected peaks in all the samples. (a) The “More >” button that bring out the panel. (b) The “Less <” button that close the panel. (c) The four buttons that control the appearance of the overlapped peaks: horizontal resume, horizontal zoom-in, vertical normalization, and vertical resume. (d) Use the horizontal resume button to resume the detected peaks to their original scale. (e) Use the vertical normalization button to normalize the peak heights of all the detected peaks to 1.0. (f) Use the horizontal zoom-in button to expand the detected peaks horizontally to the same tight RT range in order to check for the RT differences among the peaks.

Show batch effect correction

MRMQuant provides the batch effect correction function for MRM sample data contains multiple batches. To invoke such a correction, the user needs to check the “Batch Effect Correction” option in the “Quantitation Parameter” panel, as shown in Figure 34. In MRMQuant, we adopted the quality control-based robust LOESS (locally estimated scatterplot smoothing) signal correction (QC-RLSC) algorithm published by Dunn et al. and The Human Serum Metabolome (HUSERMET) Consortium. In short, for each compound, the following four steps are performed for batch effect correction. Step 1. Trend Estimation: a LOESS curve is fitted to its abundance in the QC samples. Step 2. Interpolation: the corrected abundances of the compound in other samples are estimated using cubic-spline interpolation/extrapolation of the LOESS curve (all the samples should be arranged in their analytical order). Step 3. Trend Removal: The ratios between the abundances computed from the ion chromatograms and those from the LOESS curve are calculated. Step 4: Magnitude Restoration: After trend removal, the LOESS curve becomes a flat horizontal line with intercept 1, and thus the abundances are restored by multiplying the ratios by the median abundance of the QC sample. For absolute quantitation, the conversion from abundances to concentration is performed after the batch effect correction via internal standards or standard curves.



fraction of the number of QC samples in the data. For example, when the value is 0.1, the window size is equal to 10% of the number of QC samples in the data.

The figure displays two identical software interfaces for 'Batch Effect Correction'. Each interface consists of a table of samples, a regression span input field, and three buttons at the bottom.

Table Headers:

- QC Sample
- Reference
- Batch End
- Batch
- Sample File Name

Regression Span Input Fields:

- (Left) Set a regression span: 0.1
- (Right) Set a regression span: 3

Buttons at the bottom of each interface:

- Ok
- Skip
- Cancel

Figure 35. The “Batch Effect Correction” window for the user to input batch-related information.

After the quantitation process is finished, the user can press the “Show Batch Effect Correction” button above the heatmap to inspect the effectiveness of the batch effect correction, as shown in Figure 36. Once the button is pressed, a “Batch Effect Correction” window will appear to show the concentrations of a target compound before and after the correction is made, as shown in Figure 37. In this figure, the original concentrations of the targeted compound BA100 across sample files and their concentrations after batch effect correction. As shown in the figure, the regression line of the compound concentration in the QC samples exhibit a slightly decreasing trend (slope = -0.0712) in the original data. However, such trend was eliminated and the regression line was leveled out (slope = -0.0060) after the batch effect correction. In addition, the R^2 value was reduced from 0.57 to 0.037 indicating the diminishing of fluctuation in the corrected concentrations.

At the top of the figure, there are functions for the user to change the plots. First, the user can change the displayed compound in the plot by selecting another compound name in the dropdown menu. Moreover, to reveal differences in small values or to suppress them in high values, the user can press the “Log Transform” button to change the concentration value from linear to logarithmic scale. In addition, if the user is not satisfied about the correction result, he/she can use the “Rerun” button to run the batch effect correction with different parameters. Furthermore, the user can use the “v More” button to inspect the concentration trend across samples of a particular internal standard. Similarly, the user can use the “Log Transform” button at the top of the plot to change the concentration value from linear to logarithmic scale.

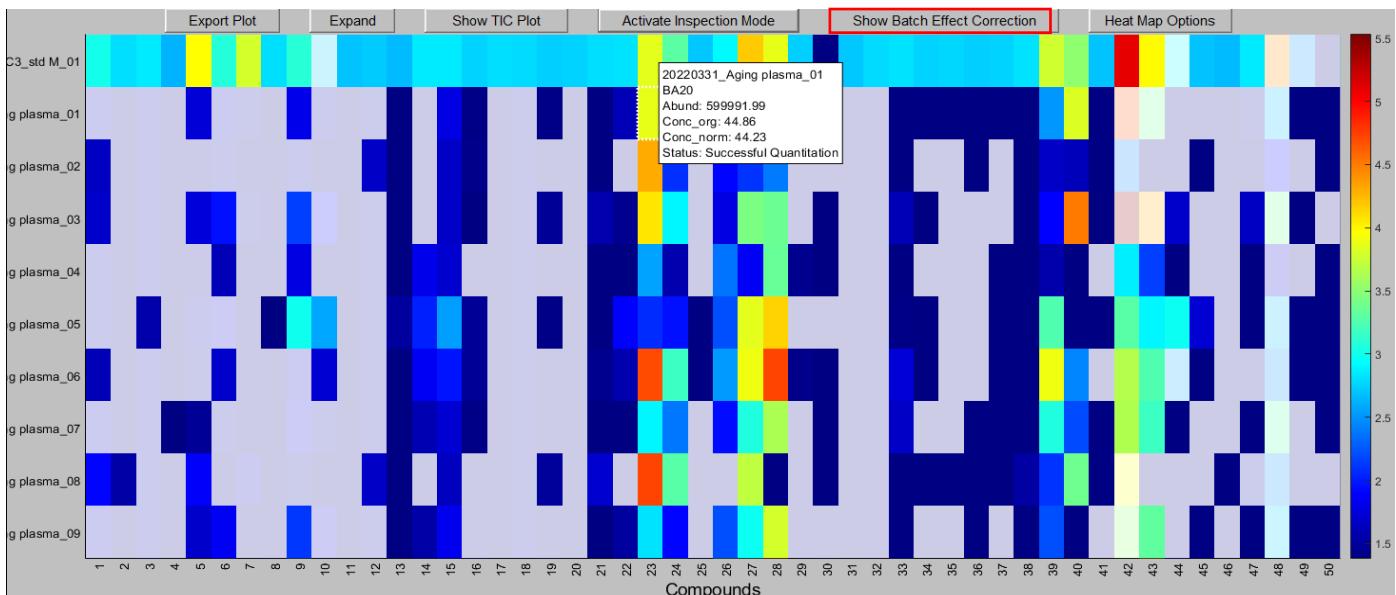


Figure 36. The “Show Batch Effect Correction” button above the heatmap.

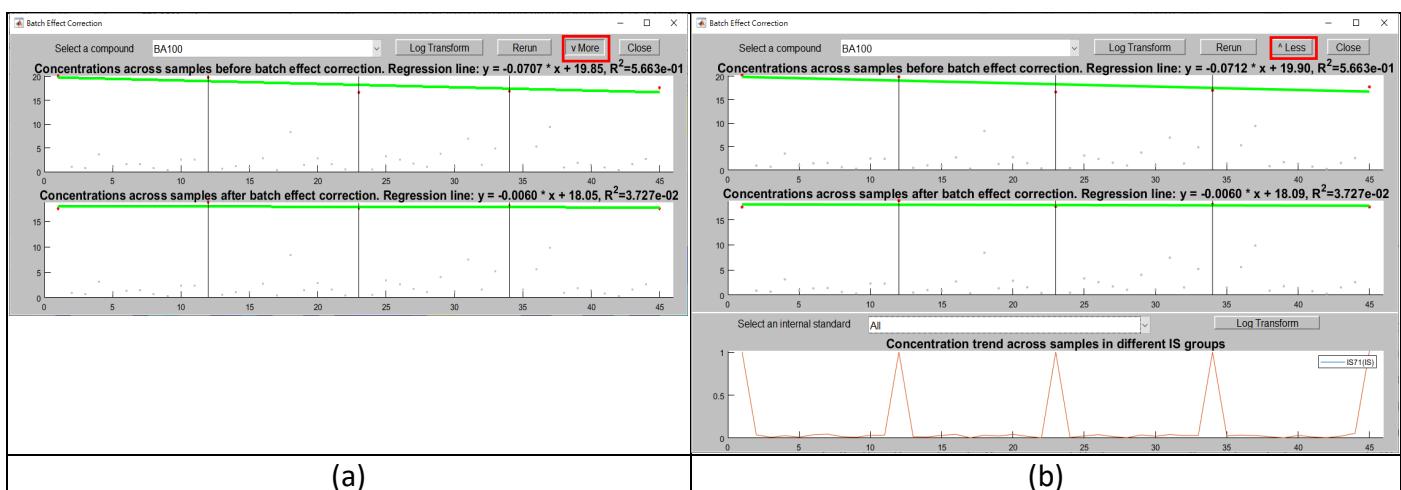
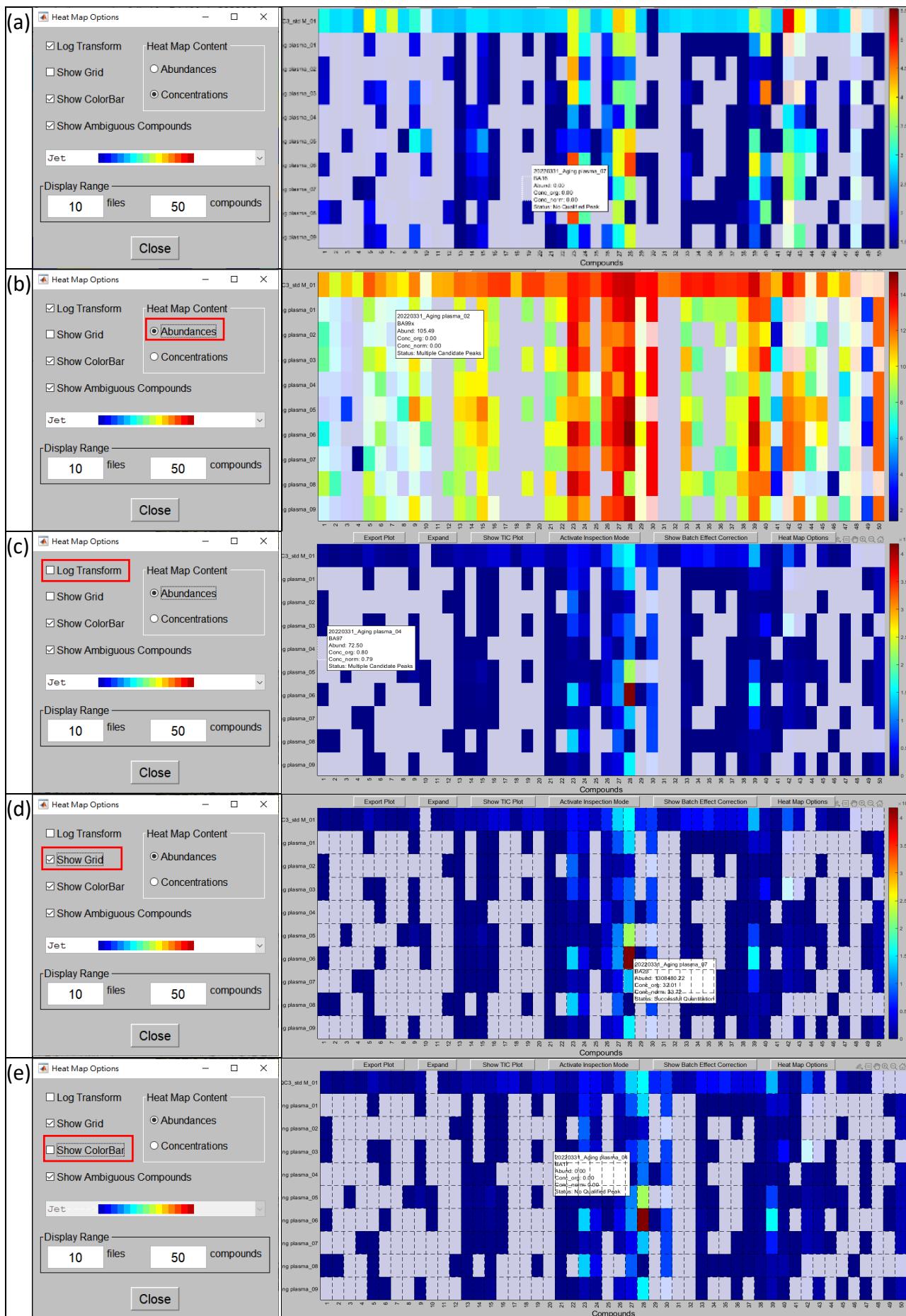


Figure 37. The regression lines of the concentrations of the QC samples before and after batch effect correction. (a) The original comparison. (b) The plot of the concentration trend across samples of a particular internal standard is revealed if the “v More” button is pressed.

Heatmap options

The aspect of the heatmap can be customized by the user using the “Heatmap Option” button, as shown in Figure 38. Figure 38(a) shows the default heatmap, in which the logarithmic concentrations of the first 50 target compounds in the first 10 samples are showed in the heatmap. From Figure 38(b) to Figure 36(g) show accordingly (b) the logarithmic concentrations being replaced by logarithmic abundances, (c) the logarithmic abundances being replaced by the original abundances, (d) the grid lines being added among cells, (e) the color bar indicating the colors in the map and their corresponding values being removed, (f) the masks denoting the problematic cells being removed, (g) the color scheme being changed from “Jet” to “Hot”, and (h) the display range being changed from 10 files and 50 compounds to 20 file and 30 compounds.



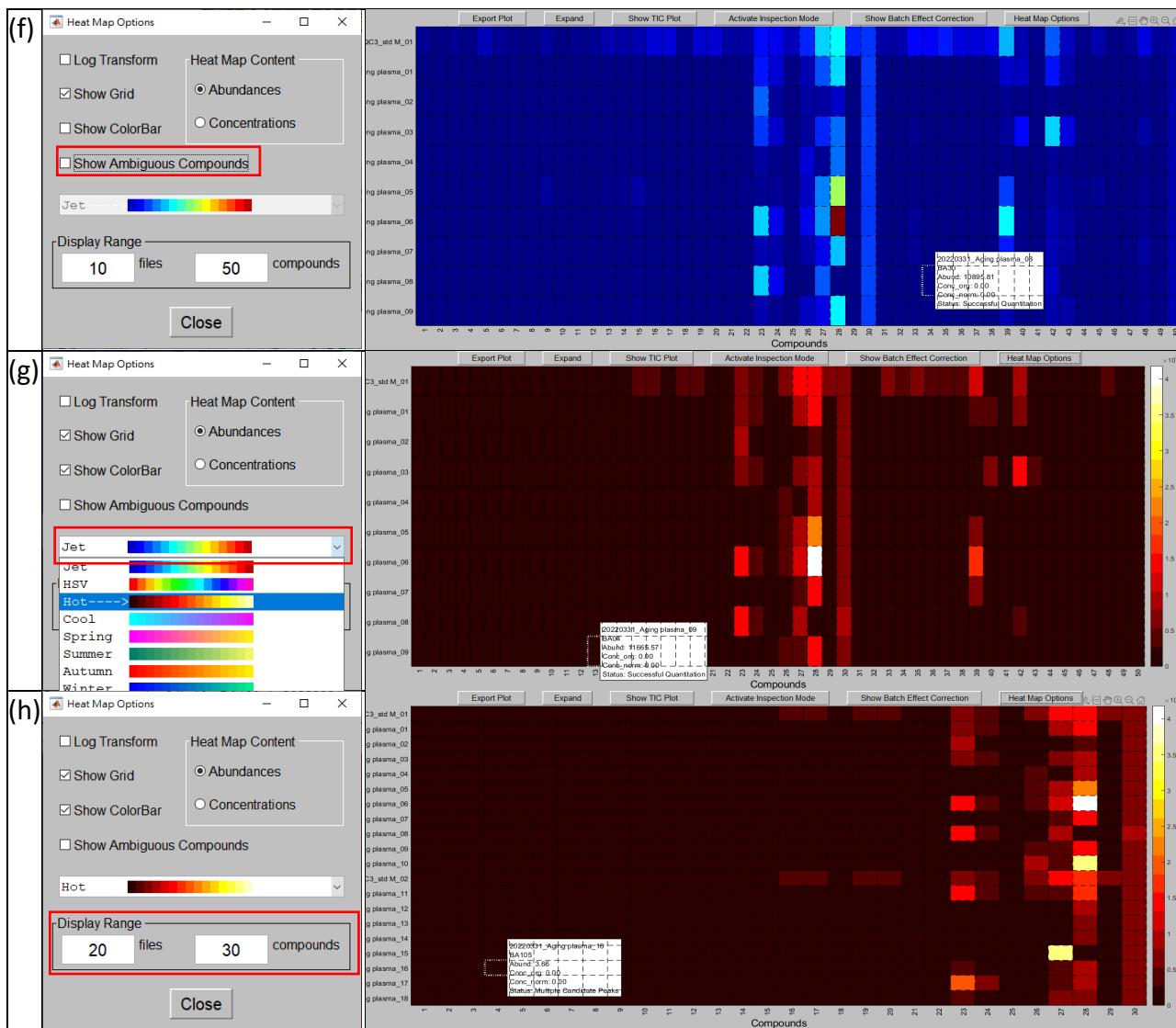


Figure 38. The heatmap options and their corresponding effects to the heatmap. (a) The heatmap of the default options (show 50 compound logarithmic concentrations in each of 10 samples with masks for ambiguous quantitations in Jet color scheme). (b) The heatmap after the content is changed from concentrations to abundances. (c) The displayed abundances are changed from logarithmic to linear scale. (d) The grid lines are added to the heatmap. (e) The colorbar indicating the associated values of the colors is removed. (f) The grey masks showing ambiguous quantitations are removed. (g) The color scheme of the heatmap is changed from “jet” to “hot” (with colorbar resumed). (h) The size of the heatmap is changed to 30 compounds in each of the 20 sample files.

Quantitation message

During the quantitation process, the “Quantitation Parameter” panel will be minimized, the “Show Message <” button will be renamed as “Hide Message >”, and the “Quantitation Message” panel will be revealed, as shown in Figure 39. This message panel is mainly designed to display a message reflecting the encountered problematic quantitation, for example, unsuccessful in peak tracing (saturated peaks, irregular peak shapes), deconvolution, standard curve generation (no suitable quadratic curve), etc. Such messages will also be saved to the output file when the quantitation result is exported. The user can determine whether to correct the quantitation of the corresponding compound using the “Multiple Reaction Monitoring” window, as mentioned in the section 6. The user can press the “Show Message <”/“Hide Message >” button to open/close the message window, as shown in Figure 40.

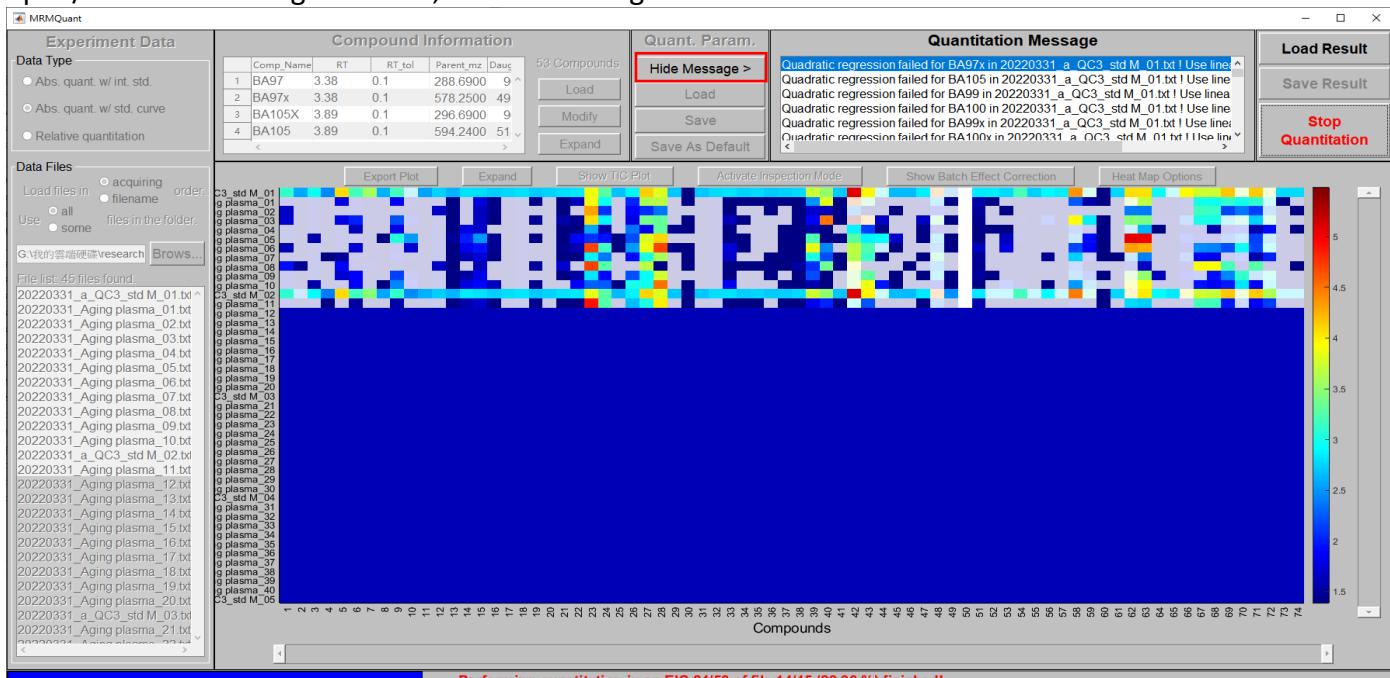


Figure 39. The “Quantitation Message” panel will appear to display a message reflecting the encountered problematic quantitation.

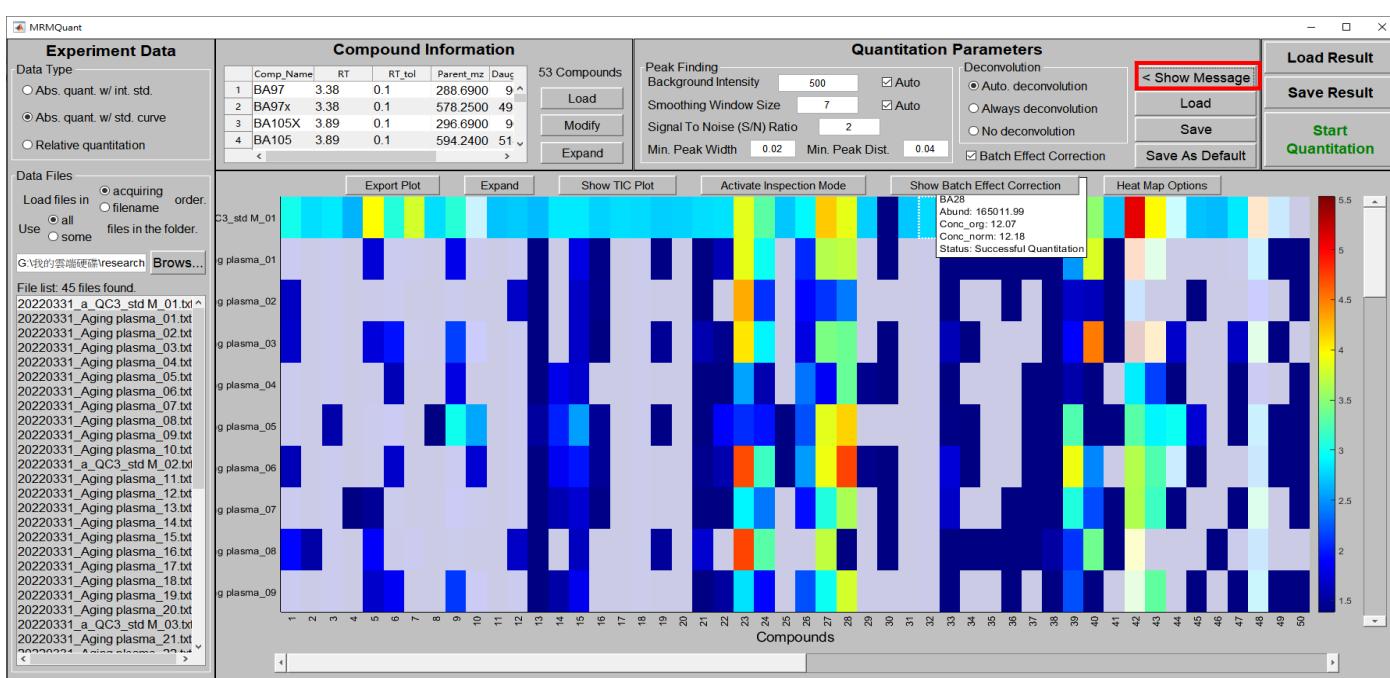


Figure 40. The user can press the “Hide Message >” button to minimize the message window and resume the parameter window.

Standard curve generation

If the user selected “Abs. Quant. w/ STD. Curve” at the Data Type, after the user clicks on the “Start Quantitation” button, the “Import Standard Curve Files” window will appear for the user to input relative information for the standard curve, as shown in Figure 41. If any quantitation result which used the same standard curves exists, the user can import the standard curve information by pressing the “load” button at the end of the window to avoid repeated computations. Otherwise, the user needs to provide (1) the sample files for the standard curve generation, (2) the method file for the targeted compounds in the sample files, and (3) the quantitation parameters in this window. Except for the first item, the latter two information can use the same ones as those for the testing sample files in steps 4 and 5. The files are suggested to be named as sample name_concentration so that MRMQuant can automatically extract the concentrations from the file names, as shown in Figure 41. Users can change curve types (quadratic or linear) and regression weights ($1, 1/x, 1/x^2$) for all compounds using the dropdown menus at the top of the “Standard Compounds and their Calibration Curve” section. These entities can also be change for individual compound using the dropdown menus at the end of each row, as shown in Figure 42. Once all the information for the standard curve generation is provided, the user can press the “Ok” button and the quantitation process of the samples for the standard curve generation will be commenced, as shown in Figure 43. The quantitation result in heat map form is shown in Figure 44. Note that the cells with the 20% transparent grey mask indicate those results that require further inspection and those with white color denote saturated peaks.

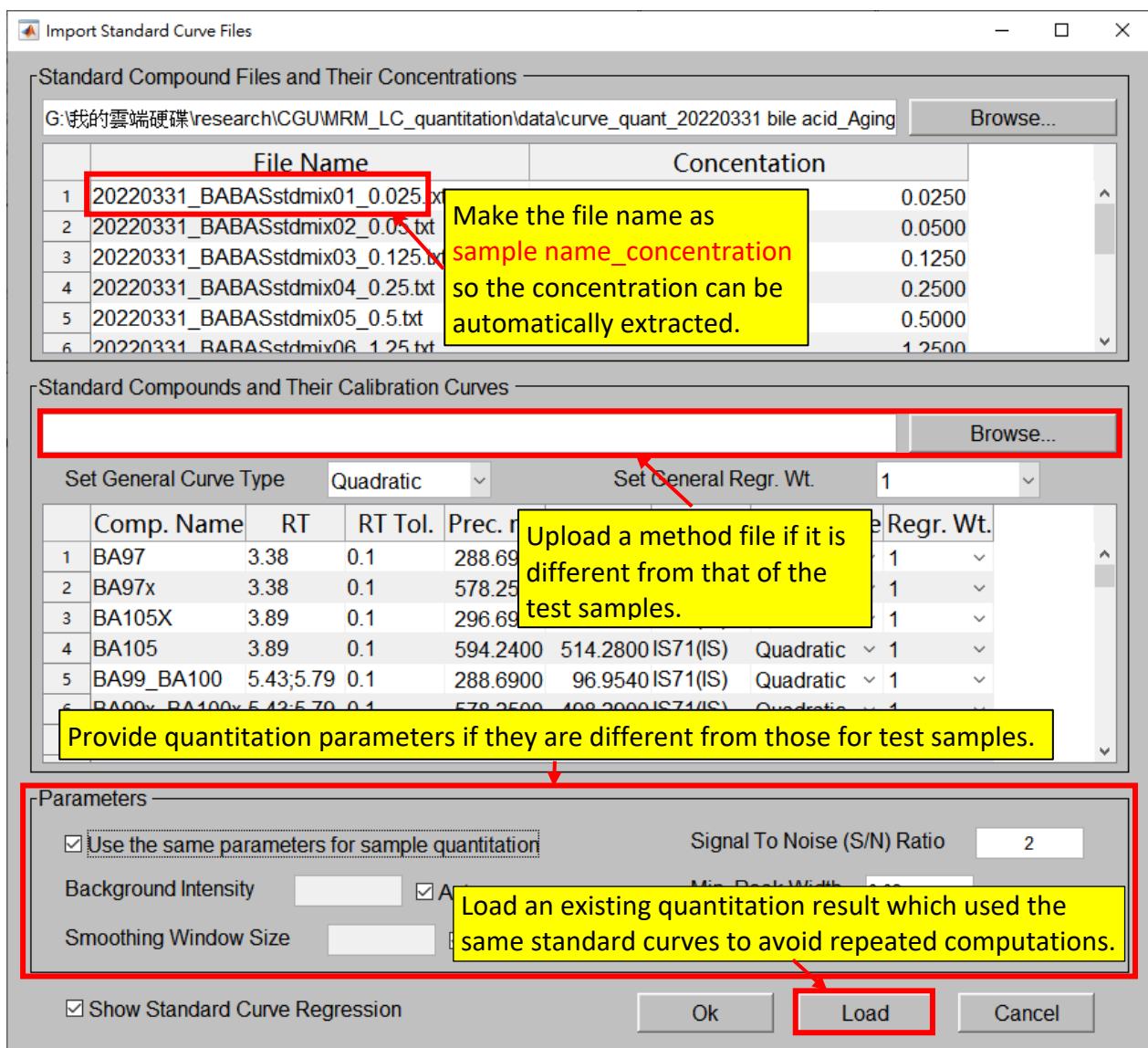


Figure 41. The “Import Standard Curve Files” window for standard curve generation.

Standard Compounds and Their Calibration Curves

Change all curves to a type at once

Set General Curve Type		Quadratic	Set General Regr. Wt.		1			
	Comp. Name	RT	Prec. mz	Prod. mz	IS	Curve Type	Regr. Wt.	
1	BA97	3.38	288.6900	96.9540	IS71(IS)	Quadratic	1	
2	BA97x	3.38	578.2500	498.2900	IS71(IS)	Quadratic	1	
3	BA105X	3.89	296.6900	96.9540	IS71(IS)	Quadratic	1	
4	BA105	3.89	594.2400	514.2800	IS71(IS)	Quadratic	1	
5	BA99_BA100	5.43;5.79	0.1	288.6900	96.9540	IS71(IS)	Quadratic	1
6	BA99x_BA100x	5.43;5.79	0.1	578.2500	498.2900	IS71(IS)	Quadratic	1
7	BA96	7.37	280.6900	96.9540	IS71(IS)	Quadratic	1	

(a)

Standard Compounds and Their Calibration Curves

Change all regression to the same weight at once

Set General Curve Type		Quadratic	Set General Regr. Wt.		1			
	Comp. Name	RT	RT Tol.	Prec. mz	Prod. mz	IS	Curve Type	Regr. Wt.
1	BA97	3.38	0.1	288.6900	96.9540	IS71(IS)	Quadratic	1
2	BA97x	3.38	0.1	578.2500	498.2900	IS71(IS)	Quadratic	1
3	BA105X	3.89	0.1	296.6900	96.9540	IS71(IS)	Quadratic	1
4	BA105	3.89	0.1	594.2400	514.2800	IS71(IS)	Quadratic	1
5	BA99_BA100	5.43;5.79	0.1	288.6900	96.9540	IS71(IS)	Quadratic	1
6	BA99x_BA100x	5.43;5.79	0.1	578.2500	498.2900	IS71(IS)	Quadratic	1
7	BA96	7.37	0.1	280.6900	96.9540	IS71(IS)	Quadratic	1

(b)

Standard Compounds and Their Calibration Curves

Change curve type individually

Set General Curve Type		Quadratic	Set General Regr. Wt.		1			
	Comp. Name	RT	RT Tol.	Prec. mz	Prod. mz	IS	Curve Type	Regr. Wt.
1	BA97	3.38	0.1	288.6900	96.9540	IS71(IS)	Quadratic	1
2	BA97x	3.38	0.1	578.2500	498.2900	IS71(IS)	Quadratic	1
3	BA105X	3.89	0.1	296.6900	96.9540	IS71(IS)	Quadratic	1
4	BA105	3.89	0.1	594.2400	514.2800	IS71(IS)	Quadratic	1
5	BA99_BA100	5.43;5.79	0.1	288.6900	96.9540	IS71(IS)	Quadratic	1
6	BA99x_BA100x	5.43;5.79	0.1	578.2500	498.2900	IS71(IS)	Quadratic	1
7	BA96	7.37	0.1	280.6900	96.9540	IS71(IS)	Quadratic	1

(c)

Standard Compounds and Their Calibration Curves

Change curve regression weight individually

Set General Curve Type		Quadratic	Set General Regr. Wt.		1			
	Comp. Name	RT	RT Tol.	Prec. mz	Prod. mz	IS	Curve Type	Regr. Wt.
1	BA97	3.38	0.1	288.6900	96.9540	IS71(IS)	Quadratic	1
2	BA97x	3.38	0.1	578.2500	498.2900	IS71(IS)	Quadratic	1
3	BA105X	3.89	0.1	296.6900	96.9540	IS71(IS)	Quadratic	1
4	BA105	3.89	0.1	594.2400	514.2800	IS71(IS)	Quadratic	1
5	BA99_BA100	5.43;5.79	0.1	288.6900	96.9540	IS71(IS)	Quadratic	1
6	BA99x_BA100x	5.43;5.79	0.1	578.2500	498.2900	IS71(IS)	Quadratic	1
7	BA96	7.37	0.1	280.6900	96.9540	IS71(IS)	Quadratic	1

(d)

Figure 42. Calibration curve types (a) and regression weights (b) for all compounds and curve types (c) and regression weights (d) for individual compound can be changed using the dropdown menus at the top of the “Standard Compounds and their Calibration Curve” section

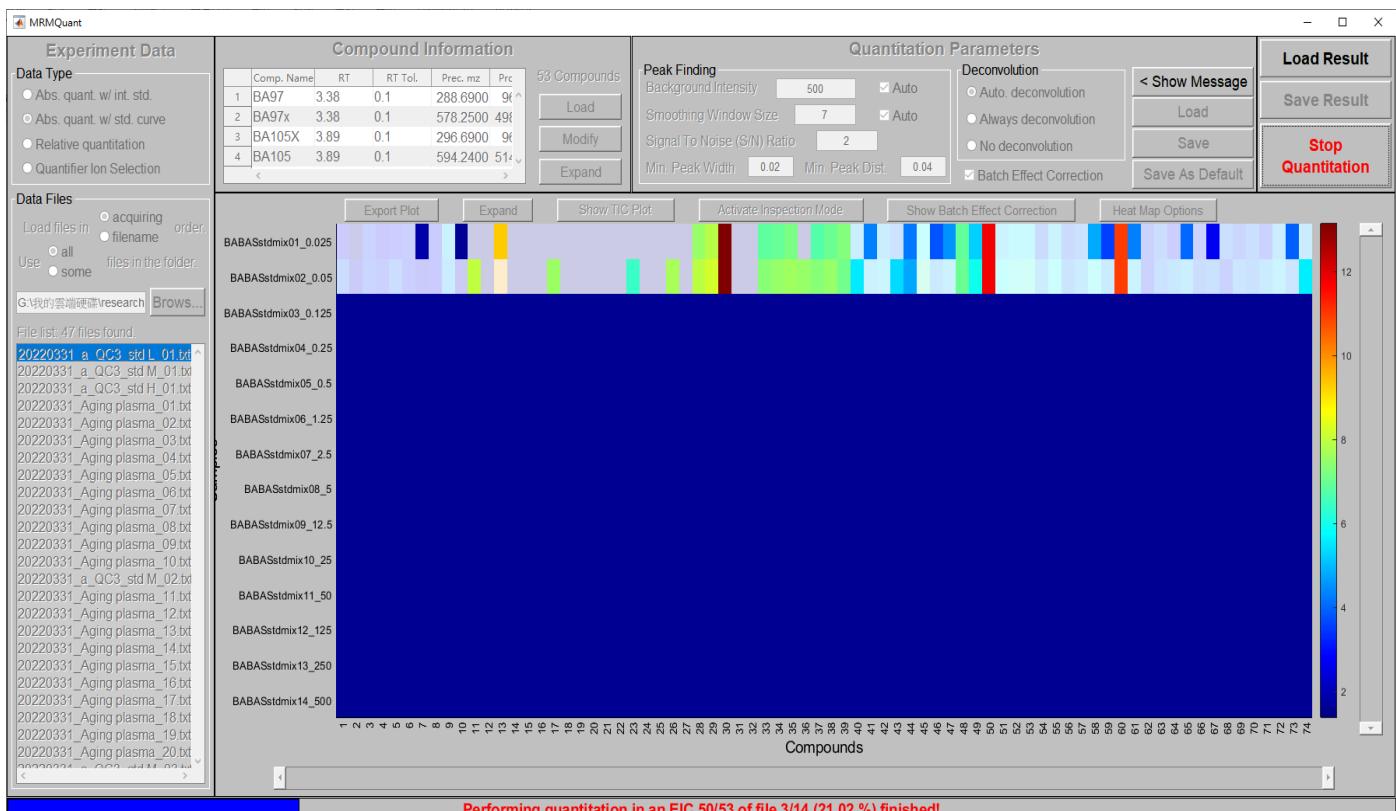


Figure 43. The quantitation is performed on the samples for standard curve generation.

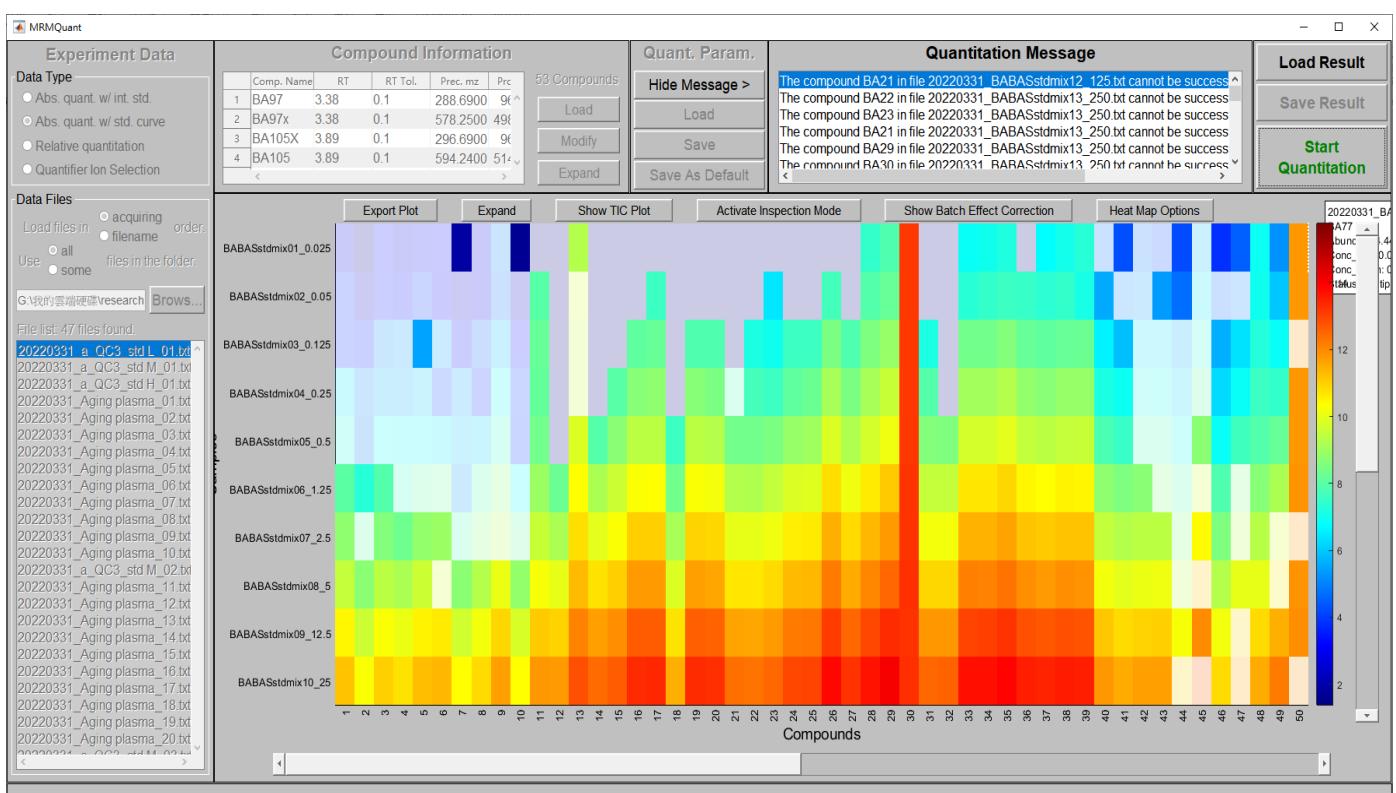


Figure 44. The quantitation result in heat map form of the data for standard curve generation.

After the quantitation of the SC files is finished, a “Standard Curve” window will appear showing the calculated SCs, as shown in panel (a) of Figure 45. Each plot in the window shows a blue line with a “+” mark at the data points indicating the designated compound concentrations (horizontal axis) and their calculated abundances (vertical axis) in. The red line in the plot shows the corresponding regression line (i.e., the SC) of the data points. The plot is shown in linear scale, but can be changed to logarithmic scale to reveal the detail in the small concentration points. By clicking on the regression plot of a compound, the ion chromatograms of the compound in different SC files will be revealed in panel (b), as shown in Figure 45. The user can also

choose to skip certain points in the regression plot by unchecking the box next to the ion chromatograms if they do not seem to fit in the curve after the corrections. Saturated peaks are automatically excluded from the regression process for their unreliable abundances, as shown in Figure 46. In panel (c), there are two options to adjust the plots in panel (a) and in panel (b), respectively. On the left-hand side, the “Show log-scaled standard curves” changes the standard curves in panel (a) from linear to logarithmic scale. This option is designed to reveal the regression details in the small values, as shown in Figure 47. The “Show absolute height for the detected height” option is designed to magnify the height of the detected peaks in all the ion chromatograms to compare their shapes across samples, as shown in Figure 48. By default, the peak heights are displayed in the same scale for amplitude comparison. But they can be displayed in the normalized scale for peak shape comparison by selecting the option in panel (c). The user can also click on an ion chromatogram to further inspect and correct the detected peak. Once the correction is made, the SC will be updated.

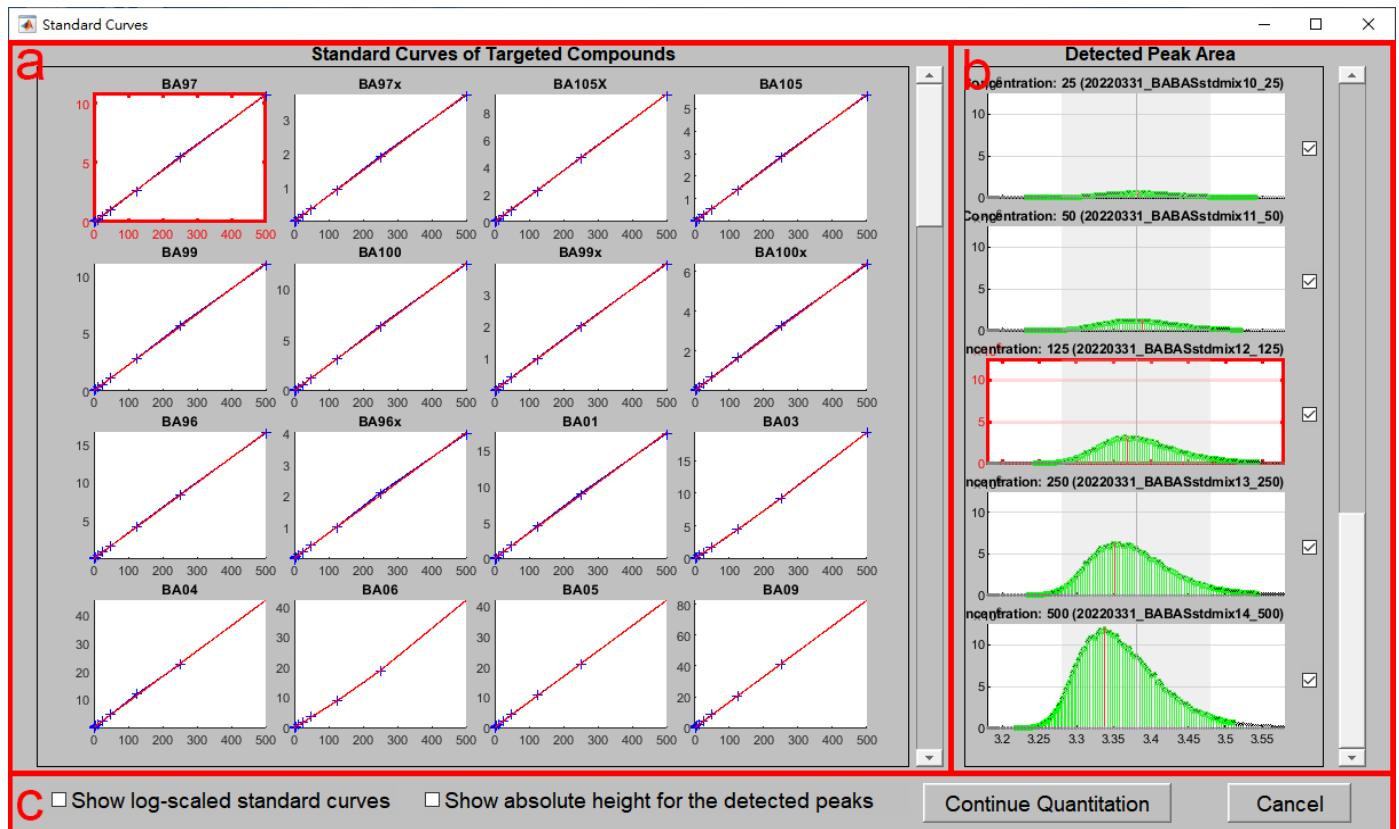


Figure 45. The standard curves for the targeted compounds generated via the sample files of known concentrations. Panel (a) shows the standard curves, whereas panel (b) shows the individual ion chromatograms of the highlighted curve in panel (a). In panel (c), the checkboxes control the linear/logarithmic scale of the standard curves and the height of the peaks in the ion chromatograms, respectively.

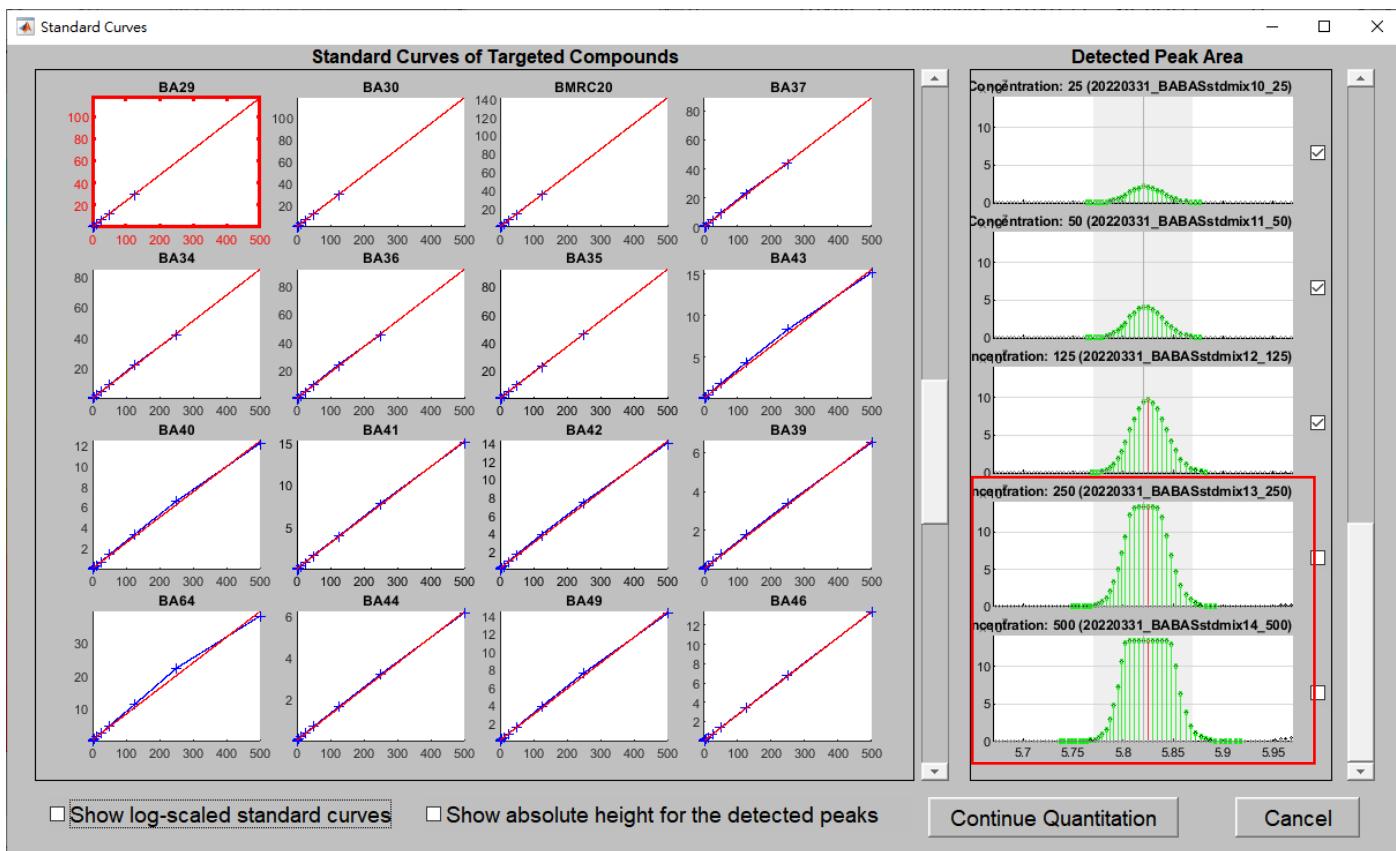


Figure 46. Saturated peaks are automatically excluded from the regression process for their unreliable abundances. The user can also choose to skip certain points in the regression plot by unchecking the box next to the ion chromatograms if they do not seem to fit in the curve after the corrections.

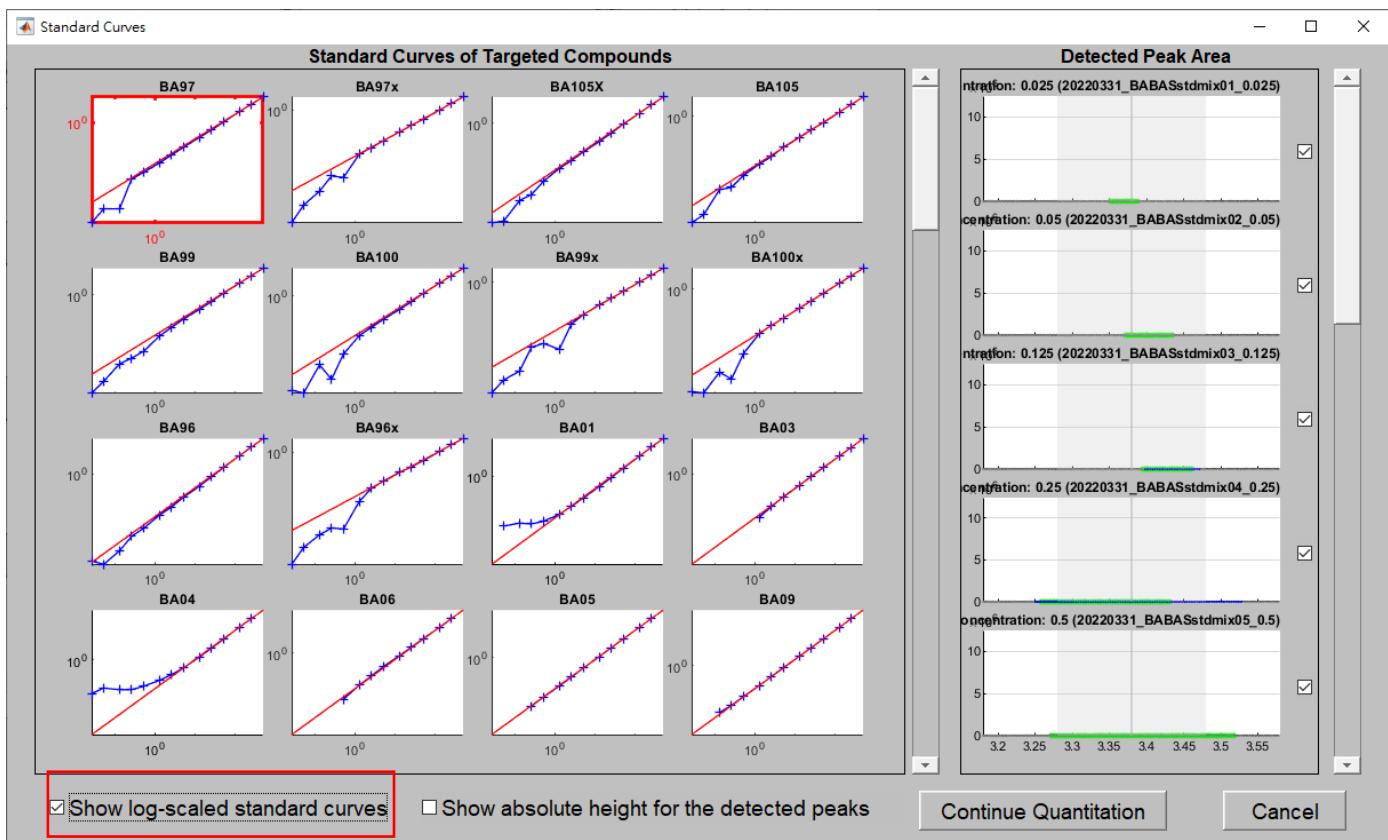


Figure 47. The “Show log-scaled standard curves” changes the standard curves from linear to logarithmic scale to reveal the regression details in the small values.

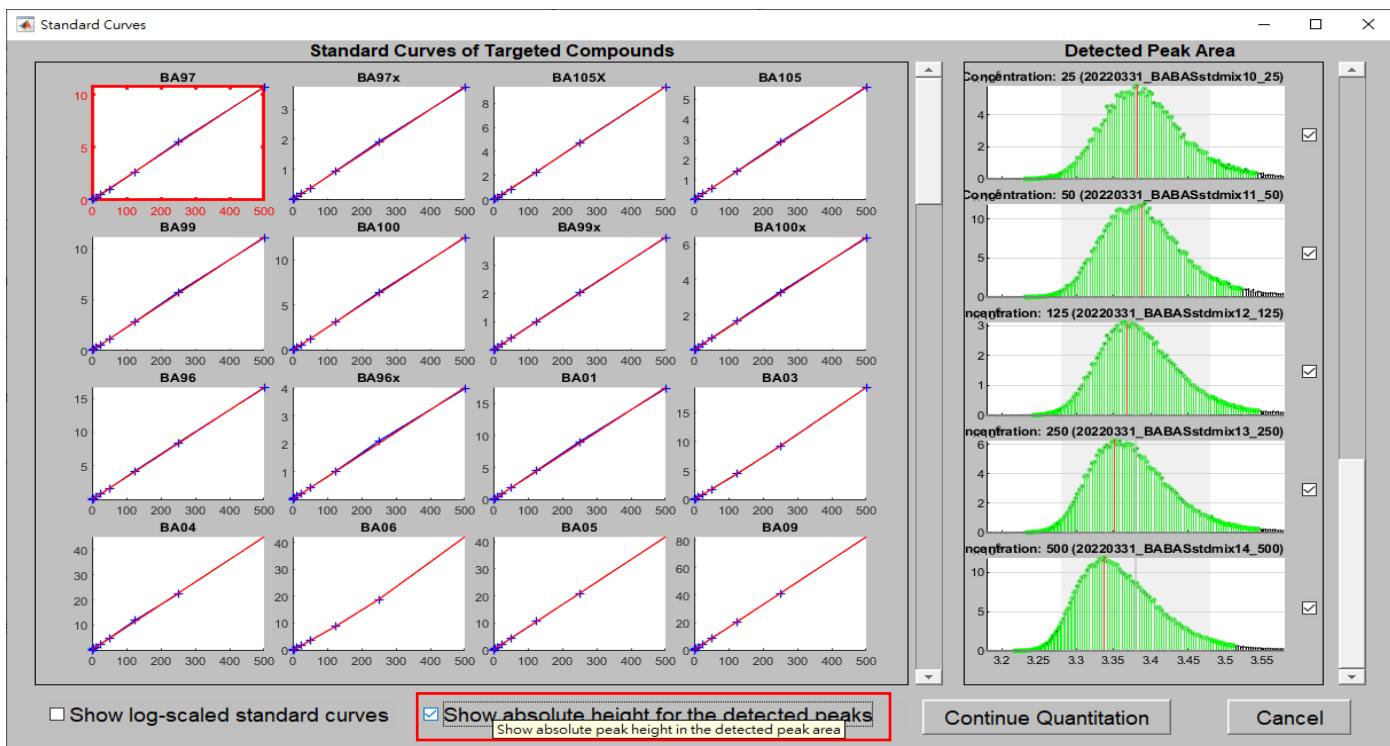


Figure 48. The “Show absolute height for the detected height” option is designed to magnify the height of the detected peaks in all the ion chromatograms to compare their shapes across samples.

However, for samples with low concentration, their signal intensities in the ion chromatogram are usually low and hence susceptible to noise and subsequently lead to inaccurate peak detection and quantitation, as shown in Figure 49. When some of the peaks are not correctly quantitated, the user can click on the corresponding ion chromatogram and it will bring up the Multiple Reaction Monitoring window, as shown in Figure 17. For details about how to use the five correction methods to correct the quantitation, please refer to section **9. Activate/deactivate inspection mode**. Here, we simply demonstrate how to use the “select a reference” method to fix the inconsistency problem of a compound across multiple samples. For example, we click on the fourth chromatogram on the right and it will bring up the Multiple Reaction Monitoring window, as shown in Figure 50.

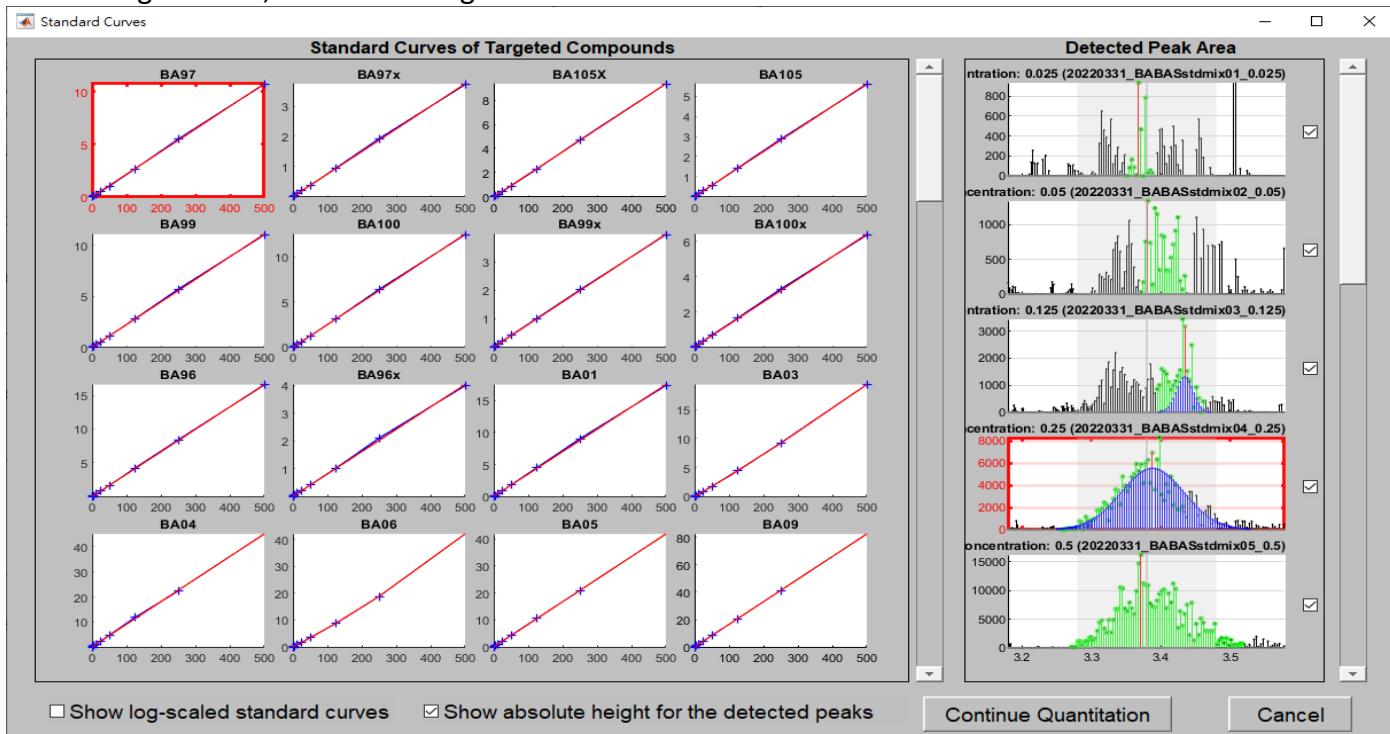


Figure 49. Samples with low concentration, their signal intensities in the chromatogram are usually low and hence susceptible to noise and subsequently lead to inaccurate peak detection and quantitation.

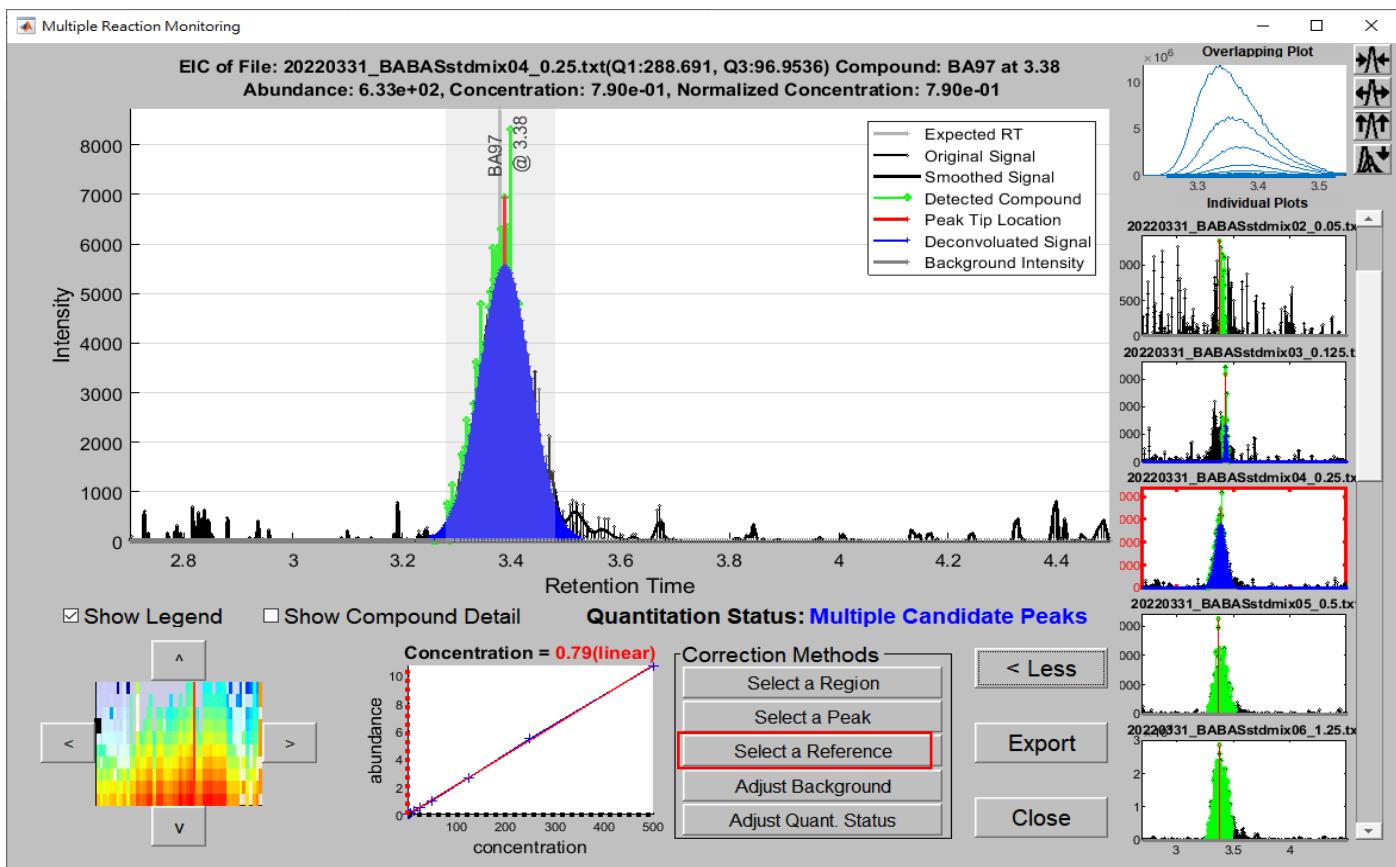


Figure 50. The Multiple Reaction Monitoring window for peak area correction.

In Figure 50, if the “Select a Reference” button is pressed, a window that allows the user to specify the reference sample will be brought up. Because there is only one batch in the standard curve samples, one sample is to be assigned as the reference, as shown in Figure 51. In this case, the sample with concentration 50 is selected as the reference since this sample has higher concentration and thus the compound herein is more likely to have higher peak signals. Although there are samples with higher concentrations (125, 250, 500), they consist of saturation peaks, as shown in Figure 46.

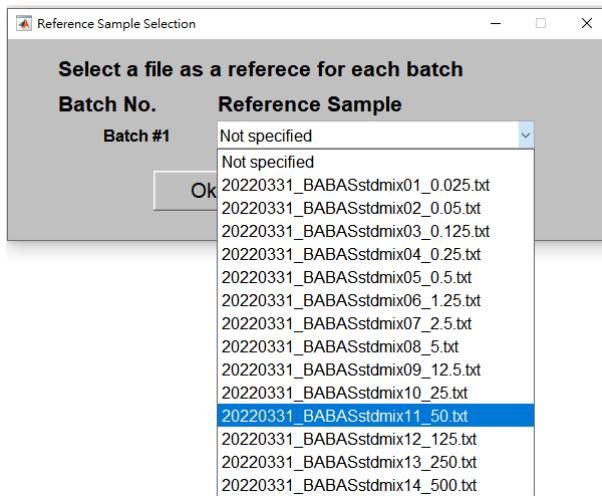


Figure 51. The window for the user to select a reference from candidate samples

As shown in Figure 52, after the correction, the compound peaks in different samples are more consistent since they were identified using a similar RT range as that in the reference. More importantly, the peak areas (abundances) are more consistent (data points are smoother along a curve) in the standard curve, as shown in Figure 53. If no more correction is required, the user can press the “Continue Quantitation” button to quantify the testing samples. Otherwise, the user can press the “Cancel” button to terminate the quantitation process, as shown in Figure 54.

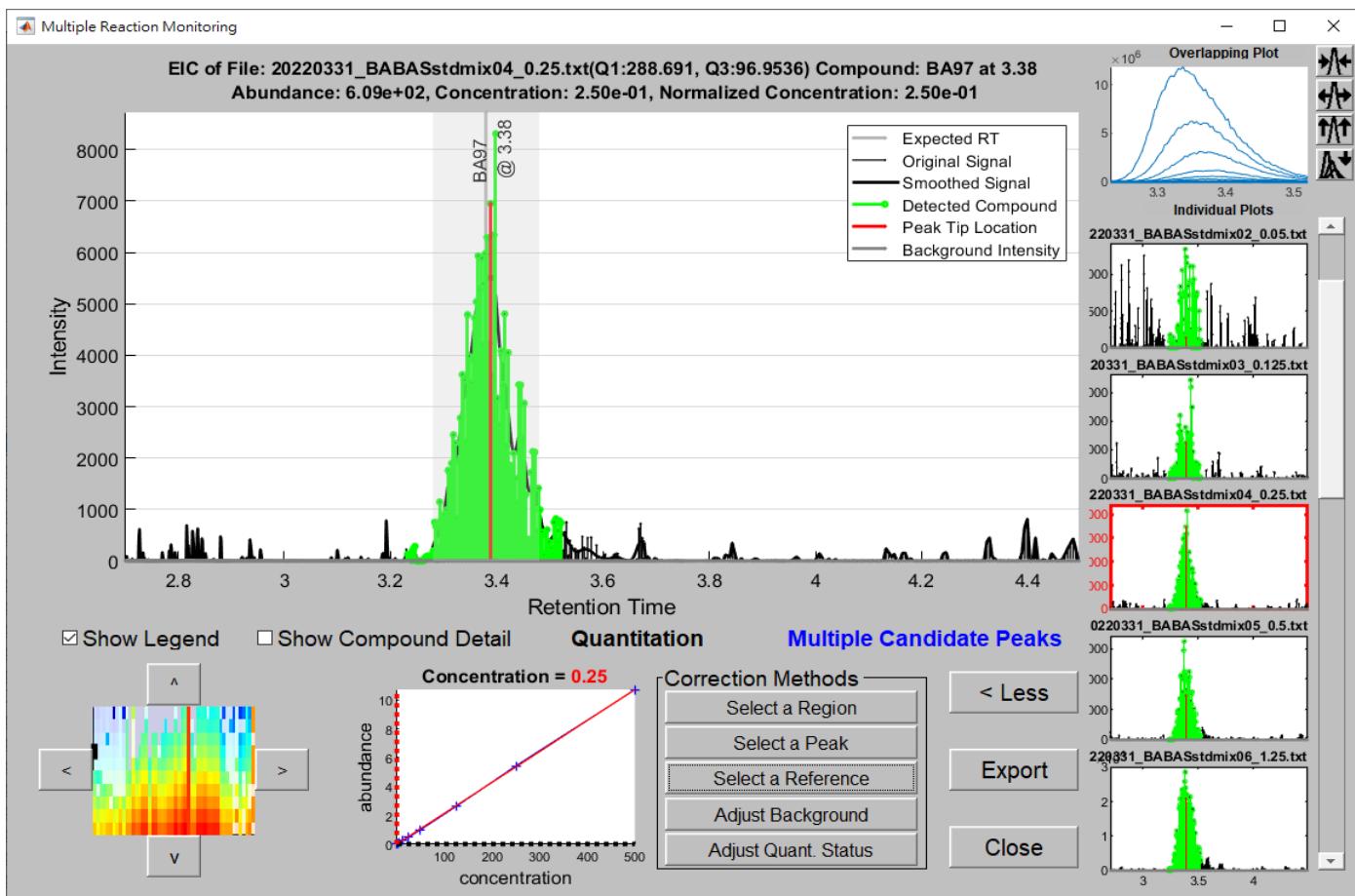


Figure 52. The quantitated peaks in the current sample (middle plot) and in the other samples (plots in the right) are more consistent in RT range after the correction.

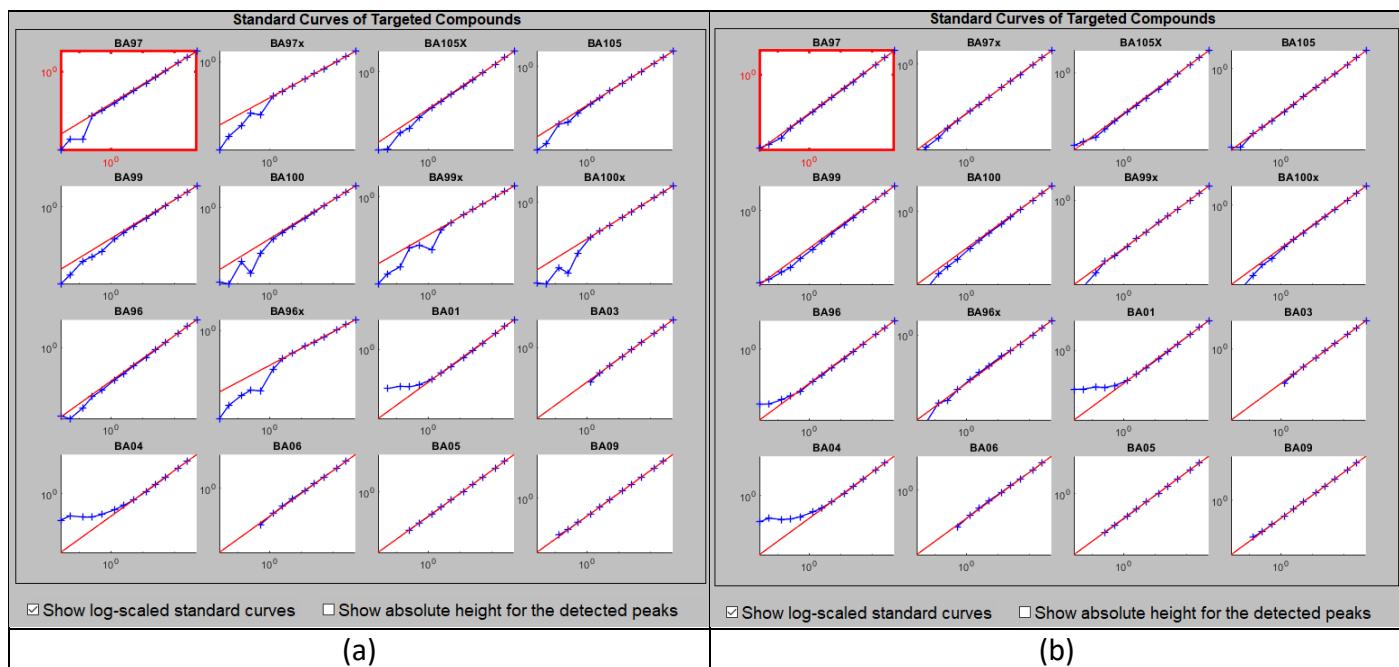


Figure 53, Standard curves before and after the “Select a Reference” correction, (a) before the correction and (b) after the correction.

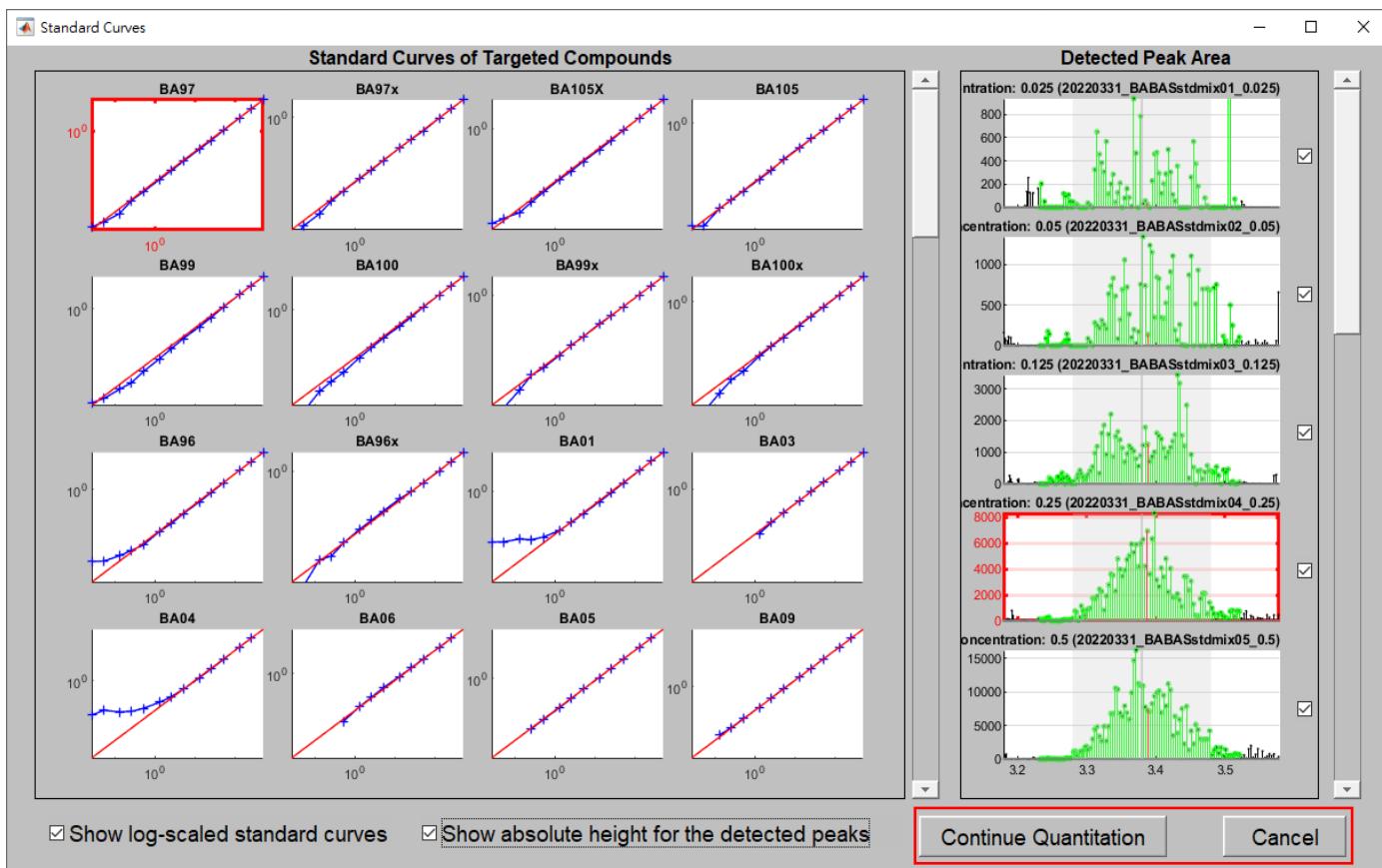


Figure 54. The user can press the “Continue Quantitation” button to quantify the testing samples or press the “Cancel” button to terminate the quantitation process.

Input/Output the quantitation result

When the quantitation process is completed, a window will appear to ask the user to provide the path and the file name to save the quantitation result. In addition, the user can save the result manually using the “Save Result” button at the upper right corner of the GUI, when a correction is made, as shown in Figure 55. Once the button is pressed, a window containing the list of all the quantitation result items (such as abundance, concentration, retention time, etc.) will pop up. Different experiment type generates different items. By default, all the generated items will be saved. Figure 56 shows the items for “Abs. quant. w/ std. curve”. However, some of the items (for example, status background color as shown in Figure 57 and regression curve plot as shown in Figure 58) may require longer time to be saved. The user can select the required items by checking the corresponding box in front of the item.

The quantitation results are saved in a Microsoft Excel file in the xlsx format, as shown in Figure 59. An empty cell in the Excel file indicates an unquantified compound, usually due to saturation. Each of the items is saved on a separate sheet in the Microsoft Excel file. For easy access to existing quantitation results, a MATLAB mat file with the same name as the Excel file will also be generated for future inspection and modification. However, it should be noted that when the mat file is larger than 2GB, the save time can increase considerably. If the quantitation mat file is larger than 2GB, a warning message will appear to warn the user for long saving time, as shown in Figure 60. It is highly recommended that the user saves such a file in local drive (e.g. drive C or drive D). Saving large mat files to cloud drive can take a very long time.

The user can use the “Load Result” button at the upper right corner of the GUI (as shown in Figure 61) to inspect and correct the existing quantitation result, and apply different parameter settings to re-compute the entire quantitation result. Once the “Load Result” button is pressed, an open file window will show up for the user to select the mat file associated with the quantitation result of interest, as shown in Figure 62.

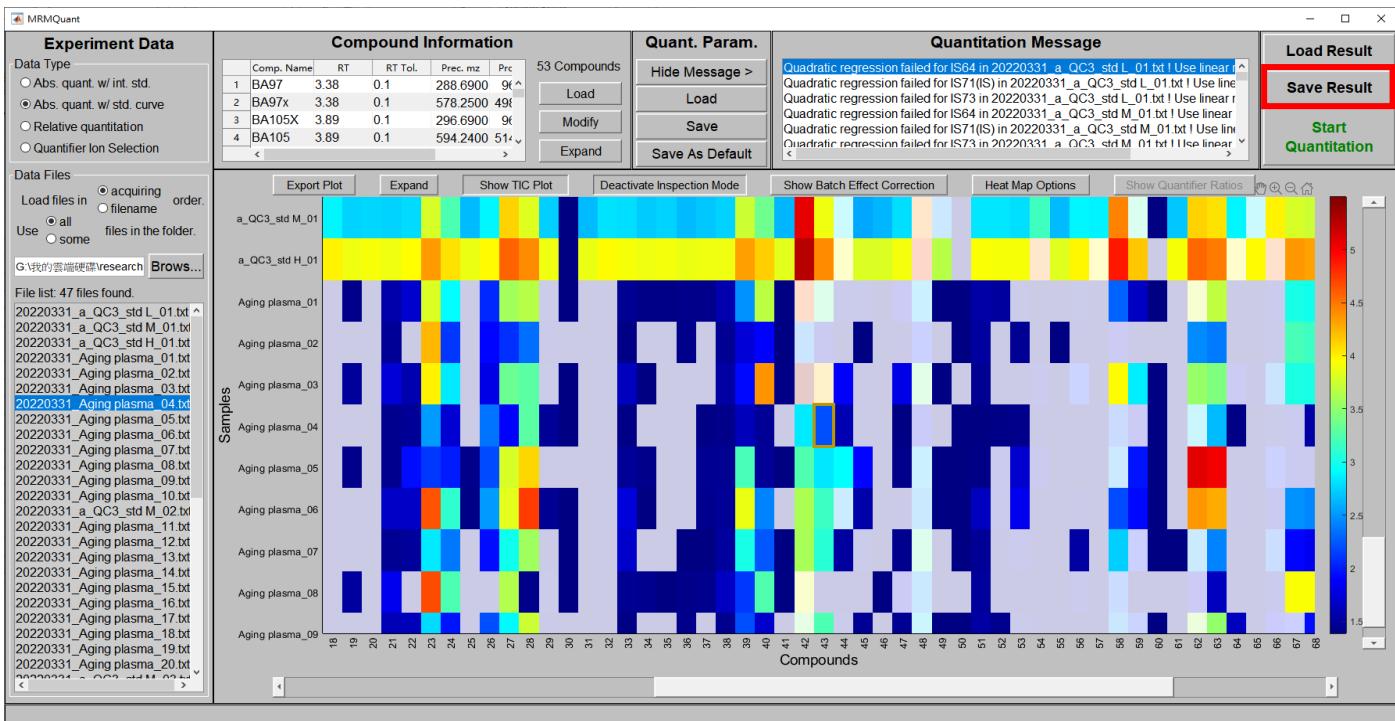


Figure 55. The “save result” button in the GUI for the user to save a new or a corrected quantitation result.

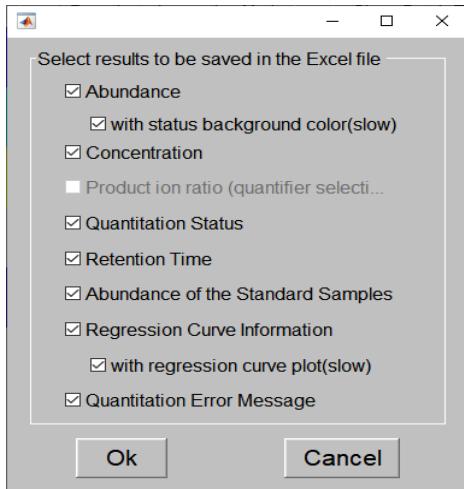


Figure 56. Output items of a quantitation result. The dark black items are default for the “Abs. quant. w/ std. curve” experiment.

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T		
Row					No Qualified Peak		Multiple Candidate Peaks		Saturated Signals		Defect Quantitation		Concentration Unconvertable								
3	20220331_a_QC3_std_L_01	28746.42728	5181.749193	1257.3536	3902.24723	41200.11161	33844.29931	10583.58181	75298.82584	3224.33247	0	14506.07387	46346.86363	31813.50637	56571.81475	7826.34426	75054.82662	10860.13323	70429.23014		
4	20220331_a_QC3_std_M_01	68899.66708	13298.66036	49346.60256	16335.64468	223704.7384	90378.00049	45938.18411	13593.11876	121691.691	15666.35661	9374.06266	68808.30179	180631.2377	149047.4475	184196.81323	337480.45239	35730.37114	57074.84363	30518.8199	
5	20220331_a_QC3_std_H_01	219699.4622	43485.09278	188914.654	63583.05146	318169.2739	274802.3327	75598.40995	78209.60304	302079.6625	36482.69350	241789.9545	20974.3208	588190.3237	92269.865	661248.1319	128330.1701	1406491.204	231223.347	1243390.804	
6	20220331_Aging plasma_01	1381.923222	255.3798995	30.0279460	0	8183.8749	3638.193731	1404.399823	723.0702592	13925.520188	0	14542.23249	0	32333.07394	7764.848308	0	0	5528.540527			
7	20220331_Aging plasma_02	1075.396457	396.1890278	18.50467873	3.852347137	1362.454252	2427.23086	125.827293	313.37621	3691.367462	52.25268212	0	4481.76196	2639.94896	0	22818.88019	9767.095706	0	0		
8	20220331_Aging plasma_03	2901.559187	639.4482686	29.6366801	3.280248462	8403.793274	13866.444588	44.10249174	3502.472207	0	0	3395.16218	0	22487.71948	5091.824971	0	0	18142.12137			
9	20220331_Aging plasma_04	72.5008183	72.5529812	14.9674187	3.665086865	1567.8595484	3881.4464949	109.8784203	884.0729389	12151.8584	321.7486603	0	0	16716.33741	24641.1914	25215.4987	0	0	0		
10	20220331_Aging plasma_05	24.8700758	53.54499376	44.10249174	9.420696792	1332.678121	90378.08909	541.1911414	1427.07108	10880.8484	13647.49705	0	0	34468.71943	42557.42758	134075.369314	15553.38192	0	0	5265.416043	
11	20220331_Aging plasma_06	869.713697	113.3336836	0	2.179966809	2238.2738	6418.09229	161.0386896	132.00355	1793.50795	0	0	1089.95176	27231.24255	51509.70487	15991.89565	0	0	0		
12	20220331_Aging plasma_07	751.0548271	156.7273874	38.00726409	0	0.043332577	3679.484681	2068.407222	40.35632849	9256.715595	873.0750802	0	0	7535.983542	9717.445845	27710.10161	8772.280168	0	0	0	
13	20220331_Aging plasma_08	8769.639941	1725.1424661	15.1532087	10.18960703	11284.19264	345.4180826	1927.042094	13.15262723	1638.88833	9.52697181	0	0	46112.712756	8159.190022	0	1929.9125	0	0	15172.54776	
14	20220331_Aging plasma_09	404.815617	48.14933515	20.4150003	7.967698209	9324.999034	10790.17929	969.680875	2052.473969	2255.781532	0	0	11665.5665	6421.601664	37049.51834	9192.361348	0	0	0		
15	20220331_Aging plasma_10	73.7453286	277.7155101	27.71317787	0	2372.801071	10480.09532	231.7689526	2779.688794	46439.5129	5032.936467	0	0	785.599903	22846.61676	46585.31003	40721.0933	0	0	8040.208285	
16	20220331_a_QC3_std_M_02	76024.76156	12908.67576	48901.08124	15906.03337	228267.7828	91646.17407	42700.34467	31809.92265	114573.5452	1187.39237	2162.80149	69325.70438	189550.9380	158486.4137	197983.5695	348390.278	382258.5333	60626.575248	333674.358	
17	20220331_Aging plasma_11	1216.335874	651.8851248	10.51675127	0.021141741	349.4273929	1126.3520428	57.8473928	2548.41108	348.6253163	0	0	7662.484064	6168.641478	20568.20168	8979.16947	0	0	7802.061233		
18	20220331_Aging plasma_12	177.980633	161.8611802	34.49815641	3.452753184	609.959473	311.01680	52.7539599	53.5581535	11425.97714	3789.31929	0	0	14741.21798	32105.5877	7158.038596	0	0	0		
19	20220331_Aging plasma_13	101.041619	156.8662042	41.62907234	0.387262562	3721.01070	2644.266947	519.679369	976.399859	29488.40528	5847.205554	0	0	9917.98121	28207.62356	34745.79768	21784.68579	0	0	0	
20	20220331_Aging plasma_14	1255.648494	482.1608342	17.79070544	12.20564046	2704.84432	1052.73798	5158.241016	4861.59498	67949.76007	13817.67023	0	0	4151.60099	13674.478236	32105.97877	9426.978568	0	0	0	
21	20220331_Aging plasma_15	615.6598729	94.83931984	7.761425836	0.760528566	31918.77794	463.701817	6258.574787	67.552258	1067.854715	866.1875157	0	0	0	0	0	0	0	0	18925.23478	
22	20220331_Aging plasma_16	11493.74004	364.826197	9.76585411	3.663752914	15186.8651	35641.806	4732.70074	17427.28833	69095.36604	14728.15612	0	0	0	9865.761587	6035.627298	16911.03831	4273.229326	0	0	7324.66895
23	20220331_Aging plasma_17	1915.01269	192.447208	27.925735	3.50326472	6223.480724	4730.71215	2006.718185	14317.748196	3282.828101	0	0	0	8474.671239	8897.056293	3478.6765	0	0	0	8813.243149	
24	20220331_Aging plasma_18	3613.622779	126.63484	67.8189184	20.32514933	3005.31774	1019.51382	10100.12153	5064.5271637	7484.940599	0	0	0	10405.82658	0	2568.55797	5267.040771	0	0	6417.57006	
25	20220331_Aging plasma_19	3298.02293	999.3978956	21.20478561	6.092860219	6069.516735	5479.655792	2542.317618	2477.3514223	14748.38974	2672.421118	0	0	0	6850.3090936	7387.997102	26758.50202	0	0	0	11657.31663
26	20220331_Aging plasma_20	256.581209	165.0012071	26.83650771	0	2697.882826	723.349479	421.5432175	165.3702522	1435.95496	109.4940811	0	0	0	8246.409888	0	2268.07307	0	0	0	
27	20220331_a_QC3_std_M_03	51926.63998	1687.76768	17.4174761	2052.78292	171091.3108	68367.71402	5498.06538	98250.02491	15662.40299	69564.44805	60348.09218	176309.8184	13614.5587	185140.6804	318641.2311	35030.8949	48924.23712	304758.9958		
28	20220331_Aging plasma_21	1378.160098	462.34905	23.92406464	4.338989106	2741.058646	967.7965097	113.0806623	547.0672653	3339.853592	115.4059377	0	0	0	0	0	0	0	0	2773.226535	
29	20220331_Aging plasma_22	946.6810026	396.625738	19.13828502	2.456957105	4308.423674	12317.25318	900.5014834	5651.887261	4387.956319	7840.6474949	0	0	0	11651.12462	0	33866.07451	12877.11987	0	0	0
30	20220331_Aging plasma_23	88.79869616	115.0464797	22.58011568	2.155128838	6712.171454	9432.25359	2282.67047	4232.381372	6282.50221	10662.96672	0	0	10405.26044	24653.25355	24159.45416	20107.09028	0	0	0	
31	20220331_Aging plasma_24	2967.405157	947.6138047	44.12491375	22.40311933	10219.04597	5987.71245	1454.855928	2223.043438	179.8611901	20.40630937	0	0	4037.904711	0	0	8995.20854	0	0	6171.909067	
32	20220331_Aging plasma_25	348.259145	217.701408	32.93714432	4.044.06868	6334.306539	1124.394716	931.7698764	16643.68648	3000.902724	0	0	0	6888.769692	0	35819.102	0	0	0	0	
33	20220331_Aging plasma_26	1500.504379	289.6612967	92.18940232	11.81960881	16681.18581	1522.02265	5374.050237	7061.600993	83518.7245	18129.12014	0	0	0	1363.86678	1601.16033	29181.7487	6720.388718	0	0	0
34	20220331_Aging plasma_27	389.531813	210.732721	0	0	3071.732931	3567.585252	664.1196196	504.3636996	35543.03198	8228.568903	0	0	3871.881917	16188.559903	13902.52165	41389.58259	30718.62551	0	0	

Figure 57. The computed abundance colored with status background.

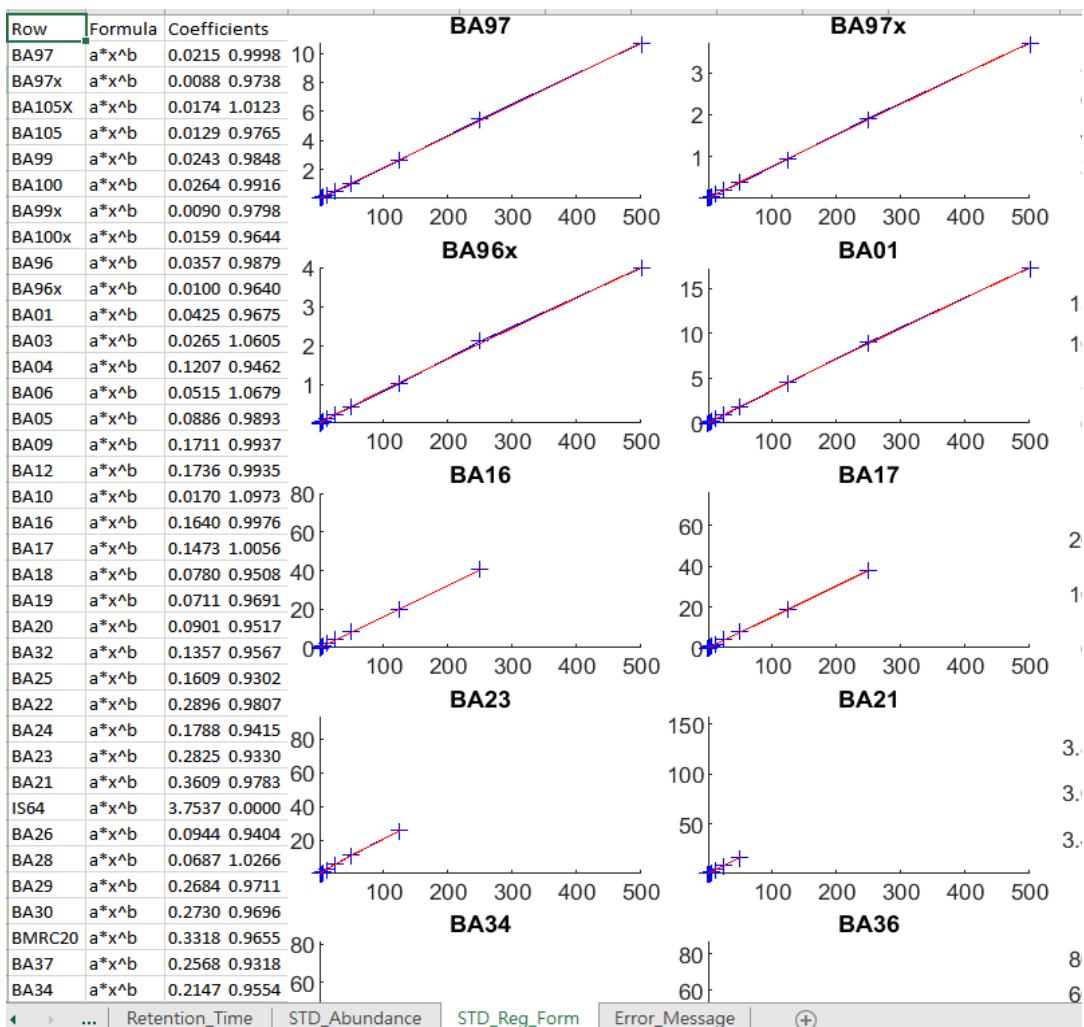


Figure 58. Part of regression curve plots shown with the regression form of the standard samples.

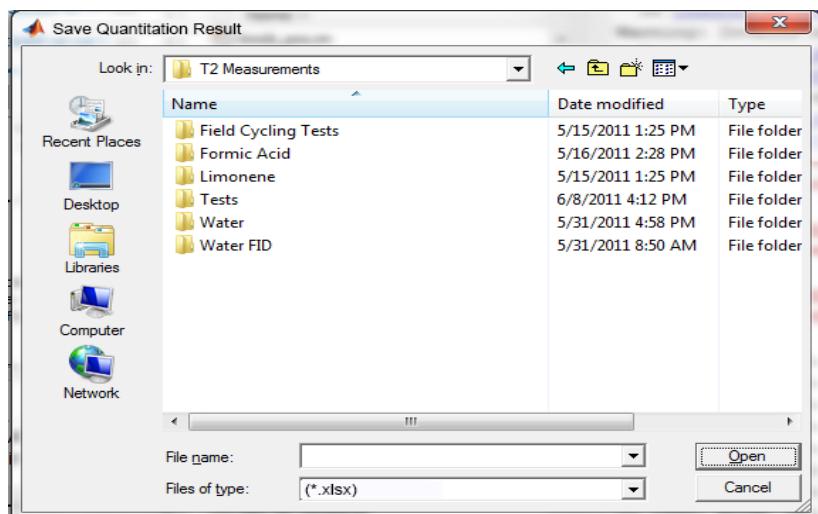


Figure 59. The window allows the user to save the quantitation result to a MS Excel xlsx file.

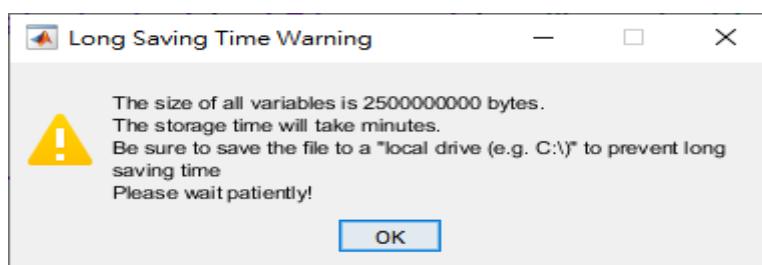


Figure 60. The warning message for long saving time of the quantitation result. It is highly recommended that the user saves such a file in local drive (e.g. drive C or drive D) instead of a cloud drive.

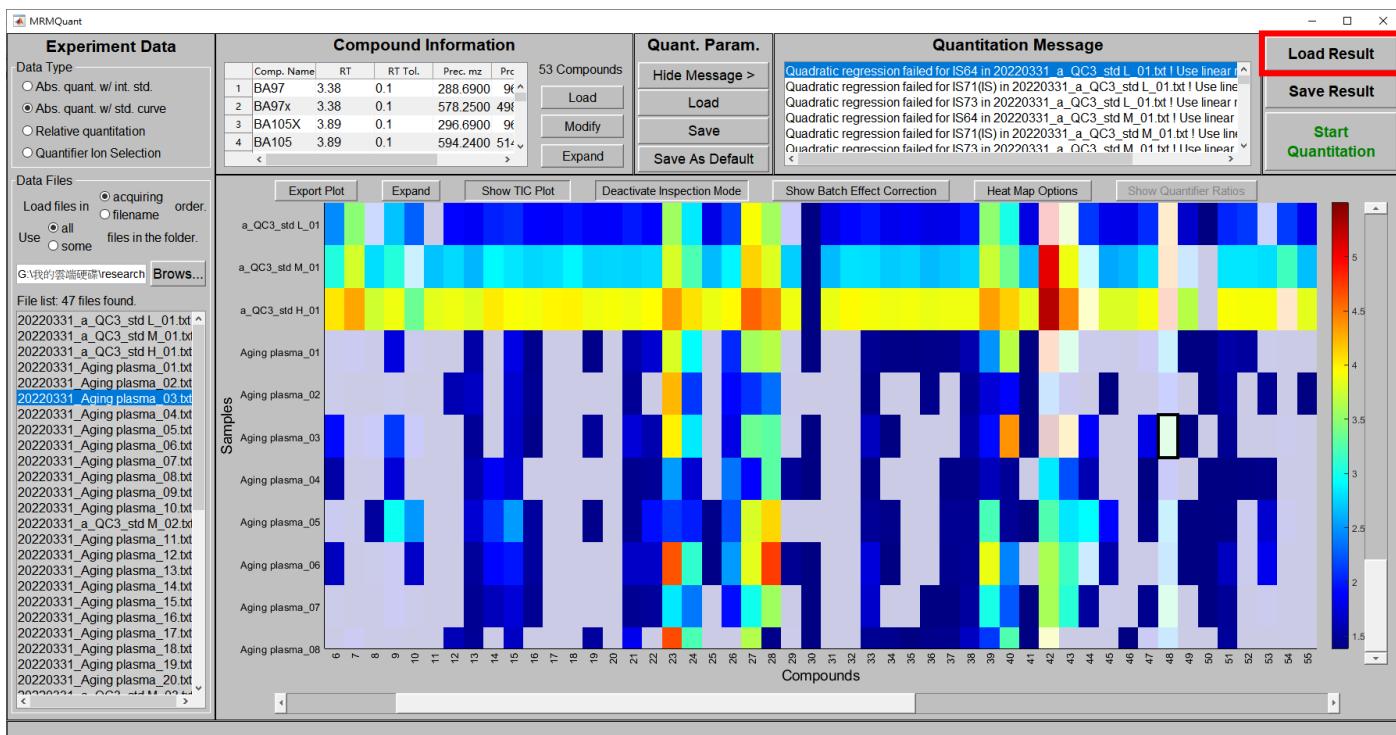


Figure 61. The “load result” button in the GUI for the user to load an existing quantitation result.

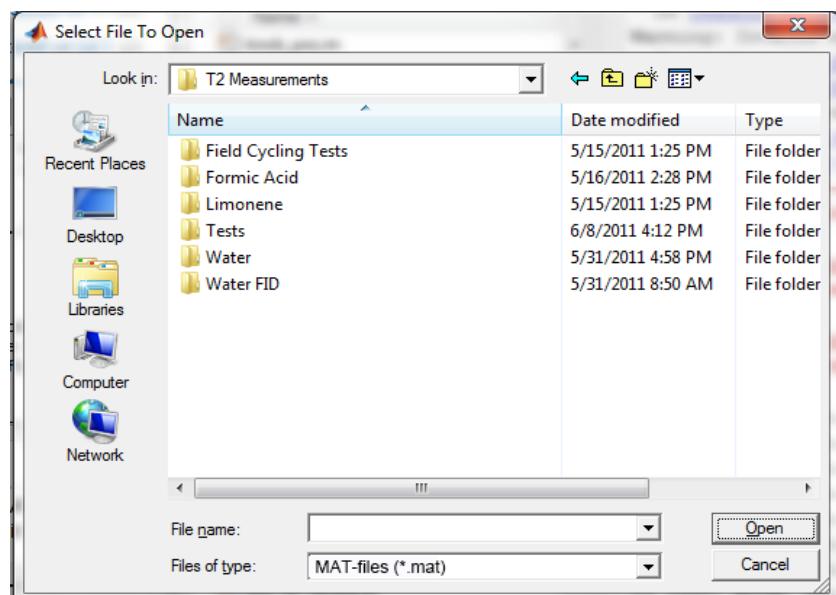


Figure 62. The window allows the user to load the quantitation result from a MATLAB mat file.

Quantifier ion selection

At the design phase of an experiment, users need to determine a quantifier ion from all fragments of a compound for quantitation. Usually such a quantifier ion should be as consistent, and probably more abundant, as possible in all samples. In MRMQuant, the “Quantifier Ion Selection” option in the “Data Type” section of the “Experiment Data” panel was designed for such a purpose.

The procedure of performing the “Quantifier Ion Selection” task is similar to the “Relative Quantitation”. Users need to provide the MRM data files and a method file. The MRM data files contain the compound of interest and their product ions and the method file provides names of the compounds and the m/z values and RTs of the compounds and their product ions. After the file are loaded and the quantitation parameters are input, the user can click on the “start quantitation” button to commence the calculations. Figure 63 shows the result of a quantifier ion selection experiment, where the abundance ratios of all product ions to the most abundant ion are shown. Users can select a suitable product ion for the quantifier of a

compound according the result. Once the “Close” button on the bottom of the window is pressed, the window will be closed. To reveal the table, users can press on the “Show Quantifier Ratios” button on the top of the heat map, as shown in Figure 64.

	Compound Name	RT	Precursor	Product Ions	Max. Ion	Abund. Ratio Ion1/Max. Ion	Abund. Ratio Ion2/Max. Ion	Abund. Ratio Ion3/Max. Ion
1	AA-D3	4.33	138.02	65.1100,92.1060,120.0800	120.08	0.12003+-0.024775	0.24931+-0.059484	1+-0
2	HAA-D3	4.33	154.07	80.1060,108.0600,136.0800	136.08	0.23996+-0.069551	0.1955+-0.048775	1+-0
3	QA-D1	4.33	167.99	78.0910,150.0600	150.06	0.45409+-0.3647	1+-0	
4	QA-13C3-15N-D1	2.96	171.99	82.0720,154.0600	82.072	1+-0	2.2585+-4.2468	
5	5HT	2.96	177.06	160.0400	160.04	1+-0		
6	5HT D4	2.96	181.07	164.0600	164.06	1+-0		
7	KA-D1	1.34	190.07	144.0500,172.0700	144.05	1+-0	0.35546+-0.016138	
8	HIAA	1.34	192.03	146.0600	146.06	1+-0		
9	Trp-D1	1.72	205.12	118.0200,188.1000	118.02	1+-0	0.17395+-0.038648	
10	Trp 15N2-D2	1.72	207.11	119.1100,189.1200	189.12	0.55784+-0.31188	1+-0	
11	KYN-D2	2.68	209.11	94.0970,192.0900	94.097	1+-0	0.53635+-0.049336	
12	5HTP-D1	2.68	221.12	162.0900,204.0900	204.09	0.67462+-0.20117	1+-0	
13	3HK-D2	3.68	225.16	110.0900,208.1300	208.13	0.36042+-0.13087	1+-0	

Figure 63. The output table for the quantifier ion selection experiment

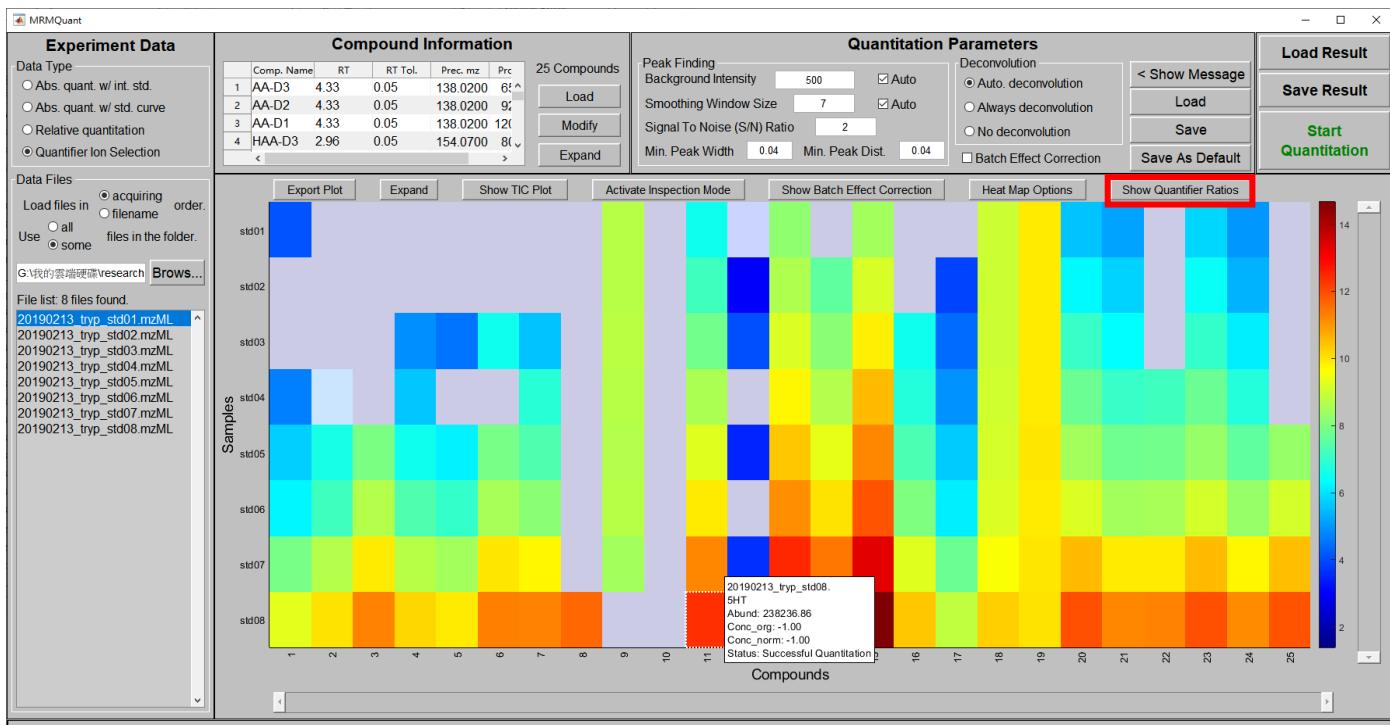


Figure 64. The quantifier ion selection can be displayed using the “Show Quantifier Ratios” button.

Starting a new quantitation during/after an existing one

MRMQuant allows users to start a new quantitation during or after an existing quantitation task. A new quantitation can be achieved by changing at least one of the following items (1) data type, (2) test sample files, (3) method file, and (4) quantitation parameters. If an existing quantitation is unsaved and the user attempt to change any of the items, a question window will pop up to inquiry whether the user intends to (1) save and proceed, (2) proceed without saving, or (3) cancel the change(s), as shown in the Figure 65. After that, the user can proceed to input required data and perform a new quantitation.

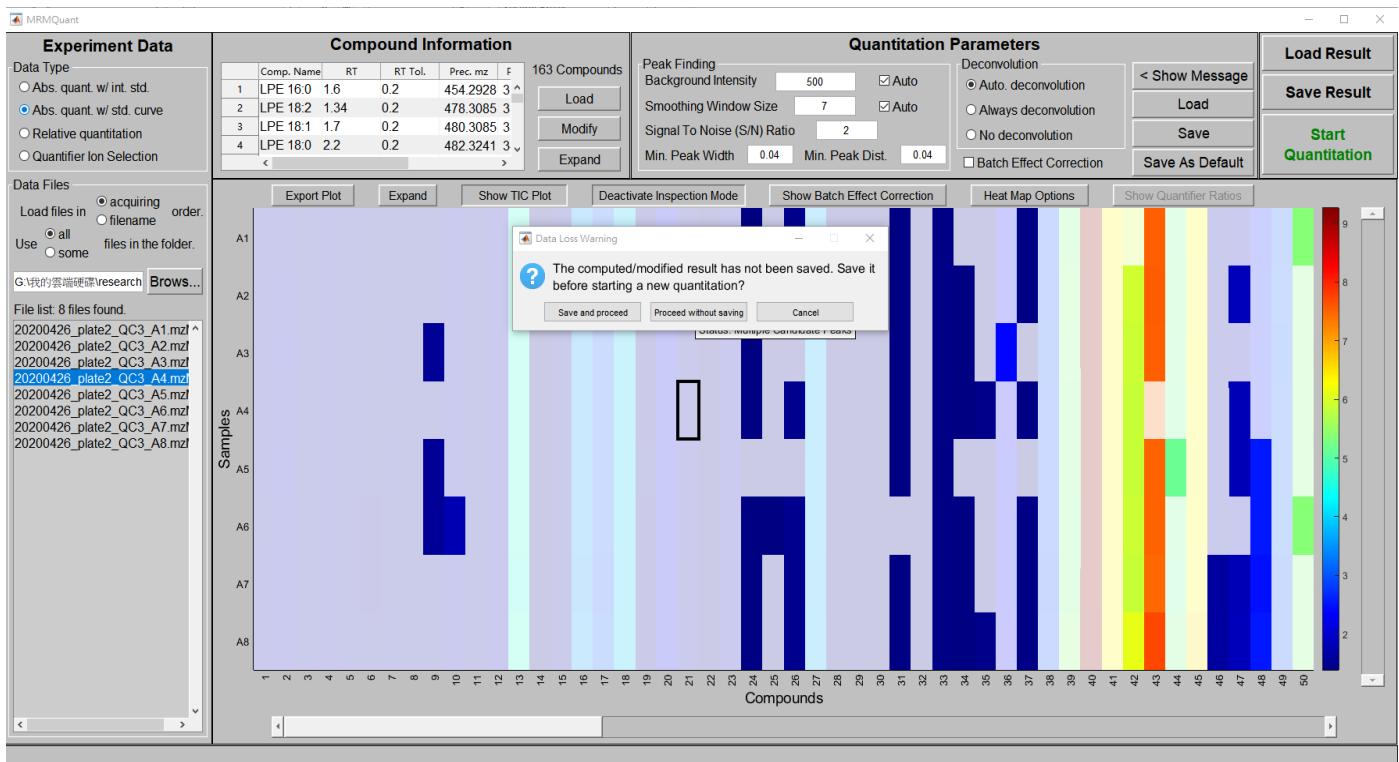


Figure 65. A question window pops up when a new data type is selected whereas the quantitation result has not been saved.

Appendix

A.1. Example method file for relative quantitation

CompNams	RT	RT_tol	Precursor_mz	Product_mz
LPE 18:1 (d7) a_LPE 18:1 (d7) b	1.63; 1.74	0.05	487.31	346.31
LPE 22:6 a_LPE 22:6 b	1.21; 1.27	0.03	526.29	385.29
LPC 18:1 (d7) a_LPC 18:1 (d7) b	1.55; 1.66	0.05	529.3	184.07
LPC 22:6 a_LPC 22:6 b	1.15; 1.22	0.03	568.34	184.07
CE 18:1 (d7)	8.72	0.1	675.61	369.35
PE 33:1 (d7)	5.61	0.1	711.48	570.54
CE 22:6	8.26	0.1	714.59	369.35
PEp 36:1 (d9)	6.55	0.1	739.63	392.2
PEp 38:6	5.53	0.1	748.53	364.2
PC 33:1 (d7)	5.49	0.1	753.54	184.07
PE 38:6	5.29	0.1	764.52	623.52
PEp 40:7	5.57	0.1	774.54	390.2
PEp 40:6	6.02	0.1	776.56	392.2
PC 36:6	4.58	0.1	778.54	184.07
PCp 36:1 (d9)	6.42	0.1	781.68	184.07
PE 40:8	4.91	0.1	788.54	647.54
PE 40:7	5.34	0.1	790.54	649.54
PE 40:6	5.8	0.1	792.55	651.55
PCe 38:6	5.47	0.05	792.59	184.07
PC 38:7	4.66	0.05	804.55	184.07
PEp 42:6	6.46	0.1	804.62	420.2
PC 38:6	5.16	0.1	806.57	184.07
TAG 48:1 (C18:1) (d7)	8.5	0.1	829.79	523.47
TAG 48:1 (C15:0) (d7)	8.5	0.1	829.79	570.5
PC 40:8	4.78	0.1	830.57	184.07
PC 40:7	5.21	0.1	832.57	184.07
PC 40:6	5.69	0.1	834.57	184.07
TAG 52:6 (C22:6)	8.15	0.1	868.74	523.4
TAG 54:8 (C22:6)	7.93	0.1	892.68	547.4
TAG 54:7 (C22:6)	8.15	0.1	894.75	549.4
TAG 54:6 (C22:6)	8.38	0.1	896.77	551.4
TAG 56:9 (C22:6)	7.95	0.1	918.72	573.44
TAG 56:8 (C22:6)	8.18	0.1	920.79	575.5

A.2. Example method file for quantifier ion selection

Comp. Names	RT	RT Tol.	Proc. mz	Prod. mz
AA-D3	4.33	0.05	138.02	65.11
AA-D2	4.33	0.05	138.02	92.106
AA-D1	4.33	0.05	138.02	120.08
HAA-D3	2.96	0.05	154.07	80.106
HAA-D2	2.96	0.05	154.07	108.06

HAA-D1	2.96	0.05	154.07	136.08
QA-D1	1.34	0.05	167.99	78.091
QA-D2	1.34	0.05	167.99	150.06
QA-13C3-15N-D1	1.72	0.05	171.99	82.072
QA-13C3-15N-D2	1.72	0.05	171.99	154.06
5HT	2.68	0.05	177.06	160.04
5HT D4	2.68	0.05	181.07	164.06
KA-D1	3.68	0.05	190.07	144.05
KA-D2	3.68	0.05	190.07	172.07
HIAA	2.9	0.05	192.03	146.06
Trp-D1	3.57	0.05	205.12	118.02
Trp-D2	3.57	0.05	205.12	188.1
Trp 15N2-D2	3.57	0.05	207.11	119.11
Trp 15N2-D1	3.57	0.05	207.11	189.12
KYN-D2	2.9	0.05	209.11	94.097
KYN-D1	2.9	0.05	209.11	192.09
5HTP-D1	2.76	0.05	221.12	162.09
5HTP-D2	2.76	0.05	221.12	204.09
3HK-D2	1.93	0.05	225.16	110.09
3HK-D1	1.93	0.05	225.16	208.13

A.3. Example method file for absolute quantitation with internal standard

CompNams	RT	RT_tol	Proc. mz	Prod. mz	IS	Conc
LPE 16:0	1.6	0.2	454.2928	313.2928	LPE 18:1 (d7)	1
LPE 18:2	1.34	0.2	478.3085	337.3085	LPE 18:1 (d7)	1
LPE 18:1	1.7	0.2	480.3085	339.3085	LPE 18:1 (d7)	1
LPE 18:0	2.2	0.2	482.3241	341.3241	LPE 18:1 (d7)	1
LPE 18:1 (d7)	1.68	0.2	487.3085	346.3085	LPE 18:1 (d7)	1
LPE 20:4	1.29	0.2	502.2928	361.2928	LPE 18:1 (d7)	1
LPE 22:6	1.23	0.2	526.2928	385.2928	LPE 18:1 (d7)	1
LPC 14:0	1.08	0.2	468.3085	184.07	LPC 18:1 (d7)	45
LPC(P-16:0/0:0)	1.72	0.2	480.3449	104.08	LPC 18:1 (d7)	45
LPC(O-16:0/0:0)	1.78	0.2	482.3605	104.08	LPC 18:1 (d7)	45
LPC 15:0	1.29	0.2	482.3605	184.07	LPC 18:1 (d7)	45
LPC 16:1	1.16	0.2	494.3241	184.07	LPC 18:1 (d7)	45
LPC 16:0	1.53	0.2	496.3398	108.07	LPC 18:1 (d7)	45
LPC 17:1	1.38	0.2	508.3398	184.07	LPC 18:1 (d7)	45
LPC 17:0	1.82	0.2	510.3554	184.07	LPC 18:1 (d7)	45
LPC 18:2	1.28	0.2	520.3398	184.07	LPC 18:1 (d7)	45
LPC 18:1	1.62	0.2	522.3554	184.07	LPC 18:1 (d7)	45
LPC 18:1 (d7)	1.61	0.2	529.3054	184.07	LPC 18:1 (d7)	45
LPC 20:5	1.05	0.2	542.3241	184.07	LPC 18:1 (d7)	45
LPC 20:4	1.24	0.2	544.3398	184.07	LPC 18:1 (d7)	45
LPC 20:3	1.43	0.2	546.3554	184.07	LPC 18:1 (d7)	45
LPC 20:2	1.75	0.2	548.3711	184.07	LPC 18:1 (d7)	45
LPC(O-18:0/0:0)	2.43	0.2	510.3554	104.08	LPC 18:1 (d7)	45
LPC 18:0	2.13	0.2	524.3711	184.07	LPC 18:1 (d7)	45
LPC 19:0	2.46	0.2	538.38	184.07	LPC 18:1 (d7)	45
LPC 20:1	2.2	0.2	550.3867	184.07	LPC 18:1 (d7)	45
LPC 20:0	2.82	0.2	552.4024	184.07	LPC 18:1 (d7)	45
LPC 22:0	3.54	0.2	580.43	184.07	LPC 18:1 (d7)	45
LPC 24:0	4.24	0.2	608.47	184.07	LPC 18:1 (d7)	45
SM C12:0	3.75	0.2	647.52	184.07	SM C18:1 (d9)	40
SM C13:0	4.09	0.2	661.53	184.07	SM C18:1 (d9)	40
SM C14:1	3.87	0.2	673.54	184.07	SM C18:1 (d9)	40
SM C14:0	4.43	0.2	675.55	184.07	SM C18:1 (d9)	40
Dihydro SM C14:0	4.66	0.12	677.53	184.07	SM C18:1 (d9)	40
SM C16:1	4.53	0.2	701.59	184.07	SM C18:1 (d9)	40

A.4. Example method file for absolute quantitation with standard curve

Comp. Names	RT	RT Tol.	Proc. mz	Prod. mz	IS
BA97	3.38	0.1	288.69	96.954	IS71(IS)
BA97x	3.38	0.1	578.25	498.29	IS71(IS)
BA105X	3.89	0.1	296.69	96.954	IS71(IS)
BA105	3.89	0.1	594.24	514.28	IS71(IS)
BA99_BA100	5.43;5.79	0.1	288.69	96.954	IS71(IS)

BA99x_BA100x	5.43;5.79	0.1	578.25	498.29	IS71(IS)
BA96	7.37	0.1	280.69	96.954	IS71(IS)
BA96x	7.37	0.1	562.25	482.29	IS71(IS)
BA01	11.6	0.02	359.29	359.28	IS71(IS)
BA03_BA04	9.96;10.61	0.05	373.27	373.27	IS71(IS)
BA06_BA05	10.37;10.75	0.05	375.29	375.28	IS71(IS)
BA09	9.14	0.1	377.27	377.26	IS71(IS)
BA12_BA10	7.46;7.75	0.05	387.25	387.24	IS71(IS)
BA16	9.43	0.1	389.26	389.25	IS71(IS)
BA17_BA18_BA19	8.01;8.17;8.52	0.05	389.27	389.26	IS71(IS)
BA20	6.91	0.1	391.28	391.27	IS71(IS)
BA32_BA25_BA22	7.7;8.21;8.52	0.05	391.28	391.27	IS71(IS)
BA24_BA23_BA21	10.07;10.22;10.51	0.05	391.28	391.27	IS71(IS)
IS64	10.22	0.1	397.37	397.36	IS71(IS)
BA26	3.52	0.1	401.23	401.22	IS71(IS)
BA28	7.81	0.1	403.25	403.24	IS71(IS)
BA29_BA30	5.82;6.88	0.05	405.26	405.25	IS71(IS)
BMRC20	4.74	0.1	407.28	407.27	IS71(IS)
BA37_BA34	6.19;6.28	0.05	407.28	407.27	IS71(IS)
BA36_BA35	7.39;7.96	0.1	407.28	407.27	IS71(IS)
BA43_BA40	5.63;6.01	0.05	448.31	74	IS71(IS)
BA41_BA42	8.17;8.68	0.1	448.2	74	IS71(IS)
BA39	10.2	0.1	432.31	74	IS71(IS)
BA64	10.19	0.1	455.25	96.953	IS71(IS)
BA44	2.12	0.05	458.25	74	IS71(IS)
BA49	5.28	0.1	464.28	74	IS71(IS)
BA46	6.16	0.1	464.3	74	IS71(IS)
BA47	11.82	0.1	466.3	80	IS71(IS)
IS71(IS)	6.16	0.1	468.43	74.1	IS71(IS)

A.5 Report program errors

Suggestions and comments about the software are welcome. If the program is terminated unintentionally without a warning or an error message, please find the “**MRMQuantErrorReport.log**” file in the folder where the MRMQuant was installed and email the content of the log file to Dr. Ke-Shiuan Lynn at 128171@mail.fju.edu.tw. The sample file when the program was evaluating before the termination and the associated method file may be needed to solve the problem. It is highly recommended that the user appends the related files alone with the problem description in the email.