Basic Instructions for Using AMDIS with MS Search

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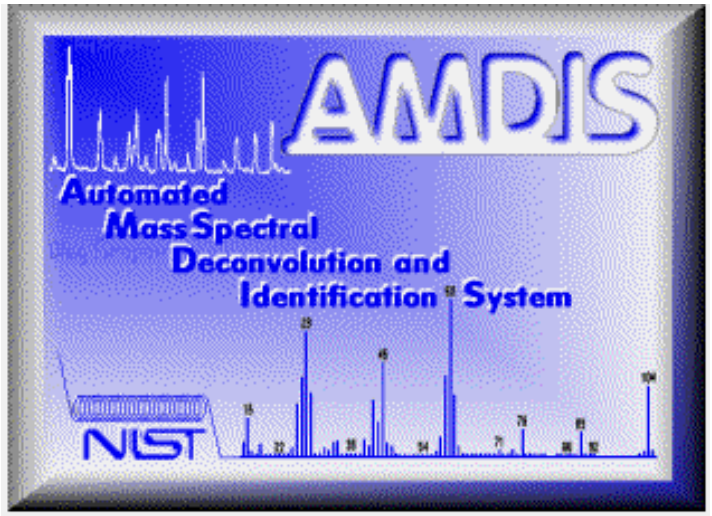
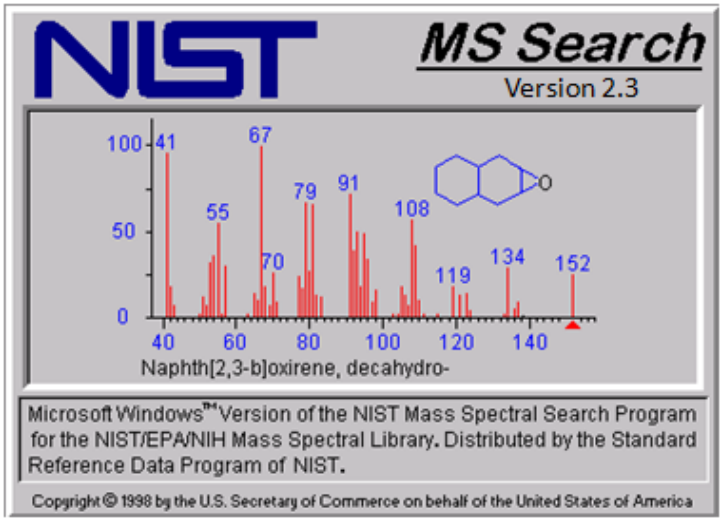
Input from Gary Mallard
11/24/2019

See AMDIS Manual for Detailed Instructions





What is AMDIS?

Automated **M**ass spectral **D**econvolution and **I**dentification **S**ystem

Developed to automatically detect chemicals in violation of Chemical Weapons Convention

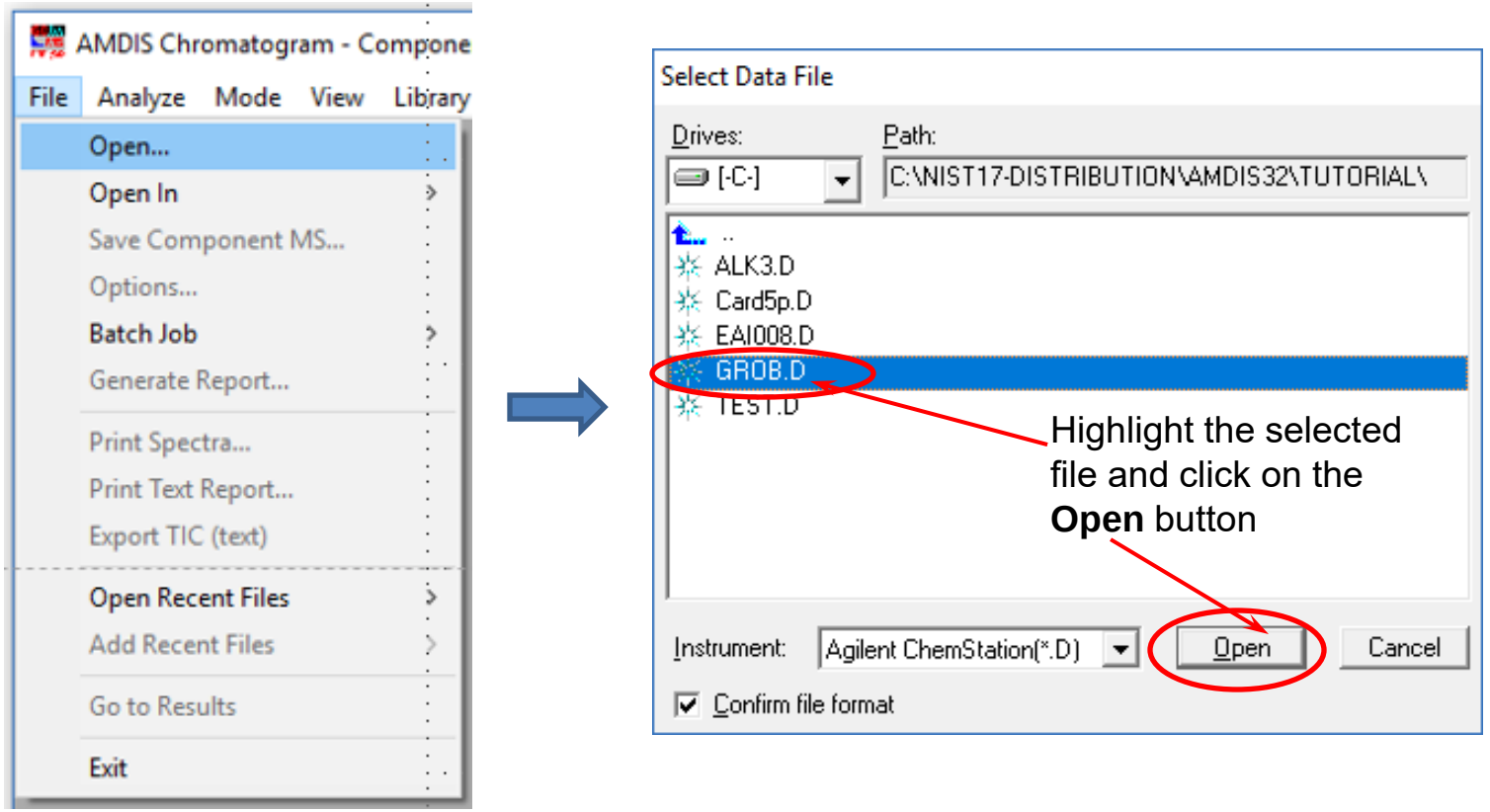
- Software to automatically separate (deconvolute) chemical background in GC/MS data from signal for sample components
- Background corrected spectra can be ***sent*** to the NIST Mass Spectral Search Program for identification
- Spectra can also be searched automatically ***within*** AMDIS to give results yielding names, but not structures
- Software can be used to automatically find targeted species in complex mixtures
- Deconvolution can be manually controlled, if necessary
- Software can be used to compare “Good” and “Bad” samples analyzed by EI GC/MS and differences categorized

Some Terminology

- AMDIS extracts the mass spectra of individual **Components** from chromatograms obtained by the repetitive spectral acquisition of a mass spectrometer during a gas chromatographic elution. These are symbolize with a ▼ on top of the chromatogram at the point of elution
- When AMDIS extracts the spectrum, that spectrum is automatically search against an integral AMDIS **Target Compound Library** (an MSL file). If a component is identified, a **T** is place above the ▼ to show that the component has been identified.
- In GC/MS, a single chromatographic peak can be one of several different **Components** of a mixture. AMDIS identifies the pure **Components** from their mass spectra.
- **RMB** and **LMB** denote right and left mouse button, respectively

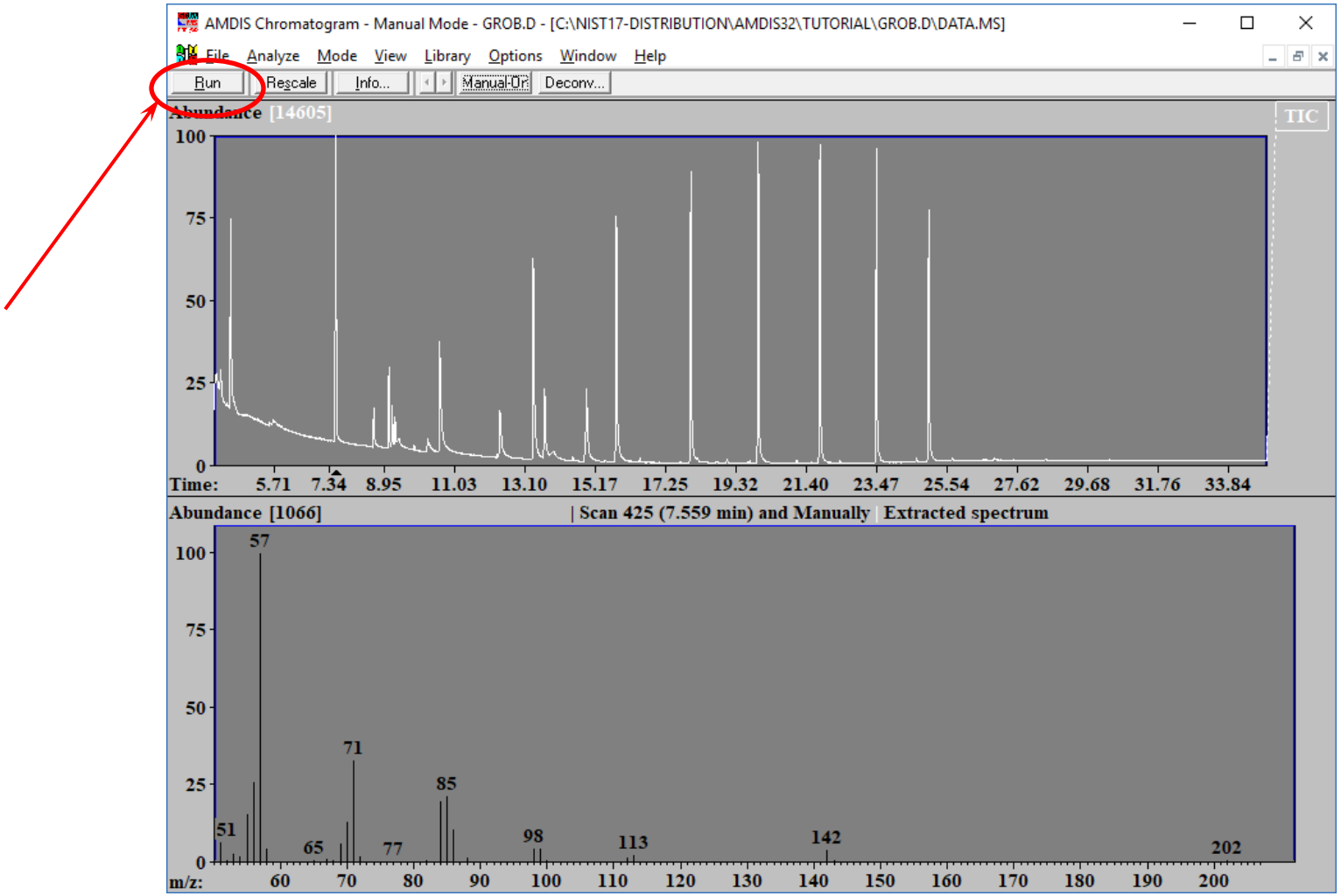
Opening File with AMDIS

- Can process many different file types with AMDIS including Agilent, netCDF, etc.
- ***Before*** sending components to library search, must open and run the file to get background corrected spectra



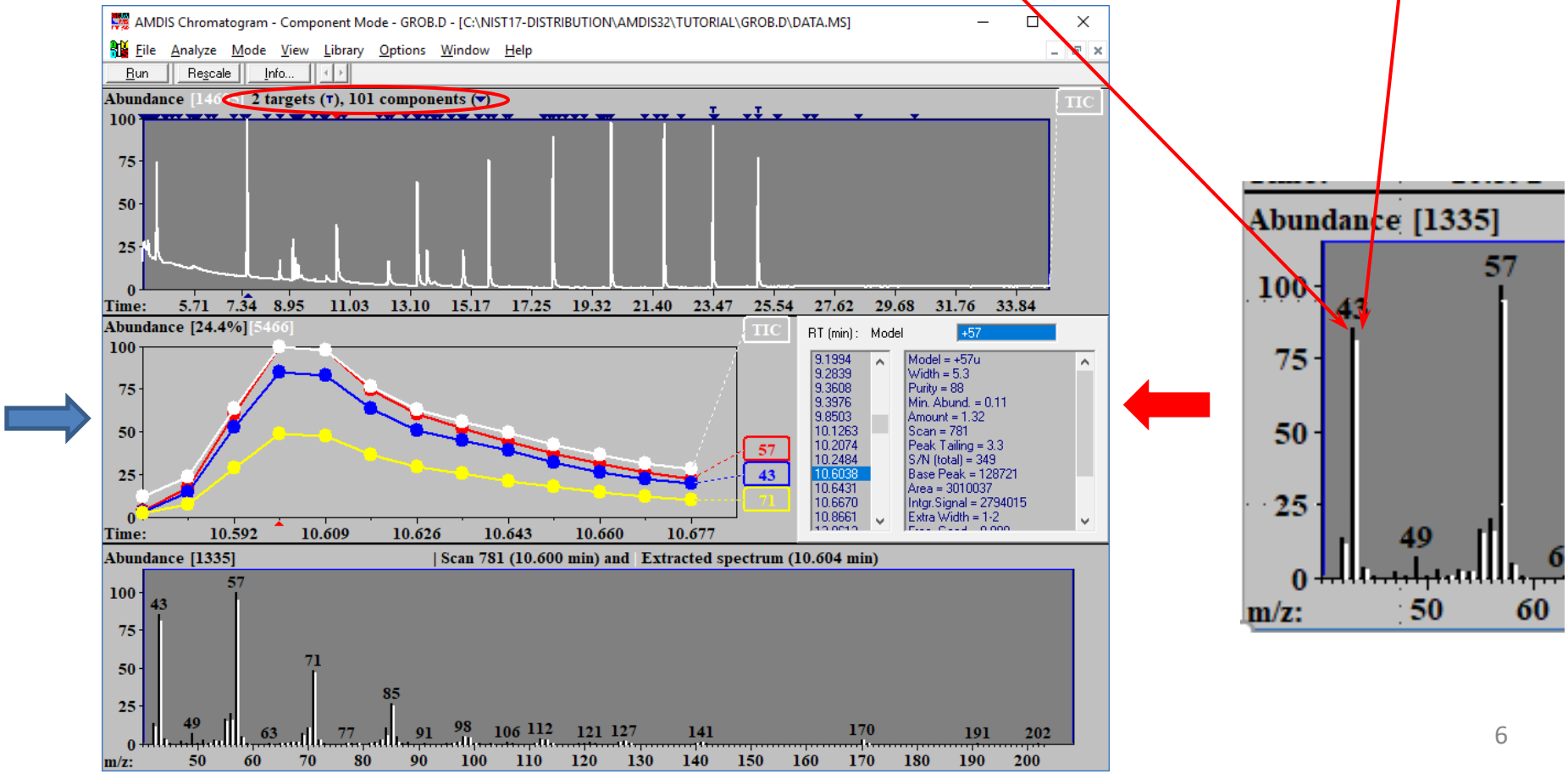
Deconvoluting Spectra

- First click the **LMB** with the **Pointer** on the **Run** button to deconvolute the file and search each spectrum against the selected **Target Compounds Library** (**Analyze\Settings\Lib**)
- The computer plots a chromatogram from every *m/z* value in the data file
- Then “looks” at the stacked plots to determine which ions “belong” with each other and subtracts out ions from air, column bleed, other nearby Components, etc.



Evaluating Deconvoluted Results

1. Note the number of **Components** found (101)
2. Note the little blue upside-down triangles, left click on any one to see deconvoluted spectrum
3. After selecting one blue triangle, can step through by using up or down arrows ***on your keyboard***
4. The left middle window shows what ions were “modeled” to define your spectrum
5. The right middle window show you the associated parameters for each peak
6. The bottom window shows the unsubtracted spectrum in black and the deconvoluted in white

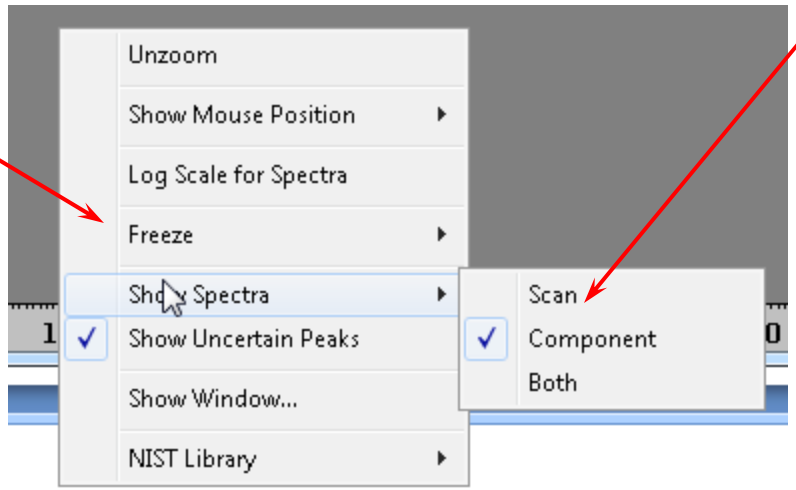


Evaluating Deconvoluted Results (continued)

- Can just show the **Component** (white peaks), the Scan (black peaks), or Both, but best to get accustomed to looking at both
- When the black matches the white, you probably have a good spectrum of a major **Component**
- For minor **Components**, possibly coeluting with a major **Component**, the white will be different than black and in many cases smaller
- With default “deconvolution parameters”, AMDIS will sometimes ID too many components
- The “deconvolution parameters” need to be adjusted to minimize this
- Very dependent on having a good stable signal from the instrument, but in my experience, just tends to do that without using the appropriate filters for processing (*more on that later*).

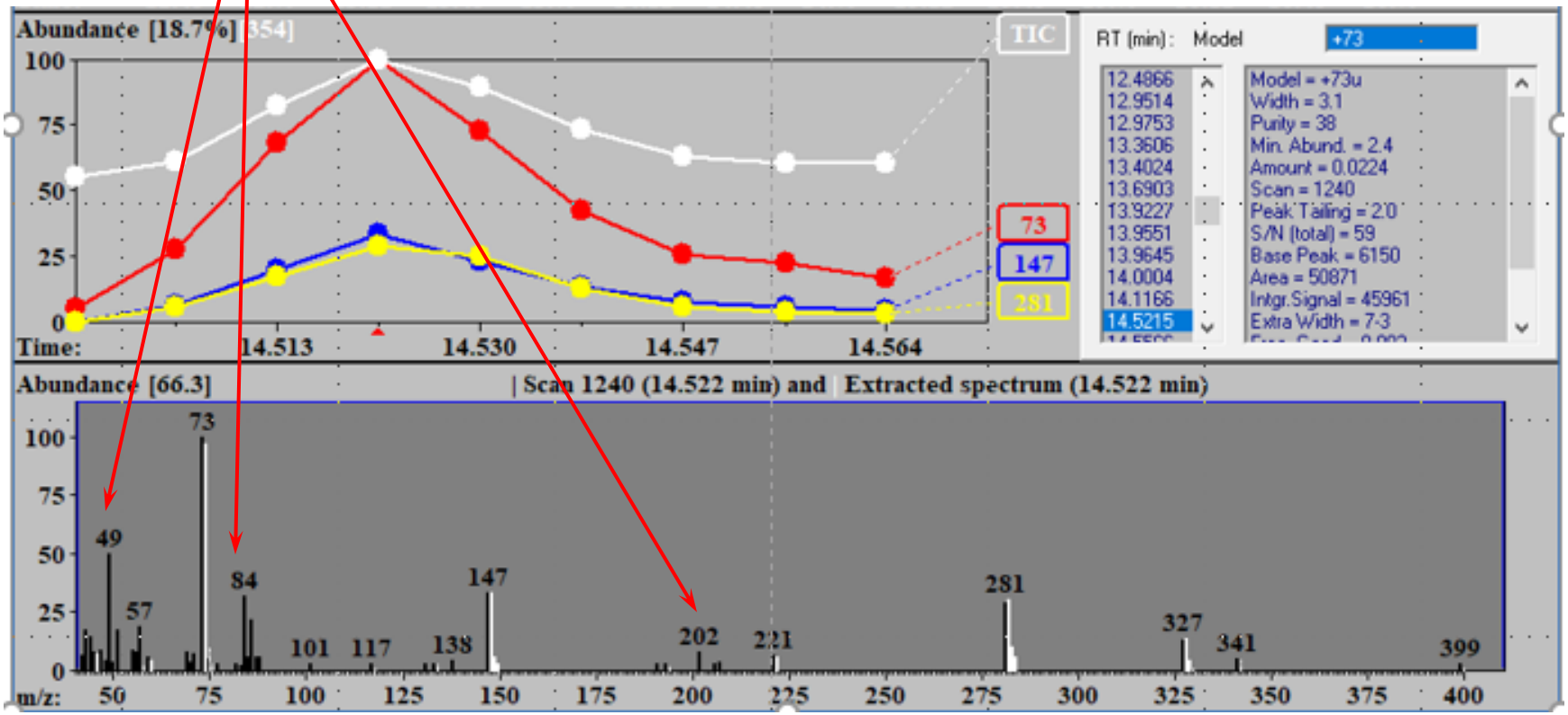
RMB Menu displayed by placing the **Pointer** on the Spectrum window and clicking the **RMB**

Which peaks will be displayed in the Spectrum window.



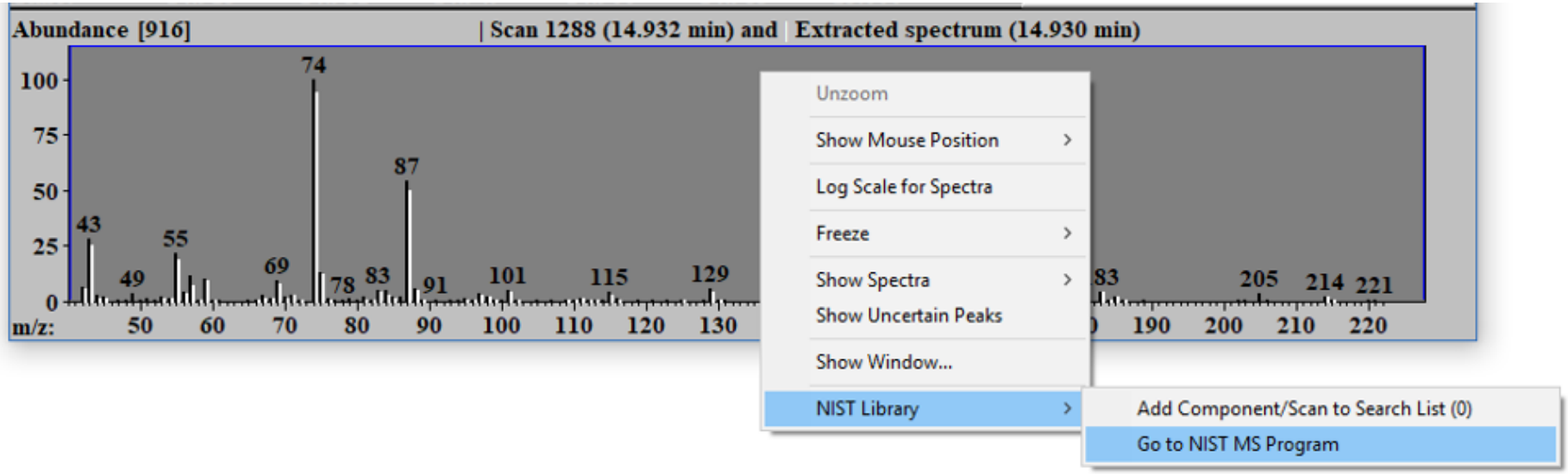
Evaluating Deconvoluted Results (continued)

- Note black (uncorrected peak with background)
- White is spectrum corrected for back ground and all non tracking ions removed



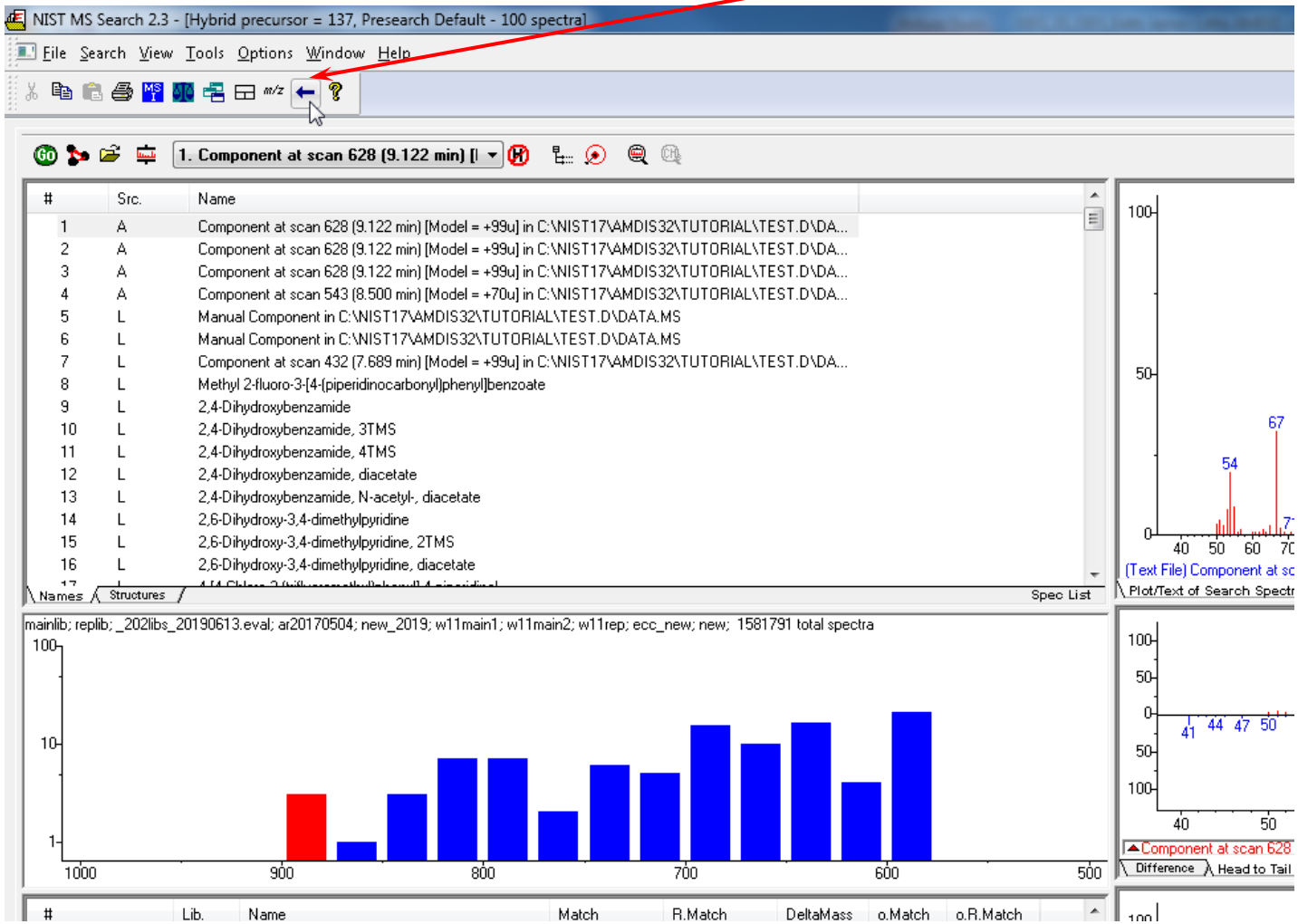
Sending Deconvoluted Spectra to NIST Search Program

- To send an individual mass spectrum to the NIST MS Search Program, click the **RMB** with the **Pointer** on the spectrum to display the **RMB** menu
- Select **Go to NIST MS Program**
- The spectrum will be sent to the NIST MS Search Program, if the Program is active; and, if not active, it will be started and the spectrum then sent
- If **Automation** is checked in the **Library Search Option's Search** tab, the search will occur automatically and the results will be displayed in the MS Search Program



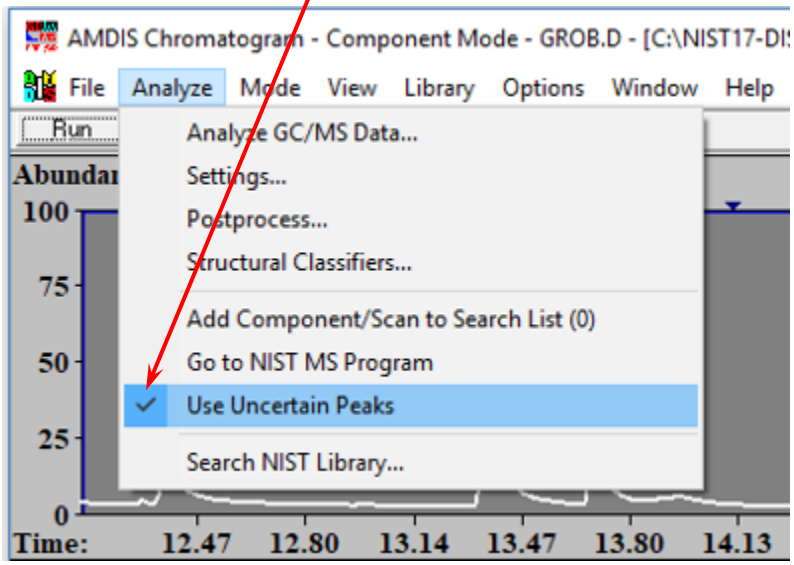
Returning to AMDIS Window after NIST Search

- After NIST search, return to AMDIS window by putting the **Pointer** on “Switch to Caller” button and click the **LMB.**

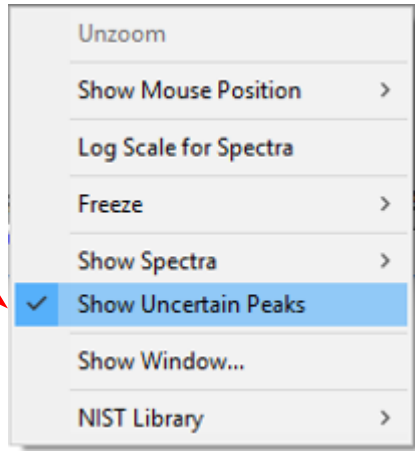


Uncertain Peaks, Dashed Lines, in Deconvoluted Spectrum

- Sometimes the AMDIS “decides” that some peaks “might” be associated with the deconvoluted spectrum, but it is not sure; you will need to change the basic settings if you want to use them
- These “uncertain peaks” are shown as dashed white lines in the spectrum
- To use them and send them for library searching, the Analyze settings have to be changed
- First, click the **RMB** with the **Pointer** on the spectrum to cause the display of the **RMB** menu and select **Show Uncertain Peaks**. Once selected, this will remain until changed.
- Then go to top of the **Analyze** menu, displayed from the Main Menu, and select **Use Uncertain Peaks**



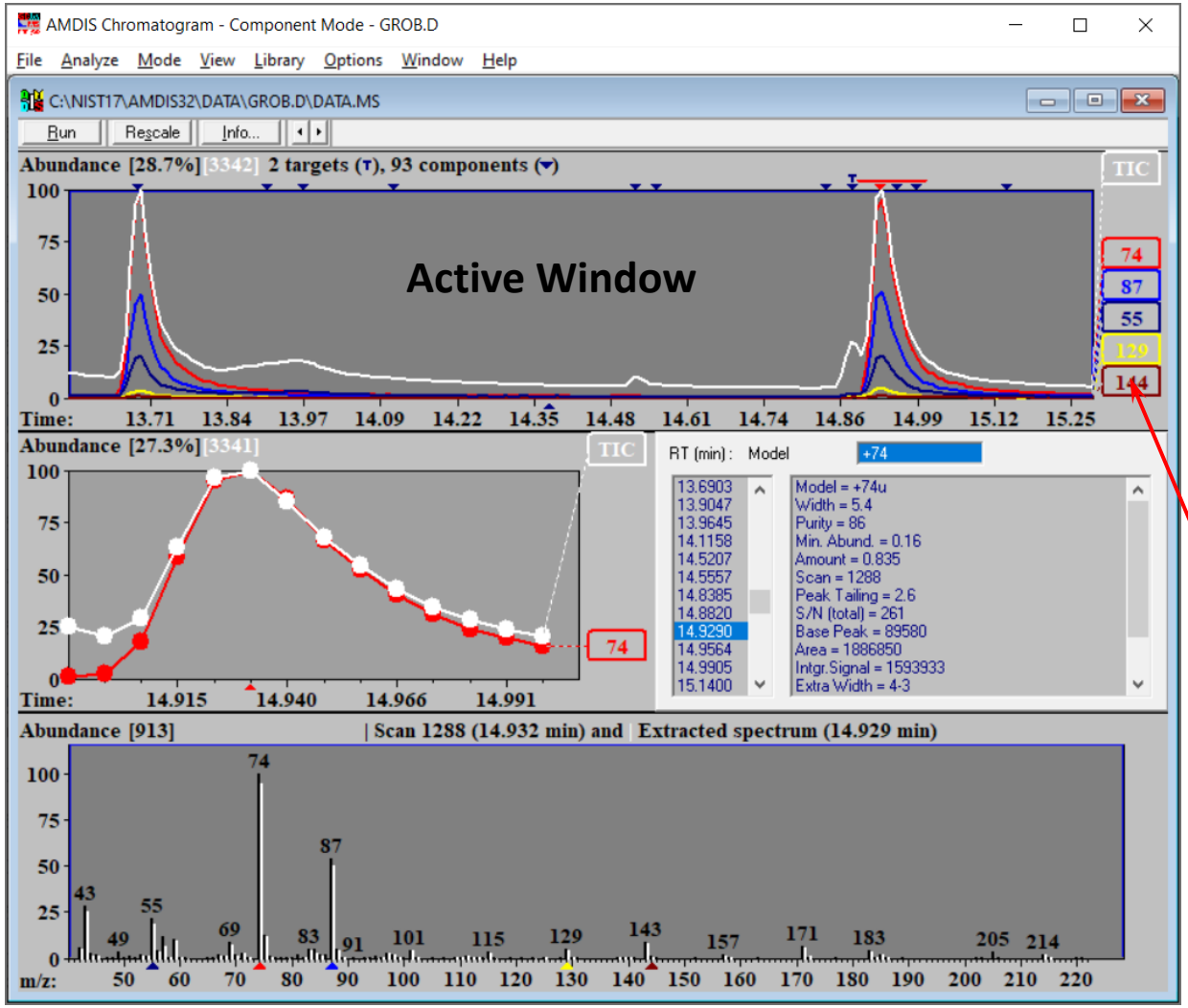
Analyze Menu



Right Mouse-button Menu with Pointer on Spectrum

Plotting Single (or Extracted) Ion or (Mass) Chromatograms

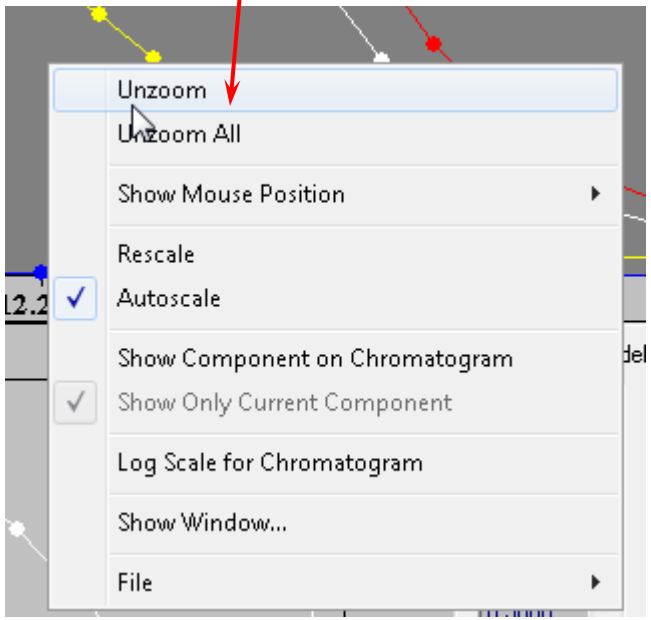
- To plot ion current *vs* time (i.e., a mass chromatogram), just click the **LMB** with the **Pointer** on the peak representing the ion in the spectrum window, and the mass chromatogram will immediately be displayed in a different color in the active window. The intensity of the peak produced by the 1st selected ion is set to be 100%. If a subsequent ion is more abundant than that 1st selected ion, its plot will be off scale

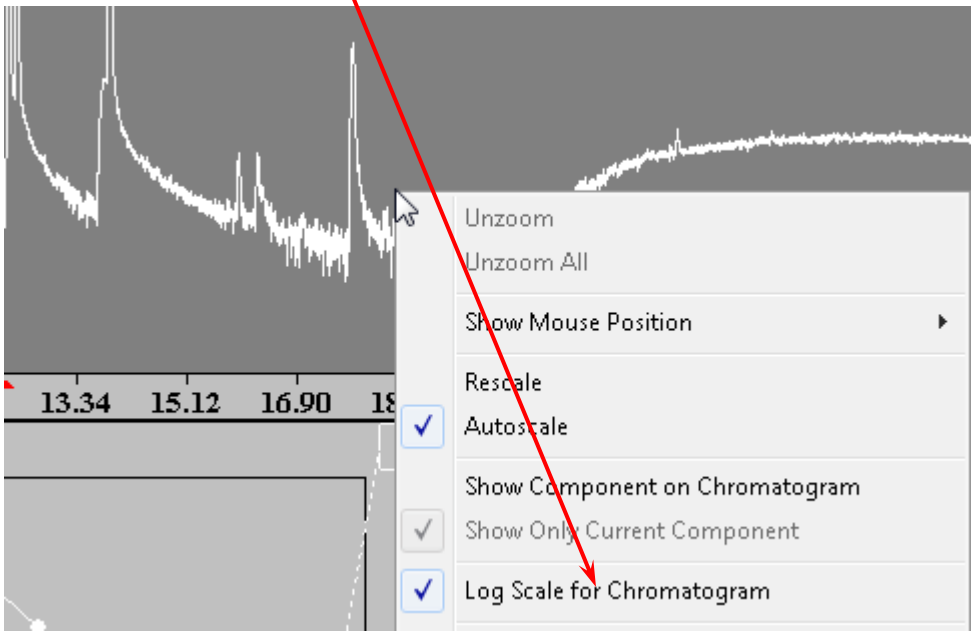


- Either the chromatogram (top) window of the model (middle left) window can be the active window
- To select the active window, put the **Pointer** on the bar above the window and click the **LMB**.
- The active window is dark gray
- To delete that mass chromatogram, just click the **LMB** with the **Pointer** on its box to the right of the top chromatogram

Expanding Chromatograms or Plotting in Log Scale to See Small Peaks

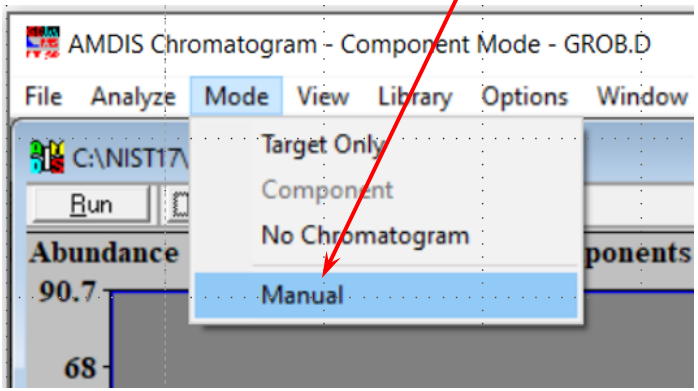
- To expand the chromatogram or spectrum, just hold down the **LMB** and drag (Drag-n-Drop)
- To unzoom, right click in the window and select **Unzoom** or **Unzoom All** from **RMB** menu
- Another way to see small peaks is to put Mouse-pointer on the chromatogram (or spectrum) window, click the **RMB**, and select **Log Scale for Chromatogram** or **Log Scale for Spectra** from the **RMB** menu

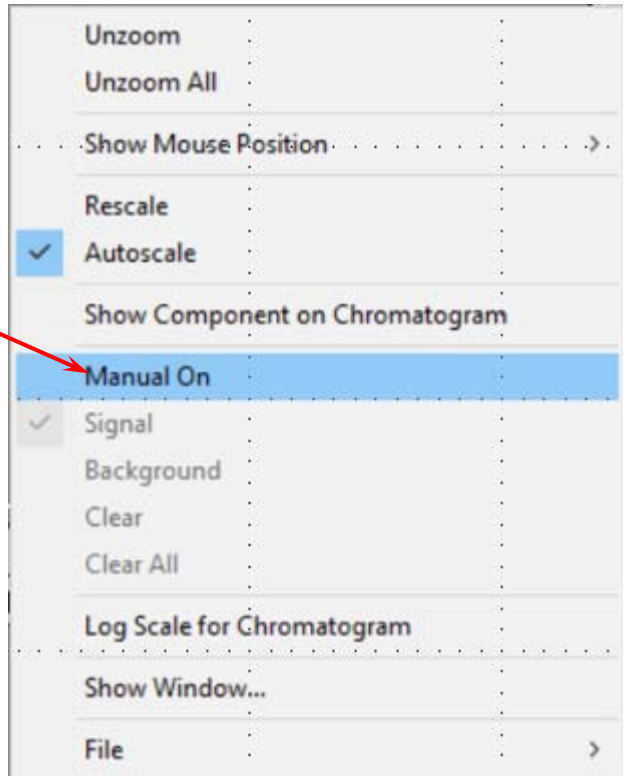




Manually Processing File in AMDIS

- If you just want an nondeconvoluted (uncorrected) spectrum of the background, click the **LMB** with the **Pointer** on the scan of interest, it can then be sent to the MS Search Program and searched against the NIST and/or other libraries
- AMDIS can produce a manual background-subtracted spectrum, typical of other MS software
- Often helpful for broad or peaks with excessive tailing
- First, go to top bar and select **Manual** from the **Mode** menu on the Main Menu bar
- Second, display the **RMB** menu and select **Manual On**

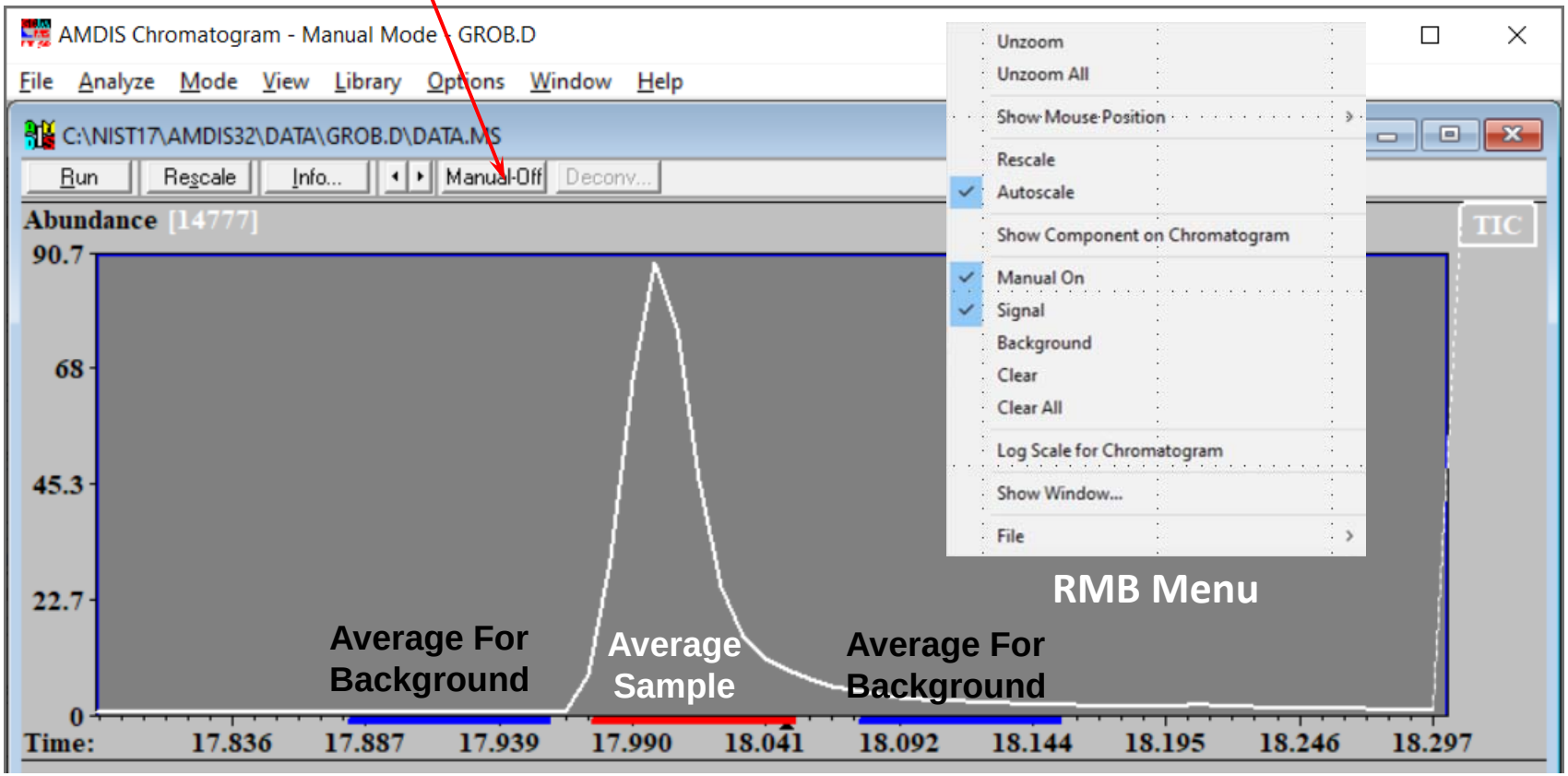




RMB Menu displayed by putting **Pointer** on Chromatogram window and clicking the **RMB**

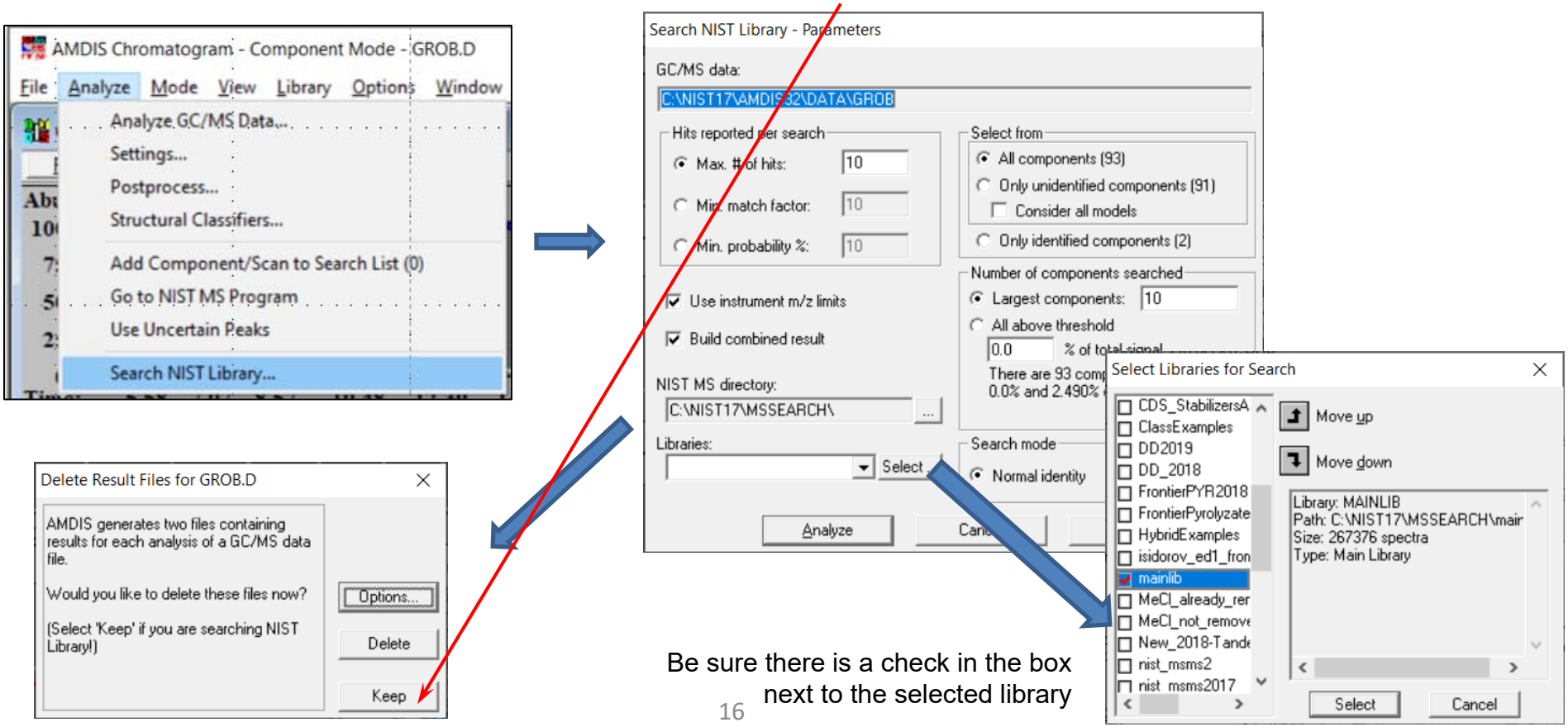
Manually Processing File in AMDIS (continued)

- From **RMB** menu displayed with **Pointe**r on the chromatogram window select (***one at a time***) in a sequence, **Signal** (one or more ranges to average) and **background** (one or more ranges)
- The manually background spectrum is shown in the spectrum window, (bottom of the two displayed windows; the model window (middle), used in deconvolution, is no longer present
- The chromatogram window can be unzoomed using the **RMB** menu; but, to zoom requires **LMB** clicking on the **Manual Off** button above the chromatogram turning it to **Manual On**
- The spectrum obtained can be sent to MS Search using the **RMB** menu



Automated Searching of Deconvoluted Spectra within AMDIS

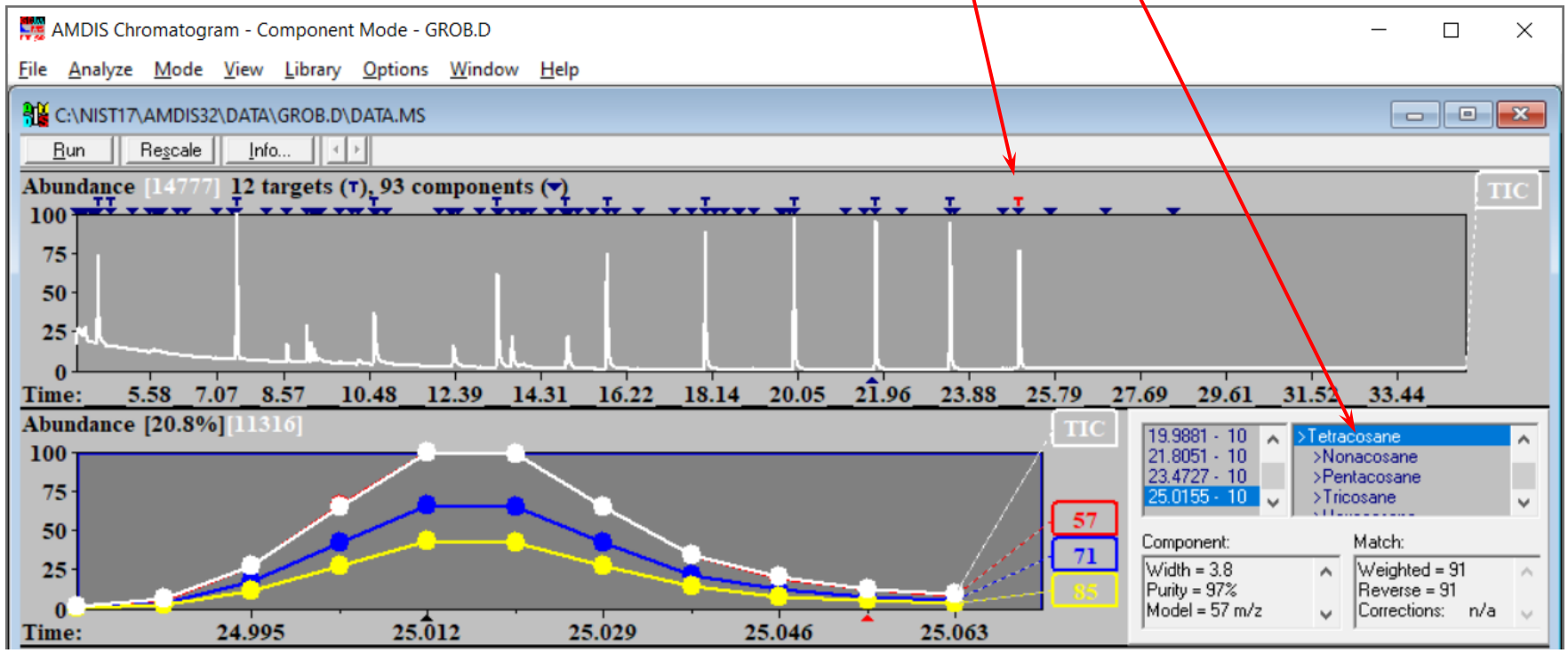
- Searching Mass Spec Libraries with Results shown in AMDIS (names only, ***no*** structures!)
- Select **Search NIST Library…** from the **Analyze** menu on the Main Menu bar to send spectra to MS Search automatically
- Can select more than one library by clicking with the Left Mouse-button on the **Select** button in the **Search NIST Library – Parameters** dialog box (**Select Libraries for Search** dialog box)
- Set parameters to limit the search (use **Help** button if necessary)
- Left click on **Analyze** button and be sure to select ***Keep*** in **Delete Results File** dialog box



Be sure there is a check in the box next to the selected library

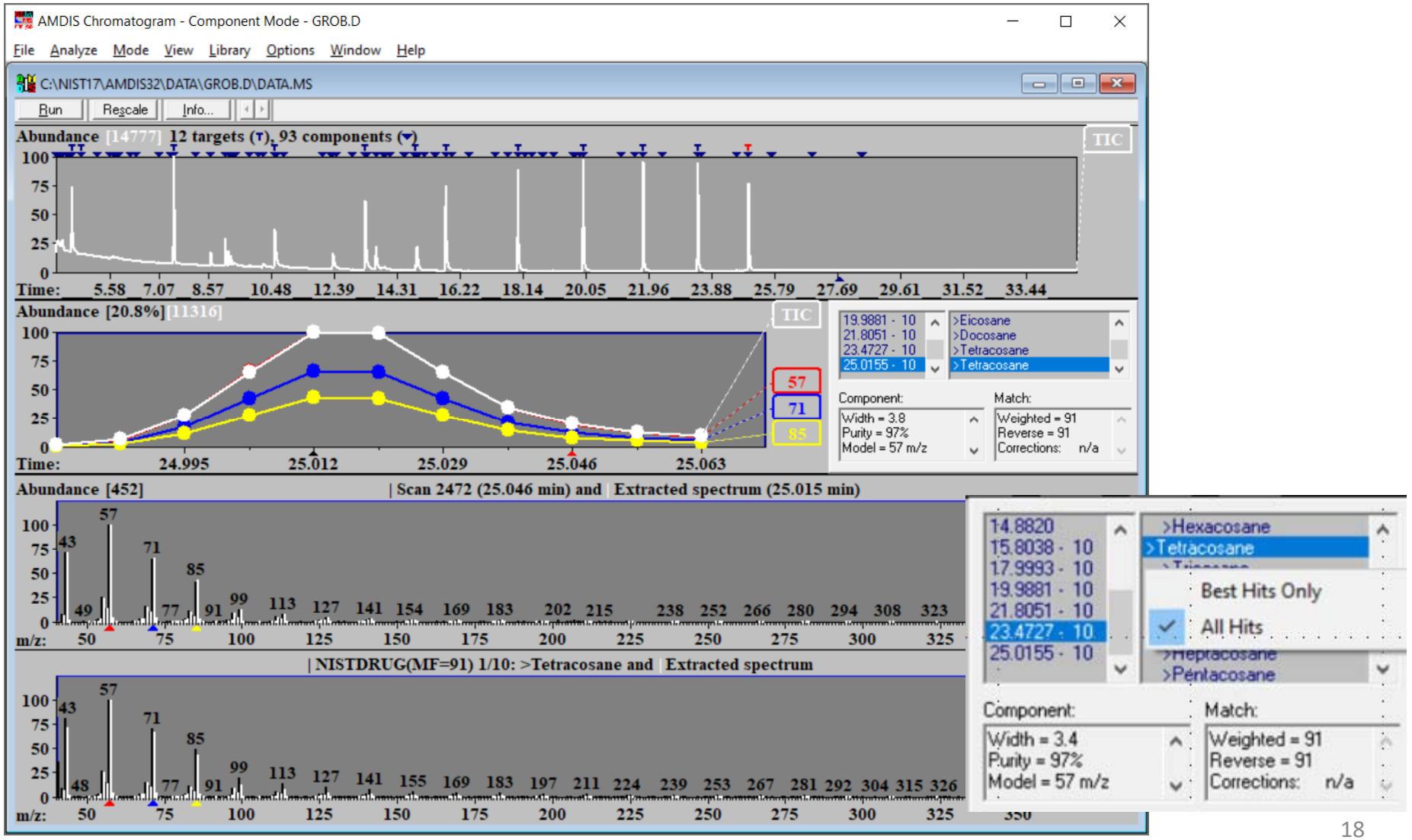
Examining Results of EI Mass Spectral Search with AMDIS

- Click **LMB** with the **Pointer** on any one blue **T** (turns **Red**) above chromatogram window
- **T** stands for target and that will be the library search results
- If the **T** furthest to the left, click on ***down arrow*** on keyboard to step through the results (L to R)
- The ***up arrow*** keys results in jumping from **T** to **T** from right to left
- The list in the Results window is from using the NIST MS Search Program and NOT the search of the Target Compounds Library



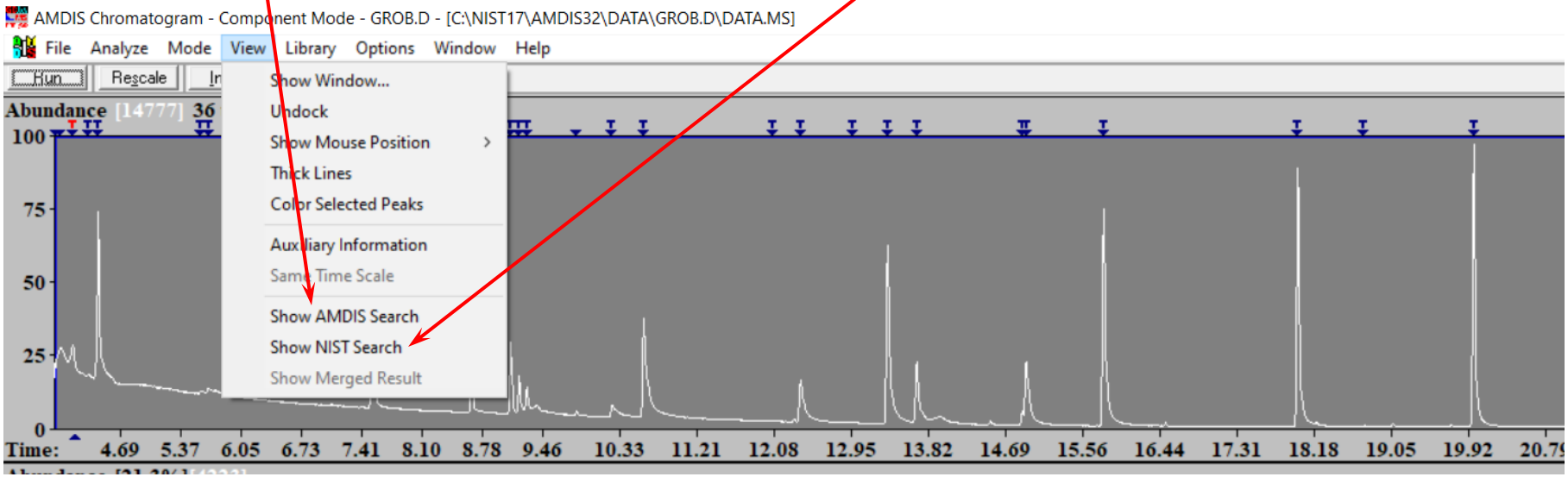
Examining Results of EI Search Results with AMDIS (cont'd)

- The unknown spectrum *vs* spectrum library hit will be displayed in the lower window as you step through the results using the **up** and **down arrow** keys
- Can also click the **RMB** with the **Pointer** on the **Results** window to see all hits for a **Component**



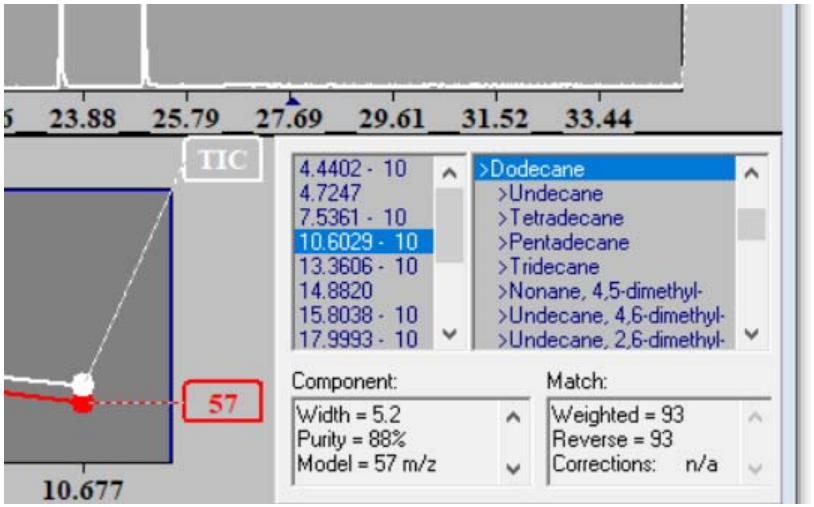
Examining Results of EI Search Results with AMDIS (cont'd)

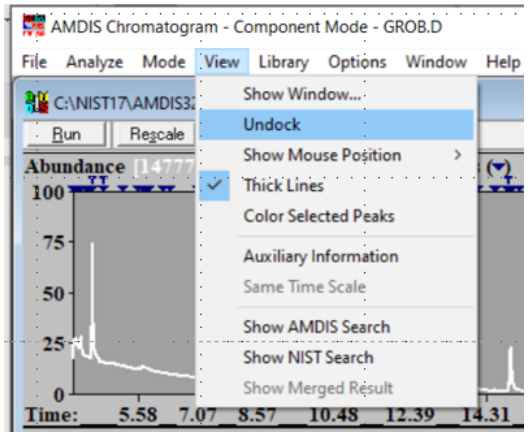
- Deconvoluted spectra can be searched using the AMDIS internal **Target Compounds Library** or libraries in the NIST MS Search Program using MS Search
- The library search results of either search can be displayed by selected **Show NIST Search** or **Show AMDIS Search from the View** menu right after the search using the NIST MS Program has been performed
- After one is selected, it will be grayed and the **Show Merged Result** selection will no longer be grayed. Once **Show Merged Result** is selected, it will be grayed and the other two will no longer be grayed
- More information can be found on these options on pg 62 of AMDIS manual (Section 3.1.4.8)

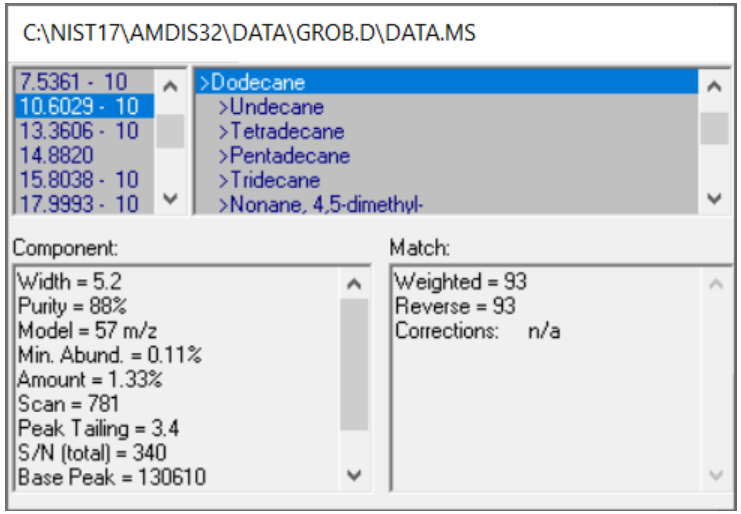


Undocking Library Results Windows for Viewing

- Viewing library search results is better done by undocking the Results window
- Undocking is accomplished by clicking the **RMB** with the **Pointer** on the frame of the window or from the **View** menu
- After **Undocking**, the window can be moved and resized as desired

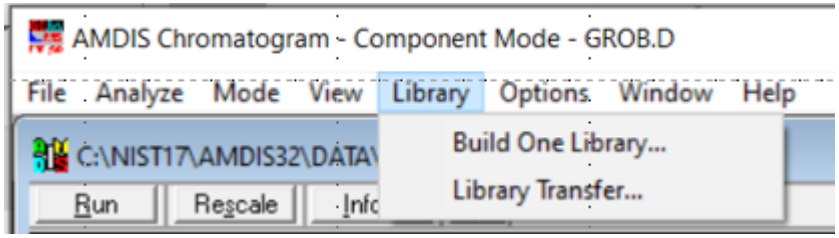


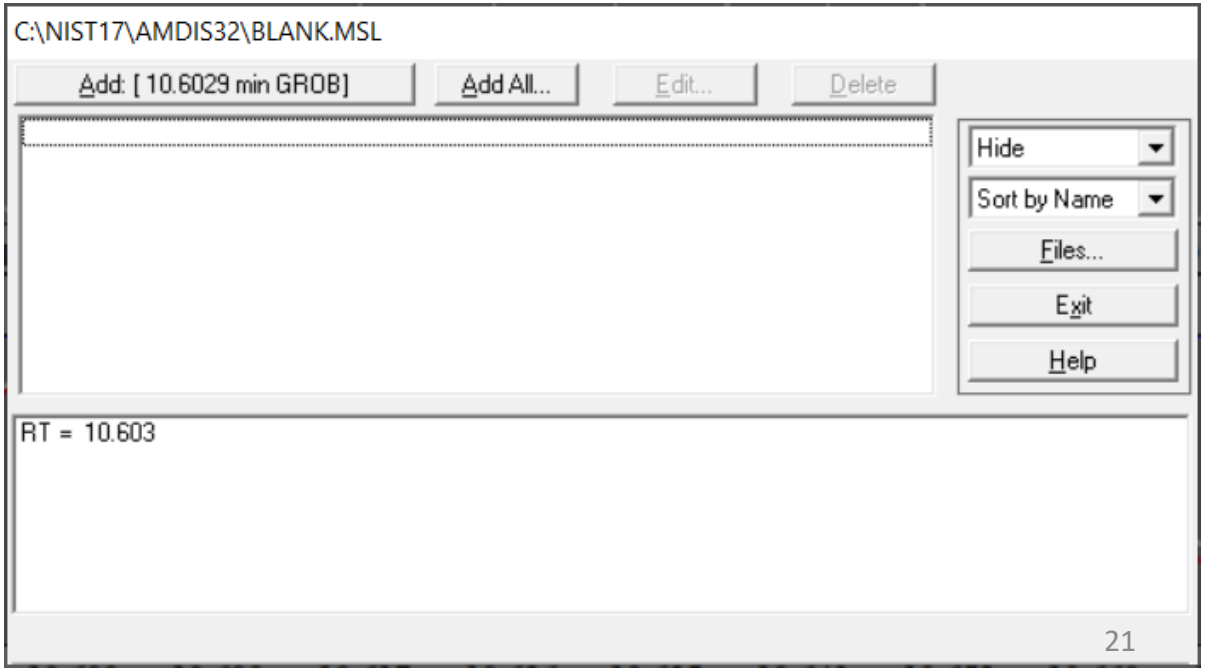




Creating Small Custom Libraries of Targeted Species within AMDIS

- Small custom libraries can be created in AMDIS and then searched like the NIST mainlib would be searched against a file to give targeted species; **T**, at top of the chromatogram
- The library can just be any spectrum of interest, not necessarily a traditional library entry

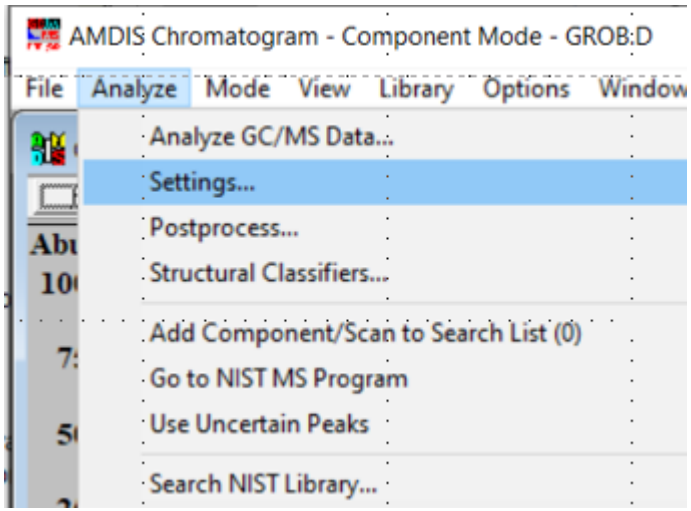


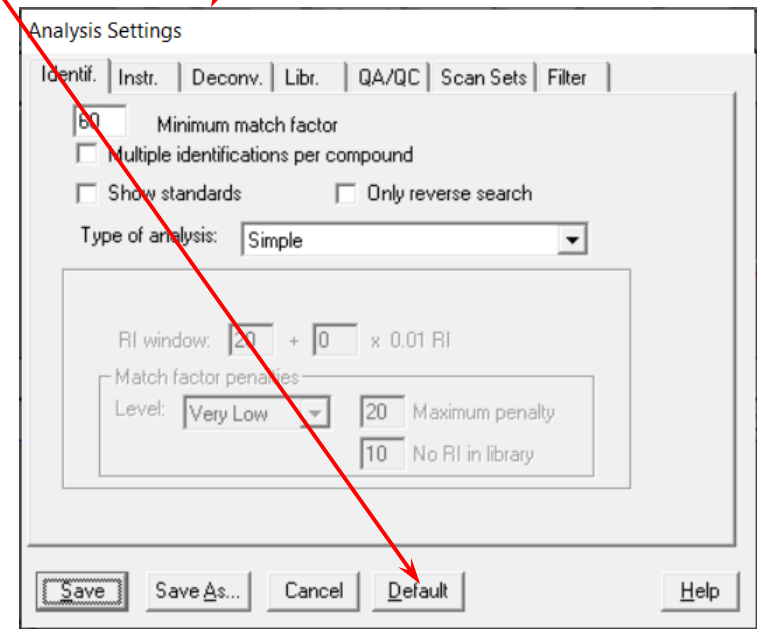


Information on Building AMDIS Custom Libraries Found in Help file or see AMDIS Manual pg 150 (Section 8.1.1)

Minimizing Marking Components in Chromatogram

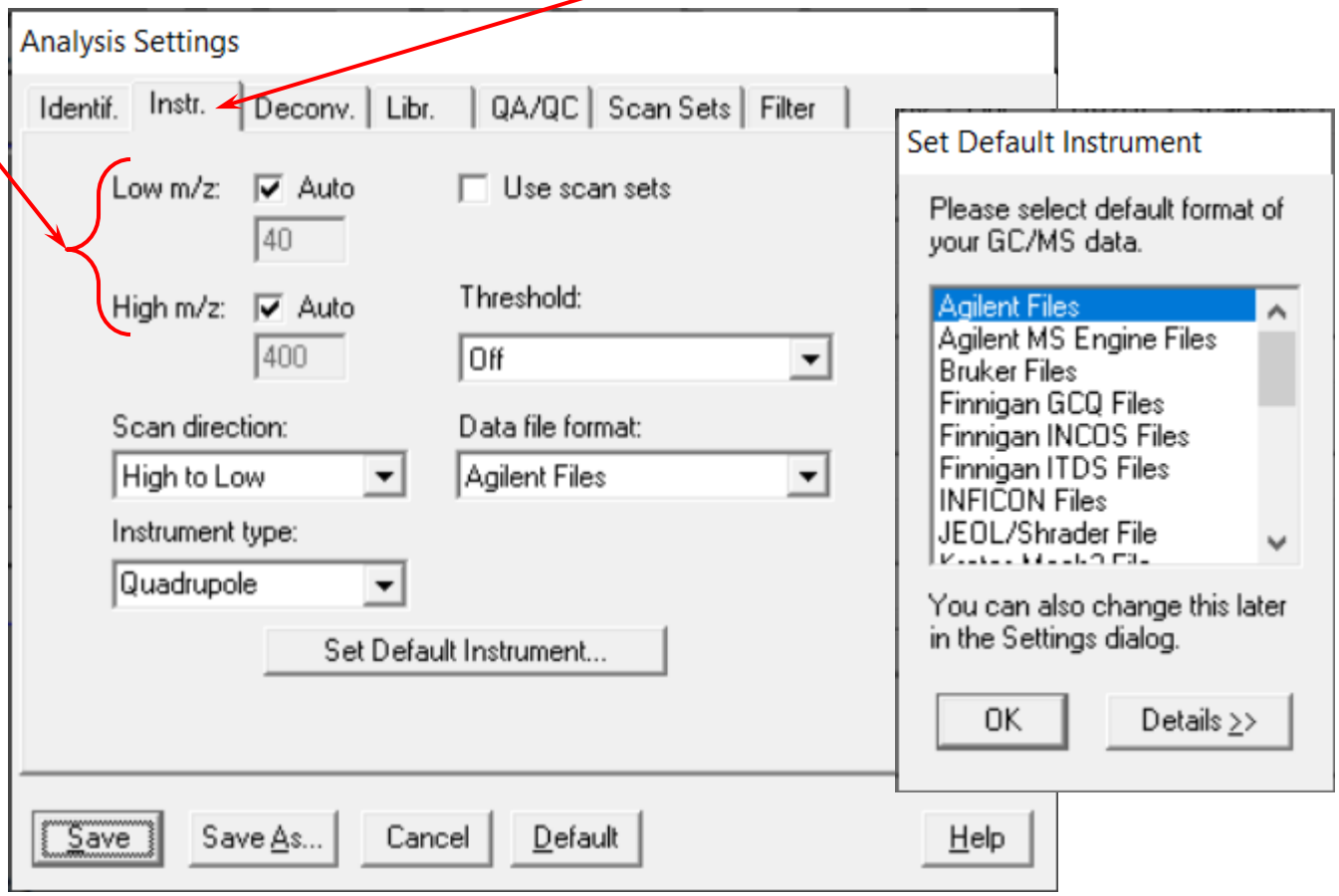
- The “multi-marking” of **Components** due to noise or instrument scanning irregularities can be annoying
- Almost all instruments under varying conditions tend to have this problem
- This can be minimized by adjusting some parameters In the **Analysis Settings** dialog box
- Note that there are multiple tabs with many parameters in this dialog box
- It is easy to restore the program’s **Default** settings





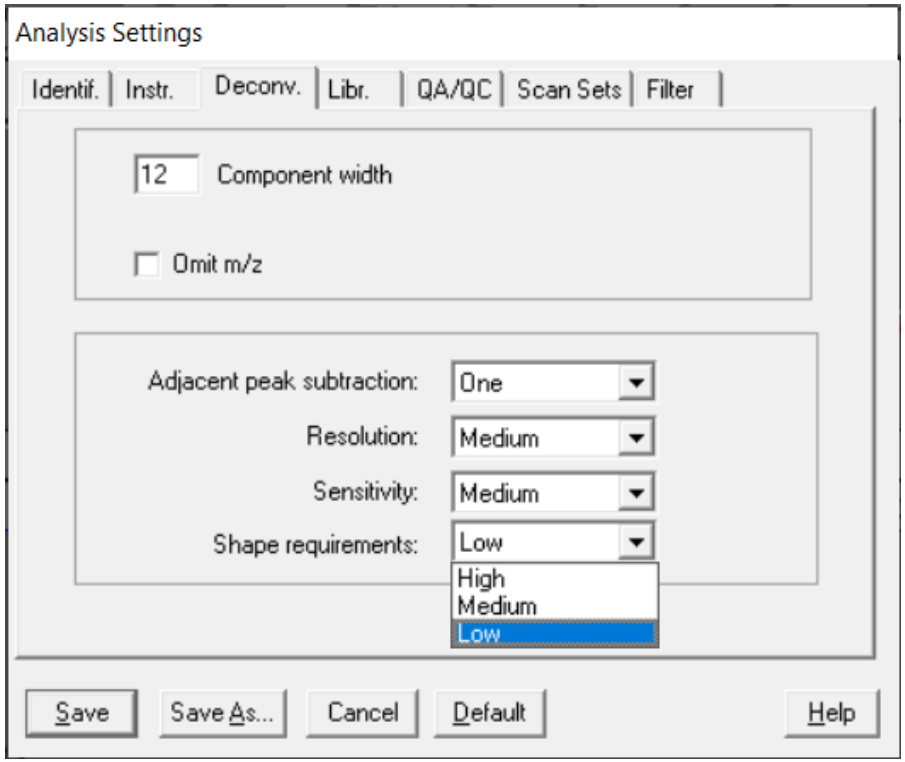
Minimizing Marking Components in Chromatogram (cont'd)

- Setup the processing parameters based on the instrument and its scan function (**Instr** tab)
- Can set the low and high m/z manually, or just automatically use those determined by AMDIS from the file



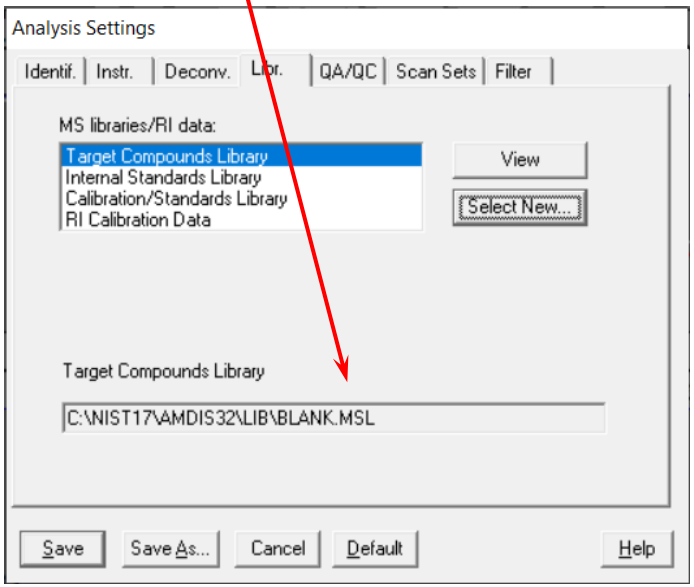
Minimizing Marking Components in Chromatogram (cont'd)

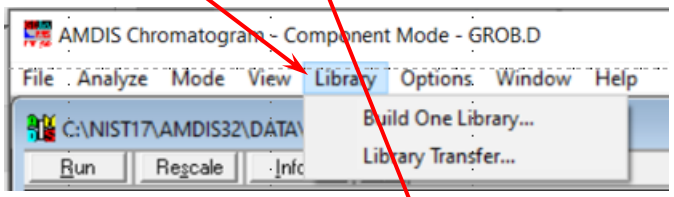
- The **Deconv**olution tab can be set to get rid of some peaks
- In particular, for tailing peaks, might want to set the **Shape** requirements to Low
- The default for **Shape** requirements is Medium
- The values show below are the **Defaults**
- In general, the **Filter** tab (*see page 28 of this presentation*) usually minimizes the multi-marking of **Components**

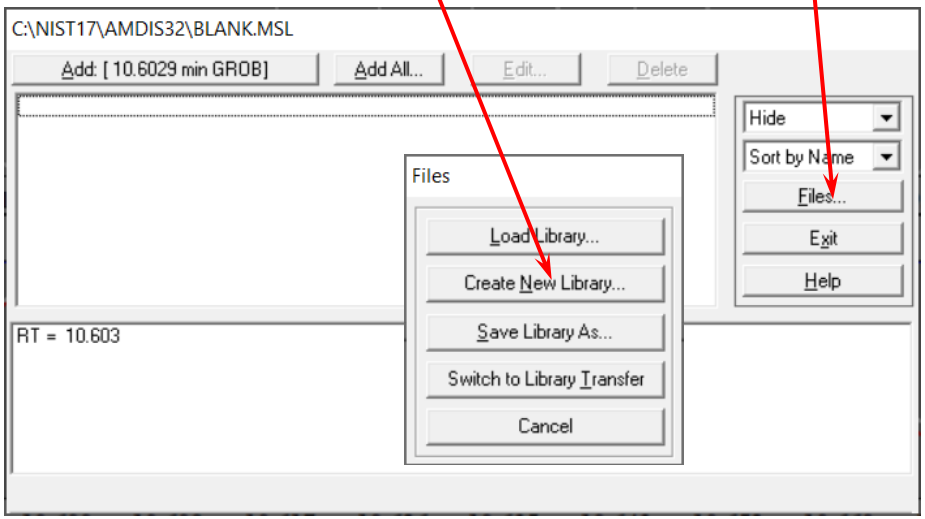


Minimizing Marking Components in Chromatogram (cont'd)

- Created a library named **Blank** so that when the file is deconvoluted, no **Components** are targeted with a **T**, they are marked with a ▼ to show that a **Component** was detected
- Created the library by first selecting **Library** from the Main Menu, then clicked on **File** button in the displayed dialog box, then click on the **Create New Library** in the **Files** dialog box

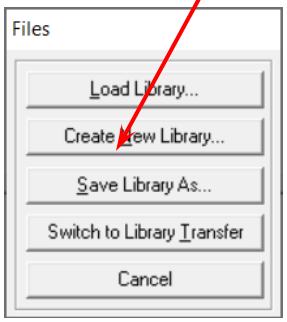


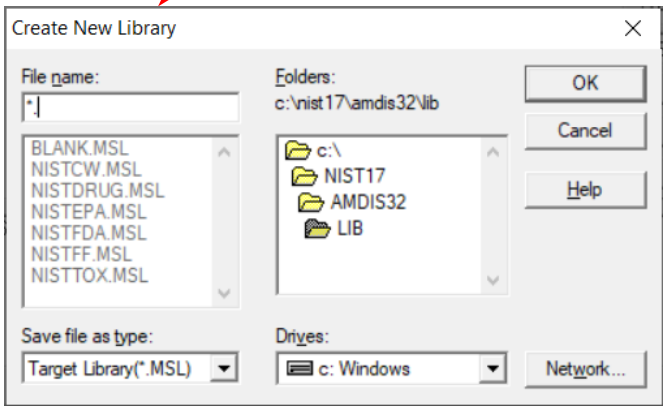




Minimizing Marking Components in Chromatogram (cont'd)

- After clicking on the **Create New Library** button in the **Files** dialog box the **Create New Library** file save dialog box will be displayed
- There is a known Bug in v.2.73 <build 149.31> (Apr 25, 2017) and earlier versions of AMDIS. In order for the file name entered in the **File name:** text entry box to have an extension, it has to be entered with the name. The dialog box has a section with the label **Save file as type:**. It shows the file should be saved as an ***.MSL** file; however, this does not work. Be sure to add the **MSL** extension to the file name or the file will be saved with no extension.

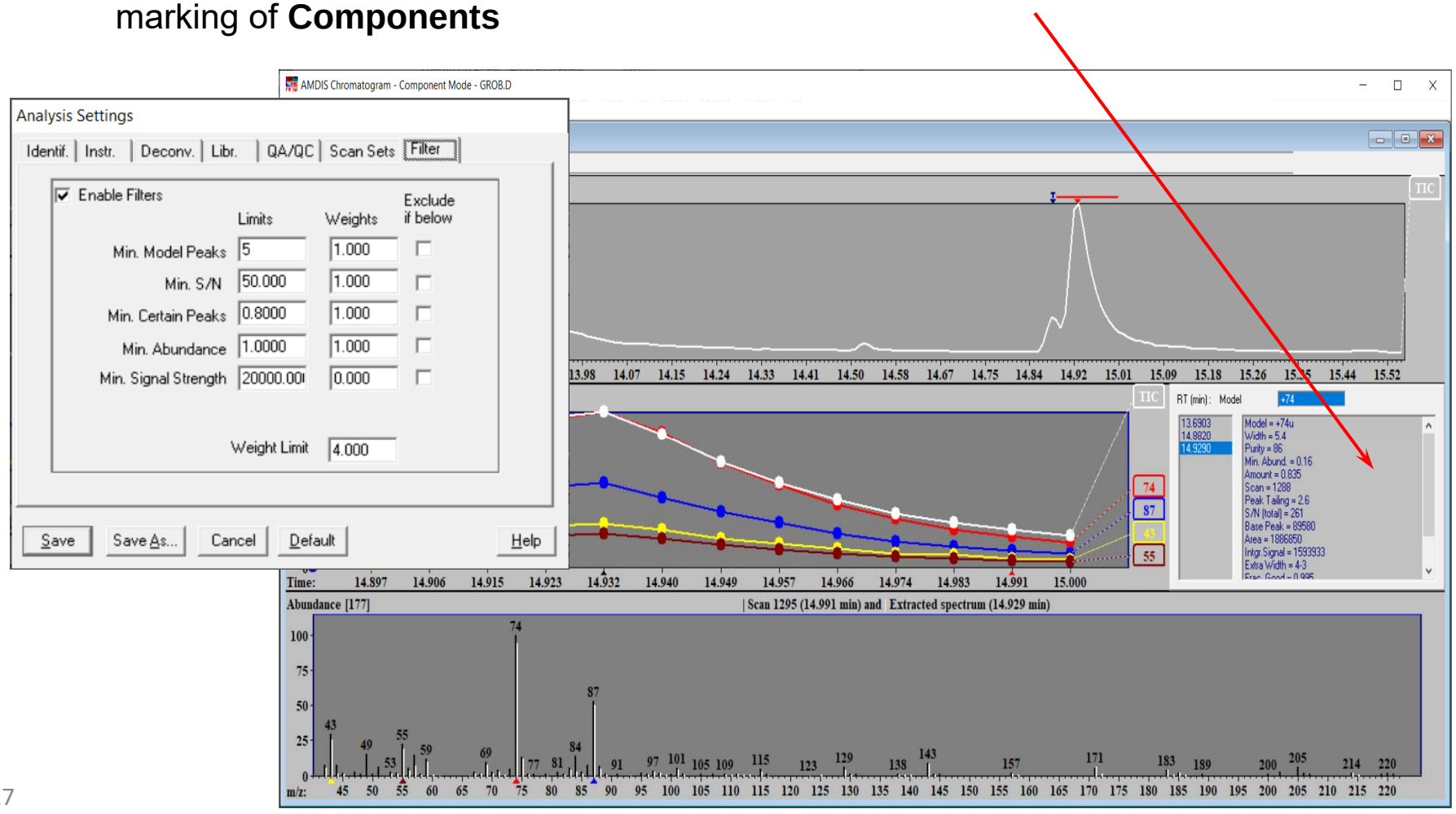




Bug Alert

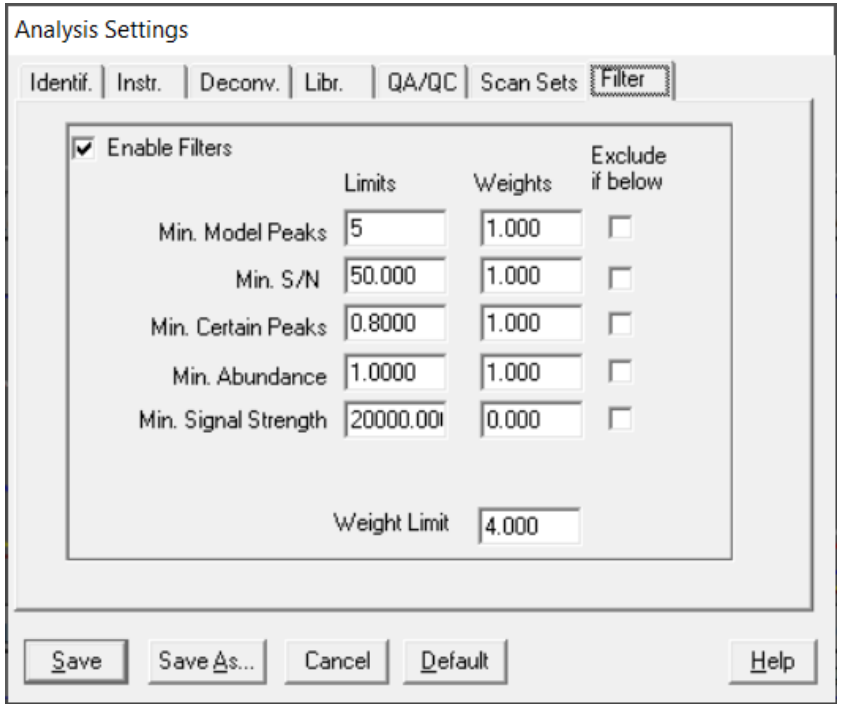
Minimizing Marking Components in Chromatogram (cont'd)

- The default filter settings are shown below; the default settings do NOT have the **Enable Filters** check box selected; unless checked, the fields are grayed
- The values associated with a particular **Component** can be viewed in the window next to the Model (middle-left) window
- Looking at these values gives an idea of how to limit parameters to minimize the marking of **Components**



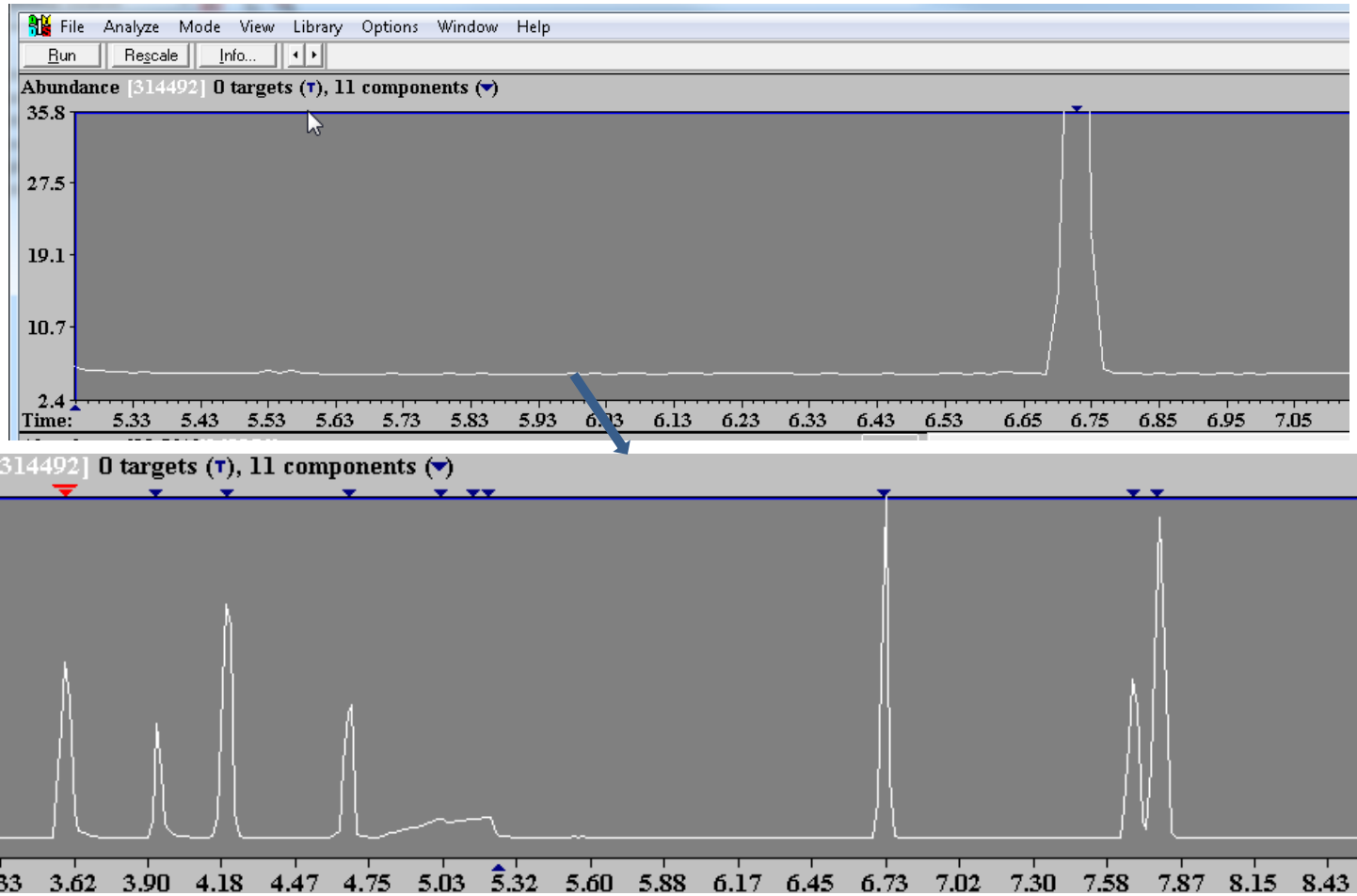
Minimizing Marking Components in Chromatogram (cont'd)

- The limits are scaled, thus if "Min Model Peaks" below is set at 5 and there are less, the weight for this parameter is decreased below 1, if >5, the weight factor for this parameter is >1
- The scaling for these parameters are not linear and there is a maximum set for each
- If a **Component's** Σ of weights is >4, it is included as a deconvoluted peak, if not, it is excluded
- An absolute limit can also be set for any one of these parameters by checking **Exclude if below** and selecting a value
- Adjusting these parameters greatly determines the number of times a chromatographic peak will be marked and the total number of marked peaks (detected components)



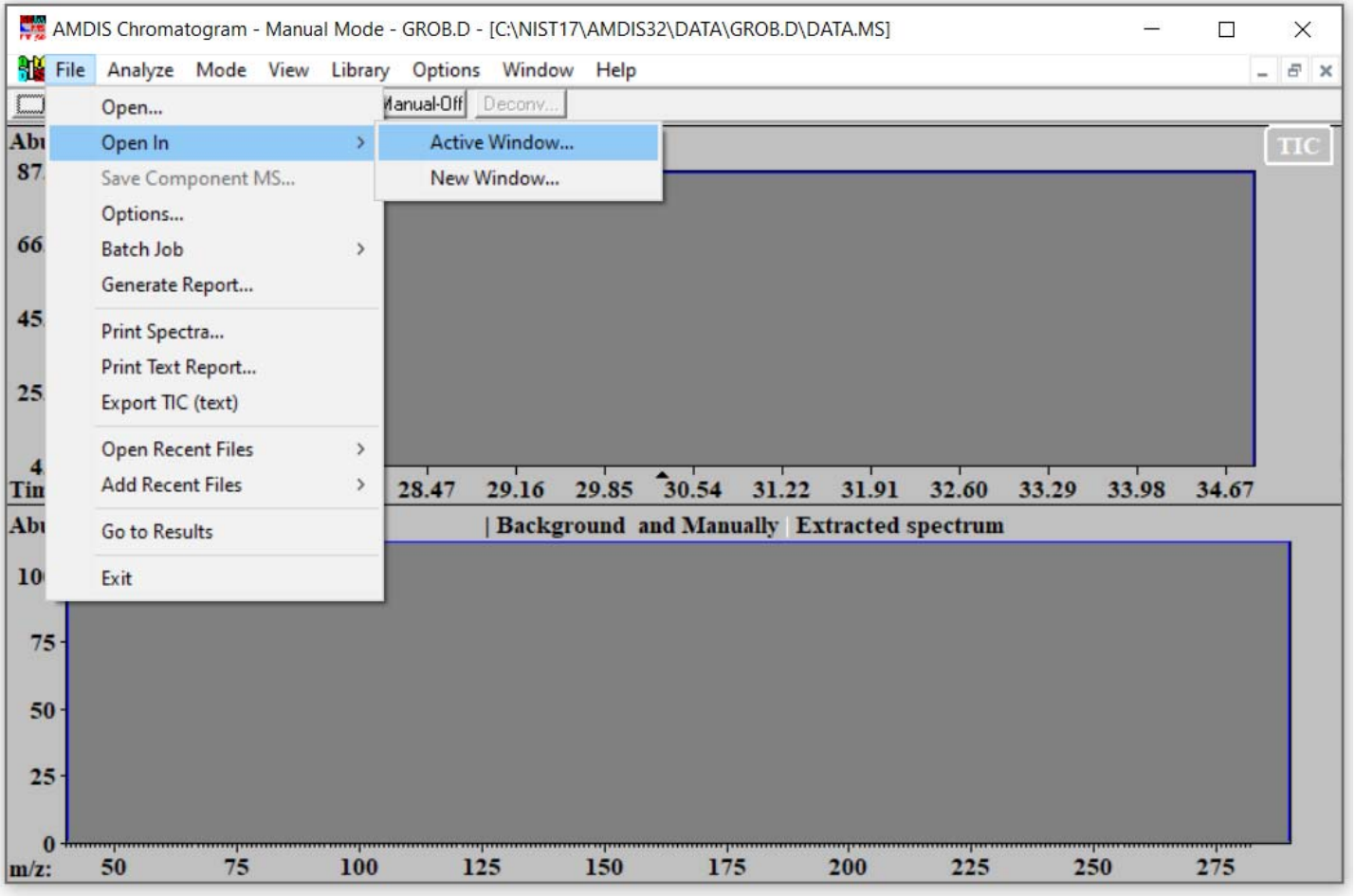
Minimizing Marking Components in Chromatogram (cont'd)

- When trying to determine the proper parameters, expand the chromatogram to only show the most difficult areas
- Change to parameter and only the area shown will be reanalyzed
- After getting all the parameters as desired, then show the whole chromatogram and **Run** (Reanalyze) again
- This will greatly speed the process!



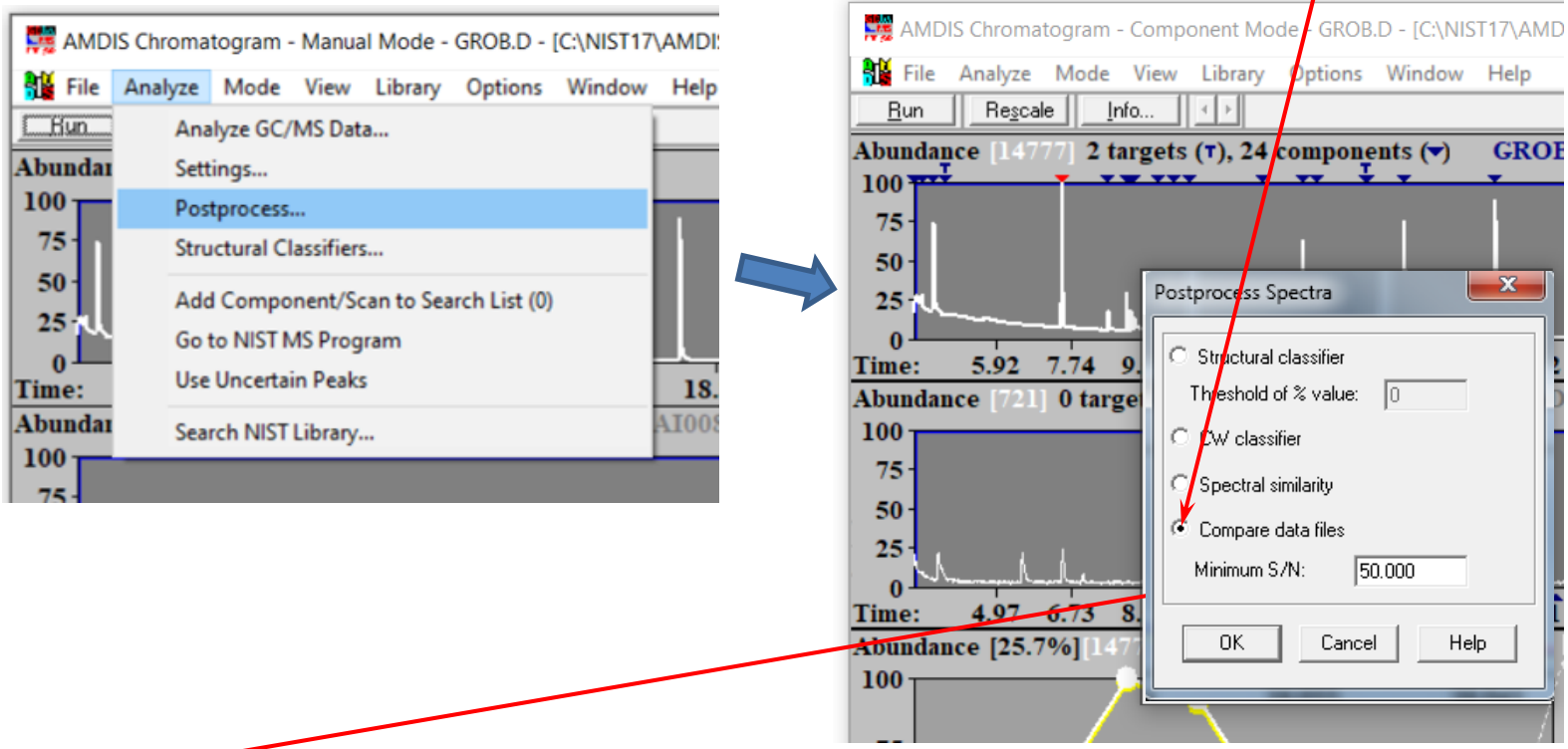
Comparing Two Chromatograms for Differences

- Two data files can be compared to determine differences, *i.e.*, "*Good*" and "*Bad*" samples
- Both ***must be loaded into the same window*** and both analyzed (run deconvolution)
- A good description of this process begins on pg 143 of the AMDIS Manual
- First, **Open** one file as normal
- Then open the file to be compared using **Open In/Active window…** as shown below



Comparing Two Chromatograms for Differences (cont'd)

- Put the **Pointer** on the top chromatogram and click the **LMB** followed by putting the **Pointer** on the **Run** button and clicking the **LMB** to deconvolute the file as normal using the appropriate settings. Repeated this process for the second chromatogram.
- Then, select **Postprocess** from the **Analyze** menu on the Main Menu and select "Compare data files"
- This process will compare both files to find differences
- Pick an appropriate S/N (bottom of pg 143 of manual)

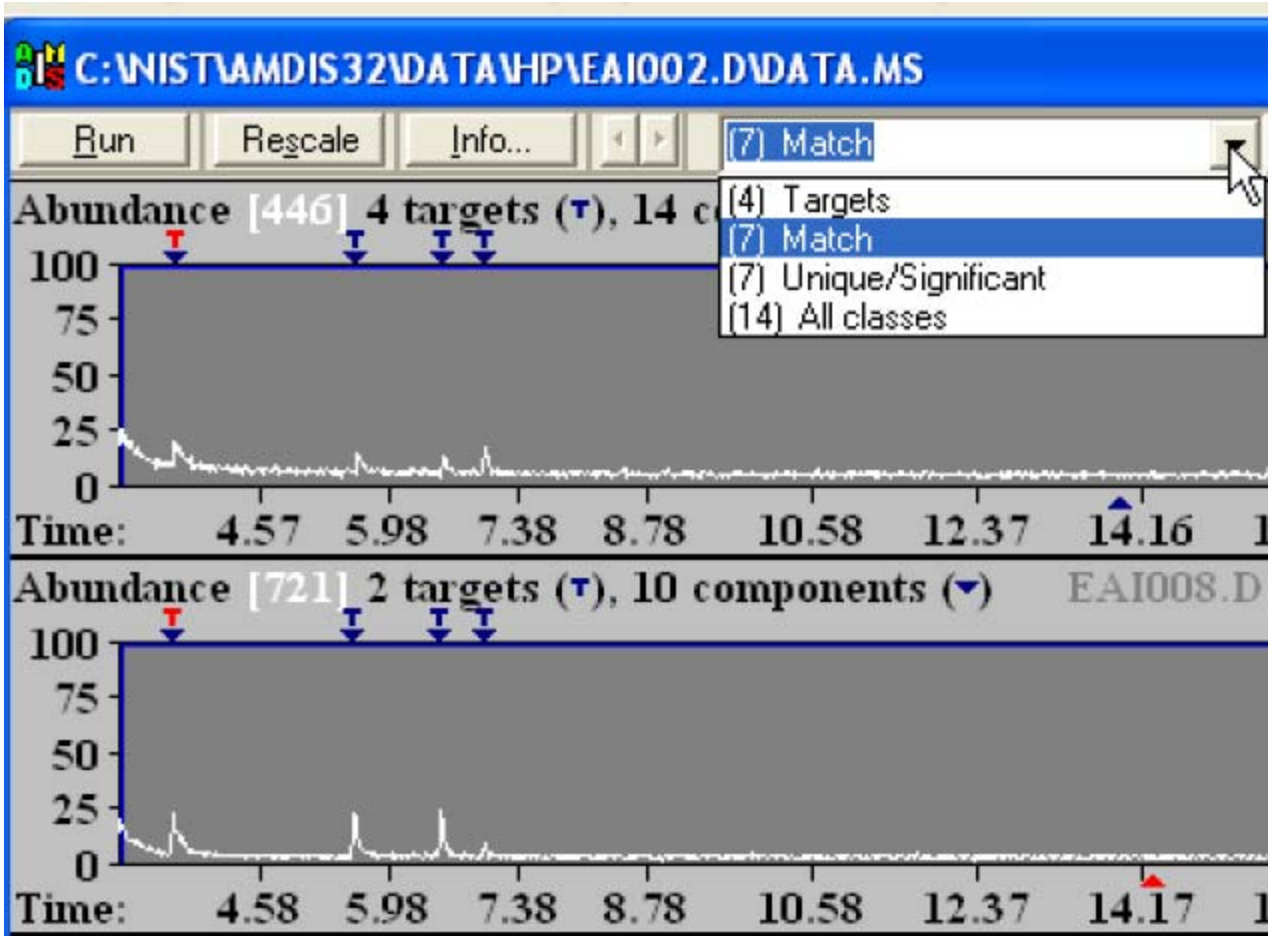


Specify a **Minimum S/N** to suit situation and then click the **OK** button

Note: The specified **Minimum S/N** should be adjusted to a level sufficiently high to prevent very low unique **Components** from appearing as **Unique/Significant** identifications

Comparing Two Chromatograms for Differences (cont'd)

- The classes will be shown with a **T** when the menu is pulled down
- Can select either the top or bottom file, and the results are then with respect to the selected file (pg 144 of manual)



Classes of Comp'ds Compared in Post Process of Two Files

- The classes that are shown in the pull down menu for each file are shown below from pg 146 of the AMDIS User's manual

To compare results for both data files, it is necessary to make each active and perform the Compare Data Files analysis technique on each. Whenever the other file is made active by putting the **Pointer** on it and clicking the **LMB**, the drop-down list box changes to reflect that file's values

A **Component** will be assigned to one of the following groupings:

Match/Larger	a pair of **Components** match, but one items is at least 3X larger than the other;
Match	a pair of **Components** match and neither is 3X, or more larger than the other
Unique/Significant	a **Component** that is only present in the active data file and whose signal is equal to or exceeds the signal-to-noise threshold describe above
Unique/Trace	a **Component** that is only present in the active data file and whose signal is less than the signal-to-noise threshold described above

Displaying Chromatograms So That Time Scales Expand Together

- To get the files to expand together, select **Same Time Scale** from the **View** menu, as shown