MS Compare

MsMetrix

Reference Peak Warping Alignment Demo

The MS Compare module contains 4 Alignment algorithms to correct for possible chromatographic shifts. Alignment correction can be very difficult and is a topic of current research. The algorithms are continuously being updated to provide the user with better and faster correction routines.

Alignment Algorithms available in MS Compare

1. Offset Correction – zoom in on the selected peak for a simple offset correction.
2. Cross Correlation – calculate offset correction based on time range
3. Correlation Optimized Warping – See the manual for an example. COW calculations can be lengthy and difficult for crowded chromatograms. The use of BPC's are preferred, but finding the right parameters can be difficult. If your files are large, try to use an offset correction first, followed by a Reference Peak Warping correction.
4. Reference Peak Warping - tries to align your data based on a number of user selected peaks (Markers)

Reference Peak Warping using Selected Peaks:
Reference Peak Warping tries to shift and correct the chromatograms based on a number of well defined peaks. Try to use the following guidelines:

- The peaks should be selected across the chromatogram to cover the time range of interest
- Ideally, all chromatograms should contain the selected peak with comparable peak heights
- If possible the extracted ion currents (EIC's) should contain only one large peak
- the peaks for a given EIC should not interfere (cross) with peaks from another EIC. If this is the case, skip the peak and select a new peak at a different retention time
- Try to find peaks that are comparable in size

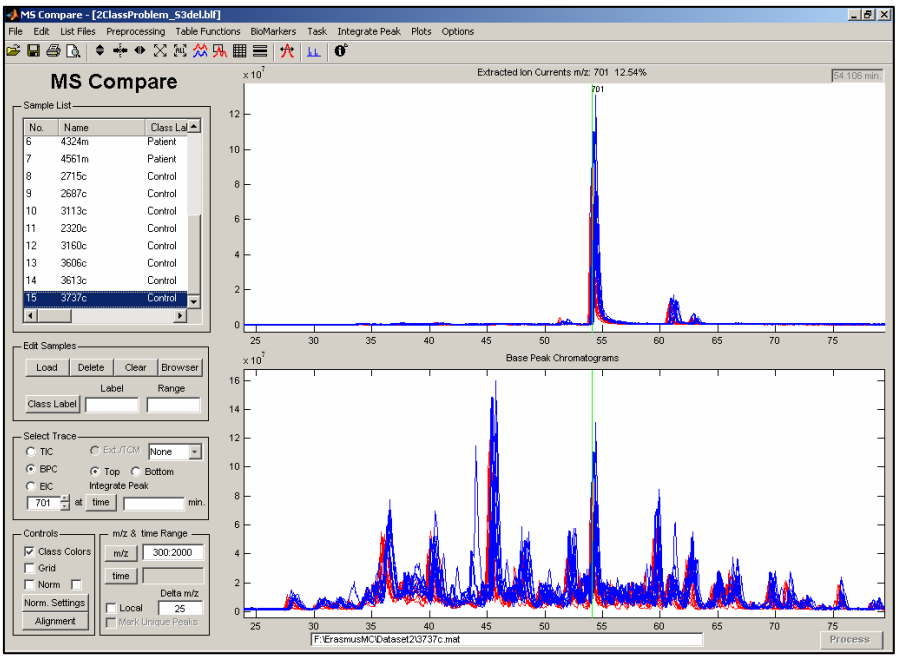
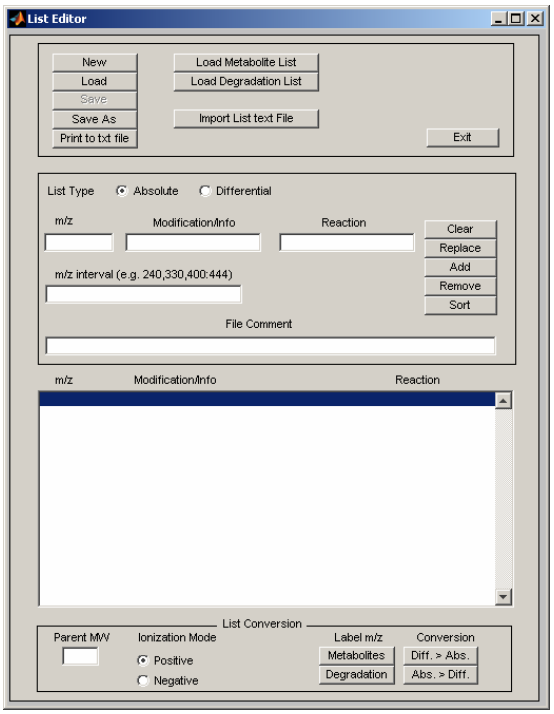


Figure 1: Data Loaded, displayed are the Base Peak Chromatograms. Top: EIC m/z 701

Step 1: Selecting the Reference Peaks.
Open the **Create Peak Tracking m/z List** from the menu (Edit). This window will be used to store the selected m/z values and to create a synthetic Total Ion Chromatogram based on the selected m/z values.
m/z values can be added in the m/z edit box. Press **Add** to add the value to the list. When all selections have been made, save the list under a new file name (e.g. Tracking.list). Close the Editor.

Proceed as follow: try to pick peaks from left to right keeping in mind the above guidelines. Do not select the very small peaks and also no EIC's very close together.
If you want to add the EIC to the list, enter the m/z value to the List Editor and press Add.
In this example 12 peaks were selected from the Base Peak Chromatograms.



Step 2:
To view the Reconstructed Ion Current based on the selected peaks use: **Menu > Plots > Peak Tracking Plot**. Select the appropriate List file. Do not apply character labeling and we will not connect the peaks using lines.

The result will be shown in stacked mode. If the result is not satisfactory, edit the peak list and redraw the Reconstructed Ion Chromatograms. From figure 2 it is concluded that a proper selection has been made.
You can estimate the maximum shift amount by using the Peak Width Measurement Tool. In this case the maximum shift was estimated to be 1.5 minutes.

Step 3: Alignment Correction
Based on Figure 2, the Reference Peak Warping will be applied. From the MS Compare Control Area, select Alignment. You will be asked if MS Xelerator should suggest a reference samples. All samples will be aligned based on the selected reference sample. Select Yes.

The Alignment Window (Figure 3) will be displayed. Select Reference Peak Warping and **enter a value of 1.5 minutes** for the maximum shift parameter. This values should be based on the estimated differences between the peaks. The other parameter will not be changed. The EIC radio button should have been selected (Synthetic Ion Chromatogram). Use the create peak list button the delete or add peaks (not used in this demo).

Press **Preview**. The result of alignment correction will be displayed after a few seconds (Figure 4). Zoom in on the region of interest. **Press the spacebar to apply the same scaling to the aligned data.** If peaks are not well aligned, try to use different parameters or delete the problematic peak. If the result is satisfactory, close the window and you will be returned to the Alignment Window. Press **Apply**, to accept the changes made to the time axis. Try to extract a few Ion Currents and check if the alignment was properly performed.

Sometimes a combination of correction algorithms can be applied in order to align difficult shifting problems. Start with offset correction to get rid of the larger shifts. Than apply a reference peak warping and finally you could try to apply a Correlation Optimized Warping (if necessary).

The next time you load the same data set, you will be asked if the saved RPW corrections should be applied. This will also be applicable when COW alignment was performed.

Contact MsMetrix for problematic Data Sets

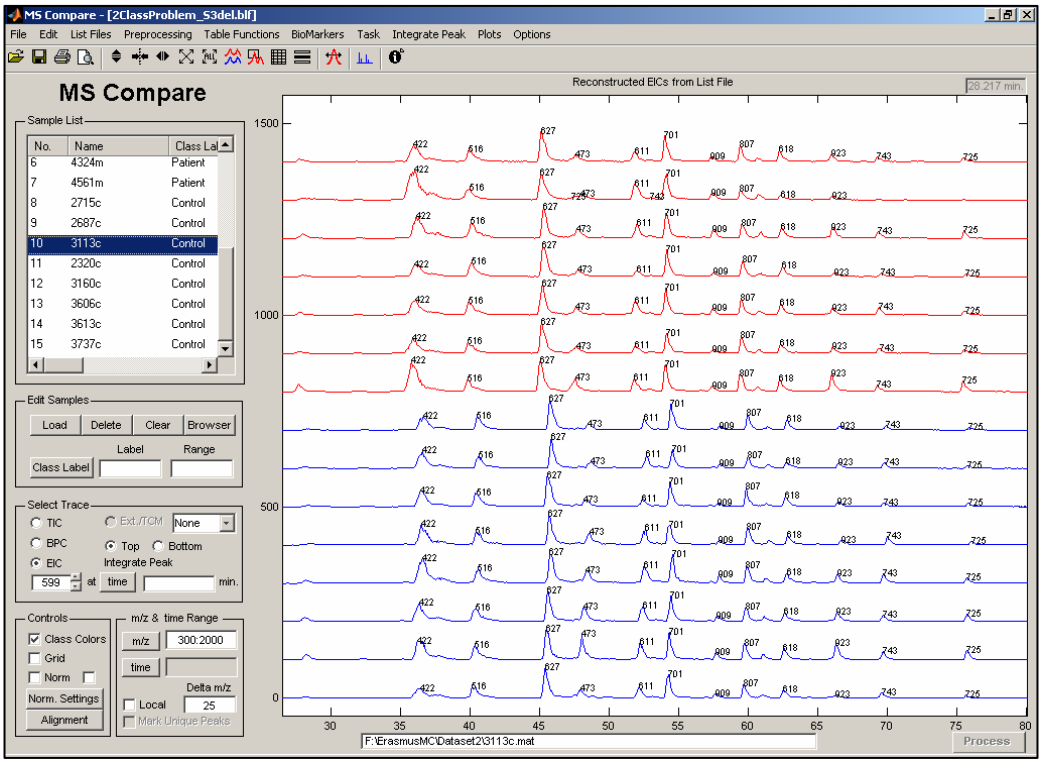
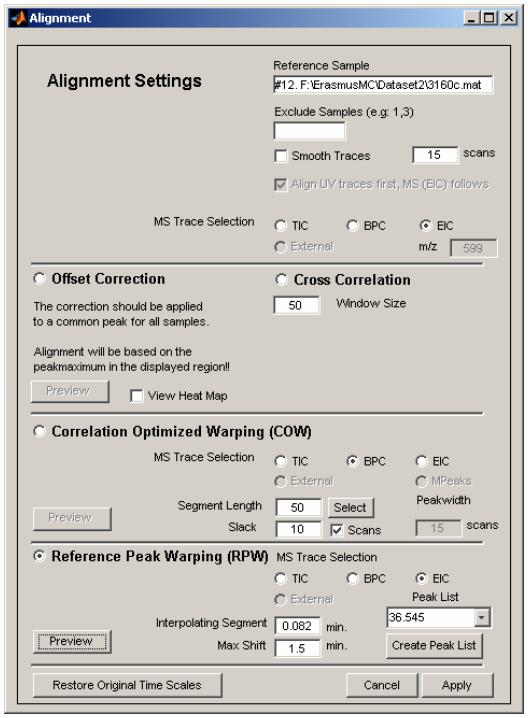


Figure 2: Reconstructed Ion Chromatograms of all samples in stacked mode. Chromatograms are created based on the selected m/z list. Peak markers are displayed on top of each peak

Figure 3: Alignment Window



Creating a RPW peak list manually:

The Alignment RPW GUI also contains a button **Create Peak List**. In the above example the peak list from a reconstructed ion chromatogram was used. However, you can also create a new list or delete or add peaks from the existing list.

Press the **Create Peak List Button**. The Create Alignment Peak List window will be shown. When entered from the above procedure, the reference sample will be plotted and the earlier chosen peaks will be marked. To better view your data select **Plot all Traces and Stacked Plot.** Your screen should look like shown below. To start from scratch delete all peaks. You may also delete a single peak, by selecting it from the list and then press delete.

Try to set the retention times in the middle of each peak group. The max shift value should be set based on the dispersion of the groups. If groups are to close, do not select it. Sometimes it will be better to leave out a peak to get a better result. If one of the samples would be very difficult to align you may try the following strategy.

Align all samples but leave out the problematic sample. Make a new list for the reference sample and the sample that was left out. Experiment using RPW alignment using only these two samples. Alignment should be easier to apply since the focus will be on two samples only.

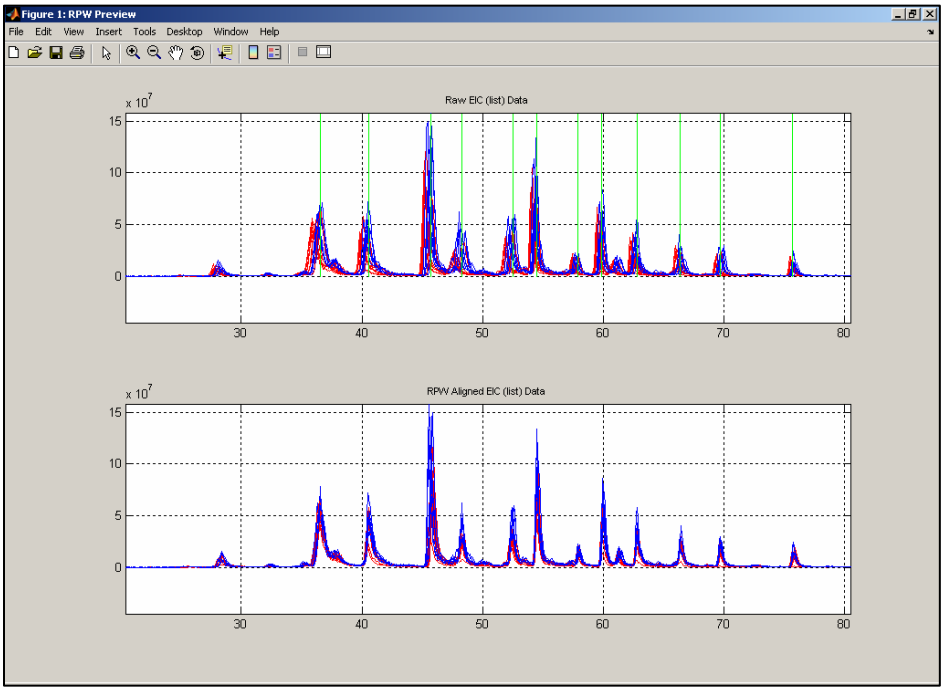


Figure 4: Preview from RPW alignment correction. Top: raw EIC's (reconstructed), Bottom: EIC's after alignment correction. Use the space bar after zooming to apply equal scaling.

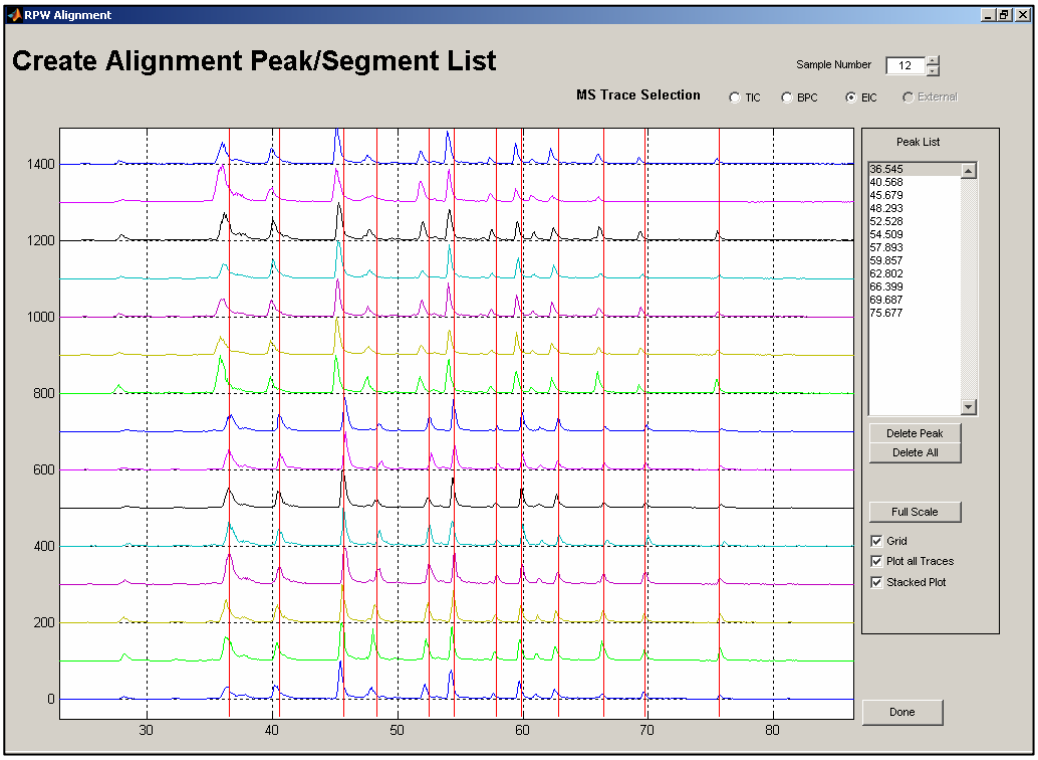


Figure 5: setting peak positions manually for RPW alignment