MsMetrix

MsXelerator Demo:
Impurity Profiling and Peak Purity Analysis

Introduction:

MsXelerator contains different tools and algorithms to perform Impurity Profiling and Peak Purity Analysis. Impurity Profiling is easy using the available tools. Peak Purity analysis based on LC/MS data is often more difficult due to fragmentation, ion suppression and the presence of a large number of ions co-eluting with the parent peak. This document describes how Impurity Profiling and Peak Purity Analysis can be performed.

Step1: Getting an overview of the data:

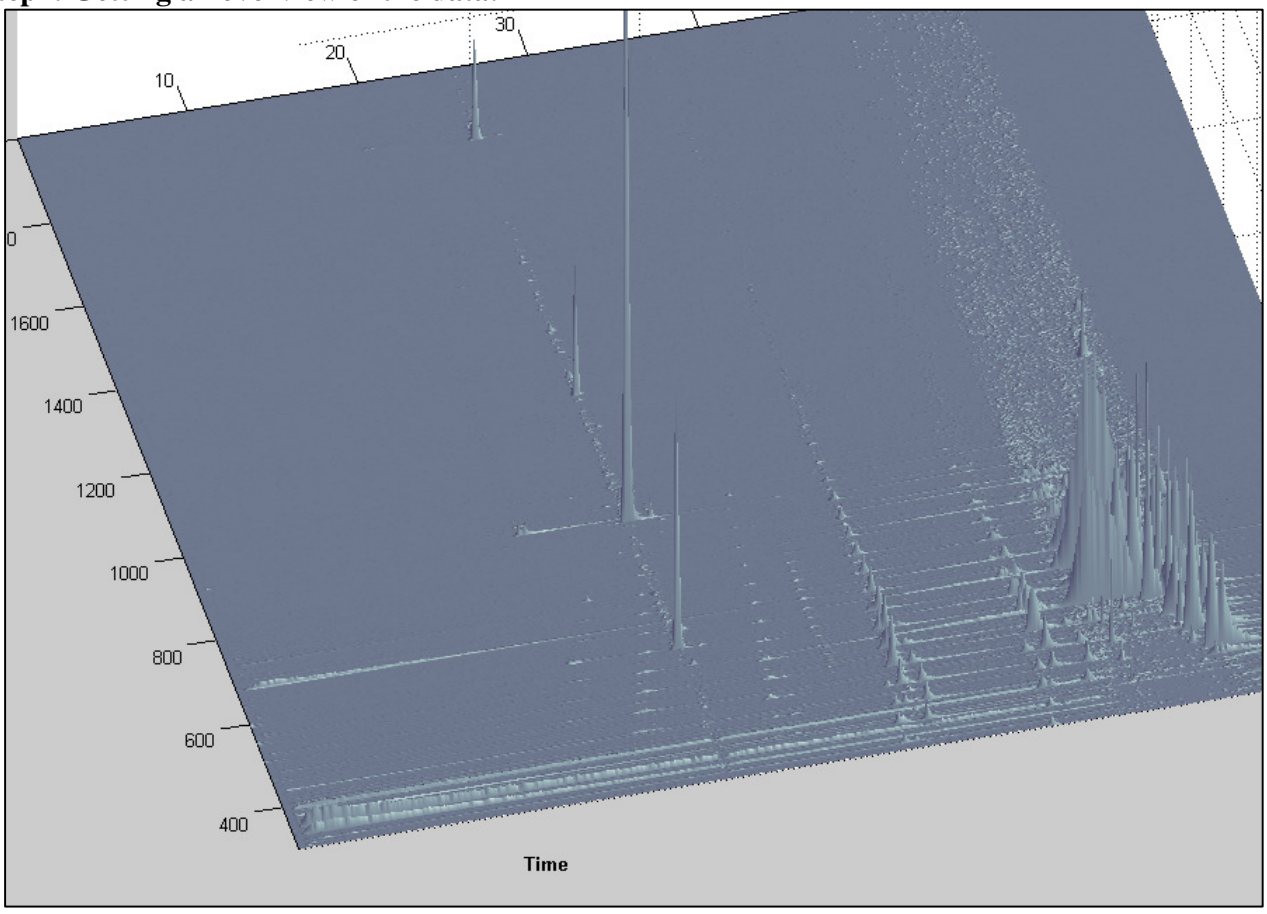

Figure 1: 3-Dimensional Overview of sample HPLC_MS 1

A good starting point will be to create a 3-dimensional plot or heatmap plot of the sample first. This was done for the first sample, see Figure 1 and 2. From Figure 1 it can be concluded that there are quite a few polymeric peaks present in the sample. The beautiful curved peak patterns observed from 22 - 35 min are indicative for the presence of polyethylene glycol. Peaks will show a mass difference of 44 or 22. If we zoom in more into this surface, more regions can be observed having

these small but clear patterns. One of them is crossing the Parent Peak. We should take care that none of these PEG peaks near the Parent Peak are by mistake taken as real impurities. The same patterns can be seen from the Heatmap plot, see Figure 2. In the MS Compare module, these heatmaps can be zoomed to any level. Clicking on one of the density regions will then automatically plot the extracted the ion currents (EIC). This can be done for multiple samples simultaneously. Shown in Figure 2 is the EIC of one of the impurities at the tail of the parent peak (m/z 990). The EIC is plotted in nominal mode.

The second conclusion made from these plots is that many ions are co-eluting with the Parent peak. Some of these are very noisy others are perfectly co-eluting and could be fragments or clusters. Making a distinction between fragments and real impurities can be a challenging task. Real impurities that perfectly co-elute might need some chemical or bio-chemical explanation. Examples how to do this are shown later.

A third phenomenon that could happen is that sometimes a co-eluting fragment "looks' like an impurity due to ion-suppression or other physical events. It will look like an impurity is on the tail or start of the parent peak because in the middle the ions are suppressed. Sometimes it will look like there are two impurities on both sides. If you see this, it is probably an artifact.

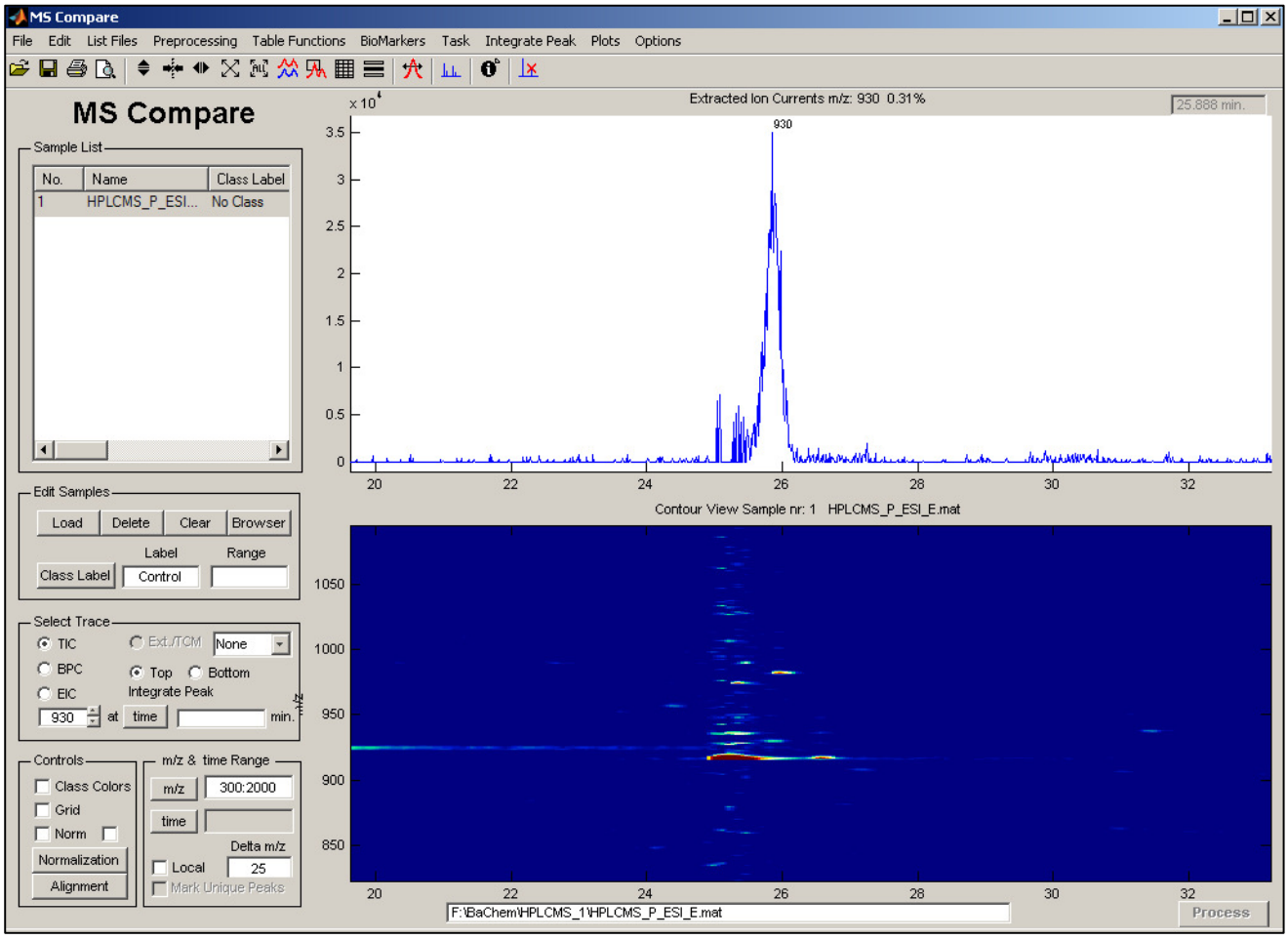

Figure 2: MS Compare: Heatmap plot combined with EIC extraction

Step 2: Peak Purity Analysis using the Browser:

The Browser is basically the portal of MsXelerator. The Browser is very powerful to perform impurity profiling or peak purity analysis. If there are many peaks present in the sample we will directly switch to the more sophisticated algorithms to perform peak detection and all that comes with it. Figure 3 displays the Browser with the sample loaded.

From the Browser you can plot the TIC or BPC in the lower window. Clicking on any time position will plot the mass spectrum in the top window. The mass chromatogram (EIC) belonging to the largest mass is then automatically plotted to the middle window. Plotting can be done in overlay, normalized, in nominal or exact mass mode etc. Clicking on any of the m/z values in the top will automatically plot the mass chromatogram. You can also enter an m/z value in the Select m/z box. The Browser has a feature called "Peak Stripping" which is often used in Peak Purity analysis. It is a sequential procedure to subtract the ions from the TIC at a certain position. After removing the parent, isotopes and larger fragments, the small impurities will show up. Another very powerful feature is called Local Screening. In this case you can automatically create new TIC's or BPC's from small m/z windows (typically 25 Dalton). These are created on the fly. Local Screening can also be performed in MS Compare on series of samples simultaneously. In this situation it is often used to detect small differences between batches or samples.

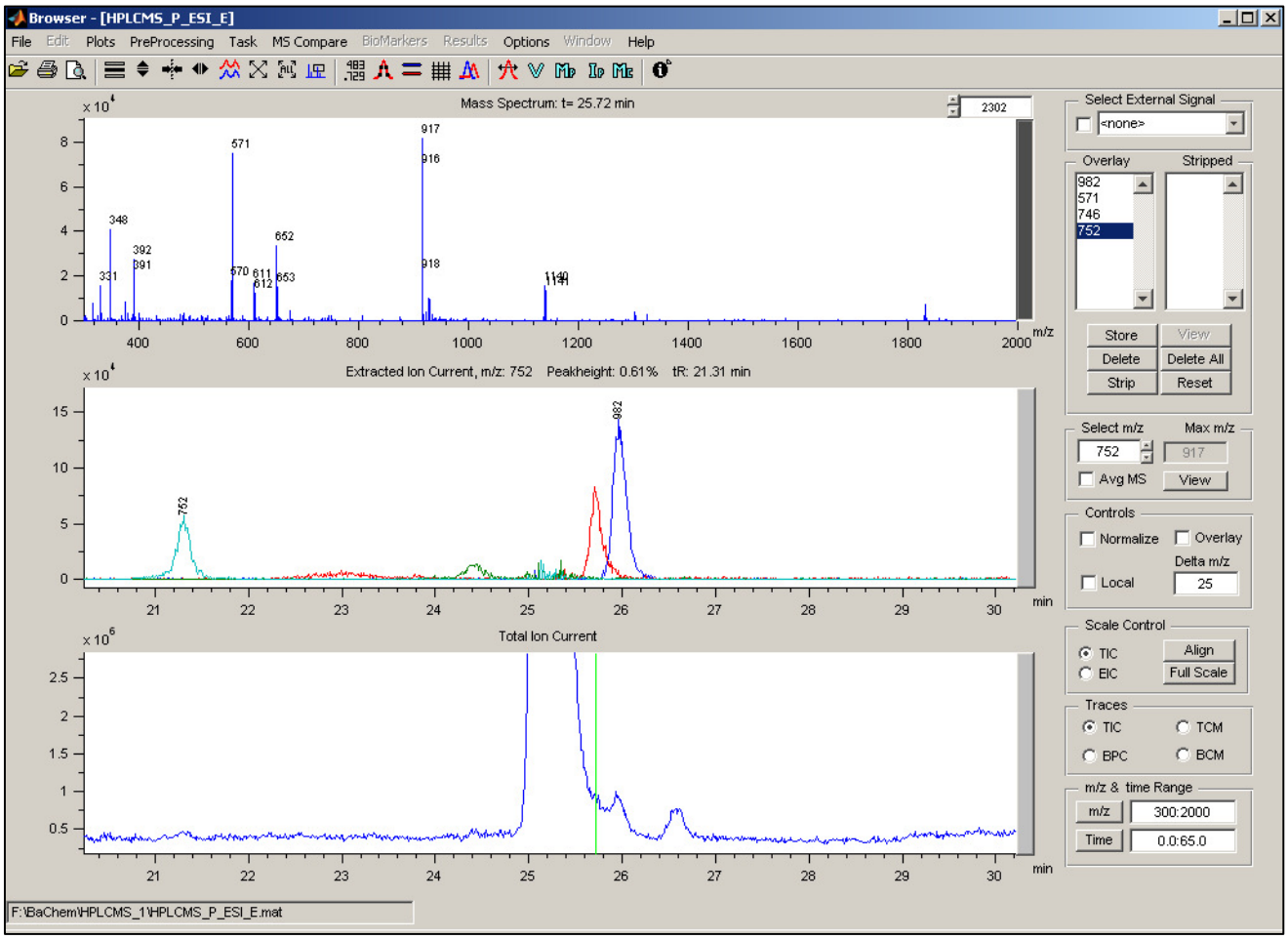

Figure 3: Some smaller peaks at the tail directly identified from the Browser.

In general (depending on the noise level) we can say that peaks present at levels below 1%, compared to the parent, will be difficult to detect from the TIC or BPC. Local Screening will dramatically improve the detection level of impurities. However, the Peak Detection algorithms available in the MPeaks module will go to any level and process all mass chromatograms in seconds.

Step 3: Impurity Profiling and Peak Purity Analysis using MPeaks:

By far the most powerful method to perform Impurity and Peak Purity Analysis is by using the MPeaks module. MPeaks will detect all significant chromatographic peaks present in your sample in probably less than 10 seconds. A full quantitative report is made. Plotting can be done directly from the MPeaks result table. The sample can be overlaid with a reference

sample, you can do fast differential analysis between two samples, delete isotopes/fragments, identify your peaks using predefined lists etc, etc. To use MPeaks peak picking you will need an estimate of your most narrow peak in the data. The algorithm tries to detect all chromatographic peaks above the noise level. You can set a threshold level, a Signal to Noise ratio etc. etc.

A default run with MPeaks in the time window 20.55 – 31.34 min. will detect more than 400 peaks in about 10 seconds. The first step hereafter is to remove the ^{13}C Isotope peaks. This will result in 275 peaks remaining. The majority of these peaks are co-eluting fragments, sometimes very noisy. We have set a rather narrow peak width of 11 scans. If we use a value of 25 scans, the table will be reduced to only 150 peaks. Figure 4 shows the MPeaks window after the run is completed. The result table is displayed in the lower part and consists of entries like: m/z value, retention time, peak height, % peak height, % area, FWHH, smoothness of peak etc. These tables can be sorted on any value from the table. By using the Editor you will be able to filter the table using specific queries. E.g. show all peaks on the right side of the parent lower than 1% based on peak height. Mass values and peak height are based on nominal values but can be converted to accurate m/z values and peak heights.

Figure 4 shows a few selected impurities in front and after the parent peak. The TIC is plotted in red in the middle (normalized), for easy comparison.

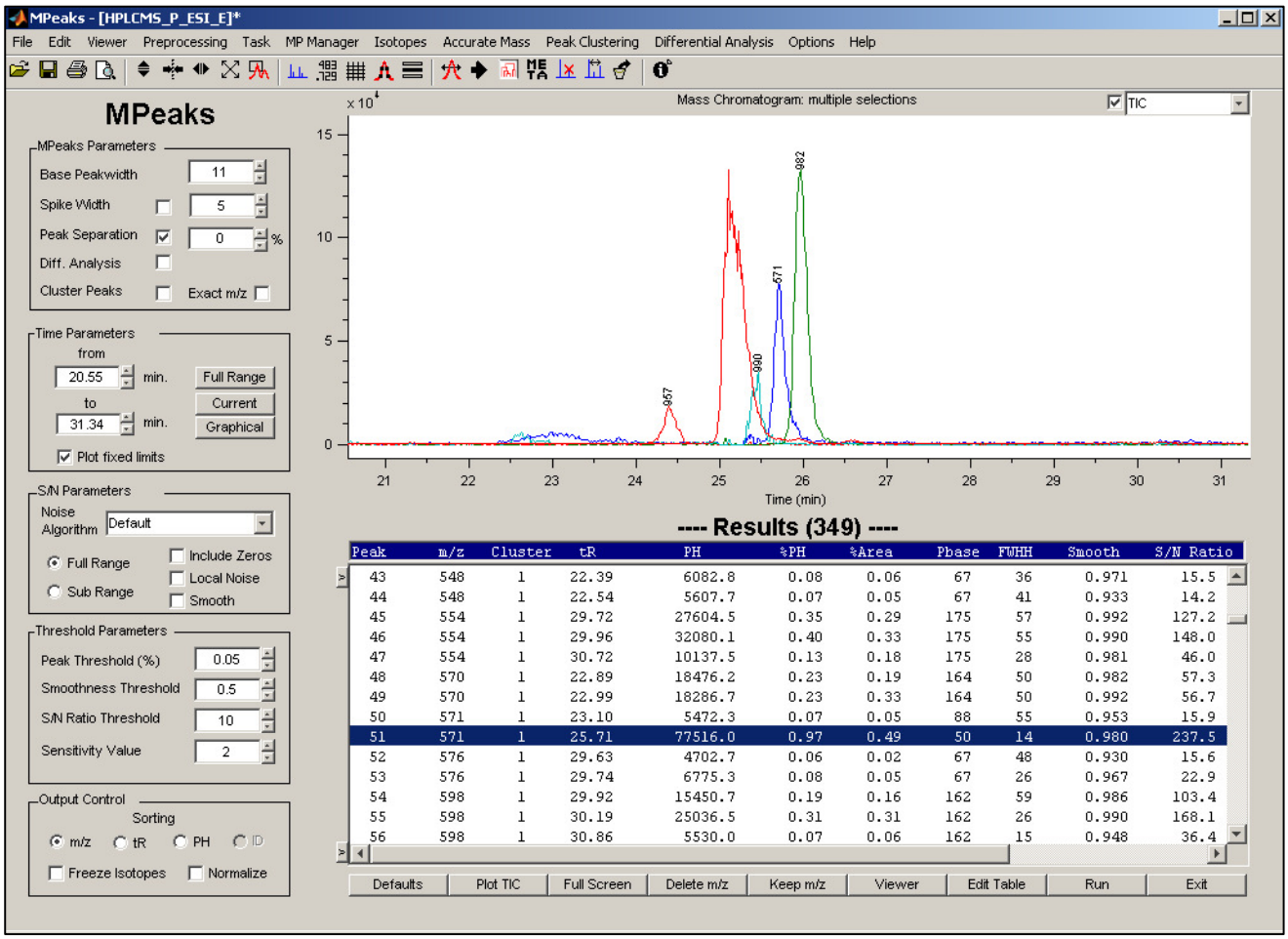

Figure 4: MPeaks Result Screen with some selected peaks plotted in overlay with the Total Ion Current

MPeaks has a specialized Viewer to plot all peaks in line mode, matrix mode or to create a dot plot. Figure 5 shows the Dot plot. The TIC is plotted in overlay, and the FWHH can be added to the dot. Peaks can be labeled by clicking on it. It is very easy to switch from one plot to the other. As you can see a large number of peaks are present under the parent peak. These

can be fragments, clusters, impurities, isotopes and peaks having different charges. A polymeric pattern is present at the left of the parent peak.

Both MPeaks and the specialized Viewer can be used to further focus on real impurities. We will further narrow down the retention time region between 23 and 27 minutes, which still gives about 100 peaks.

The Viewer will be used as follow:
Sort all peaks on retention time, so we can easily work from left to right.
Auto zoom in on the peaks in the region of interest (plot peaks +/- 4 minutes around each the retention time of the peak).
Overlay the normalized TIC, so it will be easy to distinguish impurities from fragments or noise.
Plot 10 Mass Chromatograms in one window simultaneously (maximum 100).

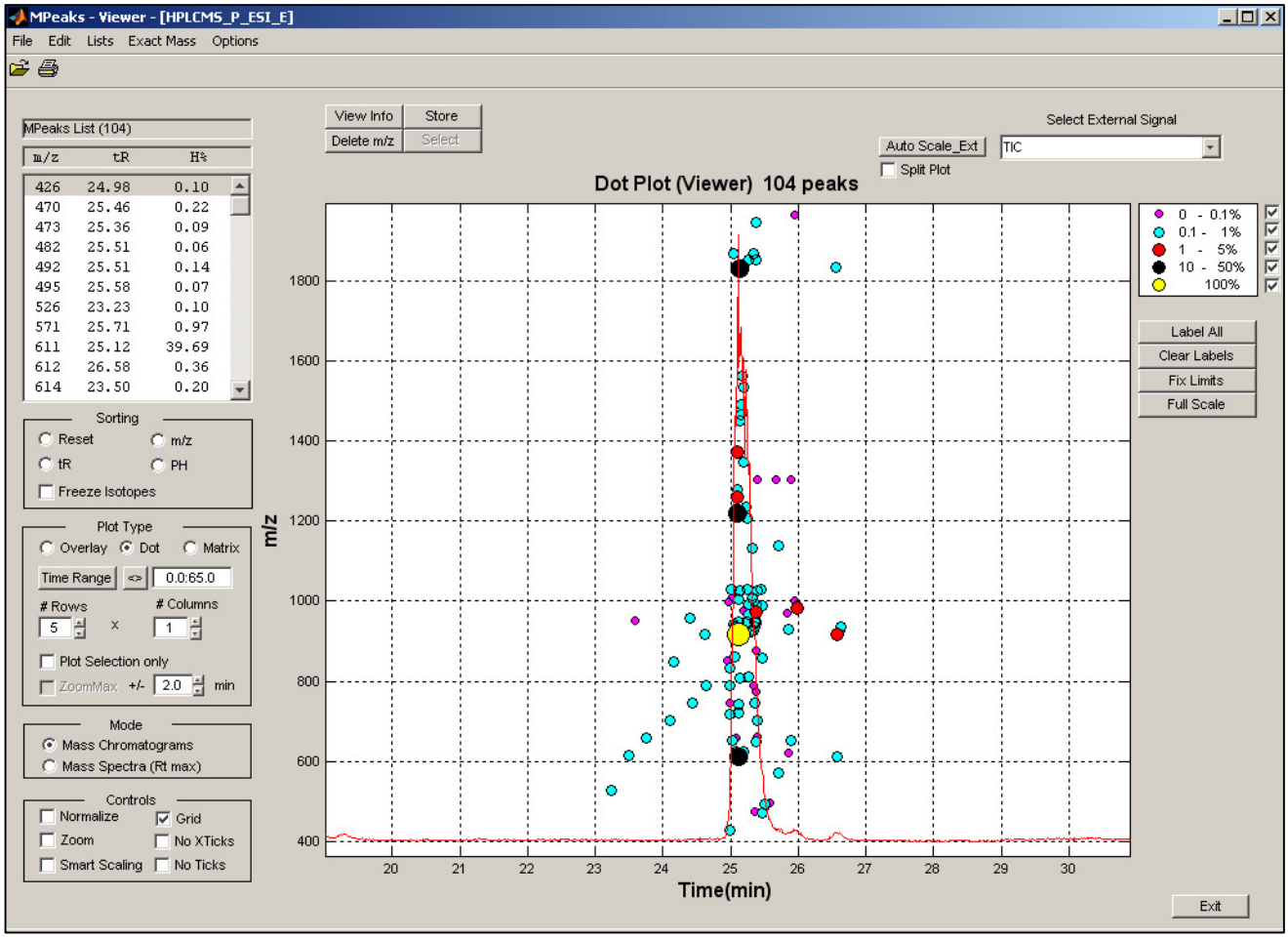

Figure 5: Dot Plot Overview from MPeak's Viewer

Three types of Matrix plots will be made: an example of peaks eluting before the parent, one plot demonstrating co-eluting peaks and an example of peaks eluting on the tail. The majority of the peaks are co-eluting or very noisy and will have to be removed. This can easily be done using the Viewer or directly in MPeaks.

Sometimes a mass chromatogram has more than one peak in the selected range. A re-scaling will be necessary. As an example see the 9th subplot in Figure 6, m/z 917. The plot refers to a small impurity of the 917 ion. However the 917 ion is actually the parent (charge 2+), as can be seen from the plot.

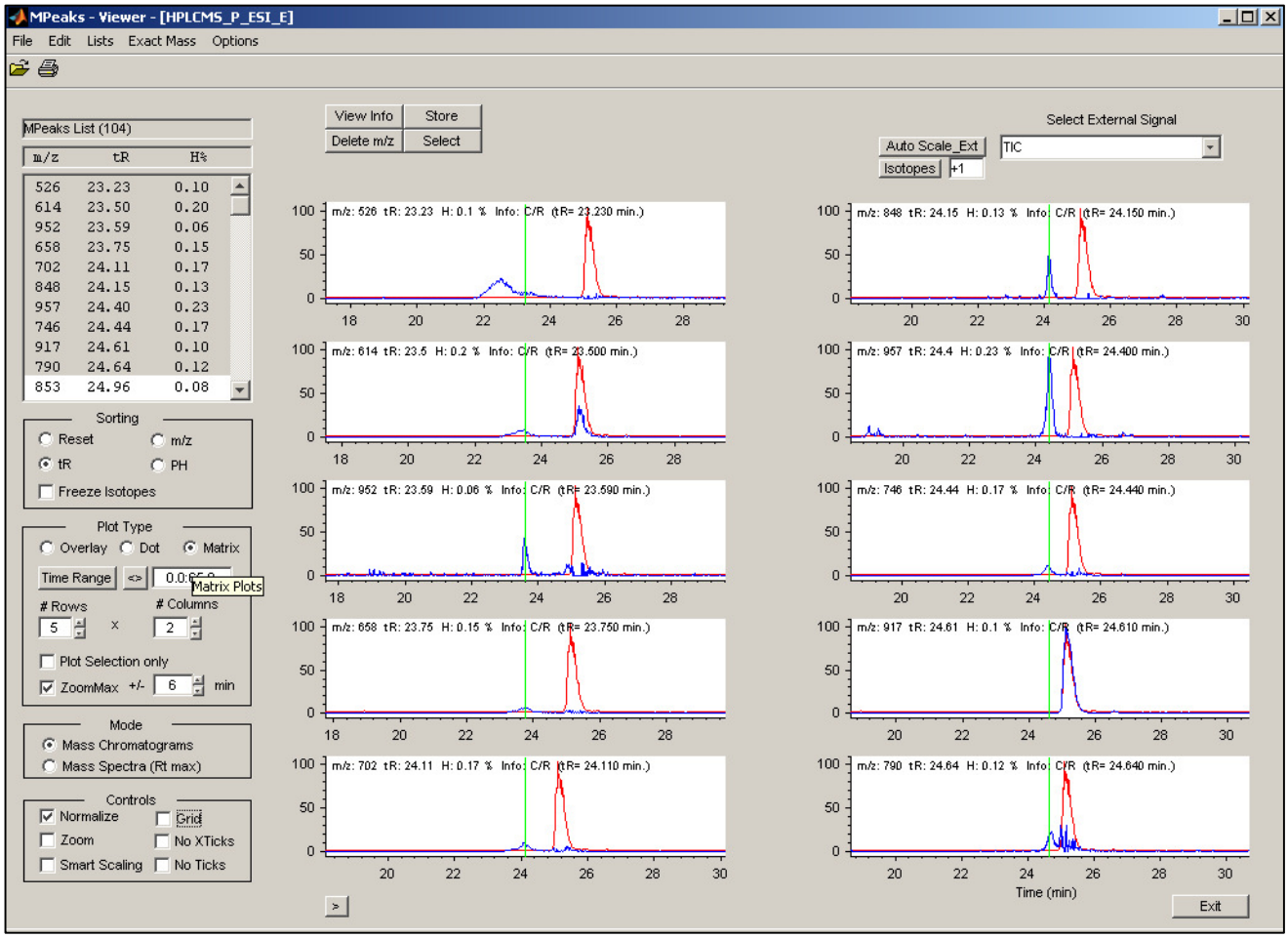

Figure 6: MPeaks Results Viewer: Matrix Plot of selected peaks eluting before parent, sorted on retention time

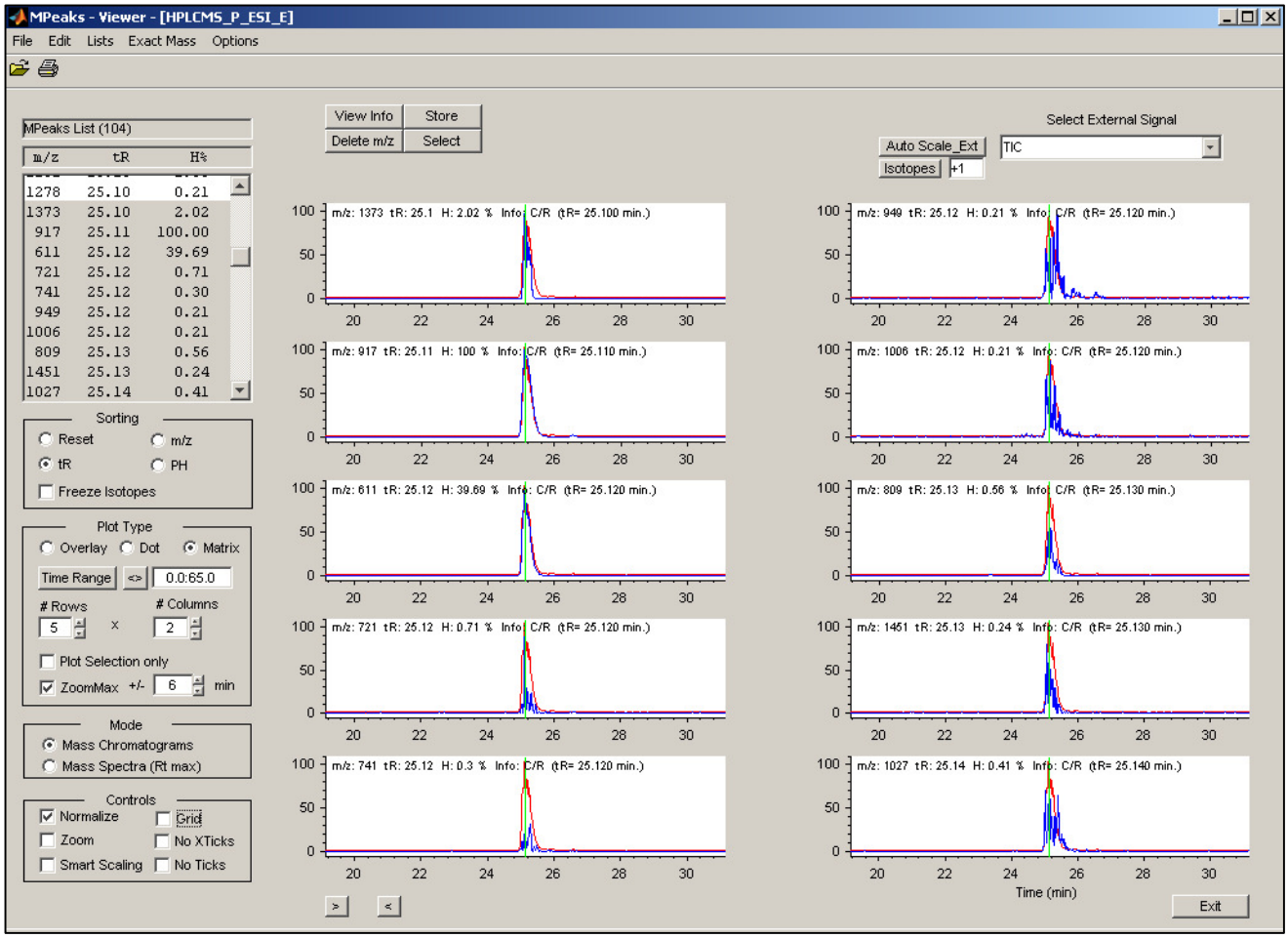

Figure 7: MPeaks Results Viewer: Matrix Plot of selected peaks co-eluting

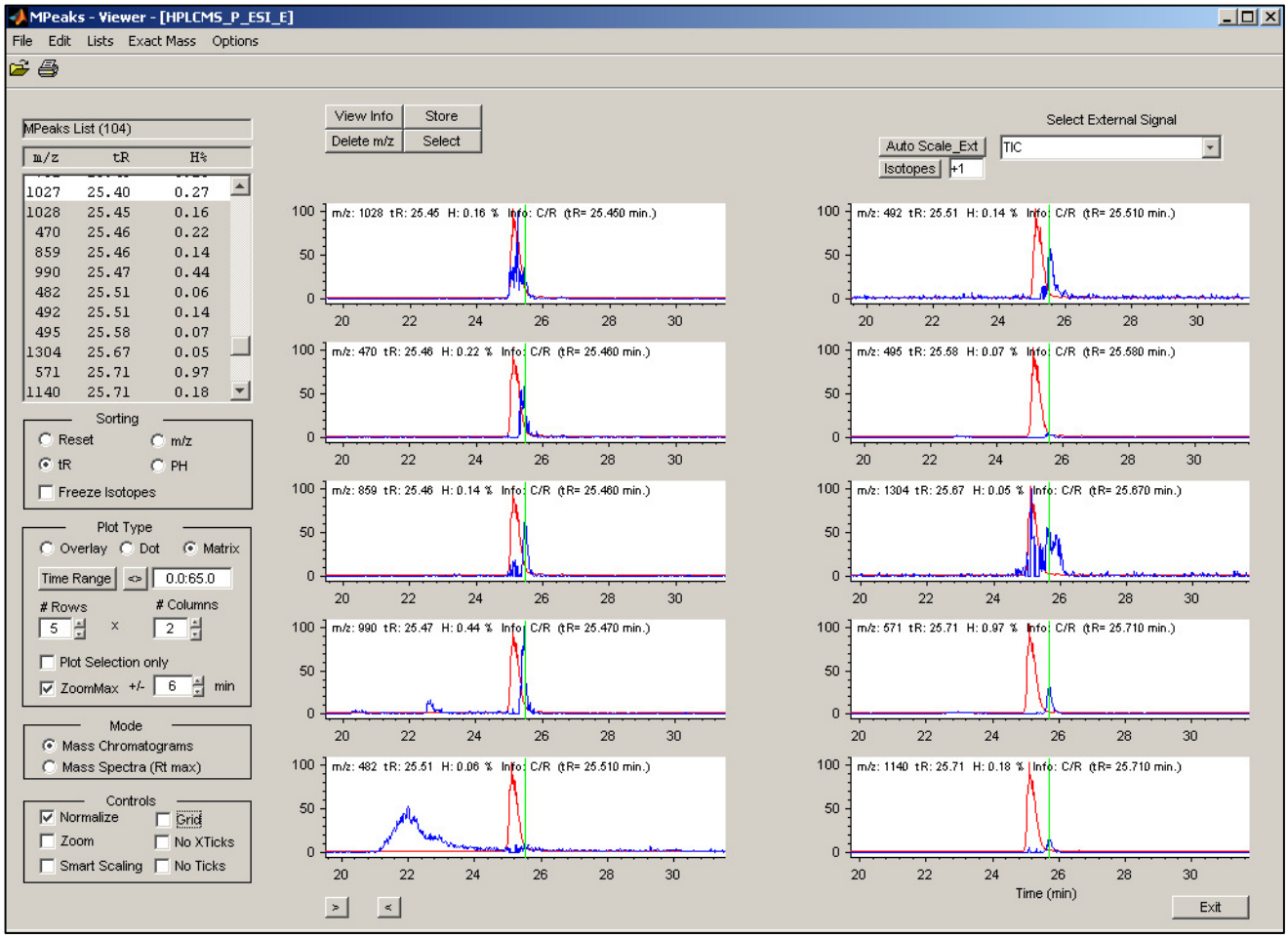

Figure 8: MPeaks Results Viewer: Matrix Plot of selected peaks eluting after parent

Step 4: Using a Targeted Approach / Identification:

The above procedure is a so-called un-targeted approach. We could also use prior knowledge to find impurities related to the parent. The MPeaks module of MsXelerator can make use of so-called differential peak lists. These lists describe the possible relative changes due to oxidation, reduction, loss of certain groups etc. MsXelerator contains two pre-defined lists, one for Degradation Profiling and one for Metabolite profiling. The user can edit these lists. Reactions can be added or deleted from the list.

Figure 9 shows the predefined Degradation Table. The m/z values give the relative change from the parent mass. Using the Viewer you are able to directly plot all mass chromatograms from this list relative to the mass of the parent product. You could also use MPeaks to do Peak Picking first followed by identification of all peaks using the list.

Lists can be built in nominal or accurate mass mode. If your instrument would have accurate mass values you are able to perform the search using accurate relative differences.

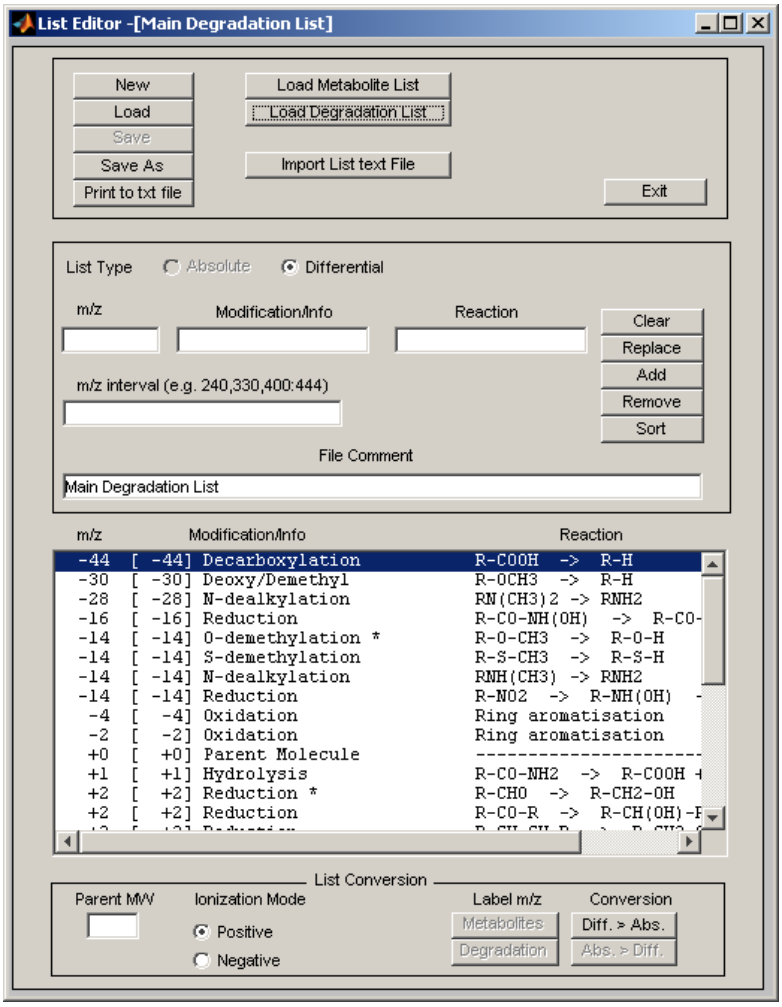

Figure 9: Prediction List Editor

MPeaks has many more possibilities to reduce or explore the results:

Conversion to accurate m/z values
Calculation of all charge states (requires high resolution instrument)
Detection of Adducts
Differential Analysis between two samples (checking against control sample)
Linking to Databases
Pre-Processing of Data: Smoothing, Baseline Correction, Despiking
Isotope Pattern Checking (e.g. check if impurities contain Chlorine or Bromine)
Batch Manager: run 100 samples using the same settings in parallel
Checking detected peaks against a list of user specified m/z values
Etc. etc.

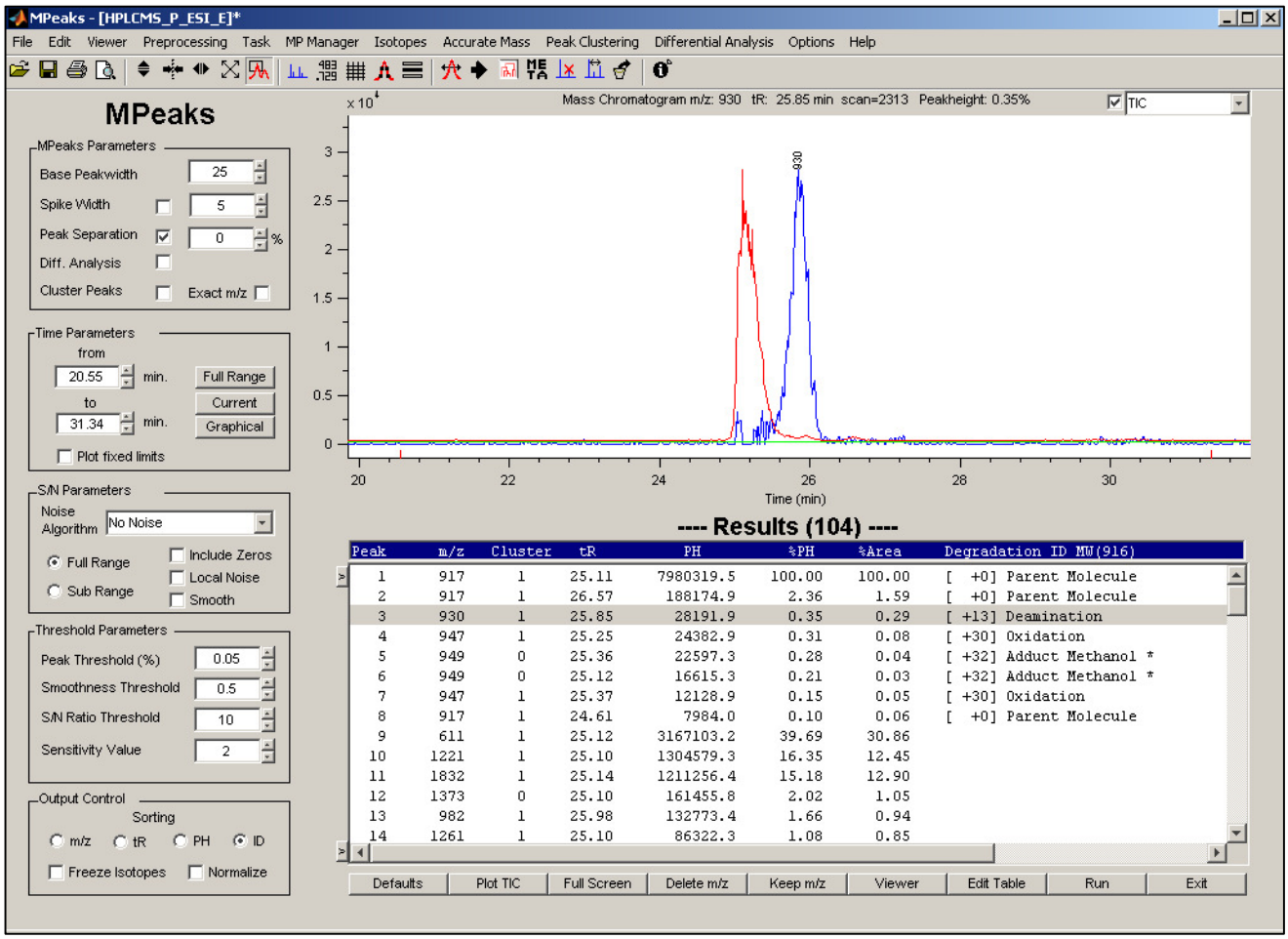

Figure 10: MPeaks: peaks are labeled based after linking with the predefined Degradation list

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