

MsXelerator

LC/MS Metabolite Profiling Examples

Detecting Chlorine containing Metabolites:

The example relates to a urine metabolite sample measured on a Waters QTof. The sample is contaminated with polyethylene glycol, which give rise to the presence of many non-drug related peaks. Figure 1 displays the Total Ion Current. The TIC for the MDF filtered data set is displayed in Figure 2. Both sets will be compared on the number of chlorine peaks found.

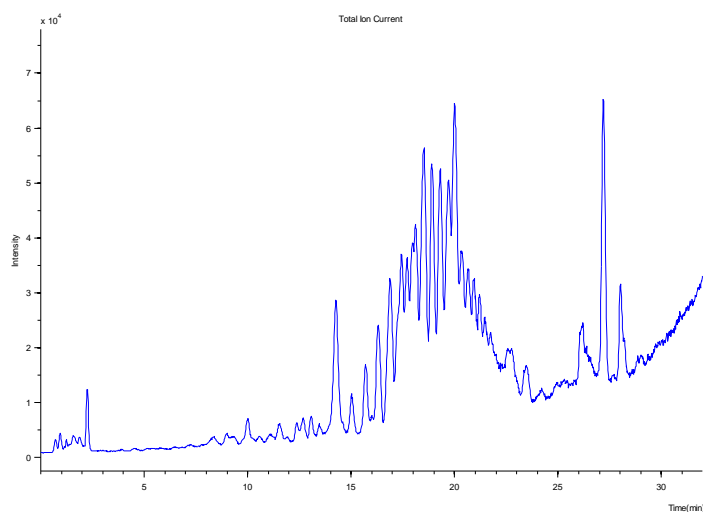


Figure 1: Total Ion Current from Urine Metabolite sample

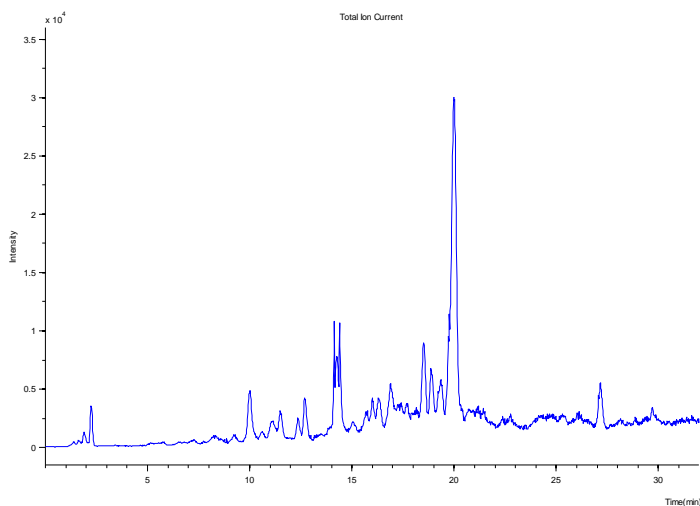


Figure 2: Total Ion Current after mass defect filtering

MPeaks Analysis:

A default MPeaks run on the raw data results in the detection of 2544 peaks (threshold 0.05%). Keeping the mono-isotopic peaks results in 1591 peaks, the majority of which are not relevant. From the dot-plot, the presence of many polyethylene

glycol peaks is evident (Figure 3). The typical polymer peak patterns having delta m/z values of 44 Dalton and eluting one after the other are easily recognized.

A further filter operation could be to keep the charge 1+ peaks only. Using the Charge Analysis algorithm available from the MPeaks module 921 peaks remain.

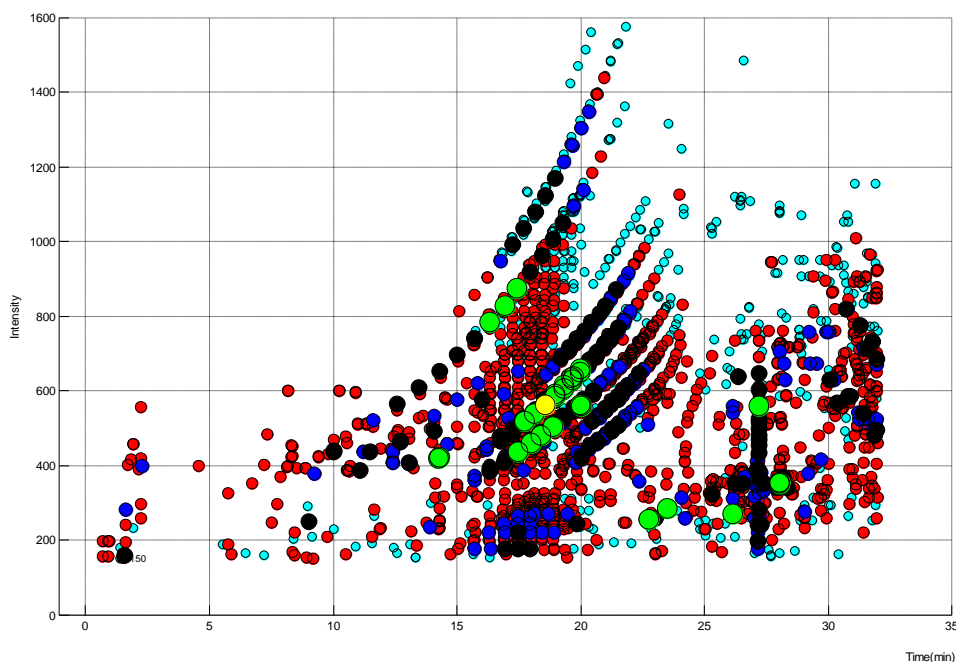


Figure 3: MPeaks Dot plot, showing all detected peaks in the time – m/z contour map

IPeaks Analysis:

The drug from this example contains 2 chlorine atoms. The specific Cl and Cl₂ isotope patterns will be used to remove the majority of peaks not related to the parent compound. A direct search for both single and double chlorine containing metabolites on the raw data set using the IPeaks algorithms results in the detection of 55 components. Figure 4 displays the chlorine containing peaks in the dot-plot. The IPeaks overview is displayed in Figure 5. Shown are the extracted ion currents of two ³⁵Cl containing peaks from the result list. The ³⁷Cl isotopes are shown in overlay for comparison.

Running the MPeaks algorithm on the mass defect filtered data set results in 332 peaks. The MDF filter can be used to delete many of the non-drug related peaks. After de-isotoping 198 peaks remain. A search on chlorine containing peaks from the MDF data set results in 35 components. However, some of the larger metabolites falling outside the MDF window were missed.

Figure 6 compares the top 16 peaks from the IPeaks results with the MDF filtered EIC's. Seven chlorine containing peaks were not found after MDF filtering was applied, because they were removed by the filter operation. One peak is "chopped" due to the fact that the peak is on the edges of the MDF filter. The mass accuracy on both flanks fall inside the mass defect filter window. However, the middle part of the peak has been removed.

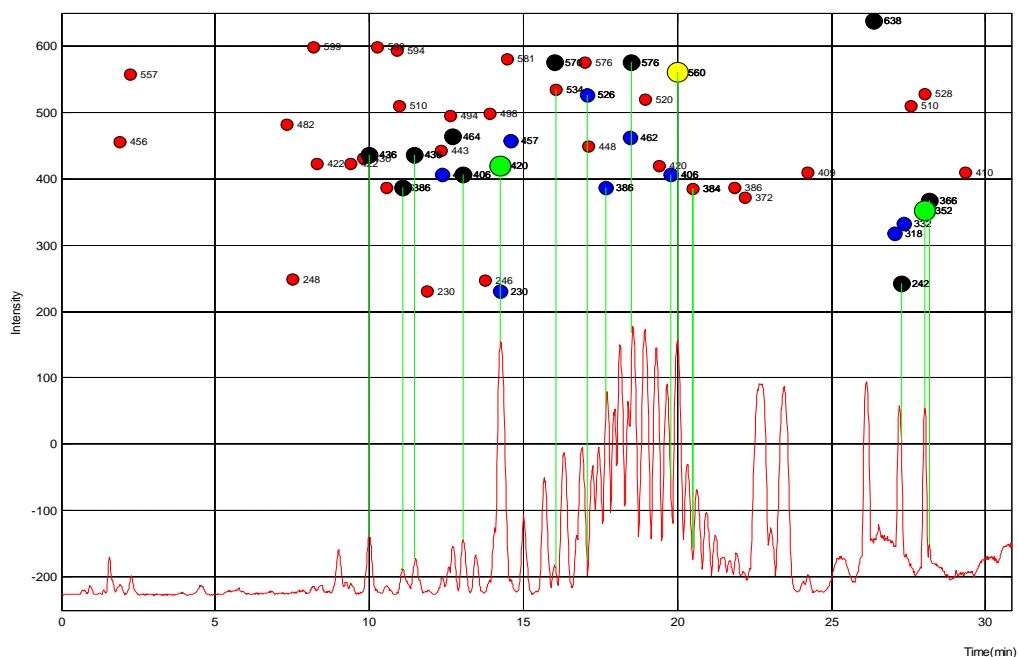


Figure 4: Ipeaks Dot plot, showing all detected chlorine containing peaks in the time - m/z map in overlay with the base peak chromatogram. Some of the larger peaks are linked to the BPC.

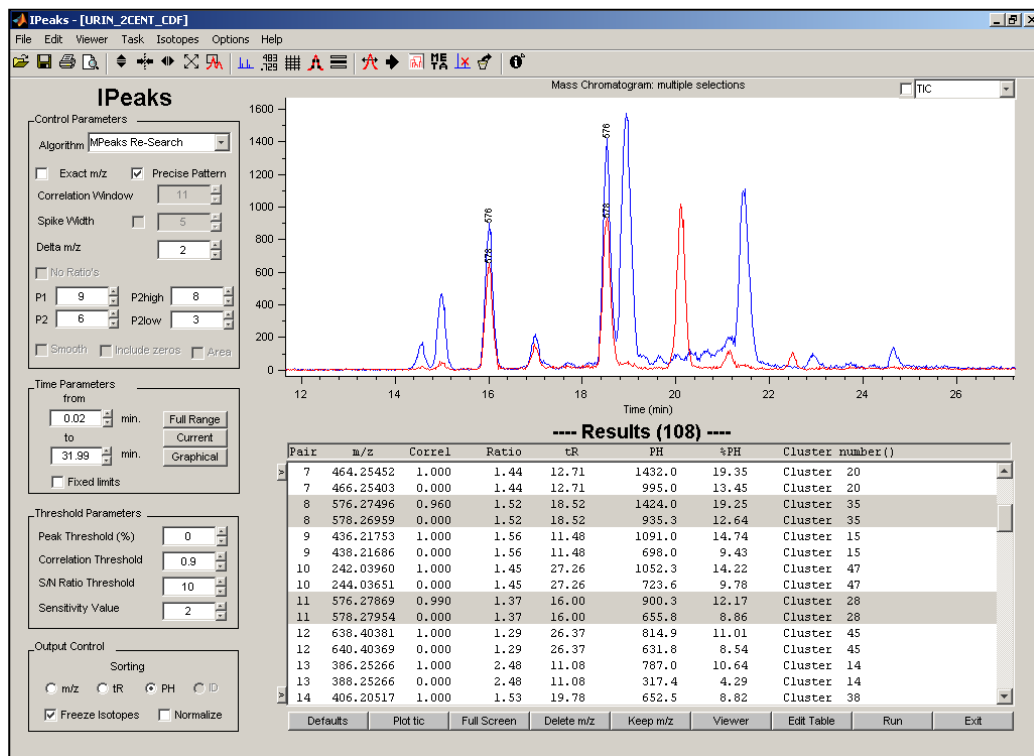


Figure 5: Ipeaks Result Screen showing two Cl₂ containing Extracted Ion Currents

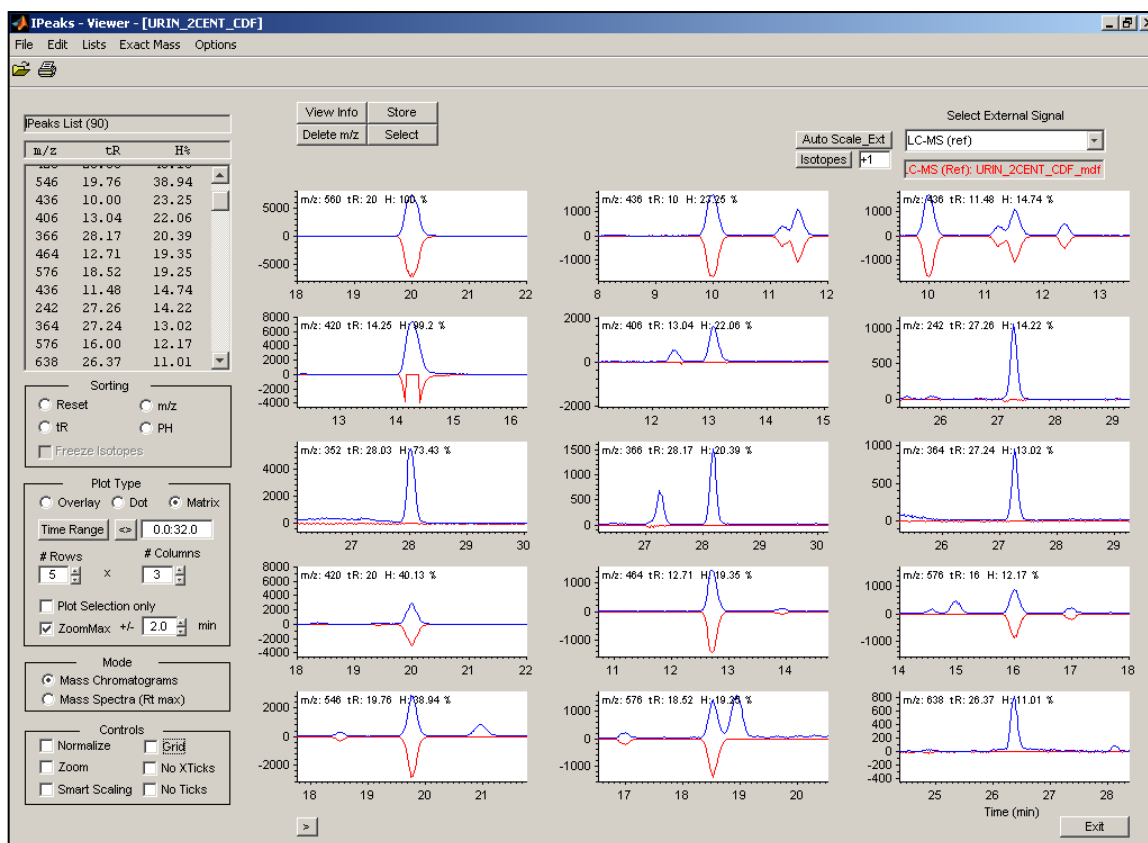


Figure 6: Chlorine containing EIC's in overlay the mass defect filtered EIC's.

Conclusions:

Both post-processing algorithms can be of added value to Metabolite Profiling studies, especially when dealing with complex matrices. The Isotope Search algorithm is extremely useful when dealing with chlorine or bromine containing drugs. Mass defect filtering is also a strong post-processing technique. MDF can be run directly on the MPeaks result table, leaving the original file intact. This greatly speeds up all MDF processing since no conversion will be required.

Metabolite Profiling: Species Comparison

The MS Compare module was used to study 12 samples from 6 species, both male and female. Sample were analyzed on two time points (t=0 and t=3 hours).

MS Compare was used to perform the following tasks:

1. Align all chromatograms using a peak matching algorithm
2. Identify, quantify and tabulate all major metabolite peaks
3. Perform species comparison using Principal Component Analysis

Figure 7 shows the MS Compare screen in which the 12 samples are loaded. In the bottom window the aligned Base Peak Chromatograms are shown in overlay. The shifted chromatograms were aligned using a peak matched warping algorithm. After alignment, comparisons of metabolites from different samples is much easier.

Clicking on any of the displayed peaks in the bottom window will identify the selected sample and plot the extracted ion currents in the top window. Any peak can easily be integrated and added to the result table. The table shown uses a semi-quantitative listing (absent – low – medium –strong). Results can also be displayed using percentage or absolute area counts. If present, the user may also select UV traces to perform the analysis.

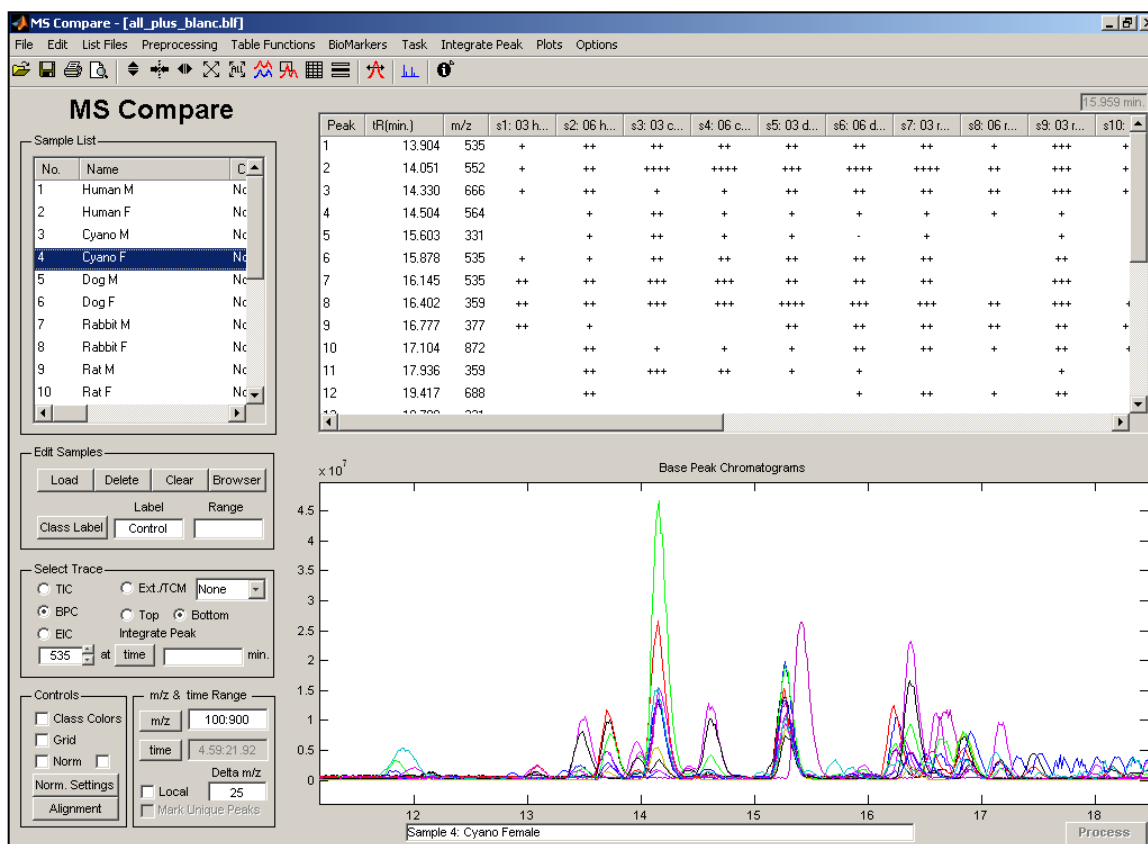


Figure 7: MS Compare screen, showing aligned Base Peak Chromatograms of 12 samples in overlay

Chromatograms may also be plotted in stacked mode or as heatmap plots. The result table was created manually in about 15 minutes. The larger peaks were selected – identified and quantified. In total 17 metabolites peaks were used in this tutorial (threshold 5%). In Figure 8, a selection of peaks is plotted as a function of the sample number. Different relative levels are easily compared in this way.

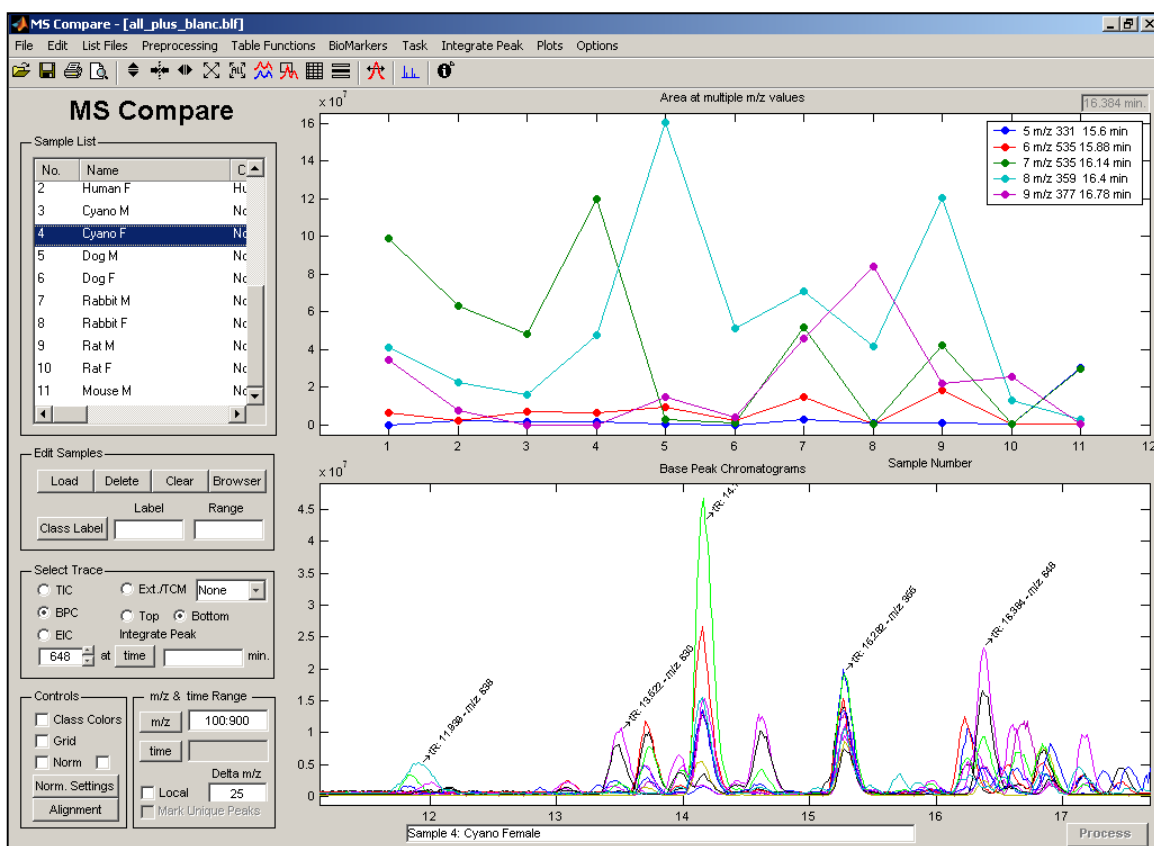


Figure 8: MS Compare – levels of 5 metabolite peaks are plotted for all samples

To better show the differences and similarities between the profiles, one can make use of the multivariate techniques Principal Component Analysis (PCA) or Clustering. Both techniques will analyze the whole table in a multivariate way.

Figure 9 shows the Scores Plot for pc1 and pc2. From this plot it can be concluded that the Metabolite profile for Human Male is closest to the Rabbit Female profile. In a similar way all other samples can be compared and visualized together. On closer inspection, it appears that the pc1 axis is correlated most with the parent compound (strongest peak present). Alternatively, the samples can also be displayed in a cluster map. The majority of samples appear to have similar profiles, but 4 samples seem to have quite different profiles.

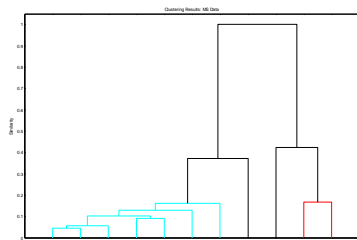


Figure 9: Principal Component Score Plot – Species Comparison

