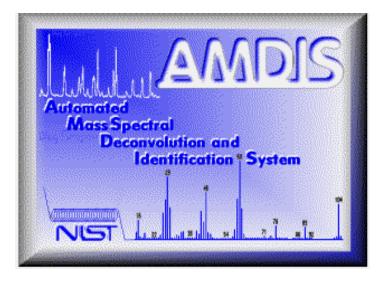
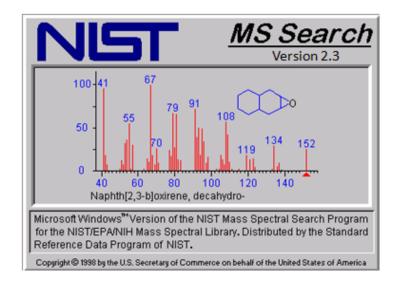
Basic Instructions for Using AMDIS with MS Search

By James L. Little, O. David Sparkman Input from Gary Mallard 11/24/2019

See AMDIS Manual for Detailed Instructions





What is AMDIS? Automated Mass spectral Deconvolution and Identification System

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
 v 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

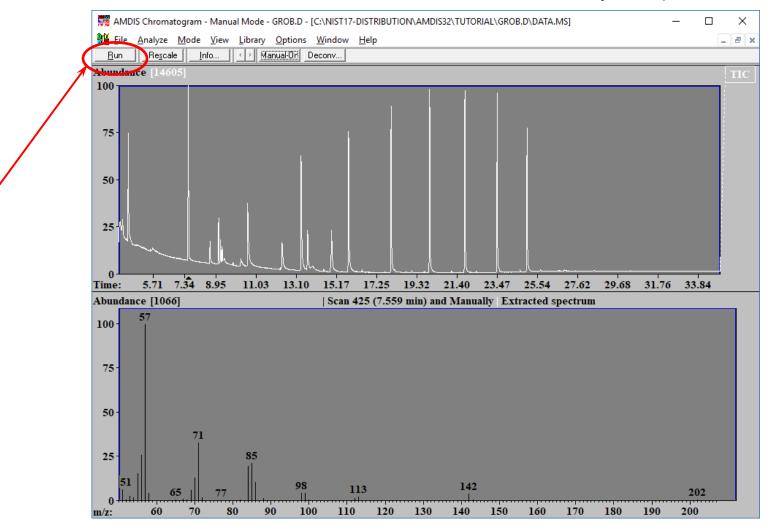
Opening File with AMDIS

- 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

1	AMDIS Chr	omatogi	ram - C	ompone
File	Analyze	Mode	View	Library
	Open			
	Open In			>
	Save Com	ponent l	MS	
	Options			
	Batch Job			2
	Generate	Report		
	Print Spec	tra		
	Print Text	Report		
	Export TIC	(text)		
	Open Rec	ent Files		>
	Add Rece	nt Files		>
	Go to Res	ults		
	Exit			

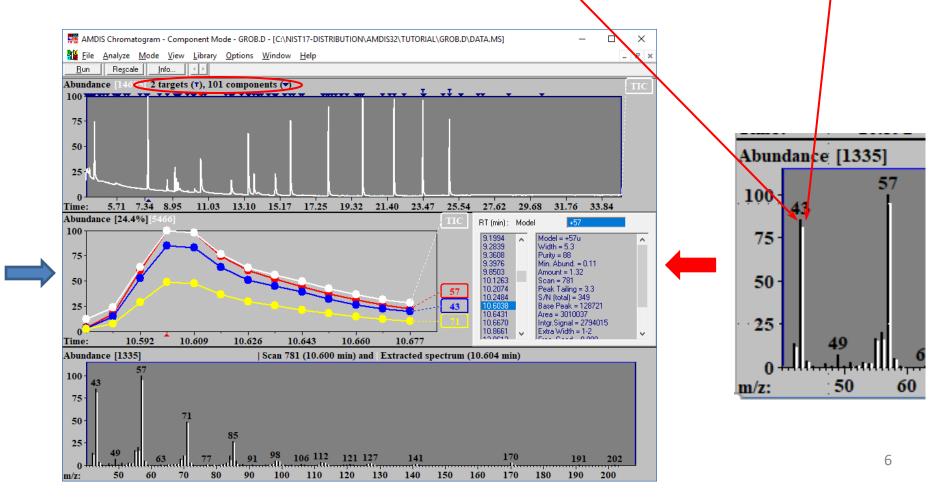
Deconvoluting Spectra

- 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)

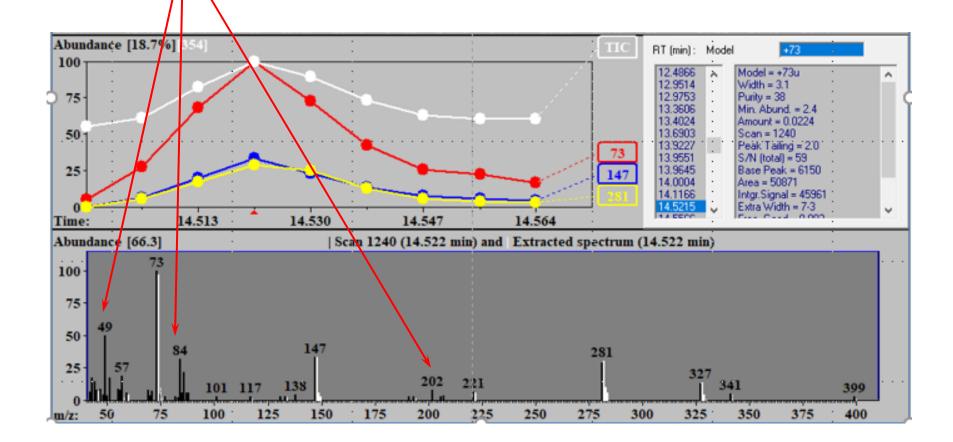
- Can just show the Component (white peaks), the Scan (black peaks), or Both, but best to get accustomed to looking at both
- When the black matches the white, you probably have a good spectrum of a major **Component**
- For minor Components, possibly coeluting with a major Component, the white will 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*).

Which peaks will be **RMB** Menu displayed by placing the displayed in the Pointer on the Spectrum window Spectrum window. and clicking the RMB Unzoom Show Mouse Position ۲ Log Scale for Spectra Freeze ۲ Shq Spectra Scan / ۲ Show Uncertain Peaks < Component Both Show Window... NIST Library ۲

7

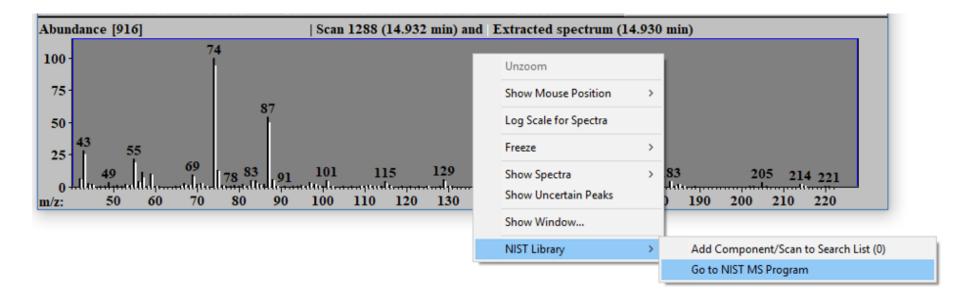
Evaluating Deconvoluted Results (continued)

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



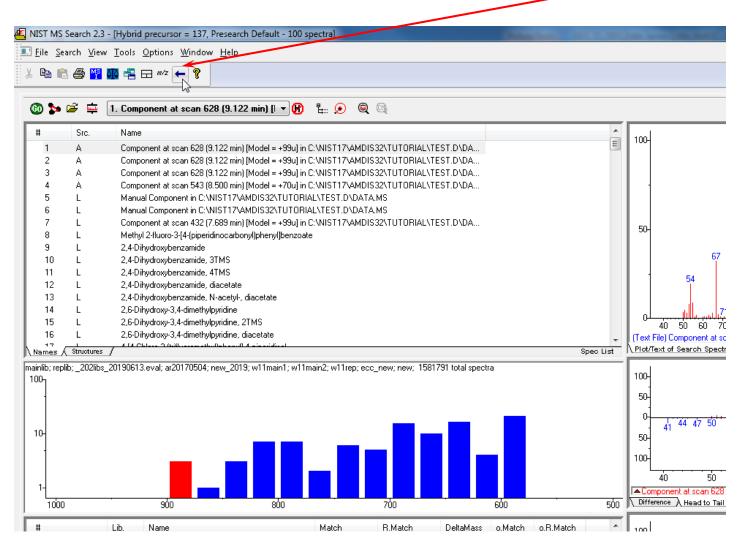
Sending Deconvoluted Spectra to NIST Search Program

- 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.



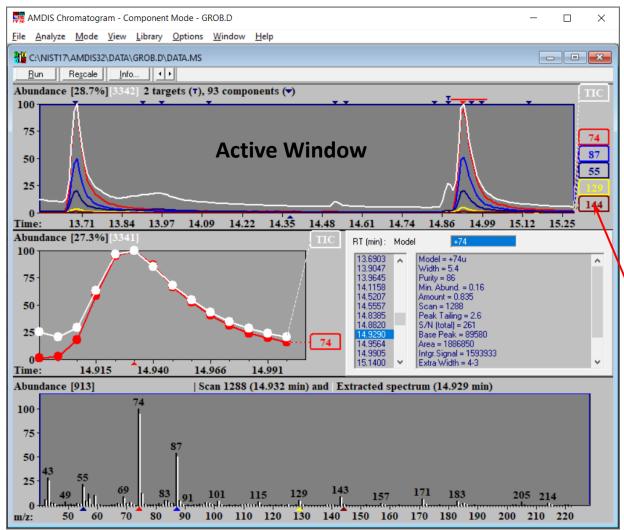
Uncertain Peaks, Dashed Lines, in Deconvoluted Spectrum

- 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



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

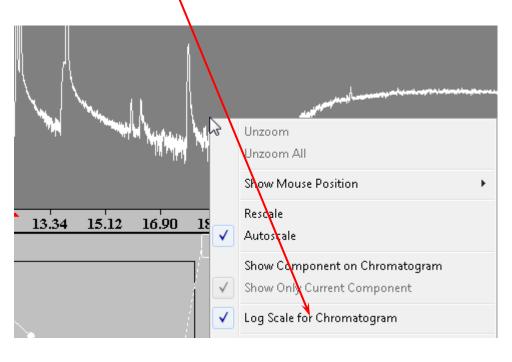


- Either the chromatogram (top) window of the model (middle left) window can be the active window
- To select the active window, put the **Pointer** on the bar above the window and click the LMB.
- The active window is dark gray
- To delete that mass chromatogram, just click the LMB with the Pointer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

		Unzoom 🗸	,
		Unzoom All	I
		Show Mous	e Position 🔹 🕨
		Rescale	
12.2	✓	Autoscale	
		Show Comp	ponent on Chromatogram
	\checkmark	Show Only	Current Component
		Log Scale fo	or Chromatogram
		Show Wind	ow
		File	•



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
MDIS Chromatogram - Component Mode - GROB.D
File Analyze Mode View Library Options Window
Image: C:\NIST17 Target Only Bun Component Abundance No Chromatogram ponents
68 - Manual

	Unzoom	1	1	0.00
	oncoon			
	Unzoom All			
	Onzoonnan	-2		
s. 1	Show Mouse	Position		.».
			- X.	
	Rescale			
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~	Autoscale			
	Show Compo	onent on Chromatogr	am	
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1	Signal			
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	Background	•		
	Clear		· .	
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	The Contractor	à		
		L. promatogram		
	Log Scale for	emonacogram		
	Log Scale for		· · · · ·	
• •				
	Show Windo		· · · · · ·	
			· · · · · ·	

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

Manually Processing File in AMDIS (continued)

- From RMB menu displayed with Pointer 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

🗱 AMDIS Chromatogram - Manual Mode GROB.D		Unzoom	1		×
<u>F</u> ile <u>A</u> nalyze <u>M</u> ode <u>V</u> iew <u>L</u> ibrary <u>O</u> ptions <u>W</u> indow <u>H</u> elp		Unzoom All	:		
		Show Mouse Position	· · · · · »·		×
Run Rescale Info • Manual-Off Deconv		Rescale			
Abundance [14777]	Ť	Autoscale	20122		пс
90.7		Show Component on Chromato	gram		
		Manual On Signal	•••••		
		Background			
68-		Clear	:		
		Clear All	:		
45.3	: :	Log Scale for Chromatogram	· · · · · · · · · · ·		
43.3		Show Window	:		
		File	>		
22.7-		RMB Menu	I		
Average For Average Avera	ae	For			
Background Sample Backg	-				
0 Time: 17.836 17.887 17.939 17.990 18.041 18.092		18.144 18.195	18.246	18.29	7

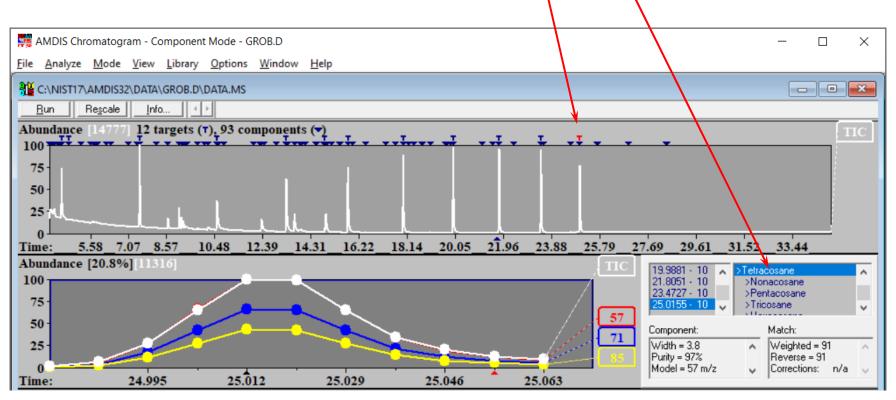
Automated Searching of Deconvoluted Spectra within AMDIS

- 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

MDIS Chromatogram - Component Mode - GROB.D	Search NIST Library - Parameters		
	GC/MS data:		
Eile Analyze Mode View Library Options Window	C:\NIST17\AMDI932\DATA\GROB		
Analyze GC/MS Data	Hits reported per search	Select from	
Settings	• Max. # of hits: 10	 All components (93) 	
Aba Postprocess	C Miz match factor: 10	 Only unidentified components (9 Consider all models 	1)
10 Structural Classifiers	C Min. probability %; 10	Only identified components (2)	
7: Add Component/Scan to Search List (0)		Number of components searched	
5 Go to NIST MS Program		Largest components: 10	
7. Use Uncertain Peaks	Build combined result	C All above threshold	
Search NIST Library		0.0 % of totalsignal There are 93 comp Select Librari	ies for Search X
	NIST MS directory: C:\NIST17\MSSEARCH\	0.0% and 2.490%	Y
	·	ClassExan	
	- Calaat	Search mode DD2019 DD_2018	Move down
Delete Result Files for GROB.D ×	·	FrontierPY	R2018
AMDIS generates two files containing results for each analysis of a GC/MS data	Analyze	and FrontierPy	rolyzate Path: C:\NIST17\MSSEARCH\main
file.		isidorov_e	
Would you like to delete these files now?		mainlib	ady rer
(Select 'Keep' if you are searching NIST		MeCl_not_	remove
Library!) Delete	Be sure there is a check ir	n the box	
Keep	next to the selecte		
	16		2 Select Cancel

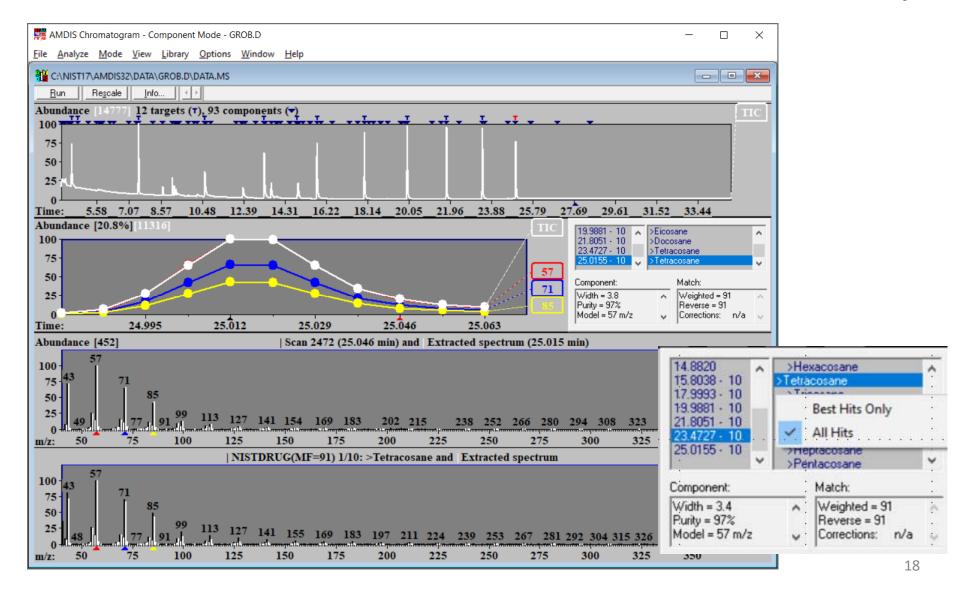
Examining Results of El Mass Spectral Search with AMDIS

- 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



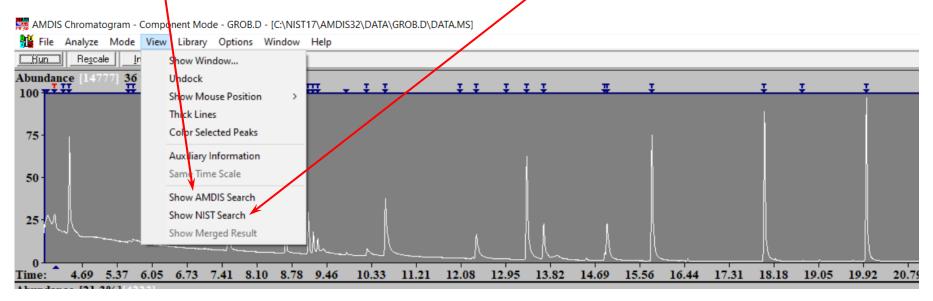
Examining Results of EI Search Results with AMDIS (cont'd)

- 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)

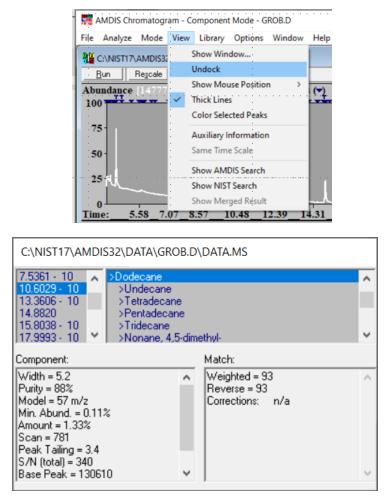
- Deconvoluted spectra can be searched using the AMDIS internal Target Compounds Library or libraries in the NIST MS Search Program using MS Search
- The library search results of either search can be displayed by selected Show NIST Search or Show AMDIS Search from the View menu right after the search 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

		<u></u>	
23.88 25.79	27.69 29.61	31.52 33.44	
	4.4402 · 10 A	>Dodecane >Undecane	^
	7.5361 - 10 10.6029 - 10	>Tetradecane >Pentadecane	
	13.3606 - 10 14.8820 15.8038 - 10 17.9993 - 10	>Tridecane >Nonane, 4,5-dimethyl- >Undecane, 4,6-dimethyl- >Undecane, 2,6-dimethyl-	~
~	Component:	Match:	
57	Width = 5.2 Purity = 88% Model = 57 m/z	Weighted = 93 Reverse = 93	^



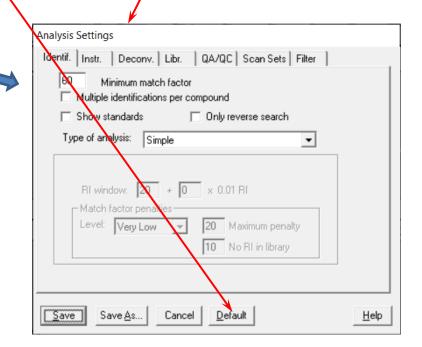
Creating Small Custom Libraries of Targeted Species within AMDIS

- 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

🛤 AMDIS Chromatogram - Component Mode - G	ROB.D	
File Analyze Mode View Library Options.	Window Help	
Build One Lib		
Information on Building AMDIS Custom Libraries Found in Help file or see AMDIS Manual pg 150 (Section 8.1.1)	C:\NIST17\AMDIS32\BLANK.MSL <u>A</u> dd: [10.6029 min GR0B] <u>A</u> dd All <u>E</u> dit <u>Delete</u> RT = 10.603	Hide ▼ Sort by Name ▼ <u>Files</u> <u>Exit</u> <u>H</u> elp
		21

- 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

11	🗱 AMDIS Chromatogram - Component Mode - GROB.D					
File	Analyze	Mode	View	Library	Options	Window
-	Ana	lyze GC/	MS Dat	a		
	Sett	ings		•		
Abi	Pos	tprocess.				:
10	Charl	ictural Cl	assifiers			
	Add	I Compo	nent/So	an to Sea	rch List (0)	· · · .
7:	Go	to NIST N	IS Prog	ram		:
5	Use	Uncertai	n Peaks			:
	Sea	rch NIST	librany			
2	Sea	ICH NIST	ciorary.	· .		



- 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

Identif. Instr.	Deconv. Libr	. QA/QC Scan Sets Filter	
			Set Default Instrument
Low m/z:	Auto	🔲 Use scan sets	Please select default format your GC/MS data.
High m/z	400 Auto	Threshold:	Agilent Files Agilent MS Engine Files Bruker Files
Scan dire	ection:	Data file format:	Finnigan GCQ Files Finnigan INCOS Files
High to I	-ow 🔻	Agilent Files 🔹	Finnigan ITDS Files
Instrumer	nt type:		INFICON Files JEOL/Shrader File
Quadrup	ole 💌		You can also change this la
	Set Defa	ult Instrument	in the Settings dialog.
			OK Details ≥>

- The Deconvolution 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 multimarking of Components

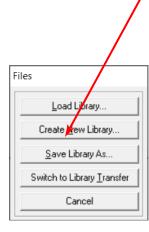
Analysis Settings						
Identif. Instr. Deconv. Libr. Q	A/QC Scan Sets Filter					
12 Component width						
☐ Omit m/z						
Adjacent peak subtraction:	One 💌					
Resolution:	Medium					
Sensitivity:	Medium					
Shape requirements:	Low					
	High Medium Low					
Save Save As Cancel	<u>D</u> efault <u>H</u> elp					

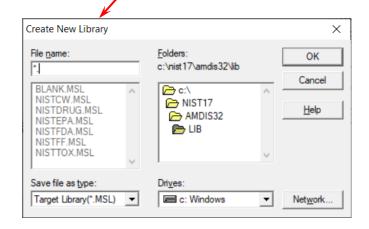
- Created a library named **Blank** so that when the file is deconvoluted, no **Components** are targeted with a **T**, they are marked with a **▼** to show that a **Component** was detected
- Created the library by first selecting **Library** from the Main Menu, then clicked on **File** button in the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

Analysis Settings
Identiř, Instr. Deconv. L <mark>p</mark> r. QA/QC Scan Sets Filter
MS libraries/RI data: Target Compounds Library Internal Standards Library Calibration/Standards Library RI Calibration Data
Target Compounds Library C:\NIST17\AMDIS32\LIB\BLANK.MSL
Save Save As Cancel Default <u>H</u> elp

C:\NIST17\AMDIS32\BLANK.MSL	All Edit Delete	Hide
RT = 10.603	Create <u>N</u> ew Library <u>Save Library As</u> Switch to Library <u>I</u> ransfer Cancel	<u>H</u> elp

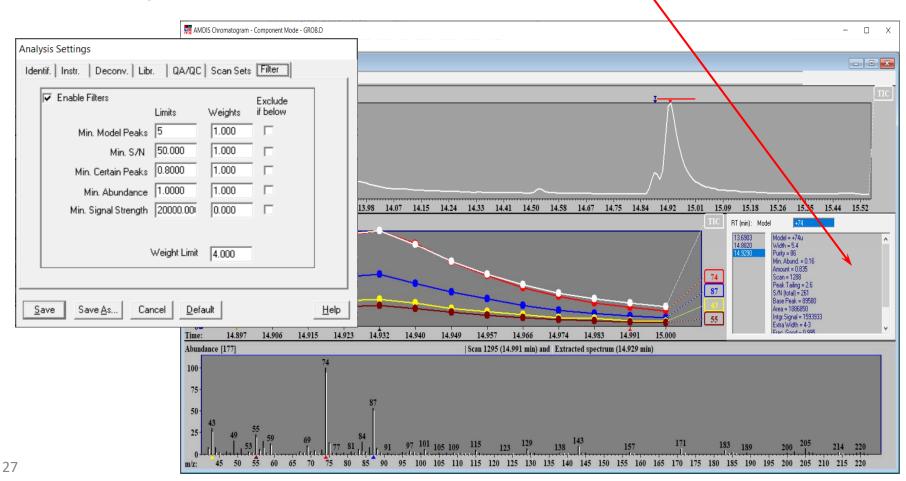
- After clicking on the **Create New Library** button in the **Files** dialog box the **Create New Library** file save dialog box will be displayed
- There is a known Bug in v.2.73 <build 149.31> (Apr 25, 2017) and earlier versions of AMDIS. In order for the file name entered in the File name: text entry 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

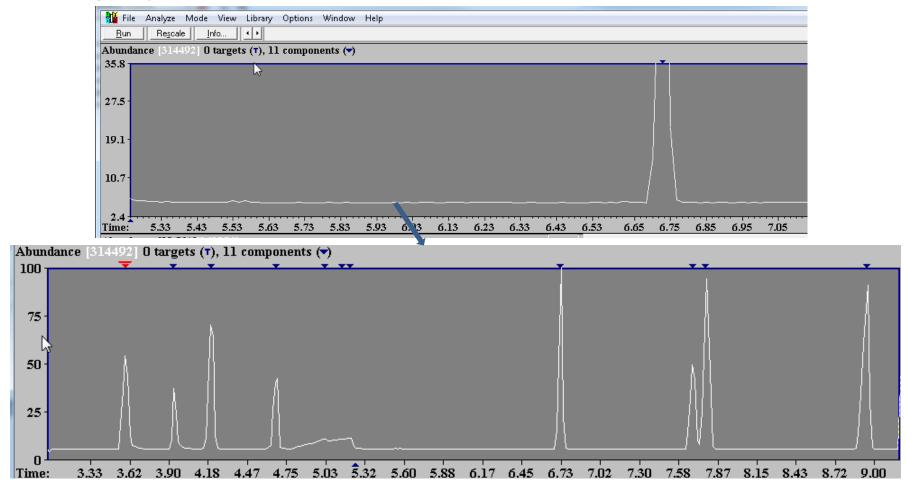
- The default filter settings are shown below; the default settings do NOT have the Enable Filters check box selected; unless checked, the fields are grayed
- The values associated with a particular Component can be viewed in the window next to the Model (middle-left) window
- Looking at these values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



- 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 ∑ 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)

E E	nable Filters	Limits	Weights	Exclude if below
	Min. Model Peaks	5	1.000	
	Min. S/N	50.000	1.000	
	Min. Certain Peaks	0.8000	1.000	
	Min. Abundance	1.0000	1.000	
	Min. Signal Strength	20000.00	0.000	
		Weight Limit	4.000	j

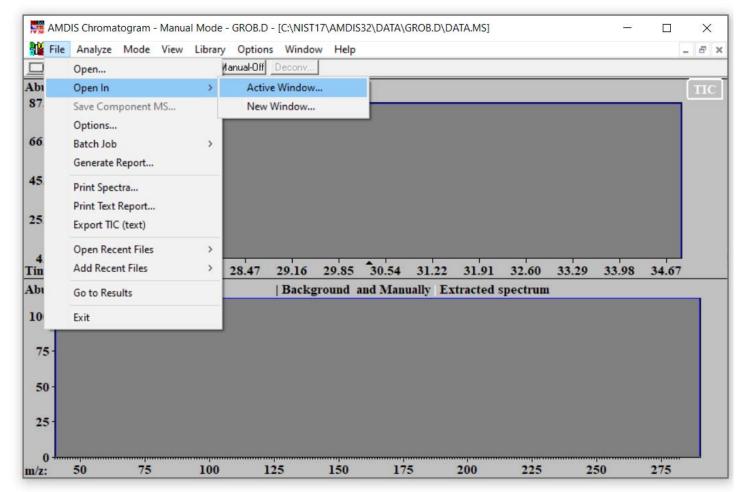
- When trying to determine the proper parameters, expand the chromatogram to only show the most difficult areas
- Change to parameter and only the area shown will be reanalyzed
- After getting all the parameters as desired, then show the whole chromatogram and Run (Reanalyze) again
- This will greatly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



29

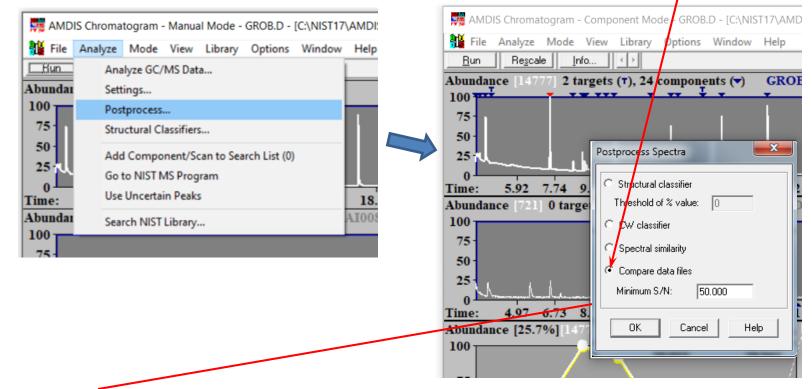
Comparing Two Chromatograms for Differences

- 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)

	STVAMDI	S32\DA	TAVHP	EA 1002	DVDATA.M	AS		
<u>R</u> un	Resc	ale	Info	4 >	(7) Match			N
Abunda	nce [446			r), 14 c	(4) Targets(7) Match			-70
100	-	* *	Ţ			/Significant sses		
50 25 0	N			0 70	10 59	12.27	14.16	
Time: Abundar	4.57 nce [72]	5.98 2 tai	7.38 gets (8.78 T), 10 c	10.58 omponen	12.37 ts (•)	EAI008	.D
100 75 50 25		• •	*					
0 Time:	4.58	5.98	7.38	8.78	10.58	12.37	14.17]

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

