**MsMetrix**

# MS-Xelerator Tutorial:
# Impurity Profiling and Peak Purity Analysis

The sample used for Impurity Profiling and Peak Purity Analysis was measured on an Agilent MSD. The Total Ion Current is displayed in Figure 1 (top), zoomed in on the smaller peaks. Many spikes (probably due to the type of chromatography that was used) are visible, making the identification of small peaks difficult.

MS-Xelerator has a number of pre-processing algorithms to enhance the quality of your data when needed: De-Spiking, Smoothing and Baseline Correction. The effect of running a de-spiking together with a slight smoothing can be viewed in the bottom plot of Figure 1. The impurities are now visible, based on the TIC.

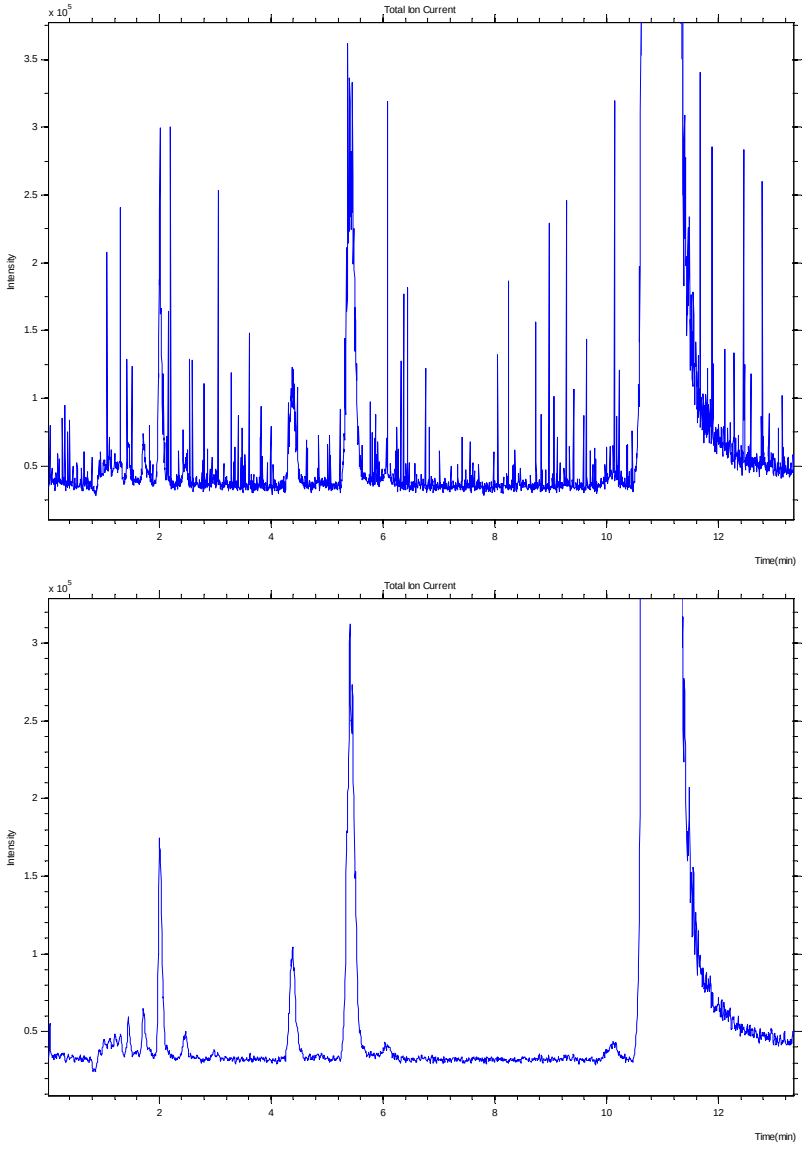


**Figure 1: Top Raw TIC, Bottom: TIC after despiking and smoothing**

The Browser Module can be used to check and identify the impurities by just clicking on the detected peaks in the TIC window. An overview of some of the peaks is shown in Figure 2: at the bottom the TIC is shown, in the middle the EIC's are plotted and the top displays the mass spectrum at the selected scan number.

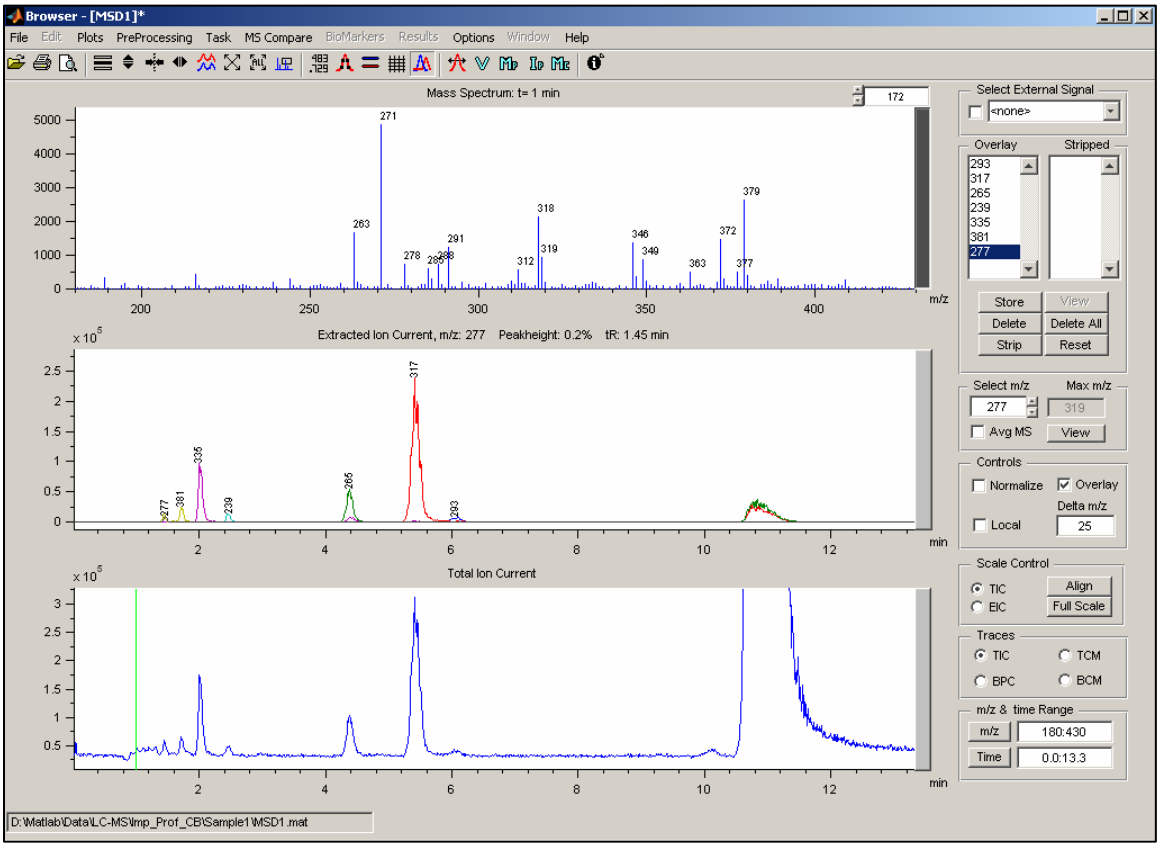


**Figure 2: Browser Overview of detected impurities.**

Although the Browser is a powerful graphical module to detect impurities, co-eluting and or very small components will not be visible based on the Total Ion Current or Base Peak Chromatogram. To detect the smaller ones, the Browser offers utilities like peak stripping and local screening. However, far more sensitive and much faster would be to let MPeaks do the peak picking.

## Running MPeaks:

MPeaks is a very fast and easy to use algorithm to detect all significant peaks in your data set. An average sample can be processed in about 5 seconds. A large number of filters and algorithms can then be used to further search for relevant peaks.

For this sample, a default run detects 69 significant peaks (threshold 0.05%) in about 2 seconds. The first filtering step will be to delete all $^{13}C$ isotopes from the result list using a de-isotoping algorithm; 44 peaks remain. Potential degradation products can be marked using the MPeaks Metabolite/Degradation ID module. The result is shown in Figure 3:

The results from MPeaks can be visualized using a number of interactive plots. To get a direct overview of all peaks use the Dot-Plot. It will plot every detected peak on a 2D contour map (time versus m/z). Each dot represents a peak. Color and size are linked to percentage peakheight. From the dot-plot you may switch to overlay plots or matrix plots using all or just a number of selected peaks.

Two groups of  nearly co-eluting peaks have been marked. From the dot-plot it will be easy to study and select nearly co-eluting peaks. Two regions in which co-elution of peaks seems to occur have been marked in Figure 4. Figure 5 shows all peaks in overlay zoomed in on the region 1-7 minutes. From the dot-plot it is also concluded that the parent component has many fragment ions.

| Peak | m/z | Cluster | tR | PH | %PH | %Area | Degradation ID MW(318) |
|---|---|---|---|---|---|---|---|
| 1 | 319 | 1 | 10.80 | 4506624.0 | 100.00 | 40.84 | [ +0] Parent Molecule |
| 2 | 317 | 0 | 5.41 | 238485.3 | 5.29 | 3.39 | [ -2] Oxidation |
| 3 | 335 | 0 | 2.00 | 97704.0 | 2.17 | 0.87 | [ +16] Hydroxylation * |
| 4 | 317 | 1 | 10.76 | 27757.3 | 0.62 | 0.41 | [ -2] Oxidation |
| 5 | 319 | 0 | 10.11 | 10146.7 | 0.23 | 0.23 | [ +0] Parent Molecule |
| 6 | 335 | 0 | 4.39 | 7861.0 | 0.17 | 0.12 | [ +16] Hydroxylation * |
| 7 | 291 | 1 | 0.92 | 3057.3 | 0.07 | 0.02 | [ -28] N-dealkylation |
| 8 | 333 | 0 | 2.97 | 2568.3 | 0.06 | 0.03 | [ +14] Ketone * |
| 9 | 265 | 0 | 4.39 | 53954.7 | 1.20 | 0.70 | |
| 10 | 265 | 1 | 10.83 | 36661.3 | 0.81 | 0.79 | |
| 11 | 265 | 1 | 10.93 | 30258.7 | 0.67 | 0.69 | |
| 12 | 381 | 1 | 1.71 | 24176.0 | 0.54 | 0.20 | |
| 13 | 381 | 1 | 1.44 | 15141.7 | 0.34 | 0.08 | |
| 14 | 239 | 0 | 2.47 | 14013.7 | 0.31 | 0.13 | |

Defaults | Plot TIC | Full Screen | Delete m/z | Keep m/z | Viewer | Edit Table | Run | Exit

**Figure 3: MPeaks peak picking results: possible degradation products have been marked**

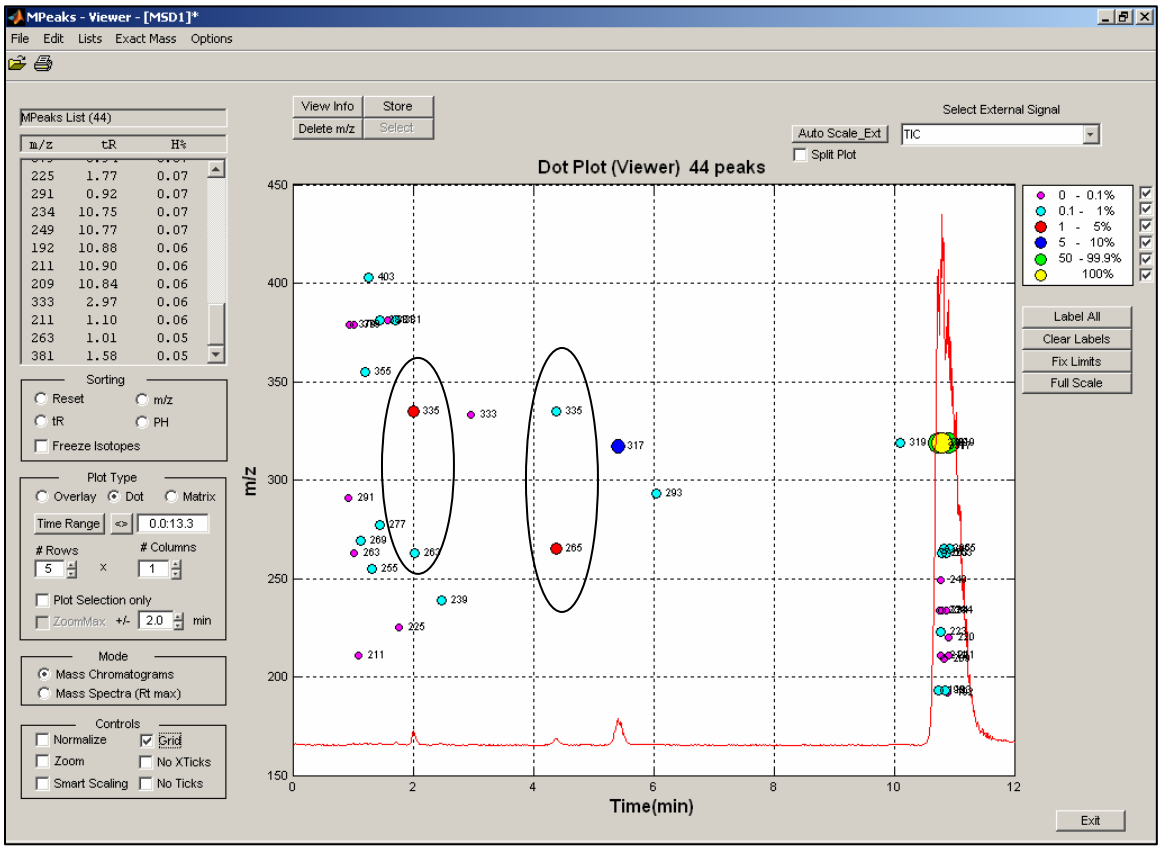


**Figure 4: MPeaks Dot-Plot in overlay with TIC, showing all detected peaks.**

## Peak Purity Analysis:

To automatically discriminate between perfectly co-eluting peaks (fragment ions and adducts) and nearly co-eluting peaks, MPeaks contains a so-called clustering algorithm. After this operation a cluster parameter will be added to the MPeaks result table. Co-eluting peaks will be in the same cluster, nearly co-eluting peaks will be in sequential numbered clusters. The MPeaks table after clustering is displayed in Figure 6.

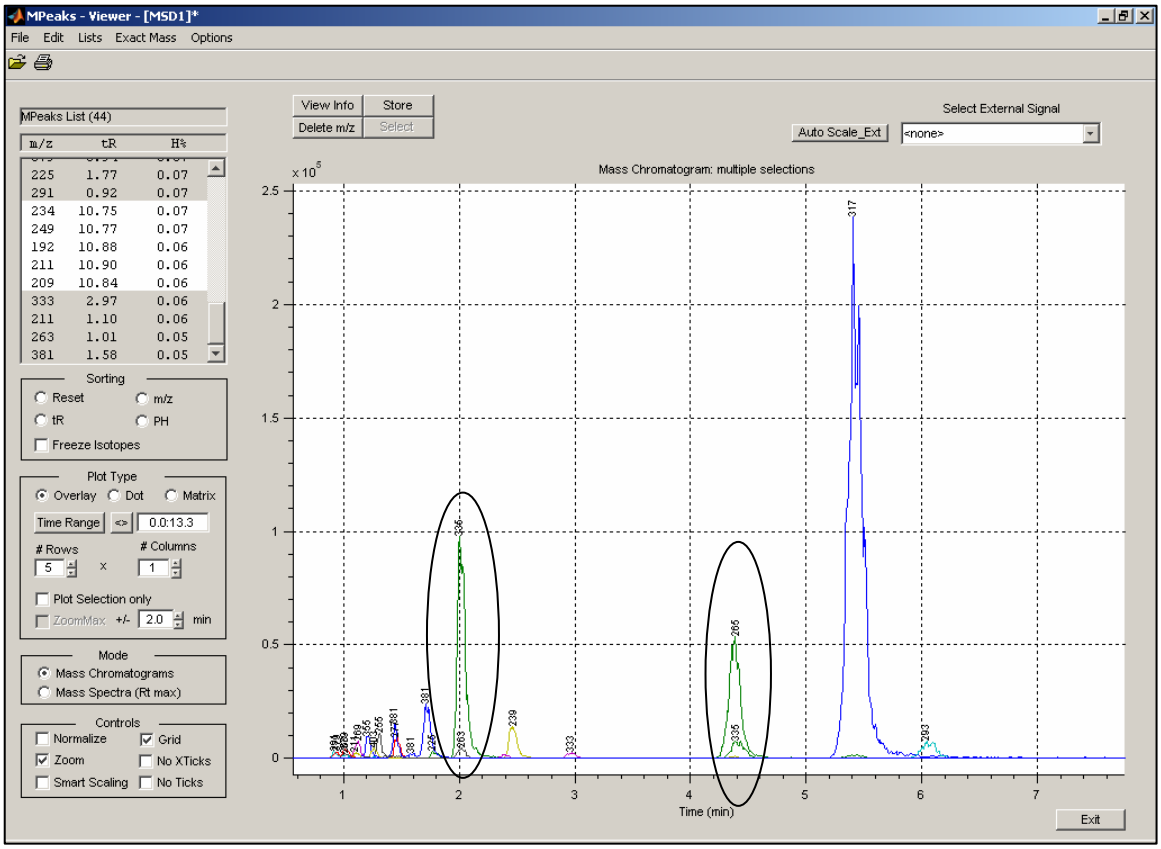


**Figure 5: overlay of all detected peaks, possible co-eluting peaks from the dot-plot have been marked**

The third column from the table now displays the calculated cluster parameter. All 4 peaks were marked and plotted. It can be concluded that m/z 335 and m/z 263 are in the same cluster and therefore show “perfect” co-elution. On the other hand, m/z 335 and m/z 265 seem to be co-eluting but are **not** in the same cluster. After zooming in and normalization it is indeed concluded that these peaks are different in retention time (Figure 7).

The smaller peaks from each cluster can be deleted from the table directly or afterwards. The clustering algorithm can also be run directly, without first deleting all $^{13}C$ isotopes. All isotopes will be in the same cluster as will also be the case for Na and K adduct peaks. The MPeaks module has separate algorithms to detect isotope and adduct peaks.

The complete analysis of this tutorial sample can be performed in less than a minute.

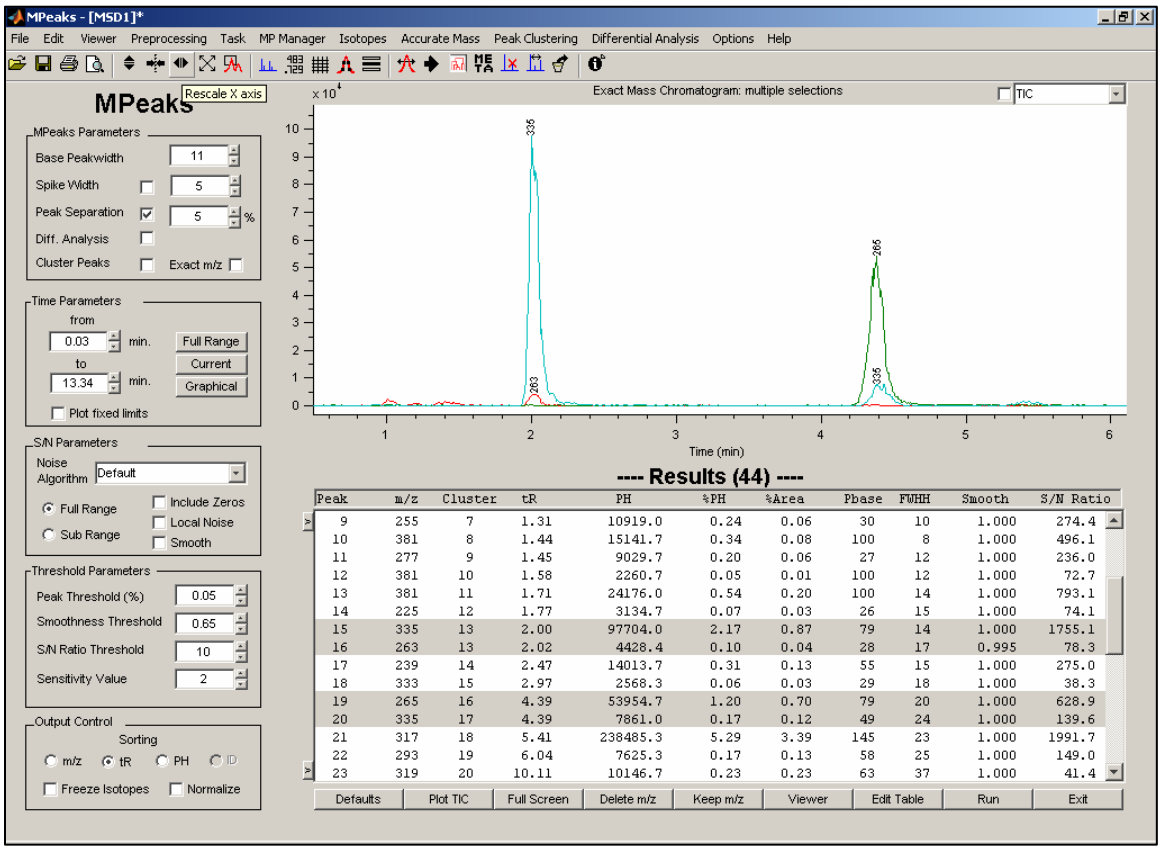


**Figure 6: Result after clustering, marked are the peaks from the dot-plot.**

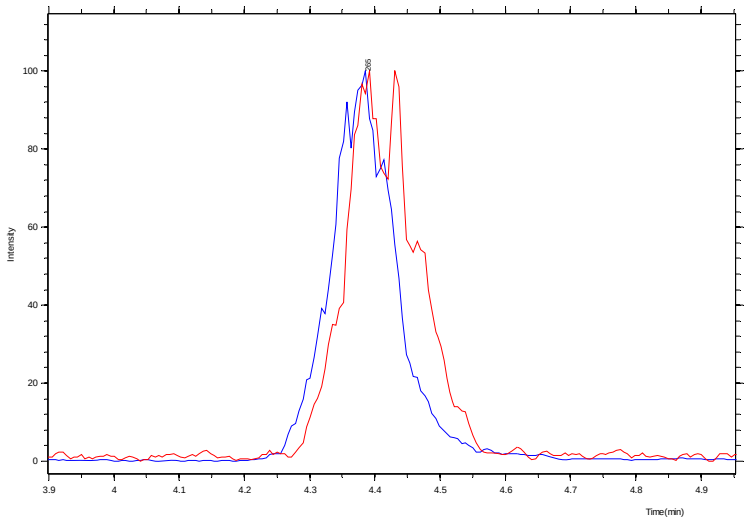


**Figure 7: m/z 335 and m/z 263 in overlay after normalization. It is evident that the peaks do not perfectly co-elute and therefore cannot be fragments or adducts.**