High Resolution Deconvolution® Technical Brief

Section 1
Pre-Requisites for Peak Finding

High Resolution Deconvolution (HRD®) finds independent chemical components in complex chromatograms that may contain multiple partially overlapped peaks. By adding the extra capability of mathematical separation to chromatographic data, *HRD* can distinguish individual analytes in regions of coelution. While *HRD* can detect more analytes than can be chromatographically baseline-separated, there are limits to what can be mathematically separated without risking chemical mis-assignment or quantitative error.

Ion statistics are an important consideration for *HRD* and the Pegasus® GC-HRT which is an instrument designed to be capable of detecting very low levels of ions. With abundant ion levels, ion statistics are typically not considered because other sources of variation (injection reproducibility, flow rate fluctuations, power supply noise, etc.) dominate. At low ion levels, however, the effects of ion statistics become one of the primary sources of noise and this variation needs to be accounted for during data handling.

Incorporating ion statistics with data processing provides advantages in handling HRT data, but there are some inherent limitations when dealing with data from these rare event situations. With HRT data and complex samples, coelutions can occur where there are not enough ions present to determine with statistical confidence if there is one analyte with a large degree of variation or multiple analytes (See Section 3 for representative examples). Ion statistics and confidence levels are utilized during peak finding to make these decisions and to try to balance over splitting masses of the same analyte into separate features and merging separate analytes as a single feature.

At higher ion levels there is more confidence, so less variability in centroid location and peak width/shape are accepted relative to a less intense analyte.

Optimized chromatography, mass spectral acquisition, and improved ion statistics will always give the highest quality data processing results and the mathematical algorithms the best likelihood of success. Towards that goal, the following best practices for data acquisition are recommended.

Chromatographic Separation: Utilize appropriate columns, temperature programs, flow rates, etc. to chromatographically separate analytes as much as possible.

Mass Spectral Acquisition: The *HRD* algorithms perform best when data are acquired at 12 points/fwhh (full width half height of chromatographic peak). If the "Use Recommended Acquisition Rate" box (Figure 1) is checked and a correct expected chromatographic fwhh peak width is entered in the MS method during data acquisition, the software will automatically use an appropriate acquisition rate. The algorithms can handle peak widths between 0.5 and 3 times the input value, so if peak width varies markedly across the acquisition, enter a peak width that is slightly less than twice that of the narrowest peak. Use the XIC function to investigate peak widths. Peaks narrower than 0.5 times the estimated chromatographic FWHH may fail to be reported. Peaks wider than 3 times the estimated chromatographic FWHH may be over-split into multiple peak markers or be susceptible to other reporting errors. If possible, chromatographic adjustments for more consistent peak widths would be recommended.

A common misconception with deconvolution is that it improves by increasing the acquisition rate. Fundamentally both over- and under-sampling reduce the effectiveness of deconvolution. Note that above the point of oversampling (>36 points/fwhh) or below the point of undersampling (<6 points/fwhh) a chromatographic peak will lead to unexpected results in *HRD*.

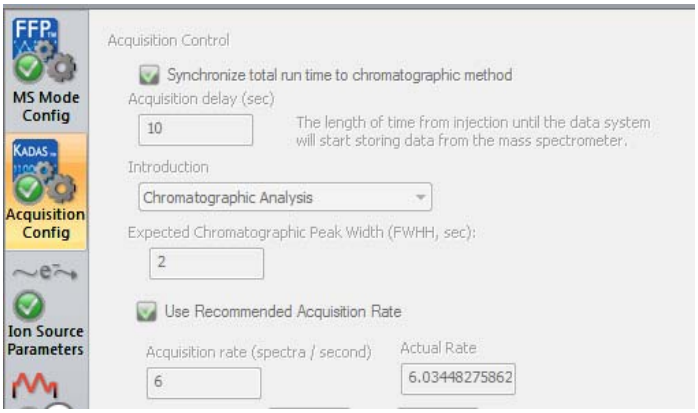


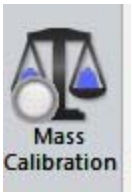
Figure 1*: Key MS method parameters for HRD*

Analyte Intensity: Ion statistics and confidence intervals are a fundamental part of HRT operation and data processing, and better confidence and ion statistics are possible with more ions (up to the upper limit of linear dynamic range). Variability in retention time and peak widths that is common in trace level analytes challenges automated Peak Finding. Target Analyte Find (see Section 4) may be useful in these situations. Also to improve peak finding, increasing the amount of ions that reach the detector by increasing on-column amounts, increasing extraction frequency, and acquiring with a recently tuned and optimized instrument is strongly recommended.

Section 2

Basic Peak Finding Method

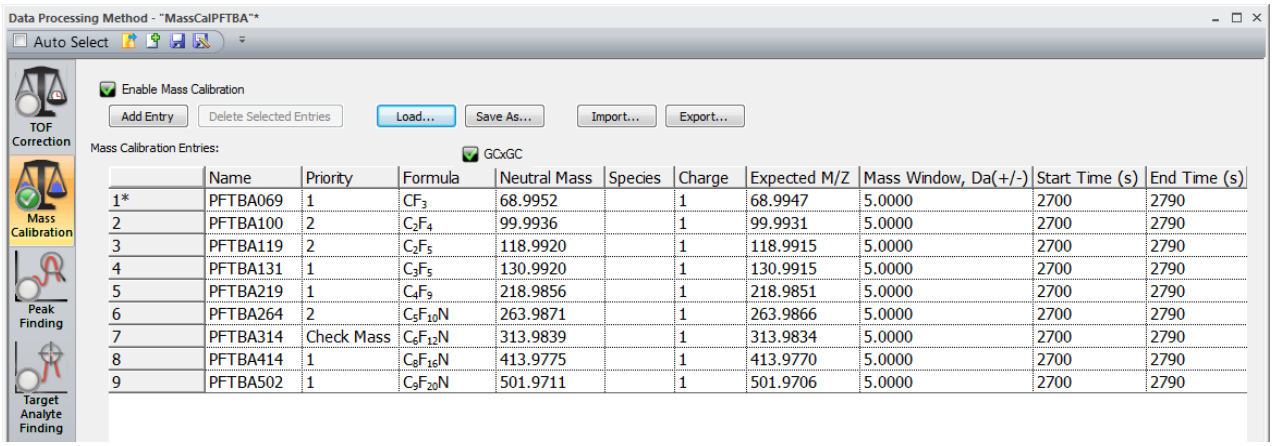
Basic identification of components in samples typically includes peak finding and library searching of mass calibrated data. The following outlines the recommended parameters (as a starting point) and guidance for each of these steps.



Mass Calibration is an essential pre-requisite and should be performed prior to other data processing steps. Mass calibration will take a few seconds, while peak finding can take much longer depending on data complexity. If mass calibration is repeated, peak finding must also be repeated, so it is recommended that these data processing steps be performed independently as a time saver if peak finding parameter adjustments are needed.

The purpose of mass calibration is to align the data based on time-of-flight (TOF) of known masses to ensure the best mass accuracy of the data overall. Mass calibration will impact peak finding results because data pre-processing incorporates noise filtering. When data are accurately calibrated electronic artifacts that inhabit chemically impossible mass defect space will be removed with filters during peak finding. Please be sure to check your mass calibration before processing as described below.

Options of mass calibration include PFTBA (if turned on for all or part of acquisition), siloxane column bleed (if operating at high GC temperatures), or other known masses. In the event that none of these are available, it is possible to mass calibrate on a known analyte peak.



	Name	Priority	Formula	Neutral Mass	Species	Charge	Expected M/Z	Mass Window, Da(+/-)	Start Time (s)	End Time (s)
1*	PFTBA069	1	CF_3	68.9952		1	68.9947	5.0000	2700	2790
2	PFTBA100	2	C_2F_4	99.9936		1	99.9931	5.0000	2700	2790
3	PFTBA119	2	C_2F_5	118.9920		1	118.9915	5.0000	2700	2790
4	PFTBA131	1	C_3F_5	130.9920		1	130.9915	5.0000	2700	2790
5	PFTBA219	1	C_4F_9	218.9856		1	218.9851	5.0000	2700	2790
6	PFTBA264	2	$C_5F_{10}N$	263.9871		1	263.9866	5.0000	2700	2790
7	PFTBA314	Check Mass	$C_6F_{12}N$	313.9839		1	313.9834	5.0000	2700	2790
8	PFTBA414	1	$C_8F_{16}N$	413.9775		1	413.9770	5.0000	2700	2790
9	PFTBA502	1	$C_9F_{20}N$	501.9711		1	501.9706	5.0000	2700	2790

Figure 2*: Example PFTBA mass calibration method*

Pre-loaded mass calibration tables for PFTBA can be loaded from the load button circled in red above (Figure 2), but must be adjusted to match the acquired data.

1. Any masses that fall outside of the acquisition range should be removed. (Highlight row and "Delete Selected Entry".)
2. The "Start Time(s)" and "End Time(s)" values should be adjusted to correspond to a time that the MS is actively acquiring data and that the calibration masses are present and stable.

Mass Calibration tables can also be built from scratch with the "Add Entry" button and adjusting parameters per mass, as described above. The masses used should span the collected mass range and, ideally, at least 5 priority one masses would be included. The

mass calibration algorithm uses masses with the smallest Priority numbers first. If the Mass Calibration Table contains Priority 1 masses and Priority 2 masses, initially only the Priority 1 masses will be regressed to establish an initial calibration. This initial calibration will be used to find the Priority 2 masses, then all Priority 1 masses and all Priority 2 masses will be regressed to establish the final mass calibration that is applied to the sample. This loop will be executed over all Priority levels defined in the table, but at most two levels of Priority are generally recommended. Since the mass calibration algorithm will always select the most intense signal within the defined mass window, multiple priority levels enable using a robust number of calibrant masses in the final calibration, even if some or most of these masses have intense neighboring signals. Within a defined Priority, masses with wider Mass Windows will be considered ahead of masses with narrower Mass Windows. If the Mass Calibration Table contains only Priority 1 masses, two of these masses have a window of ±10 Da and three of these masses have a window of ±5 Da, the initial calibration will be based only on the two masses with ±10 Da windows. This initial calibration will be used to find the masses with narrower windows, and the final calibration will be based on all masses. This loop will be executed once for every different sized Mass Window within a single Priority.

After setting the parameters, the "Mass Calibration" DP method should be applied to the data and the mass accuracy checked by selecting the data file and viewing the Mass Calibration Matrix Table (Figure 3). Right click from within the table to view and select "Coefficients" for an overall metric of the mass calibration. The equation should have been built from at least 5 masses and the resulting "Mass Accuracy RMS" should be less than 1.0 ppm.

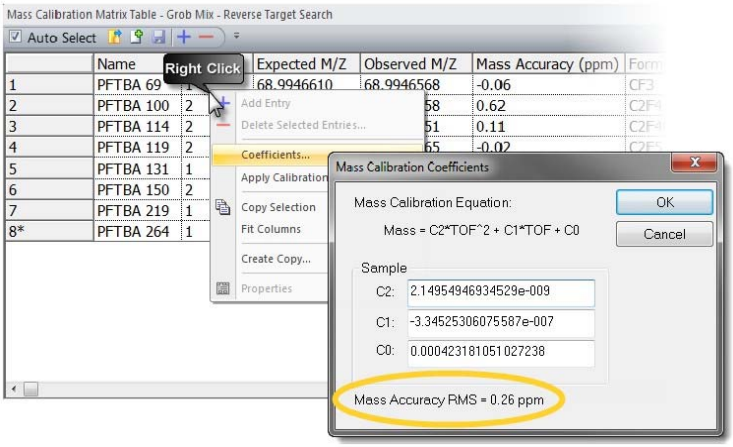


Figure 3*: Checking Mass Calibration in the Mass Calibration Matrix Table*

If "Mass Accuracy RMS" is over 1.0 ppm, adjustments should be made and mass calibration should be repeated.

1. Delete current MC (mass calibration) results. (Right click on sample, select DP results, and delete.)
2. Adjust MC table as needed based on reviewing the MC Matrix Table. Some adjustments may include:
    a. Change the retention time window to an area of better stability or one with fewer interferences
    b. Narrow or expand the mass tolerance window if the wrong mass was selected or if the true mass was outside the initial tolerance window
    c. Delete poorly performing masses (those with very high mass accuracy values).
    d. Add other persistent masses
3. Repeat data processing and check "Mass Accuracy RMS" in Mass Calibration Matrix table.

Peak Finding can then be enabled to locate and deconvolute analyte peaks within data that have been successfully mass calibrated (MC RMS <1.0 ppm).

A basic peak finding method is shown below (Figure 4) with user-input parameter recommendations and descriptions to follow.

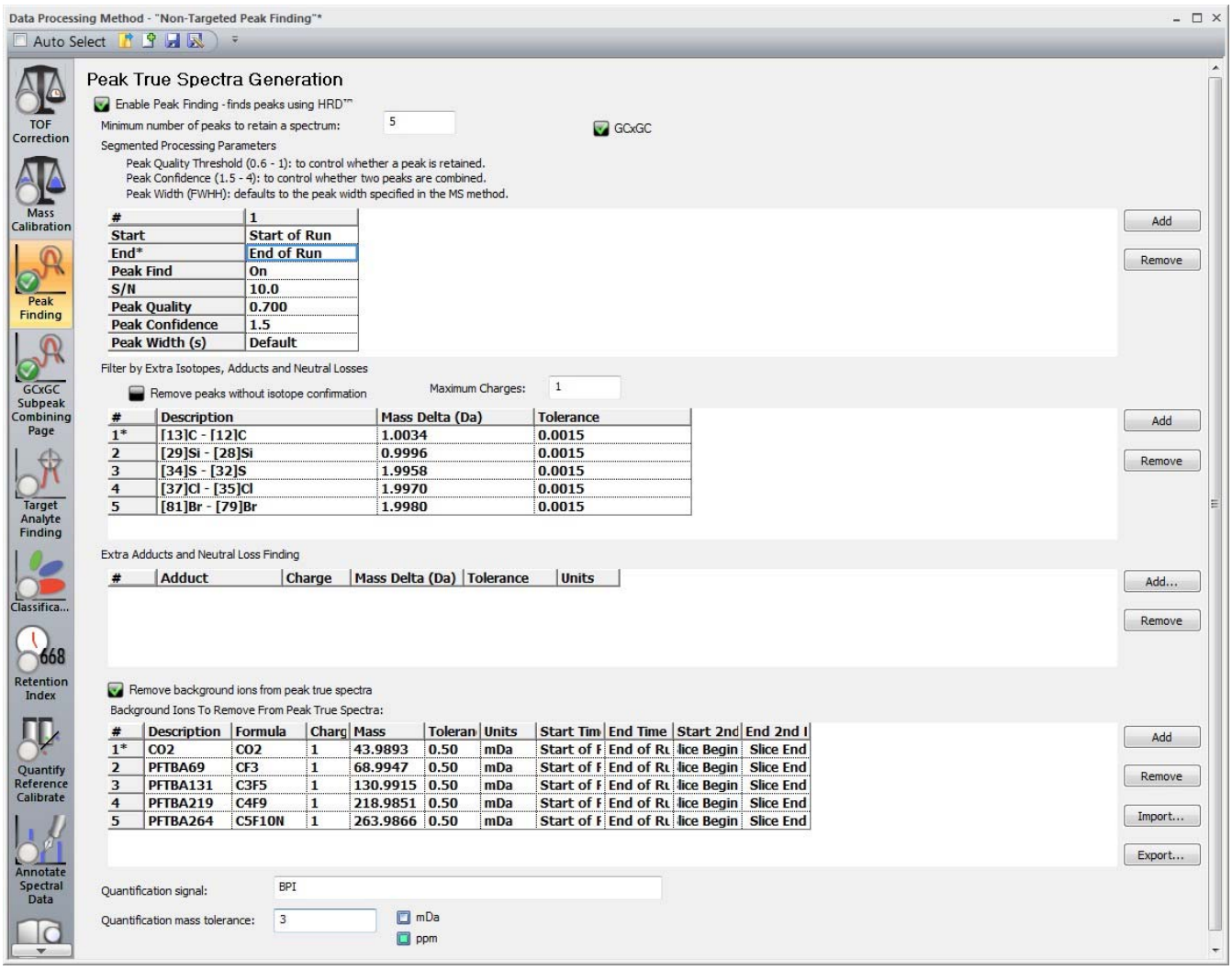


Figure 4*: HRD Data Processing Parameters*

Minimum Number of Peaks to Retain a Spectrum: This value serves as a filter and will remove peak markers from the Peak Table that have Peak True spectra that contain less than the input value of m/z in the mass spectrum. Five is a reasonable value, but this may need to be lower for CI data or data collected with a narrow mass range (for example, in UHR mode).

Start Data Processing: Peak Finding should not be turned ON until the signal is stable. If data were acquired before the filament was turned on or before the end of solvent peak elution, segmented processing is recommended, with an initial segment from start of run to a retention time when the spectral background is consistent with most of the acquisition where Peak Find is set to OFF. Refer to the following example.

#	1	2
Start*	Start of Run	250 s
End	250 s	End of Run
Peak Find	Off	On
S/N		10.0
Peak Quality		0.700
Peak Confidence		1.5
Peak Width (s)	Default	Default
Intensity Threshold		0.0

Figure 5*: Peak Find Set to OFF*

S/N: This value serves as a filter and will remove peak markers from the Peak Table that do not have any mass with a S/N above the specified value. Processing with a low S/N threshold of 10 will likely introduce false positive peaks in the results. However, post processing S/N filters can be applied to the Peak Table with the same effect as processing with a higher S/N. Initial processing with S/N of 10 allows the user to review results and determine a reasonable S/N threshold between false positives and true peaks. Post-processing filters can then be applied without requiring data processing to be repeated.

Peak Quality: This value serves as a filter and will remove peak markers from the Peak Table that do not have a peak quality above the specified value. Peak quality is a measure of how closely raw chromatographic peak shapes correlate to an ideal bigaussian chromatographic peak shape. Analyte compatibility with chromatographic conditions impacts the peak shape, so the appropriate threshold will be analyte and sample dependent. Like S/N, though, Peak Table results can be filtered for Peak Quality, so it is recommended to process with a low value of 0.7 or 0.8 and apply post-processing filters.

Peak Confidence: This value will impact peak finding and balances over-splitting fragments of the same analyte into separate features with merging separate analytes into a single feature. The statistical variation in retention time and widths for m/z eluting together can be described with confidence intervals to predict if there is one analyte with a large amount of variation or two (or more) analytes coeluting. The peak confidence can be considered similar to a t-distribution. A peak confidence value of 1.5 is similar to a t-test on chromatographic centroid locations with 90% confidence. Confidence and intensity are related, so more variability in retention time and peak width will be accepted for a low level analyte relative to a higher intensity analyte for a given Peak Confidence value. A value of 1.5 is strongly recommended, but if there are too many peak markers and masses are split into separate peak markers when they should not be, the peak confidence (i.e., confidence that you have not over split analytes) could be increased. It is not recommended to decrease the Peak Confidence below 1.5.

Peak Width: If the expected chromatographic peak width in the MS method for acquisition was inaccurate or if the data were acquired at an inappropriate sampling rate for any other reason, the preferred recourse is to reacquire data at an appropriate sampling rate. If reacquisition is impractical, useful data processing results may still be achieved by changing the Peak Width from "Default" to a truly representative peak width. As with the expected chromatographic peak width in the MS method, highly variable peak widths across the acquisition should be addressed by setting this Peak Width to slightly less than twice the narrowest expected peak width. Leaving the DP method Peak Width at "Default" means that data processing will use the expected peak width that was specified in the MS method used for acquisition.

Setting the Intensity Threshold based on Recorded Chemically Unrelated Noise Signals: The mass defect plot below shows that real chemical signals can fall in the red-outlined wedge for singly charged ions or the blue-outlined wedge for doubly charged ions. Points outside of these wedges cannot represent real GC-amenable chemically related signals, as they lie at a chemically impossible mass defect for singly or doubly charged ions. The green points that represent such chemically impossible signals are selected and plotted on the chromatogram using [Right-Click] [Show on Chromatogram]. Note that most of these points look like isolated noise spikes on the chromatogram, all of these spikes are less than 100 counts high, and most of the spikes are between about 25 counts hight and 50 counts high. Setting the intensity threshold at 50 counts would filter out most of these noise spikes. Since the detector response may change with instrument tuning, this threshold should only be applied to files acquired under identical conditions while the same tune is in effect.

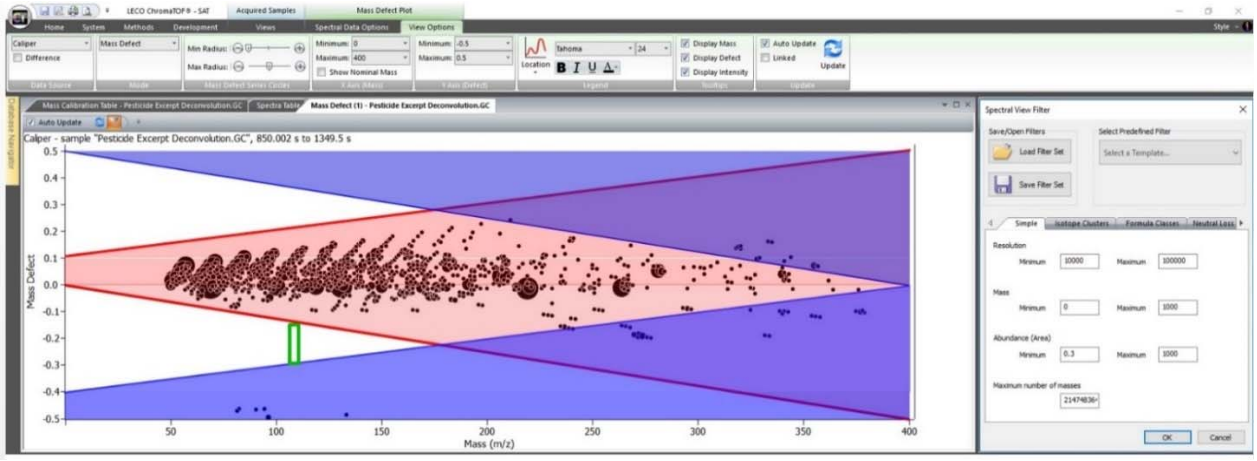


Figure 6*: Caliper Mass Defect Plot ($^{12}C \equiv 12$ scale) Summing 500 S of Acquired Data.* The spectral view filter minimum abundance threshold is set so that only real chemically attributable signals are shown. All singly-charged real chemically attributable signals fall inside the red-shaded wedge. Doubly-charged "half-mass" real chemically attributable signals lie inside the blue-shaded wedge. Note that slightly above 400 Da, the (12C ≡ 12) mass defect scale wraps around, so that very high mass defect signals of a nominal mass may overlap with very low mass defect signals of the adjacent greater nominal mass. Below about 150 Da, there are significant gaps in mass defect not occupied by any real chemically attributable signals of terrestrial isotopic composition. If the minimum abundance threshold is lowered to zero, all the points that appear within the white triangles of chemically impossible mass defect will represent chemically irrelevant noise spikes. For this example, noise points falling inside the green rectangle (110 < m/z < 115, -0.30 < Defect < -0.15) were selected and shown on the chromatogram.

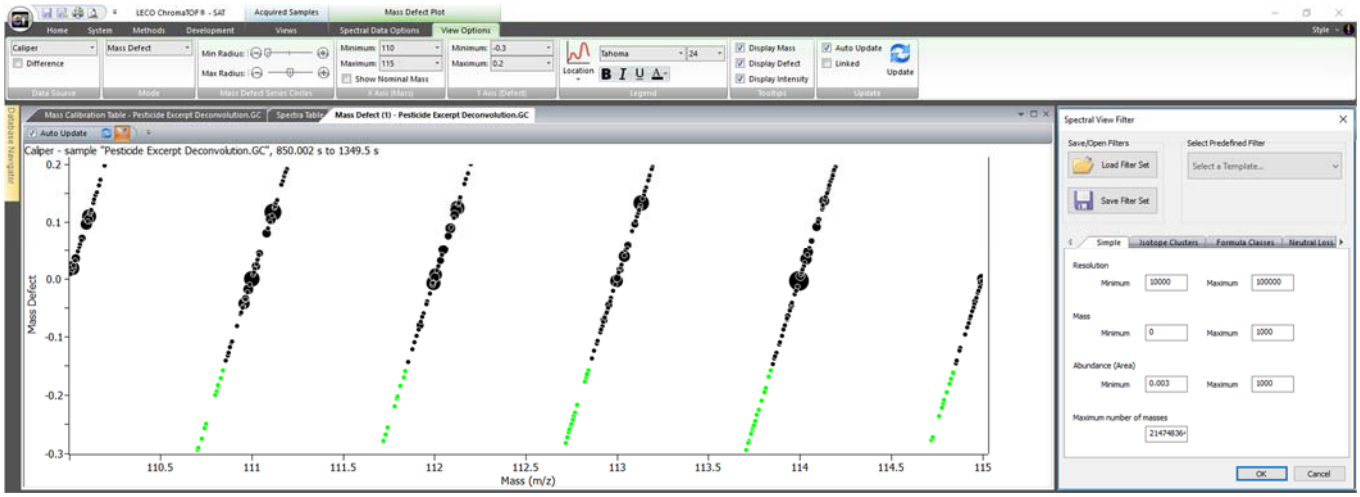


Figure 7: Mass defect Plot as in Figure 6; Minimum Abundance Threshold Reduced and Multiple Noise Points at Chemically Impossible Mass Defect Selected

Intensity Threshold: This can be used to filter weak signals from the raw data before the data are processed by the *HRD* algorithms. If the Intensity Threshold is set to an appropriate level, possible advantages include reduced data processing time and cleaner reported spectra. If the Intensity Threshold is set to zero, no intensity-based filtering occurs prior to passing the data to the *HRD* algorithm. Setting a non-zero Intensity Threshold that is too low will increase data processing time without significantly affecting the results, as the intensity threshold check would be applied to every signal and most or all signals would pass. Setting the Intensity Threshold too high may cause false negative results. There are two ways to estimate the appropriate non-zero Intensity Threshold. One way is to find the smallest signal that correctly belongs to a known analyte and set the intensity threshold to slightly less than this value. The other way is to plot several signals that cannot represent real analyte signals, and set the Intensity Threshold at about the 90th height percentile for these signals. This second method is illustrated in Figure 8, following.

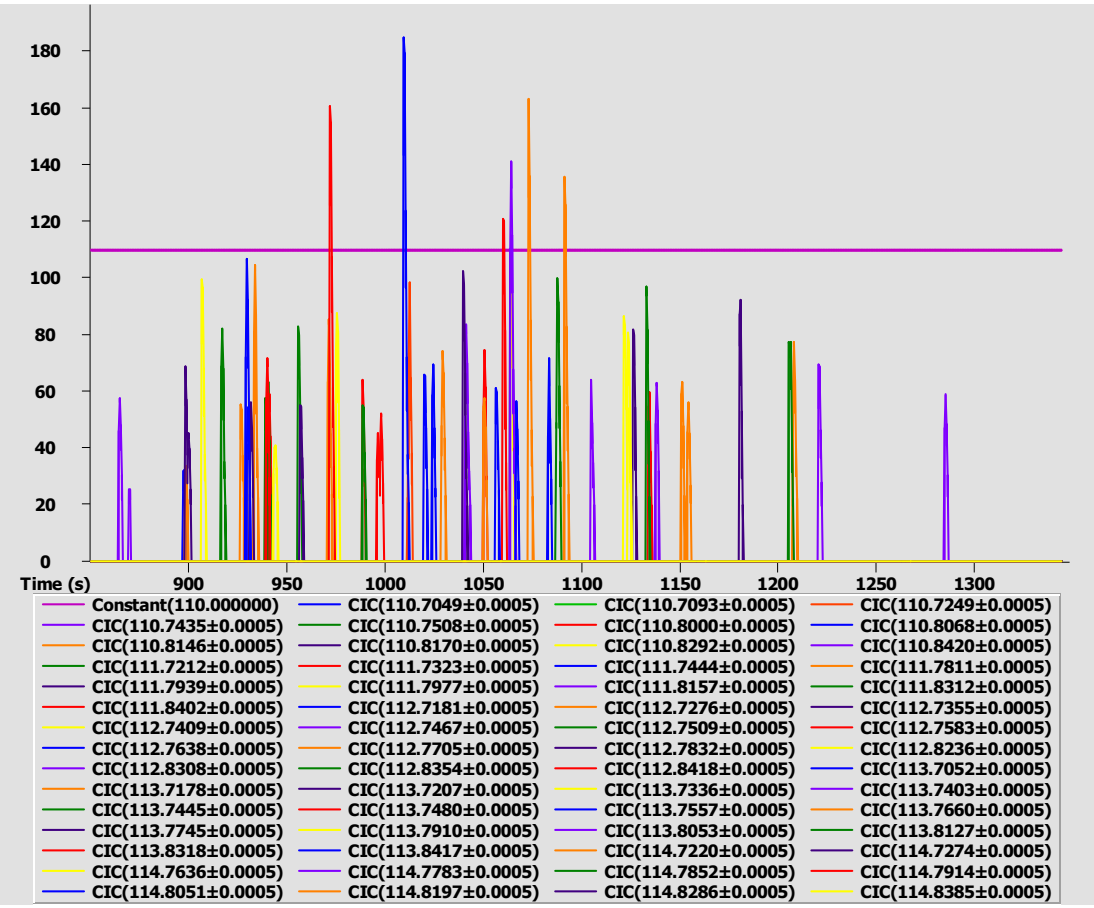


Figure 8*: Extracted Ion Chromatograms of Points Selected in* Figure 7*. The line at Constant (110) is above 90% of the noise spikes in the chromatogram, thus 110 would be a reasonable setting for the peak finding intensity threshold for this file.*

Filter by Extra Isotopes, Adducts, and Neutral Losses: In Figure 4, the checkbox is a filter that removes peak markers from the Peak Table without isotope confirmation determined by m/z spacing (not relative isotopic abundance). If "Remove peaks without isotope confirmation" is enabled, Peak True spectra lacking at least one isotope, as defined in the isotope list, are removed from the Peak Table. This option could be enabled as it is a powerful way to discriminate chemically attributable features from spurious noise events.

Populating the isotope table with reasonably expected isotopes will also improve peak finding results as extra tolerance will be allowed to include m/z in Peak True spectra when the masses are putative isotopes.

The adduct table is not recommended for routine processing, but may help combine masses for CI reagent gasses where multiple characteristic adduct species are expected, or for analytes with highly characteristic neutral losses such as loss of halogen cells.

Enable **Library Search Options** to perform a spectral similarity search. The Peak True in the entire Peak Table are searched with the parameters as specified.

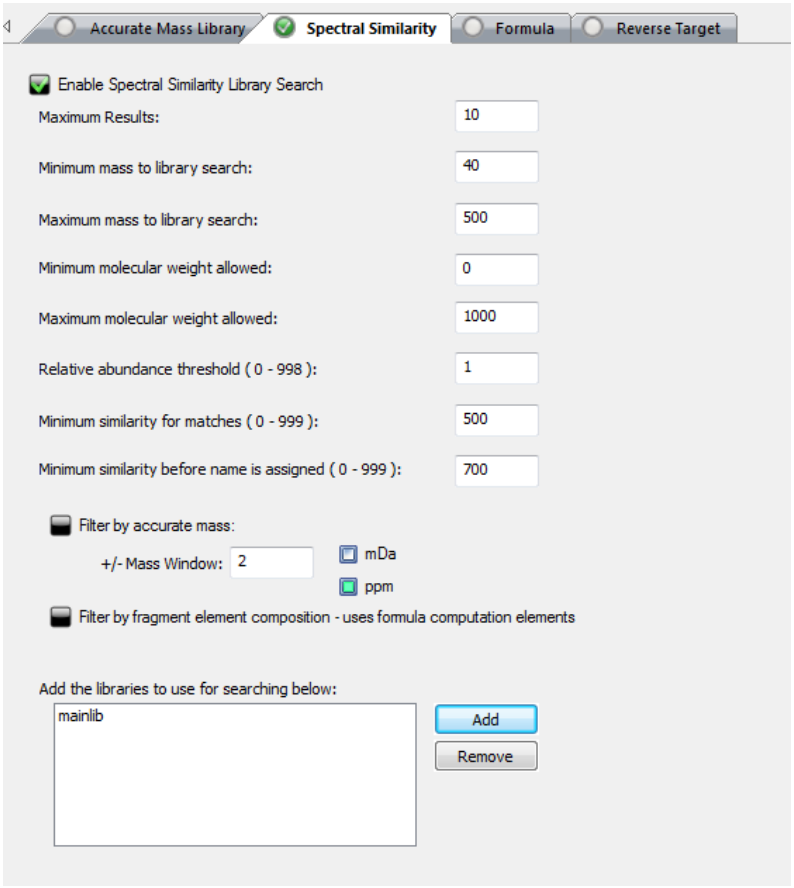
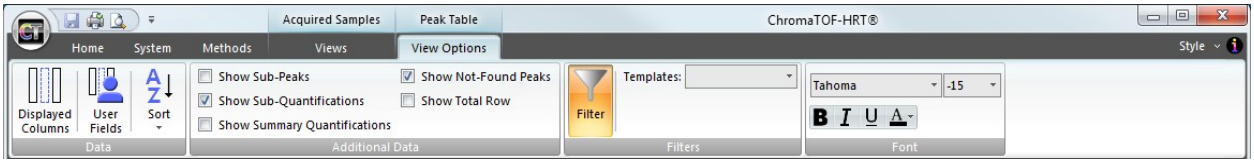


Figure 9*: Spectral Similarity Search*

Post-Processing "Advanced Filters" for Data Review: These are available for filtering peak table results without having to repeat data processing. These are applied through the Filter button, and many peak attributes are available.



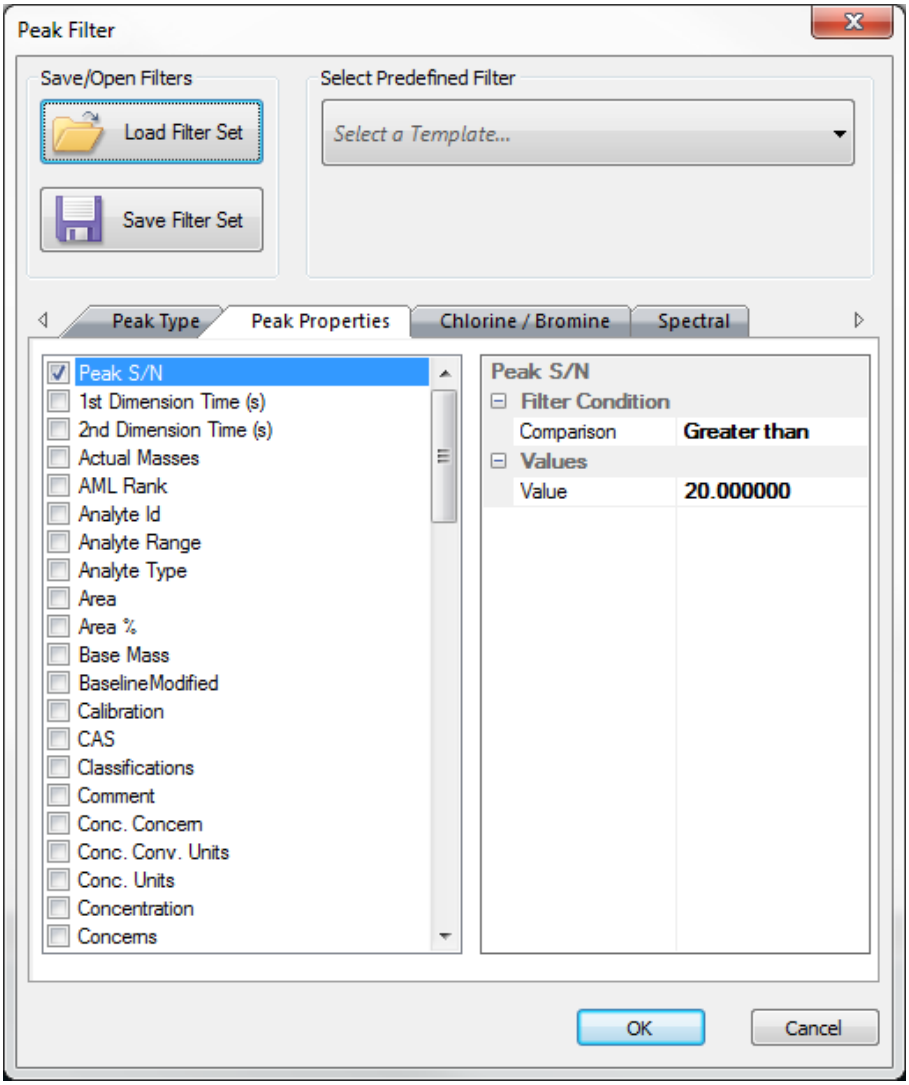


Figure 10*: Filter Button*

Section 3

Expectations, Tips, and Tricks

This section contains examples of typical cases and troubleshooting tips.

Chromatographic Separation and Ion Statistics: *HRD* peak finding uses a statistical approach when assigning peak markers within the data. There is more statistical confidence at high ion levels, so less variation in peak apex and width are permitted. More variation is anticipated and permitted for analytes with lower levels of ions where there is less statistical confidence. This allows for appropriate treatment for chromatographically isolated analytes without splitting a single analyte into multiple peak markers at low ion intensity levels, as depicted in Figure 11.

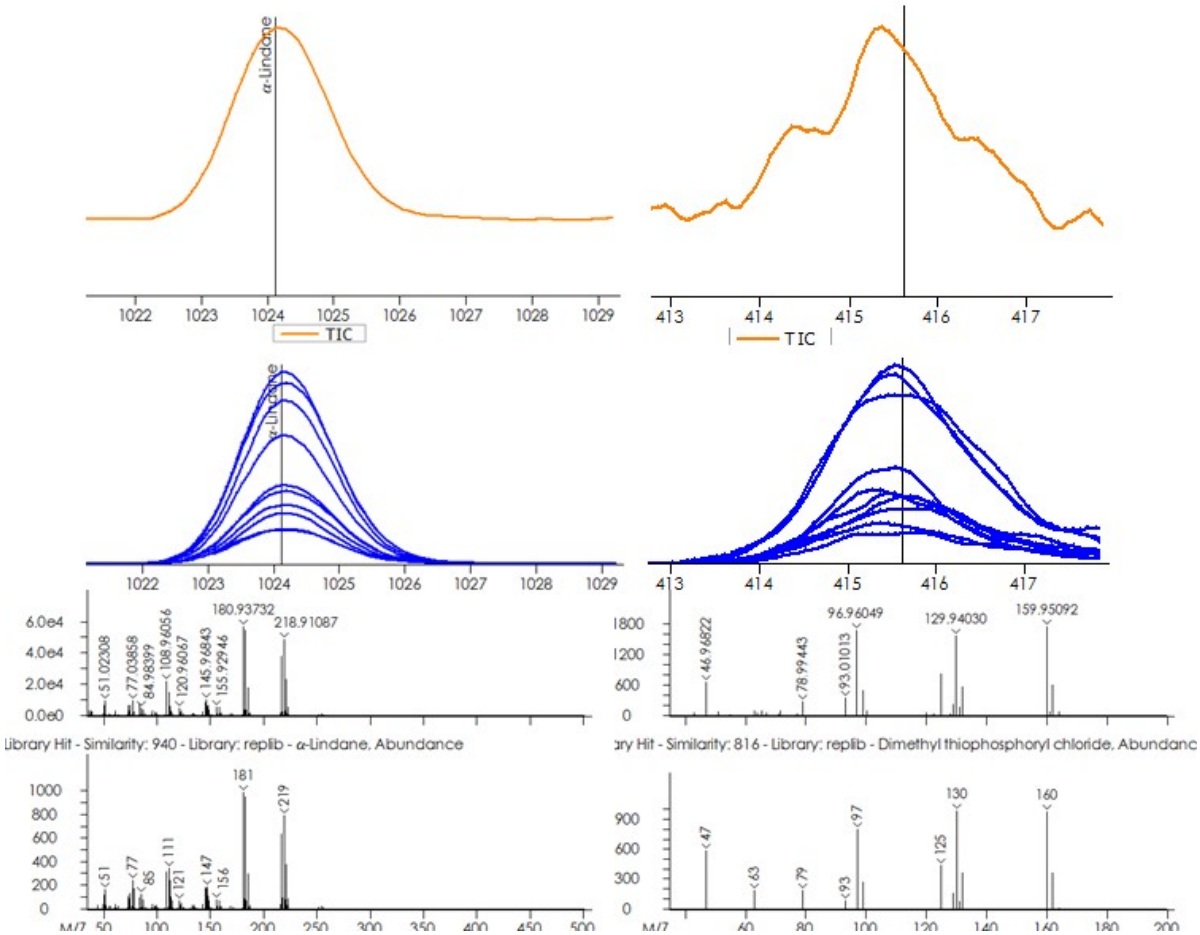


Figure 11*: Example of HRD Results for Both High Intensity and Low Intensity Peaks*

Confident Ion Statistics Allow Separation of Coelutions: When there is enough statistical confidence, coeluting analytes can be deconvoluted into separate features. At higher ion intensity levels less variability in peak apex and width between individual m/z is anticipated, so there is generally enough confidence to mathematically determine that the observed variations belong to multiple analytes and unique peak markers can be expected. An example is shown in Figure 12.

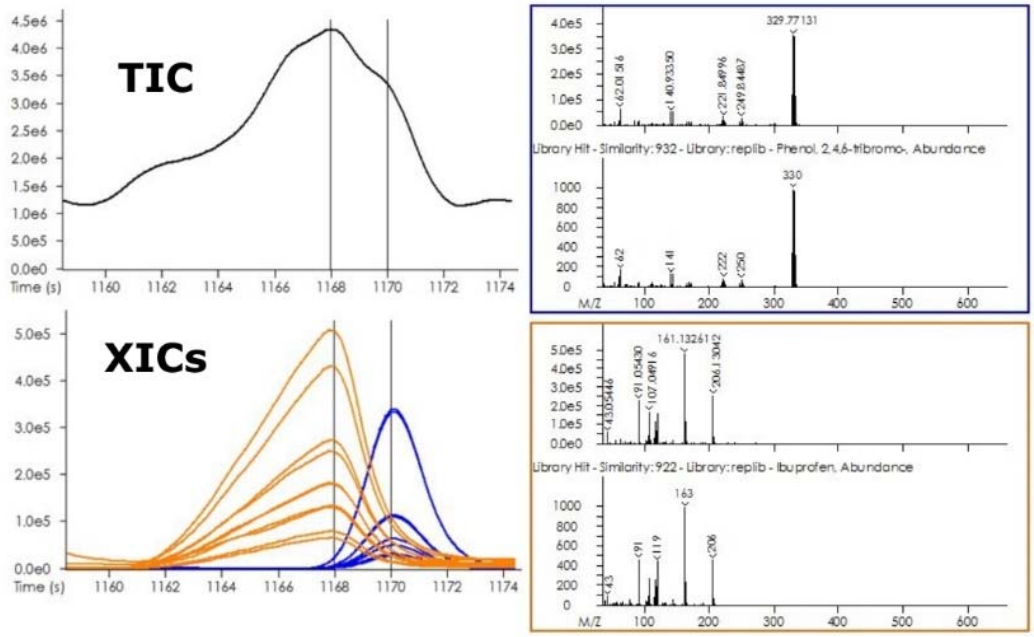


Figure 12*: Confident Separation of Coelution*

Less Confident Ion Statistics Favor Peak Merging (Multiple Features per Peak Marker): Some coelutions may exceed *HRD*'s capabilities. These are situations in complex data where there is coelution and insufficient ions to justify multiple peak markers with statistical confidence. This can occur if the ion intensity levels are low even if the difference in peak apex and width is high, or if the ion intensity levels are high and the difference is low. Mathematics can deconvolute beyond these limits, but the chemical meaning is compromised and masses from the same peak are split into multiple peak markers. To avoid this over-splitting, *HRD* is cautious to deconvolute only with good confidence and favors merging peaks over splitting into multiple peak markers.

An example of merging is shown in Figure 13. By looking at individual XICs, it may seem that there is enough retention time difference to justify unique peak markers. However, when XICs for m/z at lower intensity are added, the distinction is blurred. Because the low level masses cannot be confidently assigned between the separate peak markers, *HRD* merges these to a single Peak True instead of dividing m/z between multiple peak markers. In cases such as these the user must decide for themselves what is truly an analyte of interest and what is not.

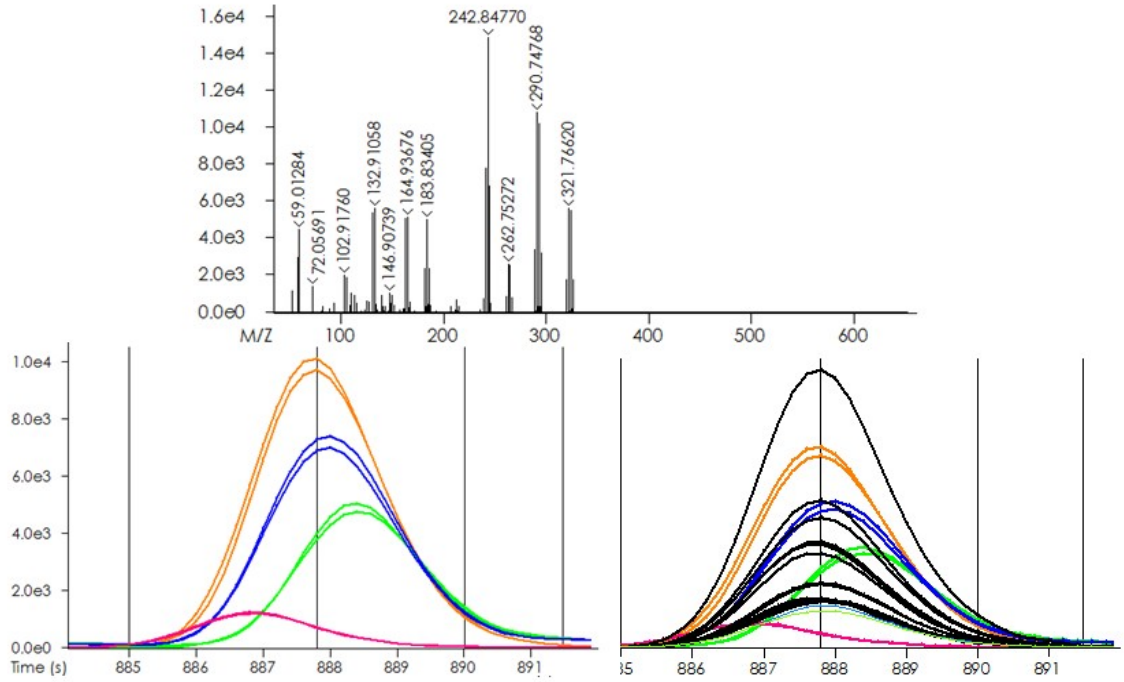


Figure 13*: Example of Lower Confidence of Deconvolution*

In these cases, the following are possible next steps:

- Target Analyte Finding (See Section 4) can be useful for adding peak markers to the data and appropriately assigning m/z to the correct Peak True.
- Reinjection of sample with different chromatography (improved separation).
- Manual data interrogation.

Over-splitting (multiple peak markers for one feature) is uncommon, but if observed can be improved by increasing the Peak Confidence value in the Data Processing method.

Peak Width (FWHH): defaults to the peak width specified in the MS method.

#	1
Start*	Start of Run
End	End of Run
Peak Find	On
S/N	10.0
Peak Quality	0.700
Peak Confidence	1.5
Peak Width (s)	Default

Figure 14*: Peak Confidence Value*

Missing Peak Markers are usually related to filters that are applied either in pre-processing or post-processing.

- Post-processing filters that were turned on for a previous sample and not disabled will be applied to other data files as well. If the filter icon is highlighted yellow, filters are enabled and being applied to the data file. This is one possibility for missing peak markers. If the data were initially processed at a lower S/N and Peak Quality, peak finding does not need to be repeated. Turning the filters off and/or adjusting the thresholds may restore the missing peak marker.

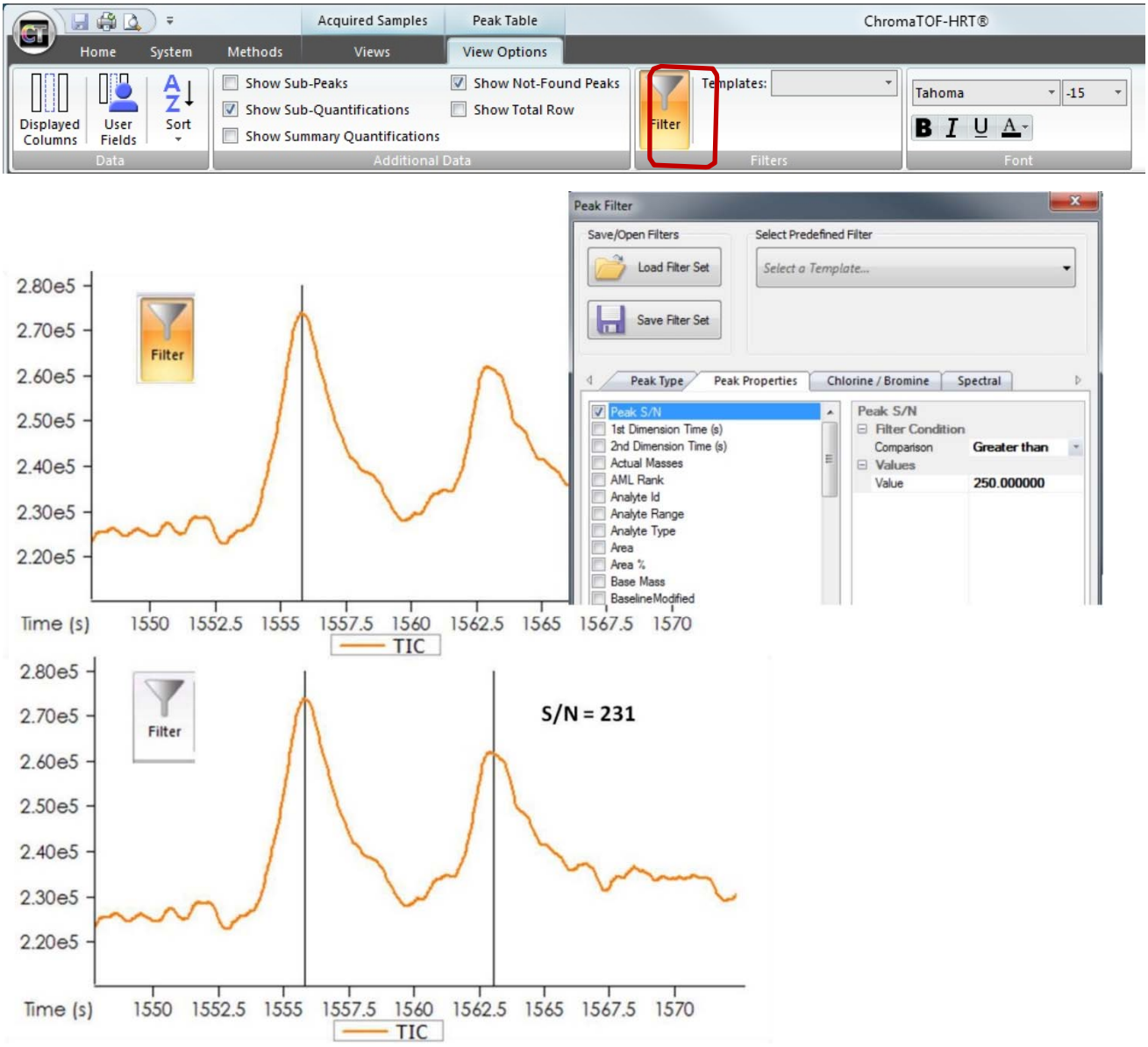


Figure 15*: Highlighted Filter Button Indicating Enabled Filters being Applied to the Data File*

- Pre-processing filters take advantage of high resolution data to remove chemically impossible noise. These are determined by mass defect and rely on accurate mass calibration (See Section 2). If data are not properly mass calibrated, it is possible to shift chemically relevant information into chemically impossible mass defect space. These data are not lost, however, mass calibration (with Mass Accuracy RMS <1 ppm) and peak finding must be repeated to correct for this.

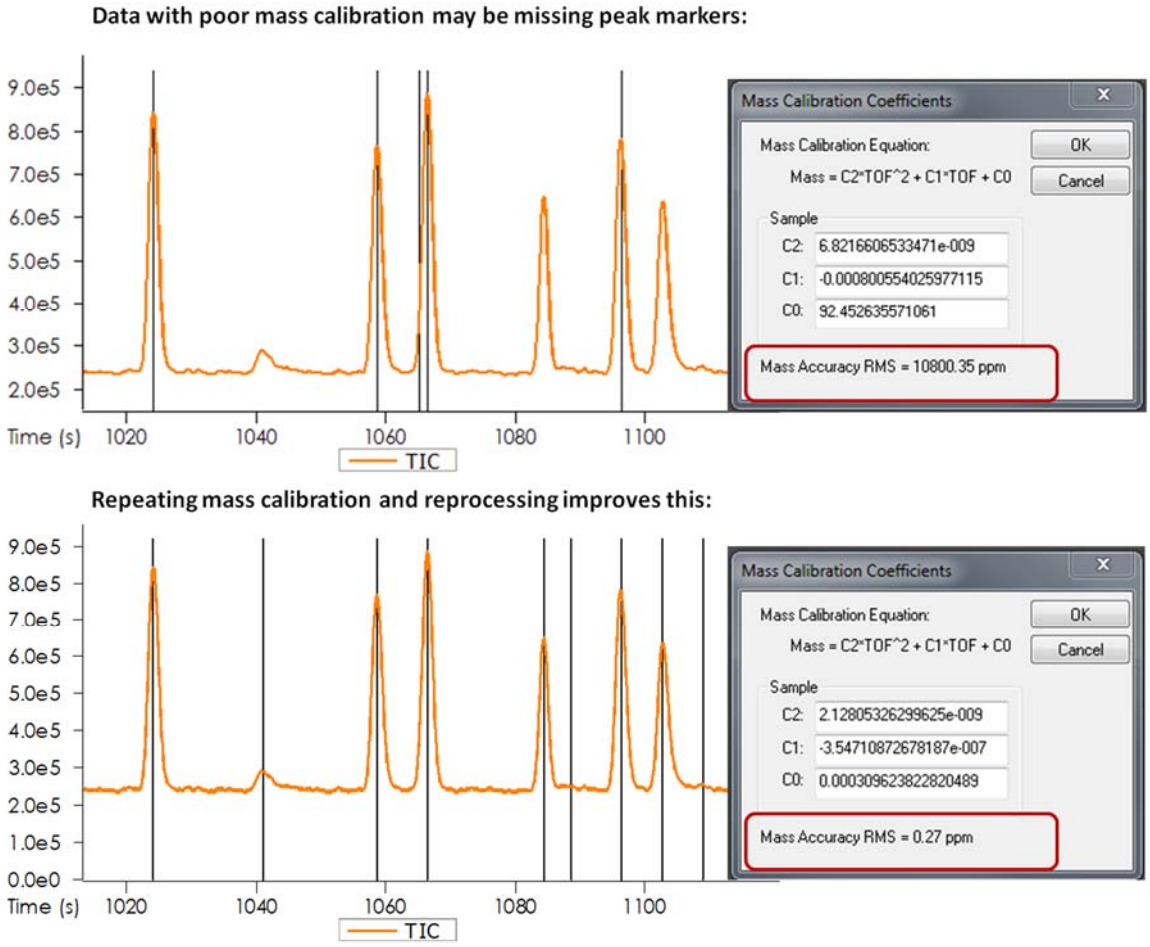


Figure 16*: Repeating Mass Calibration and Peak Finding to Improve Mass Accuracy*

Missing Masses in the Peak True Spectrum. Occasionally, masses may be observed in the raw caliper spectra that are missing from the Peak True spectrum. These can sometimes be included in the Peak True with the following adjustments.

- If missing masses are at very low levels, there may not be enough ion statistics to confidently distinguish these masses from noise. To check this scenario, plot the XICs for the missing masses. If there is no discernable peak shape, *HRD* is not likely to include these in the Peak True spectrum. Target Analyte Find can be used in these cases to assign the masses to the Peak True spectrum.

- If missing masses are at very low levels and are potential isotopes, the isotope table in the data processing method can be used to increase the tolerance and allow these masses in the Peak True spectrum even if there is less confidence. The Data Processing method can be adjusted to include the missing species and/or the tolerances in the isotope table can be increased (circled in red, following), and the data can be reprocessed.

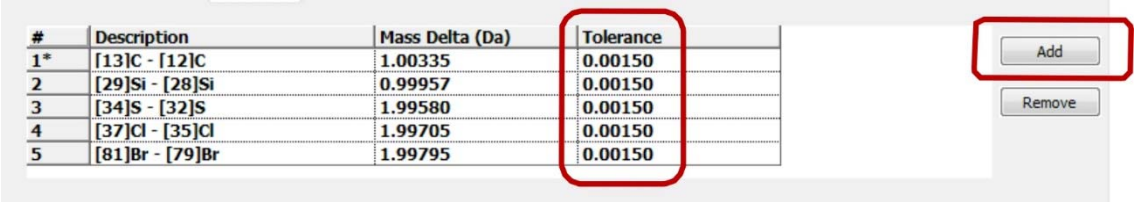


Figure 17*: Adjusting the Data Processing Method*

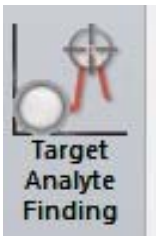
- If missing masses are intense and have clear chromatographic profiles, first check for neighboring peak markers to see if the masses were assigned to another Peak True. If the missing masses are present in one of these peak markers, Peak Confidence in the data processing method can be increased and the data reprocessed to merge the peak markers. If there are no neighboring peak markers and no filters applied, the mass calibration should be checked. If the mass calibration is off, masses may have been calibrated into chemically impossible mass defect space and filtered during pre-processing (See Section 2). In this case, mass calibration and peak finding should be repeated.

Too Many Peak Markers. If there are many peak markers in the Peak Table that do not appear to be real and are representing noise, the processing or post-processing filters for S/N and/or peak quality can be increased. These can be applied as post-processing filters through the filter tab without requiring re-processing (See Section 2). If applied in this way, the filters will need to be adjusted to the desired level each time the file is reviewed (a template can be saved for rapid application). Alternatively, the data file can be re-processed with the higher thresholds for permanent peak table adjustments.

Section 4
Advanced Data Processing Options

There are several advanced data processing options that can be employed that may be useful for specific processing needs.

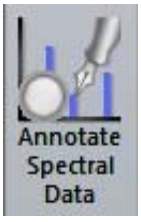
The Use of **TOF Correction** is not common practice. It is similar to automatic drift correction (done during acquisition). If drift correction during acquisition was not sufficient, TOF correction can be used after acquisition. TOF correction only uses the masses in the table and can be done with column bleed or other persistent masses. This should only be utilized as a "last resort" for mass calibration problems.



Target Analyte Find is an alternate or additional peak finding approach that can be used when the analyst knows what they are looking for. Only analytes on the target list will be searched and only the masses specified on the target list per analyte will appear in the Peak True spectrum. This is also useful for getting peak markers for coelutions that exceed *HRD* capabilities or for including low level masses into a peak marker that have insufficient ion statistics for basic peak finding. The E-TAF feature will merge signals from a reported Peak True spectrum into a Target Analyte spectrum if both peak markers are close enough and the Peak True spectrum contains all the Required Masses for the Target Analyte. The purpose of this feature is to create a richer spectrum for the Target Analyte and to eliminate duplicate peak markers for the same peak, where one peak marker comes from *HRD* deconvolution, and the other peak marker comes from Target Anlayte Finding.

Retention Index methods can be enabled and incorporated into Data Processing.

Quantification methods, including **Calibration, Reference, and Semi-Quantification**, can be enabled and incorporated to Data Processing. **Calibration** computes the absolute concentration based on a calibration. **Reference** computes the relative concentration of peaks with respect to a reference. And, **Semi-Quantification** computes concentration based on another analyte's calibration curve.



Annotate Spectral Data adds labels to spectra based on adducts expected (for example NH_4^+ with Chemical ionization).

Additional **Library Search Options** are available beyond the Spectral Similarity Search that is routinely performed. These can be selected by moving to other tabs in this window and entering the associated parameters. The following library search options are available: **Accurate Mass Library**, **Spectral Similarity**, **Formula**, and **Reverse Target**.

Peak Data ASCII Export Options are available to automatically copy processed Peak Tables to .csv files.

Section 5
GC×GC Data

All of the information provided in Sections 1–4 applies to GC×GC data processing as well. The linear chromatogram of GC×GC data is first processed the same as one-dimensional GC data—that is, the peaks are found and deconvoluted. The resulting peaks and peak true spectra are then post- processed to combine the modulated peaks (slices) of each analyte into a single analyte result as reported in the peak table and shown in the chromatogram and 3D view. This is accomplished utilizing first- and second-dimension retention times and mass spectral comparisons.

GC×GC subpeak combining is added to the DP method by checking the GC×GC box on the Peak Finding page (Figure 18). This will add the GC×GC Subpeak Combining Page as shown in Figure 19. The retention time matching of subpeaks (slices) has no user parameters in the DP method. This is handled internally in the software. For the mass spectral match of subpeaks there is a "Spectral Match Required to Combine" parameter. This specifies the minimum spectral match (same NIST algorithm as used in library spectral matching) between the subpeak and the base subpeak (largest subpeak) that is required to combine the subpeaks. The default value is 500. A value of less than 500 is not recommended. However, a larger value may be appropriate if subpeaks of different analytes with similar spectra are being combined.

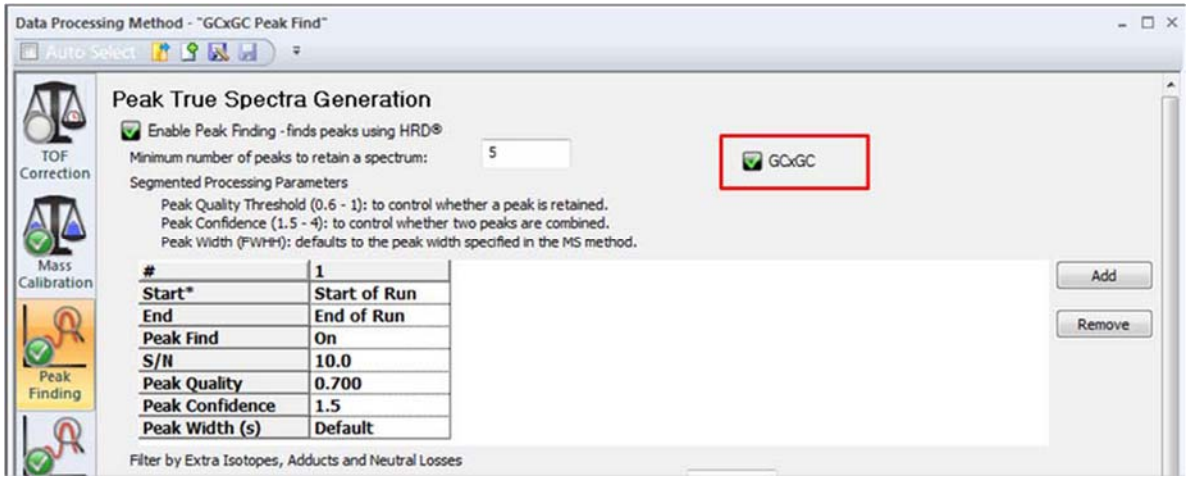


Figure 18*: Adjusting the Data Processing Method*

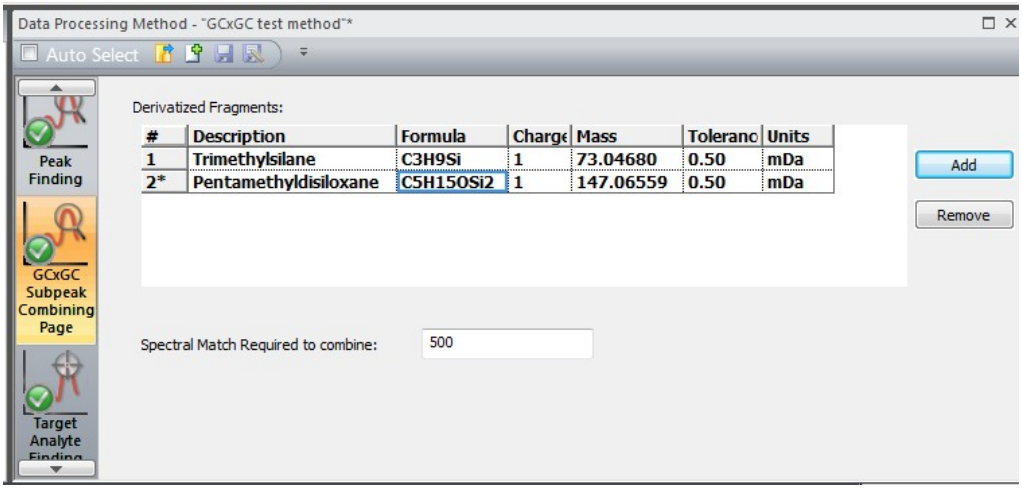


Figure 19*: GCxGC Subpeak Combining Page*

The Derivatized Fragments section in the GC×GC Subpeak Combining Page (as shown in Figure 19) can be used when a sample is derivatized and the resulting derivative products have prominent, common mass fragments due to the derivatizing reagent used. The prominent, common mass fragments can adversely affect the combining of subpeaks. By specifying these prominent, common mass fragments the subpeak combining can be improved. The following description explains conceptually how the addition of the common masses of the derivative products to the data processing method improves the results.

For GC×GC samples, after deconvolution, all subpeaks (modulations, slices) of a particular analyte are found and grouped together based on their retention times and spectra. The NIST spectral comparison function is used to compare the spectra of the modulated peaks. In the NIST algorithm, the more intense masses will affect the match result more than the less intense masses. Because the common masses of the derivatives are shared among all the derivatization products and they are usually the most intense masses, they will cause two different derivatization products to have a very good spectral match and be grouped into one peak when they should not be grouped together. For this reason, another spectral comparison is performed with the common masses of the derivatives excluded. Both match values of these comparisons must pass a threshold in order to provide confidence that the two subpeaks are really from the same compound.

For individual peaks, the issues and possible solutions described above for one-dimensional GC peaks also apply to GC×GC data. For peak combine issues, examine the Peak True spectra. If too many subpeaks are being combined, consider using a higher mass spectral match. If a derivatization has been done, examine the spectra for prominent, common mass fragments due to the derivatization reagent. If there are such fragments, consider putting them into the Derivatized Fragments table. In the rare cases where adjustments to the automatic data processing do not combine the subpeaks correctly, the manual describes how to manually combine and un-combine subpeaks.