AMDIS — USER GUIDE

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Automated

Mass Spectral

Deconvolution &

Identification

System

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1   The AMDIS Program

The **A**utomatic **M**ass Spectral **D**econvolution and **I**dentification **S**ystem (AMDIS) allows you to automatically find any of a set of target compounds in a gas chromatography/mass spectrometry (GC/MS) data file. The program first deconvolutes the GC/MS data file to find all of the separate components. Each of these components is then compared against a library of target compounds. The match factor between the target spectrum and the deconvoluted component spectrum is then reported, if it is above a user set value, and the matched target spectrum is called a library hit.

AMDIS uses a library of mass spectra with or without retention indices to identify compounds in the data file. The program can be configured to build a retention index calibration data file, to use the retention index data along with the mass spectral data, or to work without reference to any retention index data. In addition, AMDIS can be used to build a user defined library, either from GC/MS data files, or from the data in the NIST Mass Spectral Database.

The program will optionally track the results of routine performance mixture runs (such as the Grob test mixture). Data are retained from previous runs so that changes in the performance of the system can be monitored.

AMDIS also provides compound class identification, a batch processing capability and a convenient link to the NIST *MS Search* program.

Before you start to use AMDIS, please check how this User Guide can help you (**1.1 How to Use This User Guide**).

1.1   How to Use This User Guide

You should read this section first and then the following section; which describes the typographic and related conventions used in this manual (**1.2 Conventions Used in This User Guide**).

It is useful, although not essential, to understand a little of the background and methodology of AMDIS and that can be found in **1.3 An Overview of Automated GC/MS Identification by Steve Stein**.

Chapter **2 Getting Started** is essential reading. It first introduces you to the basic visuals and a few essential features of the two different user interfaces that AMDIS offers.

The rest of that chapter is then written in tutorial style and leads you one-by-one through all of the "analysis types" that AMDIS supports. Many useful topics are introduced whilst doing this and there are many references to their corresponding detailed sections for those readers who want to immediately learn a little more about a specific topic.

Even if you don't normally read manuals, the 30 minutes or so required to go through this chapter will dramatically improve your ability to quickly start productive work with AMDIS.

It is very highly recommended that you read Chapter **2**.

Before reading that you may wish to read the short Chapter **13 Glossary** to get a basic introduction to, and understanding of, key terms used in conjunction with AMDIS.

The chapters that follow the tutorials are written in the style of a technical reference and are topic-based, rather than tutorial-based. They provide full details of all AMDIS parameters and functionality.

Chapter **11** is a list of frequently asked questions (FAQs). As well as giving answers to questions, some useful tips are also introduced. It may be worth your while to read through these before finally starting productive work with AMDIS.

Chapter **12** lists the Varmuza classifiers, whose use is described in **7.1.1 Structural Classifier**.

Almost everywhere in AMDIS clicking the right mouse button will bring up a "context menu" (a list of one or more commands). These commands will vary as you move around the screen (they are "context-sensitive"). In addition, the commands presented can change as you progress through an analysis.

> **Note:**   An option that is "grayed out" (disabled) at some point may be available later; and vice versa.

1.2 Conventions Used in This User Guide

References to sections in this manual are presented in **bold Arial font** as a section number, followed by the title. For example: The conventions used in this user guide are described in **1.2 Conventions Used in This User Guide**. Occasionally a section reference may just use its section number; especially if it is being repeated close to its first definition.

Commands that can be selected from menus are in **bold Arial font**. For example: On the **View** menu click **Undock**. Sub-menu commands may also be identified from a higher level menu by specifying the two or more menu items separated by **|**. For example: Click **View | Undock**; Click **View | Show Mouse Position | On Top**.

Names of command buttons and tabs are also in **bold Arial font**. For example: To exit from the dialog click **Close**; The **Standards** tab gives information about the standards used.

Window and dialog names, plus certain specific named areas of the screen, are given in *italics*. For example: Note the information presented in the *Results* window; The *Analysis Settings* dialog has 5 tabs; The *Information Lists* area can be undocked and moved.

References to the names (but not the selection) of types of analysis will appear in italics. For example: A *Simple* analysis does not involve the use of internal standards, but the *Use RI Calibr. Data + Internal Std.* analysis type does.

References to books, or computer files, folders, documents, or programs are given in *italics*. For example: Read Chapter 1 of Microsoft's *Introduction to Windows XP* book; Refer to the *README.TXT* file in the *AMDIS32* folder.

Names of checkboxes, radio buttons, other parameters and parameter groups, values to be selected from a list, specific values or text shown on the display, warning messages and text to be entered by typing are written in **bold Times New Roman font**. For example: For **Type of analysis**, select **Use Internal Standards for RI**; After processing, **Total Signal** shows a value of **1.80e+008**; For **File name** type **NISTEPA** or another name of your choice; An invalid file produces the **Cannot read GC/MS data file** warning message.

Occasionally <u>underlining</u> will be used to highlight important information. In the Glossary (**13 Glossary**) it is used to indicate other glossary terms.

Occasionally it is necessary to refer to the folder in which AMDIS is installed. Since the default folder name is *AMDIS32* this will be used, but, if you have AMDIS installed in a different folder, please substitute that name wherever you see *AMDIS32* mentioned.

Datafiles shown in this User Guide in figures as located in *AMDIS32\HP\DATA* folder may be installed in *AMDIS32\TUTORIAL* instead.

1.3 An Overview of Automated GC/MS Identification by Steve Stein

Gas chromatography/mass spectrometry (GC/MS) has long been the method of choice for identifying volatile compounds in complex mixtures. This method can fail, however, when acquired spectra are "contaminated" with extraneous mass spectral peaks, as commonly arise from co-eluting compounds and ionization chamber contaminants. These extraneous peaks can pose a serious problem for automated identification methods where they can cause identifications to be missed by reducing the spectrum comparison factor below some pre-set identification threshold. In addition, the presence of spurious peaks in a spectrum adds to the risk of making false identifications. Perhaps worst of all, the added uncertainty leads to a general loss of confidence in the reliability of making identifications by GC/MS, especially for trace components in complex mixtures, a key application area for this technique.

The most common method for extracting "pure" spectra for a chromatographic component from acquired spectra is to subtract spectra in a selected "background" region of the chromatogram from spectra at the component maximum. This, however, is only appropriate when background signal levels are relatively constant (ionization chamber contamination, for example). Moreover, highly complex chromatograms may have no identifiable "background" region.

An automated approach for dealing with contaminated spectra is to assume that acquired mass spectral peaks that do not match a reference spectrum originate from impurities. While this method can identify trace components embedded in complex background spectra, it can also produce false positive identifications for

target compounds having simple spectra (i.e., when target compounds have spectra which are, in effect, embedded in the spectra of other compounds in the analyzed mixture).

AMDIS is an integrated set of procedures for first extracting pure component spectra and related information from complex chromatograms and then using this information to determine whether the component can be identified as one of the compounds represented in a reference library. The practical goal is to reduce the effort involved in identifying compounds by GC/MS while maintaining the high level of reliability associated with traditional analysis.

Previous Work

Since the inception of GC/MS, there has been a continuing interest in extracting "pure" component spectra from complex chromatograms. Biller and Biemann [1] devised a simple method in which the extracted spectrum is composed of all mass spectral peaks that maximize simultaneously. Colby [2] improved the resolution of this method by computing more precise ion maximization times. Herron, Donnelly and Sovocol [3] demonstrated the utility of Colby's method in the analysis of environmental samples.

Another computationally facile approach for extracting spectra based on subtraction of adjacent scans ("backfolding") has been recently proposed [4]. An advantage of this approach is that it does not explicitly require maximization.

A more computationally intensive approach developed by Dromey et al [5], called the "model peak" method, extracts ion profiles that have similar shapes. As in the Biller/Biemann procedure, this method uses maxima in ion chromatograms to detect chromatographic components. The shape of the most prominent of these maximizing ion chromatograms is used to represent the shape of the actual chromatographic component. Ion chromatograms with this shape are extracted by a simple least-squares procedure. This method was successfully used for target compound identification in a large-scale EPA study [6]. Rosenthal [7] proposed an improvement to the peak perception logic for this method.

A number of matrix-based approaches have been proposed that make no assumptions concerning component peak shape. These methods generally process an abundance data matrix consisting of m/z, elution time pairs. Sets of ions whose abundances are correlated with each another are extracted. While diverse approaches have been described, to our knowledge none of them have been fully implemented and tested for general-purpose use.

Method

The model peak method of Dromey et al. [5] was selected as the basis for spectrum extraction both because it has been shown to produce reliable results in large-scale tests and because it followed an approach similar to that of an analyst. However, its ability to extract weak signals was found to be poor. The origin of this problem was its inability to establish thresholds to enable it to distinguish signal from noise. This problem was solved in the present work by processing ion abundances in signal-to-noise units rather than as absolute abundances. This permitted the rational setting of the thresholds throughout the spectrum extraction process. Chemical identification was based on an optimized spectrum comparison function described earlier [8], and extended to incorporate other information derived from GC/MS data. Analysis of test results led to the development of further refinements in the spectrum comparison process. The overall process involves four sequential steps: 1) noise analysis, 2) component perception, 3) spectral "deconvolution", 4) compound identification.

References

[1] "Reconstructed Mass Spectra, A Novel Approach for the Utilization of Gas Chromatograph—Mass Spectrometer Data", Biller, J.E.; Biemann, K. Anal. Lett. 1974 7 515-528.

[2] "Spectral Deconvolution for Overlapping GC/MS Components" Colby, B. N. J. Amer. Soc. Mass Spectrom. 1992 3 558-562.

[3] "Software-Based Mass Spectral Enhancement to Remove Interferences from Spectra of Unknowns", Herron, N.R.; Donnelly, J.R.; Sovocool, G.W. J. Amer. Soc. Mass Spectrom. 1996 7 598-604.

[4] "Automated Extraction of Pure Mass Spectra from Gas Chromatographic / Mass Spectrometric Data", Pool, W.G.; Leeuw, J.W.; van de Graaf, B. J. Mass Spectrom. 1997 32 438-443.

[5] "Extraction of Mass Spectra Free of Background and Neighboring Component Contributions from Gas Chromatography/Mass Spectrometry Data" Dromey, R.G; Stefik, M.J.; Rindfleisch, T.C; Duffield, A.M. Anal. Chem. 1976 48 (9) 1368-1375.

[6] "An Evaluation of Automated Spectrum Matching for Survey Identification of Wastewater Components by Gas Chromatography-Mass Spectrometry" Shackelford, W.M.; Cline, D.M.; Faas, L; Kurth, G. Analytica Chim. Acta 1983 146 25-27.

[7] "Improvement of Algorithm for Peak Detection in Automatic Gas Chromatography-Mass Spectrometry Data Processing" Hargrove, W.F.; Rosenthal, D.; Cooley, P.C. Anal. Chem. 1981 53 538-539.

[8] "Optimization and Testing of Mass Spectral Library Search Algorithms for Compound Identification" Stein, S.E.; Scott, D.R. J. Amer. Soc. Mass Spectrom. 1994 5 859-866.

2   Getting Started

This chapter is written in "tutorial style" and it is <u>very highly recommended</u> that you read it and follow through the worked examples. The whole process should take about 30 minutes and will provide you with an excellent appreciation of the capabilities of AMDIS.

This chapter starts off by explaining how to simultaneously view AMDIS and its *Help* window (**2.1 Simultaneously Viewing AMDIS and its Help Window**) and how to get AMDIS into a known state for running a tutorial (**2.2 Getting AMDIS into a Known State**).

This is followed by a general introduction to, and brief description of, the two basic window modes of AMDIS (**2.3 Confirm Window and Results Window Overview**) and then a short introduction to the **various** analysis types that AMDIS supports (**2.6 Analysis Types Overview**).

The two windows are the *Confirm* window (**2.4 Confirm Window Appearance**) and the *Results* window (**2.5 Results Window Appearance**). You are given just enough information about these to get the most benefit from the tutorial sections that follow; one for each analysis type:

Simple	**2.7 Analysis Type - Simple**
Use Retention Time	**2.8 Analysis Type - Use Retention Time**
RI Calibration/Performance	**2.9 Analysis Type - RI Calibration/Performance**).
Use Retention Index Data	**2.10 Analysis Type - Use Retention Index Data**).
Use Internal Standards for RI	**2.11 Analysis Type - Use Internal Standards for RI**
Use RI Calibr. Data + Internal Std.	**2.12 Analysis Type - Use RI Calibr. Data + Internal Std.**).
Performance Check for RI	**2.13 Analysis Type - Performance Check for RI**).

Each tutorial describes any pre-requisites, the operational flow and some post-analysis options relevant to the particular analysis type. Each tutorial utilizes demo data files and will mainly use default settings of AMDIS parameters.

Throughout the tutorials, the opportunity is occasionally taken to briefly introduce a related topic.

Although each tutorial section is self-contained, it is recommended to initially go through them in order as they do to a certain extent lead on from one to the other. If later you want to simply refresh your memory on one section, go to it directly.

Finally, this chapter recaps what you have learned (**2.14 Recap**).

2.1   Simultaneously Viewing AMDIS and its Help Window

If at any time you wish to simultaneously view AMDIS and its help window, do the following:

1)   Activate the help window using the **Help | Contents** menu command.

2)   Ensure that AMDIS and its help window are not minimized.

3)   Ensure that all other windows are minimized.

4)   For operating systems from *Windows 2000* onwards, right-click in any "unused" area in the *Windows* task bar to bring up its menu and click **Tile Windows Vertically**. The task bar is usually at the bottom of the screen and has the **start** button at the left.

>   **Note:**   For earlier *Windows* operating systems, there will be an equivalent operation.

To exit from this mode, do the following:

5)   Right-click in any "unused" area in the *Windows* task bar and click **Undo Tile**.

2.2 Getting AMDIS into a Known State

To ensure that the worked examples described in this chapter can be smoothly followed, it is <u>essential</u> that AMDIS is in a known state (the same as when it has just been installed). To establish this known state, please do the following:

1) If AMDIS is already active, exit from it.

2) Using standard Windows functionality, locate file *onsite.ini* in the *AMDIS32* folder.

3) Delete the *onsite.ini* file.

4) Activate AMDIS in your normal manner and for the purposes of the tutorials select **Agilent Files** in the *Set Default Instrument* dialog.

At this point all of the AMDIS parameters are set at default values, but unless AMDIS has never been used before it is possible that some of the tutorials' data files already have result files in existence. This is not a problem because the tutorials will recreate result files. However, there may be small discrepancies between what the tutorial describes as the initial state of the display and what you actually observe. If you want to avoid this, proceed as follows:

5) When you are told to open a file and results are immediately displayed (because they already exist), click **File | Open** and select any other file. Click **Open**. The *Delete Result Files* dialog appears.
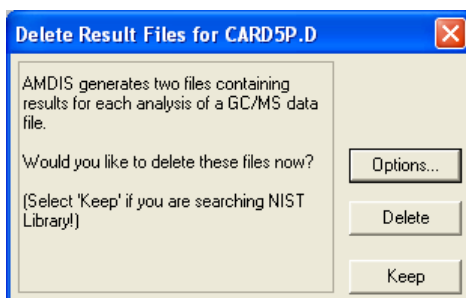
Figure 1 - Delete Results File dialog

6) Click **Delete** and the newly selected file opens.

7) Click **File | Open** and select the file you originally opened according to the tutorial's instructions. Click **Open**.

You are now in the correct state for the tutorial.

2.3 Confirm Window and Results Window Overview

AMDIS offers two significant user interfaces (windows) for you to work with.

The *Confirm* window is a full-functionality window, offering a menu bar and one or more included "child" windows (for example, **Figure 4 - Confirm window showing the graphical results of an analysis for a target**). Each child window has a button bar, several graphical areas and a text area. The menu bar provides access to the full functionality of AMDIS and the graphical areas permit interactive manipulations and detailed analysis of the results of AMDIS processing.

The *Confirm* window is fully described in Chapter **3 AMDIS Confirm Window** and will be the window of choice for most users.

The *Results* window offers a more limited set of functionality than the *Confirm* window and in particular does not display graphics, nor permit library manipulations. It can be useful when it is sufficient to simply review the results of AMDIS processing (for example, **Figure 7 - Results window shows only the non-graphical results of an analysis**).

The *Results* window is fully described in Chapter **4 AMDIS Results Window**.

> **Note:** It is straightforward to swap between these two windows:

In the *Confirm* window, click **File | Go to Results** to go to the *Results* window.

In the *Results* window, click **Confirm** to go to the *Confirm* window.

Note: AMDIS will always start up in the *Confirm* window, irrespective of the prevailing window selection when it was last closed down.

2.4   Confirm Window Appearance

The *Confirm* window has two basic appearances according to whether or not it is showing the results of an analysis.

> **Note:**   When results are being shown, the contents of the *Confirm* window are also regulated using the **Mode** command (**3.1.3 Mode Menu**).

When a new data file is opened that does not have previously kept results, the *Confirm* window has two areas.

The two areas are:

- *Chromatogram Display*

- *Mass Spectral Display*
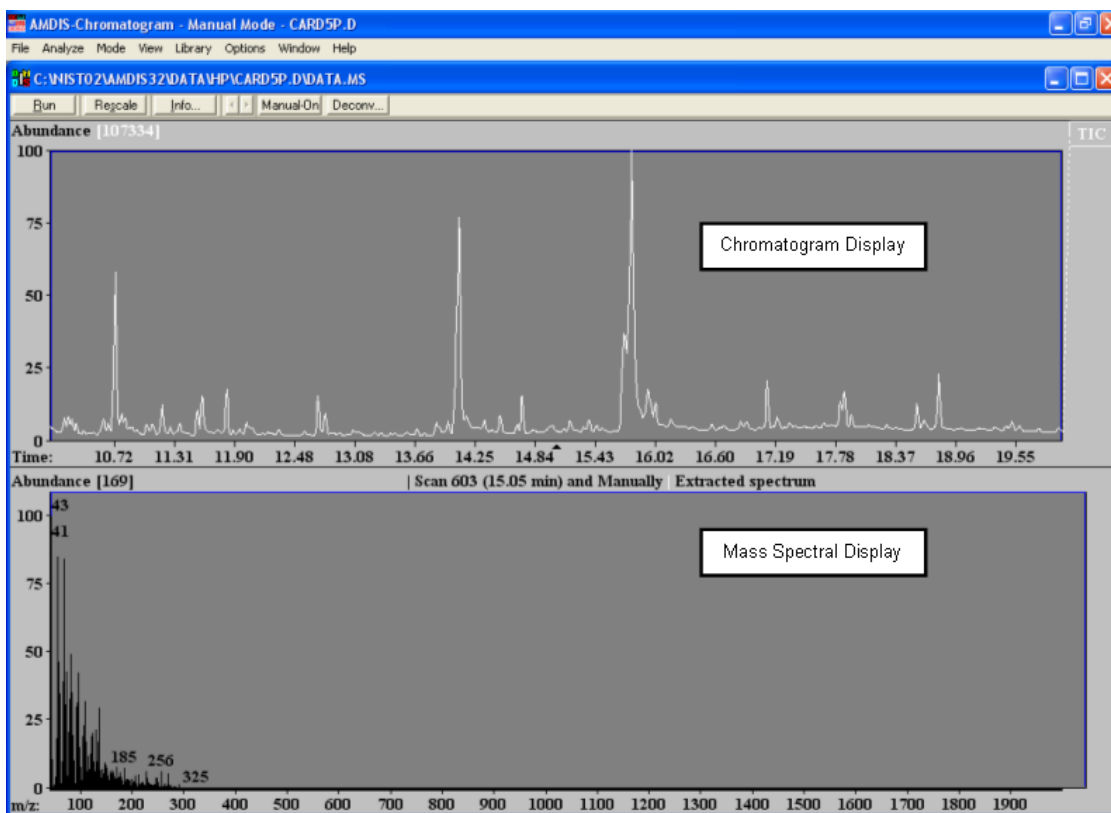
Figure 2 - Confirm window showing unprocessed data

When a data file has been analyzed, or a file is opened with previously kept results, the *Confirm* window can now have four basic areas (**Figure 3 - Confirm window showing the graphical results of an analysis for a component** and **Figure 4 - Confirm window showing the graphical results of an analysis for a target**).

The four areas are:

- *Chromatogram Display*   see **2.4.1 Chromatogram Display**

- *Profile Display*   see **2.4.2 Profile Display**

- *Information Lists*   see **2.4.3 Information Lists**

- *Mass Spectral Display*   see **2.4.4 Mass Spectral Display**

If you wish to adjust the scaling of the *Chromatogram Display* or *Mass Spectral Display* areas you can do this interactively (**2.4.5 Changing the Display Range**).
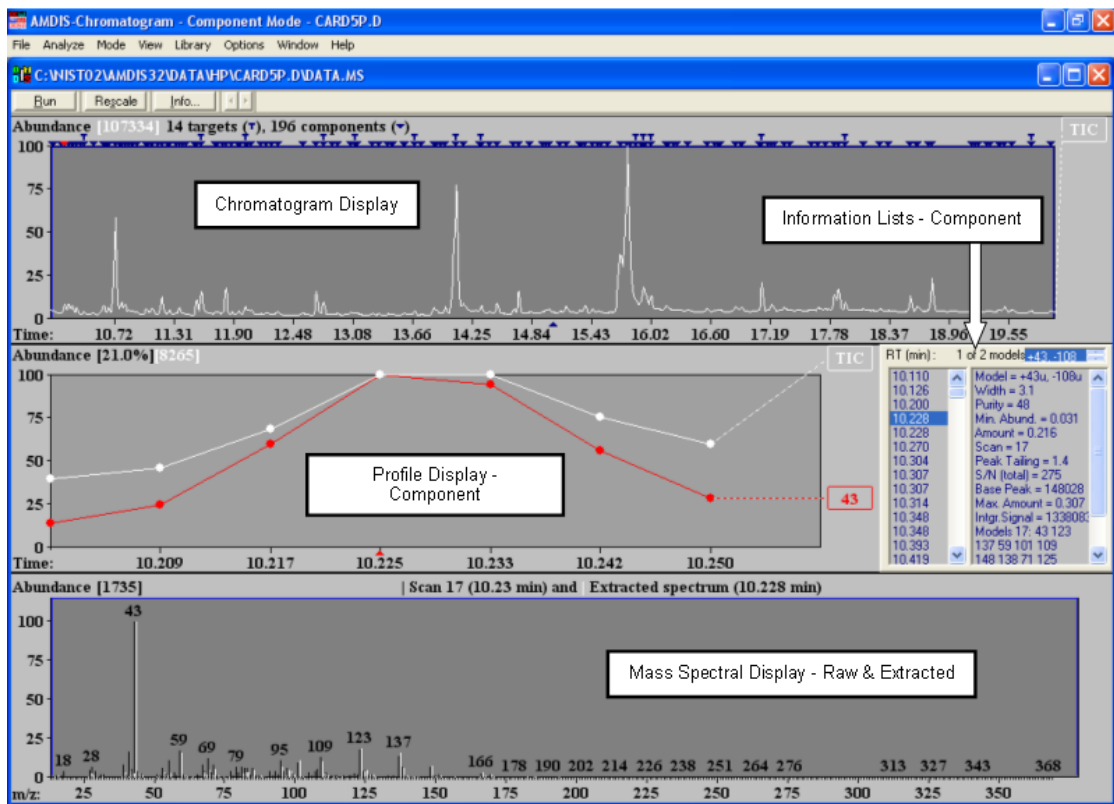
Figure 3 - Confirm window showing the graphical results of an analysis for a component
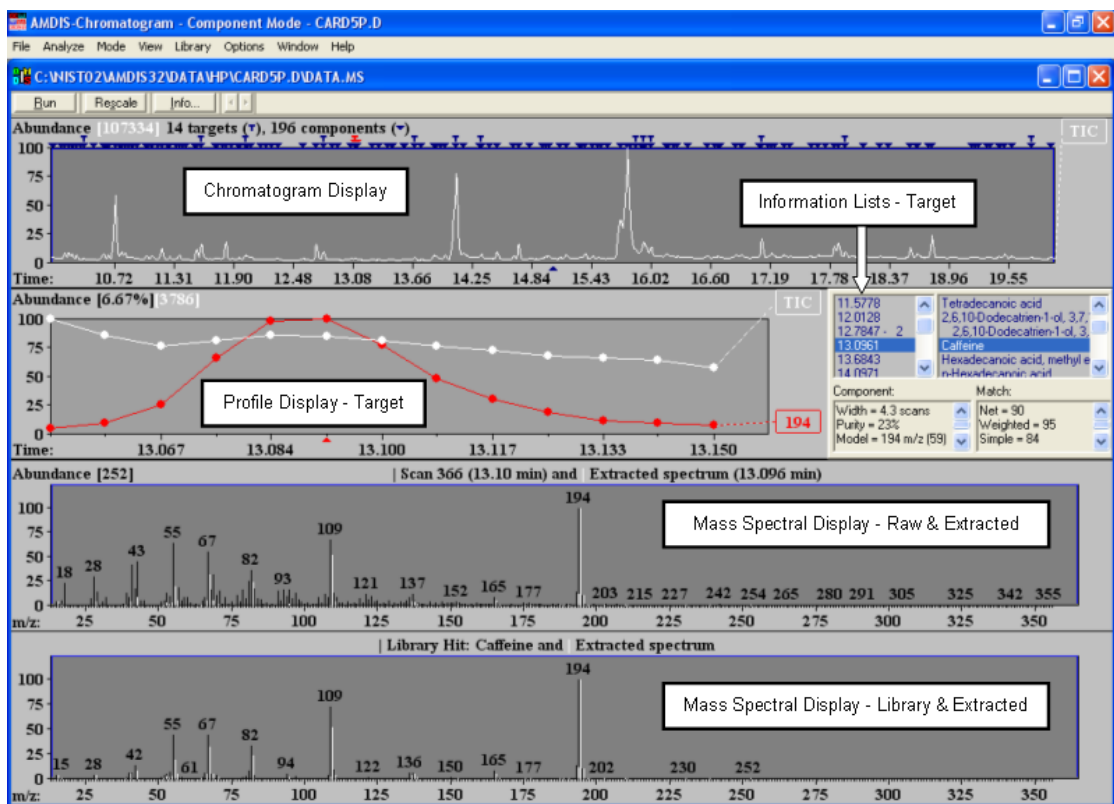
Figure 4 - Confirm window showing the graphical results of an analysis for a target

To give yourself a little more more screen space for viewing the displayed AMDIS data, you may wish to click the child window's **Maximize** button. You can swap back at any time by clicking the **Restore Down** button.
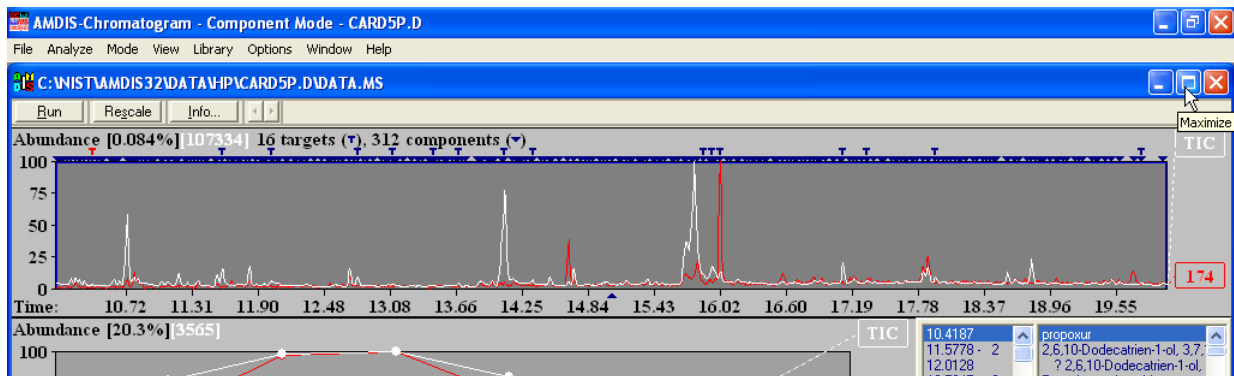
Figure 5 - Before Maximize
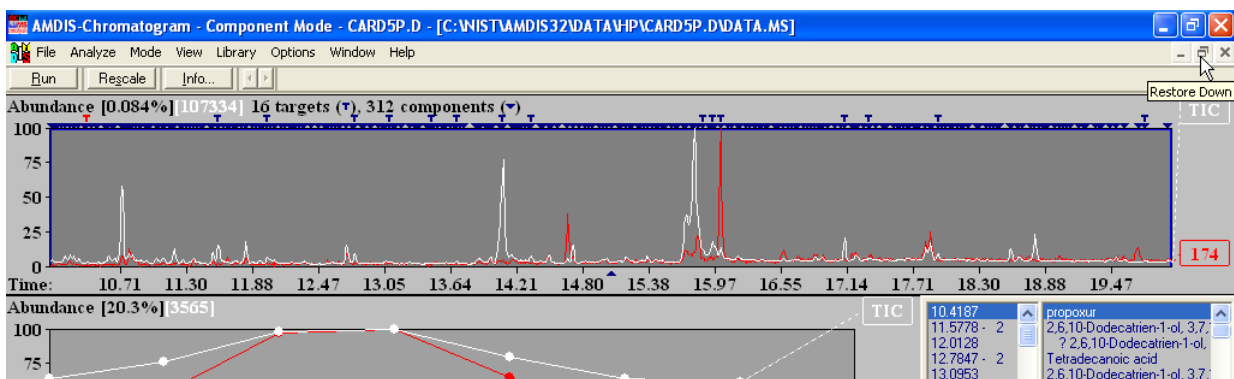
Figure 6 - After Maximize

2.4.1   Chromatogram Display

The *Chromatogram Display* area generally shows the TIC chromatogram, but may also show one or more mass chromatograms.

The x-axis shows retention time in decimal minutes and the y-axis is a relative abundance percentage scale with the absolute abundance value representing 100% given at the top left of the display; eg, **107334** in **Figure 3 - Confirm window showing the graphical results of an analysis for a component** and **Figure 4 - Confirm window showing the graphical results of an analysis for a target**.

To the right of the abundance value for analyzed data (and as appropriate) are the numbers of found targets (**T** symbol) and components (▼ symbol); where the count of components includes the targets.

Along the top of the chromatogram graphical display area are rows of **T** and ▼ symbols, which are colored dark blue and are positioned at the retention times at which these compounds were determined by the deconvolution process.

The currently selected target or component is signified by its symbol being colored red and the rest of the *Confirm* window's data relate to this compound.

You can click on any of the symbols to make it the current selection.

For full details of the *Chromatogram Display* area see **3.3.1 Chromatogram Display**.

2.4.2   Profile Display

The *Profile Display* area generally shows the TIC chromatogram and major characteristic ion(s) of the deconvoluted compound over the retention time window in which is was determined. The y-axis is a relative abundance percentage scale with the absolute abundance value representing 100% given at the top left of the display; eg, **3786** in **Figure 4 - Confirm window showing the graphical results of an analysis for a target**.

For full details of the *Profile Display* area see **3.3.2 Profile Display**.

2.4.3   Information Lists

The *Information Lists* area changes somewhat according to whether the currently selected compound is being displayed as a component (▼) or as a target (т).

For a component there are two sections side-by-side (**2.4.3.1 Component**).

For a target there are four sections arranged more or less as quadrants (**2.4.3.2 Target**).

For full details of the *Information Lists* area see **3.3.3 Information Lists**.

2.4.3.1   Component

The left-hand section is a list of retention times for all components.

The right-hand section provides various details about the component as determined during the deconvolution process.

You can click on a retention time value and all areas of the whole display will change to reflect the chosen component.

2.4.3.2   Target

The upper-left quadrant is a list of retention times for all targets.

The upper-right quadrant is a list of "hits" (identifications) from a target library.

The lower two quadrants provide various details about the target as determined during the deconvolution process.

You can click on a value in either upper section and all areas of the whole display will change to reflect the chosen retention time or hit.

2.4.4   Mass Spectral Display

The *Mass Spectral Display* area will change according to whether the currently selected compound is being displayed as a target (т) or as a component (▼).

The x-axis shows m/z values and mass spectral peaks are displayed rounded to integer m/z.

For a component there is simply one *Mass Spectral Display* showing the raw (original) spectrum overlaid with the extracted spectrum resulting from the deconvolution process. Ions from the raw spectrum are shown in black and those from the extracted spectrum in white. Note that, for clarity they are slightly offset from each other along the x-axis.

The y-axis is a relative abundance percentage scale with the absolute abundance value representing 100% given at the top left of the display; eg, **1735** in **Figure 3 - Confirm window showing the graphical results of an analysis for a component**.

For a target there are two *Mass Spectral Displays*. The upper display shows the same information as just described for a component. The lower display is similar, but shows a library hit (black ions) overlaid with the extracted spectrum (white ions) and again they have a slight offset for visual clarity.

Since library spectra are only stored with relative ion abundances, there is no absolute abundance value representing 100% given at the top left of this display.

For full details of the *Mass Spectral Display* area see **3.3.4 Mass Spectral Display**.

2.4.5   Changing the Display Range

Often the *Chromatogram Display* will be very "crowded" with target and component symbols overlapping. In order to see the detail of the chromatogram(s) and to easily choose (by clicking on it) a specific target or component symbol, it is necessary to first "zoom" (magnify) the display. Similarly, you may wish to zoom the *Mass Spectral Display* to see greater detail of the mass spectral peaks.

By using the "click-drag-release" technique, the display can be zoomed individually in either the x-direction or the y-direction, or in both directions simultaneously.

Note: "Click-drag-release" is as follows. First click and hold the left mouse button at one end of the desired linear range, or one corner of the desired rectangular area. Now drag the mouse pointer to the opposite end or corner of the desired selection and finally release the button.

To zoom in the x-direction, do a click-drag-release operation just below the x-axis. On releasing the mouse button, the selected area is redrawn to fill the whole width of the display area.

To zoom in the y-direction, do a click-drag-release operation just to the left of the y-axis. On releasing the mouse button, the selected area is redrawn to fill the whole height of the display area.

To zoom a rectangular area of the display, do a click-drag-release operation within the display area to "rubber band" (mark out) the area to be magnified. On releasing the mouse button, the selected area is redrawn to fill the whole display area.

To cancel the effects of a zoom, right-click anywhere in the relevant display and click **Unzoom** or, if available, **Unzoom All** on the offered menu (for more details see **3.3.1.1 Context Menu** for the *Chromatogram Display* and **3.3.2.1 Context Menu** for the *Mass Spectral Display*).

2.5 Results Window Appearance

Whereas the *Confirm* window shows results for targets and components, the *Results* window only shows information for targets.

The *Results* window has 3 basic areas and is fully described in Chapter **4 AMDIS Results Window**.
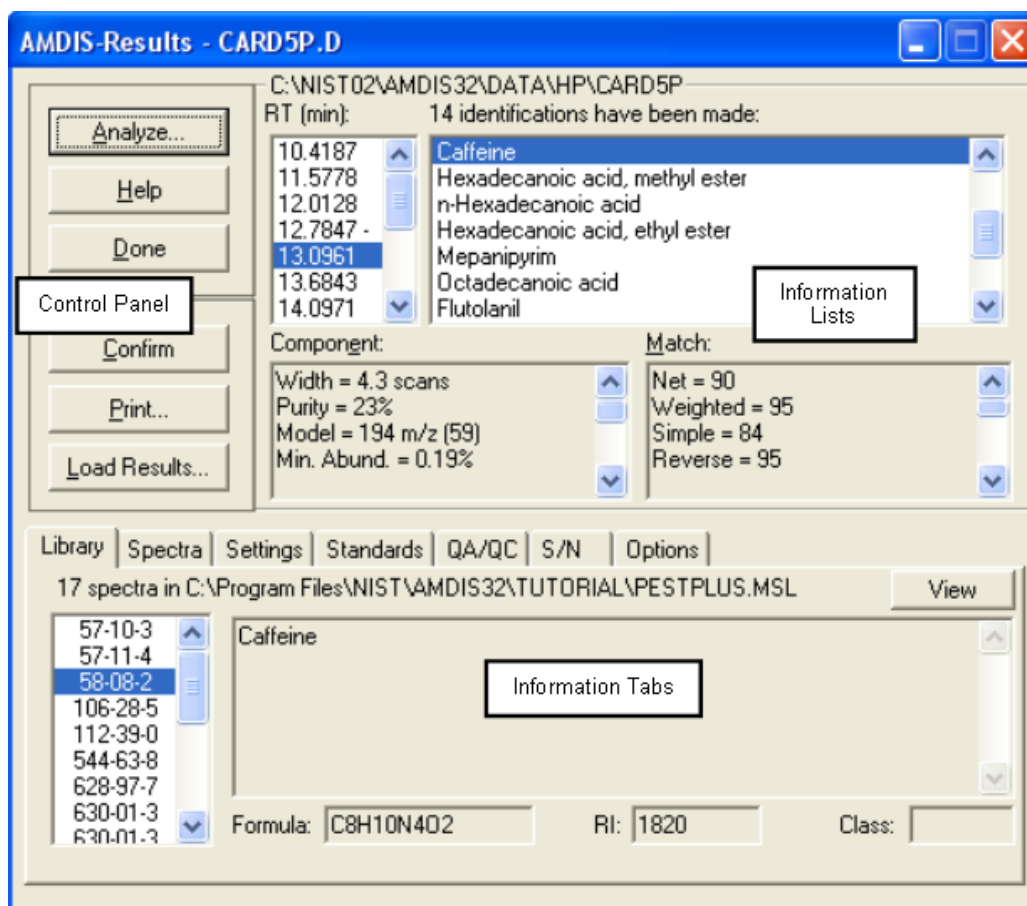
Figure 7 - Results window shows only the non-graphical results of an analysis

The three areas of the *Results* window are:

- *Control Panel*

- *Information Lists*

- Information Tabs

The *Control Panel* is a series of command buttons that control the operations available in the *Results* window.

The *Information Lists* area has four sections arranged more or less as quadrants. The upper-left quadrant is a list of retention times for all targets. The upper-right quadrant is a list of "hits" (identifications) from a target library. The lower two quadrants provide various details about the target as determined during the deconvolution process. When you click on a value in either upper quadrant, the lower two quadrants and certain of the tabs in the *Information Tabs* area will change to reflect the chosen retention time or hit.

The *Information Tabs* area has a number of selectable tabs. The **Library** and **Spectra** tabs change to reflect the selection in the *Information Lists*. Other tabs show data that are independent of the particular targets and so do not change as different selections are made.

2.6 Analysis Types Overview

AMDIS supports several different "analysis types" for determining whether or not deconvoluted spectra from samples match any of the target compounds (targets) stored in a referenced "target library". The analysis type you choose will depend to a large extent on the type of data that you are working with. Of particular significance is whether or not you are using retention indices and, if you are, whether you are utilizing internal standard compounds within a sample for their computation, or whether you are using an external calibration file holding retention index values.

For full details of the analysis process and analysis types see **6 Analysis**.

The analysis types are:

→   *Simple*

Only mass spectra are used in determining target matches; so no use is made of retention time or retention indices (**2.7 Analysis Type - Simple**).

→   *Use Retention Time*

Following a target match determined by mass spectral comparison, the compound's retention time is compared against the library value to confirm the match. (**2.8 Analysis Type - Use Retention Time**).

→   *RI Calibration/Performance*

A clean mixture of known composition is acquired and the data used to create an *RI Calibration Data* file for use by other appropriate analysis types (**2.9 Analysis Type - RI Calibration/Performance**).

→   *Use Retention Index Data*

Following a target match determined by mass spectral comparison, the compound's retention index is computed using an external, *RI Calibration Data* file, such that the quality of the match can be further assessed (**2.10 Analysis Type - Use Retention Index Data**).

→   *Use Internal Standards for RI*

Known compounds (the "internal standards") are co-injected with the sample. Mass spectral matching determines which targets and internal standards are present and the retention times of the found internal standards are used to compute retention indices for the targets, in order that the quality of their match can be further assessed (**2.11 Analysis Type - Use Internal Standards for RI**).

→   *Use RI Calibr. Data + Internal Std.*

Internal standards are co-injected with the sample, but target matching is determined as for the *Use Retention Index Data* type and does not take any account of the internal standard retention times. The internal standards are simply used for reporting purposes and permit you to check that the acquisition system is functioning correctly (**2.12 Analysis Type - Use RI Calibr. Data + Internal Std.**).

→   *Performance Check for RI*

A clean mixture of known composition is acquired and the data for "performance check" compounds are recorded and reported so that you can determine how well the acquisition system is performing (**2.13 Analysis Type - Performance Check for RI**).

2.7 Analysis Type - Simple

This example illustrates the most basic operation of AMDIS, which is to locate and extract significant spectra from a GC/MS data file and to then compare them against an AMDIS *Target Compounds Library*.

It also describes the interactive ability to search a component's spectrum against the main NIST mass spectral database and any other NIST-format libraries (**2.7.1 Searching the NIST Database**).

> **Note:** A *Target Compounds Library* has a ".MSL" file extension.

For more detail about this analysis type see **6.1.1 Simple**.

It is a prerequisite of this type that a *Target Compounds Library* exists. For the purpose of this example, one is provided, but if you want to learn more about them, refer to section **8.1 Library Types**.

Before following the instructions below, ensure that AMDIS is in a known state by following the instructions in **2.2 Getting AMDIS into a Known State**. There should now be an "empty" *Confirm* window displayed.

Proceed as follows:

1) Click **File | Open** and in the *Select Data File* dialog, navigate to the *\NIST08\AMDIS32\TUTORIAL* folder, select the file *CARD5P.D* and click **Open**. The window shows the file's TIC in the upper half (*Chromatogram Display*) and the spectrum at the center of the displayed TIC range is shown in the lower half (*Mass Spectral Display*); **Figure 2 - Confirm window showing unprocessed data**.

2) Click **Analyze | Analyze GC/MS Data** to invoke the *Analyze GC/MS Data* dialog.
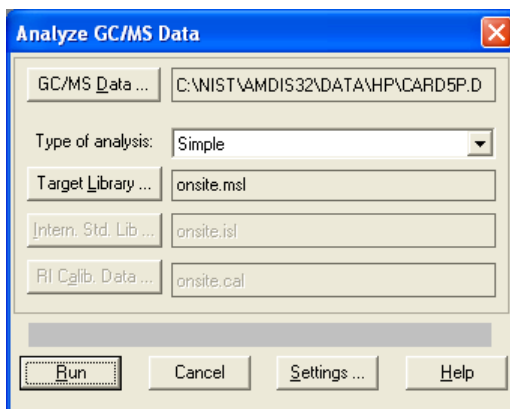
Figure 8 - Analyze GC/MS Data dialog (Simple)

Note that, the text box to the right of the **GC/MS Data** button shows the current data file selection and **Type of analysis** shows **Simple**.

3) Click **Target Library** to invoke the *Analysis Settings* dialog, showing the **Libr.** tab.
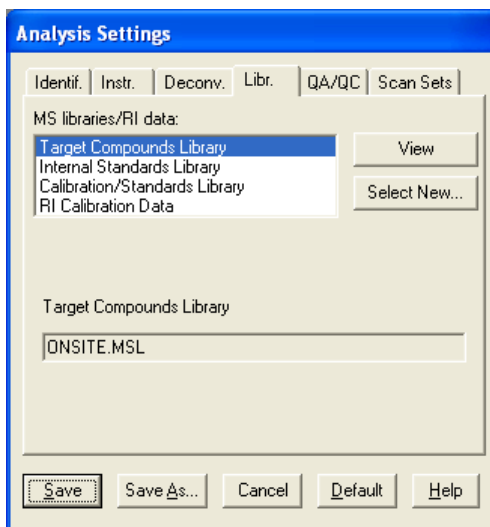
Figure 9 - Analysis Settings dialog (Libraries tab)

Click **Select New** and in the *Target Compounds Library* dialog, locate and select the file *PESTPLUS.MSL*.

> **Note:** *PESTPLUS.MSL* and other supplied libraries are normally installed in *AMDIS32\LIB*.

Click **Open** to return to the *Analysis Settings* dialog with the full path of the *PESTPLUS.MSL* file shown after **Target Compounds Library** in the lower half of the dialog.

4) Click **Save** to return to the *Analyze GC/MS Data* dialog.

Figure 10 - Analyze GC/MS Data dialog readied (Simple)

The **Intern. Std. Lib** and **RI Calib. Data** buttons in the dialog are disabled. A *Simple* analysis does not involve the use of internal standards, nor does it take any account of retention index information. We are now ready to perform the *Simple* analysis. Click **Run**.

5) Progress dialogs indicate the processing operations being performed and within a few seconds the *Confirm* window is redisplayed showing the results of the deconvolution process for a *Simple* analysis.
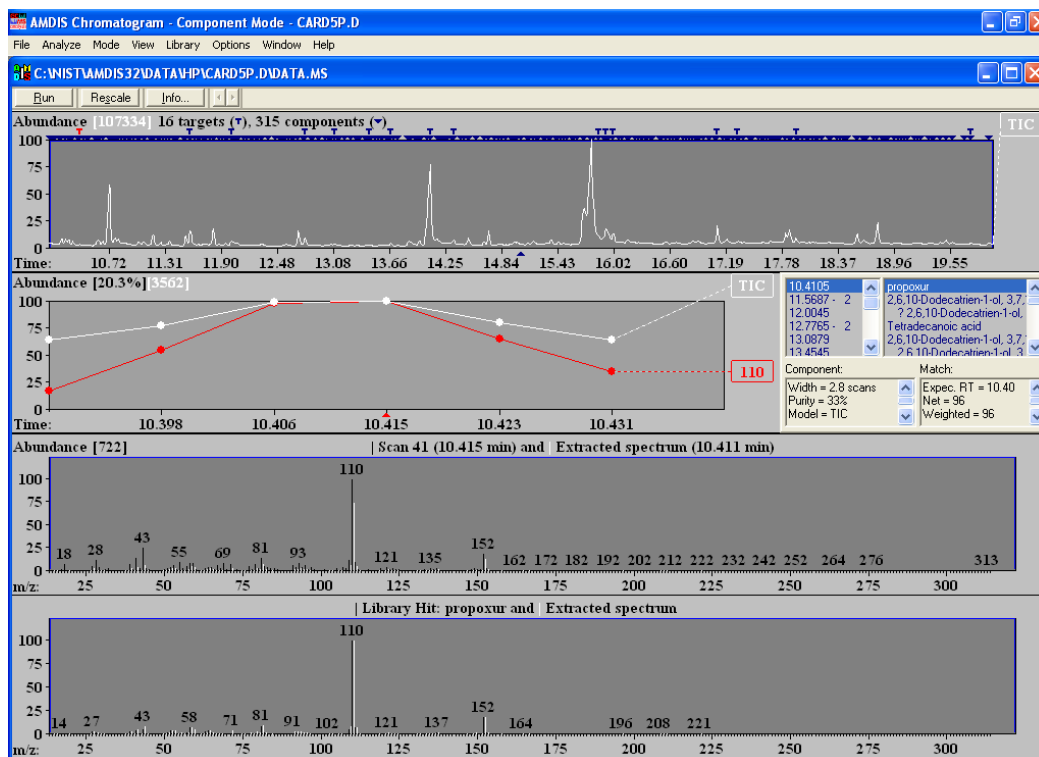
Figure 11 - The results of a Simple analysis

The file's TIC is presented in the *Chromatogram Display* area and you will see that some, but not all, of the strong peaks have a **т** mark above them. These are the components that have been identified as target compounds in the *PESTPLUS Target Compounds Library*; where 16 of its 17 entries have been identified.

There are also a large number of other components, as shown by the ▼ symbols without a **т** above them. These are compounds that have been extracted from the data file, but that have no match in the library.

6)   To see components in more detail, it is necessary to expand the display. As an example, position the mouse pointer at the top of the TIC graphical display area at about 15.5 min. While holding down the left mouse button, drag the mouse to the bottom of the TIC window at about 16.5 min. You will see a "rubber band" box as you drag the mouse and when you let go of the left mouse button the display expands and two unidentified and relatively significant peaks can be easily seen (at times 15.7060 min and 15.7768 min).
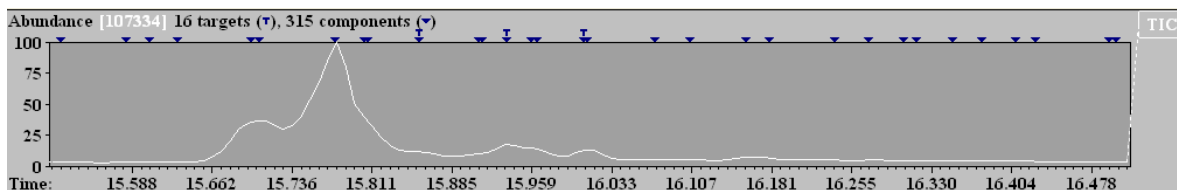
Figure 12 - Expanded chromatogram

If you make a mistake with the rubber-banding, you can always right-click anywhere in the *Chromatogram Display* area and then click **Unzoom** to correct the most recent rubber-banding, or if appropriate **Unzoom All** to immediately restore the display to its full range.

Individual components can be selected by either clicking on the blue ▼ above them, or by clicking on their retention time value in the *Information Lists* area. The currently selected component is identified by its ▼ changing in color from blue to red.

Note: The same selection mechanism and color changes apply when viewing targets (**т**). Also, as a component or target is selected, all of the other information in the *Confirm* window changes to reflect the new selection.

7) The deconvolution process does have a few parameters that can be changed by the user and so let's see where these are.

On the **Analyze** menu click **Settings** and in the *Analysis Settings* dialog, click **Deconv.** to select its tab.
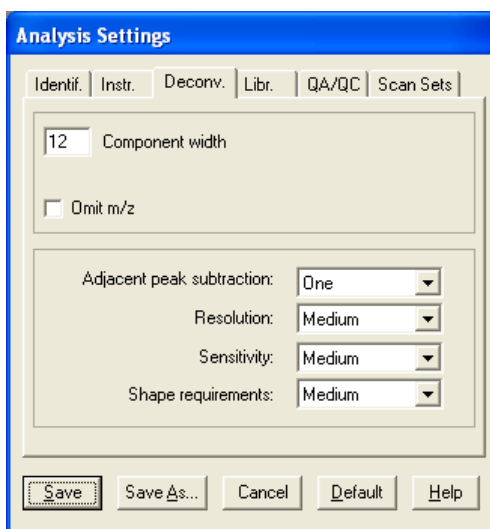
Figure 13 - Deconv. tab of Analysis Settings dialog

The *Analysis Settings* dialog is described in detail in **6.3 Analysis Settings**, but for now simply change **Sensitivity** to **Low** and click **Save**. A message appears noting that the parameters have changed and asking if you want to reanalyze. Click **Yes**.
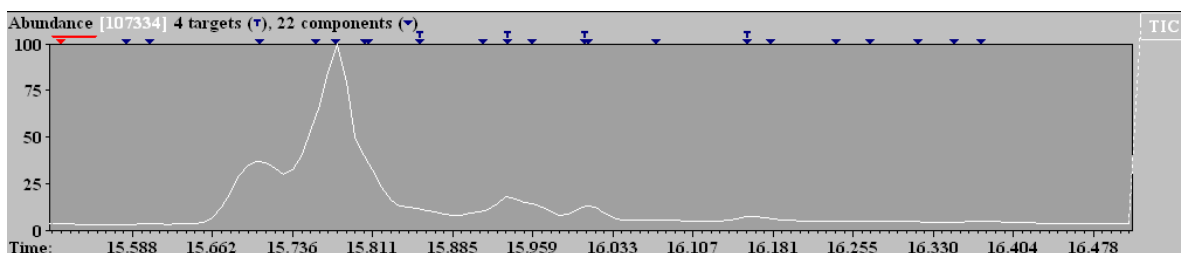
Figure 14 - After adjusted deconvolution parameters

You will see that the resulting display differs in 2 respects to the previous display.

First of all the header says **4 targets** and **22 components** (compared previously to 16 and 315, respectively). This is because when a deconvolution analysis is run, it applies to the current display range only. In the first instance the analysis was run when the full chromatogram was being displayed.

Note: The exact numbers reported for this range will depend on the actual retention time range you previously selected by rubber-banding.

Secondly there are anyway less components now in the displayed range (22 compared to about 33). Note in particular that a few closely paired components have been reduced to a single component (eg, around 15.91 min and 15.96 min). In some cases, additional targets might appear.

There are many parameters on the various tabs in the *Analysis Settings* dialog that control deconvolution, detection and reporting in AMDIS. Fortunately, in most cases the default values will either work "as is", or will only require minor changes.

2.7.1   Searching the NIST Database

From AMDIS you can search the full NIST mass spectral database with a component.

Continue as follows:

1) Click the ▼ above the large peak at 15.7768 min (or select it from the retention time list).

2) On the **Analyze** menu, click **Go to NIST MS Program**.

3) The NIST *MS Search* program is invoked and a dialog shows the progress of the search of the selected component's spectrum (this can take up to a minute or two when first invoked). The component will be identified as **Oleic Acid**, a component of the analyzed oil.

> **Note:** The exact library result here and elsewhere will depend on the installed libraries and the search parameters currently set in the NIST *MS Search* program. The result here occurs when using default search parameters and searching the *Main* and *Replib* libraries.

4) From the *MS Search* program use the ALT+TAB key, or the *Windows* task bar, to get back to AMDIS. Select the peak at 15.7060 min and click **Analyze | Go to NIST MS Program** to again search the NIST database. This peak will be identified as **9,12-Octadecadienoic acid (Z,Z)-**.

For further details see **3.1.2.6 Analyze | Go to NIST MS Program**.

2.8   Analysis Type - Use Retention Time

If you wish to use retention times as an additional aid in identifying targets, AMDIS can utilize those stored in a *Target Compounds Library*.

For more detail about this analysis type see **6.1.2 Use Retention Time**.

It is a prerequisite of this type that a *Target Compounds Library* exists. For the purpose of this example, one is provided, but if you want to learn more about them, refer to section **8.1 Library Types**.

Before following the instructions below, ensure that AMDIS is in a known state by following the instructions in **2.2 Getting AMDIS into a Known State**. There should now be an "empty" *Confirm* window displayed.

Proceed as follows:

1) Click **File | Open** and in the *Select Data File* dialog, navigate to the *\NIST08\AMDIS32\TUTORIAL* folder, select the file *CARD5P.D* and click **Open**. As normal, the window shows the file's TIC in the upper half (*Chromatogram Display*) and the spectrum at the center of the displayed TIC range is shown in the lower half (*Mass Spectral Display*); **Figure 2 - Confirm window showing unprocessed data**.

2) Click **Analyze | Analyze GC/MS Data** to invoke the *Analyze GC/MS Data* dialog and set **Type of analysis** to **Use Retention Time**.
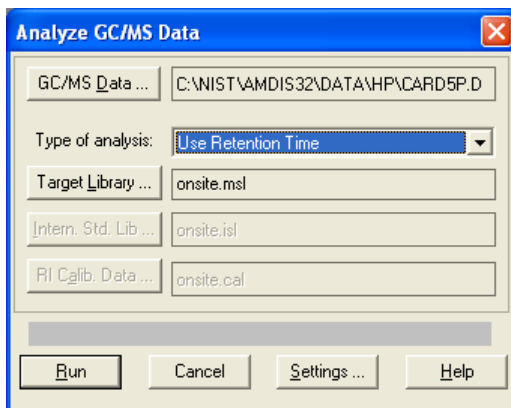
Figure 15 - Analyze GC/MS Data dialog (Use Retention Time)

3) Click **Target Library** to invoke the *Analysis Settings* dialog and on the **Libr.** tab (with **Target Compounds Library** highlighted) click **Select New**. In the *Target Compounds Library* dialog, navigate to the *AMDIS32\LIB* folder, select the file *PESTPLUS.MSL* and click **Open** to return to the *Analysis Settings* dialog.

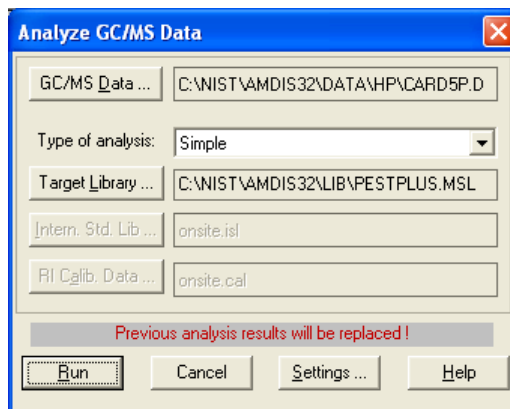4) Click **Save** to return to the *Analyze GC/MS Data* dialog.
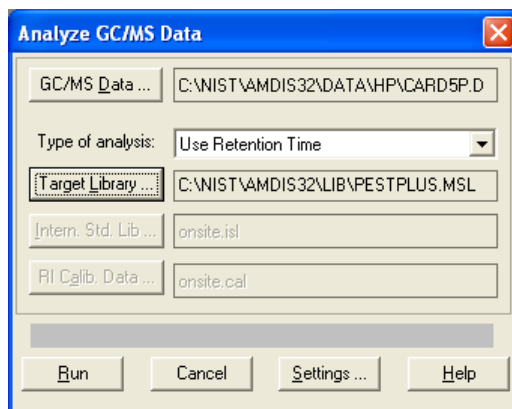
Figure 16 - Analyze GC/MS Data dialog readied (Use Retention Time)

The **Intern. Std. Lib** and **RI Calib. Data** buttons in the dialog are disabled. A *Use Retention Time* analysis does not involve the use of internal standards, nor does it take any account of retention index information, but there are a few analysis parameters that are specific to the *Use Retention Time* analysis.

5) Click **Settings** and in the *Analysis Settings* dialog click **Identif.**.
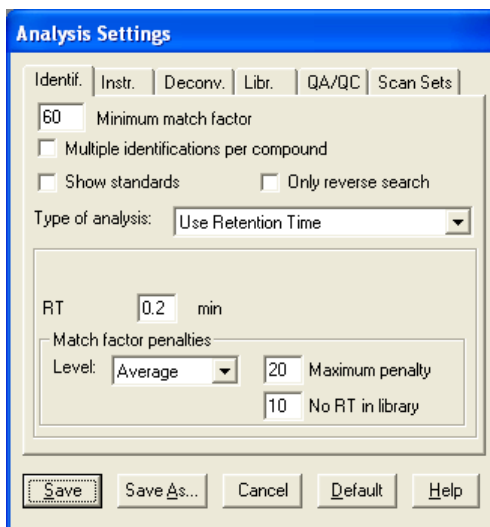
Figure 17 - Analysis Settings dialog (Use Retention Time)

RT and its associated **Match factor penalties** are used by this analysis type only. These and the other parameters on this tab are fully described in **6.3.1 Identif. (Identification) Tab**, but for now note that **No RT in library** is set to **10**.

6) We are now ready to perform the analysis. Click **Save** to exit the *Analysis Settings* dialog and return to the *Analyze GC/MS Data* dialog.

7) Click **Run** to start the analysis and within a few seconds the results are displayed.
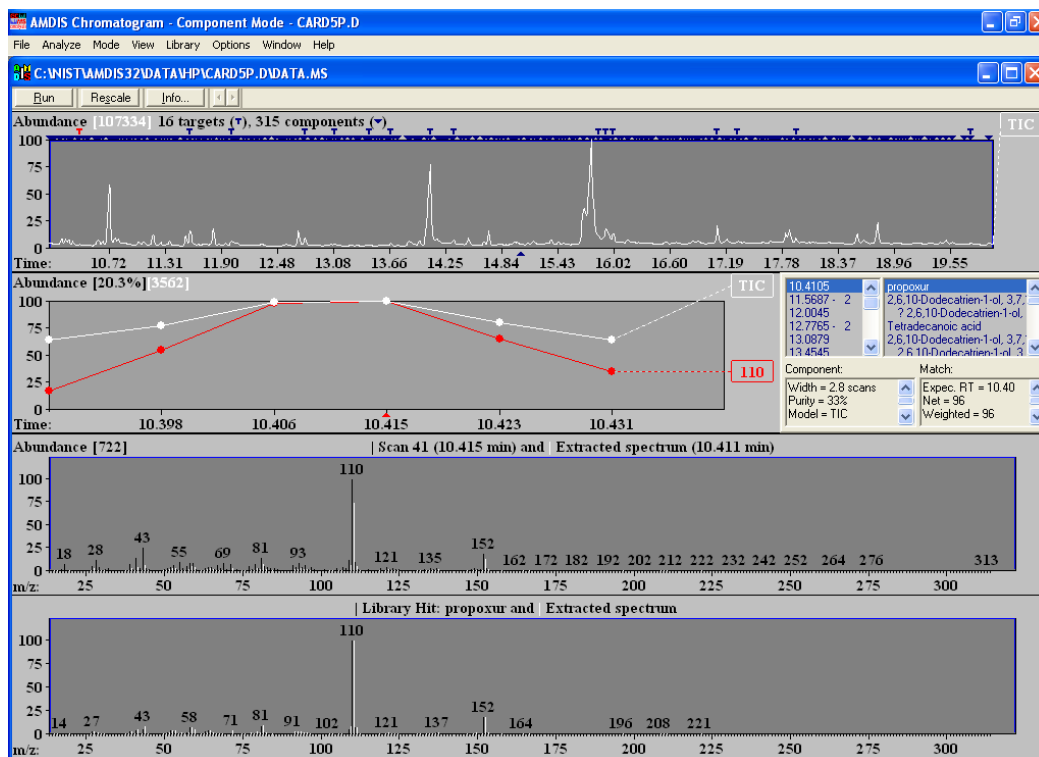
Figure 18 - Use Retention Time analysis results

8)   So that we can more easily examine the AMDIS results, on the **Mode** menu click **Target Only**. You will see that only targets are now displayed.

> **Note:**   You would switch back to viewing both by clicking **Mode | Component** (for more details see **3.1.3 Mode Menu**).

Whilst viewing targets, right-click anywhere in the upper right-hand quadrant of the *Information Lists* area and you will be presented with a 2-entry menu.
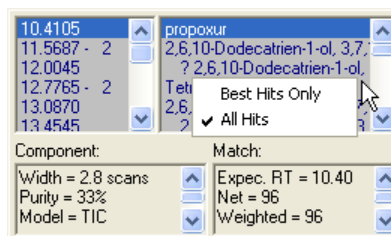
Figure 19 - Controlling the displayed hits

Click **Best Hits Only** to display only the single best hit for each target.

Now click on the target symbols above the TIC chromatogram display, or click on retention times in the *Information Lists* area, and you will conveniently see just the "best hit" name of each target is displayed; with the currently selected one highlighted. Further, the displayed **Component** and **Match** information relates to the selected target.

9)   If you scroll the **Match** information for a selected target, you will see a **RETENTION** value reported under **Corrections**.

Figure 20 - RETENTION correction to Net value

For most targets you will find that **RETENTION** is **0.0**; for example **propoxur**. However, **Mepanipyrim** has a value of **-10.0** and **Hexacosane** a value of **-1.0**.

A value of **0.0** means that the target has been found within the **RT** window you specified on the **Identif.** tab of the *Analysis Settings* dialog. The **No RT in library** value of 10.0 is given to **Mepanipyrim** because it does not have an RT value in the *PESTPLUS.MSL* library. When the library's expected retention time for **Hexacosane** is compared to the found retention time for it, the difference is greater than the **Identif.** tab's **RT** window and hence a penalty is computed (**-1.0** in this case).

The **RETENTION** penalty is applied to the computed **Net** (match) value for the target and so will reduce the latter's magnitude; hence making the component a less favorable hit for the target. In this way, as a target is found further away from its expected time, a larger penalty will be applied (up to the **Maximum penalty** value on the **Identif.** tab of the *Analysis Settings* dialog).

If at this time you are interested in further details of this logic, see **6.3.1 Identif. (Identification) Tab**.

2.9   Analysis Type - RI Calibration/Performance

If you wish to use retention indices as an additional aid in identifying targets, or for checking the performance of the acquisition system, AMDIS can utilize an *RI Calibration Data* file that holds a correspondence between retention time and retention index. The compounds used when creating an *RI Calibration Data* file are referred to as the "retention index standards". If compounds are used for performance checking purposes, they are referred to as "calibration check compounds".

Note: An *RI Calibration Data* file has a ".CAL" file extension.

For more detail about this analysis type see **6.1.4 Use Retention Index Data**.

You will learn that an *RI Calibration Data* file is used in three of the analysis types that we will soon examine.

This tutorial section illustrates how an *RI Calibration Data* file is created by analyzing a clean mixture of known composition (often a "C-series" or "normal hydrocarbons" sample).

It is a prerequisite of this analysis type that a *Calibration & Standards Library* exists. For the purpose of this example, one is provided, but if you want to read more about them, refer to section **8.1 Library Types**.

Note: A *Calibration & Standards Library* has a ".CSL" file extension.

Before following the instructions below, ensure that AMDIS is in a known state by following the instructions in **2.2 Getting AMDIS into a Known State**. There should now be an "empty" *Confirm* window displayed.

Proceed as follows:

1)   Click **File | Open** and in the *Select Data File* dialog, navigate to the *\NIST08\AMDIS32\TUTORIAL* folder, select the file *ALK3.D* and click **Open**.

2)   Click **Analyze | Analyze GC/MS Data** to invoke the *Analyze GC/MS Data* dialog and set **Type of analysis** to **RI Calibration/Performance**.
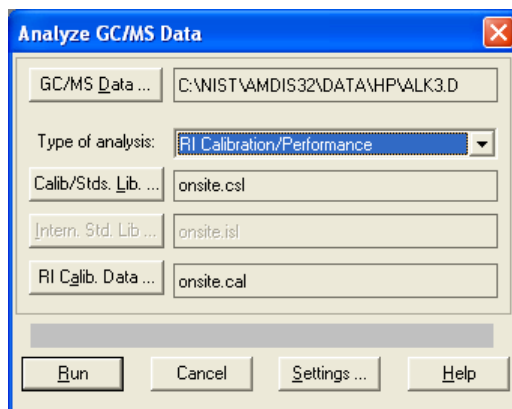
Figure 21 - Analyze GC/MS Data dialog on entry (RI Calibration/Performance)

Note: The button that was labeled **Target Library** for a *Simple* and *Use Retention Time* analysis is now labeled **Calib/Stds. Lib.**.

3) Click **Calib/Stds. Lib.** to invoke the *Analysis Settings* dialog and on the **Libr.** tab (with **Calibration/Standards Library** highlighted) click **Select New**. In the *Calibration/Standards Library* dialog, navigate to the *AMDIS32\LIB* folder, select the file *ALKANES.CSL* and click **Open** to return to the *Analysis Settings* dialog with the full path of the *ALKANES.CSL* file shown as the **Calibration/Standards Library** in the lower half of the dialog. Click **Save** to return to the *Analyze GC/MS Data* dialog.

4) Click **RI Calib. Data** to invoke the *Analysis Settings* dialog and on the **Libr.** tab (with **RI Calibration Data** highlighted) click **Select New**. Type **ALKANES.CAL** as the **File name**. Click **Open** to return to the *Analysis Settings* dialog with the full path of the *ALKANES.CAL* file shown as the **RI Calibration Data** in the lower half of the dialog. Click **Save** to return to the *Analyze GC/MS Data* dialog.
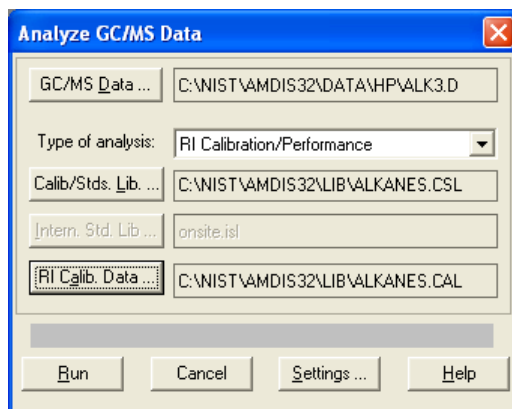
Figure 22 - Analyze GC/MS Data dialog readied (RI Calibration/Performance)

Note: If *ALKANES.CAL* already exists, the message **Previous RI calibration data will be replaced!** appears above the bottom row of buttons; otherwise this area is blank. When first running this example, the *ALKANES.CAL* file should not exist. In any case, it can be overwritten for this example, but should you in the future not want a file to be overwritten, simply click **RI Calib. Data**, follow the process again and choose a **File name** that does not already exist.

5) The **Intern. Std. Lib** button in the dialog is disabled. An *RI Calibration/Performance* analysis does not involve the use of internal standards. We are now ready to perform the analysis. Click **Run**.

A confirmation dialog appears for you to approve the file selections.
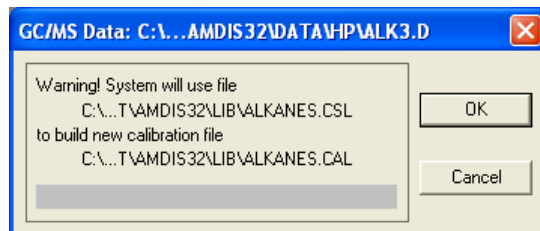
Figure 23 - Confirmation dialog (RI Calibration/Performance analysis)

If you saw an overwrite warning in the previous dialog, it will reappear here (at the bottom); otherwise the warning area is blank. Click **OK** to proceed.

6) Within a few seconds the *Confirm* window is redisplayed showing the results of the analysis.
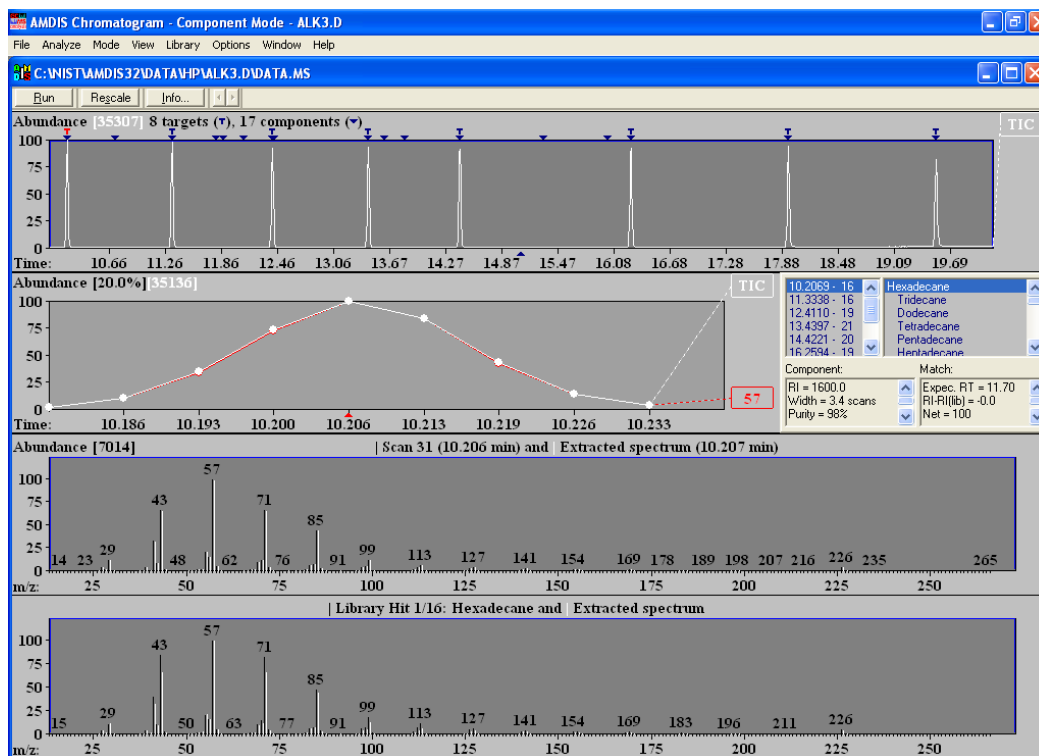
Figure 24 - RI Calibration/Performance analysis results

You will see that all of the TIC peaks have been detected as Targets.

7) The *RI Calibration Data* file (.CAL) that we have created is not directly viewable within AMDIS, but is a simple text file and can be easily viewed; for example, using *Notepad*.
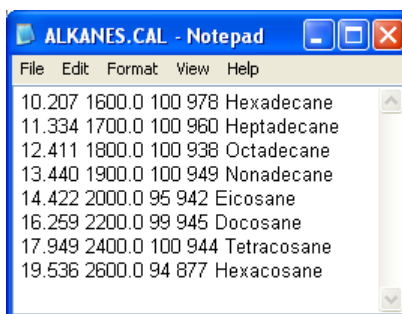
Figure 25 - RI Calibration Data file listing

The pertinent information that it contains per entry is a retention time and the corresponding retention index (for example, 10.207 and 1600.0, respectively, in the figure). The retention time is that of a deconvoluted peak and the retention index is taken from the corresponding entry in the *Calibration & Standards Library* (.CSL).

8) Finally, let's take a quick look at the *Calibration & Standards Library* that we started with.

On the **Library** menu click **Build One Library**. In the *Build One Library* editor that appears, click **Files** and in the next dialog click **Load Library**. Change **Files of type** to **Calibr. & Stds Library(*.CSL)** and navigate to the *AMDIS32/LIB* folder. Finally click *ALKANES.CSL* and **Open** to load the library into the *Build One Library* editor.
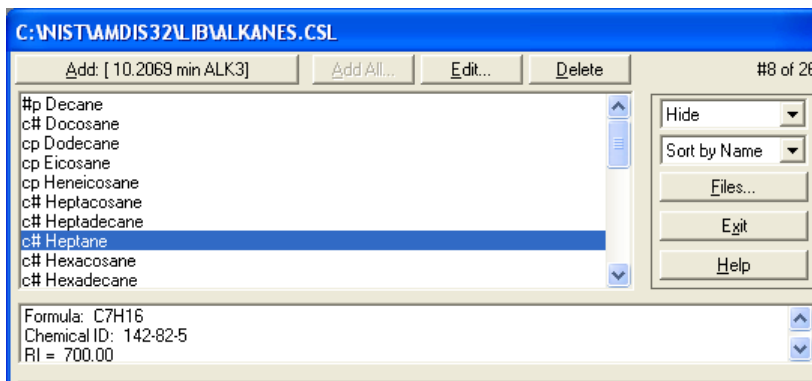
Figure 26 - Calibration & Standards Library dialog

Here you can see some of the target compounds that we have just successfully located in the *ALK3.D* data file.

Note: The "**c**" to the left of the compound name shows that the compound is a "calibration check" compound and so will be used in this analysis type. A "**p**" signifies that a compound will be used as a "performance check" compound.

If you click **Edit** you get the *Spectrum Editor (Calibration/Standards Library)* dialog for the currently highlighted compound and you can see the sort of information stored for an entry. Click **Cancel** to exit from the dialog and leave the values unchanged.

It's outside the scope of this section to discuss any further library details. As mentioned before, full details can be found in section **8 Libraries**; although we will mention a little more about this in the final tutorial section (**2.13 Analysis Type - Performance Check for RI**).

2.10   Analysis Type - Use Retention Index Data

If you have retention index (RI) information stored in a *Target Compounds Library*, an *RI Calibration Data* file can be used as an additional quality check on the results determined by the standard AMDIS spectral comparison process.

Note: A *Target Compounds Library* has a ".MSL" file extension and an *RI Calibration Data* file has a ".CAL" file extension.

For more detail about this analysis type see **6.1.4 Use Retention Index Data**.

The *RI Calibration Data* file is created as we have just seen using the *RI Calibration/Performance* analysis type (**2.9 Analysis Type - RI Calibration/Performance**).

In the *Use Retention Index Data* analysis type the *RI Calibration Data* file is acting as an "external calibration" file. In practice you only need to create a new one of these when the chromatographic conditions you are using have changed significantly such that the correlation between retention time and retention index in the calibration file is no longer accurate enough.

The *Use Retention Index Data* analysis type first identifies target compounds using spectral comparisons only (exactly as in the *Simple* analysis type). Next it utilizes the actual retention times of the identified targets to

compute their retention index values; by linear interpolation techniques utilizing the calibration data held in the *RI Calibration Data* file. Only then does it determine what to do with these retention indices.

If you have elected to utilize RI information (see below), for each identified target it will compare its calibration-computed RI value with the value held in the *Target Compounds Library* and will adjust the target's match value according to how good the fit is.

If you are not utilizing RI information, the target match value is not adjusted and the RI information is simply used in reporting the deviation between computed and library RI values.

It is a prerequisite of this analysis type that a *Target Compounds Library* and an *RI Calibration Data* file exist. Further, the calibration information can only be of any use for compounds in the library that have RI values stored for them. For the purpose of this example, suitable libraries are provided, but if you want to learn more about them, refer to **8.1 Library Types**.

Before following the instructions below, ensure that AMDIS is in a known state by following the instructions in **2.2 Getting AMDIS into a Known State**. There should now be an "empty" *Confirm* window displayed.

Proceed as follows:

1) Click **File | Open** and in the *Select Data File* dialog, navigate to the *\NIST08\AMDIS32\TUTORIAL* folder, select the file *CARD5P.D* and click **Open**.

2) Click **Analyze | Analyze GC/MS Data** and in the *Analyze GC/MS Data* dialog set **Type of analysis** to **Use Retention Index Data**. Use the **Target Library** and **RI Calib. Data** buttons to select the *PESTPLUS.MSL* and *ALKANES.CAL* files, respectively. The dialog should now resemble the following:
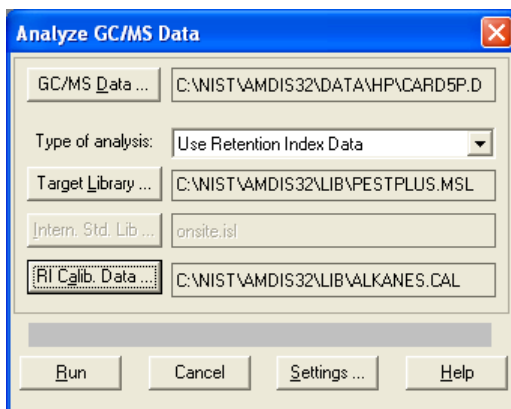
Figure 27 - Analyze GC/MS Data dialog (Use Retention Index Data)

Note: The **Intern. Std. Lib** button in the dialog is disabled. This type of analysis does not involve the use of internal standards.

3) Although retention index values are automatically computed in this analysis type, nevertheless we are able to tell AMDIS exactly how to use them. Click **Settings** and in the *Analysis Settings* dialog click **Identif.**.

It's outside the scope of this section to discuss the details of the parameters in the bottom half of the dialog, but they are fully described in **6.3.1 Identif. (Identification) Tab**. For now, note that the **Match factor penalties** group has its **Maximum penalty** value set at **20**.
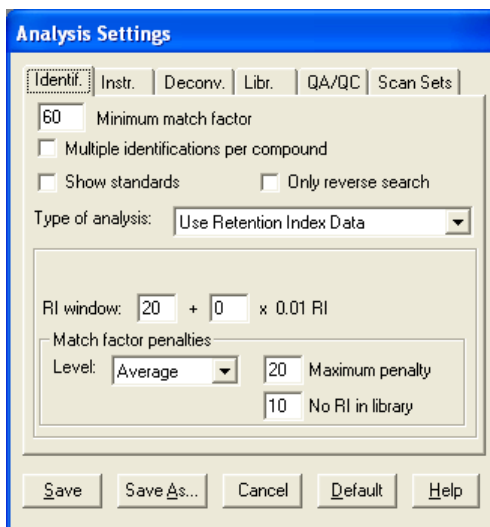
Figure 28 - Settings dialog (Use Retention Index Data)

4) We are now ready to perform the analysis. Click **Save** to exit the *Analysis Settings* dialog and return to the *Analyze GC/MS Data* dialog.

> **Note:**   If the selected GC/MS data file has previously been analyzed, the message **Previous analysis results will be replaced!** appears above the bottom row of buttons; otherwise this area is blank.
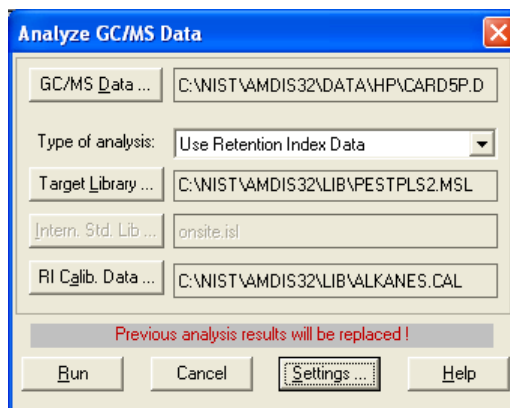
Figure 29 - Analyze GC/MS Data dialog showing warning (Use Retention Index Data)

When first running this example, the analysis results file for *CARD5P.D* should not exist. In any case, previous analysis results for a sample are always overwritten when a new analysis is performed and so this warning is completely normal as you reanalyze samples with different parameters. Click **Run** to start the analysis. Within a few seconds the results are displayed in the format you are now accustomed to.

5) We will now take this opportunity to learn a little more about the *Information Lists* area of the display. We previously saw how to control the displaying of all hits or just the best hits. Now we shall see how to undock the *Information Lists* area so that we can conveniently view all of a hit's results information at once without needing to scroll.

First select the target at retention time 14.3360 minutes.

Now right-click in the upper left-hand quadrant of the *Information Lists* area and you will be presented with a 2-entry menu.
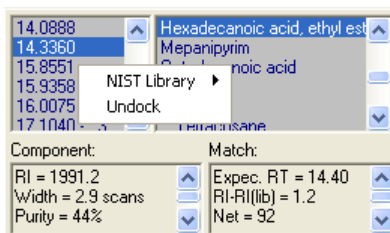
Figure 30 - Controlling docking and undocking

Click **Undock**. The *Information Lists* area now becomes a moveable window.
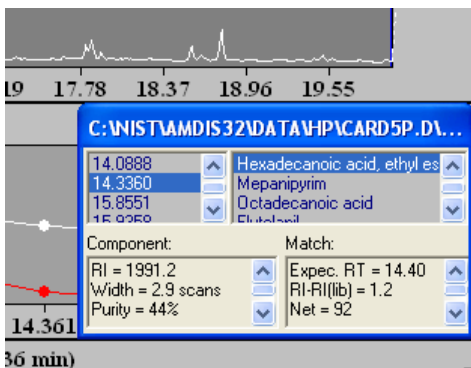
Figure 31 - Information Lists area in "Undock" mode

You can now use *Windows* techniques to drag and expand the window. Experiment moving and stretching it. Note especially that, if you stretch the window at the top edge, it increases the relative size of the upper quadrants, whereas if you stretch it at the bottom edge, it increases the relative size of the lower quadrants.

Expand the window until you can see all of the **Match** information for the target; for example:
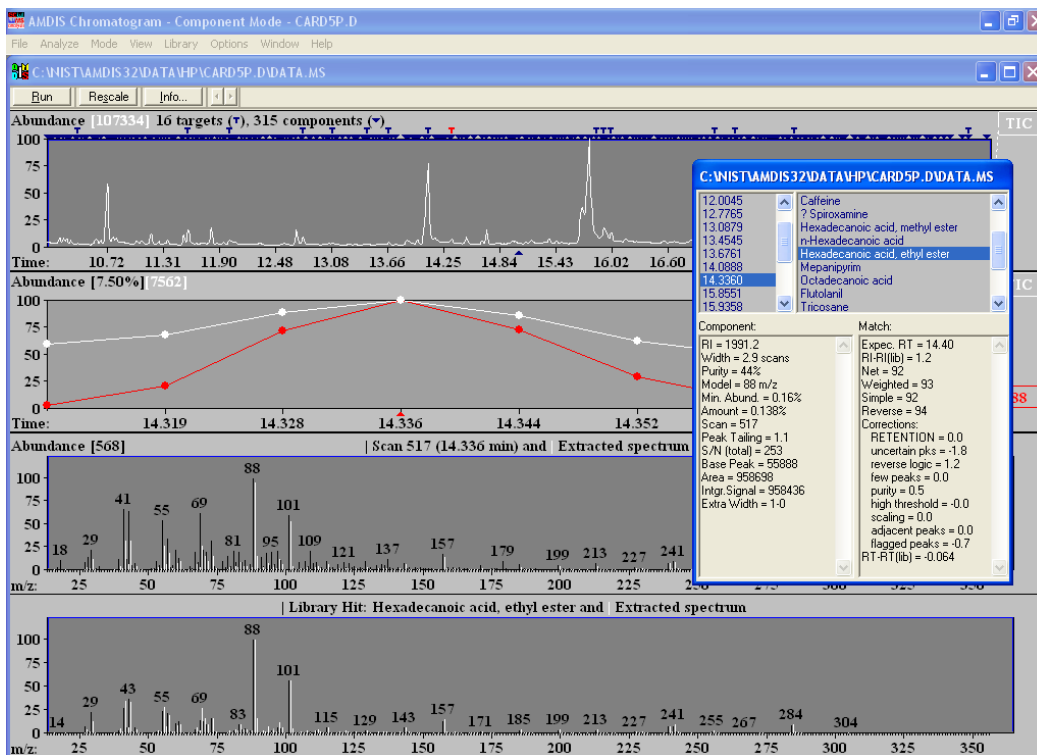
Figure 32 - Undocked Information Lists area showing all Match information

The *Information Lists* area is fully explained in **3.3.3 Information Lists**, but for now we shall just highlight a few values relating to RI.

In the **Match** area **Hexadecanoic acid, ethyl ester** has an **RI-RI(lib)** value of **1.2**, indicating how close the RI match is. This value is the difference in RI between the value computed for this component from the *RI Calibration Data* file (.CAL) and the value held in the *Target Compounds Library* (.MSL). The calculated value is shown in the **Component** area and is **1991.2** in this case; with 1900.0 being the library value.

> **Note:** If the **RI-RI(lib)** label and value are absent, this will be because the library entry does not have an RI value specified.

The **Net** value (**92** in this case) is an overall indicator of the matching process and takes all **Corrections:** into account. Finally note that in the **Corrections:** list, the **RETENTION** value is **0.0**. We will learn more about this in a moment.

6) In the upper-left pane select **13.0879** and observe the values in the lower panes change as it is selected.
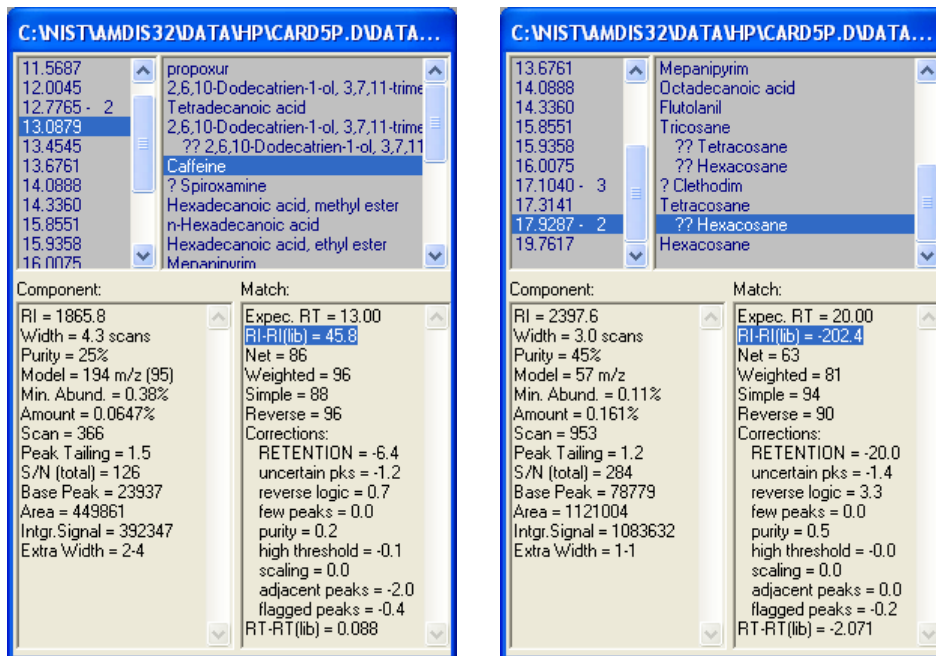
Figure 33 - Caffeine and ?? Hexacosane

The **RI-RI(lib)** value of **45.8** is again the difference between the **RI** value computed for this target (**1865.8**) and the value stored in the library (1820); and so 1865.8-1820.0 yielding **-45.8**. Finally note that the **RETENTION** value is **-6.4**. Since the **Net** value is adjusted by the summation of correction values, this relatively large negative penalty has had a significant impact on the final **Net** value.

In the upper-left pane select **17.9287**. and in the upper-right pane select **?? Hexacosane**. The first point to note about this hit is the preceding **??**. A double ? indicates a **Net** match value of 60-69 and in our case the value is **63**.

A large RI difference gives rise to a large **RETENTION**; but remember that in the *Analysis Settings* dialog you can set a maximum limit to the **RETENTION** value (**Maximum penalty = 20** in our case); which is always applied in a negative sense.

It's outside the scope of this section to discuss any more of the details of the *Information Lists* area or the *Analysis Settings* dialog's values. However, if you wish to quickly see the effect of changing the **Maximum penalty** value, go into the *Analysis Settings* dialog and change it to **10**. Now save the changes and reanalyze the sample. You will see that **Hexacosane** hit for the target at **17.9287** minutes now has a **Net** value of **73** and appears with only one **?** (since the match value is now between 70 and 79). **RETENTION** has a value of **-10.0**; the maximum allowed this time.

2.11 Analysis Type - Use Internal Standards for RI

If you have retention index (RI) information stored in a *Target Compounds Library*, an *Internal Standards Library* (.ISL) can be used to aid the target identification process. To take advantage of this it is also necessary that one or more of the internal standard compounds represented by the library are co-injected with the sample.

> **Note:** A *Target Compounds Library* has a ".MSL" file extension and an *Internal Standards Library* has a ".ISL" file extension.

For more detail about this analysis type see **6.1.5 Use Internal Standards for RI**.

The *Use Internal Standards for RI* analysis type first identifies target and internal standard compounds using spectral comparisons only (exactly as in the *Simple* analysis type). Next, using the known RI values and actual retention times of the internal standards that have been found, it computes RI values for all of the identified library targets using linear interpolation techniques.

Only now does it determine what to do with these retention indices. If you have elected to utilize RI information (see below), for each identified target it will compare its computed RI value with the value held in the *Target Compounds Library* and will adjust the target's match value according to how good the fit is. Otherwise the target match value is not adjusted and the RI information is simply used in reporting the deviation between computed and library RI values.

At the completion of every analysis, a QA/QC report is created that provides information about all of the internal standards that have been found, as well as those that were not found.

It is a prerequisite of this analysis type that a *Target Compounds Library* and an *Internal Standards Library* exist. Further, the internal standards' information can only be of any use for compounds in the library that have RI values stored for them. For the purpose of this example, suitable files are provided, but if you want to learn more about them, refer to **8.1 Library Types**.

Before following the instructions below, ensure that AMDIS is in a known state by following the instructions in **2.2 Getting AMDIS into a Known State**. There should now be an "empty" *Confirm* window displayed.

Proceed as follows:

1)   Click **File | Open** and in the *Select Data File* dialog, navigate to the *\WIST08\AMDIS32\TUTORIAL* folder, select the file *CARD5P.D* and click **Open**.

2)   Click **Analyze | Analyze GC/MS Data** and in the *Analyze GC/MS Data* dialog set **Type of analysis** to **Use Internal Standards for RI**. Use the **Target Library** and **Intern. Std. Lib** buttons to select the *PESTPLUS.MSL* and *INTSTD2.ISL* files, respectively. The dialog should now resemble:
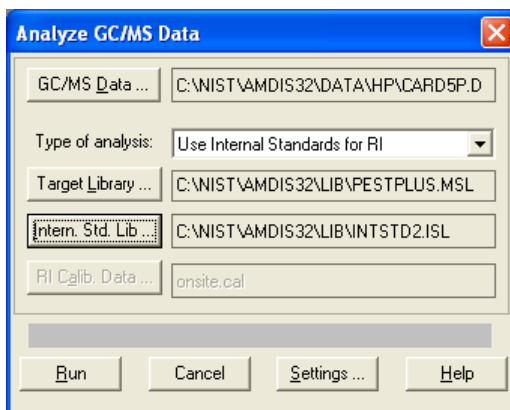
Figure 34 - Analyze GC/MS Data dialog (Use Internal Standards for RI)

> **Note:** The **RI Calib. Data** button in the dialog is disabled. This type of analysis does not involve the use of an *RI Calibration Data* file.

3)   Although retention index values are automatically computed in this analysis type, we still need to tell AMDIS exactly how to use them.

Click **Settings** and in the *Analysis Settings* dialog click **Identif.**. Now set **Minimum match factor** to **65**.

4) We are now ready to perform the analysis. Click **Save** to exit the *Analysis Settings* dialog and return to the *Analyze GC/MS Data* dialog.

Click **Run** to start the analysis. Within a few seconds the results are displayed in the usual format.

Set the *Information Lists* area to view only the best hits and then examine **RI-RI(lib)** values for the found targets.

> **Note:** If the **RI-RI(lib)** label and value are absent, this will be because the library entry does not have an RI value specified.

You will see that **Caffeine** (found at 13.0879 minutes) has a value of **0** and is a perfect match. In fact **Caffeine** is one of the two internal standard compounds in the *Internal Standards Library* (*INTSTD2.ISL*) and is also a target compound in the *PESTPLUS.MSL Target Compounds Library*. When an internal standard is also found as a target it will always have a value of exactly **0**. The other internal standard is pentacosane, but this is not in the target library; in fact it has been deconvoluted as a component at 18.783 minutes (**Figure 37 - AMDIS Results - QA/QC report**).

5) Now let's look at the QA/QC report that was mentioned earlier and take the opportunity to briefly discuss the *AMDIS Results* dialog.

Click **Info** (found near the top left of the *Confirm* window) to display the *AMDIS Results* dialog.
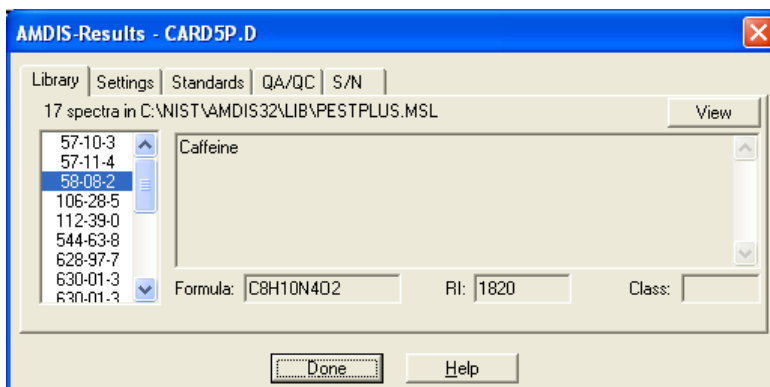
Figure 35 - AMDIS Results dialog (Library tab)

When it appears, the dialog presents the **Library** tab and shows information from the *Target Compounds Library* for the currently selected target (**Caffeine** in our example). Note the textual header giving the name of the *Target Compounds Library* and saying how many spectra there are in it.

Click **Standards** to display a tab listing pertinent non-spectral information about all of the internal standards in the *Internal Standards Library*.
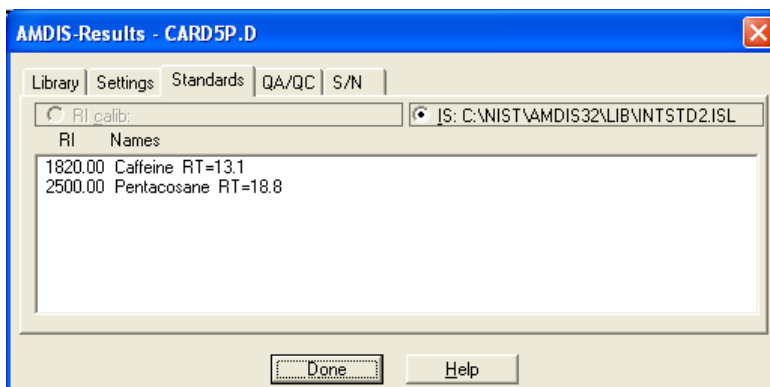
Figure 36 - AMDIS Results dialog (Standards tab)

Note: The **RI calib** radio button is disabled. The "RI Calibration" information is only available when an *RI Calibration Data* file (.CAL) has been used in an analysis (we will briefly examine this in the next tutorial section).

Now click **QA/QC** to view the QA/QC "Results" report and scroll down to the bottom of the report.
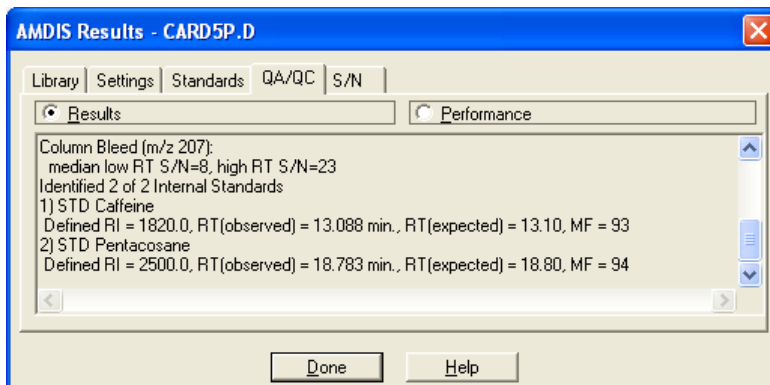
Figure 37 - AMDIS Results - QA/QC report

Information about the internal standards that have been found is presented. If any of the internal standards have not been found, a separate list of those would also be presented. We will learn about the **Performance** radio button later (**2.13 Analysis Type - Performance Check for RI**).

The **Settings** tab presents important parameter values used for the most recent analysis and the **S/N** tab provides various information relating to the signal-to-noise analysis performed on the data file. The discussion of these tabs and further details of the other tabs is outside the scope of this section, but they are fully described in Chapter **5 Auxiliary Information**.

Note: It is important to understand that much of the information presented in the *AMDIS Results* dialog relates to the analysis results underlined being examined. As such, these values are not necessarily those that would be used for a new analysis, as analysis settings may have been modified in the *Analysis Settings* dialog (**3.1.2.2 Analyze | Settings**). The exception is that the library information is taken from the current state of the library; which in general may have been edited since the analysis.

Click **Done** to close the dialog.

6) Finally, let's take a quick look at our *Internal Standards Library*.

On the **Library** menu click **Build One Library**. In the *Build One Library* editor that appears, click **Files** and in the next dialog click **Load Library**. Change **Files of type** to **Internal Std. Library(*.ISL)** and navigate to the *AMDIS32/LIB* folder. Finally click *INTSTD2.ISL* and **Open** to load the library into the *Build One Library* editor.
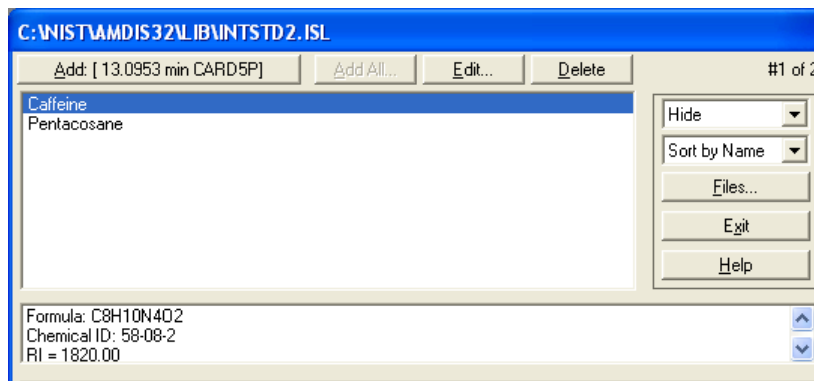
Figure 38 - Internal Standards Library dialog

Here you can see the list of internal standard compounds that we have just successful located in the *CARD5P.D* data file.

If you click **Edit** you get the *Spectrum Editor (Internal Standards Library)* dialog for the currently highlighted compound and you can see the sort of information stored for an entry. Click **Cancel** to exit from the dialog and leave the values unchanged.

It's outside the scope of this section to discuss library details any more. Full details can be found in **8.1 Library Types**.

Click **Exit** to return to the *Confirm* window.

2.12 Analysis Type - Use RI Calibr. Data + Internal Std.

The *Use RI Calibr. Data + Internal Std.* analysis type is equivalent in operation to the *Use Retention Index Data* analysis type that you have already learned about. Therefore, to derive maximum benefit, you need a *Target Compounds Library* with retention index (RI) information stored in it and an *RI Calibration Data* file.

An *Internal Standards Library* is also required, but unlike in the *Use Internal Standards for RI* analysis type we have just examined, the internal standards are used only as a functional check of the acquisition system and are not used in predicting target RI values; since this is done using the *RI Calibration Data* file.

> **Note:** A *Target Compounds Library* has a ".MSL" file extension, an *RI Calibration Data* file has a ".CAL" file extension and an *Internal Standards Library* has a ".ISL" file extension.

For more detail about this analysis type see **6.1.6 Use RI Calibr. Data + Internal Std.**.

As in the *Use Internal Standards for RI* analysis type, internal standards are co-injected with the sample.

The *Use RI Calibr. Data + Internal Std.* analysis type first identifies target and internal standard compounds using spectral comparisons only and then computes retention index values for the identified targets using the calibration data held in the *RI Calibration Data* file.

If you have elected to utilize RI information (using the *Analysis Settings* dialog), for each identified target it will compare its calibration-computed RI value with the value held in the *Target Compounds Library* and will adjust the target's match value according to how good the fit is. If you are not utilizing RI information, the target match value is not adjusted and the RI information is simply used in reporting the deviation between computed and library RI values.

The deviation between the expected and observed retention times of each internal standard is given in the QA/QC report (see below for details).

It is a prerequisite of this analysis type that a *Target Compounds Library*, an *RI Calibration Data* file and an *Internal Standards Library* exist. Further, the calibration information can only be of any use for compounds in the target library that have RI values stored for them. For the purpose of this example, suitable files are provided.

Before following the instructions below, ensure that AMDIS is in a known state by following the instructions in **2.2 Getting AMDIS into a Known State**. There should now be an "empty" *Confirm* window displayed.

Proceed as follows:

1) Click **File | Open** and in the *Select Data File* dialog, navigate to the *\WIST08\AMDIS32\TUTORIAL* folder, select the file *CARD5P.D* and click **Open**.

2) Click **Analyze | Analyze GC/MS Data** and in the *Analyze GC/MS Data* dialog set **Type of analysis** to **Use RI Calibr. Data + Internal Standard.**. Use the **Target Library**, **Intern. Std. Lib** and **RI Calib. Data** buttons to select the *PESTPLUS.MSL*, *INTSTD2.ISL* and *ALKANES.CAL* files, respectively. The dialog should now resemble:
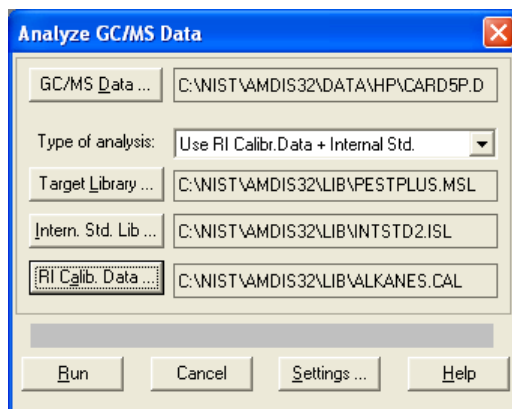
Figure 39 - Analyze GC/MS Data dialog (Use RI Calibr. Data + Internal Std.)

3) Although retention index values are automatically computed in this analysis type, we still need to tell AMDIS exactly how to use them.

    Click **Settings** and in the *Analysis Settings* dialog click **Identif.**. Now set **Minimum match factor** to **65**.

4) We are now ready to perform the analysis. Click **Save** to exit the *Analysis Settings* dialog and return to the *Analyze GC/MS Data* dialog.

    Click **Run** to start the analysis. Within a few seconds the results are displayed in the usual format.

    Set the *Information Lists* area to view only the best hits of the targets and then examine **RI-RI(lib)** values for the found targets.

    Note: If the **RI-RI(lib)** label and value are absent, this will be because the library entry does not have an RI value specified.

If you check the value for the **Caffeine** internal standard you can see that it is no longer exactly **0**; and so is unlike the previous *Use Internal Standards for RI* analysis method where it was exactly **0**. This time the internal standards are being treated more or less the same as normal targets and in this case are subject to the normal RI computation (from the *RI Calibration Data* file) and RI adjustments.

We will soon discuss this in a little more detail, but before we do click **Info** to display the *AMDIS Results* dialog and then click **Standards** to display **RI calib** data. This is a list of all compounds in the *RI Calibration Data* file and shows their stored **RT**, **RI** and **Names** information.
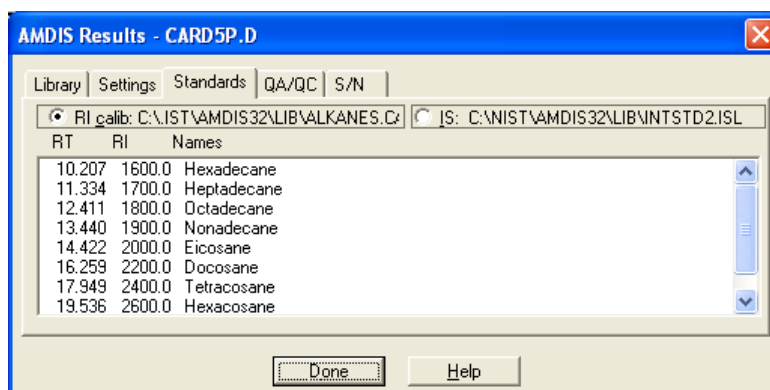
Figure 40 - AMDIS Results dialog (Standards tab, RI calib)

It is this **RT** and **RI** calibration information that is used to predict the RI of a target from its found RT (see below).

If you click **IS**, you will see the same type of information as described previously for the *Use Internal Standards for RI* analysis type.

Similarly, click **QA/QC** and scroll down to the bottom of the **Results** section. You will again see that both of the internal standards have been found. The difference between **RT(observed)** and **RT(expected)**, together with the match value (**MF**), give some indication of how well the overall acquisition system is performing.
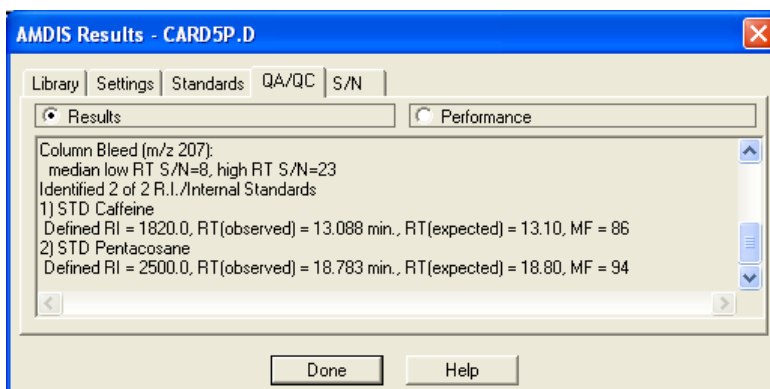
Figure 41 - QA/QC results showing the internal standards

The discussion of these tabs and further details of the other tabs is outside the scope of this section, but they are fully described in Chapter **5 Auxiliary Information**.

> **Note:** It is important to understand that much of the information presented in the *AMDIS Results* dialog relates to the analysis results <u>currently being examined</u>. As such, these values are not necessarily those that would be used for a new analysis, as analysis settings may have been modified in the *Analysis Settings* dialog (**3.1.2.2 Analyze | Settings**). The exception is that the library information is taken from the current state of the library; which in general may have been edited since the analysis.

5) Now let's see how the *RI Calibration Data* file's RT and RI information are used.

Click **Done** to exit from the *AMDIS Results* dialog and then select **Tetradecanoic acid** in the undocked *Information Lists* area. It will look similar to:
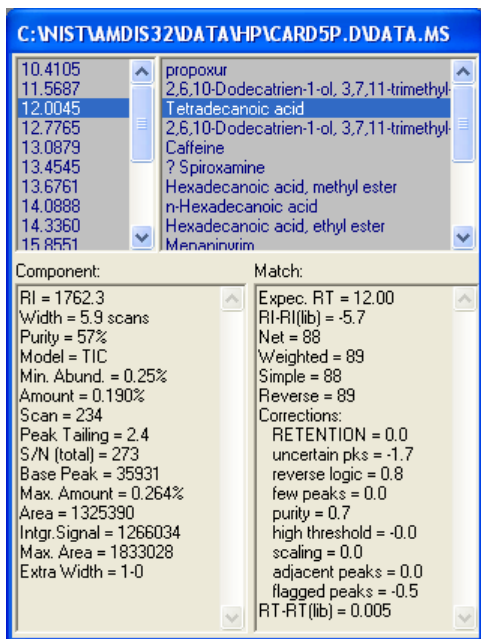
Figure 42 - Results for Tetradecanoic acid
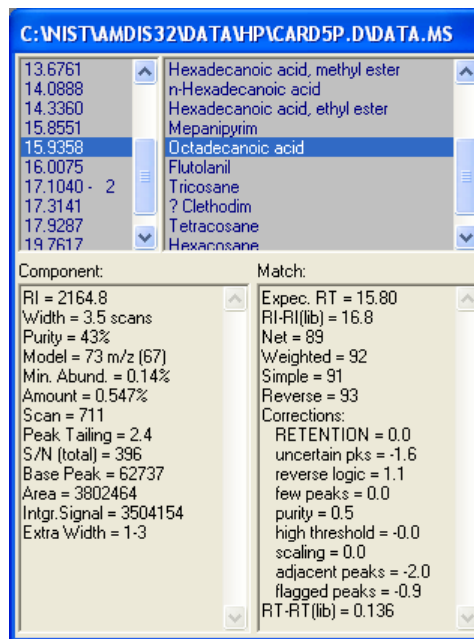
Figure 43 - Results for Octadecanoic acid

The RT of **Tetradecanoic acid** is **12.0045** and so the *RI Calibration Data* file is examined for the closest **RT** value below this and the closest above (in this case **11.334** and **12.411 - Figure 40 - AMDIS Results dialog (Standards tab, RI calib**). These two values and their corresponding RI values (**1700.0** and **1800.0**)

are used to predict the RI of **Tetradecanoic acid** by simple interpolation (yielding **RI = 1762.3**). The difference between the computed RI value for **Tetradecanoic acid** and its value in the *Target Compounds Library* (1768) is presented as **RI-RI(lib)** in the **Match** results area (1762.3 - 1768 = **-5.7**).

Further details of this calculation are outside the scope of this section, but they are fully described in **6.1.6 Use RI Calibr. Data + Internal Std.**.

Now select **Octadecanoic acid** and note its **RT** of **15.9358** (**Figure 43 - Results for Octadecanoic acid**). The RT pair now used from the *RI Calibration Data* file are **14.422** and **16.259** (**Figure 40 - AMDIS Results dialog (Standards tab, RI calib**).

2.13   Analysis Type - Performance Check for RI

The *Performance Check for RI* analysis type is used as a check to see how well the acquisition system is performing. You do this by first acquiring a clean mixture of known composition containing one or more "performance check" compounds. You then analyze the results utilizing a *Calibration & Standards Library* and an *RI Calibration Data* file; both of which we have already learned about in **2.9 Analysis Type - RI Calibration/Performance**.

> **Note:**   A *Calibration & Standards Library* has a ".CSL" file extension and an *RI Calibration Data* file has a ".CAL" file extension.

For more detail about this analysis type see **6.1.7 Performance Check for RI**.

The *Calibration & Standards Library* is a list of compounds that can be used in the creation of a *RI Calibration Data* file (as described in **2.9 Analysis Type - RI Calibration/Performance**) and/or for performance checking purposes.

First of all the *Performance Check for RI* analysis type attempts to identify all compounds in the *Calibration & Standards Library* using spectral comparisons only and then computes retention index values for the identified targets using the calibration data held in the *RI Calibration Data* file.

If you have elected to utilize RI information (using the *Analysis Settings* dialog), for each identified target it will compare its calibration-computed RI value with the value held in the *RI Calibration Data* file and will adjust the target's match value according to how good the fit is. If you are not utilizing RI information, the target match value is not adjusted and the RI information is simply used in reporting the deviation between computed and library RI values.

Finally, any "performance check" compounds that have been identified have their data added to the Performance Log file. This file can be inspected at any time and by examining the details of the information, it is possible to detect any gradual (or sudden) deterioration in the acquisition system that might indicate the need for remedial action, or in some cases simply the creation of a new *RI Calibration Data* file.

It is a prerequisite of this analysis type that a *Calibration & Standards Library* and an *RI Calibration Data* file exist. Further, the *Calibration & Standards Library* should have at least one compound identified as a "performance check" compound. For the purpose of this example, suitable files are provided.

Before following the instructions below, ensure that AMDIS is in a known state by following the instructions in **2.2 Getting AMDIS into a Known State**. There should now be an "empty" *Confirm* window displayed.

Since this tutorial illustrates the Performance Log, it would also be beneficial to start with that in a known state. The Performance Log is actually a collection of 3 files that are located in the folder in which AMDIS is installed. Their names are *perfom.idx*, *perform.tDB* and *perform.txt*. If these files already exist and you know you want to preserve them, use standard *Windows* techniques to rename each of them before running this tutorial and then afterwards delete the newly-created files and rename your saved files back to their original names.

Proceed as follows:

1)   Click **File | Open** and in the *Select Data File* dialog, navigate to the *\NIST08\AMDIS32\TUTORIAL* folder, select the file *CARD5P.D* and click **Open**.

2)   Click **Analyze | Analyze GC/MS Data** and in the *Analyze GC/MS Data* dialog set **Type of analysis** to **Performance Check for RI**. Use the **Calib/Stds. Lib.** and **RI Calib. Data** buttons to select the *ALKANES.CSL* and *ALKANES.CAL* files, respectively. The dialog should now resemble:
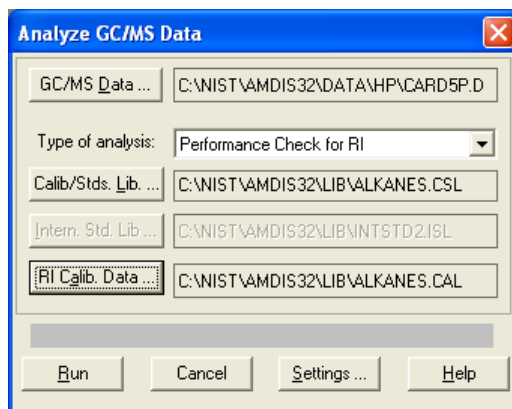
Figure 44 - Analyze GC/MS Data dialog (Performance Check for RI)

> **Note:** The **Intern. Std. Lib** button in the dialog is disabled. This type of analysis does not involve the use of internal standards.

3) Click **Settings** and in the *Analysis Settings* dialog click **Identif.**. Now set **Minimum match factor** to **65**.

4) We are now ready to perform the analysis. Click **Save** to exit the *Analysis Settings* dialog and return to the *Analyze GC/MS Data* dialog.

   Click **Run** to start the analysis. Within a few seconds the results are displayed in the usual format.

   If you undock and expand the *Information Lists* area and set it to show only best-hit data, the target list will look something like:

Figure 45 - Information Lists area after Performance Analysis

5) Before we discuss the actual results of the *Performance Check for RI* analysis, let's quickly examine the *Calibration & Standards Library* that we used.

   On the **Library** menu click **Build One Library**. In the *Build One Library* editor that appears, click **Files** and in the next dialog click **Load Library**. Change **Files of type** to **Calibr. & Stds Library(*.CSL)** and navigate to the *AMDIS32/LIB* folder. Finally click *ALKANES.CSL* and **Open** to load the library into the *Build One Library* editor.

   Select **Sort by RI** and examine the full list of compounds by scrolling.
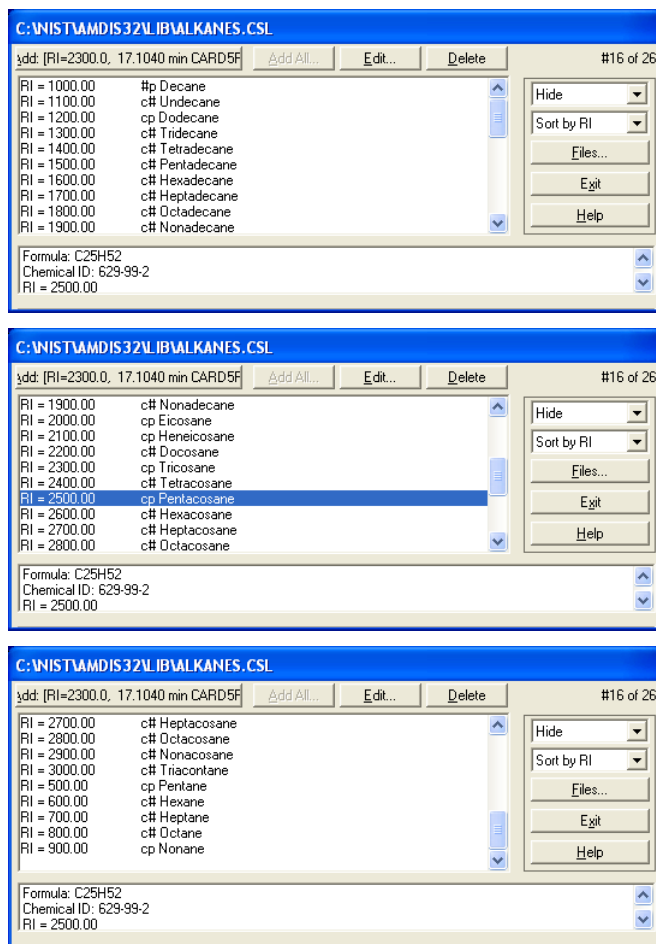
Figure 46 - Calibration & Standards Library (Performance Check)

The compound entries that are flagged with **cp** or **#p** are the "performance check" compounds and in this case you can see that there are a total of 8. Click **Exit** to leave the dialog.

6) Click **Info** to display the *AMDIS Results* dialog and then click **QA/QC**, followed by **Performance** to display data for the "performance check" compounds that have been found.
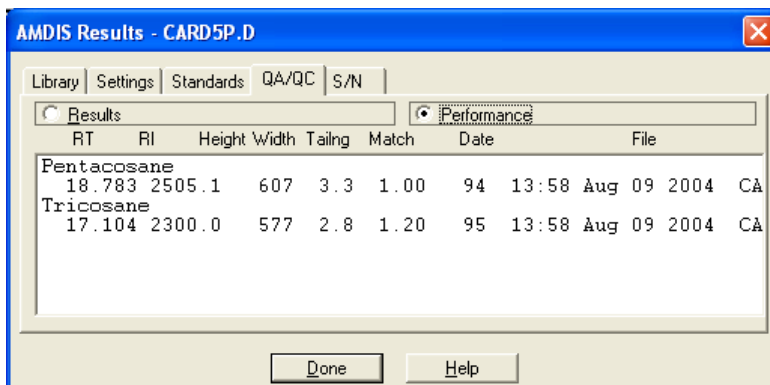
Figure 47 - AMDIS Results dialog (Performance Check)

In our case *CARD5P.D* has two "performance check" compounds in it and these have been found as targets; which is what you would hope for. The absence of a compound known to be in the acquired mixture would be a cause for some concern and would lead to further investigation.

In our case we currently only have one set of performance data recorded. As this analysis is repeated over time, the data build up until we have many lines of data for each compound. By observing the reported **RT**, **RI**, **Height**, **Width**, **Tailing** and **Match** data, it is possible to spot a gradual change over time (or a sudden larger change) of one or more of these values.

Further details relating to performance checks are outside the scope of this section, but they are fully described in **5.5.1.3 Scan Sets Information**.

Click **Done** to exit.

7)   Before we finally leave the tutorial sections we will take the opportunity to quickly see how we print or save the results of <u>any</u> analysis type.

On the **File** menu click **Print Spectra** to invoke the *Print Spectra* dialog.
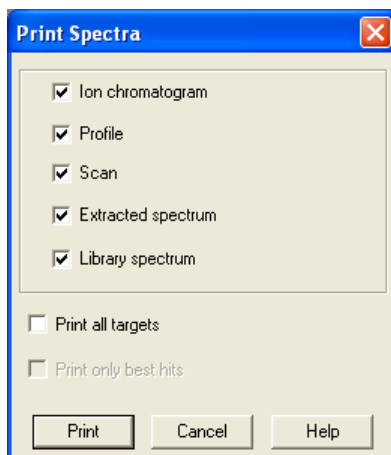
Figure 48 - Print Spectra dialog

The settings allow you some control over the graphical items that are printed and are fully described in **3.1.1.9 File | Print Spectra**. Experiment if you wish by using the **Print** button, but finally return to the *Confirm* window.

8)   On the **File** menu click **Print Text Report** to invoke the *Print Text Report* dialog.
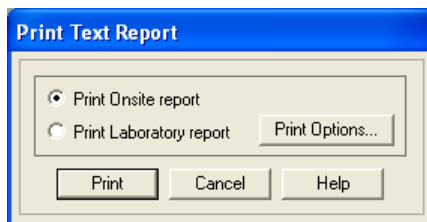
Figure 49 - Print Text Report dialog

If you click **Print Options** the ensuing dialog's settings allow you some control over the textual items that can be printed and are fully described in **3.1.1.9.1 Layout Details**. Experiment if you wish by using the **Print** button, but finally return to the *Confirm* window.

9)   It is possible to save most of the results of an analysis in textual format for subsequent examination, or simply for historical recording, or any other reason. On the **File** menu click **Generate Report** to invoke the *Generate Report* dialog.
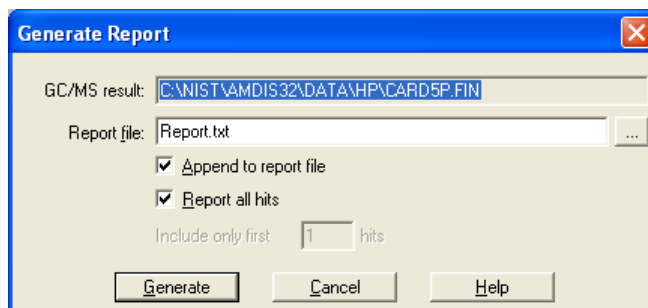
Figure 50 - Generate Report dialog

The details of report generation are outside the scope of this section, but they are fully described in **3.1.1.8 File | Generate Report**. Click **Cancel** to return to the *Confirm* window.

2.14   Recap

In this section you were introduced to the most commonly used main window of AMDIS (the *Confirm* window) and the other major window was briefly described (the *Results* window). You have learned the basics of manipulating the display of the *Confirm* window and have worked through each analysis type in turn. In doing this you have utilized all of the file types that AMDIS supports and you have been introduced to many of the most significant commands and some parameters. You have been shown how to view and perform basic interpretation of results and it was mentioned how to print and create reports.

All of this should give you the confidence to begin "serious" work with AMDIS.

The rest of this User Guide is written in the style of a reference document giving full details of all features with little or no reference made to supplied example files. However, it does cross-reference tutorial sections where relevant to enable you to refresh your memory of a technique that you perhaps use only occasionally.

3 AMDIS Confirm Window

AMDIS offers two significant user interfaces (windows) for you to work with.

The *Confirm* window is a full-functionality window, offering a menu bar and one or more included "child" windows (for example, **Figure 51- Confirm window showing its various display areas**). Each child window has a "button bar", several graphical areas and a text area. The menu bar provides access to the full functionality of AMDIS and the graphical areas permit interactive manipulations and, together with the text area, detailed analysis of the results of AMDIS processing.

The *Confirm* window will be the window of choice for most users.

The *Results* window offers a more limited set of functionality than the *Confirm* window and in particular does not display graphics, nor permit library manipulations. It can be useful when it is sufficient to simply review the results of AMDIS processing.

The *Results* window is fully described in Chapter **4 AMDIS Results Window**.

> **Note:**   It is straightforward to swap between these two windows:

> In the *Confirm* window, click **File | Go to Results** to go to the *Results* window.

> In the *Results* window, click **Confirm** to go to the *Confirm* window.

> **Note:**   AMDIS will always start up in the *Confirm* window, irrespective of the prevailing window selection when it was last closed down.
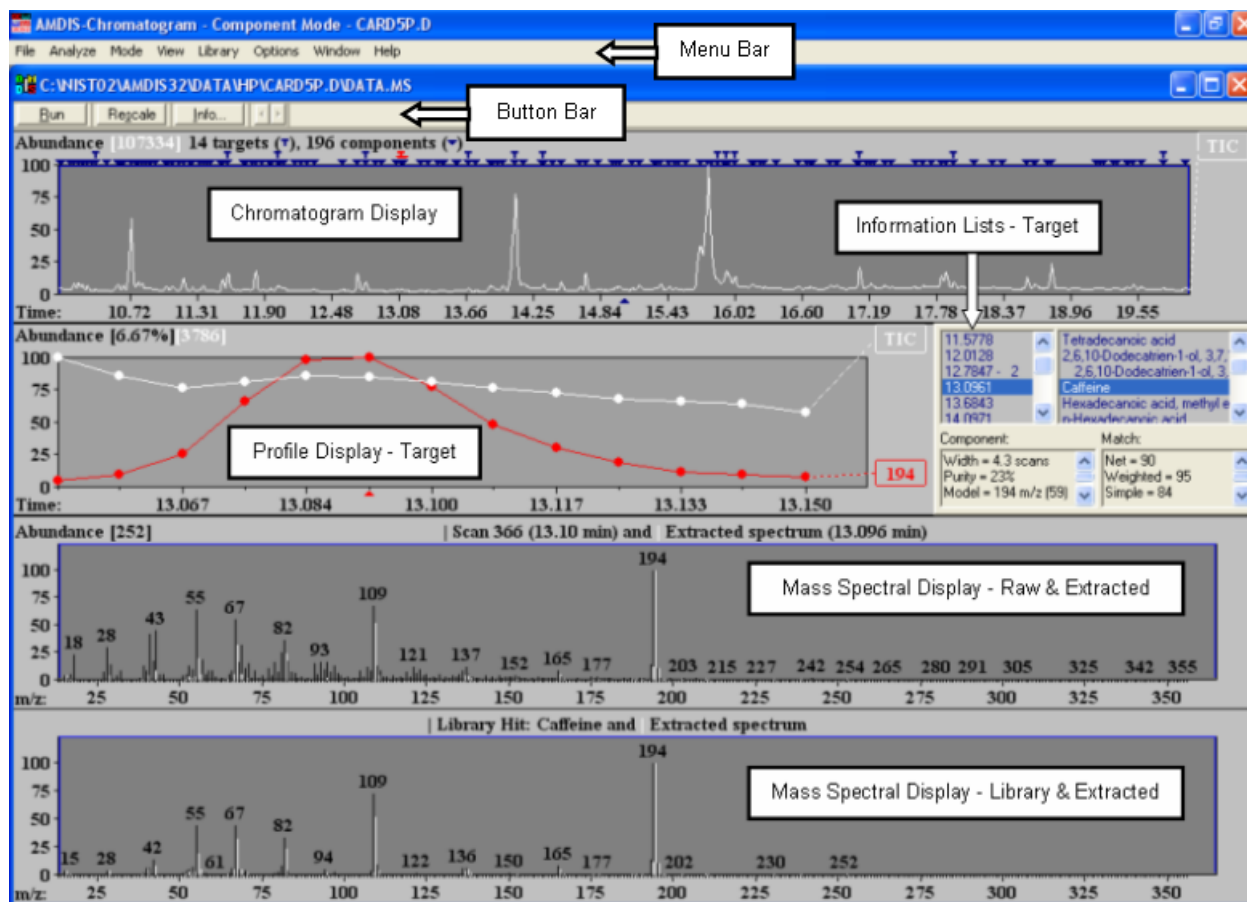
Figure 51 - Confirm window showing its various display areas

A brief overview of the *Confirm* window and its display areas, as well as some basic commands for manipulating the display, is given in **2.4 Confirm Window Appearance**.

In the *Confirm* window you can view either a single chromatogram (the most common mode of operation), or more than one chromatogram. In general, all of the features discussed for the *Confirm* window are relevant to both of these modes. Specific information relating to multiple chromatograms can be found in **3.4 Display of Multiple Chromatograms**.

The "top level" commands available in the *Confirm* window are described in **3.1 Menu Bar**.

The command buttons available in the button bar are detailed in **3.2 Button Bar**.

The various display areas and their manipulation are explained in **3.3 Data Displays**.

3.1 Menu Bar

The menu bar presents the following options:

File	see **3.1.1 File Menu**
Analyze	see **3.1.2 Analyze Menu**
Mode	see **3.1.3 Mode Menu**
View	see **3.1.4 View Menu**
Library	see **3.1.5 Library Menu**
Options	see **3.1.6 Options Menu**
Window	see **3.1.7 Window Menu**
Help	see **3.1.8 Help Menu**

Although menu bar options are apparently always available, the menus they present when selected will mostly be disabled if there is no data file open in the *Confirm* window.

3.1.1 File Menu

The **File** menu is used to:

→ open new files

Open	see **3.1.1.1 File | Open**
Open In	see **3.1.1.2 File | Open In**
Open Recent Files	see **3.1.1.3 File | Open Recent Files**
Add Recent Files	see **3.1.1.4 File | Add Recent Files**

→ save components for use with the NIST mass spectral database

Save Component MS	see **3.1.1.5 File | Save Component MS**

→ set result file options

Options	see **3.1.1.6 File | Options**

→ handle batch Job files

Batch Job	see **3.1.1.7 File | Batch Job**

→ save and print results

Generate Report	see **3.1.1.8 File | Generate Report**
Print Spectra	see **3.1.1.9 File | Print Spectra**
Print Text Report	see **3.1.1.10 File | Print Text Report**

→ swap to the *Results* window

Go to Results	see **3.1.1.11 File | Go to Results**

→   exit from AMDIS

Exit   see **3.1.1.12 File | Exit**

3.1.1.1   File | Open

The **Open** command is used to select a data file for display within AMDIS.

Click **File | Open** to invoke the *Select Data File* dialog.
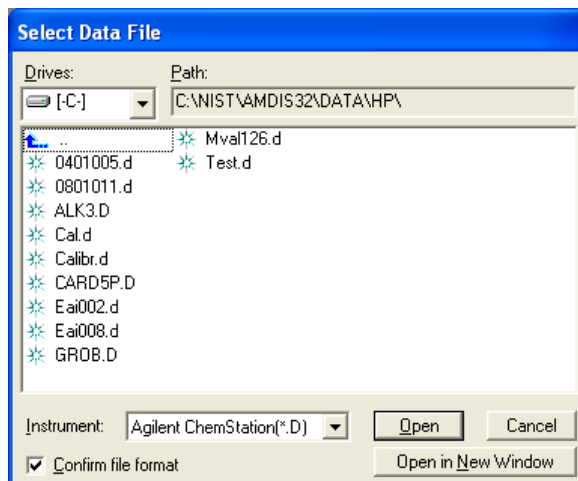
Figure 52 - Select Data File dialog

The dialog is used to select a data file of a specified instrument type and optionally have its format verified for that instrument type.

Ensure **Instrument** is set to the type of data you wish to analyze.

Since AMDIS supports a variety of instrument file formats, you would normally ask AMDIS to check that a selected data file is of the correct format for the selected **Instrument**. To do this select the **Confirm file format** checkbox.

> **Note:**   When you select **Confirm file format** AMDIS will not display files that it determines to be of an invalid format. If you clear the checkbox, you are able to select any file. However, if the file is subsequently determined to have an invalid format when you attempt to open it, you will get a **Cannot read GC/MS data file** warning message.

Use **Drives** to select the drive to be accessed and then navigate the folder structure by double-clicking on closed folders to expand them and by double-clicking ⬆️.. to move one step back up the folder hierarchy; noting that **Path** shows you where you currently are in the folder hierarchy. When you see the file you want, either click it to highlight it and then click **Open** or **Open in New Window**, or double-click it to select it and immediately exit from the dialog (equivalent to using **Open**).

If there are currently no files open in AMDIS, **Open** will place the new file in a "child" window, which will occupy the full display area. If one or more files already exist, **Open** will replace the "current" file with the new file as described in **3.1.1.2.1 File | Open In | Active Window**.

Open in New Window, however, places the new file in a new child window of its own as described in **3.1.1.2.2 File | Open In | New Window**.

> **Note:**   If there is currently no file displayed in AMDIS, **Open in New Window** is equivalent to **Open**.

3.1.1.2   File | Open In

The **Open In** menu is used to:

→   specify the window in which the opened data file will be placed

Active Window   see **3.1.1.2.1 File | Open In | Active Window**

New Window   see **3.1.1.2.2 File | Open In | New Window**

3.1.1.2.1   File | Open In | Active Window

The **Active Window** command is used to select a data file to replace the "current" data file. It will be enabled when one or more files already exist, whether in a single window or multiple windows.

Click **File | Open In | Active Window** to invoke the *Select Data File* dialog (**Figure 52 - Select Data File dialog**) and then select a file as described in **3.1.1.1 File | Open**.

> **Note:**   The **Open in New Window** button will not appear in the dialog.

The selected file will replace the "current" file (see **3.4.2 Two Chromatograms in One Window** for an explanation as to which file is the current file when more than one is being displayed).

If the file being replaced has been analyzed, you may be asked about keeping its results before it is replaced (**3.1.1.6 File | Options**).

Analogously, if the replacement file has been previously analyzed and its results kept, it will be displayed together with its previous results.

3.1.1.2.2   File | Open In | New Window

The **New Window** command is used to select a data file to be displayed in a new child window. It will usually be enabled when one or more files already exist, whether in a single window or multiple windows. If several windows already exist and it is not enabled, that is because the maximum number of windows has already been reached.

Click **File | Open In | New Window** to invoke to invoke the *Select Data File* dialog (**Figure 52 - Select Data File dialog**) and then select a file as described in **3.1.1.1 File | Open**.

> **Note:**   The **Open in New Window** button will not appear in the dialog. Instead the dialog's **Open** button will achieve the same effect.

The selected file will be displayed in a new window and the displayed windows will be "tiled" (equivalent to the **Window | Tile** command; **3.1.7.2 Window | Tile**). If the file has been previously analyzed and its results kept (**3.1.1.6 File | Options**), it will be displayed together with its previous results.

3.1.1.3   File | Open Recent Files

Unless AMDIS has just been installed, you will already have opened one or more data files. In that case, the **Open Recent Files** command will be enabled for you to conveniently select one of the most recently opened files to replace the "current" data file.

Click **File | Open Recent Files** and note that a list numbered from **1** upwards shows in reverse chronological order the most recently opened data files. Simply click on the name of the file that you want and it will replace the "current" file with the new file as described in **3.1.1.2.1 File | Open In | Active Window**.

3.1.1.4   File | Add Recent Files

Unless AMDIS has just been installed, you will already have opened one or more data files. In that case and provided you haven't already got two data files displayed in the current window, the **Add Recent Files** command will be enabled for you to conveniently select one of the most recently opened files to be added into the current window.

Click **File | Add Recent Files** and note that a list numbered from **1** upwards shows in reverse chronological order the most recently opened data files. Simply click on the name of the file that you want to add as the second file in the window. To learn more about having two files in one window, see **3.4.2 Two Chromatograms in One Window**.

3.1.1.5   File | Save Component MS

The **Save Component MS** command allows you to save the spectrum of an AMDIS component in the *Mass Spectral Transfer* (MSP) file format of the NIST *MS Search* program for subsequent library search by that program. It will be enabled only when a data file has been analyzed.

Click **File | Save Component MS** command to invoke the *Save Component As* dialog.
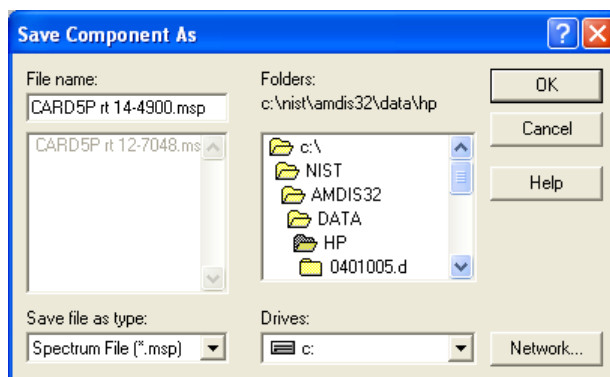
Figure 53 - Save Component As dialog

Use **Drives** and **Folders** to select the destination for the *Mass Spectral Transfer* file and provide a **File name** for it. Now click **OK** to complete the operation.

> **Note:** Any uncertain peaks in the component will not be included in the MSP file even if **Analyze | Use Uncertain Peaks** has its check mark applied (for more about uncertain peaks, see **3.1.2.7 Analyze | Use Uncertain Peaks**).

> **Note:** To subsequently search this *Mass Spectral Transfer* file, first activate the NIST *MS Search* program. Now click **File | Open**, find and select the *Mass Spectral Transfer* file and click **Open**. In the next dialog highlight the relevant spectrum and click **Import Selected**. Answer the next dialog's question and the spectrum will then be imported, automatically searched and the search results displayed.

3.1.1.6   File | Options

The **Options** command allows you to tell AMDIS what to display when AMDIS is activated and how to deal with the analysis results of a data file when that file is being removed from the display, including when you exit from AMDIS.

Click **File | Options** to invoke the *AMDIS Options* dialog.
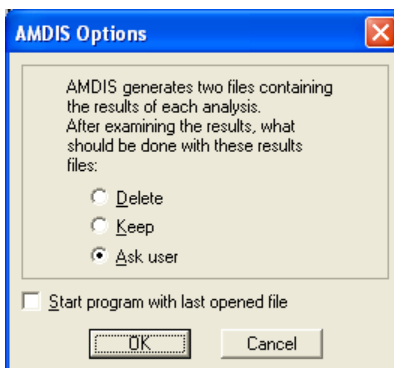
Figure 54 - AMDIS Options dialog

You can choose to automatically **Delete** or **Keep** results files, or to always be asked (**Ask user** - see below). If you keep results, AMDIS will automatically display those results when the corresponding data file is next opened; otherwise by deleting the results, you will need to reanalyze it to obtain results.

If you want AMDIS to always start with the last opened data file when AMDIS was last being used, select **Start program with last opened file**. If you have also chosen to keep its results, they will be automatically displayed with it.

If you select **Ask User**, each time a file with analysis results is about to be removed from the display, the *Delete Result Files* dialog will appear.
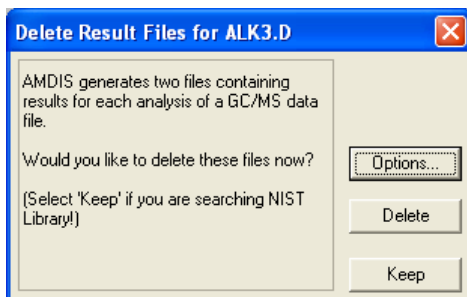
Figure 55 - Delete Result Files dialog

Click **Delete** or **Keep** according to your requirements.

3.1.1.7   File | Batch Job

The **Batch Job** menu is used to:

→   create, edit and run Job files

      Create and Run Job   see **9.1 Create and Run Job**

→   show the results created by the most recent Job file run

      Show Results of Last Job   see **9.2 Show Results of Last Job**

A Job file is essentially a list of data files, where each data file has its associated analysis instructions. Job files are created, modified and initiated to run using the **Create and Run Job** command. During the automated processing a report can be automatically created. The results of the last run Job file can be examined at any time using the **Show Results of Last Job** command.

Job files and the **Batch Job** command are fully described in Chapter **9 Batch Jobs**.

3.1.1.8   File | Generate Report

The **Generate Report** command writes the analysis results of the currently active file to a tab-delimited format, text file; suitable for import into data analysis programs.

> **Note:**   Only found targets are reported. As such, if no targets have been found during the analysis, the **Generate** command will be disabled. Section **3.1.2.1 Analyze | Analyze GC/MS Data** discusses the production of analysis results.

Click **File | Generate Report** to invoke the *Generate Report* dialog.
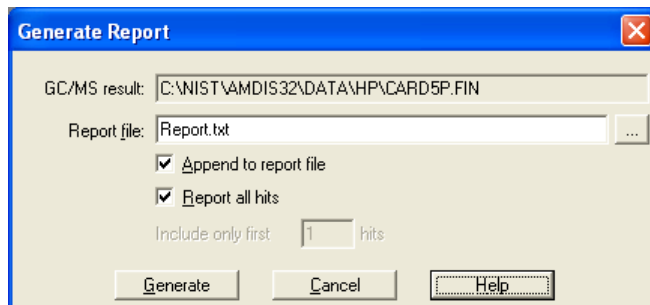
Figure 56 - Generate Report dialog

GC/MS result is a reminder of the data file, for which the report will be generated and **Report file** is the name you choose for the results. You can either enter a name here directly or use the browse button ( ) to invoke a standard *Open* dialog for you to select the folder and file name to be used.

If you select **Append to report file**, the current data file's analysis results will be written at the end of the specified **Report file**.

> **Note:**   If the file does not already exist, it will be created with just the current results in it.

You can choose to report all hits for each target by selecting **Report all hits**. If instead, you clear its checkbox, **Include only first <n> hits** is enabled for you to select how many of each target's top hits you want reporting.

Click **Generate** to write the analysis results to the specified report file.

3.1.1.9 File | Print Spectra

The **Print Spectra** command prints a fixed-format graphical report, according to what information is available and what you select.

Click **File | Print Spectra** to invoke the *Print Spectra* dialog.
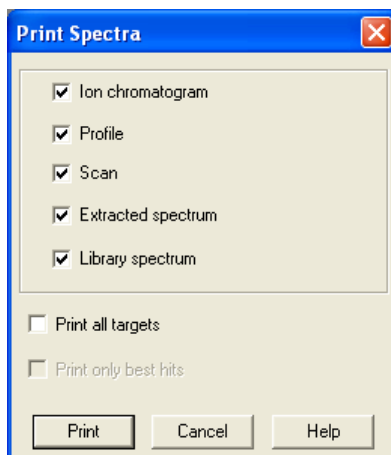
Figure 57 - Print Spectra dialog

Checkboxes are available for you to select what type of information is to be printed (for details see **3.1.1.9.1 Layout Details**). If something cannot be printed because it does not exist, its checkbox will be disabled. For example, **Library spectrum** will be disabled, if a target is not currently being displayed. Similarly, if no analysis has been performed, the only selections enabled are **Ion chromatogram** and **Scan**.

> **Note:** The selections available reflect to a certain extent what could be displayed, which is not necessarily the same as what is being displayed, since the latter is controlled by the **View | Show Window** command (**3.1.4.1 View | Show Window**).

Click **Print** to print the requested selections.

> **Note:** If you have not selected anything to print, a *Warning* dialog appears telling you that **No selection was made**.

3.1.1.9.1 Layout Details

The checkboxes in the *Print Spectra* dialog (**Figure 57 - Print Spectra dialog**) control the content and layout of the requested report; subject to the requested information being appropriate and so available.

The first five checkboxes determine the type of information that is reported for each target and the final two control which targets are reported.

Ion chromatogram displays the contents of the *Chromatogram Display*.

Profile displays the contents of the *Profile Display*.

> **Note:** If **Print all targets** is clear and a component is currently selected in the *Confirm* window, certain textual component information is also presented (a subset of that described in **3.3.3.1 Component Mode Details**).

> **Note:** If **Print all targets** is selected, **Profile** is disabled. If you want profiles of multiple targets printed, you will have to print them one at time.

Scan displays the original data file spectrum in the *Mass Spectral Display - Raw & Extracted*.

Extracted spectrum displays the extracted spectrum in the *Mass Spectral Display - Raw & Extracted*.

Library spectrum displays the library spectrum in the *Mass Spectral Display - Library & Extracted*.

The report will contain the selected information items and in the same order as they are in the dialog.

If **Print all targets** is clear, **Print only best hits** is disabled and the report will present the requested information on a single page for the currently displayed target or component.

If **Print all targets** is selected, **Print only best hits** is enabled. If **Ion chromatogram** is selected, the first page of the report will simply be the chromatogram and each subsequent page will show the other selected spectral information; one hit per page. With **Print only best hits** selected, only the information for each target's best hit is presented. If **Print only best hits** is clear, all hits for all targets are reported (one per page) and so this represents the most extensive report format available.

3.1.1.10 File | Print Text Report

The **Print Text Report** command prints analysis results for components and targets in a textual format and according to which options you select.

Examples of printed reports and details of all report variations are given in Chapter **15 Text Report Details**.

Click **File | Print Text Report** to invoke the *Print Text Report* dialog.
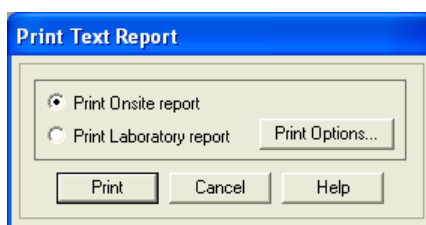
Figure 58 - Print Text Report dialog

Note: If there are no results to print, a warning dialog will appear instead.

There are two types of report that can be selected. However, they have many similarities and the Onsite report features are predominantly a subset of those of the Laboratory report.

According to whether **Print Onsite report** or **Print Laboratory report** is selected, the **Print Options** button will invoke the *Onsite Report Print Options* dialog, or the *Print Options* dialog, respectively.
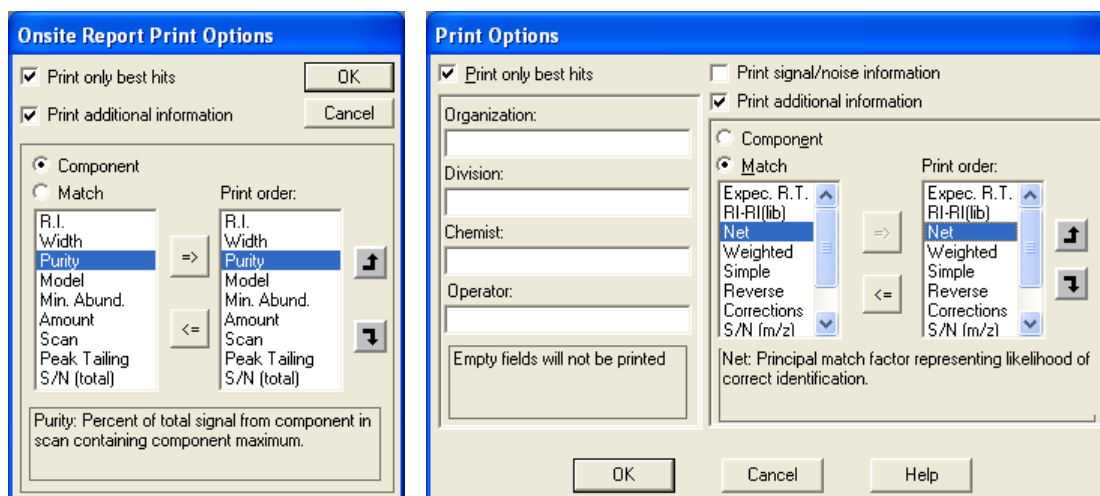
Figure 59 - Onsite Report Print Options and Print Options dialogs

The Laboratory report has extra information that can be reported, as compared to the Onsite report.

The extra information includes textual input fields for **Organization**, **Division**, **Chemist** and **Operator** that can be specified. If a particular field is not required, it should be left blank and it will not appear at all on the report. If specified, the **Organization** and **Division** information appears in the report header and the **Chemist** and **Operator**

names appear at the bottom of each report page with the intention that each page can be signed off by the relevant person(s).

> **Note:** The Onsite report always has signoffs on each page.

The Laboratory report also has a **Print signal/noise information** checkbox to control whether or not signal and noise data are printed (select it to print the information).

> **Note:** The Onsite report always prints the signal/noise information.

The remaining features of the two dialogs are identical in functionality.

Select the **Print only best hits** checkbox to print only the highest ranked hit for each target. Leave it clear if you want all hits printed for each target.

If selected, the **Print additional information** checkbox enables the **Component** and **Match** radio buttons to allow the selection here, and hence subsequent printing, of additional **Component** <u>and</u> **Match** values for each hit. Click **Component** to see the specific "component-type" values that can be selected for printing. Similarly, click **Match** to see the "match-type" values that can be selected. Both of these are illustrated by the dialogs in **Figure 59 - Onsite Print Options and Print Options dialogs**.

The additional values to be printed are those in the **Print order** lists (one list for **Component** and one for **Match**) and their selection and ordering are controlled as described below (**3.1.1.10.1 Value Selection and Ordering**).

Once you have finished setting up the list and any other parameters in the dialog, click **OK** to return to the *Print Text Report* dialog and then click **Print** to print the text report.

> **Note:** The **Component** and **Match** values that are selected are shared by the *Onsite Report Print Options* dialog <u>and</u> the *Print Options* dialog and so any changes to the selections and print order in one dialog, will be there if you invoke the other dialog. It is also important to understand that the **Component** and **Match** radio buttons are used in the dialog only to select and manipulate the two different sets of **Print order** values to be printed. It does not matter which radio button is selected when you exit from the dialog, since the values for both sets of data will always be printed if the **Print additional information** checkbox is enabled. Finally, the two sets of values represent the superset of all possible information that can be provided from all analysis types. As such, there are a few values that can be selected for printing that are only calculated for particular analysis types and so will not be printed unless the corresponding analysis type was used to process the data file.

3.1.1.10.1 Value Selection and Ordering

There are 2 list boxes in the dialog. The left list box holds all of the values that can be chosen. The right list box (**Print order**) holds all of the values that have been chosen and allows their order to be set. There are 4 "arrow" buttons in the dialog to control these operations.

=>	<=	⬆	⬇
Right-Arrow	*Left-Arrow*	*Up-Arrow*	*Down-Arrow*

Figure 60 - "Arrow" buttons

The right list box is set up as follows:

1) To add a value to the right list box, first highlight it in the left list box; for example, by clicking on it. If the value is already in the right list box, the dialog's right-arrow button will be disabled; otherwise it will be enabled.

   Click the right-arrow button to copy the value to the right list box and repeat as necessary to copy other values.

2) To remove a value from the right list box, first highlight it in the right list box and then click the left-arrow button. Repeat as necessary to remove other values.

3)   The right list box values will be presented in the order they occur within the list, starting from the top. To change the order of the list, highlight a value to be moved and then click the up-arrow or down-arrow button, one or more times as appropriate.

3.1.1.11   File | Go to Results

AMDIS offers two significant user interfaces for you to work with as described in **2.3 Confirm Window and Results Window Overview**.

The **Go to Results** command swaps you from the *Confirm* window to the *Results* window.

The *Results* window is described in detail in Chapter **4 AMDIS Results Window**.

3.1.1.12   File | Exit

The **Exit** command is used to exit from AMDIS.

When you click **File | Exit**, you will be asked to confirm that you want to exit. Click **Yes** to confirm, or **No** to return to AMDIS.

Before exiting, AMDIS will check the setting of the *AMDIS Options* dialog (**Figure 54 - AMDIS Options dialog**). If its Ask user radio button is selected and analysis results exist for one or more of the files currently being displayed, the *Delete Result Files* dialog (**Figure 55 - Delete Result Files dialog**) will appear to ask you what you want to do with the current result files.

> **Note:**   If there are multiple data files on display with results, this dialog will appear for each of those.

3.1.2   Analyze Menu

The **Analyze** menu is used to:

$\rightarrow$ prepare for and analyze data files

Analyze GC/MS Data	see **3.1.2.1 Analyze | Analyze GC/MS Data**
Settings	see **3.1.2.2 Analyze | Settings**
Postprocess	see **3.1.2.3 Analyze | Postprocess**
Structural Classifiers	see **3.1.2.4 Analyze | Structural Classifiers**

$\rightarrow$ interact with the NIST *MS Search* program

Add Component/Scan to Search List (n)	see **3.1.2.5 Analyze | Add Component/Scan to Search List (n)**
Go to NIST MS Program	see **3.1.2.6 Analyze | Go to NIST MS Program**
Use Uncertain Peaks	see **3.1.2.7 Analyze | Use Uncertain Peaks**
Search NIST Library	see **3.1.2.8 Analyze | Search NIST Library**

3.1.2.1   Analyze | Analyze GC/MS Data

The **Analyze GC/MS Data** command first of all analyzes a GC/MS data file to locate components. According to the selected analysis type, it may then compare these components against a list of target compounds and determine any matches; which themselves may then be used, for example, to create a calibration file. In all cases the analysis results are presented in the *Confirm* window for further examination and processing.

The various analysis types and their operation, including parameter selection and results presentation, are described in detail in **6.1 Analysis Types**.

Click **Analyze | Analyze GC/MS Data** to invoke the *Analyze GC/MS Data* dialog.
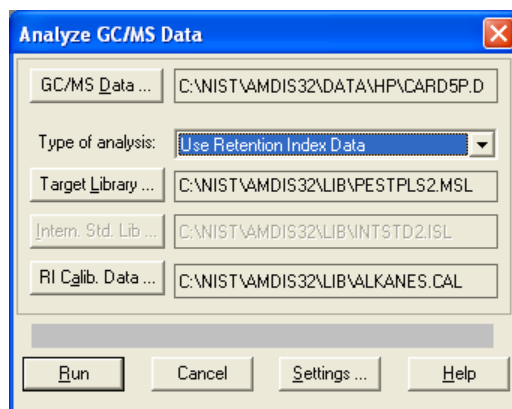
Figure 61 - Analyze GC/MS Data dialog

Of particular note in the dialog are **Type of analysis** and **Settings**.

The value selected for **Type of analysis** determines how many of the 3 file buttons immediately below it are enabled and also how they are labeled. **Settings** invokes the multi-tabbed *Analysis Settings* dialog with parameters both for controlling the analysis and for providing feedback from it.

The current data file will be shown to the right of the **GC/MS Data** button. If you wish to select a different file, click **GC/MS Data** to invoke the *Select Data File* dialog (**Figure 52 - Select Data File dialog**) and select a file as described in **3.1.1.1 File | Open** and exit from the dialog.

Select the required **Type of analysis**. According to that selection, from 1 to 3 of the buttons immediately below it will be enabled for file selection(s). Further, the enabled button(s) will be labeled according to the type of file(s) that are needed for your chosen analysis type.

Click on a file button (for example, **Target Library** or **RI Calib. Data**) to invoke the *Analysis Settings* dialog showing its **Libr.** tab.
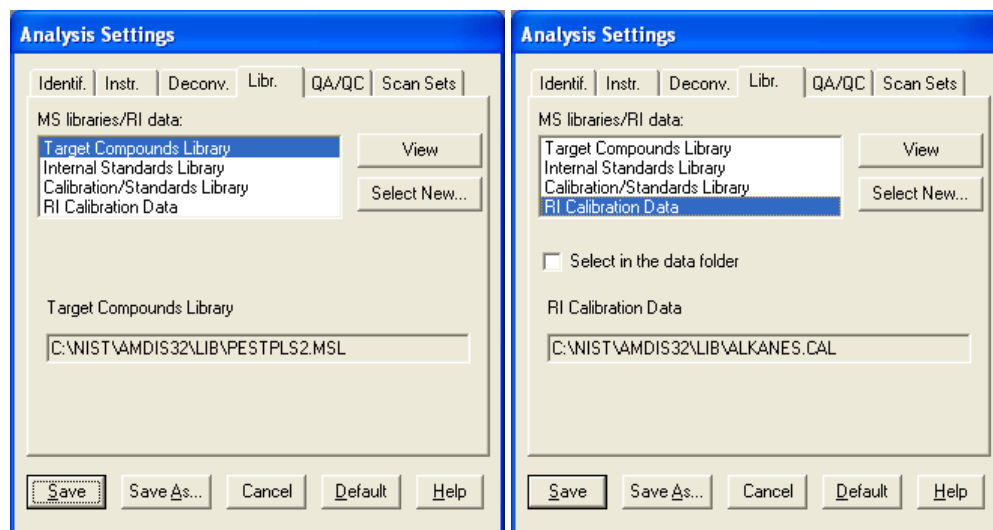
Figure 62 - Analysis Settings dialog; Libr. tab

The **MS libraries/RI data** list will have a file type highlighted appropriate to the button used to invoke the dialog. Further, the text box below the list will be labeled according to the file type (for example, **Target Compounds Library** or **RI Calibration Data** in **Figure 62 - Analysis Settings dialog; Libr. tab**) and for convenience shows the current file selection for that type of file.

For the **RI Calibration Data** file type only, the **Select in the data folder** checkbox appears. If you select this checkbox, it affects the default folder utilized when clicking the **Select New** button; as described below.

To select a different file click **Select New** to invoke a file selection dialog; noting that both the dialog title and **Files of type** will be appropriately set. For example:
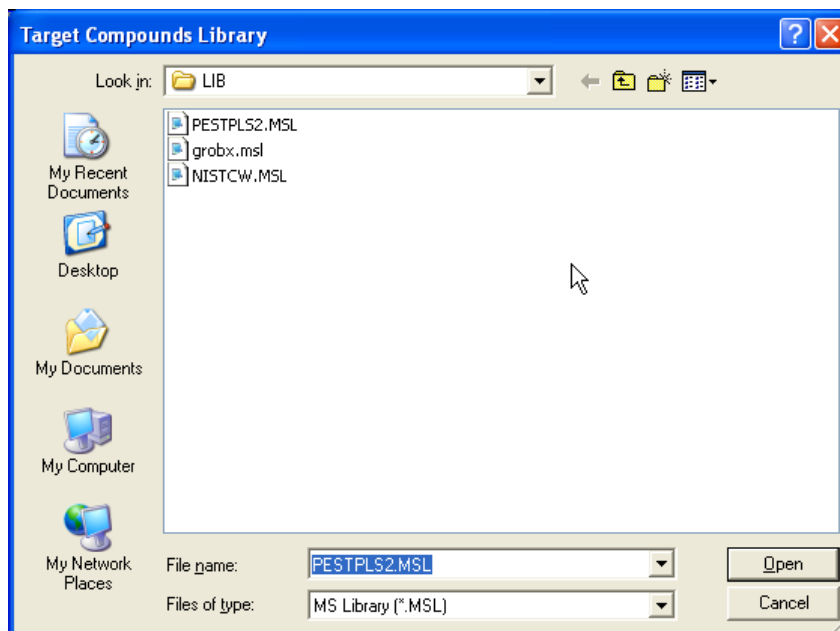
Figure 63 - File selection dialog

The default folder (**Look in**) will depend on the setting of the **Select in the data folder** checkbox in the preceding *Analysis Settings* dialog (**Figure 62 - Analysis Settings dialog; Libr. tab**). If the checkbox is clear, **Look in** and **File name** will show the location and name of the most recently selected file of that type (as had been shown in the previous dialog). However, if **Select in the data folder** is selected, **File name** will be the same, but now **Look in** will show the folder where the current data file is located. For example, based on **Figure 61 - Analyze GC/MS Data dialog** this would be **\NIST08\AMDIS32\TUTORIAL**.

Select a file using standard *Windows* techniques and exit from this dialog back to the *Analysis Settings* dialog.

If you want to examine the detail of the chosen file, click **View** to invoke a dialog appropriate to the selected file.

If an *RI Calibration Data* file is being examined, the dialog will be like **Figure 64 - RI Calibration Data file (CAL) dialog**. Otherwise it will be similar to **Figure 65 - Calibration & Standards Library (CSL) dialog**.
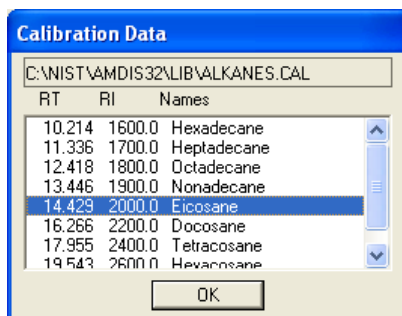
Figure 64 - RI Calibration Data file (CAL) dialog
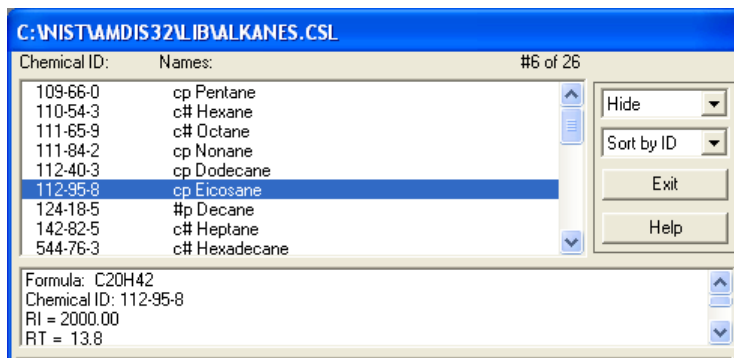
Figure 65 - Calibration & Standards Library (CSL) dialog

The dialog for most library files has two filters (to its top-right) that control what is displayed (**Figure 65 - Calibration & Standards Library (CSL) dialog**). These and the contents of the dialog are fully described in **8.2.3 Library Editor Features**. Click **Exit** to return to the *Analysis Settings* dialog.

The *RI Calibration Data* file dialog is far simpler (**Figure 64 - RI Calibration Data file (CAL) dialog**). It is a scrollable list of the calibration file compounds showing the retention time (**RT**), retention index (**RI**) and name of each compound (**Names**). Calibration files are described in **8.1.4 RI Calibration Data File (CAL)**. Click **OK** to return to the *Analysis Settings* dialog.

The *Analysis Settings* dialog has other tabs containing parameters controlling the analysis and these, together with the command buttons along the bottom of the dialog, are fully described in **3.1.2.2 Analyze | Settings**.

When you have finished selecting files and modifying parameters in the *Analysis Settings* dialog, exit from it (for example, by clicking **Save**) back to the *Analyze GC/MS Data* dialog (**Figure 61 - Analyze GC/MS Data dialog**).

> **Note:** The **Settings** button of the *Analyze GC/MS Data* dialog is provided as an alternative way to get to the *Analysis Settings* dialog.

Click **Run** to activate the analysis.

> **Note:** In some cases, where previous results for the current data file already exist, you will be given a warning message and you will have to confirm that those results will be overwritten.

> **Note:** If AMDIS finds m/z values in the data that exceed the **High m/z** setting on the Instrument tab of the *Analysis Settings* dialog (**6.3.2 Instr. (Instrument) Tab**) the *Maximum m/z Mismatch* warning dialog informs you.
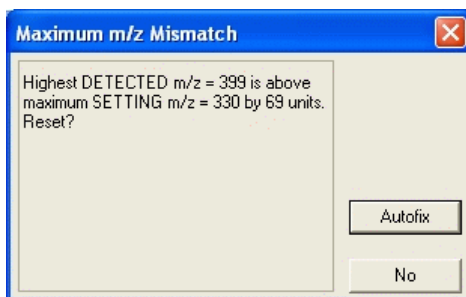
Figure 66 - Maximum m/z Mismatch dialog

> Click **Autofix** to have the **High m/z** value in the *Analysis Settings* dialog automatically adjusted; otherwise click **No** to leave the value unchanged.

Depending on the chosen analysis type and the complexity and amount of the GC/MS data, the analysis could take several seconds. In that case you would see the progress dialogs that are generated.

When the analysis is complete the results are presented graphically in the *Confirm* window and are described fully in **3.3 Data Displays**.

Note: In the case of the *Results* window, there are no graphical results. Instead results are presented textually as described in **4.2 Information Lists**.

Note: In the case of the *Confirm* window the analysis is performed on the portion of the data file currently shown in the *Chromatogram Display* area of the window and replaces any existing results for the file. For the *Results* window the whole file is always used.

3.1.2.2   Analyze | Settings

The **Settings** command invokes a dialog that enables you to make selections and provide values, including file names, to all of the user-settable parameters involved in the analysis process.

Click **Analyze | Settings** to invoke the *Analysis Settings* dialog showing one of its tabs; for example:
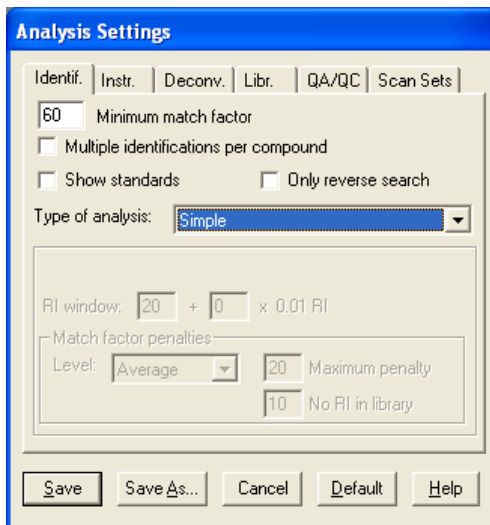
Figure 67 - Analysis Settings dialog; Identif. tab

The *Analysis Settings* dialog has several tabs and these are described in detail in **6.3 Analysis Settings**.

The **Cancel** button works in the normal manner. It cancels any changes made on all tabs and exits from the dialog.

The **Default** button restores all settings on all tabs to default values (the same as when AMDIS was first installed). This is particularly useful when you are unsure about the impact of recent changes you have made and want to return to a known usable state.

Click **Save** if you have made changes and are ready to leave the dialog; parameter changes made on all tabs are preserved.

Note: In most cases you will now get a message informing you that you have changed parameters affecting the analysis and will be asked if you want to reanalyze the data. Click **Yes** or **No** accordingly.

Before exiting the dialog, it is possible to save the current analysis settings for future reuse. Click **Save As** to invoke the *Save Setting As* dialog, enter a **File name** and **Save in** location of your choice and click **Save** to create your named INI file and to exit from that dialog back to the *Analysis Settings* dialog.

Note: If you wish to subsequently reuse the settings in a saved INI file, proceed as follows:

1)   Exit from AMDIS

2)   Using standard *Windows* functionality, locate and delete the file *onsite.ini* in the *AMDIS32* folder.

3)   Locate the saved INI file, whose values you wish to restore for use in AMDIS.

4)   Make a copy of it in the *AMDIS32* folder and then rename it as *onsite.ini*.

5)   Restart AMDIS. The restored values will now be in use.

It is important to understand that the *Analysis Settings* dialog reflects the values you last set in it and not always therefore the values used to analyze the data file, whose results you are currently examining. If you want to see the pertinent values used for the current file, use the **View | Auxiliary Information** command and select its dialog's **Settings** tab (for full details, see **3.1.4.6 View | Auxiliary Information**).

> **Note:** In the *Results* window you would instead examine the **Settings** tab of the *Information Tabs* area for the current file's values (**5.3 Settings Tab**).

3.1.2.3 Analyze | Postprocess

The **Postprocess** command provides access to additional processing on all of the components found by the most recent analysis and subsequently provides the results of that processing for you to examine. It will only be enabled when you are displaying the results of an analysis.

Its facilities are fully described in **7.1 Postprocess**.

3.1.2.4 Analyze | Structural Classifiers

The **Structural Classifiers** command applies to the currently selected component or target and invokes a dialog to show its Varmuza Classifier values. It will only be enabled when you are displaying the results of an analysis.

Its facilities and the Varmuza Classifiers, which were developed by Prof. Varmuza's research group, are fully described in **7.1.1 Structural Classifier**.

3.1.2.5 Analyze | Add Component/Scan to Search List (n)

The **Add Component/Scan to Search List (n)** command takes the spectrum in the *Mass Spectral Display - Raw & Extracted* area and adds it to a list of spectra for subsequent searching with the NIST *MS Search* program; by using the **Analyze | Go to NIST MS Program** command (**3.1.2.6 Analyze | Go to NIST MS Program**). The value **(n)** shows how many spectra are currently in the list and will reset to zero after the **Analyze | Go to NIST MS Program** command has been used.

The status of the **Analyze | Use Uncertain Peaks** command determines whether or not uncertain peaks are included in the spectrum to be searched (**3.1.2.7 Analyze | Use Uncertain Peaks**).

> **Note:** The **Add Component/Scan to Search List (n)** command will only be of use if the NIST *MS Search* program and NIST mass spectral database are installed.

> **Note:** In the NIST *MS Search* program the origin of each spectrum is identified. An AMDIS component's spectrum will end up being listed similar to the following:

Component at scan 865 (10.861 min) [Model = +57u] in \NIST08\AMDIS32\TUTORIAL\TEST.D\DATA.MS

> and a non-component scan similar to the following:

Scan 1189 (13.236 min) in \NIST08\AMDIS32\TUTORIAL\TEST.D\DATA.MS

3.1.2.6 Analyze | Go to NIST MS Program

The **Go to NIST MS Program** command invokes the NIST *MS Search* program to either search the current spectrum, or, if a list of pre-selected spectra exists, that list of spectra will be searched instead. The search is performed against standard NIST-format libraries.

> **Note:** If the NIST *MS Search* program is not installed on your system, this command will simply fail to function without any warning message.

The list to be searched is created using the **Analyze | Add Component/Scan to Search List (n)** command (**3.1.2.5 Analyze | Add Component/Scan to Search List (n)**).

The status of the **Analyze | Use Uncertain Peaks** command determines whether or not uncertain peaks are included in the spectrum to be searched (**3.1.2.7 Analyze | Use Uncertain Peaks**).

If automatic searching has been preset in the NIST *MS Search* program, a search will immediately take place of the single spectrum or of all spectra in the search list and the *MS Search* program will display the results of the search. Otherwise, the search will need to be manually initiated.

To do a manual search from within the NIST *MS Search* program, double-click on the spectrum identifier of interest in the section of the window showing the list of spectrum identifiers (either the top-left or bottom-left

section of the window, depending on the window layout that is currently selected). Alternatively highlight the desired spectrum identifier and click the button bar's **Go** button.

> **Note:** To turn on automation in the NIST *MS Search* program, click **Tools | Library Search Options** within the NIST *MS Search* program and in the dialog that appears click the **Automation** tab and select **Automatic Search On**.

> **Note:** If it seems that the NIST *MS Search* program is not functioning, because it does not appear and because control stays in AMDIS (probably accompanied by a single, rapid refresh of the display), it may either be because the NIST *MS Search* program is not installed on your system, or because of an inappropriate setting in the *MS Search* program. In either case this command will simply <u>fail to function without any warning message</u>. If you know it exists, in the *MS Search* program click **Tools | Library Search Options** and in the dialog that appears click the **Automation** tab and clear the **Return Focus to Caller upon Completion** checkbox.

3.1.2.7 Analyze | Use Uncertain Peaks

The **Use Uncertain Peaks** command determines whether or not "uncertain peaks" are included in the spectra selected for library searching using the **Analyze | Go to NIST MS Program** and **Analyze | Add Component/Scan to Search List (n)** commands (**3.1.2.6** and **3.1.2.5**, respectively).

As you click on this command it will alternately apply and clear a check mark (tick) to the left of it. When the check mark is applied, uncertain peaks will be used; otherwise they will be ignored.

Uncertain peaks are those ions in a mass spectrum that AMDIS finds impossible to assign to a specific component due to the uncertain deconvolution of the mass spectral data. For display information about uncertain peaks, see **3.3.4 Mass Spectral Display**.

> **Note:** In general usage uncertain peaks should not be utilized (because it is not certain that they belong to the component's spectrum!) and so **Use Uncertain Peaks** should be left clear.

3.1.2.8 Analyze | Search NIST Library

The **Search NIST Library** command allows you to search, using the NIST *MS Search* program, some or all of the components detected during the most recent analysis. The search is performed against standard NIST-format libraries. When the search has completed, the results are available for viewing in the normal AMDIS manner.

> **Note:** The status of the **Analyze | Use Uncertain Peaks** command (**3.1.2.7 Analyze | Use Uncertain Peaks**) is ignored here as uncertain peaks are never included in a spectrum searched using the **Search NIST Library** command.

Click **Analyze | Search NIST Library** to invoke the *Search NIST Library Parameters* dialog.
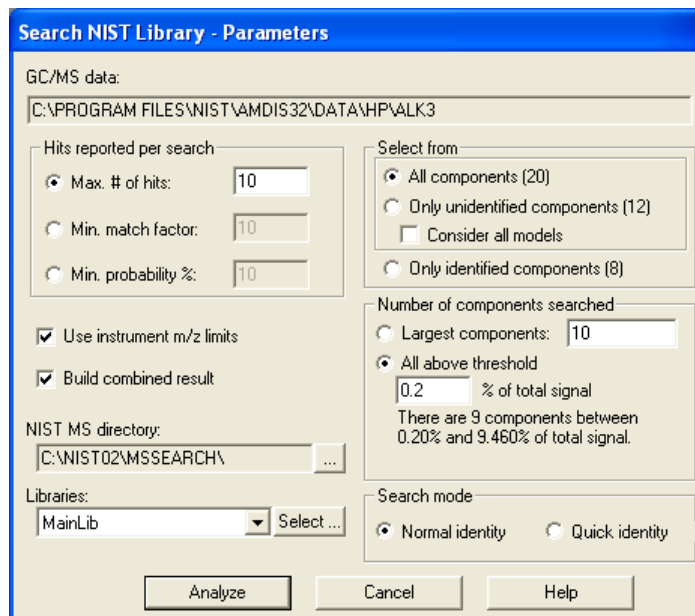
Figure 68 - Search NIST Library Parameters dialog

There are several groups of parameters in the dialog that determine what is searched, how the search is performed and how the results are reported.

The **Select from** group of parameters allow you to select which components are to be searched.

Click **All components (n)** to select all of the components identified by AMDIS; where **(n)** tells you how many of them there are. To select only those components that AMDIS did not identify in its library, click **Only unidentified components (n)**. Otherwise, click **Only identified components (n)** to select only the targets found by AMDIS. In the case of the first two options, you can also choose to **Consider all models** by selecting its checkbox. If the checkbox is clear, only the model listed first for each component will be used.

Having selected the component group that you want to search, you can now further limit how many of those components will be searched using the **Number of components searched** parameter group and choosing one of two magnitude thresholds. In both cases it is the component's "amount" value that is being utilized (**3.3.3.1 Component Mode Details**).

Click **Largest components** and enter how many components are required of the **Select from** group's type (**10** in **Figure 68 - Search NIST Library Parameters dialog**). This imposes an absolute maximum on the number of components that will be searched. The selection process starts with the largest component and works its way down in amount until the specified number has been reached, or there are no more components remaining; based on the **Select from** group's selection.

Alternatively click **All above threshold** and enter a percentage of total signal amount (**0.2** in **Figure 68 - Search NIST Library Parameters dialog**). This equates to an absolute amount threshold above which all selected components will be searched. The text below the threshold shows you how many components will be selected by the specified value and as you are entering a new value this text will change, as appropriate.

The **Search mode** parameter group determines the type of NIST library search to be performed. Click **Quick identity** or **Normal identity** as required.

> **Note:**   Both of these search options are fully described in the help file and manual for the NIST *MS Search* program.

> In summary, **Quick identity** uses a single prescreen (or presearch) algorithm prior to the main search, whereas **Normal identity** uses several prescreens. The **Normal identity** search will typically identify more library spectra for comparison to the submitted spectrum than will the **Quick identity** search.

The **NIST MS directory** and **Libraries** parameters together show you the libraries that will be searched and the **Select** button invokes the *Select Libraries for Search* dialog that allows you to modify the search list as well as the order in which they are searched.
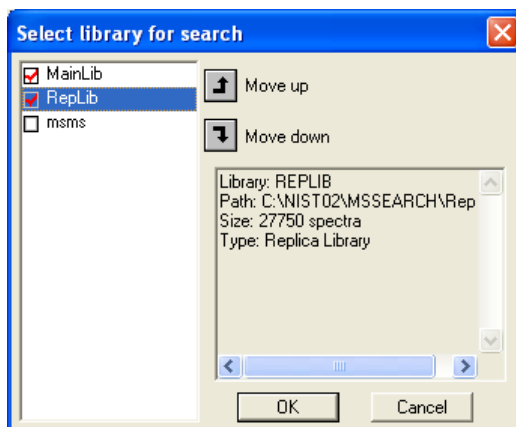
Figure 69 - Select Library for Search dialog

The dialog shows the list of available libraries. For each displayed library, select its checkbox to include it in the search, or clear its checkbox to exclude it. To change the search order, click on a library name and use the **Move up** or **Move down** button as appropriate. As you click on a library name, the dialog also shows some basic details about it; including its number of spectra.

The displayed libraries reside in the folder indicated by the **NIST MS directory** parameter. To change this folder, click on the browse button (...) to invoke the *Browse for Folder* dialog, locate the desired folder and click **OK**.

The **Use instrument m/z limits** checkbox should be selected if you want the search process to consider the full mass range of the instrument that acquired the data. Otherwise the actual mass range of each spectrum is utilized.

The **Hits reported per search** group of parameters provide alternative ways of determining how many hits are reported for each search.

Click **Max. # of hits** and enter the maximum number of hits to be reported. Alternatively, click **Min. match factor** and enter the match factor threshold, such that only those hits with "weighted" match factors greater than this value will be reported. Otherwise click **Min. probability %** and enter the percentage probability threshold that hits must exceed to be reported.

> **Note:**   **Weighted** match factors are shown in the *Information Lists* area (**3.3.3.2 Target Mode Details**) and are identical to those computed in the NIST *MS Search* program. The **Reverse** match factor result from the NIST search is also presented there. The probability values computed in the NIST *MS Search* program are the ones compared to the **Min. probability %** threshold set here, but the actual values are not reported in AMDIS.

Finally, select the **Build combined result** checkbox, if you want the results of this search to be added to the currently displayed results. Otherwise, leave it clear and the new results will replace the current results.

When you have the parameters set appropriately, click **Analyze** to start the search process.

The *Analyze with NIST MS* progress dialog appears to show the progress of the search. When the search has finished and depending on the setting in the *AMDIS Options* dialog (**3.1.1.6 File | Options**), you may be asked by the *Delete Result Files* dialog if you want to keep the results. Click **Keep**, else the results will not be displayed.

> **Note:**   If the NIST *MS Search* program is not installed on your system, this command will simply <u>fail to function without any warning message</u>.

The actual results that are finally displayed will depend on which of the **View** menu's **Show AMDIS Search**, **Show NIST Search** and **Show Merged Result** commands is currently active (**3.1.4 View Menu**).

If desired, a search result may now be added to an AMDIS library (**8.2.3.2.2 Add Button**)

3.1.3 Mode Menu

The **Mode** menu is used to:

→ select how analysis results are displayed

Target Only	see **3.1.3.1 Mode	Target Only**
Component	see **3.1.3.2 Mode	Component**
No Chromatogram	see **3.1.3.3 Mode	No Chromatogram**

→ perform manual processing of data

Manual	see **3.1.3.4 Mode	Manual**

All of the **Mode** menu commands act in a similar manner to radio buttons in that only one is active at any particular time. As such the active one is unavailable for selection, whereas the others are available. The active mode is also identified by name in the title bar (above the menu bar).

None of the commands are available if there are no analysis results. In that case the display will be as illustrated in **Figure 2 - Confirm window showing unprocessed data**.

3.1.3.1 Mode | Target Only

The **Target Only** command sets up the *Confirm* window such that only targets can be viewed and manipulated.

In this mode the *Confirm* window has its full complement of 5 areas as illustrated in **Figure 4 - Confirm window showing the graphical results of an analysis for a target**. The *Confirm* window is fully described in Chapter **3 AMDIS Confirm Window**.

3.1.3.2 Mode | Component

The **Component** command sets up the *Confirm* window such that targets and components can be viewed and manipulated.

In this mode the *Confirm* window has its full complement of 5 areas when viewing a target (as illustrated in **Figure 4 - Confirm window showing the graphical results of an analysis for a target**), or only 4 areas if viewing a component (as illustrated in **Figure 3 - Confirm window showing the graphical results of an analysis for a component**). The *Confirm* window is fully described in Chapter **3 AMDIS Confirm Window**.

3.1.3.3 Mode | No Chromatogram

The **No Chromatogram** command sets up the *Confirm* window such that only targets can be viewed and manipulated.

In this mode the *Confirm* window has only 4 areas, which are the 4 areas left after the *Chromatogram Display* is omitted from the full complement of 5 areas that are illustrated in **Figure 4 - Confirm window showing the graphical results of an analysis for a target**. The *Confirm* window is fully described in Chapter **3 AMDIS Confirm Window**.

Since the *Chromatogram Display* is absent, targets can only be selected for display in the *Information Lists* (**2.4.3.2 Information Lists**).

3.1.3.4 Mode | Manual

The **Manual** command sets up the *Confirm* window such that two manual operations can be performed when analysis results are present.

The first is a normal background subtraction. The second is a true deconvolution using a model peak selected by you. In the latter mode you may also draw the model peak and select a mass or a drawing to form the basis of subtraction for adjacent peaks.

The facilities available when the **Manual** command is selected are fully described in Chapter **10 Manual Background Subtraction and Manual Deconvolution**.

3.1.4 View Menu

The **View** menu is used to:

$\rightarrow$ control the appearance of the display

      Show Window   see **3.1.4.1 View | Show Window**

      Undock   see **3.1.4.2 View | Undock**

      Show Mouse Position   see **3.1.4.3 View | Show Mouse Position**

      Thick Lines   see **3.1.4.4 View | Thick Lines**

      Color Selected Peaks   see **3.1.4.5 View | Color Selected Peaks**

      Same Time Scale   see **3.1.4.7 View | Same Time Scale**

$\rightarrow$ examine additional information relating to the analysis results

      Auxiliary Information   see **3.1.4.6 View | Auxiliary Information**

$\rightarrow$ control which results are shown after performing a NIST library search of AMDIS analysis results

      Show AMDIS Search   see **3.1.4.8 View | Show AMDIS Search**

      Show NIST Search   see **3.1.4.9 View | Show NIST Search**

      Show Merged Result   see **3.1.4.10 View | Show Merged Results**

3.1.4.1   View | Show Window

The **Show Window** command allows you to customize certain aspects of the screen contents.

Click **View | Show Window** to invoke the *Show Window* dialog.
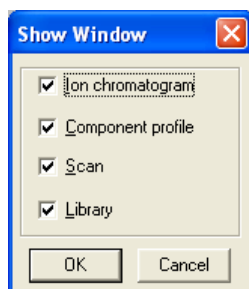
Figure 70 - Show Window dialog

The dialog has checkboxes for you to select the screen areas of the display that you wish to see, but, depending on the current display mode (**3.1.3 Mode Menu**) and screen contents, one or more may be unavailable for selection.

     Note: The various screen areas are introduced in **2.4 Confirm Window Appearance**.

This command is particularly useful if you need to see data on a small screen, or are trying to show multiple chromatograms (**3.4 Display of Multiple Chromatograms**).

Ion chromatogram displays the *Chromatogram Display* area to show the data file's total ion chromatogram (Target Only and Component modes).

Component profile displays the *Profile Display* and *Information Lists* areas, showing chromatogram profiles and textual information appropriate to the currently selected component or target (all modes).

     Note: If the *Information Lists* area is currently undocked from the *Profile Display* (**3.1.4.2 View | Undock**), the *Information Lists* area will remain on view when the **Component Profile** checkbox is cleared.

Scan displays the *Mass Spectral Display - Raw & Extracted* area to show the raw and deconvoluted scan (all modes).

Library displays the *Mass Spectral Display - Library & Extracted* area to show the deconvoluted scan and its associated library spectrum (Target Only mode and, provided a target is selected for viewing, Component mode).

3.1.4.2   View | Undock

The **Undock** command controls whether or not the *Information Lists* area of the screen is fixed in position to the right of the *Profile Display* area, or whether it is free to be moved around the screen and to be resized.

>   **Note:**   The various screen areas are introduced in **2.4 Confirm Window Appearance**.

If the **View | Undock** command does not have a check mark applied to its left, it is not active and the *Information Lists* area will be in its fixed position to the right of the *Information Lists* area.

If, however, it does have a check mark applied to its left, it is active and the *Information Lists* area may be positioned anywhere within the main AMDIS display area. One advantage of undocking is that better use can be made of the screen space. For example, it is possible to not show the *Profile Display* area and so allow the *Mass Spectral Display* area(s) to be larger. Another advantage is that the undocked *Information Lists* area may be expanded to show more information at one time.

Click **View | Undock** to alternate between applying and removing the menu item's check mark.

Refer also to **3.3.3.3.2 Undock** to learn more about undocking.

3.1.4.3   View | Show Mouse Position

The **Show Mouse Position** menu is used to:

→   control whether or not information is presented on the screen that relates to the current mouse position:

>   **On Cursor**   see **3.1.4.3.1 View | Show Mouse Position | On Cursor**
>
>   **On Top**   see **3.1.4.3.2 View | Show Mouse Position | On Top**
>
>   **Off**   see **3.1.4.3.3 View | Show Mouse Position | Off**

When a specific menu command is clicked, a check mark is applied to its left. However, these menu commands act like radio buttons and so are mutually exclusive. As such, only one command can have a check mark applied at any one time and so the check mark next to the previously active command is removed.

Whichever command is active will have its action applied to all graphical display areas.

>   **Note:**   If there is no check mark applied to any command, this is because a context menu has been used to change the **Show Mouse Position** status of one or more individual graphical areas to a different setting. Clicking the menu command resets all individual selections to its setting. See **3.3.1.1 Context Menu** for more information about the context menu.

3.1.4.3.1   View | Show Mouse Position | On Cursor

When the mouse pointer is within any of the graphical display areas and the **On Cursor** command is selected, the current x-axis position of the pointer will be shown close to the pointer.

When the pointer is within an area having a retention time axis, the pointer position is expressed as the closest scan number.

When the pointer is within an area having an m/z axis, the pointer position is expressed as the closest integer m/z value.

3.1.4.3.2   View | Show Mouse Position | On Top

When the mouse pointer is within any of the graphical display areas and the **On Top** command is selected, the current x- and y-axis positions of the pointer will be shown at a fixed position directly above the top right of the graphical area.

When the pointer is within an area having a retention time axis, the pointer position is expressed as the closest scan number, followed by the pointer's actual retention time position and ending with its actual absolute intensity value.

When the pointer is within an area having an m/z axis, the pointer position is expressed as the closest integer m/z value, followed by the pointer's actual intensity axis value. The intensity will be expressed in percentage terms for a library spectrum and otherwise as an absolute value.

3.1.4.3.3　View | Show Mouse Position | Off

When the **Off** command is selected, no information is shown relating to the mouse pointer position.

3.1.4.4　View | Thick Lines

The **Thick Lines** command controls the width with which chromatogram and mass spectral peaks are drawn.

If the **View | Thick Lines** command does not have a check mark applied to its left, it is not active and lines are drawn with their default thickness.

If it does have a check mark applied to its left, it is active and lines will be drawn several times thicker. This may have advantages under normal operation, but can be especially useful when taking screen captures for use in presentations.

Click **View | Thick Lines** to alternate between applying and removing the menu item's check mark.

3.1.4.5　View | Color Selected Peaks

The **Color Selected Peaks** command controls whether or not mass spectral peaks are color-matched with their corresponding ion chromatograms.

If the **View | Color Selected Peaks** command does not have a check mark applied to its left, it is not active and all mass spectral peaks are always drawn in their single assigned color (in default, black).

However, if the command does have a check mark applied to its left, it is active. In this case, as a mass spectral peak is clicked in either of the *Mass Spectral Display* areas, the peak changes to the same color as the single mass chromatogram trace that is drawn in whichever of the *Chromatogram Display* or *Profile Display* areas is active. Clicking on a peak that is already colored, removes the corresponding ion chromatogram trace from the active display area and reverts the peak to its normal color. The m/z values and their corresponding colors are maintained separately for the *Chromatogram Display* and *Profile Display* areas. As such, any mass spectral peak coloring will reflect the currently active area of these two display areas.

Irrespective of the state of the **Color Selected Peaks** command, the small triangle shown below the axis for each selected m/z peak will match the color of the corresponding mass chromatogram trace.

Click **View | Color Selected Peaks** to alternate between applying an removing the menu item's check mark.

See **3.1.6.3 Options | Color** for details of how to control these various colors.

See also **3.3.1 Chromatogram Display** for additional information relating to this command.

3.1.4.6　View | Auxiliary Information

The **Auxiliary Information** command invokes the multi-tabbed *AMDIS Results* dialog (**Figure 71 - AMDIS Results dialog**) that presents various information relating to the current analysis. As such, these values are not necessarily those that would be used for a new analysis, as these may have been modified in the *Analysis Settings* dialog as described in **3.1.2.2 Analyze | Settings**.
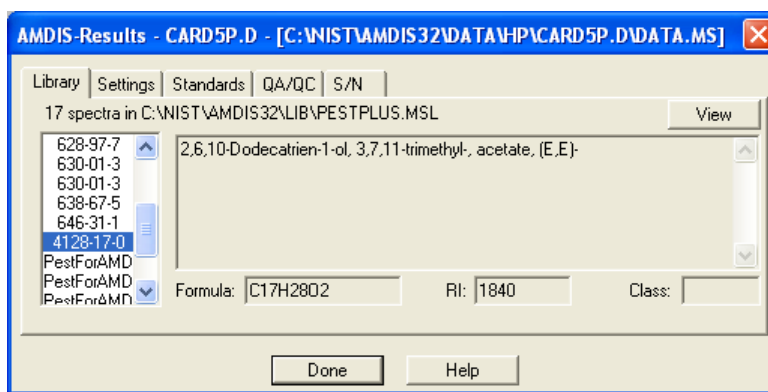
Figure 71 - AMDIS Results dialog

The *AMDIS Results* dialog is fully explained in Chapter **5 Auxiliary Information**.

Click **Done** to exit from the dialog.

3.1.4.7   View | Same Time Scale

The **Same Time Scale** command is only available if two or more chromatograms are being displayed and determines whether or not they all have aligned retention time axes. See **3.4 Display of Multiple Chromatograms** for details about displaying multiple data files.

If the **View | Same Time Scale** command does not have a check mark applied to its left, it is not active and each data file fully occupies the width of its *Chromatogram Display* area.

If the command does have a check mark applied to its left, it is active and the various retention time axes will have the same range, which in default will be from the lowest of all initial retention times to the highest of all final retention times of the displayed data files. If any one of the chromatograms is "zoomed" using the mouse (**2.4.5 Changing the Display Range**), the retention time range of all chromatograms will change to maintain the same time scale.

This functionality is particularly useful if you want to conveniently compare the same retention time range of different data files.

Click **View | Same Time Scale** to alternate between applying and removing the menu item's check mark.

3.1.4.8   View | Show AMDIS Search

The **Show AMDIS Search** command is only available after AMDIS analysis results have been further analyzed using the **Analyze | Search NIST Library** command with its dialog's **Build combined result** checkbox selected (**3.1.2.8 Analyze | Search NIST Library**).

The **View | Show AMDIS Search**, **View | Show NIST Search** and **View | Show Merged Results** commands act in a similar manner to radio buttons in that only one is active at any particular time. As such the active one is unavailable for selection, whereas the other two are available.

Click **View | Show AMDIS Search** to display only those targets from the combined results that were found by the AMDIS analysis.

3.1.4.9   View | Show NIST Search

The **Show NIST Search** command is only available after AMDIS analysis results have been further analyzed using the **Analyze | Search NIST Library** command with its dialog's **Build combined result** checkbox selected (**3.1.2.8 Analyze | Search NIST Library**).

The **View | Show AMDIS Search**, **View | Show NIST Search** and **View | Show Merged Results** commands act in a similar manner to radio buttons in that only one is active at any particular time. As such the active one is unavailable for selection, whereas the other two are available.

Click **View | Show NIST Search** to display only those targets from the combined results that were found by the NIST *MS Search* program.

3.1.4.10   View | Show Merged Results

The **Show Merged Results** command is only available after AMDIS analysis results have been further analyzed using the **Analyze | Search NIST Library** command with its dialog's **Build combined result** checkbox selected (**3.1.2.8 Analyze | Search NIST Library**).

The **View | Show AMDIS Search**, **View | Show NIST Search** and **View | Show Merged Results** commands act in a similar manner to radio buttons in that only one is active at any particular time. As such the active one is unavailable for selection, whereas the other two are available.

Click **View | Show Merged Results** to display all targets from the combined results; ie, those that were found by the AMDIS analysis together with those found by the NIST *MS Search* program.

3.1.5   Library Menu

The **Library** menu is used to:

$\rightarrow$ create and modify AMDIS library files

| **Build One Library** | see **3.1.5.1 Library | Build One Library** |
| **Library Transfer** | see **3.1.5.2 Library | Library Transfer** |

You may edit libraries or add data to libraries from current experiments, from text files, or from files created using the NIST *MS Search* program. For details about building and editing libraries, see Chapter **8 Libraries**.

3.1.5.1   Library | Build One Library

The **Build One Library** command allows you to construct a new library, or add to an existing library, by copying one or more component spectra to it from the current AMDIS analysis results. You may also modify the entries of an existing library.

AMDIS libraries are introduced and described in Chapter **8 Libraries** and the **Build One Library** command is fully described in **8.2.1 Build One Library Editor**.

3.1.5.2   Library | Library Transfer

The **Library Transfer** command allows you to construct a new library, or add to an existing library, by copying one or more entries from an existing library to it. You may also copy component spectra to a library from the current AMDIS analysis results. Individual entries in the library that is being created or modified can also be edited.

AMDIS libraries are introduced and described in Chapter **8 Libraries** and the **Library Transfer** command is fully described in **8.2.2 Library Transfer Editor**.

3.1.6   Options Menu

The **Options** menu is used to:

→   control the display of mass chromatograms

| **Select M/z** | see **3.1.6.1 Options | Select M/z** |

→   control fonts and colors

| **Font** | see **3.1.6.2 Options | Font** |
| **Color** | see **3.1.6.3 Options | Color** |

3.1.6.1   Options | Select M/z

The **Select M/z** command allows you to control the mass chromatograms that are displayed in the *Chromatogram Display* and *Profile Display* areas. It also interrelates with the manual selection of mass chromatograms by clicking on a displayed spectrum as described in **3.3.4 Mass Spectral Display**.

Note: This command is only available when analysis results are being displayed.

Click **Options | Select M/z** to invoke the *Select M/z* dialog.
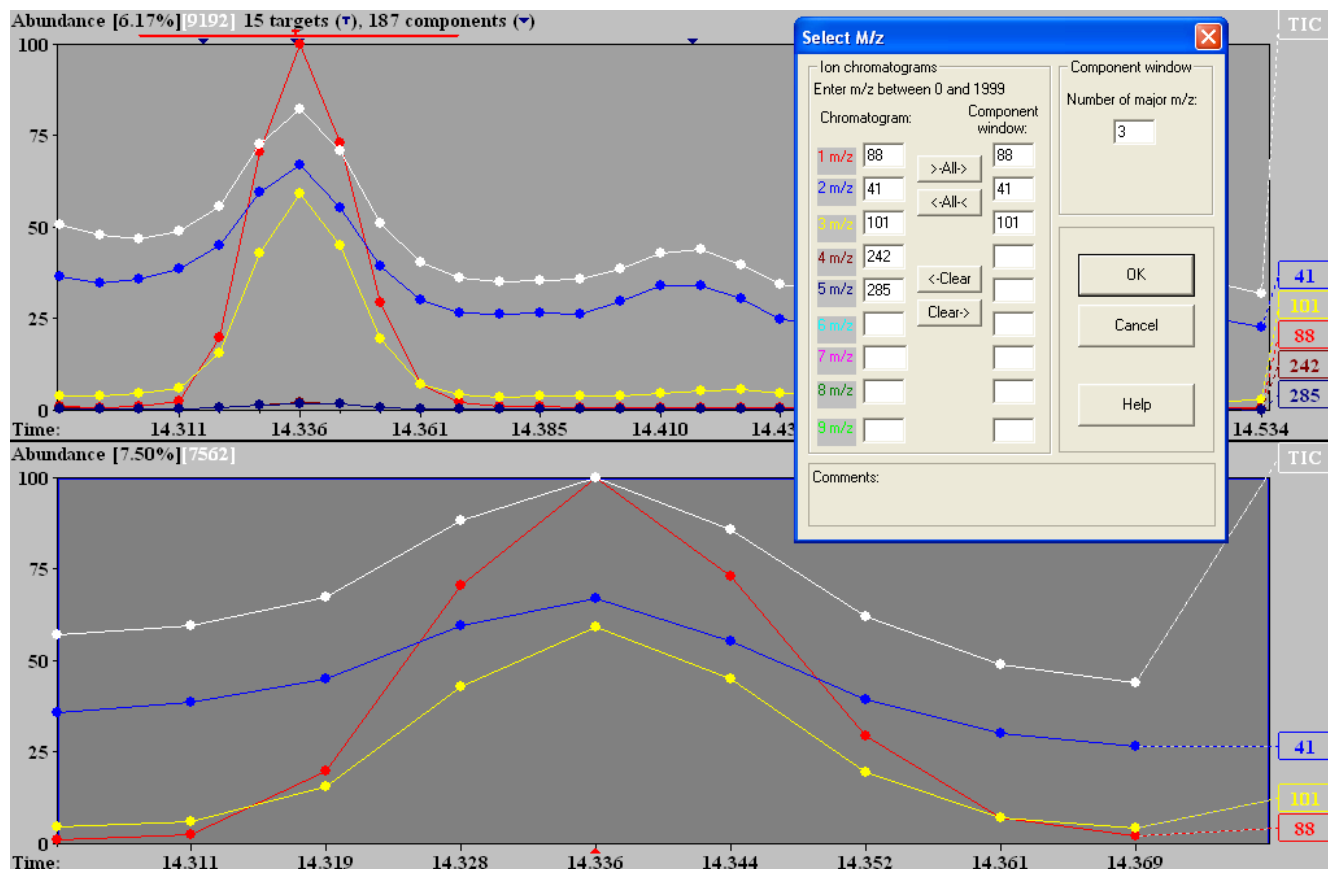
Figure 72 - Select M/z dialog

The dialog has two m/z lists. On entry to the dialog, the **Chromatogram** list shows the m/z values of any mass chromatogram(s) currently in the *Chromatogram Display* area and similarly the **Component window** list shows the value(s) in the *Profile Display* area. To the left of the **Chromatogram** list is the color that will be used for each m/z, for both the label and the ion chromatogram trace; as illustrated in **Figure 72 - Select M/z dialog**.

Within the **Ion chromatograms** parameter group are 4 command buttons:

>–All–> takes all of the m/z values in the **Chromatogram** list and copies them to the **Component window** list, such that only these values are now in that list.

<–All–< takes all of the m/z values in the **Component window** list and copies them to the **Chromatogram** list, such that only these values are now in that list.

<–Clear removes all m/z values from the **Chromatogram** list.

Clear–> removes all m/z values from the **Component window** list.

It is also possible to manually delete, replace or add new m/z values to either list using keyboard input.

On clicking **OK** to exit the dialog, the display changes to reflect the dialog's m/z values.

> **Note:** If the **Component window** list is clear when **OK** is clicked, the most intense m/z of the component spectrum is automatically put into the list and so is displayed.

The **Number of major m/z** parameter is utilized whenever a different component is selected for display. When that happens the specified number of the most intense ions in the component spectrum display their mass chromatograms in the *Profile Display* area. If you then enter this dialog you will see those same masses (and only those) now occupying the **Component window** list. However, the **Chromatogram** list is independent of the selected component and will remain the same until altered by any of the mechanisms described above.

3.1.6.2 Options | Font

The **Font** command allows you to select the details of the font that is used for all text in all display areas, except for *Information Lists*.

Click **Options | Font** to invoke the *Font* dialog.
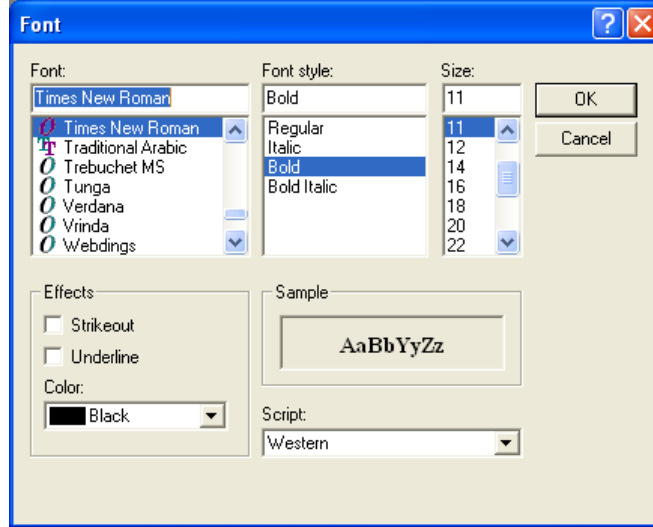
Figure 73 - Font dialog

As you click in the dialog to select any parameter value, the **Sample** area gives you an immediate feedback as to what your current selection would look like. Click **OK** to make your selections take effect.

3.1.6.3 Options | Color

The **Color** command allows you to choose virtually all of the colors that AMDIS utilizes and further offers the facility to store several different sets of these colors as "color schemas".

Click **Options | Color** to invoke the *Color* dialog.
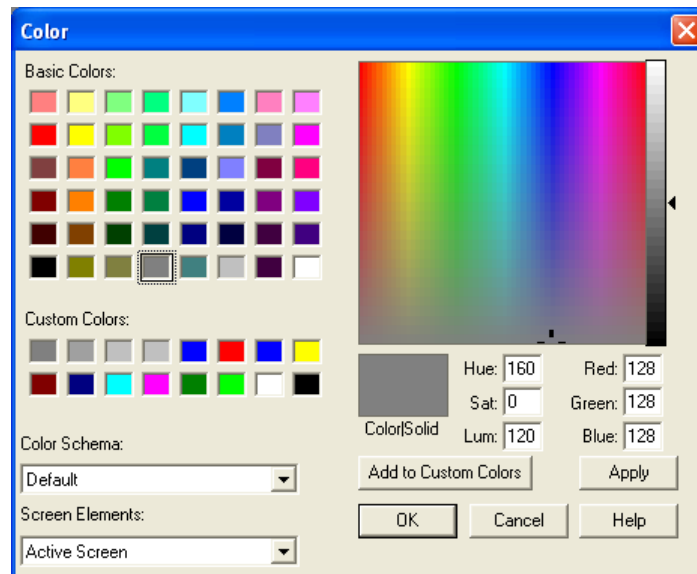
Figure 74 - Color dialog

There are a number of named color sets that can be defined and their names can be seen in the **Color Schema** drop-down list box. Each schema holds a color definition for each of the different drawing components that AMDIS utilizes in its various displays and these are shown in the **Screen Elements** drop-down list box (for a description of these, see **3.1.6.3.1 Screen Elements**).

To view current color assignments, select a **Color Schema** and then one of the **Screen Elements**. The stored color is displayed visually in the **Color|Solid** box, with the various numeric values defining it to the right.

The current **Screen Elements** color can be changed in a variety of ways. You can click on one of the **Basic Colors** or **Custom Colors**, or click anywhere in the large color box, or modify the numeric values. The selected color is immediately shown in the **Color|Solid** box. Click **Apply** to see the impact this new color has on the current display (assuming that the **Screen Elements** selection is currently visible). When you are satisfied with the current selections, click **OK** to save the values as the selected **Color Schema**; otherwise click **Cancel** to leave the original values as they were.

> **Note:** Although **Cancel** does not adjust the original color values, any changes made to the display using **Apply** will continue to be utilized in that window until it is closed.

Once you have found a color you particularly like, you may want to store it for subsequent convenient reuse. In that case, click **Add to Custom Colors** and note that it replaces the currently highlighted selection in **Custom Colors**, or the next in sequence if you are repetitively adding multiple colors.

Click **OK** to save any changes, or **Cancel** to retain the original values.

> **Note:** The rows of **T** and ▼ symbols along the top of the *Chromatogram Display* area and mass spectral peaks (except uncertain peaks) in all *Mass Spectral Display* areas are of a fixed color and so cannot be changed.

3.1.6.3.1 Screen Elements

The **Screen Elements** drop-down list box shows the various drawing components that AMDIS utilizes in its various displays. These are:

Active screen	The **Active screen** areas are all *Mass Spectral Displays* plus whichever of the *Chromatogram Display* or the *Profile Display* most recently had the input focus. An active screen area is one that can respond to an action in another screen area; for example, to display a mass chromatogram or spectrum.
Inactive screen	The **Inactive screen** area is whichever of the *Chromatogram Display* or the *Profile Display* has not most recently had the input focus. It is the screen area that will not display a mass chromatogram when an ion is selected in a *Mass Spectral Display*.
Background	The **Background** is the main background for the window, in which the active and inactive screen areas sit.
Buttons	**Buttons** refers to the background of the mass chromatogram label (rectangular area with number) that appears to the right of the *Chromatogram Display* and *Profile Display* areas and which can be clicked on to remove both it and the trace it is labeling .
Active frame	The **Active frame** is a border line drawn up both sides and across the top of each active screen area to further identify an active screen area.
1...9 m/z color	An **m/z color** refers to one of the possible 9 unique single ion chromatogram traces that can be drawn in each of the *Chromatogram Display* and *Profile Display* areas. The color selection will be used for the trace itself and also its associated button's text and border.
TIC and Component color	The **TIC and Component color** is used for the TIC chromatograms drawn in the *Chromatogram Display* and *Profile Display* areas.
Uncertain peaks color	The **Uncertain peaks color** is used for uncertain peaks, which appear in a *Mass Spectral Display* and are drawn dashed (**3.1.2.7 Analyze | Use Uncertain Peaks**).

3.1.7 Window Menu

The **Window** menu is used to:

→ control how child windows are displayed

Cascade	see **3.1.7.1 Window | Cascade**
Tile	see **3.1.7.2 Window | Tile**
Arrange Icons	see **3.1.7.3 Window | Arrange Icons**
Close All	see **3.1.7.4 Window | Close All**
<window list>	see **3.1.7.5 List of Open Windows**

It is possible to simultaneously have multiple files open in different child windows (**3.4.1 Multiple Chromatograms in Independent Windows**). Each window can either be "active" (not minimized) or "minimized". If a window is minimized it is represented by an icon at the bottom of the main AMDIS window. It is also possible to have two chromatograms in a single window (**3.4.2 Two Chromatograms in One Window**).

The arrangement of the child windows within the main AMDIS window is controlled by the **Cascade** and **Tile** commands. If windows are minimized, the **Arrange Icons** command can be used.

3.1.7.1 Window | Cascade

Cascade is a standard *Windows* command to arrange active child windows in a cascading manner such that all window title bars can be read.

3.1.7.2 Window | Tile

Tile is a standard *Windows* command to neatly arrange active child windows in a tiled manner.

3.1.7.3 Window | Arrange Icons

Arrange Icons is a standard *Windows* command to neatly arrange any minimized child windows.

3.1.7.4 Window | Close All

The **Close All** command will close all child windows that are currently open.

3.1.7.5 List of Open Windows

At the bottom of the **Window** command's menu is a list numbered from **1** up to however many child windows are currently in use. Each list member is a full file descriptor of the data file in the child window and the window currently having the input focus will have a check mark to the left of its numeric identifier.

To give the input focus to a different window, simply click on the descriptor of the window that you want.

3.1.8 Help Menu

The **Help** menu is used to:

→ provide help on AMDIS

Contents	see **3.1.8.1 Help | Contents**
Help on Current	see **3.1.8.2 Help | Help on Current**
About	see **3.1.8.3 Help | About AMDIS**
Go to AMDIS Website	see **3.1.8.4 Help | Go to AMDIS Website**

3.1.8.1 Help | Contents

The **Contents** command invokes the full AMDIS help system, which offers comprehensive help on AMDIS; including index and search facilities.

The help system is navigated in the normal *Windows* manner.

3.1.8.2 Help | Help on Current

The **Help on Current** command invokes the full AMDIS help system, but at an appropriate topic for the *Confirm* window.

The help system is navigated in the normal *Windows* manner.

3.1.8.3 Help | About AMDIS

The **About AMDIS** command invokes a dialog showing version information for AMDIS.

3.1.8.4 Help | Go to AMDIS Website

Provided that you have an internet connection, the **Go to AMDIS Website** command takes you to the http://chemdata.nist.gov/ web page (Chemical Data from NIST) where you can find links to various chemical data products, updates, publications and presentations that are available from NIST.

These include a link to the AMDIS Download Page , where you can find the latest information about AMDIS.

3.2 Button Bar

Each child window in the AMDIS *Confirm* window has its own "button bar" positioned between the child window's title bar and its main graphics display.

The button bar presents the following options:

Run	see **3.2.1 Run**
Rescale	see **3.2.2 Rescale**
Info	see **3.2.3 Info**
◄ ►	see **3.2.4 Arrowheads**
Manual-On and Manual-Off	see **3.2.5 Manual-On and Manual-Off**
Deconv	see **3.2.6 Deconv**

3.2.1 Run

The **Run** button first of all analyzes a GC/MS data file to locate components. According to the selected analysis type, it may compare these against a list of target compounds and determine any matches; which themselves may then be used, for example, to create a calibration file. In all cases the analysis results are presented in the *Confirm* window for further examination and processing.

The parameters controlling the analysis are those set up in the *Analyze GC/MS Data* dialog (**Figure 61 - Analyze GC/MS Data dialog**) and are described in **3.1.2.1 Analyze | Analyze GC/MS Data**.

The various analysis types and their operation, including parameter selection and results presentation, are described in detail in Chapter **6 Analysis**.

Depending on the chosen analysis type and the complexity and amount of the GC/MS data, the analysis could take several seconds. In that case you would see progress dialogs.

When the analysis is complete the results are presented graphically in the *Confirm* window and are described fully in **3.3 Data Displays**.

> **Note:**   The analysis is performed on the portion of the data file currently shown in the *Chromatogram Display* area of the window and replaces any previous results for the file.

> **Note:**   When you click **Run** it is possible for the *Maximum m/z Mismatch* warning dialog to appear. Normally you would click **Autofix** to proceed (**High m/z** in **6.3.2 Instr. (Instrument) Tab**).
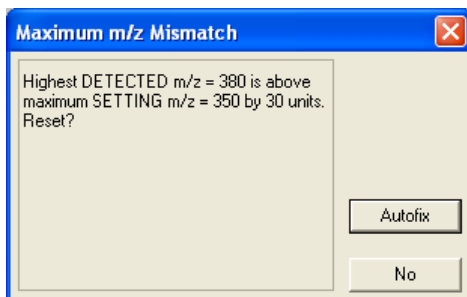
Figure 75 - Maximum m/z Mismatch dialog

3.2.2 Rescale

The **Rescale** button will readjust the vertical axis of the *Chromatogram Display* area such that the largest mass chromatogram peak in the displayed retention time range is displayed at full height. The TIC chromatogram is also renormalized if necessary and the intensity axis maximum is labeled with **100**.

See also **3.3.1.1.3 Rescale**.

3.2.3 Info

The **Info** button invokes the *AMDIS Results* dialog, which has a number of tabs holding various information relating to the current data file, analysis settings and analysis results.

The identical dialog is invoked by the **View | Auxiliary Information** command and the dialog is fully described in Chapter **5 Auxiliary Information**.

3.2.4 Arrowheads

The and "arrowhead" buttons are used to move the currently "zoomed" retention time axis of the *Chromatogram Display* area, respectively, left (lower in retention time) or right (higher in retention time). Each click of a button adjusts the start and end of the displayed retention time in the appropriate direction by approximately one sixteenth of the displayed retention time range. Once the minimum (for), or maximum (for), retention time is reached, the corresponding button no longer functions.

The arrowhead buttons are only enabled when the display is in a zoomed state (**2.4.5 Changing the Display Range**).

3.2.5 Manual-On and Manual-Off

The **Manual-On** button toggles to **Manual-Off** when selected and vice versa, and works in conjunction with the **Deconv** button.

In its **Manual-Off** state the **Deconv** button is not available for selection and manual, mass-spectral background subtraction can be performed (**10.1 Manual Background Subtraction**).

In its **Manual-On** state the **Deconv** button is available for selection and when clicked permits manual deconvolution (**3.2.6 Deconv**).

> **Note:** The **Manual-On/Manual-Off** button only appears if the *Confirm* window holds no analysis results for the current data file.

3.2.6 Deconv

The **Deconv** button invokes the *Deconvolution* dialog to permit manual deconvolution. It is only available for selection if the **Manual-On/Manual-Off** button is showing **Manual-On** (**3.2.5 Manual-On and Manual-Off**).

Manual deconvolution is described in **10.2 Manual Deconvolution**.

> **Note:** The **Deconv** button only appears if the *Confirm* window holds no analysis results for the current data file.

3.3   Data Displays

The *Confirm* window has two basic appearances according to whether or not it is showing the results of an analysis and these are introduced in **2.4 Confirm Window Appearance**.

Further, the contents of the *Confirm* window can be regulated using the **View | Show Window** command (**3.1.4.1 View | Show Window**), its context-sensitive **Show Window** equivalent (**3.3.4.1.7 Show Window**), or when results are being shown, the **Mode** command (**3.1.3 Mode Menu**).

In all display areas except the *Profile Display*, it is possible to use the mouse to zoom in to see more detail and to subsequently zoom out again (**2.4.5 Changing the Display Range**).

In all display areas a context-sensitive menu is always available by right-clicking the mouse and these menus are described in each display area's section below.

The 4 main display areas are:

Chromatogram Display   see **3.3.1**

Profile Display   see **3.3.2**

Information Lists   see **3.3.3**

Mass Spectral Display   see **3.3.4**

3.3.1   Chromatogram Display

The *Chromatogram Display* area generally shows the TIC chromatogram on a retention time x-axis labeled in decimal minutes. It may also show one or more single-mass chromatograms on the same time axis.

If you right-click anywhere within the *Chromatogram Display* area, a command menu appears that permits you to control many aspects of the display (**3.3.1.1 Context Menu**).

As an example of the *Chromatogram Display* area, consider:
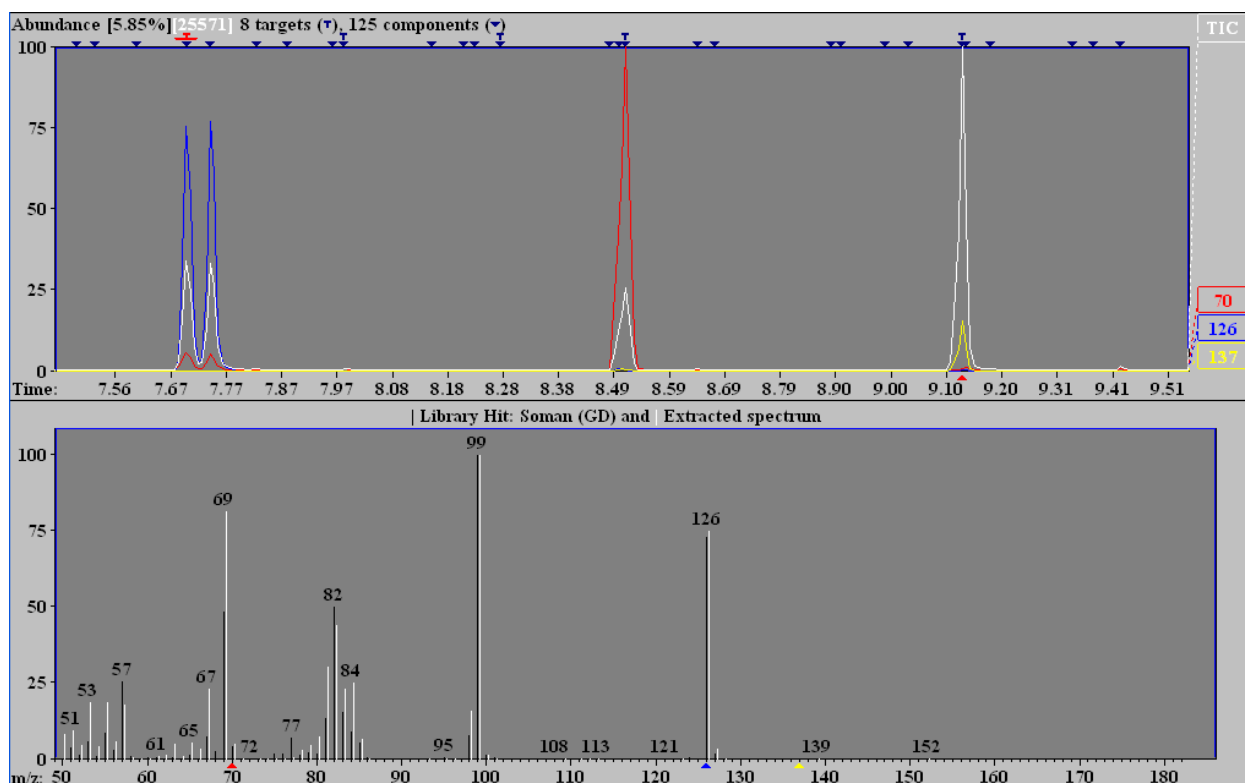
Figure 76 - Chromatogram Display chromatograms

The display shows a TIC chromatogram (white) and 3 single-mass chromatograms; m/z 70 (red), 126 (blue) and 137 (yellow). You can see that the chromatogram traces and their numeric identifiers to the right of the display are color-coded. The colors utilized are selected using the **Options | Color** command (**3.1.6.3 Options | Color**).

The *Chromatogram Display* always shows the TIC chromatogram unless it has been explicitly turned off by clicking on its identifier. In that case, the trace is removed, but the TIC identifier still remains (colored black) and the trace can be turned back on again by clicking on the identifier.

Single-mass chromatogram traces are interactively added to the display by clicking in any *Mass Spectral Display* area. The integer m/z value closest to the mouse click is chosen. If the selected m/z chromatogram is not already displayed, it will be added to the display and a ▲ symbol of the same color positioned at the appropriate m/z position below the x-axis (as shown in **Figure 76 - Chromatogram Display chromatograms**).

> **Note:** If two spectral displays are active, the colored ▲ symbol appears below both m/z axes.

> **Note:** The selected m/z peak may also change color (**3.1.4.5 View | Color Selected Peaks**).

A chromatogram trace (and its numeric identifier) can be removed from the display by clicking either on its numeric identifier in the *Chromatogram Display*, or on the corresponding m/z value position in the *Mass Spectral Display*.

Single mass chromatograms can also be added and removed using the **Options | Select M/z** command (**3.1.6.1 Options | Select M/z**) and the *Mass Spectral Display* area will reflect any changes in the same manner as interactive additions and removals.

Often the *Chromatogram Display* will be very "crowded" with target and component symbols overlapping. In order to see the detail of the chromatogram(s) and to easily choose (by clicking on it) a specific target or component symbol, it is necessary to first "zoom" (magnify) the display (**2.4.5 Changing the Display Range**).

To the top left of the *Chromatogram Display* area is **Abundance** information. Consider again **Figure 76 - Chromatogram Display chromatograms**. The **[25571]** is the absolute value multiplier for the intensity percentage value of the y-axis and relates to the maximum TIC value within the displayed retention time range, irrespective of whether or not the TIC chromatogram is currently turned on or off and irrespective of any vertical scaling. For example, the absolute value at 100% on the intensity axis would in this case be 25571 * 100 = 2557100.

If one or more single ion chromatograms are displayed, an additional value is presented; for example, **[5.85%]** in **Figure 76 - Chromatogram Display chromatograms**. This is a multiplier that enables you to determine the absolute values of the displayed single ion chromatograms. Chromatogram m/z 70 has the largest single ion chromatogram intensity in **Figure 76 - Chromatogram Display chromatograms** and we can calculate that its corresponding absolute value at 100% on the y-axis is 5.85% of 25571 * 100; approximately 149590.

The displayed value(s) will be preserved through any vertical zooming, since each is simply a multiplier of the percentage value. For example, if the vertical axis has been zoomed such that it is showing 20%, the absolute value represented by a signal at that 20% will now be 511420 (ie, 20% of 25571 * 100).

For analyzed data (and as appropriate) you can see to the right of the abundance information the number of found targets (T symbol) and components (▼ symbol); where the count of components includes the targets.

Along the top of the chromatogram graphical display area are rows of T and ▼ symbols, where a T will appear immediately above its corresponding ▼. These symbols are colored dark blue and are positioned at the retention times determined for these compounds by the deconvolution process. You can click on any of the symbols to make it the current selection, at which point the *Confirm* window will redisplay in target or component mode, according to your selection.

> **Note:** If you have chosen to only view targets (**3.1.3.1 Mode | Target Only**), only T symbols will be displayed for selection.

The currently selected target or component is signified by its symbol being colored red and the rest of the *Confirm* window's data relates to this compound. For example, consider:
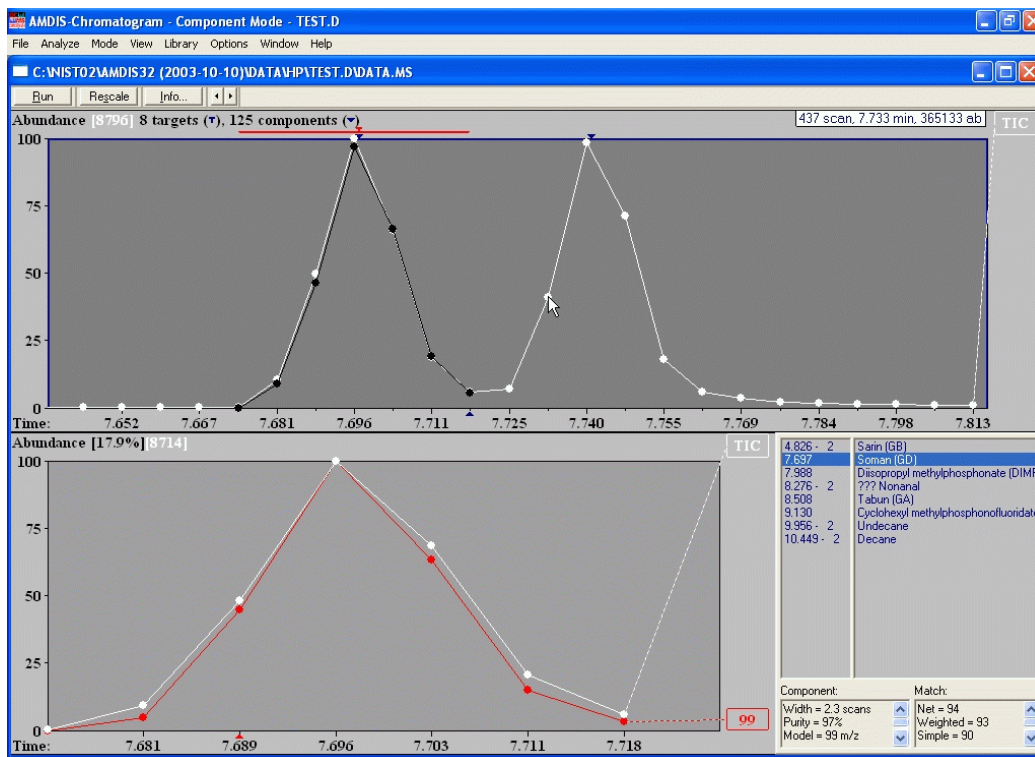
Figure 77 - Expanded Chromatogram

In the figure the target at 7.697 minutes has been selected and the *Profile Display* and *Information Lists* areas show information for that. Notice first of all that the retention time display range is small enough such that individual scan values can easily be seen and they are represented as white circles. Also, there is a horizontal red line drawn to either side of the highlighted target and this represents the baseline width of the target as identified by the deconvolution process. Finally, there is a black trace corresponding to the red horizontal line and this shows the deconvoluted peak's actual profile (**3.3.1.1.7 Show Component on Chromatogram**). In this case there is an almost perfect correspondence of the two traces indicating good chromatographic separation and virtually no chemical noise background.

> **Note:** The m/z 99 trace in the *Profile Display* shows an equivalent close fit to the TIC chromatogram as might be expected in these circumstances.

The horizontal red line and black trace are also relevant for components.

You will often be able to see a ▲ symbol below the x-axis. This will be colored black, blue or red and is a marker reflecting what is being viewed in the *Mass Spectral Display* area (**3.3.4 Mass Spectral Display**).

There may also be positional information displayed for the mouse, either within the chromatogram trace area, or to the top right of the display as illustrated in **Figure 77 - Expanded Chromatogram**. This is described in **3.1.4.3 View | Show Mouse Position**.

In some cases, mainly when preparing to show results in presentations, you may wish to draw thicker chromatogram lines. This is achieved using the **Thick Lines** command (**3.1.4.4 View | Thick Lines**).

3.3.1.1   Context Menu

If you right-click anywhere within the *Chromatogram Display* area, a command menu appears (the "context-sensitive" menu).

The context-sensitive menu is used to:

→   adjust the scaling of the displayed chromatograms

Unzoom   see **3.3.1.1.1 Unzoom**

Unzoom All	see **3.3.1.1.2 Unzoom All**	
Rescale	see **3.3.1.1.3 Rescale**	
Autoscale	see **3.3.1.1.4 Autoscale**	
Log Scale for Chromatogram	see **3.3.1.1.5 Log Scale for Chromatogram**	

→ control any feedback from the mouse pointer's position

Show Mouse Position	see **3.3.1.1.6 Show Mouse Position**	

→ control how components are shown

Show Component on Chromatogram	see **3.3.1.1.7 Show Component on Chromatogram**	
Show Only Current Component	see **3.3.1.1.8 Show Only Current Component**	

→ control manual, mass-spectral background subtraction

Manual On	see **3.3.1.1.9 Manual On**	
Signal	see **3.3.1.1.10 Signal**	
Background	see **3.3.1.1.11 Background**	
Clear	see **3.3.1.1.12 Clear**	
Clear All	see **3.3.1.1.13 Clear All**	

→ control which areas of the *Confirm* window are shown

Show Window	see **3.3.1.1.14 Show Window**	

→ determine the data file(s) displayed

File	see **3.3.1.1.15 File**	

Since this menu is context sensitive, not all commands will always be available.

3.3.1.1.1 Unzoom

The **Unzoom** command is available when the display has been zoomed and will restore the display to its state prior to the previous zoom. If several zooms have taken place in succession, **Unzoom** will be able to be used repeatedly until the starting condition is attained.

Zooming is described in **2.4.5 Changing the Display Range**.

3.3.1.1.2 Unzoom All

The **Unzoom All** command is available when the display has been zoomed more than once and will restore the display to its initial state prior to the first zoom in any succession of zooms.

Zooming is described in **2.4.5 Changing the Display Range**.

3.3.1.1.3 Rescale

The **Rescale** command will readjust the vertical axis of the *Chromatogram Display* area such that the largest mass chromatogram peak in the displayed retention time range is displayed at full height. The TIC chromatogram is also renormalized if necessary and the intensity axis maximum is labeled with **100**.

This feature can be particularly useful when you have been adjusting the display by zooming (**2.4.5 Changing the Display Range**) and have "cut off" the top of a mass chromatogram that you now wish to see fully. Alternatively you may have been viewing a very intense chromatogram together with less intense ones and you have just deleted the most intense one in order that you can see more of the detail of the less intense chromatograms.

This command performs the same action as the button bar's **Rescale** button (**3.2.2 Rescale**).

If you always want mass chromatograms to be automatically rescaled, use **Autoscale** (**3.3.1.1.4 Autoscale**).

3.3.1.1.4 Autoscale

The **Autoscale** command is used to specify the vertical scaling effect of adding and removing single ion mass chromatograms. Its use does not affect the operation of TIC chromatogram scaling.

Each time you click on **Autoscale**, it will alternate between having and not having a check mark applied to its left.

With a check mark applied, each time a mass chromatogram is added or removed from the *Chromatogram Display* area all mass chromatogram traces are examined and then redrawn if there is a new maximum signal value within the displayed retention time range.

Without a check mark applied, the addition and removal of mass chromatograms does not affect the display normalization, except that as mass chromatograms are removed to leave only one displayed, this will always be redrawn to full scale. In the case where no mass chromatograms currently exist, the addition of the first will determine the scale of the abundance axis.

> **Note:** Whether or not the **Autoscale** command has a check mark applied, the TIC trace will always scale itself to its maximum value within the displayed range.

See also **Rescale** (**3.3.1.1.3 Rescale**), which can be used when **Autoscale** does not have a check mark applied.

3.3.1.1.5 Log Scale for Chromatogram

The **Log Scale for Chromatogram** command is used to adjust the scaling of chromatogram intensities.

Each time you click on **Log Scale for Chromatogram**, it will alternate between having and not having a check mark applied to its left.

With a check mark applied, the axis is labeled as **Log abundance** and intensities are displayed in log (base 10) format.

Without a check mark applied, intensities are displayed in linear format and the axis is labeled as **Abundance**.

In both cases the y-axis label is followed by the absolute intensity (in square brackets) represented by **100** on the y-axis.

3.3.1.1.6 Show Mouse Position

The **Show Mouse Position** menu is used to:

$\rightarrow$ control whether or not information is presented on the screen that relates to the current mouse position (**On Cursor**, **On Top** and **Off** commands).

When a specific menu command is clicked, a check mark is applied to its left. However, these menu commands act like radio buttons and so are mutually exclusive. As such, only one command can have a check mark applied at any one time and so the check mark next to the previously active command is removed.

Whichever command is active will have its action applied to the current graphical display area only.

These context menu commands are identical in name and operation to those of the main menu's **Show Mouse Position** menu commands and are also affected by their selection.

Their operation and interactions are described fully in **3.1.4.3 View | Show Mouse Position**.

3.3.1.1.7 Show Component on Chromatogram

The **Show Component on Chromatogram** command determines whether or not the reconstructed TIC profile of a component is shown together with the standard TIC trace.

Each time you click on **Show Component on Chromatogram**, it will alternate between having and not having a check mark applied to its left.

With a check mark applied, a component's trace is shown in black at the same scaling as the TIC trace (**Figure 78 - Chromatogram showing components**). Otherwise without a check mark applied, the trace is not shown.

Note: **Show Component on Chromatogram** will display either a single component's trace, or multiple traces, according to the setting of **Show Only Current Component** (**3.3.1.1.8 Show Only Current Component**).
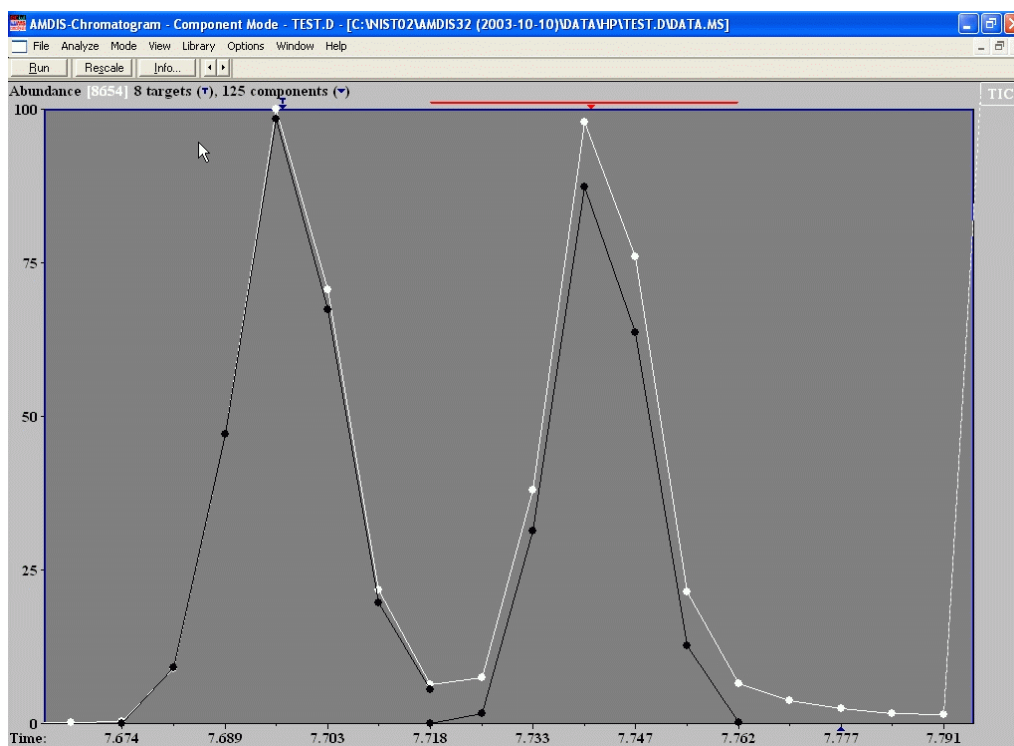
Figure 78 - Chromatogram showing components

When the deconvolution algorithm finds components, it stores abundance information for each ion of each scan of the component. It is these abundances that are added together for each scan to reconstruct its TIC value. The range of scans forming the component is represented at the top of the display by the horizontal red line to either side of the selected target or component symbol; as illustrated for the right-hand component in **Figure 78 - Chromatogram showing components**. The displayed traces also provide visual feedback as to how much of the total signal is taken up by a component.

Note: The red line will not appear if the displayed range is too wide and so zooming would be necessary if you wished to see it.

3.3.1.1.8 Show Only Current Component

The **Show Only Current Component** command determines whether a single component profile is shown within the displayed range, or all component profiles are shown.

Each time you click on **Show Only Current Component**, it will alternate between having and not having a check mark applied to its left.

With a check mark applied, only a single component profile is shown. Otherwise without a check mark applied, all component profiles are shown (for example, **Figure 78 - Chromatogram showing components**).

Note: **Show Only Current Component** is only available for selection and utilized when **Show Component on Chromatogram** has a check mark applied to its left (**3.3.1.1.7 Show Component on Chromatogram**).

In normal usage **Show Only Current Component** would have its check mark applied. However, with it removed you may find some benefit in seeing how components overlap; but you will normally need to use high magnification for this to be effective.

3.3.1.1.9   Manual On

The **Manual-On** command determines whether or not manual, mass-spectral background subtraction mode is active.

Each time you click on **Manual-On**, it will alternate between having and not having a check mark applied to its left.

With a check mark applied, manual mass-spectral background subtraction mode is active and **Signal** (**3.3.1.1.10**), **Background** (**3.3.1.1.11**), **Clear** (**3.3.1.1.12**) and **Clear All** (**3.3.1.1.13**) are all enabled.

If **Signal**, **Background**, or **Clear** is clicked, a check mark is applied to its left. However, these three menu commands act like radio buttons and so are mutually exclusive. As such, only one command can have a check mark applied at any one time and so the check mark next to the previously active command is removed. The mouse cursor will also change in appearance to reflect which command of the three has the check mark applied.

Without a check mark applied to **Manual-On**, manual mass-spectral background subtraction mode is inactive.

> **Note:**   **Manual-On** is only available for selection if the *Confirm* window holds no analysis results for the current data file.

This command performs the same action as the button bar's **Manual-On** button (**3.2.5 Manual-On and Manual-Off**) and its operation is fully described in **10.1 Manual Background Subtraction**.

3.3.1.1.10   Signal

The **Signal** command is only active when **Manual-On** has a check mark applied to its left (**3.3.1.1.9 Manual On**). When **Signal** has a check mark applied to its left, the shape of the mouse cursor changes to signify the **Signal** command.

The mouse pointer is then used to manually select a series of spectra, which are immediately averaged together to yield a single spectrum, from which an averaged background spectrum will then be subtracted to provide a higher quality spectrum representation of the chromatographic peak.

Its operation is fully described in **10.1 Manual Background Subtraction**.

3.3.1.1.11   Background

The **Background** command is only active when **Manual-On** has a check mark applied to its left (**3.3.1.1.9 Manual On**). When **Background** has a check mark applied to its left, the shape of the mouse cursor changes to signify the **Background** command.

The mouse pointer is then used to manually select a series of spectra, which are immediately averaged together to yield a single spectrum. This is then subtracted from the single averaged spectrum representing the signal, to provide a higher quality spectrum representation of the chromatographic peak.

Its operation is fully described in **10.1 Manual Background Subtraction**.

3.3.1.1.12   Clear

The **Clear** command is only active when **Manual-On** has a check mark applied to its left (**3.3.1.1.9 Manual On**). When **Clear** has a check mark applied to its left, the shape of the mouse cursor changes to signify the **Clear** command.

The mouse pointer is then used to manually clear any spectra selected by the **Signal** or **Background** command. After each use of the mouse, the manually extracted spectrum is recalculated using all remaining signal and background spectra.

Its operation is fully described in **10.1 Manual Background Subtraction**.

3.3.1.1.13   Clear All

The **Clear All** command is only active when **Manual-On** has a check mark applied to its left (**3.3.1.1.9 Manual On**).

When **Clear All** is clicked, all spectra currently chosen by the **Signal** or **Background** command are cleared; such that no manually extracted spectrum now exists.

Its operation is fully described in **10.1 Manual Background Subtraction**.

3.3.1.1.14 Show Window

The **Show Window** command allows you to customize certain aspects of the screen contents.

The operation of this command is identical to the **View | Show Window** command and is fully described for it (**3.1.4.1 View | Show Window**).

3.3.1.1.15 File

The **File** menu is used to:

→ open or close data files

Open	see **3.3.1.1.15.1 File	Open**
Add	see **3.3.1.1.15.2 File	Add**
Close	see **3.3.1.1.15.3 File	Close**

3.3.1.1.15.1 File | Open

The **Open** command is used to select a data file for display within AMDIS.

The operation of this command is identical to the main menu's **File | Open** command and is fully described for it (**3.1.1.1 File | Open**).

3.3.1.1.15.2 File | Add

The **Add** command is used to select a second data file for display within a single window in AMDIS.

Add is not available for selection when 2 files already exist in a single window.

The operation of this command is identical to the main menu's **File | Open In | Active Window** command and is fully described for it (**3.1.1.2.1 File | Open In | Active Window**).

3.3.1.1.15.3 File | Close

The **Close** command is used to remove a data file from display within AMDIS.

Close is only available for selection when 2 files already exist in a single window and will remove the file in the display area selected by this command.

3.3.2 Profile Display

The *Profile Display* area shows the TIC chromatogram and major characteristic ion(s) of the deconvoluted compound (component or target) over the retention time window in which is was determined.

If you right-click anywhere within the *Profile Display* area, a command menu appears that permits you to control aspects of the display (**3.3.2.1 Context Menu**).

As an example of the *Profile Display* area, consider:
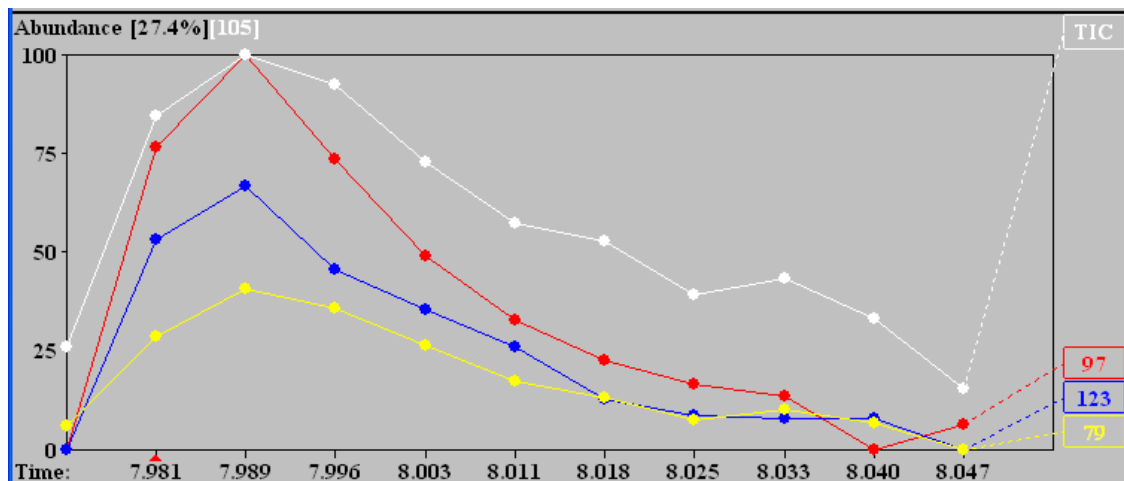
Figure 79 - Profile Display

The TIC chromatogram (white trace) and most abundant of the displayed component ions (red trace) are always drawn to full scale on the y-axis. In this case, two additional significant ions are also shown (blue and yellow traces).

The number of ion chromatograms shown is controlled using the **Options | Select M/z** command (**3.1.6.1 Options | Select M/z**).

The model peak, the peak showing the most rapid rise and fall, is always shown; it is assumed to be the most characteristic peak for a particular component. The model peak is often the TIC, but in complex mixtures with many coeluting peaks, the individual ion signals are far more useful as a model. In cases where a specific peak has been subtracted during the deconvolution process it will always be shown, even if that peak does not occur in the component's spectrum.

> **Note:** In the rare situation where the requested number of ions to be displayed is 1 and the model peak is not the TIC and there is a subtracted peak, the model peak and subtracted peak will both be shown.

> **Note:** If less than the expected number of chromatogram traces are drawn it will be because the deconvoluted spectrum does not have that many ions in it.

The y-axis is a relative abundance, percentage scale. The absolute abundance value given at the top left of the display is for the TIC chromatogram and is equivalent to 100% on the y-axis; eg, **105** in **Figure 79 - Profile Display**. The percentage abundance value there shows the abundance relationship between the TIC and the most abundant ion. In this case m/z **97** has an absolute abundance of approximately **29** (27.4% of 105).

In general, the *Chromatogram Display* is active. As a result, when a peak is selected in a *Mass Spectral Display* (by clicking on it with the mouse) the individual ion chromatogram will be shown in the *Chromatogram Display*. However, if you click the mouse in the *Profile Display* area, that will become active and any m/z selected in a *Mass Spectral Display* will now show up as a single ion chromatogram in the *Profile Display*. This is especially useful if you want to look at peaks at high resolution while at the same time being able to see the entire chromatogram.

3.3.2.1 Context Menu

If you right-click anywhere within the *Profile Display* area, a command menu appears (the "context-sensitive" menu).

The context-sensitive menu is used to:

→   adjust the scaling of the displayed chromatograms

    Unzoom   see **3.3.2.1.1 Unzoom**

    Log Scale for Component   see **3.3.2.1.2 Log Scale for Component**

→   control any feedback from the mouse pointer's position

    Show Mouse Position   see **3.3.2.1.3 Show Mouse Position**

→   control how components are shown

    Show Component   see **3.3.2.1.4 Show Component**

→   control which areas of the *Confirm* window are shown

    Show Window   see **3.3.2.1.5 Show Window**

Since this menu is context sensitive, not all commands will always be available.

3.3.2.1.1 Unzoom

The **Unzoom** command is available when the display has been zoomed (once, or multiple times in succession) and will restore the display to its original state prior to any zooming.

Zooming is described in **2.4.5 Changing the Display Range**, but in this case note that it is only possible to zoom the intensity axis.

3.3.2.1.2 Log Scale for Component

The **Log Scale for Component** command is used to adjust the scaling of chromatogram intensities. Click the command to alternate between applying and removing the check mark to its left.

If **Log Scale for Component** has a check mark applied, the axis is labeled as **Log abundance** and intensities are displayed in log (base 10) format. Otherwise, intensities are displayed in linear format and the axis is labeled as **Abundance**.

In both cases the y-axis label is followed by the absolute intensity (in square brackets) represented by **100** on the y-axis.

3.3.2.1.3 Show Mouse Position

The **Show Mouse Position** menu is used to:

→   control whether or not information is presented on the screen that relates to the current mouse position (**On Cursor**, **On Top** and **Off** commands).

When a specific menu command is clicked, a check mark appears to its left. However, since the menu commands are mutually exclusive, only one can be checked at any one time and so the check mark next to the previously checked command is removed.

When a command is checked, its action will apply to the current graphical display area only.

These context menu commands are identical in name and operation to those of the main menu's **Show Mouse Position** menu commands and are also affected by their selection.

Their operation and interactions are described fully in **3.1.4.3 View | Show Mouse Position**.

3.3.2.1.4 Show Component

The **Show Component** command determines whether or not the reconstructed profile of a component is shown together with the standard TIC and ion traces. Click the command to alternate between applying and removing the check mark to its left.

If **Show Component** has a check mark applied, a component's trace is shown in black at the same scaling as the TIC trace. Otherwise, the trace is not shown.

3.3.2.1.5　　Show Window

The **Show Window** command allows you to customize certain aspects of the screen contents.

The operation of this command is identical to the **View | Show Window** command and is fully described for it (**3.1.4.1 View | Show Window**).

3.3.3　Information Lists

The *Information Lists* area shows textual information for the components and targets (as relevant) resulting from the current analysis.

The *Information Lists* area can be "docked" or "undocked" from the *Profile Display* (**3.3.3.3.2 Undock**). If undocked, it is no longer tied to the *Profile Display* and its position and size can be altered; typically to enable clearer or more efficient viewing of the data.

If you right-click anywhere within the *Information Lists* area, a command menu appears that permits you to control aspects of the display (**3.3.3.3 Context Menu**).

The *Information Lists* area layout changes somewhat according to whether the currently selected compound is a component (▼) or is a target (ᴛ). For a component there are two "sections" side-by-side and for a target there are four sections arranged more or less as quadrants.

To switch between viewing component data and target data, simply click in the *Chromatogram Display* on the symbol (▼ or ᴛ) for the desired mode. The layout varies according to the selection.

Component　　　　　　　　　　　　　　　　　　Target

Figure 80 - Component and Target Mode Layouts (Docked)

In component` mode there are 2 sections (**3.3.3.1 Component Mode Details**). The left-hand section is a list of retention times for all components and the right-hand section provides various details about the component as determined during the deconvolution process.

In target mode there are 4 sections (**3.3.3.2 Target Mode Details**). The upper-left quadrant is a list of retention times for all targets. The upper-right quadrant is a list of "hits" (identifications) from a target library. The lower two quadrants provide various details about the target as determined during the deconvolution process.

If you click on a retention time in either mode, or additionally a hit in target mode, all of the other sections plus all of the other AMDIS screen areas change to reflect the chosen component or target.

3.3.3.1　　Component Mode Details
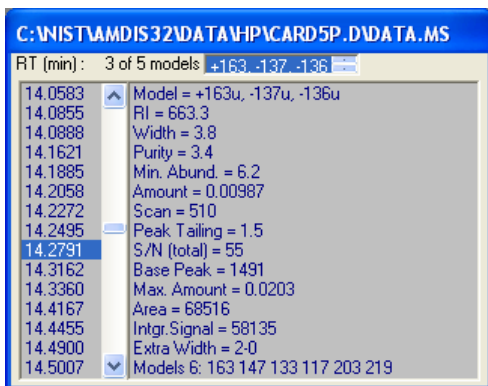
In component mode there are 2 sections. The left-hand section is a list of retention times for all components and the right-hand section provides various details, as determined during the deconvolution process, about the currently highlighted component. The contents of the right-hand section will differ slightly for TIC and m/z peak models.
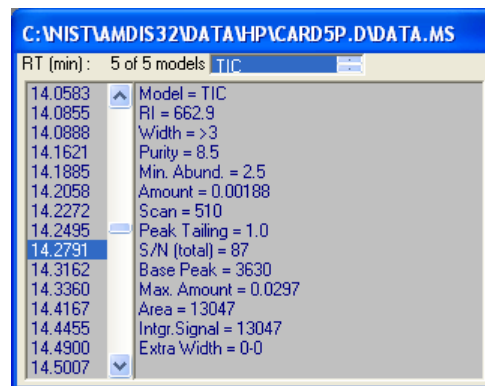
| M/z Model | TIC Model |

Figure 81 - Component Layout Details (Undocked)

To change the highlighted component, either click on the list of retention times here, or click on a component symbol (▼) in the *Chromatogram Display*. At the top of the window is information on the model peak; which may be a single m/z value, an m/z value combination or the TIC. If there are multiple model peaks, an appropriate label is presented (eg, **1 of 5 models** in the figure) and a single-line scroll box allows selection of the desired model and the component details change to reflect the selection.

> **Note:** You can also see the different models of components by using the down-arrow key to progressively move through the retention time list (once a value has just been highlighted there). The up-arrow key acts in the opposite direction and somewhat differently by skipping any models other than the first.

Unless otherwise noted, the following are listed for a component irrespective of the analysis type used in generating the components:

RT (min): The retention time in minutes of the component peak at its maximum intensity point (this being the value highlighted in the left column).

Model The m/z value (or TIC) used for determining the component peak shape. If adjacent peak subtraction has been activated (**6.3.3 Deconv. (Deconvolution) Tab**), the m/z value may be followed by one or two additional m/z values. These are masses, whose profiles have been subtracted from the model peak's profile, to yield the component profile. The model peak is generally the ion (or TIC) that rises and falls the fastest.

RI The computed retention index of the component.

> **Note:** This only appears for those analysis types where retention index values are computed for a component.

Width The full width at half maximum height of the chromatographic component peak; where the width is given in scans, not time. A > symbol can sometimes occur (eg, >**4** in **Figure 81 - Component Layout Details (Undocked)**). This indicates some uncertainly about the peak width due to one or both peak "ends" not being below, or close enough to, the baseline. Typically quadrupole mass spectrometers scan about once a second.

Purity The percentage of the total ion signal at the component's maximum intensity scan (**Scan**) that belongs to the deconvoluted component. AMDIS determines this by first extracting all of the ions associated with a component and then summing them to yield the total ion signal of the component (**Intgr.Signal**).

Min. Abund. The abundance of the smallest observable mass spectral peak (as a percentage of the **Base Peak**).

Amount The area of the deconvoluted component (**Area**) relative to the total ion count for the entire chromatogram, expressed as a percentage.

> **Note:** The total ion count can be found (but expressed to only 2 significant figures) as **Total signal** on the **S/N** tab of the *AMDIS Results* dialog (**5.6.1 Signal**).

Scan	The number of the scan <u>nearest</u> to the maximum intensity point of the component; rarely does the component maximize exactly at the retention time of a specific scan.

Peak Tailing   The ratio of the area to the right (later time) of the center of the peak to the area on the left of the peak (earlier time). A well formed peak has a value near unity. Excessive tailing will yield values much greater than 1.

S/N (total)   The total signal-to-noise value as measured by utilizing all ions in a component.

Base Peak   The abundance of the most intense mass spectral peak in the deconvoluted spectrum.

Max. Amount   In some cases (usually when a component cannot be fully isolated) AMDIS determines that the area associated with the signal is actually much larger than the standard computation of **Area**. In that situation a maximum area is predicted (**Max. Area**, **3.3.3.2 Target Mode Details**) and expressed as a percentage of the total ion count for the entire chromatogram.

> **Note:** The total ion count can be found (but expressed to only 2 significant figures) as **Total signal** on the **S/N** tab of the *Analyze Results* dialog (**5.6.1 Signal**).

Area   This area is expressed in the units of the instrument that the component was acquired on and is computed by starting from the extracted component shape, but then using any baseline extension that seems reasonable to one or both sides of the actual extracted peak (**Extra Width**). As such, **Area** will often be larger than **Intgr.Signal**.

Intgr.Signal   The area under the actual extracted component shape expressed in the units of the instrument it was acquired on.

Extra Width   The number of scans by which the extracted component's baseline is extended to yield **Area**. It is expressed in the format **a-b**; where **a** and **b** are the number of scans extended to the left and right, respectively.

Models n   A list of all the model peaks utilized when determining the model peak for this component (**Model**); where **n** is the number listed.

> **Note:** This does not appear, if the selected model is TIC.

Class =   The class designator for the component.

> **Note:** This only appears after a postprocess analysis (**3.1.2.3 Analyze | Postprocess**).

3.3.3.2   Target Mode Details

In target mode there are 4 sections. The upper-left quadrant is a list of retention times for all targets. The upper-right quadrant is a list of the compound names of the corresponding target "hits" (identifications) from a target library. The lower two quadrants provide various details about the target; as determined during the deconvolution process.
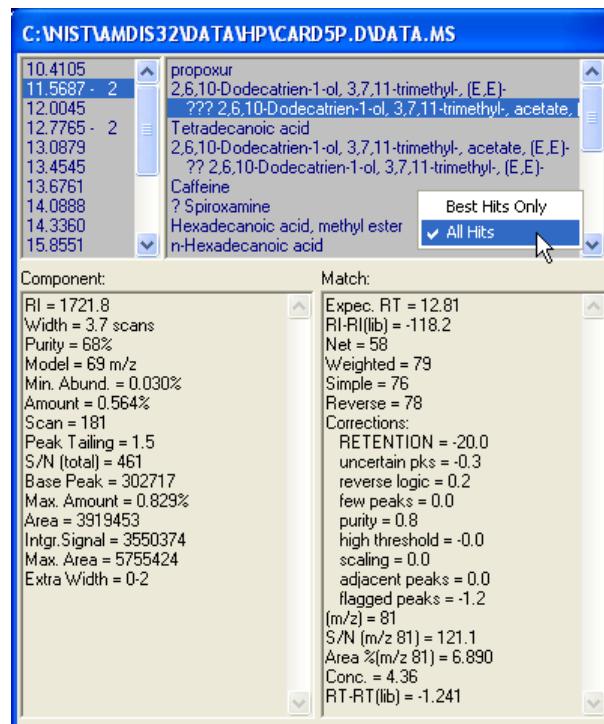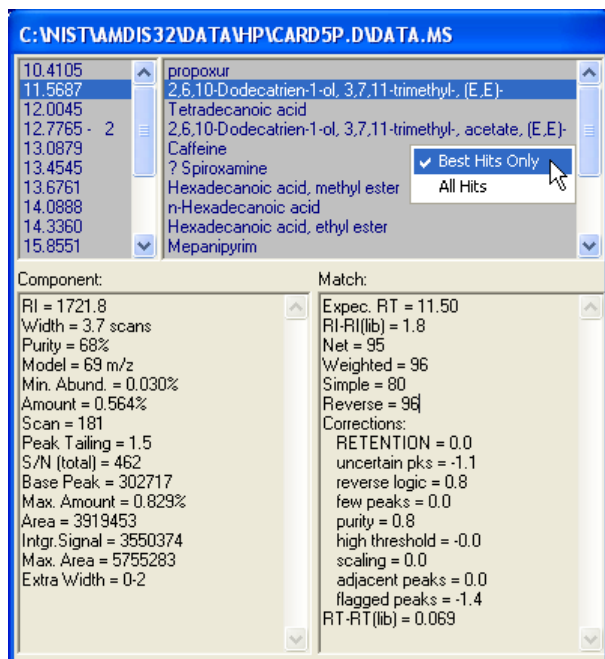
Figure 82 - Target Layout Details (Undocked)

To change the highlighted target, either click on the list of retention times or targets here, or click on a target symbol (**T**) in the *Chromatogram Display*. Alternatively, click on one of the hits.

However you select a target, the other sections change to show values for the new selection.

The **Component** parameters listed for a target are identical to those shown for a component in component mode (**3.3.3.1 Component Mode Details**), with the following addition:

Max. Area In some cases (usually when a component cannot be fully isolated) AMDIS determines that the area associated with the signal is actually much larger than the standard computation of **Area**. In that situation a maximum area is predicted.

The following **Match** parameters are always listed for a target, irrespective of the analysis type:

Net The final match quality value for the match between the deconvoluted component and the library spectrum; where its maximum value of 100 shows a perfect match.

It is computed using a number of factors, including the **Weighted** and **Reverse** match factors (below). It may be higher or lower than either of these match factors; since it is adjusted by **Corrections** values; for example, a very pure component will generate a small, positive **purity** value, which will add to and so increase the **Net** value somewhat.

In essence **Net** is computed as: 0.7 * **Weighted** + 0.3 * **Reverse** + **Corrections**

> **Note:** The **Net** value ultimately determines whether a component is considered a hit (match) for a target library spectrum. Either the best hit only, or all hits, can be viewed in the upper right quadrant (**3.3.3.3.3 Best Hits Only and All Hits**). If all hits are being displayed, they are listed in **Net** value order (starting with the highest). As the **Net** value successively decreases, it is possible for 1, 2 or even 3 question marks (?) to precede the name of a hit.

A hit is only reported if its **Net** value is above the **Minimum match factor** setting (**6.3.1 Identif. (Identification) Tab**). In that case the number of question marks depends on the actual **Net** value as follows:
80 or above None

70 - 79	?
60 - 69	??
<60	???

> **Note:** A hit may sometimes be of low quality if it is present at low concentration, or sometimes if there is a high background.

Weighted   The scaled dot product of the library and deconvoluted components using optimized weighting and scaling.

> **Note:** In all cases (ie, **Reverse**, **Simple** and **Weighted**) the dot product is expressed as the cosine of the angle between the "vectors"; ie, it is normalized by the square of the library spectrum.

Simple   The simple dot product of the library and deconvoluted components that considers all m/z peaks in both spectra.

Reverse   The dot product in which the deconvoluted component m/z peaks that are not present in the library spectrum are ignored (ie, they are treated as impurities).

Corrections   There are a series of corrections applied to the **Weighted** match factor to adjust it before it is used to calculate the **Net** value. Some of these corrections relate to the other match factors discussed above. Other corrections attempt to adjust for the overall confidence of the data. For example, if a particular ion cannot be completely assigned to the current component, a small negative adjustment is made. Most of these corrections cannot be controlled by the user; they have been determined by testing the algorithm on a very large set of GC/MS data files. However, you do have control over one important correction and that is the amount of penalty (ie, negative correction) assessed for retention mismatches between the observed peak and the library value (**Match factor penalties**; **6.3.1 Identif. (Identification) Tab**).

uncertain pks and
flagged peaks   It is sometimes impossible to determine which spectral peaks go with which component due to uncertain deconvolution of the data. This can be due to many things including: complicated background; multiple components eluting at similar times; or unresolved tailing. In these cases, the peaks are flagged as "uncertain" and are treated differently from un-flagged peaks. Two different penalties are applied to reduce the quality of the match based on whether or not these uncertain peaks match the library component:

1) If uncertain peaks match a target compound they are accepted, and if they do not match the target no penalty is applied. Since this gives a slightly higher level of possible false identifications, an **uncertain pks** penalty is applied based on the fraction of uncertain peaks that <u>do not match</u> the target compound.

2) If a match to a target compound is based predominantly on uncertain peaks, a penalty is applied to reflect the lowered confidence in this match. The **flagged peaks** penalty is based on the fraction of uncertain (flagged) peaks that <u>do match</u> the target compound.

Note that, these corrections are only calculated if there are uncertain deconvolutions in the data.

reverse logic   This is typically a positive correction. As discussed above, the net match factor **Net** is computed utilizing 0.7 * **Weighted** + 0.3 * **Reverse**. Since **Reverse** is almost always greater than or equal to **Weighted,** the use of **Reverse** will increase the net match factor. The **reverse logic** correction shows how much of an effect this has on the net match factor.

few peaks   If non-zero, this will always be a negative correction and is generated in cases where the deconvoluted spectrum has only a few peaks. Note that, for deconvolutions with only one peak a match is not possible.

purity   This is a small correction that may be positive or negative and accounts for the purity of the component. Confidence in the hit assignment is greatest if all of the TIC is accounted for by this component, but becomes progressively lower as more of the TIC is taken up by other components mixed in with this component.

high threshold If non-zero, this will always be a negative correction. In cases where the instrument threshold was set very high when the data were acquired, small peaks cannot be determined. Since the absence of small peaks that are normally below the threshold do not penalize a match, very high thresholds can produce match factors that are better than they should be.

scaling In cases where a single peak or even two or three peaks dominate a deconvoluted mass spectrum, the peak intensities are rescaled in such a way that the relative intensities of the small peaks are unchanged, but the ratio between the large and small peaks is reduced. The scaled component spectrum is now compared to the scaled target spectrum. If the match is worse than in the un-scaled case, this penalty is applied.

adjacent peaks If specific adjacent peak subtraction has been used (**Adjacent peak subtraction**, **6.3.3 Deconv. (Deconvolution) Tab**) it is assumed that the confidence in the resulting match is lowered. This negative correction reflects that lowered confidence.

RETENTION This will have the value **n/a** (not applicable) unless **Type of analysis** is set to **Use Retention Time** or computes retention index data (ie, it is set to **Use Retention Index Data**, **Use Internal Standards for RI**, **Use RI Calibr. Data** + **Internal Std.** or **Performance Check for RI**). In these cases, **RETENTION** will be a negative correction, or zero, as applicable. It is computed as a function of the difference between the measured retention time/retention index and the library's stored value. Alternatively a fixed penalty will be applied if the library entry does not hold a retention time/index value. All aspects of this penalty are specified by the user, including the maximum computed value that it may have; and these are described in section **6.3.1 Identif. (Identification) Tab**.

In addition to the above and depending on the type of analysis run and the data available, the following additional **Match** parameters may be calculated:

Expec. RT The computed expected retention time. This is only shown if retention time information is present for this compound in the target library and the analysis type causes retention times to be computed.

RI-RI(lib) The computed retention index minus the retention index value stored in the target library. This is only shown if the analysis type makes use of retention indices and the identified compound has a retention index value in the target library.

(m/z) The (**m/z**) value is only shown if a **Ref. Ion** m/z value has been specified for this compound in the target library and it is the specified library value (**8.2.4 Compound Information Editor** and **8.2.6 Combination Editors**).

S/N (m/z n) The signal-to-noise value of the indicated m/z peak (**m/z n**). This is only shown if a **Ref. Ion** m/z value has been specified for this compound in the target library and it is the specified library value (**8.2.4 Compound Information Editor** and **8.2.6 Combination Editors**).

Area %(m/z n) The percentage of the target's area attributed to the indicated m/z peak (**m/z n**). This is only shown if a **Ref. Ion** m/z value has been specified for this compound in the target library and it is the specified library value (**8.2.4 Compound Information Editor** and **8.2.6 Combination Editors**).

Conc The computed concentration.

This is only shown if the analysis type makes use of internal standards (*Use Internal Standards for RI* or *Use RI Calibr. Data + Internal Std.*) and the *Internal Standards Library* and *Target Compounds Library* are appropriately set up as described in **8.4 Using a Library for Concentration Calculations**).

It is computed as follows (for further details see **8.4.1 Calculating a Concentration**):

$$\text{Conc} = (\text{Conc}_{standard} * RF_{standard} / RF_{target}) * (\text{Area}_{target} / \text{Area}_{standard})$$

RT-RT(lib) The computed retention time minus the retention time value stored in the target library. This is only shown if the identified compound has a retention time value in the target library.

3.3.3.3 Context Menu

If you right-click anywhere within the *Information Lists* area in component mode, a command menu appears (the "context-sensitive" menu).

The context-sensitive menu is used to:

→   interact with the NIST *MS Search* program using the component spectrum

NIST Library   see **3.3.3.3.1 NIST Library**

→   control the appearance of the display

Undock   see **3.3.3.3.2 Undock**

In target mode, the context-sensitive menu varies according to the section you right-click in.

For the retention time list, the menu is identical to the component mode menu.

For the hits list, the context-sensitive menu is used to:

→   control how many hits are displayed

Best Hits Only   see **3.3.3.3.3 Best Hits Only and All Hits**

All Hits   see **3.3.3.3.3 Best Hits Only and All Hits**

For the component and match lists, the context-sensitive menu is used to:

→   control the type and order of information displayed or printed for each target

View Options   see **3.3.3.3.4 View Options**

Print Options   see **3.3.3.3.5 Print Options**

3.3.3.3.1 NIST Library

The **NIST Library** menu is used to:

→   interact with the NIST *MS Search* program using the component spectrum (**Add Component/Scan to Search List (n)** and **Go to NIST MS Program** commands).

The **Add Component/Scan to Search List (n)** command takes the deconvoluted component spectrum for the currently highlighted retention time and adds it to a list of spectra for subsequent searching with the NIST *MS Search* program when using the **Go to NIST MS Program** command.

The **Go to NIST MS Program** command invokes the NIST *MS Search* program to either search the deconvoluted component spectrum for the currently highlighted retention time, or, if a list of pre-selected spectra exists (created using the **Add Component/Scan to Search List (n)** command), that list of spectra will be searched instead. The search is performed against standard NIST-format libraries.

For more details of each command see **3.1.2.5 Analyze | Add Component/Scan to Search List (n)** and **3.1.2.6 Analyze | Go to NIST MS Program**.

3.3.3.3.2 Undock

The **Undock** command controls whether or not the *Information Lists* area of the screen is fixed in position to the right of the *Profile Display* area, or whether it is free to be moved around the screen and to be resized.

If **Undock** does not have a check mark to its left, it is not active and the *Information Lists* area will be in its fixed position to the right of the *Profile Display*. For an example of the docked *Information Lists* layouts, see **Figure 80 - Component and Target Mode Layouts (Docked)**

If **Undock** does have a check mark to its left, it is active and the *Information Lists* area now becomes a moveable window and may be positioned anywhere within the AMDIS display area. For examples of the undocked *Information Lists* layouts, see **Figure 81 - Component Layout Details (Undocked)** and **Figure 82 - Target Layout Details (Undocked)**.

Click **Undock** to alternate between applying and removing the menu item's check mark.

If the *Information Lists* area is docked, it disappears from the screen when the *Profile Display* area is removed; for example, by clearing the **Component Profile** checkbox in the *Show Window* dialog (**3.1.4.1 View | Show Window**).

However, if the *Information Lists* area is undocked, it will remain on the screen, irrespective of whether or not the *Profile Display* area is present. This has the advantage of you being able to make the other display areas larger and also to move the *Information Lists* area anywhere on the screen to avoid obscuring data.

When the *Information Lists* area is undocked, you can use *Windows* techniques to drag and expand the window. Note that, if you adjust the window at the top edge, it changes the relative size of the upper two quadrant(s), whereas if you adjust it at the bottom edge, it changes the relative size of the lower two quadrant(s).

3.3.3.3.3 Best Hits Only and All Hits

AMDIS will often identify a single component as a number of different compounds. For example, a high molecular weight member of a homologous series will often have a spectrum that closely matches a smaller member of the same series. If there are multiple hits (identifications), the actual number of hits is shown after the retention time in the retention time list section.

The **Best Hits Only** and **All Hits** commands allow you to choose how many hits (identifications) you view per target in the upper-right quadrant when in target mode (**Figure 82 - Target Layout Details (Undocked)**).

When a specific menu command is clicked, a check mark is applied to its left. However, these two commands act like radio buttons and so are mutually exclusive. As such, only one command can have a check mark applied at any one time and so the check mark next to the previously active command is removed.

If **All Hits** is selected, all of the target library compounds with a match factor (**Net** value) greater than the minimum will be shown in order of match factor (**Minimum match factor**, **6.3.1 Identif. (Identification) Tab**).

If **Best Hits Only** is selected, only the target compound hit with the highest match factor is shown.

3.3.3.3.4 View Options

The **View Options** command determines the type and order of information displayed for each target in the component and match lists when in target mode.

The *View Options* dialog has 2 scrollable list boxes.
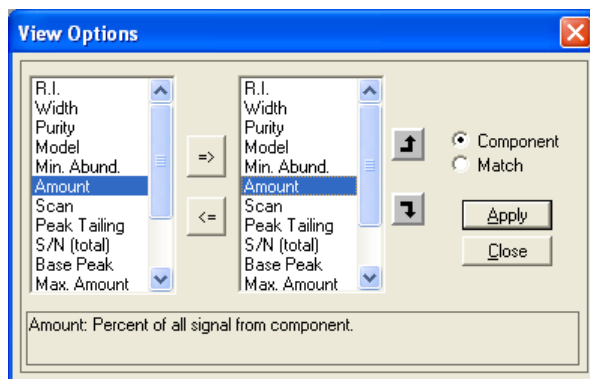
Figure 83 - View Options dialog

The left list box holds the complete selection of values that can be viewed and the right list box shows the values that have been selected and the order in which they will be displayed.

Click **Component** to see the "component-type" values that can be selected for viewing. Similarly, click **Match** to see the "match-type" values that can be selected.

In both cases the right list box is set up as described in **3.1.1.10.1 Value Selection and Ordering**.

When you have made your selections, click **Close**. The chosen order is now reflected in the display.

3.3.3.3.5 Print Options

The **Print Options** command determines the type and order of information that will be printed for each target when subsequently using the **File | Print Text Report** command.

When you click **Print Options** one of two dialogs will appear; according to the currently selected report type in the *Print Text Report* dialog of the **File | Print Text Report** command (**3.1.1.9.1 Layout Details**). The dialogs that appear and the facilities they offer are identical to the additional dialogs described for that command (**Figure 59 - Onsite Print Options and Print Options dialogs**).

3.3.4 Mass Spectral Display

The *Mass Spectral Display* area will change according to whether or not AMDIS results exist. If results do exist, the display will also change based on whether the currently selected compound is a target (**T**) or a component (▼).

> **Note:** You may control whether or not a specific *Mass Spectral Display* is actually presented for a particular mode (**3.1.4.1 View | Show Window**). Within a particular *Mass Spectral Display* you can also control, where relevant, which particular spectra are displayed (**3.3.4.1.5 Show Spectra**).

If you right-click anywhere within the *Mass Spectral Display* area, a command menu appears that permits you to control aspects of the display (**3.3.4.1 Context Menu**).

For a component there is simply one *Mass Spectral Display* showing the raw (original) spectrum overlaid with the extracted spectrum resulting from the deconvolution process.
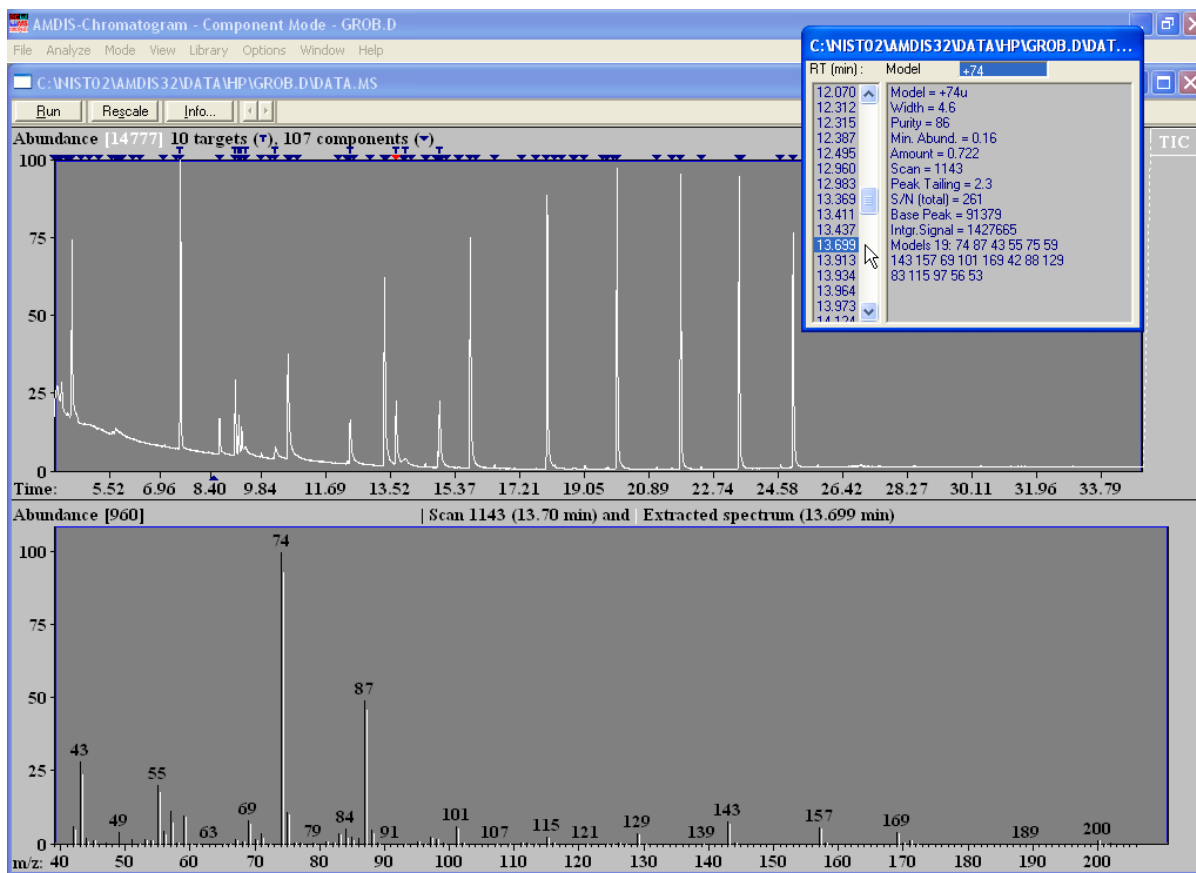

Figure 84 - Mass Spectral Display - component mode

In default, ions from the raw spectrum are shown in black and those from the extracted spectrum in white, but the latter may be changed (**3.1.6.3 Options | Color**). Note that, for visual clarity the different ions are slightly offset from each other along the x-axis.

Note: For appropriate acquisition modes of certain mass spectrometers, spectra are "deskewed" before presentation as raw spectra in AMDIS. This can result in small differences in absolute peak intensities between those displayed (and used) in AMDIS compared to those in the instrument's own data system.

The y-axis is a relative abundance, percentage scale with the absolute abundance value representing 100% given at the top left of the display; eg, **960** in **Figure 84 - Mass Spectral Display - component mode**.

Note: In fact the presented absolute value is the actual absolute value divided by 100.

For a target there are two *Mass Spectral Display*s.
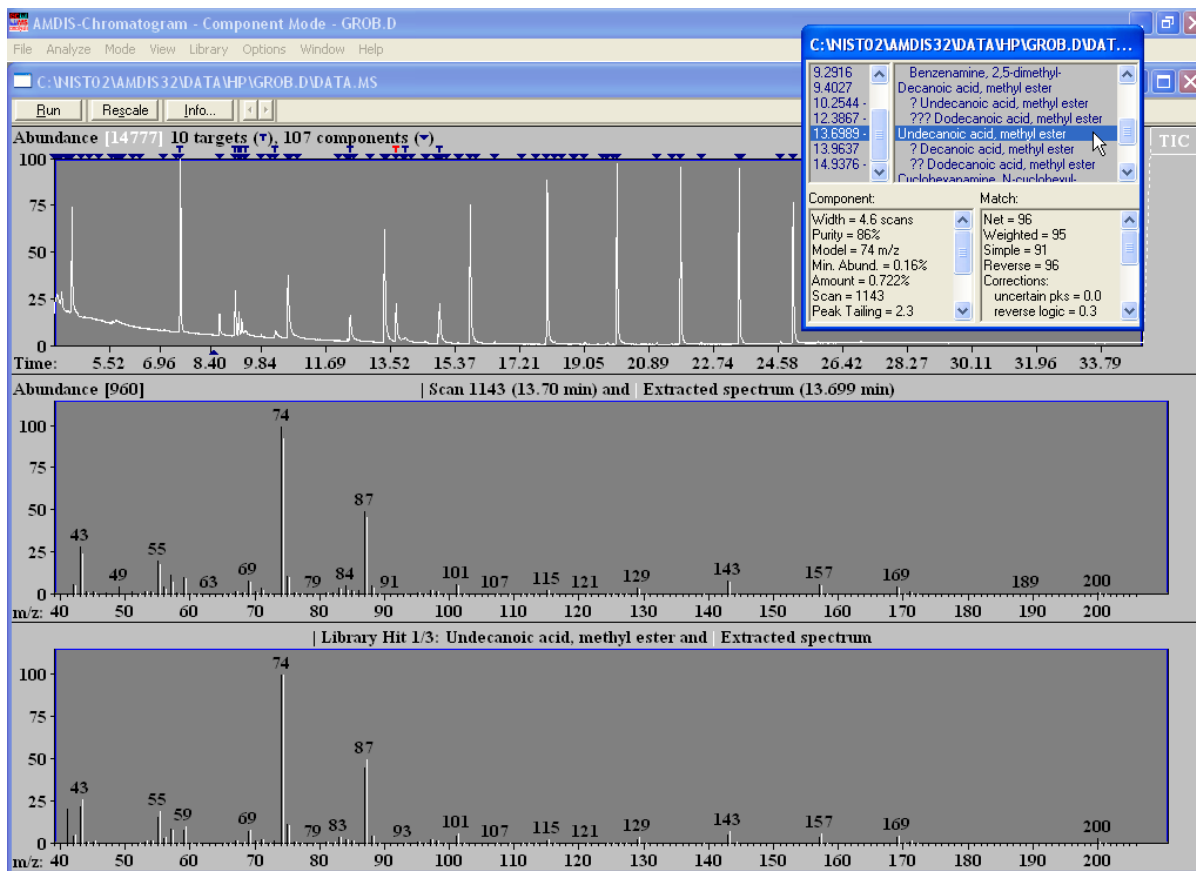
Figure 85 - Mass Spectral Display - target mode

The upper display shows the same information as just described for a component. The lower display is similar, but shows a library hit (black ions) overlaid with the extracted spectrum (white ions) and again they have a slight offset for visual clarity.

Since library spectra ions are only stored with relative abundances, there is no absolute abundance value representing 100% given at the top left of this display.

In all cases the x-axis shows m/z values and mass spectral peaks are always displayed rounded to integer m/z.

Sometimes you might want to see more detail of the mass spectral peaks. You can do this by "zooming" (magnifying) the display (**2.4.5 Changing the Display Range**).

As well as displaying spectra by selecting a target or component using its symbol in the *Chromatogram Display* or its entry in the *Information Lists*, it is also possible to simply display any spectrum by clicking in the main *Chromatogram Display* area. The selected raw spectrum replaces the raw spectrum currently being displayed and no extracted spectrum is shown.

The selected spectrum is reflected in the *Chromatogram Display* area with a red ▲ symbol positioned at the appropriate RT position below its x-axis. If a component or target is now selected for display, the ▲ symbol remains where it is, but changes color to blue.

If no previous results exist when a new file is opened, there is simply one *Mass Spectral Display* showing the raw spectrum at the mid-point of the file's retention time range.
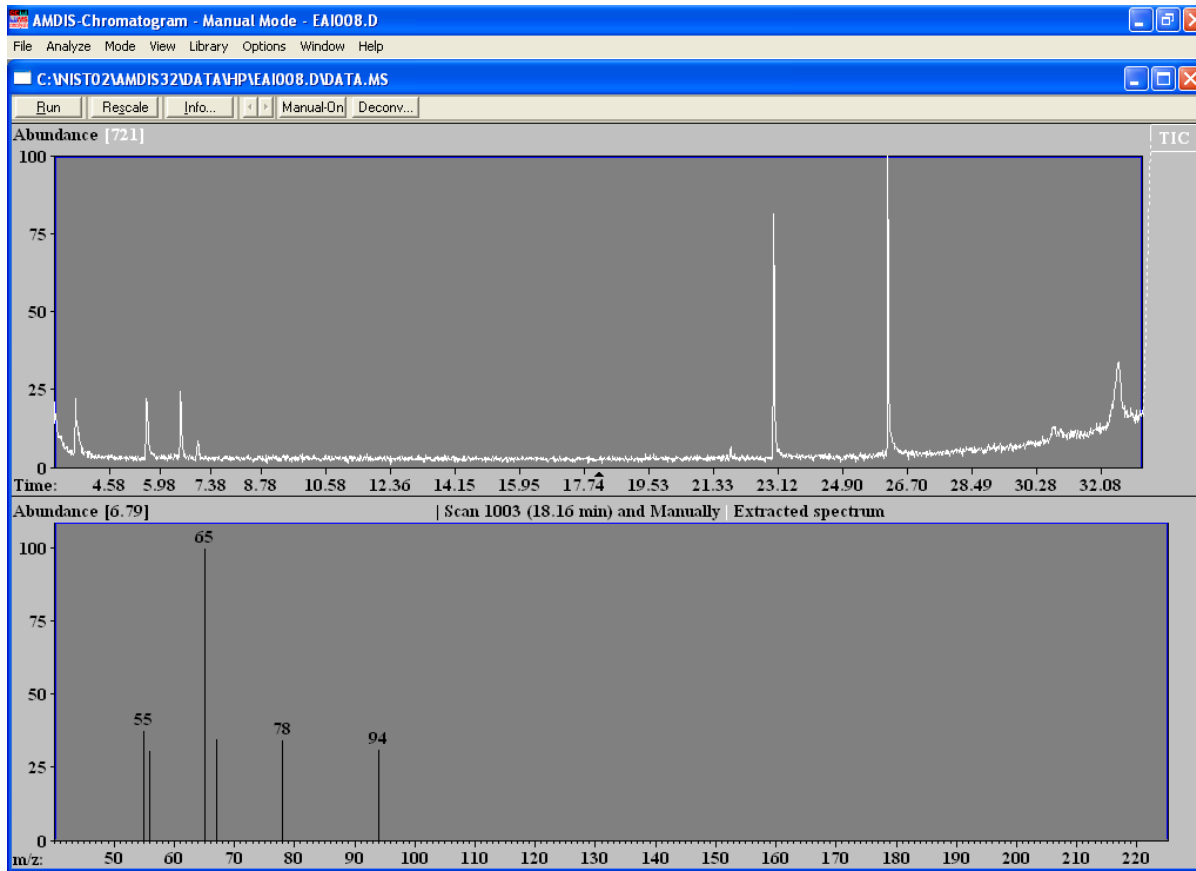

Figure 86 - Mass Spectral Display - no results mode

Again it is possible to simply select any raw spectrum for display by clicking in the main *Chromatogram Display* area and the selected spectrum is reflected in the *Chromatogram Display* area with a black ▲ symbol positioned at the appropriate RT position below its x-axis.

Whenever a spectrum is displayed in any mode, it will normalize its absolute abundance values such that the largest m/z peak is always equivalent to 100 on the y-axis. In default, the y-axis will show 100 as its maximum and, even if the display has been vertically zoomed such that a value lower than 100 is displayed, the introduction of a new spectrum will normally reset the axis scaling and maximum to 100. If you wish to compare a few spectra in absolute abundance terms and so do not want this automatic rescaling to occur, you can "freeze" the abundance scale at a particular absolute value (**3.3.4.1.3.2 Abundance**).

In an analogous manner for the display of a new spectrum, the m/z axis will in default automatically renormalize to a mass range determined by the **Low m/z** and **High m/z** parameter settings on the **Instr.** tab of the *Analysis Settings* dialog (**6.3.2 Instr. (Instrument) Tab**). Again, if you wish to compare a few spectra in absolute m/z terms and so do not want this automatic resizing to occur, you can "freeze" the m/z scale at a particular absolute range (**3.3.4.1.3.1 M/z Interval**).

It is possible to generate single mass chromatograms in the *Chromatogram Display* by clicking in any of the *Mass Spectral Display* areas. This operation and the way in which it affects both types of display are described in **3.3.1 Chromatogram Display**.

In all display modes you may sometimes wish to draw thicker mass spectral peaks; for example, when preparing to show results in presentations. This is achieved using the **Thick Lines** command (**3.1.4.4 View | Thick Lines**).

Each spectrum on display has a title above the corresponding display area. For raw spectra the title shows its scan number and retention time, whereas an extracted spectrum only has a retention time since a scan number is inappropriate. A target library spectrum shows the library hit's name. If there are multiple hits for a target, the header indicates which one it is and how many there are.

There may also be positional information displayed for the mouse, either within the graphical spectrum area, or to the top right of the display as illustrated in **Figure 87 - Uncertain Peaks**. This is described in **3.1.4.3 View | Show Mouse Position**.

Often the extracted spectrum will contain some "uncertain peaks" and these are displayed as dashed lines. For example, m/z 42, 43, 47, 54 and 55 in the following:
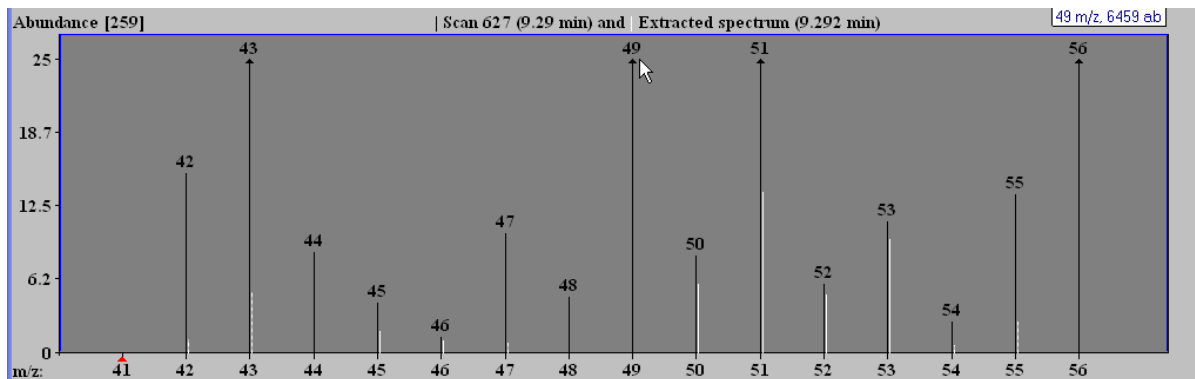

Figure 87 - Uncertain Peaks

For additional information about uncertain peaks, see **3.1.2.7 Analyze | Use Uncertain Peaks**.

Figure 87 - Uncertain Peaks also shows how peaks have an upwards-pointing arrowhead on them if their intensity is off-scale; in this case, m/z 43, 49, 51 and 56.

3.3.4.1   Context Menu

If you right-click anywhere within a *Mass Spectral Display* area, a command menu appears (the "context-sensitive" menu).

The context-sensitive menu is used to:

→   adjust the scaling and presentation of the displayed mass spectra

    Unzoom   see **3.3.4.1.1 Unzoom**

    Log Scale for Spectra   see **3.3.4.1.2 Log Scale for Spectra**

    Freeze   see **3.3.4.1.3 Freeze**

→   control any feedback from the mouse pointer's position

    Show Mouse Position   see **3.3.4.1.4 Show Mouse Position**

→   control which spectra are shown and their content

    Show Spectra   see **3.3.4.1.5 Show Spectra**

    Show Uncertain Peaks   see **3.3.4.1.6 Uncertain Peaks**

→   control which areas of the *Confirm* window are shown

    Show Window   see **3.3.4.1.7 Show Window**

→   interact with the NIST *MS Search* program

    NIST Library   see **3.3.4.1.8 NIST Library**

Since this menu is context sensitive, not all commands will always be available.

3.3.4.1.1 Unzoom

The **Unzoom** command is only available when the display has been zoomed one or more times and will always restore the display to its initial state prior to the first zoom.

Zooming is described in **2.4.5 Changing the Display Range**.

3.3.4.1.2 Log Scale for Spectra

The **Log Scale for Spectra** command is used to adjust the scaling of mass spectral peak intensities. Click the command to alternate between applying and removing the check mark to its left.

If **Log Scale for Spectra** is selected, the intensity axis is labeled as **Log abundance** and intensities are displayed in log (base 10) format.

Otherwise, intensities are displayed in linear format and the axis is labeled as **Abundance**.

In both cases the y-axis label is followed by the absolute intensity (in square brackets) represented by **100** on the y-axis.

3.3.4.1.3 Freeze

The **Freeze** menu is used to:

→   control the displayed mass and intensity range

| M/z Interval | see **3.3.4.1.3.1 M/z Interval** |
| Abundance | see **3.3.4.1.3.2 Abundance** |

When a specific menu command is clicked, a check mark is applied to its left. Since these commands are independent of one another, any combination of the two can be selected.

3.3.4.1.3.1 M/z Interval

The **M/z Interval** command is used to fix the start and end m/z values of the x-axis. Click the command to alternate between applying and removing the check mark to its left.

If **M/z Interval** is cleared, the introduction of any new spectrum into the current *Mass Spectral Display* will cause an automatic x-axis rescale to a mass range determined by the **Low m/z** and **High m/z** parameter settings on the **Instr.** tab of the *Analysis Settings* dialog (**6.3.2 Instr. (Instrument) Tab**).

However, if **M/z Interval** is selected, the current x-axis m/z limits are "frozen" and all subsequent spectra are displayed using this x-axis setting. This can be useful, for example, to look at a narrow range of masses over a number of scans. Even when "frozen" the mass range can be zoomed.

> **Note:**   The scan and library *Mass Spectral Display*s always have the same mass range and so the **M/z Interval** command applies to both, irrespective of where it is used.

3.3.4.1.3.2 Abundance

The **Abundance** command is used to fix the top of the y-axis at a specific absolute value. Click the command to alternate between applying and removing the check mark to its left.

If **Abundance** is cleared, the introduction of any new spectrum into any *Mass Spectral Display* will cause an automatic y-axis rescale such that 100 is the top of the y-axis and the most intense peak in the spectrum has been normalized to that.

However, if **Abundance** is selected, the current absolute y-axis range is "frozen" and all subsequent spectra are displayed using this y-axis setting. This can be useful, for example, to look at the absolute intensity of a set of masses as you examine a number of scans. Even when "frozen" the intensity range can be zoomed.

> **Note:**   Each of the raw and library *Mass Spectral Display*s has its own independent **Abundance** command.

3.3.4.1.4 Show Mouse Position

The **Show Mouse Position** menu is used to:

$\rightarrow$ control whether or not information is presented on the screen that relates to the current mouse position (**On Cursor**, **On Top** and **Off** commands).

When a specific menu command is clicked, a check mark appears to its left. However, since the menu commands are mutually exclusive, only one can be checked at any one time and so the check mark next to the previously checked command is removed.

When a command is checked, its action will apply to the current graphical display area only.

These context menu commands are identical in name and operation to those of the main menu's **Show Mouse Position** menu commands and are also affected by their selection.

Their operation and interactions are described fully in **3.1.4.3 View | Show Mouse Position**.

3.3.4.1.5 Show Spectra

The **Show Spectra** menu is used to:

$\rightarrow$ control which spectra are presented in the *Mass Spectral Display*

Scan	see **3.3.4.1.5.1 Scan**
Library	see **3.3.4.1.5.2 Library**
Component	see **3.3.4.1.5.3 Component**
Both	see **3.3.4.1.5.4 Both**

Since this menu is context sensitive, not all commands will always be available.

When a specific menu command is clicked, a check mark appears to its left. However, since the menu commands are mutually exclusive, only one can be checked at any one time and so the check mark next to the previously checked command is removed.

In normal use, **Both** would be selected to show 2 spectra. However, for some complex spectra or to prepare data for publication you may want to show only one or the other.

Each of the scan and library *Mass Spectral Display*s has its own independent **Show Spectra** menu.

3.3.4.1.5.1 Scan

The **Scan** command determines whether or not the raw spectrum is displayed. It is not available for the library display area in component mode. Click the command to alternate between applying and removing the check mark to its left.

If **Scan** is selected, the raw spectrum only is displayed. Otherwise the command that is selected will determine what is displayed.

3.3.4.1.5.2 Library

The **Library** command determines whether or not the library hit spectrum is displayed. It is only available for the library display area in component mode. Click the command to alternate between applying and removing the check mark to its left.

If **Library** is selected, the library spectrum only is displayed. Otherwise the command that is selected will determine what is displayed.

3.3.4.1.5.3 Component

The **Component** command determines whether or not the extracted (component) spectrum is displayed. Click the command to alternate between applying and removing the check mark to its left.

If **Component** is selected, the extracted spectrum only is displayed. Otherwise the command that is selected will determine what is displayed.

3.3.4.1.5.4 Both

The **Both** command determines whether or not both of the other selectable spectra on the current menu are displayed. Click the command to alternate between applying and removing the check mark to its left.

If **Both** is selected, both spectra are displayed. Otherwise the command that is selected will determine what is displayed.

3.3.4.1.6   Uncertain Peaks

The **Uncertain Peaks** command determines whether or not uncertain peaks are displayed in the extracted (component) spectrum. Click the command to alternate between applying and removing the check mark to its left.

If **Uncertain Peaks** is selected, any uncertain peaks that have been assigned in the extracted spectrum will be displayed as dashed lines and in their own color (**3.1.6.3 Options | Color**). Otherwise uncertain peaks will not be displayed.

For more about uncertain peaks, see **3.1.2.7 Analyze | Use Uncertain Peaks**.

3.3.4.1.7   Show Window

The **Show Window** command allows you to customize certain aspects of the screen contents.

The operation of this command is identical to the **View | Show Window** command and is fully described for it (**3.1.4.1 View | Show Window**).

3.3.4.1.8   NIST Library

The **NIST Library** menu is used to:

→   interact with the NIST *MS Search* program using the component spectrum (**Add Component/Scan to Search List (n)** and **Go to NIST MS Program** commands).

These commands are described fully in **3.1.2.5 Analyze | Add Component/Scan to Search List (n)** and **3.1.2.6 Analyze | Go to NIST MS Program**.

3.4   Display of Multiple Chromatograms

There are many cases where it is useful to have two or more chromatograms open at once and in AMDIS there are two ways to achieve this.

You may display multiple chromatograms as completely separate child windows within the *Confirm* window; each with its own full set of display areas (**3.4.1 Multiple Chromatograms in Independent Windows**).

Alternatively you may restrict yourself to having only two chromatograms in a single window. In this case, there are two separate *Chromatogram Display*s, but only one set of the other display areas, which will show data for the currently selected chromatogram of the two (**3.4.2 Two Chromatograms in One Window**).

3.4.1   Multiple Chromatograms in Independent Windows

When you are displaying multiple independent child windows within the *Confirm* window, each child window has all of the display attributes that a single window would have.

Similarly, all of the functionality of a single window is supported fully and independently in each child window, but with one exception. If you undock or dock the *Information Lists* area in any of the windows, the same will be applied to all windows.

When undocked, the *Information Lists* area may be moved anywhere on the screen (even outside of the *Confirm* window). Whenever a particular child window is selected, the undocked *Information Lists* area window will stay where it is, but its contents will change to reflect the selection (including its title showing which data file it represents).

There are different ways to open a file in a separate window, but all achieve exactly the same effect. You can use:

a)   The main menu command to directly select and open a file in a new window (**3.1.1.2.2 File | Open In | New Window**);

b)   The main menu command to open a recently accessed second file into a single window (**3.1.1.3 File | Open Recent Files**);

c) The main menu command to first invoke a file selection dialog (**3.1.1.1 File | Open**) and then use that dialog's **Open in New Window** command button;

d) The *Chromatogram Display*'s context menu command to first invoke a file selection dialog (**3.3.1.1.15.1 File | Open**) and then use that dialog's **Open in New Window** command button.

When multiple child windows are being displayed, the one that is currently "active" (and so operated on by main menu commands) is distinguishable by having its title bar and frame in the standard "active window" color used by *Microsoft Windows.*

As well as the commands just mentioned for opening a file in a new window, the following main menu facilities are specifically utilized in a multiple windows environment:

→ The window manipulation commands (**3.1.7 Window Menu**);

→ Forcing the same time scale for chromatograms (**3.1.4.7 View | Same Time Scale**).

3.4.2 Two Chromatograms in One Window

When displaying two chromatograms (ie, data files) in a single window, there are two separate *Chromatogram Display*s, but only one set of the other display areas, which will show data for the currently selected chromatogram of the two.

Although this mode offers more screen space for the various display areas, as compared to the multiple window mode, it has the disadvantage that spectra from the different files cannot be simultaneously displayed since the display areas other than the *Chromatogram Display* only show data for the currently active of the 2 chromatograms.

There are different ways to open a second file in a single window, but all achieve exactly the same effect. You can use:

a) The main menu command to select and add a second file into a single window (**3.1.1.2.1 File | Open In | Active Window**);

b) The main menu command to add a recently accessed second file into a single window (**3.1.1.4 File | Add Recent Files**);

c) The *Chromatogram Display*'s context sensitive command to add a second file into a single window (**3.3.1.1.15.2 File | Add**).

If you have undocked the *Information Lists* area you can easily determine which file's data it contains since its title shows the filename.

In any case, the currently active *Chromatogram Display* is easily distinguishable by the filename in its title bar being bold, whereas the inactive filename is subdued.

4 AMDIS Results Window

AMDIS offers two significant user interfaces (windows) for you to work with.

The *Confirm* window is a full-functionality window, offering a menu bar and one or more included child windows. Each child window has a button bar, several graphical areas and a text area. The menu bar provides access to the full functionality of AMDIS and the graphical areas permit interactive manipulations and, together with the text area, detailed analysis of the results of AMDIS processing.

The *Confirm* window is fully described in Chapter **3 AMDIS Confirm Window** and will be the window of choice for most users.

The *Results* window offers a more limited set of functionality than the *Confirm* window and in particular does not display graphics, nor permit library manipulations. It can be useful when it is sufficient to simply review the results of AMDIS processing.

An introduction to the *Results* window is given in **2.5 Results Window Appearance**.

> **Note:**   It is straightforward to swap between these two windows:

> In the *Results* window, click **Confirm** to go to the *Confirm* window.

> In the *Confirm* window, click **File | Go to Results** to go to the *Results* window.

> **Note:**   AMDIS will always start up in the *Confirm* window, irrespective of the prevailing window selection when it was last closed down.

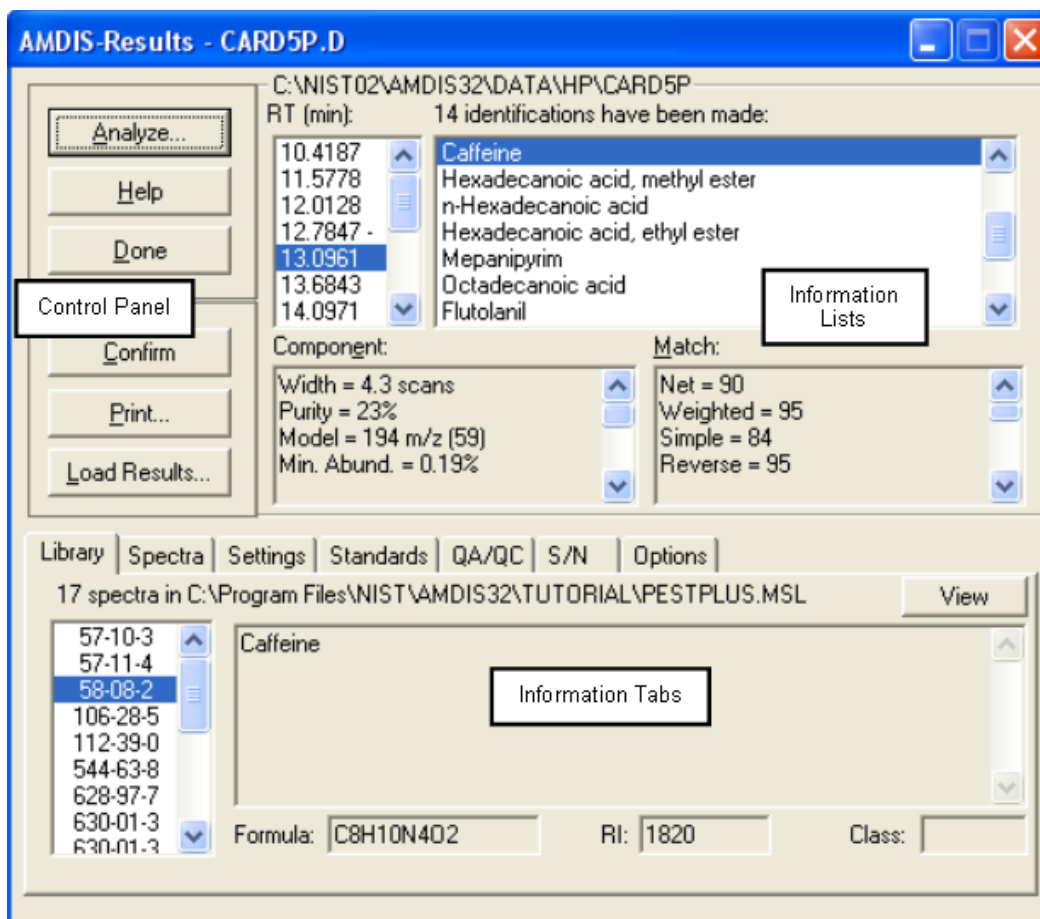The *Results* window has 3 basic areas.

Figure 88 - Results window showing its various display areas

The three areas are:

Control Panel   see **4.1 Control Panel**
Information Lists   see **4.2 Information Lists**
Information Tabs   see **4.3 Information Tabs**

4.1   Control Panel

The *Control Panel* is a series of command buttons that control the operations available in the *Results* window.

The *Control Panel* buttons are:

Analyze   see **4.1.1 Analyze**

Help   see **4.1.2 Help**

Done   see **4.1.3 Done**

Confirm   see **4.1.4 Confirm**

Print   see **4.1.5 Print**

Load Results   see **4.1.6 Load Results**

4.1.1   Analyze

The **Analyze** command first of all analyzes a GC/MS data file to locate components. According to the selected analysis type, it may compare these components against a list of target compounds and determine any matches; which themselves may then be used, for example, to create a calibration file. In all cases the analysis results are presented in the *Results* window for further examination and processing.

The various analysis types and their operation, including parameter selection and results presentation, are described in detail in Chapter **6 Analysis**.

Click **Analyze** to invoke the *Analyze GC/MS Data* dialog.
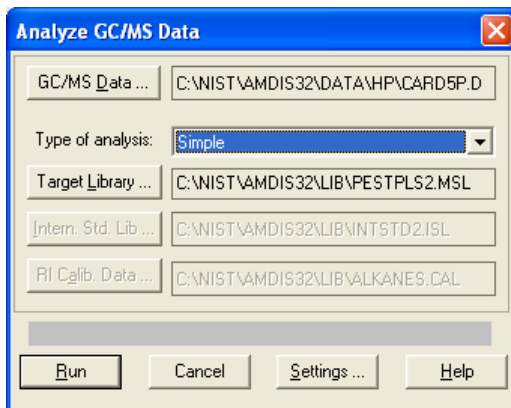

Figure 89 - Analyze GC/MS Data dialog

This dialog is identical to that invoked in the *Confirm* window and is fully described in **3.1.2.1 Analyze | Analyze GC/MS Data**.

After using **Run** to perform an analysis, the results are presented in the *Information Lists* area (**4.2 Information Lists**).

4.1.2   Help

Click **Help** to invoke the AMDIS help system at the topic describing the *Analyze GC/MS Data* dialog.

The help system is navigated in the normal *Windows* manner.

4.1.3   Done

The **Done** command is used to exit from AMDIS.

When you click **Done**, you will be asked to confirm that you want to exit. Click **Yes** to confirm, or **No** to return to AMDIS.

Before exiting, AMDIS will check the settings of the *AMDIS Options* dialog (**Figure 54 - AMDIS Options dialog**).

If its **Ask User** radio button is selected, the *Delete Result Files* dialog (**Figure 55 - Delete Result Files dialog**) will appear to ask you what you want to do with the current result file. In that case, click **Delete** or **Keep** according to your requirements. If you click **Options** you invoke the *AMDIS Options* dialog and may change its settings as described in **3.1.1.6 File | Options**.

4.1.4   Confirm

AMDIS offers two significant user interfaces for you to work with as described in **2.3 Confirm Window and Results Window Overview**.

Click **Confirm** to swap you from the *Results* window to the *Confirm* window.

The *Confirm* window is described in detail in Chapter **3 AMDIS Confirm Window**.

4.1.5   Print

The **Print** command prints analysis results for components and targets in a textual format and according to which options you select.

When you click **Print** you are presented with the *Print Text Report* dialog to set up for and print the results.
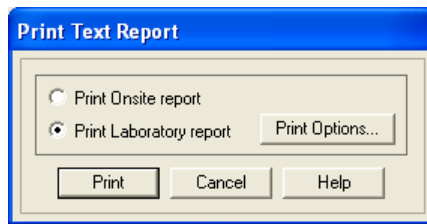

Figure 90 - Print Text Report dialog

This dialog is identical to that invoked in the *Confirm* window and is fully described in **3.1.1.9.1 Layout Details**.

4.1.6   Load Results

The **Load Results** command allows you to select the results of a previous analysis to be displayed.

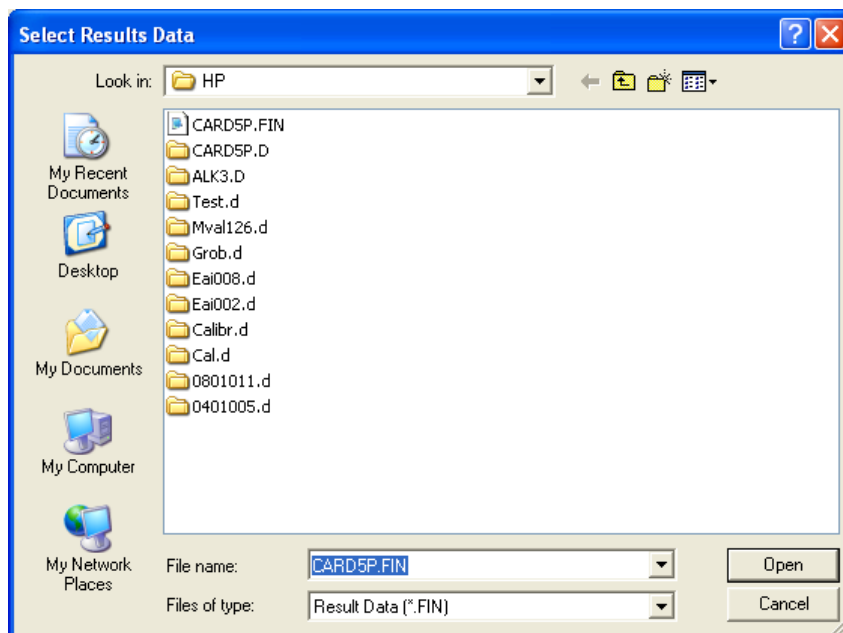Click **Load Results** to invoke the *Select Results Data* dialog.

Figure 91 - Select Results Data dialog

Use standard *Windows* techniques to locate the results file you want (.FIN extension) and click **Open** to display its results in the *Results* window.

4.2   Information Lists

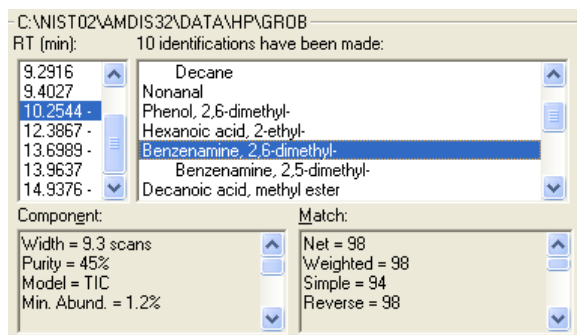The *Information Lists* area has four sections arranged more or less as quadrants.

Figure 92 - Information Lists area

The upper-left quadrant is a list of retention times for all targets.

The upper-right quadrant is a list of the compound names of the corresponding target "hits" (identifications) from a target library.

The lower two quadrants (**Component** and **Match**) provide various details about the target; as determined during the deconvolution process.

When you click on a value in either upper quadrant, the other upper quadrant, the two lower quadrants and the **Library** and **Spectra** tabs in the *Information Tabs* area will change to reflect the chosen retention time or hit.

This *Information Lists* area is similar to the *Information Lists* area of the *Confirm* window (fully described in **3.3.3 Information Lists**), but with the following significant differences:

a)   This area only displays results for targets (and so does not offer "component mode");

b)   The context menu for this retention times quadrant is the same as for its hits quadrant; ie, **Best Hits Only** and **All Hits** (and so does not offer **NIST Library** and **Undock**).

Note: As well as the context-sensitive **View Options** command in the lower two quadrants, the order in which information is presented in these quadrants can also be controlled here by the **Options** tab in the *Information Tabs* area (**5.7 Options Tab**).

4.3   Information Tabs

The *Information Tabs* area utilizes a number of tabs to present various information relating to the current analysis. As such, these values are not necessarily those that would be used for a new analysis, as these may have been modified using the **Settings** button in the *Analyze GC/MS Data* dialog as described in **4.1.1 Analyze**.
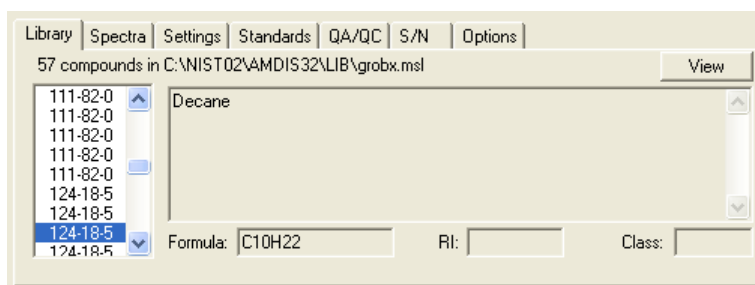

Figure 93 - Information Tabs area

The **Library** and **Spectra** tabs change to reflect the target selected in the *Information Lists* area. The other tabs show data that are independent of the particular target and so their data do not change for the results being viewed.

The *Information Tabs* area is fully explained in Chapter **5 Auxiliary Information**.

5 Auxiliary Information

Although the main focus after an AMDIS analysis is on the targets it has found, there is also a large amount of additional information that is available via a multi-tabbed dialog (*Confirm* window) or multi-tabbed area (*Results* window).

From here you may examine the library, compare the library spectrum with the extracted spectrum, examine the settings for the analysis, view the retention index standards and internal standards, examine the QA/QC report or S/N for the run, and set options for printing results.

> **Note:** It is important to understand that much of the presented information relates to the analysis results currently being examined. As such, these values are not necessarily those that would be used for a new analysis, as analysis settings may have been modified in the *Analysis Settings* dialog (**3.1.2.2 Analyze | Settings**). The exception is that the library information is taken from the current state of the library; which may have been edited since the analysis.

The *Confirm* window has two ways of getting access to this information (**3.1.4.6 View | Auxiliary Information** and **3.2.3 Info**), both of which present the *AMDIS Results* dialog.
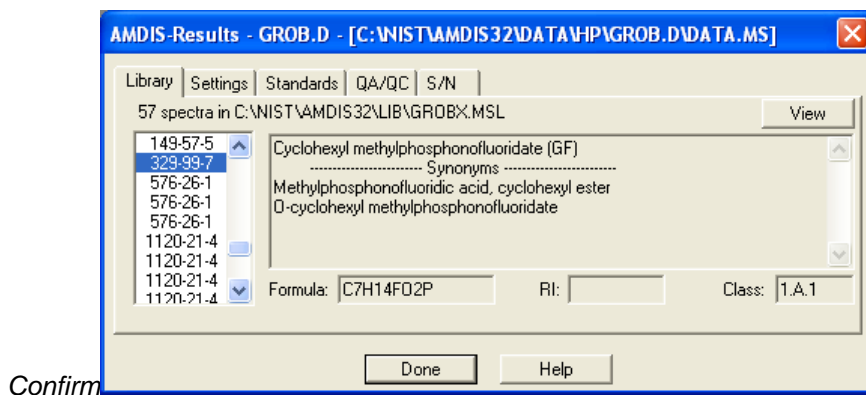

Confirm

Figure 94 - Auxiliary Information (*Confirm* Window)

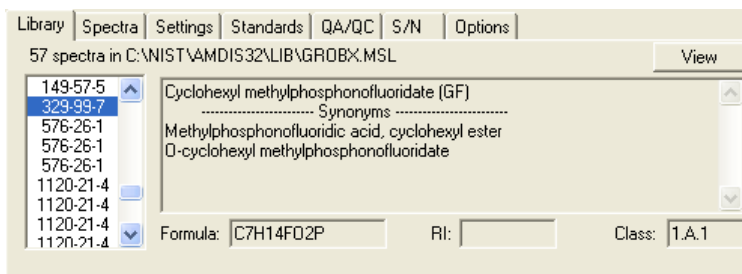The *Results* window has a dedicated information area (**4.3 Information Tabs**).

Figure 95 - Auxiliary Information (*Results* Window)

The following tabs are presented in both windows:

Library	see **5.1 Library Tab**
Settings	see **5.3 Settings Tab**
Standards	see **5.4 Standards Tab**
QA/QC	see **5.5 QA/QC Tab**
S/N	see **5.6 S/N Tab**

The following tabs are presented in the *Results* window only:

Spectra	see **5.2 Spectra Tab**

Options see **5.7 Options Tab**

5.1 Library Tab

The **Library** tab's header identifies the library being presented and tells you how many spectra it holds (**Figure 94 - Auxiliary Information (Confirm Window)**).

> **Note:** The library's name and location are taken from the analysis results' file and reflect what was used at the time of analysis. However, the library information presented is taken directly from the library now at that location. As such, it is possible that the library information you are viewing may have been modified since the results were created. If the library cannot be found, the results section will say so and the header will be blank.

The list box on the left contains the chemical identification numbers for the compounds. These numbers are the Chemical Abstracts Service registry numbers if assigned; otherwise they can be any numbers and may have been allocated arbitrarily. As you click on one of these, the rest of the tab shows, if available, its name and any synonyms, formula (**Formula**), retention index (**RI**) and class (**Class**).

In the *Confirm* window the only interaction between this dialog's information and the main *Information Lists* area is that the dialog highlights the current *Information Lists* selection on entry.

In contrast, in the *Results* window the auxiliary information will change each time you select a new entry in its *Information Lists* area.

In either window, as you select a new list box entry to display, the list box will highlight the selection and the rest of the tab's values will reflect that; as available, the compound name and any synonyms, formula, retention index and class.

The **View** button allows you to see the details of the target library and its operation is the same as the **View** button described in **3.1.2.1 Analyze | Analyze GC/MS Data**.

The **Done** button is used to exit from the dialog (*Confirm* window only).

5.2 Spectra Tab

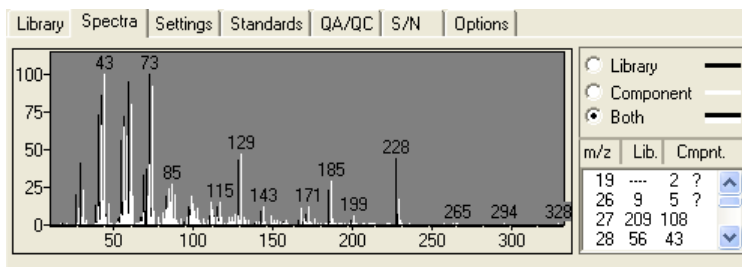The **Spectra** tab is only available in the *Results* window.

Figure 96 - Spectra tab

The deconvoluted component spectrum is shown along with the associated library spectrum. The data shown reflect the highlighted selection in the *Information Lists* area of the *Results* window.

The 3 radio buttons to the top right of the tab allow you to control what is displayed graphically. You have the option of showing just the library spectrum (**Library**), just the component spectrum (**Component**) or both together (**Both**).

Irrespective of that setting, the scrollable area to the bottom right of the tab shows the peak masses (**m/z**) and their relative intensities (scaled to 1000) in the library (**Lib.**) and component (**Cmpnt.**) spectra. A peak that is absent from one or the other spectrum is represented with dashes (**---**). Peaks with a question mark (**?**) next to them signify that the algorithm de-weighted these peaks since their deconvolution was uncertain. These are the flagged/uncertain peaks discussed in **3.1.2.7 Analyze | Use Uncertain Peaks**.

5.3   Settings Tab

The **Settings** tab displays experiment information taken from the data file plus details of the settings used in AMDIS to analyze it.
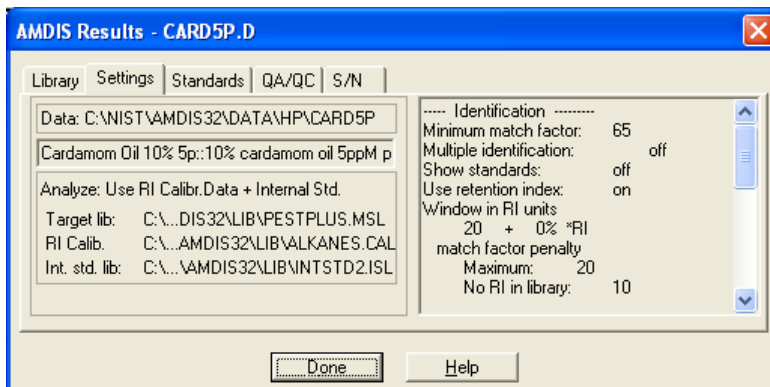

Figure 97 - Settings tab

At the top left of the tab is the name of the data file whose results are being examined together with its location when it was analyzed (**Data**). Immediately below that is a scrollable line of text, recorded with the file when it was acquired, showing experimental information. Finally, below that is the AMDIS analysis type (**Analyze**) and the one to three files used for that type of analysis (for example, **Target lib**, **RI Calib** and **Int. std. lib** in **Figure 97 - Settings tab**).

On the right of the tab is a scrollable list showing the values of all of the user-settable parameters used by AMDIS for the analysis; as set in the *Analysis Settings* dialog and fully described in **6.3 Analysis Settings**.

In summary, there are three groups of settings for which information is given:

Identification   Parameters used to control which hits are reported;

> **Note:**   The actual number of parameters shown will vary according to the settings chosen.

Instrument   Instrument values that are either stored directly in, or determined from, the data file;

Deconvolution   Parameters used to control the deconvolution process.

5.4   Standards Tab

The **Standards** tab displays information about any external and internal standards utilized in the analysis of the data file; as determined by the setting of the **Type of analysis** parameter in the *Analyze GC/MS Data* or *Analysis Settings* dialogs (respectively, **3.1.2.1 Analyze | Analyze GC/MS Data** and **6.3.1 Identif. (Identification) Tab**).

The tab has two radio buttons at the top and the information displayed below them depends on which is selected.

The **RI calib** button is used to display external standard information (**5.4.1 External Standard Mode**).

The **IS** radio button is used to display internal standard information (**5.4.2 Internal Standard Mode**).

5.4.1   External Standard Mode

The **RI calib** button is used to display external standard information. These are the standards that are used to convert retention time data to retention index.
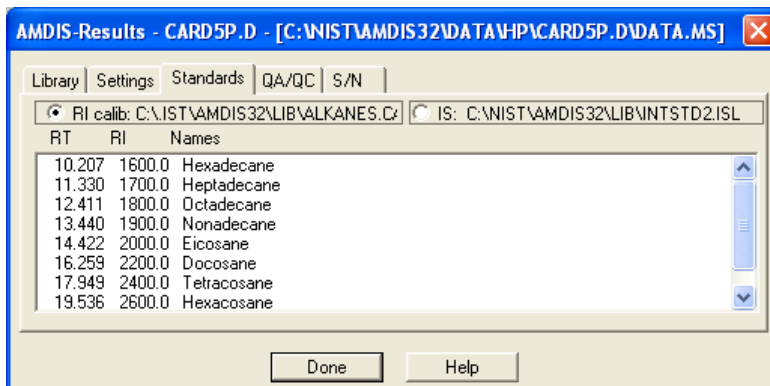

Figure 98 - Standards tab; external standard mode

The name and location of the retention index calibration file (.CAL), either created or used in the analysis, is shown to the right of the radio button and the list of retention index calibration standards (external standards) presented below is exactly its contents and their data at that time.

The retention time, retention index and compound name of each standard are given.

5.4.2   Internal Standard Mode

The **IS** radio button is used to display internal standard information.
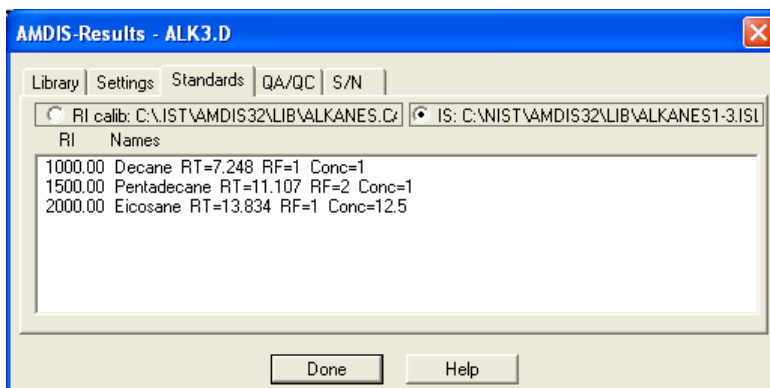

Figure 99 - Standards tab; internal standard mode

The name and location of the *Internal Standards Library* (.ISL) used in the analysis is shown to the right of the radio button and the list of internal standards presented below is exactly its contents and their data at that time.

The retention index and compound name of each standard are given, together with retention time and, if defined, response factor and concentration information.

5.5   QA/QC Tab

The **QA/QC** tab displays a variety of information about the overall quality of the data.

The tab has two radio buttons at the top and the information displayed below them depends on which is selected.

By clicking the **Results** radio button you can see data relating to the current analysis of the current file (**5.5.1 Results**).

By clicking the **Performance** radio button you can see the cumulative historical results of designated performance check compounds from previous "performance check" analyses; including the current analysis if it is a "performance check" (**5.5.2 Performance**).

5.5.1 Results

AMDIS determines and utilizes many values whilst performing an analysis and some of these values are presented here when the **Results** radio button is clicked.

The presented information can be broadly grouped as:

→ Instrument and m/z details	see **5.5.1.1 Instrument and M/z Details**
→ Background information	see **5.5.1.2 Background S/N Information**
→ Scan sets' information	see **5.5.1.3 Scan Sets Information**
→ Solvent tailing information	see **5.5.1.4 Solvent Tailing Information**
→ Column bleed information	see **5.5.1.5 Column Bleed Information**
→ Miscellaneous warnings	see **5.5.1.6 Miscellaneous Warnings**
→ Internal standards	see **5.5.1.7 Internal Standards**

5.5.1.1 Instrument and M/z Details

The first few lines of the **Results** information contain mass spectrometer instrument details from the analyzed data file; including feedback on the specified m/z range for analysis versus the actual acquired range found in the data file.
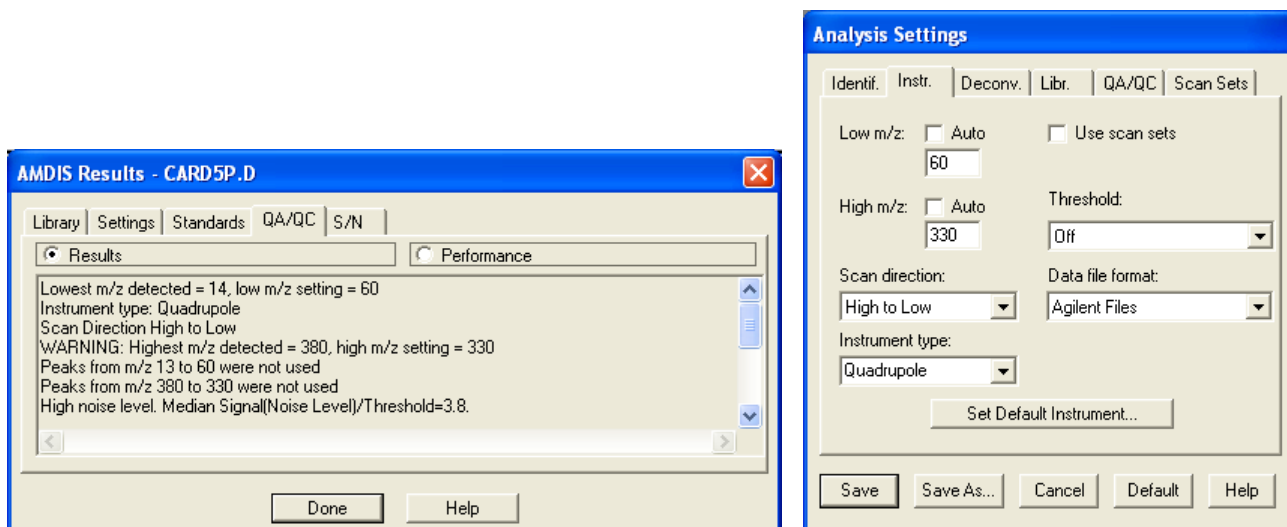

Figure 100 - QA/QC tab's instrument and m/z details plus original analysis settings

For example, if the lowest acquired peak was m/z=14, but the analysis had been requested to start at m/z=60, the window will report the data file's lowest m/z value and also the discarding of the data between that and the requested m/z value. Similarly, if the actual highest m/z peak acquired is greater than the setting for the high end of the m/z range in the analysis, the discrepancy is reported.

Also reported are very high or very low noise levels; since these are often characteristic of inappropriately set instrument threshold settings during the acquisition of the data file.

> **Note:** The instrument and m/z parameters are set on the **Instr.** tab of the *Analysis Settings* dialog (**6.3.2 Instr. (Instrument) Tab**).

5.5.1.2 Background S/N Information

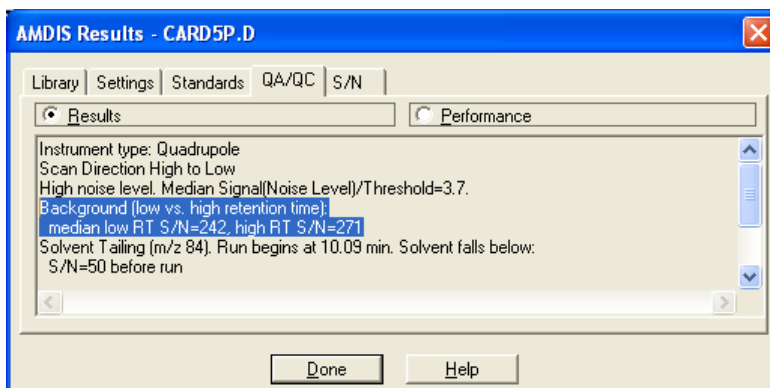Background S/N information is displayed at low and high retention times.

Figure 101 - QA/QC tab's background S/N information

Two median values are reported in S/N units and represent data taken over the first half of the run (**low RT S/N**) and over the last 10% (**high RT S/N**). A median value is the level below which the background lies for 50% of the time in its respective RT range.

For a very dirty sample, you would expect to see a large **high RT S/N** value and this would be confirmed by large values in the next run.

5.5.1.3   Scan Sets Information

If "scan sets" have been utilized in the analysis (**6.3.6 Scan Sets Tab**), their values will be reported in order.
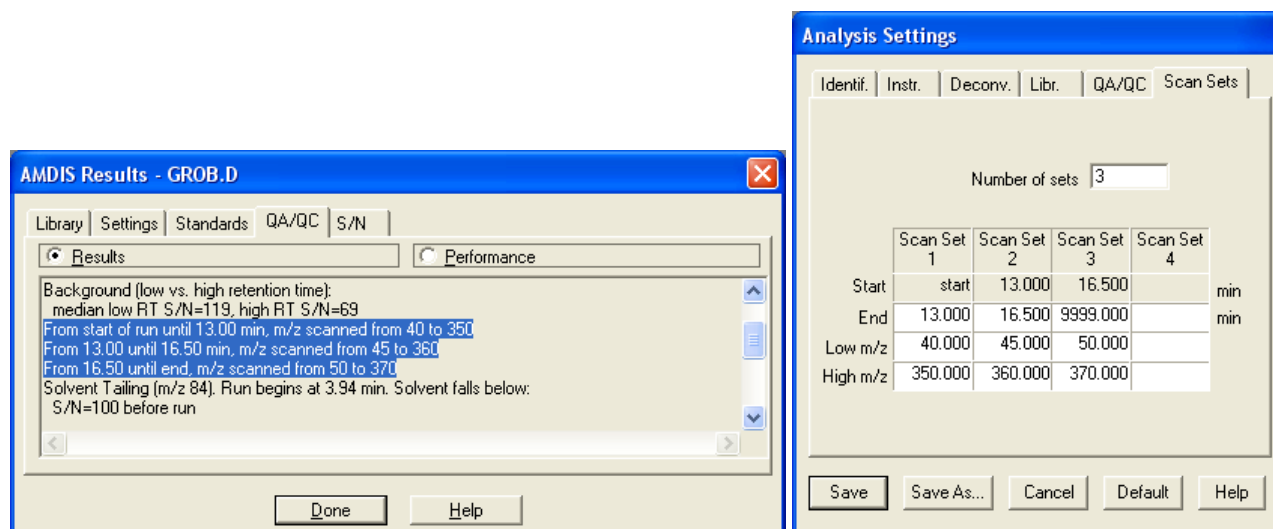

Figure 102 - QA/QC tab's scan sets' information plus original analysis settings

Each scan set used is reported on its own line and will show its retention time range and m/z range.

5.5.1.4   Solvent Tailing Information

Solvent tailing information is displayed for the **Solvent tailing** m/z value set in the **QA/QC** tab of the *Analysis Settings* dialog (**6.3.5 QA/QC (Quality Control) Tab**); where the chosen m/z should be the principal ion, or a unique ion, of the solvent.
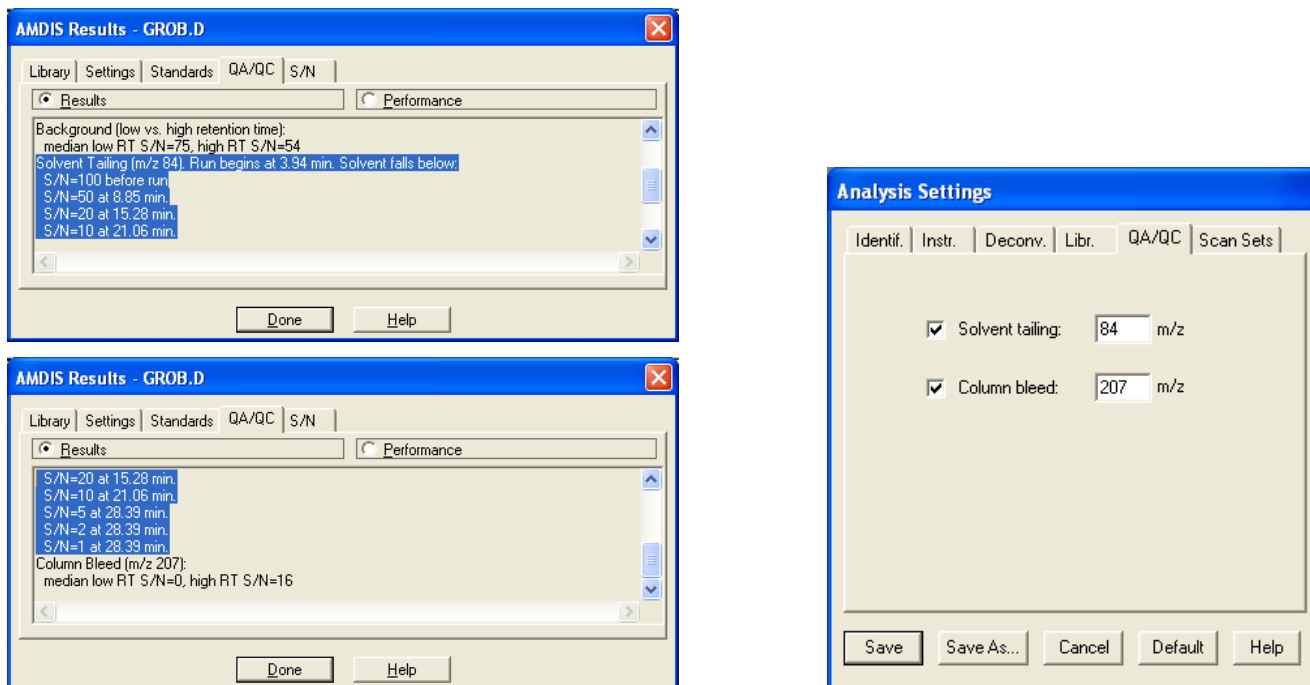

Figure 103 - QA/QC tab's solvent tailing information plus original analysis settings

The header line states the chosen ion and also says when the run begins. Subsequent lines give the time at which the m/z signal falls below a series of S/N unit values.

The reported values are given in one or more "10, 5, 2, 1" sequences (where a sequence may be partial). For example, if the first point detected has the solvent peak at an S/N of between 20 and 50, then the time at which it falls below 20, 10, 5, 2 and 1 will be reported. The fact that the S/N of the solvent fell below 50 before the run will also be noted. In the figure, the first S/N value is between 100 and 50.

> **Note:** The definition of "falling below a level" requires that the signal be below that level for at least two successive scans.

5.5.1.5   Column Bleed Information

Column bleed information is displayed for the **Column bleed** m/z value set in the **QA/QC** tab of the *Analysis Settings* dialog (**6.3.5 QA/QC (Quality Control) Tab**); where the chosen m/z should be that of an ion characteristic of the decomposition of the column.
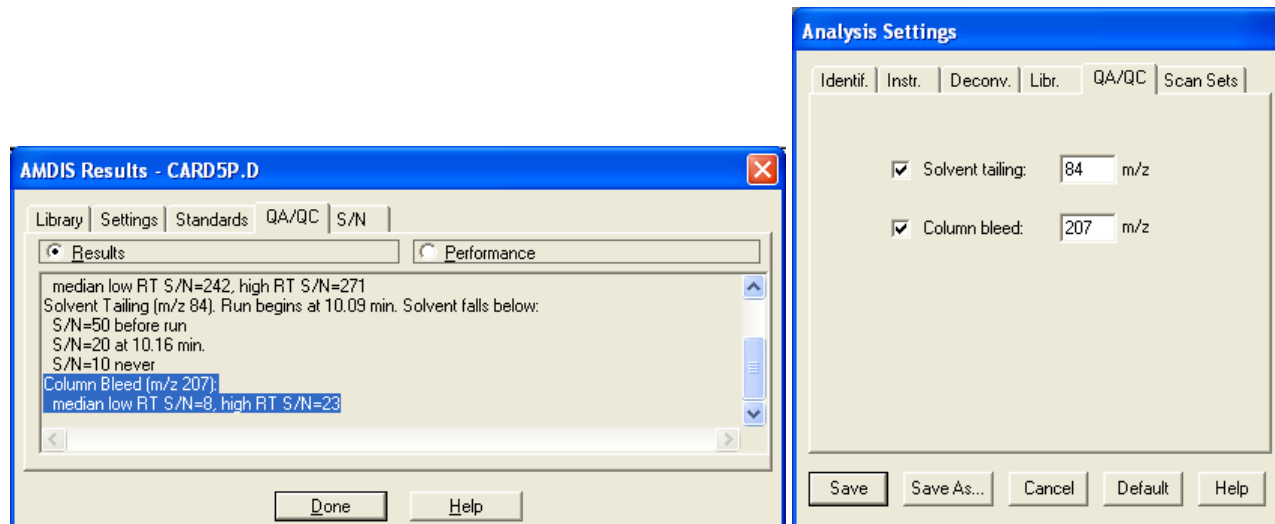

Figure 104 - QA/QC tab's column bleed information plus original analysis settings

Two median values are reported in S/N units and represent the average over the first half of the run (**low RT S/N**) and the average over the last 10% (**high RT S/N**). Very high column bleed would be indicated by high values of the **high RT S/N** value.

5.5.1.6   Miscellaneous Warnings

Miscellaneous warnings may be included at the end of the text and are self-explanatory.

For example, if high numbers of non-integral masses are found in the run, that will be reported with a note that the electron multiplier may be running at too high a voltage.

5.5.1.7   Internal Standards

If internal standards have been utilized in the analysis, all of them will be reported with found ones preceding any missed ones.
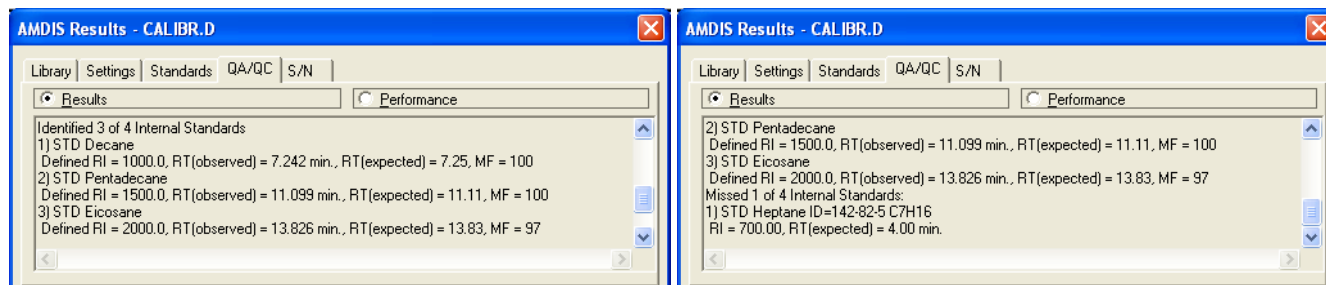
Figure 105 - QA/QC tab's internal standards' information
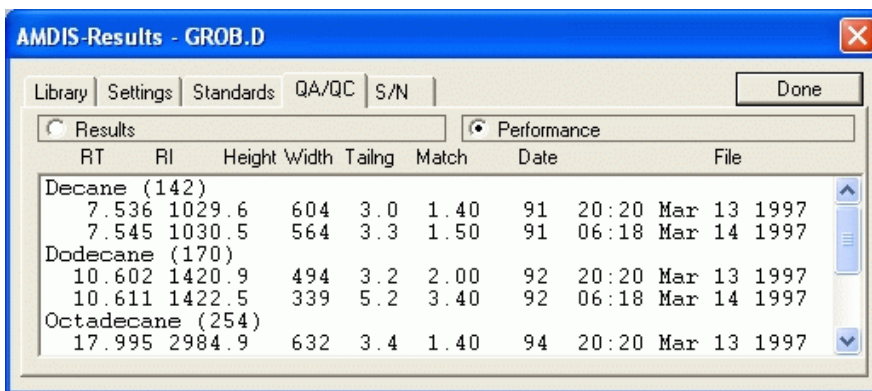
Identified internal standards are presented on 2 lines. On the first line is the compound name (**STD**). The second line shows library RI and RT values (**Defined RI** and **RT(expected)**) and values from the analysis (**RT observed** and **MF**); where **MF** is the match factor.

Missed internal standards are presented on 2 lines. On the first line are the compound name (**STD**) and the library's chemical id value (**ID**). The second line shows library RI and RT values (**RI** and **RT(expected)**).

5.5.2 Performance

AMDIS provides the facility in a *Calibration & Standards Library* (CSL) to designate a compound as a "performance check" compound (**8.5 Using the Performance Log to Track Instrument Performance**).

Whenever you analyze calibration/performance mixture runs, using analysis type *RI Calibration/Performance* or *Performance Check for RI*, the results for these compounds are added to the Performance Log. It is the values in this log that are presented here when the **Performance** radio button is clicked.

Figure 106 - QA/QC tab; performance mode

Each compound is shown using its name as the header and with multiple lines beneath in chronological order of when the data file was acquired (**Date**). This enables you to easily track changes in each compound's values over time.

Preceding the date on each line are values determined during the analysis. These are the observed retention time (**RT**), the computed retention index (**RI**), the model peak's height (**Height**), width (**Width**) and tailing (**Tailing**) values and finally the match factor (**Match**).

> **Note:** **Width** is the full width at half maximum height of the chromatographic component peak (where the width is given in scans, not time) and **Tailing** is the ratio of the area to the right (later time) of the center of the peak to the area on the left of the peak (earlier time).

For additional information on this feature, see **6.1.7 Performance Check for RI** and **6.1.3 RI Calibration/Performance**.

> **Note:** The Performance Log is actually a collection of 3 files that are located in the folder in which AMDIS is installed. Their names are *perfom.idx*, *perform.tDB* and *perform.txt*. Should you ever require to restart the Performance Log you can do so by deleting these files using standard *Windows* techniques; however, it is imperative that you delete all 3 files at the same time.

5.6   S/N Tab

The **S/N** tab displays various diagnostic information about the data file in relation to the signal (**5.6.1 Signal**) and its noise (**5.6.2 Noise**).
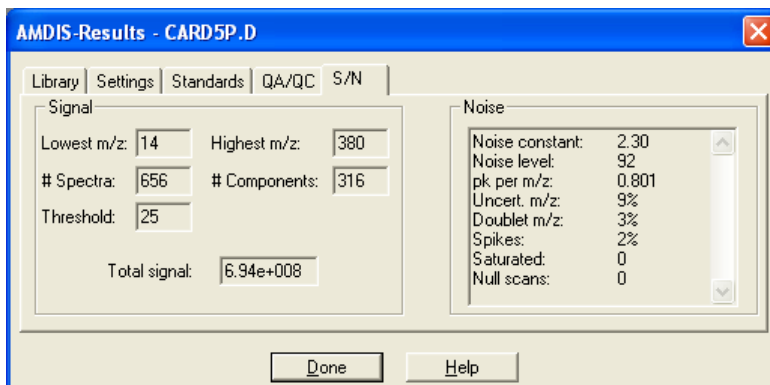

Figure 107 - S/N tab

5.6.1   Signal

The signal is analyzed for the whole run and various information presented. The lowest mass (**Lowest m/z**) and the highest mass detected (**Highest m/z**) are presented, together with the threshold (**Threshold**) and the number of distinct spectra (**# Spectra**) and components (**# Components**) found in the analysis. For some components, the number of spectra is more than one, so the number of spectra is at least as great as the number of components, and usually larger. The total signal (ion current) summed over all ions is also reported (**Total signal**). **Total signal** and **Threshold** are reported in the native units of the instrument.

5.6.2   Noise

Noise parameters in some cases suggest possible problems with the instrument.

Noise constant

The proportionality constant between the square root of the signal and the noise for any signal. If this value changes over time for a given instrument, it often indicates a developing problem. If it suddenly increases, it is certainly showing a problem that needs fixing.

Noise level

The median signal level for all ions. Since most of the signals are very small and mostly noise, the median will always be small. If the threshold level has been set too high, the signal level may be distorted.

pk per m/z

The number of peaks per mass unit averaged for all scans. A large value indicates a high noise level.

Uncert. m/z

The percentage of peaks that are more than 0.25 units away from an integer m/z. A large value is usually associated with a high noise level and often with a dirty ion source or failing electron multiplier.

Doublet m/z

The percentage of unit m/z regions where more than one peak appears. A large value indicates a high noise level.

Spikes

The number of "spike" peaks, expressed as a percentage of all peaks detected. A spike occurs when the signal is zero (or below threshold, which is considered the same), rises to a nonzero value and then goes back to zero in successive scans. It is not a spike if it just rises and falls from nonzero values.

Saturated

The number of times a single ion peak had a signal level equal to the maximum the instrument can deliver. Note that, this is only applicable when the maximum value is known; otherwise it is not reported.

Null scans

The number of scans for which there is no signal at any mass. These scans are typically points where the ion multiplier has shut down for some reason.

5.7   Options Tab

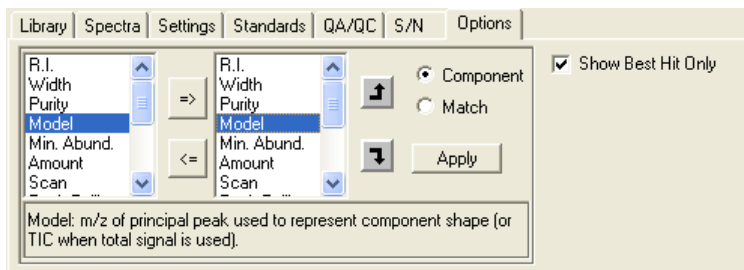The **Options** tab is only available in the *Results* window.

Figure 108 - Options tab

The **Show Best Hit Only** checkbox determines the number of hits shown in the upper-right quadrant of the *Information Lists* area (**4.2 Information Lists**). When it is selected, only a single hit (the best one) is shown for each target; equivalent to selecting **Best Hits Only** on the context menu in that quadrant. When it is cleared, all hits for each target are shown; equivalent to selecting **All Hits** on the context menu.

In the main body of the tab are two list boxes. As you click on the **Component** or **Match** radio button, the contents of the two boxes change to show items appropriate to the selected radio button. This is equivalent to selecting the **View Options** context menu in the corresponding lower quadrant of the *Information Lists* area.

In both cases the left box shows all possible items. However, the right box shows only those items that you have selected to be displayed and their order of display in the corresponding lower quadrant of the *Information Lists* area.

In both cases the right list box is set up using the "arrow" buttons as described in **3.1.1.10.1 Value Selection and Ordering**.

6   Analysis

An "analysis" is the deconvolution and subsequent processing that AMDIS performs on a data file to ultimately yield component and target results.

The analysis parameters can be set from both the *Results* window and the *Confirmation* window.

In the *Results* window select the **Analyze** button in the *Control Panel* (**4.1 Control Panel**).

In the *Confirmation* window, select the **Analyze | Analyze GC/MS Data** command (**3.1.2.1 Analyze | Analyze GC/MS Data**).

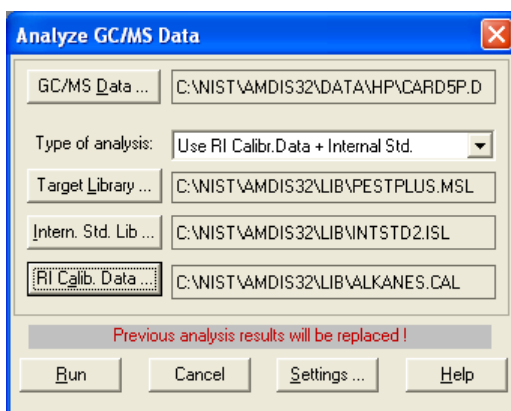In each case, the *Analyze GC/MS Data* dialog is invoked to allow you to set all of the parameters of the analysis.

Figure 109 - Analyze GC/MS Data dialog

The basics of the operation of this dialog are explained in **3.1.2.1 Analyze | Analyze GC/MS Data**, including the use of the **GC/MS Data** button to select the data file to be analyzed and the **Run** button to initiate the analysis. However, the details for **Type of analysis** (**6.1 Analysis Types**) and for the three library file buttons (**6.2 Library File Buttons**) are explained below.

The **Settings** button invokes the *Analysis Settings* dialog, which is introduced in **3.1.2.2 Analyze | Settings**, but whose parameters are described in detail below (**6.3 Analysis Settings**).

6.1   Analysis Types

AMDIS offers several different ways of analyzing data files that reflect the use or otherwise of internal and/or external standards:

Simple	see **6.1.1 Simple**
Use Retention Time	see **6.1.2 Use Retention Time**
RI Calibration/Performance	see **6.1.3 RI Calibration/Performance**
Use Retention Index Data	see **6.1.4 Use Retention Index Data**
Use Internal Standards for RI	see **6.1.5 Use Internal Standards for RI**
Use RI Calibr. Data + Internal Std.	see **6.1.6 Use RI Calibr. Data + Internal Std.**
Performance Check for RI	see **6.1.7 Performance Check for RI**

In all cases target matches are subject to parameters set on the **Identif** tab of the *Analysis Settings* dialog (**Figure 110 - Identification parameters**); for a detailed description of these parameters see **6.3.1 Identif. (Identification) Tab**.

Initial target identifications are governed by the **Minimum match factor** and **Multiple identifications per compound** parameters (the "match parameters").

For those analysis types that compute RI information for a target, the **RI window** parameters are presented together with RI-related **Match factor penalties** values (the "RI parameters").

For the *Use Retention Time* analysis type only, the **RT** parameter is presented together with RT-related **Match factor penalties** values (the "RT parameters").

As relevant, the RI parameters or RT parameters will be utilized to compute, respectively, a **RETENTION** correction to the **Net** match value (**3.3.3.2 Target Mode Details**).

The different analysis types are introduced in **2.6 Analysis Types Overview** and are described in detail in the following sections.
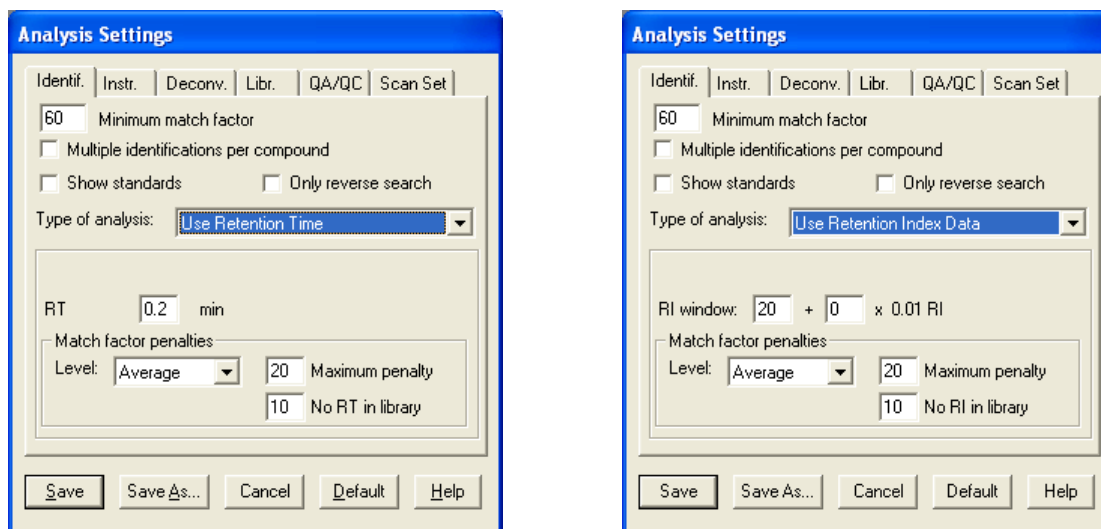
Figure 110 - Identification parameters

6.1.1   Simple

A *Simple* analysis uses only the mass spectral data for the individual target compounds in the *Target Compounds Library* (MSL) to identify the compounds. The calculated match factor depends only on the quality of the match between the deconvoluted component spectra and the target library spectra.

See **2.7 Analysis Type - Simple** for a tutorial illustrating this analysis type.

A *Target Compounds Library* is described in detail in **8.1.1 Target Compounds Library (MSL)**.
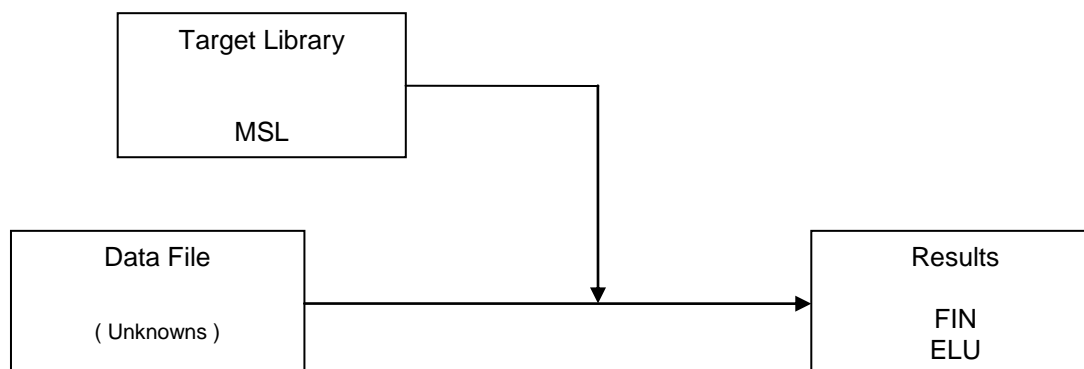
Figure 111 - Simple analysis schematic

The analysis proceeds as follows (for the parameters mentioned below refer to **Figure 110 - Identification parameters**):

a)   The deconvolution process extracts pure component spectra from the data file.

b)   In turn, each compound in the *Target Compounds Library* (MSL) is compared to the first component spectrum and a target match factor value is computed. If a target match factor is equal to or greater than the **Minimum match factor** value, the target is added to the "hit list" for that component. The hit list is ordered by match factor.

c)   The same comparison process is then repeated for each component to create a hit list for each component. Note that, a hit list can be (and often is) empty.

d)   Each target is now considered in turn and all hit lists examined to determine in which component's hit list that target has its maximum match factor. That target is now assigned as the "best hit" for that component.

e)   If **Multiple identifications per compound** is clear, a particular target can only be assigned as the best hit for a single component.

However, the target can still be assigned as a non-best hit for another component, but only where its match factor for the other component is less than the match factor of the best hit target assigned to that other component.

If **Multiple identifications per compound** is selected, there are no such "best hit" restrictions on assigning a target to a component and so the hit list adjustment process described above does not take place.

f)   Since RI parameters and RT parameters are not relevant to this analysis type they are disabled and a **RETENTION** = **n/a** is shown in the results.

g)   The target and component result files are created (FIN, ELU).

6.1.2   Use Retention Time

A *Use Retention Time* analysis first utilises the mass spectral data for the individual target compounds in the *Target Compounds Library* (MSL) to identify the compounds and assigns each compound its retention time from the data file.

Based on user settings, the difference between an assigned RT and any stored library RT value may be subsequently used to adjust the target's match value (see below).

See **2.8 Analysis Type - Use Retention Time** for a tutorial illustrating this analysis type.

A *Target Compounds Library* is described in detail in **8.1.1 Target Compounds Library (MSL)**.

Figure 112 - Use Retention Time analysis schematic

The analysis proceeds as follows (for the parameters mentioned below refer to **Figure 110 - Identification parameters**):

a)   The deconvolution process extracts pure component spectra from the data file.

b)   In turn, each compound in the *Target Compounds Library* (MSL) is compared to the first component spectrum and a target match factor value is computed. If a target match factor is equal to or greater than the **Minimum match factor** value, the target is added to the "hit list" for that component. The hit list is ordered by match factor.

c) The same comparison process is then repeated for each component to create a hit list for each component. Note that, a hit list can be (and often is) empty.

d) Each target is now considered in turn and all hit lists examined to determine in which component's hit list that target has its maximum match factor. That target is now assigned as the "best hit" for that component.

e) If **Multiple identifications per compound** is clear, a particular target can only be assigned as the best hit for a single component.

However, the target can still be assigned as a non-best hit for another component, but only where its match factor for the other component is less than the match factor of the best hit target assigned to that other component.

If **Multiple identifications per compound** is selected, there are no such "best hit" restrictions on assigning a target to a component and so the hit list adjustment process described above does not take place.

f) The **RETENTION** correction is now computed and the **Net** match value adjusted accordingly (**3.3.3.2 Target Mode Details**).

g) The target and component result files are created (FIN, ELU).

6.1.3 RI Calibration/Performance

Unlike other analysis types, an *RI Calibration/Performance* analysis is presumed to be run on a clean mixture of known composition. This analysis establishes the correlation between retention time and retention index by using mass spectral data to match the components of the data file with the standards specified in the *Calibration & Standards Library* (CSL). Usually, the normal hydrocarbons (n-alkanes) are used as retention index standards, but there is no requirement that this be the case. Retention standards that are not uniform can even be used.

See **6.1.3.1 Practical Considerations** for a discussion of practical considerations.

See **2.9 Analysis Type - RI Calibration/Performance** for a tutorial illustrating this analysis type.

A *Calibration & Standards Library* is described in detail in **8.1.2 Calibration & Standards Library (CSL)**.

> **Note:** Since you have the option of selecting the name for the *RI Calibration Data* file (CAL) that is created, you might consider naming it to reflect the current date and/or the column type.
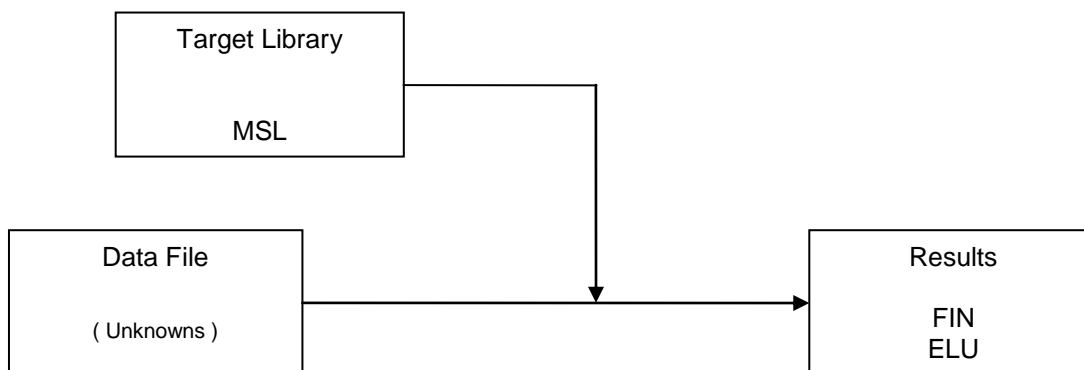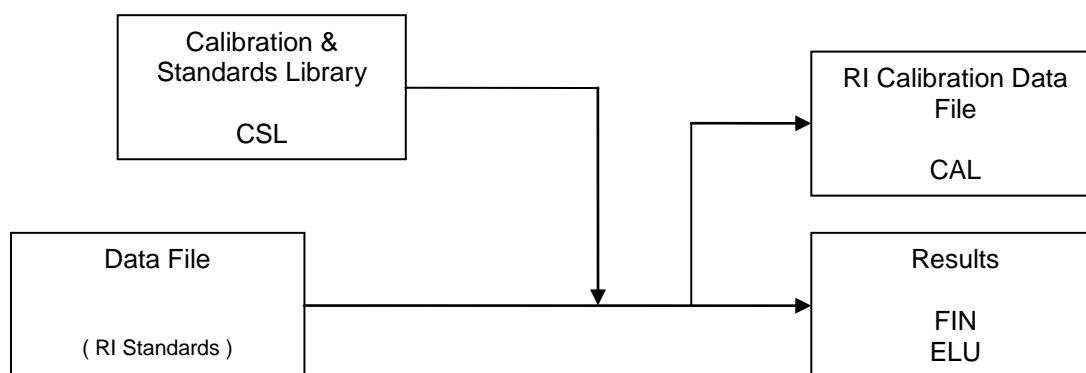
Figure 113 - RI Calibration/Performance analysis schematic

The analysis proceeds as follows (for the parameters mentioned below refer to **Figure 110 - Identification parameters**):

a) The deconvolution process extracts pure component spectra from the data file.

b) In turn, each compound in the *Calibration & Standards Library* (CSL) is compared to the first component spectrum and a calibration compound (target) match factor value is computed. If a target match factor is equal to or greater than the **Minimum match factor** value, the target is added to the "hit list" for that component. The hit list is ordered by match factor.

c) The same comparison process is then repeated for each component to create a hit list for each component. Note that, a hit list can be (and often is) empty.

d) Each target is now considered in turn and all hit lists examined to determine in which component's hit list that target has its maximum match factor. That target is now assigned as the "best hit" for that component.

e) If **Multiple identifications per compound** is clear, a particular target can only be assigned as the best hit for a single component.

However, the target can still be assigned as a non-best hit for another component, but only where its match factor for the other component is less than the match factor of the best hit target assigned to that other component.

If **Multiple identifications per compound** is selected, there are no such "best hit" restrictions on assigning a target to a component and so the hit list adjustment process described above does not take place.

f) The **RETENTION** correction is now computed and the **Net** match value adjusted accordingly (**3.3.3.2 Target Mode Details**).

g) The target and component result files are created (FIN, ELU).

h) Information for performance compounds is added to the Performance Log file (**5.5.1.3 Scan Sets Information**).

i) Since the *RI Calibration Data* file will <u>always</u> be created with its entries in RT <u>and</u> RI order, the target results are now examined and any that disobey this rule are discounted and the remaining ones are used to create the *RI Calibration Data* file (CAL).

6.1.3.1 Practical Considerations

There are a number of special features that are built into the *RI Calibration/Performance* analysis. These are based on the assumption that the *Calibration & Standards Library*'s entries will be in increasing RI value order. Ideally they will be a somewhat regularly spaced chemical series (for example, the n-alkanes, or methyl esters), as this will normally provide more reliable interpolation results; however, it is not critical.

If you are using the n-alkanes series, their highly significant molecular ions are usually present, but small, and so are not always treated with the importance that they merit. In fact, in a noisy environment it is easy, for example, for the C10 alkane to be mistaken for its C11 or C12 neighbor (and the same argument would apply to the methyl esters).

It is good practice, thereof, to utilize the **Require peak** capabilities of the *Calibration & Standards Library* to necessitate that a specific m/z peak be in an identified spectrum (**8.2.6.1 Saving Changes**).

The results of the calibration are shown in the Standards tab of the *AMDIS Results* dialog when the **RI Calib** radio button is selected (**5.4.1 External Standard Mode**).

Note that, each time an *RI Calibration/Performance* analysis is run, the *RI Calibration Data* file will be recalculated so that long term gradual drifts do not affect the calibration. The Performance Log records the retention time in order to observe these long term trends (**5.5.1.3 Scan Sets Information**).

6.1.4 Use Retention Index Data

A *Use Retention Index Data* analysis utilizes an *RI Calibration Data* file (CAL) as an external calibration file to compute RI's of located targets.

When a target from the *Target Compounds Library* (MSL) is identified using mass spectral data, its RT value will be interpolated in the *RI Calibration Data* file (CAL) to yield a computed RI value for the target. Based on user settings, the computed RI may be subsequently used to adjust the target's match value (see below).

Note: An *RI Calibration Data* file is a correlation between retention time and retention index for a set of retention index standards (**6.1.3 RI Calibration/Performance**).

See **2.10 Analysis Type - Use Retention Index Data** for a tutorial illustrating this analysis type.
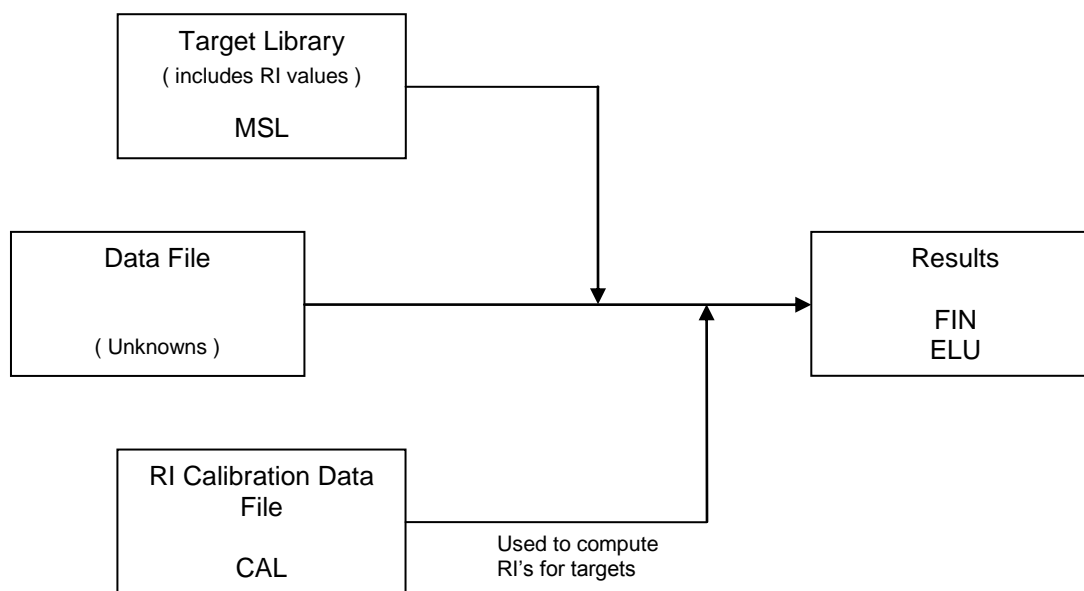
Figure 114 - Use Retention Index Data analysis schematic

The analysis proceeds as follows (for the parameters mentioned below refer to **Figure 110 - Identification parameters**):

a) The deconvolution process extracts pure component spectra from the data file.

b) In turn, each compound in the *Target Compounds Library* (MSL) is compared to the first component spectrum and a target match factor value is computed. If a target match factor is equal to or greater than the **Minimum match factor** value, the target is added to the "hit list" for that component. The hit list is ordered by match factor.

c) The same comparison process is then repeated for each component to create a hit list for each component. Note that, a hit list can be (and often is) empty.

d) Each target is now considered in turn and all hit lists examined to determine in which component's hit list that target has its maximum match factor. That target is now assigned as the "best hit" for that component.

e) If **Multiple identifications per compound** is clear, a particular target can only be assigned as the best hit for a single component.

   However, the target can still be assigned as a non-best hit for another component, but only where its match factor for the other component is less than the match factor of the best hit target assigned to that other component.

   If **Multiple identifications per compound** is selected, there are no such "best hit" restrictions on assigning a target to a component and so the hit list adjustment process described above does not take place.

f) The actual retention time of each target (RT_{act}) is now compared against the *RI Calibration Data* file (CAL) list of RT and RI pairs and its RT value interpolated linearly between the closest RT value below it (RT_{lo} and RI_{lo}) and the closest RT value above it (RT_{hi} and RI_{hi}) in order to compute an RI for it (RI_{comp}) as follows:

$$RI_{comp} = RI_{lo} + ((RI_{hi} - RI_{lo}) * (RT_{act} - RT_{lo}) / (RT_{hi} - RT_{lo}))$$

   Note: If RT_{act} is outside of the range of the calibration library's RT values, the nearest RT_{hi} and RT_{lo} pair will be used.

g) The **RETENTION** correction is now computed and the **Net** match value adjusted accordingly (**3.3.3.2 Target Mode Details**).

h) The target and component result files are created (FIN, ELU).

6.1.5   Use Internal Standards for RI

A *Use Internal Standards for RI* analysis utilizes an *Internal Standards Library* (ISL) to compute RI's of located targets. For the experiment, a set of internal standards of known retention time and retention index are co-injected with the unknown mixture.

> **Note:**   Using this analysis type it is possible to compute basic concentration values for a found target (**8.4.1 Calculating a Concentration**).

First, the internal standards of the *Internal Standards Library* (ISL) are located using mass spectral data in exactly the same way as for target compounds. When a target from the *Target Compounds Library* (MSL) is now identified using mass spectral data, its RT value will be interpolated using the located internal standards to yield a computed RI value for the target. Based on user settings, the computed RI may be subsequently used to adjust the target's match value (see below).

See **2.11 Analysis Type - Use Internal Standards for RI** for a tutorial illustrating this analysis type.
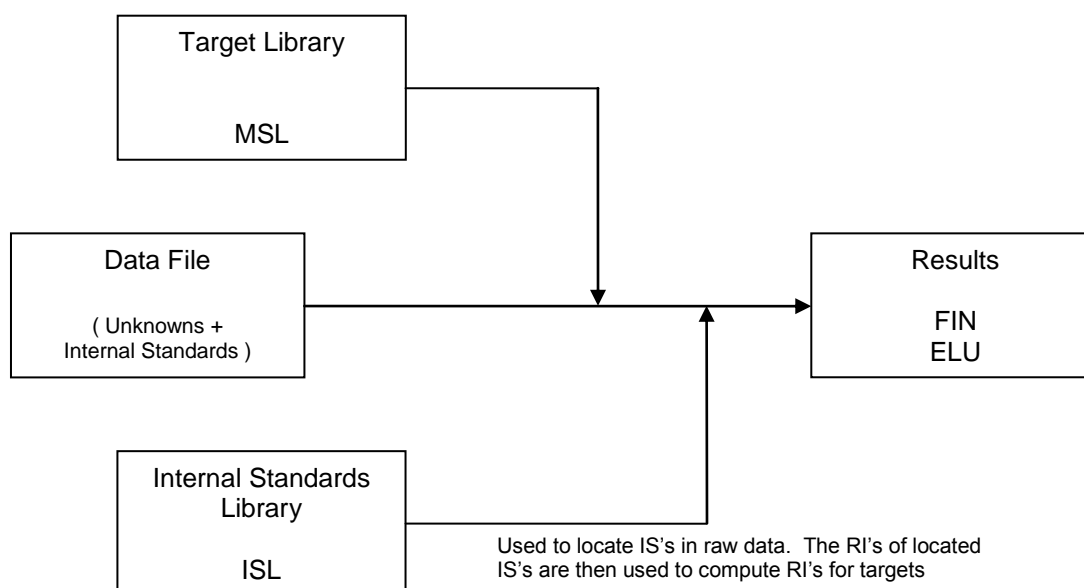

Figure 115 - Use Internal Standards for RI analysis schematic

The analysis proceeds as follows (for the parameters mentioned below refer to **Figure 110 - Identification parameters**):

a)   The deconvolution process extracts pure component spectra from the data file.

b)   In turn, each compound in the *Internal Standards Library* (ISL) and the *Target Compounds Library* (MSL) is compared to the first component spectrum and a target match factor value is computed. If a target match factor is equal to or greater than the **Minimum match factor** value, the target is added to the "hit list" for that component. The hit list is ordered by match factor.

c)   The same comparison process is then repeated for each component to create a hit list for each component. Note that, a hit list can be (and often is) empty.

d)   Each target is now considered in turn and all hit lists examined to determine in which component's hit list that target has its maximum match factor. That target is now assigned as the "best hit" for that component.

e)   If **Multiple identifications per compound** is clear, a particular target can only be assigned as the best hit for a single component.

However, the target can still be assigned as a non-best hit for another component, but only where its match factor for the other component is less than the match factor of the best hit target assigned to that other component.

If **Multiple identifications per compound** is selected, there are no such "best hit" restrictions on assigning a target to a component and so the hit list adjustment process described above does not take place.

f) The actual retention time of each target (RT_{act}) is now compared against the *Internal Standards Library* (ISL) list of RT and RI pairs and its RT value interpolated linearly between the closest RT value below it (RT_{lo} and RI_{lo}) and the closest RT value above it (RT_{hi} and RI_{hi}) in order to compute an RI for it (RI_{comp}) as follows:

$$RI_{comp} = RI_{lo} + ((RI_{hi} - RI_{lo}) * (RT_{act} - RT_{lo}) / (RT_{hi} - RT_{lo}))$$

> **Note:** If RT_{act} is higher than the highest RT of all located internal standard compounds, the nearest RT_{hi} and RT_{lo} pair will be used.

> **Note:** If RT_{act} is lower than the lowest RT (RT_{lowest} and RI_{lowest}) of all located internal standard compounds, the following equation is used:

$$RI_{comp} = RI_{lowest} * RT_{act} / RT_{lowest}$$

> **Note:** If only one internal standard is located (RT_{single} and RI_{single}), the following equation is used:

$$RI_{comp} = RI_{single} * RT_{act} / RT_{single}$$

g) The **RETENTION** correction is now computed and the **Net** match value adjusted accordingly (**3.3.3.2 Target Mode Details**).

h) The target and component result files are created (FIN, ELU).

The found or missing internal standards will be reported in the QA/QC report that is created for each analysis (**5.4.2 Internal Standard Mode** and **5.5.1 Results**).

6.1.6 Use RI Calibr. Data + Internal Std.

A *Use RI Calibr. Data + Internal Std.* analysis utilizes an *RI Calibration Data* file (CAL) as an external calibration file to adjust RI's of located targets; exactly as in a *Use Retention Index Data* analysis (**6.1.4 Use Retention Index Data**). For the experiment, a set of internal standards of known retention time and retention index are co-injected with the unknown mixture.

> **Note:** Using this analysis type it is possible to compute basic concentration values for a found target (**8.4.1 Calculating a Concentration**).

When a target from the *Target Compounds Library* (MSL) is identified using mass spectral data, its RT value will be interpolated in the *RI Calibration Data* file (CAL) to yield a computed RI value for the target. Based on user settings, the computed RI may be subsequently used to adjust the target's match value (see below).

> **Note:** An *RI Calibration Data* file is a correlation between retention time and retention index for a set of retention index standards (**6.1.3 RI Calibration/Performance**).

An *Internal Standards Library* (ISL) is also utilized, but only to insure that the instrument is functioning correctly, or that the sample preparation has been performed correctly. As such, the internal standards are not used to calculate retention indices, but are simply reported in the QA/QC report that is created for each analysis (**5.4.2 Internal Standard Mode** and **5.5.1 Results**).

See **2.12 Analysis Type - Use RI Calibr. Data + Internal Std.** for a tutorial illustrating this analysis type.

Target Library

MSL

Internal Standards
Library

ISL

Used for QC only

Data File

(Unknowns +
Internal Standards)

Results

FIN
ELU

RI Calibration Data
File

CAL

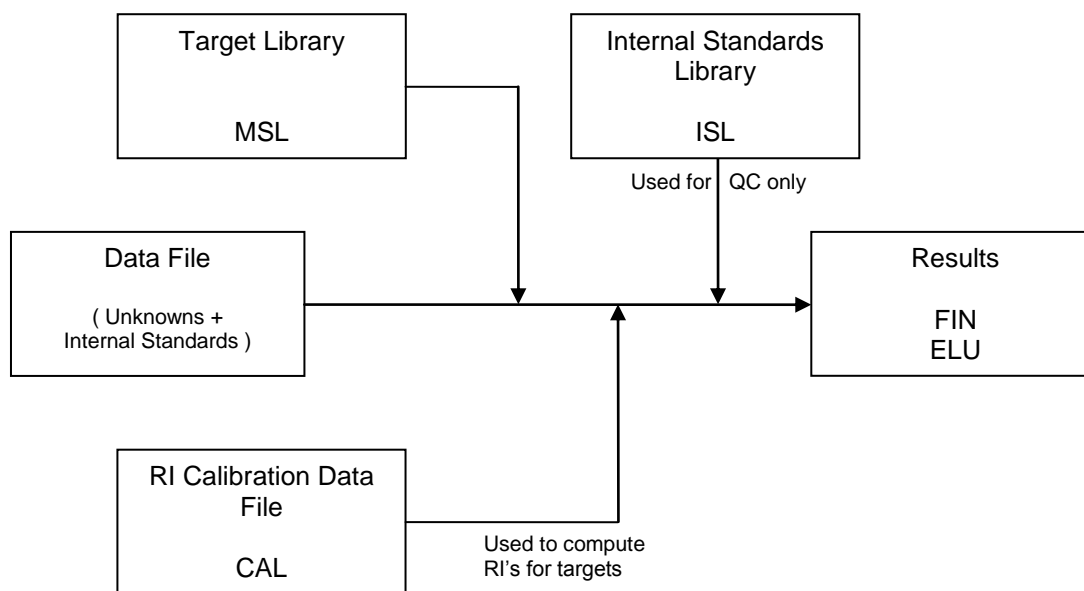Used to compute
RI's for targets

Figure 116 - Use RI Calibr. Data + Internal Std. analysis schematic

The analysis proceeds as follows (for the parameters mentioned below refer to **Figure 110 - Identification parameters**):

a)   The deconvolution process extracts pure component spectra from the data file.

b)   In turn, each compound in the *Internal Standards Library* (ISL) and the *Target Compounds Library* (MSL) is compared to the first component spectrum and a target match factor value is computed. If a target match factor is equal to or greater than the **Minimum match factor** value, the target is added to the "hit list" for that component. The hit list is ordered by match factor.

c)   The same comparison process is then repeated for each component to create a hit list for each component. Note that, a hit list can be (and often is) empty.

d)   Each target is now considered in turn and all hit lists examined to determine in which component's hit list that target has its maximum match factor. That target is now assigned as the "best hit" for that component.

e)   If **Multiple identifications per compound** is clear, a particular target can only be assigned as the best hit for a single component.

However, the target can still be assigned as a non-best hit for another component, but only where its match factor for the other component is less than the match factor of the best hit target assigned to that other component.

If **Multiple identifications per compound** is selected, there are no such "best hit" restrictions on assigning a target to a component and so the hit list adjustment process described above does not take place.

f)   The actual retention time of each target (RT_{act}) is now compared against the *RI Calibration Data* file (CAL) list of RT and RI pairs and its RT value interpolated linearly between the closest RT value below it (RT_{lo} and RI_{lo}) and the closest RT value above it (RT_{hi} and RI_{hi}) in order to compute an RI for it (RI_{comp}) as follows:

$$RI_{comp} = RI_{lo} + ((RI_{hi} - RI_{lo}) * (RT_{act} - RT_{lo}) / (RT_{hi} - RT_{lo}))$$

Note: If RT_{act} is outside of the range of the calibration library's RT values, the nearest RT_{hi} and RT_{lo} pair will be used.

g)   The **RETENTION** correction is now computed and the **Net** match value adjusted accordingly (**3.3.3.2 Target Mode Details**).

h)   The target and component result files are created (FIN, ELU).

6.1.7   Performance Check for RI

A *Performance Check for RI* analysis utilizes an *RI Calibration Data* file (CAL) as an external calibration file to adjust RI's of located *Calibration & Standards Library* (CSL) targets. Diagnostic values for those compounds identified as performance compounds are stored in the Performance Log file.

When a target from the *Calibration & Standards Library* (CSL) is identified using mass spectral data, its RT value will be interpolated in the *RI Calibration Data* file (CAL) to yield a computed RI value for the target. Based on user settings, the computed RI may be subsequently used to adjust the target's match value (see below).

The diagnostic values determined for the performance check compounds are recorded in the Performance Log file and that log can be subsequently viewed at any time (**5.5.1.3 Scan Sets Information**).

> **Note:**   An *RI Calibration Data* file is a correlation between retention time and retention index for a set of retention index standards (**6.1.3 RI Calibration/Performance**).

See **6.1.7.1 Practical Considerations**for a discussion of practical considerations.

See **2.13 Analysis Type - Performance Check for RI** for a tutorial illustrating this analysis type.
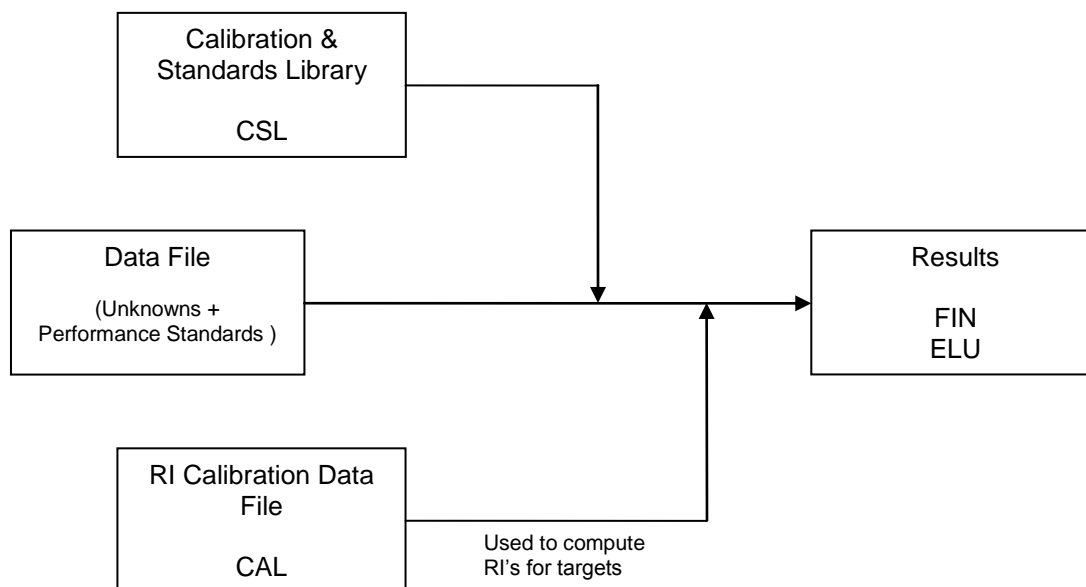
Figure 117 - Performance Check for RI analysis schematic

The analysis proceeds as follows (for the parameters mentioned below refer to **Figure 110 - Identification parameters**):

a)   The deconvolution process extracts pure component spectra from the data file.

b)   In turn, each compound in the *Calibration & Standards Library* (CSL) is compared to the first component spectrum and a calibration compound (target) match factor value is computed. If a target match factor is equal to or greater than the **Minimum match factor** value, the target is added to the "hit list" for that component. The hit list is ordered by match factor.

c)   The same comparison process is then repeated for each component to create a hit list for each component. Note that, a hit list can be (and often is) empty.

d)   Each target is now considered in turn and all hit lists examined to determine in which component's hit list that target has its maximum match factor. That target is now assigned as the "best hit" for that component.

e) If **Multiple identifications per compound** is clear, a particular target can only be assigned as the best hit for a single component.

However, the target can still be assigned as a non-best hit for another component, but only where its match factor for the other component is less than the match factor of the best hit target assigned to that other component.

If **Multiple identifications per compound** is selected, there are no such "best hit" restrictions on assigning a target to a component and so the hit list adjustment process described above does not take place.

f) The actual retention time of each target (RT_{act}) is now compared against the *RI Calibration Data* file (CAL) list of RT and RI pairs and its RT value interpolated linearly between the closest RT value below it (RT_{lo} and RI_{lo}) and the closest RT value above it (RT_{hi} and RI_{hi}) in order to compute an RI for it (RI_{comp}) as follows:

$RI_{comp} = RI_{lo} + ((RI_{hi} - RI_{lo}) * (RT_{act} - RT_{lo}) / (RT_{hi} - RT_{lo}))$

> **Note:** If RT_{act} is outside of the range of the calibration library's RT values, the nearest RT_{hi} and RT_{lo} pair will be used.

g) The **RETENTION** correction is now computed and the **Net** match value adjusted accordingly (**3.3.3.2 Target Mode Details**).

h) The target and component result files are created (FIN, ELU).

i) Information for performance compounds is added to the Performance Log file (**5.5.1.3 Scan Sets Information**).

6.1.7.1   Practical Considerations

The *Performance Check for RI* analysis type is used as a check to see how well the acquisition system is performing. It does this by identifying, in the analyzed sample, those compounds flagged as "performance check compounds" in the *Calibration & Standards Library* and then stores "performance" information for these; for subsequent review.

The practical steps involved will be similar to the following:

a) Initially acquire a clean mixture of known composition containing one or more compounds that you wish to use for performance check purposes. For example, a classic and widely used performance mixture is the "Grob Mixture"; which can be purchased ready-made.

b) Manually identify and add these compounds to a *Calibration & Standards Library* that has already been constructed for "calibration check compounds" and flag the added compounds as "performance check compounds" (**8.3.1 Creating a Calibration & Standards Library from a Data File**).

For the identification you may wish to utilize the **Analyze | Search NIST Library** (**3.1.2.8 Analyze | Search NIST Library**) or **Analyze | Go to NIST MS Program** (**3.1.2.6 Analyze | Go to NIST MS Program**) commands.

c) Whenever you are ready to record performance data, prepare a sample with all (or some) of the performance check compounds in it and then do a *Performance Check for RI* analysis. If a performance check compound is found as a target, relevant information is determined and stored cumulatively for subsequent examination via the **QA/QC** tab of the *AMDIS Results* dialog when the **Performance** radio button is selected (**5.5.1.3 Scan Sets Information**).

For each performance check compound, data for retention time, retention index, height, width and tailing of the peaks, as well as the match factor, are stored. The date and data file name are also stored. This allows you to see if there are any changes in the performance of the instrument over time.

6.2   Library File Buttons

There are three different library types, a retention index calibration file and one additional type of file generated by the NIST *MS Search* program, that AMDIS can utilize for an analysis. For the purpose of this documentation they are all considered to be AMDIS "library types":

→ Target Compounds Library (.MSL)

A *Target Compounds Library* holds a list of target compound information and associated spectra. Unlike the other libraries which only have one spectrum per compound, this library may have multiple spectra per compound. This library type is utilized in several AMDIS analysis types and is what deconvoluted spectra are compared against to see if there is a match (a "hit").

→ Calibration & Standards Library (.CSL)

A *Calibration & Standards Library* is a list of calibration/performance compound information and associated spectra; one per compound. This library type is used to identify calibration compounds when creating an *RI Calibration Data* file, or when doing a performance analysis.

→ Internal Standards Library (.ISL)

An *Internal Standards Library* is a list of internal standard compound information and associated spectra; one per compound. This library type is to identify internal standards for those analyses where internal standard compounds have been added to the sample.

→ RI Calibration Data File (.CAL)

An *RI Calibration Data* file is essentially a paired list of retention time (RT) and retention index (RI) values. In those analysis types where an RI value is utilized, it is used to compute an RI from the RT of a found target compound.

→ Mass Spectral Transfer File (.MSP)

A *Mass Spectral Transfer File* is created by the NIST *MS Search* program and holds a list of target compound information and associated spectra. It is similar to a *Target Compounds Library* and can be used wherever that is used, but it only has a single spectrum per compound.

The usage of each library type is described in detail in **6.1 Analysis Types** and the libraries themselves are explained in detail in **8.1 Library Types**.

In the *Analyze GC/MS Data* dialog (**Figure 109 - Analyze GC/MS Data dialog**) the value selected for **Type of analysis** determines how many of the 3 file buttons immediately below it are enabled and also how they are labeled.

When you select a button, it invokes the *Analysis Settings* dialog showing its **Libr.** tab (**6.3.4 Libr. (Libraries) Tab**) and enables you to conveniently select or view a library of the appropriate type.

The buttons that are available in the *Analyze GC/MS Data* dialog and how they are labeled depends on the type of analysis.

Type of Analysis	Buttons		Type of Analysis	Buttons
Simple	Target Library		RI Calibration/Performance	Calib/Stds. Lib.
	-			-
	-			RI Calib. Data

Type of Analysis	Buttons		Type of Analysis	Buttons
Use Retention Index Data	Target Library		Performance Check for RI	Calib/Stds. Lib.
	-			-
	RI Calib. Data			RI Calib. Data

Type of Analysis	Buttons		Type of Analysis	Buttons
Use Internal Standards for RI	Target Library		Use Retention Time	Target Library
	Intern. Std. Lib			-
	-			-

Type of Analysis	Buttons
Use RI Calibr.Data + Internal Std	Target Library
	Intern. Std. Lib
	RI Calib. Data

Figure 118 - Analysis types and library buttons

6.3   Analysis Settings

All of the user-settable parameters controlling an analysis are located in the multi-tabbed *Analysis Settings* dialog.
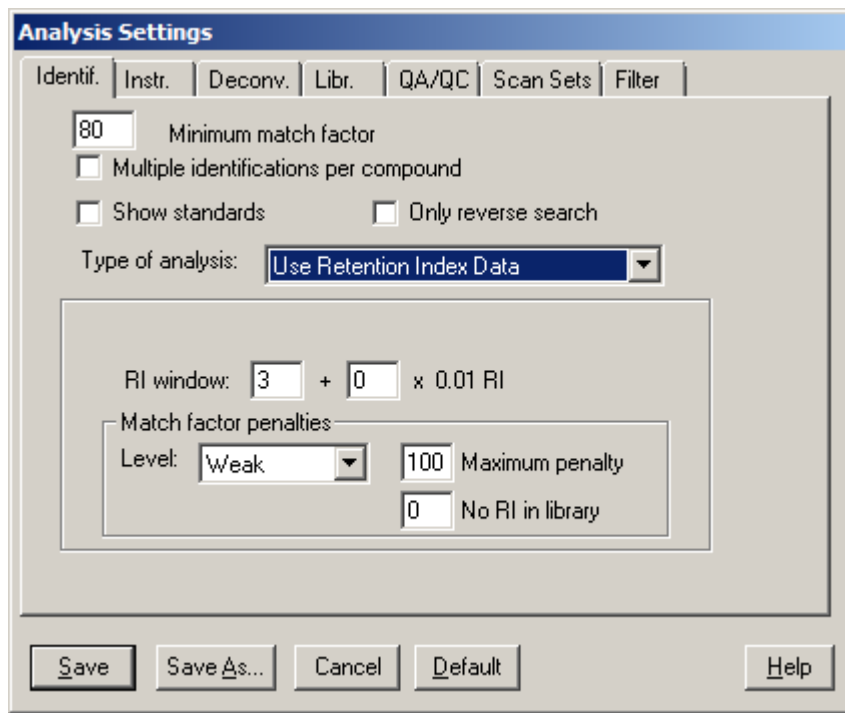

Figure 119 - Multi-tabbed Analysis Settings dialog

Each of the individual tabs controls a different set of parameters and these are grouped by function.

The **Identif.** (Identification) tab allows you to set (**6.3.1 Identif. (Identification) Tab**):

- – limits determining what results will be shown;
- – the analysis type;
- – how retention index data are utilized in adjusting overall match values.

The **Instr.** (Instrument) tab allows you to set (**6.3.2 Instr. (Instrument) Tab**):

- – the instrument type and related values;
- – the data file type and related values.

The **Deconv.** (Deconvolution) tab allows you to set parameters relating to the deconvolution process (**6.3.3 Deconv. (Deconvolution) Tab**).

The **Libr.** (Libraries) tab allows you to select and view the various library types (**6.3.4 Libr. (Libraries) Tab**).

The **QA/QC** (Quality Control) tab allows you to set parameters that are used in checking for the proper running of the instrument (**6.3.5 QA/QC (Quality Control) Tab**).

For those instruments that can acquire data files using different m/z ranges in consecutive retention time ranges during the run, the **Scan Sets** tab allows you to explicitly specify those "scan set" values such that the AMDIS analysis algorithm will function most effectively for that particular file (**6.3.6 Scan Sets Tab**).

The last tab **Filter**, provides a set of tools for reducing the number of unidentified components found in complex chromatograms. The filters set do not have any effect on the identification of target compounds. However, for the components that are not identified and show characteristics of highly overlapped or very noisy spectra, the use of filters can remove them from the final results**. (6.3.7 Filter Tab)**.

The *Analysis Settings* dialog can be activated by using the **Analyze | Settings** command (**3.1.2.2 Analyze | Settings**), or by using the **Settings** button of the *Analyze GC/MS Data* dialog (**3.1.2.1 Analyze | Analyze GC/MS Data**).

This will bring up all of the settings that are controlled by the user. Each of the individual tabs controls a different set of parameters. These are grouped by function.

The dialog's command buttons are described in **3.1.2.2 Analyze | Settings**.

6.3.1 Identif. (Identification) Tab

The **Identif.** (Identification) tab allows you to set:

- − limits determining what results will be shown;
- − the analysis type;
- − how retention index, or retention time, data are utilized in adjusting overall match values.

Its features will vary somewhat based on the selected **Type of analysis**.
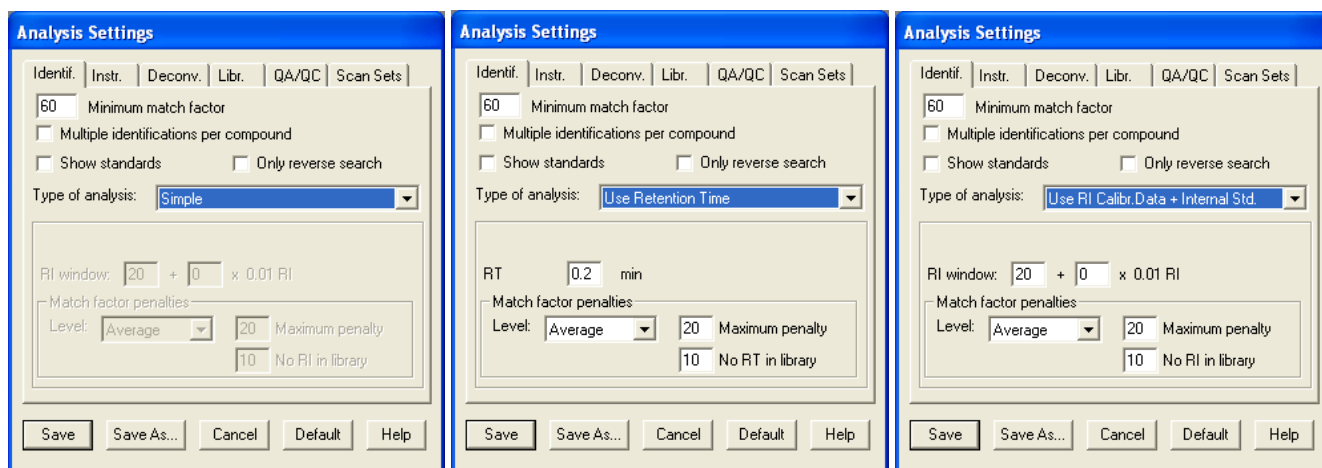

Figure 120 - Identif. tab

Minimum match factor   Default = 60

This is the lowest match factor that the program will accept to print out a target identification. Its default value of 60 has been derived from extensive testing for false positive results and in normal use you would not set it lower than this. If you set a value less than 80, all data below 80 and down to the chosen value will be reported with between one and three question marks in front of the name (for an explanation of these, see **Net** in **3.3.3.2 Target Mode Details**).

Multiple identifications per compound   Default is clear

If selected, a target compound may be identified and reported for more than one component. In the case of some isomers this is desirable.

If clear, a target compound will be reported only once and will be associated with the best match found for it within the data file.

Show standards   Default is clear

This is only applicable when the selected **Type of analysis** utilizes internal standards (ie, it is set to **Use Internal Standards for RI** or **Use RI Calibr. Data + Internal Std.**).

If selected, all internal standards found during the analysis will be reported in the *Information Lists* area of the *Results* window (**4.2 Information Lists**), or of the *Confirm* window when it is in target mode (**3.3.3 Information Lists**). They are reported in retention order at the end of all of the other identifications, regardless of where they appear in the chromatogram. They are easily identified by the **STD** preceding their names (**Figure 121 - Identified internal standards**).

Note also that, in the **Match** information a reported internal standard will also show its expected retention time (**Expec. R.T.**) and the delta in RI between the component and the library compound (**RI-RI(lib)**).

If clear, any internal standards found during the analysis will not be reported in the *Information Lists* area.

> **Note:** Irrespective of how **Show standards** is set, the internal standards' information is always presented in the *AMDIS Results* dialog (**5.4.2 Internal Standard Mode**).
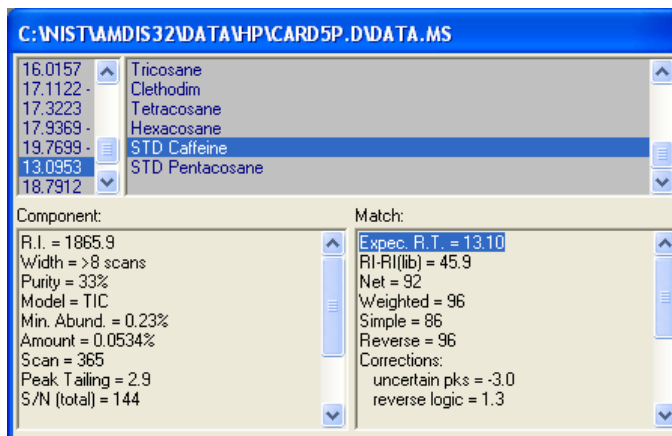

Figure 121 - Identified internal standards

Type of analysis   Default = **Type of analysis** value in the *Analyze GC/MS Data* dialog

This allows the same set of choices as for **Type of analysis** in the *Analyze GC/MS Data* dialog (**Figure 109 - Analyze GC/MS Data dialog**) and shares the same value. It is here as a convenience.

Only reverse search   Default is clear

If clear, the computed **Reverse** value will be used in calculating the **Net** value (**3.3.3.2 Target Mode Details**); which is subsequently compared against the **Minimum match factor** value to determine whether or not this component is a hit.

If selected, the **Reverse** value is still used in the same manner in computing the **Net** value, but it is the **Reverse** value itself that is compared against the **Minimum match factor** value to determine whether or not this component is a hit.

RT   Default = 0.2

RI window...   Default = 20

RT only appears when **Type of analysis** is set to **Use Retention Time**.

RI window only appears and is enabled when the selected **Type of analysis** computes retention index data (ie, it is set to **Use Retention Index Data**, **Use Internal Standards for RI**, **Use RI Calibr. Data** + **Internal Std.** or **Performance Check for RI**).

RT and **RI window** are utilized when match factors are calculated; as follows.

A target's match factor will not be enhanced if its retention time/retention index falls within a retention window, but will be reduced if it falls outside (see below). As such the use of retention time/retention index data can in general reduce false positive identifications.

Respectively, **RT/RI window** is used to specify a retention time/index window on each side of a compound's retention time/index value stored in a library. Within this window no retention time/index penalty is applied as a correction to a target's computed **Net** value (see **Net**, **Corrections** and **RETENTION** in **3.3.3.2 Target Mode Details**).

However, outside of the window, a penalty is applied that depends upon the **Match factor penalties** you set (see **Level**, **Maximum penalty** and **No RT in library**/**No RI in library** below).

The RI (but not RT) penalty can also be linearly scaled by using **+ <nnn> x 0.01 RI** (see below).

...+ <nnn> x 0.01 RI   Default = 0

This is only applicable when **RI window** is applicable.

When an **RI window** is being utilized (see above) you can set a non-zero value here (**nnn**) to vary its value linearly as the retention index value changes.

For example, if **RI window** is 20 and **nnn** is set to 5, the actual retention index window used would be:
    20 at RI=0   ie, 20 + (5 * 0.01 * 0)
    25 at RI=100   ie, 20 + (5 * 0.01 * 100)
    70 at RI=1000   ie, 20 + (5 * 0.01 * 1000)

Level   Default = Average

This is only applicable when **RT** or **RI window** is applicable.

It determines the point at which the **Maximum penalty** is applied (see below). It has values of **Weak**, **Average**, **Strong**, **Very Strong** and **Infinite**.

Its value sets the rate at which a retention time/index penalty is applied when the measured RI is outside of the initial **RI window**; as follows:

RI*windowcount* = abs(RI*measured* - RI*library*) / RI*window*

RETENTION = (RI*windowcount* - 1) * LevelFactor

where:

RI*library* is the RI value of the target in the library

RI*measured* is the RI value determined during the AMDIS analysis

abs() is the difference of two values expressed as a positive number

RI*window* is the **RI window** value

RI*windowcount* is, therefore, the number of RI*window*'s by which RI*measured* differs from RI*library*

LevelFactor is a numeric value corresponding to the value of **Level** as follows:
    Weak   1
    Average   5
    Strong   10
    Very Strong   20
    Infinite   1000

RETENTION is the computed RT/RI penalty value. However, it will be set to zero if it is negative, or will be truncated to **Maximum penalty**, if it exceeds **Maximum penalty**.

Finally, the reported **RETENTION** is expressed and used as the negative of **RETENTION**.

For example, with **Level** = **Strong** and the measured RI being 2.5 **RI window**'s away from the library RI value, RI*windowcount* will be 1.5 and a penalty of 15 will be computed (1.5 * 10).

As another example, consider the measured RI being 2.2 **RI window**'s away from the library RI value, but this time with **Level** = **Very Strong**. The computed penalty will be 24 (1.2 * 20).

For both examples, consider **Maximum penalty** set to 20. In the first case, 15 is less than 20 and so 15 will be used as the penalty and reported as **RETENTION** (**3.3.3.2 Target Mode Details**). However, in the second case 24 is greater than 20 and so the **RETENTION** used will be 20.

Maximum penalty   Default = 20

This is only applicable when **RT** or **RI window** is applicable.

It sets the maximum computed **RETENTION** that can be applied to a target and is utilized as described above for **Level**. Even when an almost perfect target match is determined (based on mass spectral and related considerations), if the retention index difference is large enough to cause the default value of 20 to be assigned to **RETENTION**, it is usually enough to make the **Net** match value fall below the level of 80; which is usually considered the minimum for a good identification (**3.3.3.2 Target Mode Details**).

Note: If the **No RT in library** or **No RI in library** penalty is applied (see below), the **Maximum penalty** value is completely ignored.

No RT in library   Default = 10

No RI in library   Default = 10

No RT in library is only applicable when **RT** is applicable. **No RI in library** is only applicable when **RI window** is applicable.

Respectively, when retention time/index data are being used for the analysis and there are no retention time/index data in the target library for a particular compound, the **Level** and **Maximum penalty** values (see above) cannot be used to compute a penalty for that compound (**RETENTION 3.3.3.2 Target Mode Details**); instead, the penalty is simply assigned the **No RT in library**/**No RI in library** value.

If the default value of 10 is used, it will require a very good match (determined based on mass spectral and related considerations) for the resultant **Net** match value to be above the level of 80; which is usually considered the minimum for a good identification (**3.3.3.2 Target Mode Details**).

Note: If the **No RT in library** or **No RI in library** penalty is applied, the **Maximum penalty** value (see above) is completely ignored.

6.3.2   Instr. (Instrument) Tab

The **Instr.** tab allows you to set:

- the instrument type and related values;
- the data file type and related values.
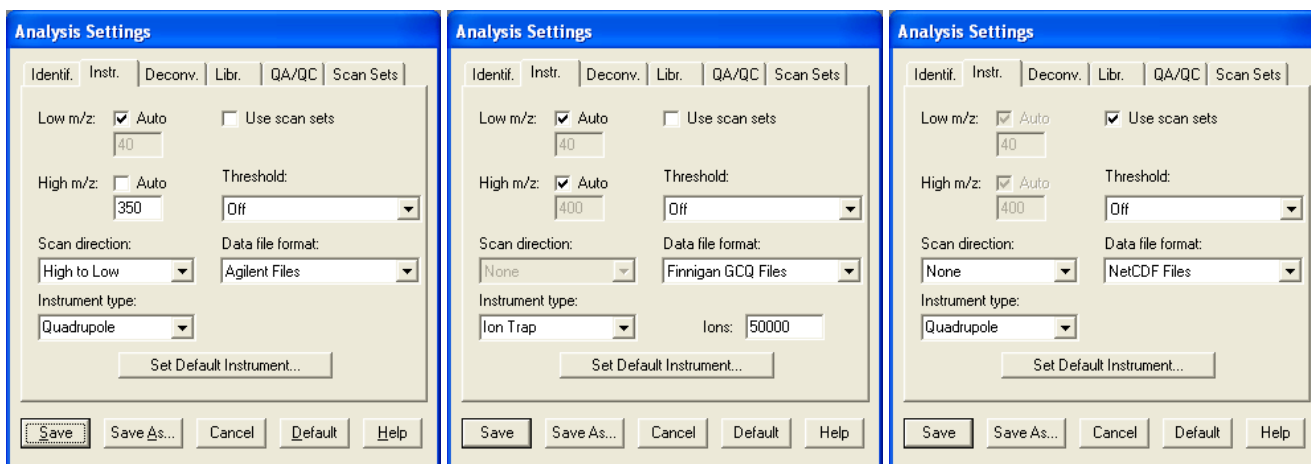
Figure 122 - Instrument tab

Low m/z...   Default = 40 (disabled)

...Auto   Default is selected

These are only enabled and applicable when **Use scan sets** is clear.

The **Low m/z** value should correspond to the low m/z instrument setting used when acquiring the type of data file being analyzed. However, if the **Auto** checkbox is selected, the **Low m/z** value is disabled and the analysis will automatically select the minimum m/z value found in the data.

If **Auto** is clear and you select a specific **Low m/z** value that is significantly higher than the experimental value, you can cause the analysis to improperly correct for the scan bias. In any case the difference will be reported in the **Results** section of the **QA/QC** tab in the *AMDIS Results* dialog (see also **5.5.1 Results**).
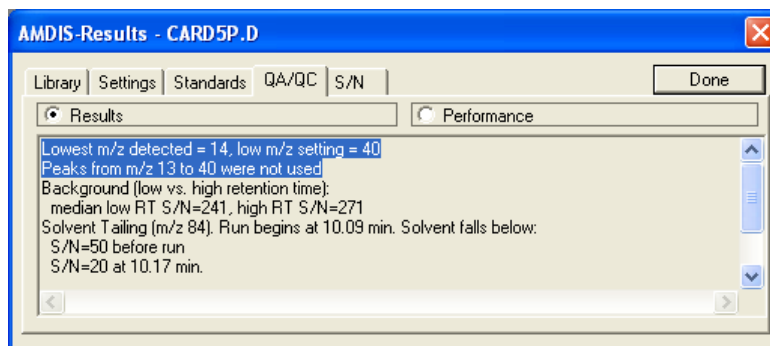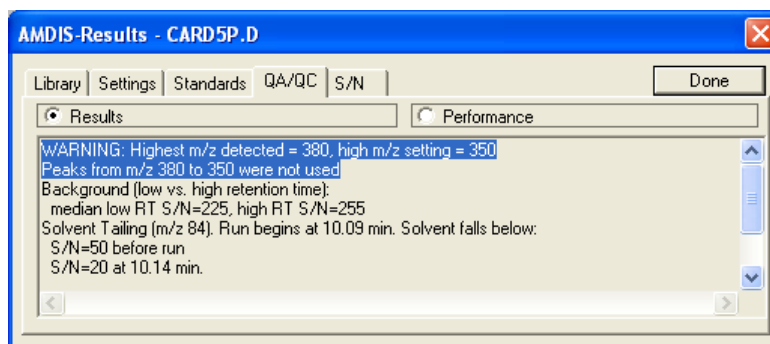
Figure 123 - Low m/z setting too high

High m/z...   Default = 400 (disabled)

...Auto   Default is selected

These are only enabled and applicable when **Use scan sets** is clear.

The **High m/z** value should correspond to the high m/z instrument setting used when acquiring the type of data file being analyzed. However, if the **Auto** checkbox is selected, the **High m/z** value is disabled and the analysis will automatically select the maximum m/z value found in the data.

If **Auto** is clear and you select a specific **High m/z** value that is significantly lower than the experimental value, you can cause the analysis to improperly correct for the scan bias. In any case the difference will be reported in the **Results** section of the **QA/QC** tab in the *AMDIS Results* dialog (see also **5.5.1 Results**).

Figure 124 - High m/z setting too low

Additionally, when you do an analysis the *Maximum m/z Mismatch* warning dialog will be presented giving you the opportunity to correct the problem.
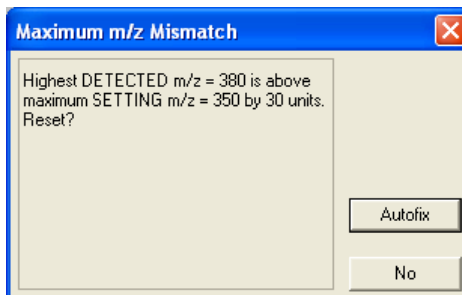
Figure 125 - Maximum m/z Mismatch warning

Click **No** to leave **High m/z** as it is and proceed. Otherwise, click **Autofix** to automatically set **High m/z** to the detected value and then use it.

Use scan sets   Default is clear

When **Use scan sets** is clear, the **Low m/z**, **High m/z** and associated **Auto** settings (see above) are used to determine the m/z range that AMDIS uses for its analysis.

When **Use scan sets** is selected, the **Low m/z**, **High m/z** and **Auto** settings are disabled and AMDIS utilizes the settings on the **Scan Set** tab for analysis purposes (**6.3.6 Scan Sets Tab**).

Threshold   Default = Off

This specifies how the signal threshold value is determined during an analysis. It has values of **Off**, **Low**, **Medium** and **High**.

With the default value of **Off**, the analysis will use the signal threshold value stored in the data file; and this normally works well.

However, in some cases there may be a benefit to setting a different value. For example, in especially noisy chromatograms setting the threshold higher can reduce the effect of noise and so may speed up processing and also might affect the number of components found.

By selecting **Low**, **Medium** or **High**, the automatically determined noise level will be utilized. For **Low** the signal threshold will then be set slightly lower than the determined noise level. For **Medium** it will be set at or near to the noise level and for **High** it will be a little above.

Scan direction   Default is determined by **Data file format**

The scan direction, or lack of, in which the data file's peaks were acquired is utilized by the AMDIS analysis process. It has values of **None**, **Low to High** and **High to Low**.

Since the direction is often instrument dependent, it is coupled to the **Data file format** selection and so will change as that changes. Further, if **Instrument type** is **Ion Trap**, it is disabled and shows **None**.

Having selected a **Data file format**, you would only normally change **Scan direction** away from its default, if that default is incorrect for the actual data file being analyzed.

If not set to **None**, its value determines how the spectrum deskew process adjusts time-to-ion positions within a scan. If set to **None**, there is no adjustment. Since deskewing can change the relative position of peaks and their intensities somewhat, and can also change which ions maximize together, it is capable of having an impact on the number of components determined.

Data file format   Default is determined using the **Set Default Instrument** button

This is a list of all of the native file formats that AMDIS can read. At the time of writing these are:

Agilent Files
Agilent MS Engine Files
Bruker Files
Finnigan GCQ Files
Finnigan INCOS Files
Finnigan ITDS Files
INFICON Files
JEOL/Schrader File
Kratos Mach3 File
MassLynx NT File
Micromass Files
mzXML/mzData Files
NetCDF Files
PerkinElmer Files
Shimadzu MS Files
Schrader/GCMate File
Varian MS Files
Varian SMS File
Varian XMS File
Xcalibur Raw File

Note: NetCDF is an industry standard exchange format and not a specific instrument company file format.

Note: The presence of these file formats is not an endorsement by NIST of the instruments, nor is the omission of a file format to be seen as criticism. NIST seeks to have all file formats in AMDIS.

In some cases software from the instrument manufacturer must be present on the computer for AMDIS to read and process a data file.

The **Data file format** selection is simply used to set the default for **Instrument type** and so indirectly for **Scan direction** and **Ions**.

Instrument type   Default is determined by **Data file format**

The default type of instrument is determined by the selection of **Data file format** and so **Instrument type** may change value as a different **Data file format** is selected. It has values **Quadrupole**, **Ion Trap**, **Magnetic Sector** and **SIM**.

Since its setting is significant for an AMDIS analysis and since in some cases the default selection may be inappropriate for the current data file, it should be changed, if it is not appropriate; otherwise, you should not normally change it.

If **Ion Trap** is selected, the **Ions** parameter appears and **Scan direction** is disabled; and shows **None**.

Ions   Default = 50000

This only appears if **Instrument type** is set to **Ion Trap** and is the count of ions collected to form a mass spectrum.

Note: When it appears, **Scan direction** is disabled; and shows **None**

Set Default Instrument

The **Set Default Instrument** button invokes the *Set Default Instrument* dialog.
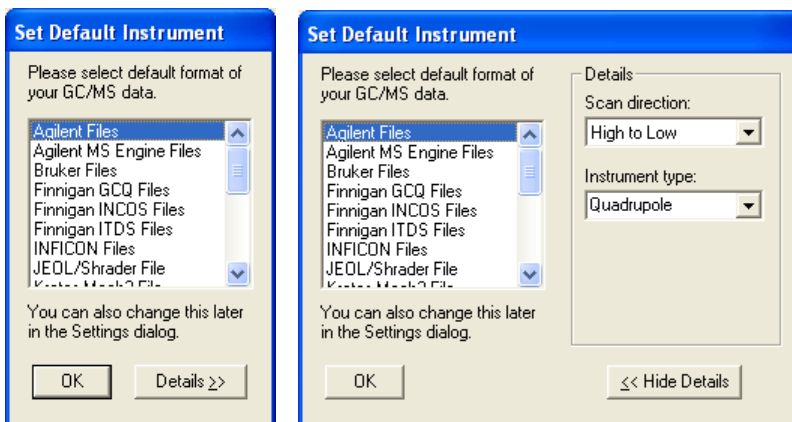
Figure 126 - Set Default Instrument dialog

Click the **Details >>** button to expand the dialog to the right and **<< Hide Details** to collapse it back.

When expanded, the **Scan direction** and **Instrument type** parameters are shown and these correspond to the **Instr.** tab's parameters of the same name, but have the default values for those parameters, as opposed to the values those parameters currently hold on the **Instr.** tab when entering this dialog. The same is true for the **Please select default format of your GC/MS data** selection in this dialog, which corresponds to the **Instr.** tab's **Data file format** parameter's default value.

It is important to understand that the values you select here (and confirm using the **OK** button) are the values used to set the **Instr.** tab's corresponding parameters but only when a new data file is opened; thereby overriding the existing values if they had been changed from their defaults.

6.3.3   Deconv. (Deconvolution) Tab

The **Deconv.** (Deconvolution) tab allows you to set parameters relating to the deconvolution process.
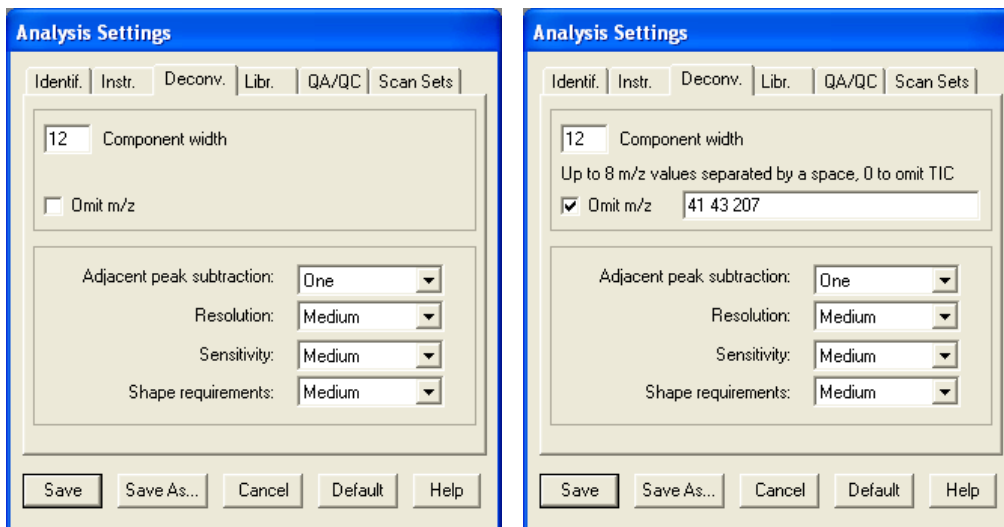

Figure 127 - Deconv. tab

Component width   Default = 12

This is the width that a component is assumed to be and is expressed in terms of mass spectral scans. If you are analyzing data with very wide peaks, you may want to increase the default value, or if all of your data are very narrow, you may wish to decrease it slightly. In general, larger settings will slightly slow down the analysis, but the effect is small. In any case the analysis will automatically adjust for wider peaks to some degree.

Omit m/z...   Default = clear

... Up to 8 m/z values separated by a space, 0 to omit TIC   Default = <blank>

If **Omit m/z** is selected, the **Up to 8 m/z values separated by a space, 0 to omit TIC** parameter appears. Any m/z values specified for it should be entered as integers, separated by a space, and will be ignored for consideration as a "model peak" when determining what constitutes a chromatographic peak. However, m/z values will be considered when the analysis process extracts a spectrum and so the m/z peak may end up in the extracted spectrum.

Since the use of the total ion current (TIC) as a model can be confusing for a very complex data file, it is also possible to ignore the TIC as a model peak. To do this, specify an m/z value of **0** (zero) as one of the 8 values.

> **Note:**   Since the TIC will often yield components when no single ion will provide a model, if you omit the use of the TIC for models, you lose the ability to extract these small peaks.

Adjacent peak subtraction   Default = One

This sets the maximum degree to which "interfering" ions may be subtracted in the analysis to yield additional model peaks for the current component. It has values **Two**, **One** and **None**. The analysis algorithm determines the appropriateness of performing any subtraction.

> **Note:**   An "interf ering" ion is one whose abundance maximizes nearby.

In general you would only use **None** when the chromatography is extremely "clean" and **Two** when it is very "congested".

If **None**, there will be no subtractions and so this yields the fastest processing.

The default value of **One** considers subtracting the most appropriate interfering ion; looking either side of the primary ion. Since the primary ion and TIC of the current peak are both potentially valid models, a maximum of 3 models is possible following subtraction; the original 2 models, plus one subtraction from the primary ion.

For example, in the figure primary ion 231 has interfering ion 235 subtracted from it. Together with TIC, the maximum of 3 models are found.
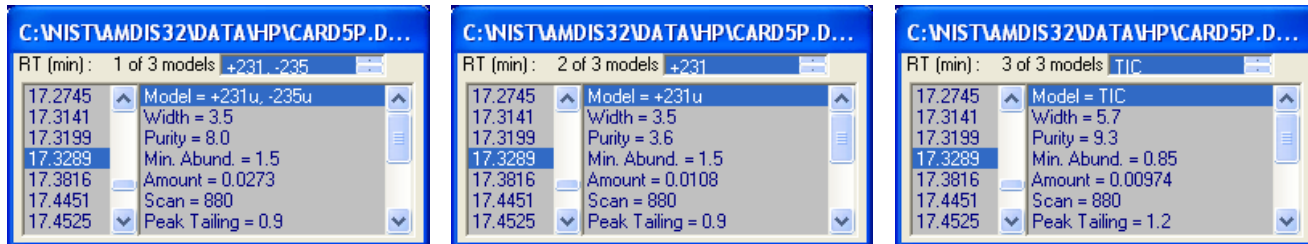

Figure 128 - Adjacent peak subtraction = 1

The value **Two** permits the subtraction of one or two interfering ions; looking either side of the primary ion and looking a little further away than when the value is set to **One**. If only a single interfering ion is found, the results are as described above for when **Adjacent peak subtraction** is set to **One**; a maximum of 3 models being reported.

However, if a second interfering ion is found, it will also be subtracted from the primary ion by itself to yield a model and then both interfering ions will be subtracted together from the primary ion to form another model. This yields 4 results and if TIC was also a primary model, the maximum of 5 models are reported.

For example, in the figure primary ion 231 has interfering ions 164 and 235 each subtracted individually and then in combination. Together with TIC, the maximum of 5 models are found and reported.
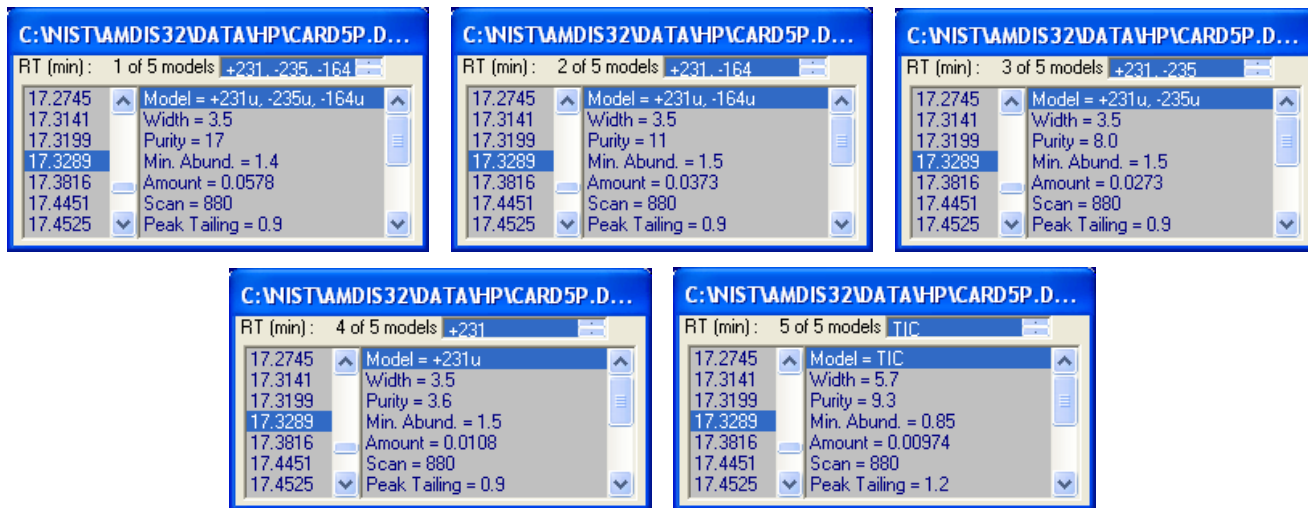
Figure 129 - Adjacent peak subtraction = 2

In all cases multiple models are ranked with the best fit being model 1.

Resolution   Default = Medium

This determines how the analysis algorithm considers the chromatographic separation of peaks. It has values of **High**, **Medium** and **Low**.

As the **Resolution** value goes up (from **Low** to **High**), the algorithm separates peaks that are closer together and so will generally increase the number of components found; and also takes slightly longer to run.

Sensitivity   Default = Medium

This determines how the analysis algorithm considers chromatographic peak width and noise. It has values of **Very High**, **High**, **Medium**, **Low** and **Very Low**.

As the **Sensitivity** value increases (from **Very Low** to **Very High**), increasingly noisy and broad peaks are determined at the expense of analysis time and an increased risk of false positives; smaller multiples of the noise factor are accepted as distinct peaks and the number of components found increases.

Shape requirements   Default = Medium

This determines how the analysis algorithm considers peak shape and is a way of forcing all of the deconvoluted peaks to more closely have the same shape. It has values of **High**, **Medium** and **Low**.

As the **Shape requirements** value goes up (from **Low** to **High**), the shape of the individual ions must be more nearly the same and so will generally tend to reduce the number of components found. The value does not have a significant effect on analysis time.

6.3.4   Libr. (Libraries) Tab

The **Libr.** tab allows you to select and view the various library types relating to an analysis.
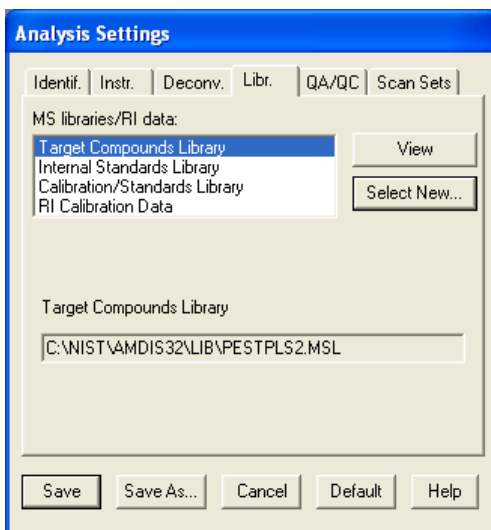
Figure 130 - Libraries tab

The operation and use of this tab is fully explained for the **Analyze | Analyze GC/MS Data** command (**3.1.2.1 Analyze | Analyze GC/MS Data**).

6.3.5   QA/QC (Quality Control) Tab

The **QA/QC** (Quality Control) tab allows you to set parameters that are used in checking for the proper running of the instrument.
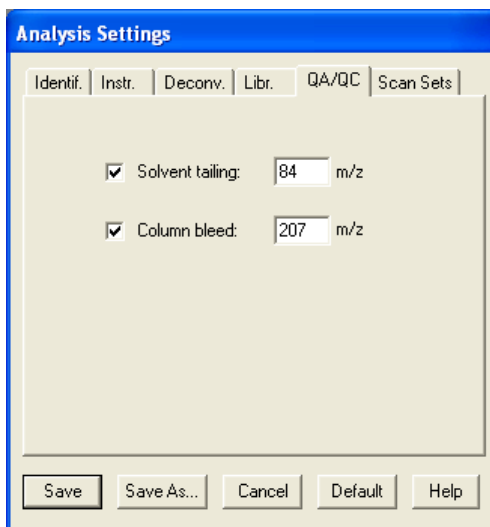
Figure 131 - QA/QC tab

While many of the parameters that are determined for each component or target can be generally used for quality control, there are two areas with particular significance for QA/QC. These concern the degree of solvent tailing and the amount of column bleed. Both of these parameters are assessed by the use of characteristic ions.

Solvent tailing...   Default is selected

...m/z   Default = 84

   m/z is only enabled if **Solvent tailing** is selected and the selected **m/z** value should either be the principal ion, or a unique ion, of the solvent.

   During an analysis various signal-to-noise (S/N) values are computed and reported in the the **Results** section of the **QA/QC** tab in the *AMDIS Results* dialog (see also **5.5.1 Results**).
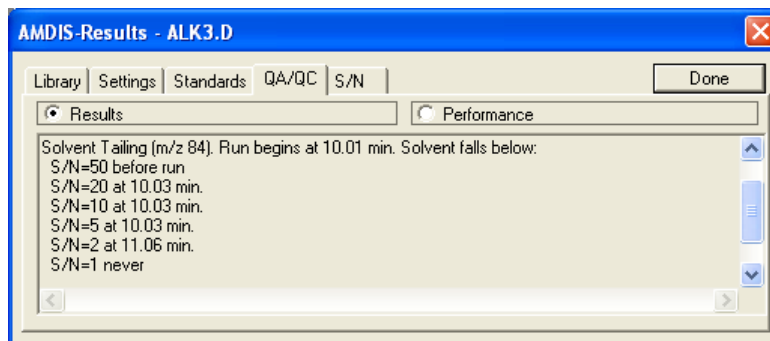

Figure 132 - Signal-to-noise (S/N) determinations

   The header line states when the run begins (ie, the end of the "solvent delay" period) and subsequent lines give the time at which the **m/z** ion's signal falls below a series of S/N values.

   The reported S/N values are given in one or more "10, 5, 2, 1" sequences and are reported until either the S/N goes below 1, or the chromatogram ends. For example (refer to **Figure 132 - Signal-to-noise (S/N) determinations**), if the first point detected has the solvent peak at an S/N of between 20 and 50, the time at which it falls below 20, 10, 5, 2 and 1 will be reported. The fact that the S/N of the solvent peak fell below 50 before the run will also be noted.

   Note: The definition of "falling below a level" requires that the signal be below that level for at least two successive scans.

Column bleed...   Default is selected

...m/z   Default = 207

m/z is only enabled if **Column bleed** is selected and the selected **m/z** value should be that of the characteristic ion of the decomposition of the column. The default value of 207 is chosen, since this is a characteristic decomposition ion of many of the common GC columns.

Two median values are calculated in S/N units and represent the average over the first half of the run (**low RT S/N**) and the average over the last 10% (**high RT S/N**). They are reported in the the **Results** section of the **QA/QC** tab in the *AMDIS Results* dialog (see also **5.5.1 Results**).
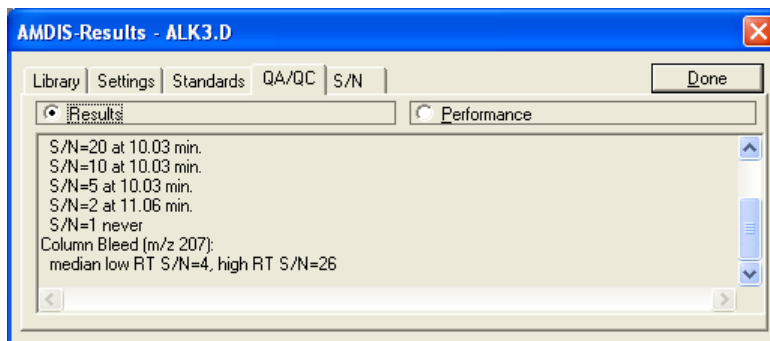
Figure 133 - Column bleed determinations

Very high column bleed would be indicated by high values of the **high RT S/N** value.

6.3.6   Scan Sets Tab

For those instruments that can acquire data files using different m/z ranges in consecutive retention time ranges during the run, the **Scan Sets** tab allows you to explicitly specify those "scan set" values such that the AMDIS analysis algorithm will function most effectively for that particular file.

Since the use of scan sets removes the need for the single **Low m/z** and **High m/z** values and their associated **Auto** checkboxes (**6.3.2 Instr. (Instrument) Tab**), the two approaches are mutually exclusive. As such, the **Use scan sets** checkbox on the **Instr.** tab should be selected to enable the use of scan sets; and cleared otherwise.

Each scan set has a **Start** and **End** retention time and **Low m/z** and **High m/z** values to specify the m/z range to be used for analysis purposes during that retention time. The **Number of sets** to be used must also be specified.
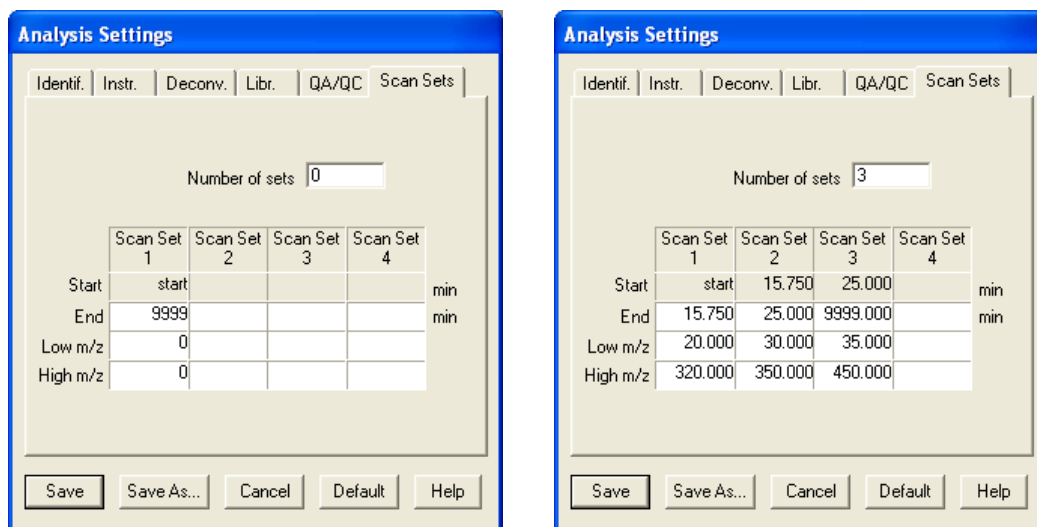
Figure 134 - Scan Sets tab

To initialize the tab ready for a new set of values, set **Number of sets** to **0** and click **Save**. Now reenter the *Analysis Settings* dialog and the **Scan Sets** tab will be in its initialized state (**Figure 134 - Scan Sets tab**); showing **Scan Set 1** having a **Start** retention time as **start**, an **End** retention time as **9999** and with **Low m/z** and **High m/z** both set to **0**.

Values for each **Scan Set n** should be entered in sequence, since the **Start** value for the next one is automatically set from the **End** value of the current one. **End** has a maximum value of **9999** and this value will be automatically set for the last scan set's **End** value when you finally **Save** the settings.

When you have defined the scan sets, remember to set **Number of sets** accordingly as this is not automatically determined. Finally click **Save** to make the settings permanent. This also has the effect of producing a consistent formatting for the values you have assigned; for example, **Figure 134 - Scan Sets tab** shows 3 scan sets and their formatting.

6.3.7   Filter Tab

The Filter Tab allows you set limits on the components that the program will report which do not match the target library.

Once **Enable Filters** has been checked you have the option of setting filters based on the **Number of Model Peaks** that the program extracts for a component, the **Signal to Noise (S/N)** level of a component, **the Fraction of Certain Peaks** (as opposed to uncertain peaks), the **Minimum Abundance** extractable in a spectrum (relative to the base peak of 100) and **Minimum Signal Strength** (area) of the peak.

The **Number of Model Peaks** increases as the number of ions that can be clearly deconvoluted increases, for some very simple spectra there are only a few model peaks even when the spectra are well resolved. However, for most data having fewer than 3-5 model peaks is a strong indication of weak or poorly defined extractions of the component.

The minimum **Signal to Noise ratio S/N** is a measure of the overall noise background and the total size of the peak.

The fraction of **Certain Peaks** (indicated in the AMDIS output as Frac. Good – fraction good) is a measure of how well the deconvolution can be done. If peaks are poorly resolved either due to noise or abundant impurities, then some ions will be uncertain (dotted in the AMDIS display). If the fraction of the total extracted ion current is too large then the confidence in the spectra and area of the component is reduced.

The **Minimum Abundance** is also related to the S/N of the data, but it is a measure of the smallest peak (relative to a base peak of 100) that can be statistically extracted. Note that smaller peaks are sometimes extracted but the confidence in the peaks is lower.

Finally, **Signal Strength** is the value of total ion current extracted. This can be high in a noisy background and not yield good spectra or low in a very quite region and yield very good spectra. It is not suggested that this

parameter be used, but it has been included to aid in processing for cases where the size of the absolute signal is critical.

Note: Except for the last item all of these items are not directly dependent upon the total signal. However, the values for the signal strength are instrument and experimental concentration dependent. The other variables are dependent on S/N and thus typically independent of the instrument.

Typically these filters are used for complex data files such as metabolomic, natural product, urine, or plasma studies where the signals cannot always be fully resolved. The filter set works by assigning weights to each of the parameters, in the example above, a weight of 1 is assigned to each of 3 parameters, and a total weight of 3 must be achieved for a component to be accepted.   For each of the weighting parameters, the full weight is achieved at the limit, and for each of the parameters the maximum weight (regardless of the value of the parameter) is twice the weight.   For all parameters the value of the weight is calculated as:

Weight = Weight Factor * (Parameter value/Limit value)^2 for Parameter value < Limit value

Weight =Weight Factor* MAX (2,$\sqrt{}$ (Parameter value/Limit value)) for Parameter value > Limit value

The sum of the net weights must be greater than the Weight Limit or the component will not be reported. Using the parameters shown above on a typical data file of TMS derivatized urine, the number o f components found was 213 (120 hits), if no filter was used the number of components was 285 (again 120 hits), and if the S/N ratio was doubled to 40, the number of components was 194 (again 120 hits). The constancy of the number of hits is a consequence of the filters being applied after the library matches are done. Thus the targets will not be affected by these changes.

It is also possible to exclude a peak if it does not meet the criteria. To do this the "**Exclude if Below**" box is checked. When this is done if the component fails on the criteria for a particular parameter, the component is rejected even if the other criteria would have given sufficient weight to keep the component.

The default condition is to have filtering off.

It is essential that if you use filtering you examine your own data carefully to see what filters provide the best results for your experiments. It is suggested that you examine a small portion of a "typical" data file and see the effect that filtering has on the outcome of the analysis.

7 Post Analysis Classification of Spectra

Under the **Analyze** menu in the *Confirm* window there are two post analysis options; **Postprocess** and **Structural Classifiers** (**3.1.2.3 Analyze | Postprocess** and **3.1.2.4 Analyze | Structural Classifiers**).

Both of these allow you to go beyond target analysis, and even beyond using the entire NIST mass spectral database to analyze the data. They allow classification of the individual components by chemical classes.

They are only available for a datafile that has already been analyzed in the standard AMDIS manner (for example, using **3.1.2.1 Analyze | Analyze GC/MS Data**) and always process all of the components resulting from that analysis; irrespective of the current display range.

The **Postprocess** command is fully described in **7.1 Postprocess** and the **Structural Classifiers** command in **7.2 Structural Classifiers**.

> **Note:** These facilities are not available in the *Results* window.

7.1 Postprocess

When you already have results (components) from a standard AMDIS analysis, the **Analyze | Postprocess** command is available in the *Confirm* window and will invoke the *Postprocess Spectra* dialog.
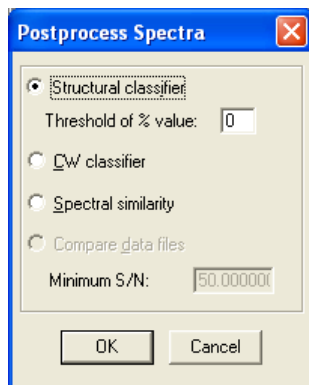
Figure 135 - Postprocess Spectra dialog

This allows you to further analyze the existing components with three distinct classifiers and to optionally compare two separately analyzed files:

Structural classifier	see **7.1.1 Structural Classifier**;
CW classifier	see **7.1.2 CW Classifier (Chemical Weapons Related Classifiers)**;
Spectral similarity	see **7.1.3 Spectral Similarity**;
Compare data files	see **7.1.4 Compare Data Files**.

In brief, **Structural classifier** and **CW classifier** classify components according to the probable functional groups within them.

Spectral similarity and **Compare data files** classify components into groups having similar spectra; where **Compare data files** operates across 2 data files.

> **Note:** **Compare data files** will only be enabled when you are displaying the results of 2 analyzed files in the same window (**3.4.2 Two Chromatograms in One Window**).

Click a radio button to select the required process operation. If applicable, specify the associated parameter's value and then click **OK** to start the processing.

> **Note:** All post-process operations analyze all existing components, irrespective of the currently displayed retention time range.

Once the processing is complete, a drop-down list box appears to the right of the button bar at the top of the *Confirm* window. You can use this to view the components in each class (**7.1.5 Viewing Classes**).

7.1.1   Structural Classifier

The Structural Classifer analysis technique classifies components according to the probable functional groups within them. See Chapter **12 Compound Classification Analysis** for a definition of the groups.

Having already done a standard AMDIS analysis, select **Structural classifier** in the *Postprocess Spectra* dialog (**7.1 Postprocess**), specify a **Threshold of % value** and click **OK**.

When you now view components in the *Information Lists*, **Class** = information is shown at the bottom of the component's information in the right pane and is usually spread over several lines.
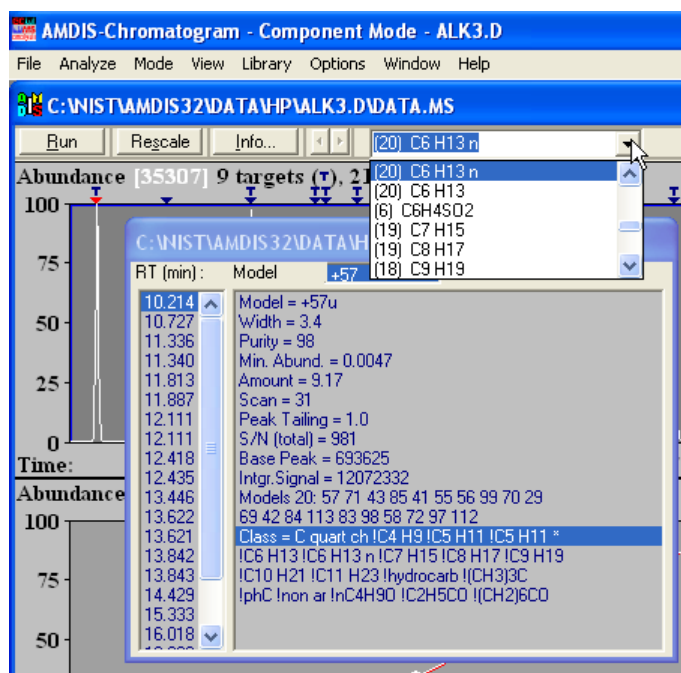
Figure 136 - Structural classifiers processing

You can also see a drop-down list box that has appeared to the right of the button bar at the top of the *Confirm* window.

For an explanation of the **Class** information and how you use the pull down selector to view the components in each class see **7.1.5 Viewing Classes**.

The **Threshold of % value** controls the degree of confidence of the results. As you set it lower, it allows additional results with less confidence to be reported.

7.1.2   CW Classifier (Chemical Weapons Related Classifiers)

There are certain specific patterns that are characteristic of many of the chemical weapons and related compounds covered under the Chemical Weapons Convention. These are classified here using a very specific set of algorithms developed at NIST by Boyu Yang and Steve Stein.

The CW Classifier analysis technique classifies components according to the probable functional groups within them.

Having already done a standard AMDIS analysis, select **CW classifier** in the *Postprocess Spectra* dialog (**7.1 Postprocess**) and click **OK**.

When you now view components in the *Information Lists*, **Class** = information is shown at the bottom of the component's information in the right pane and and shows the specific class type.
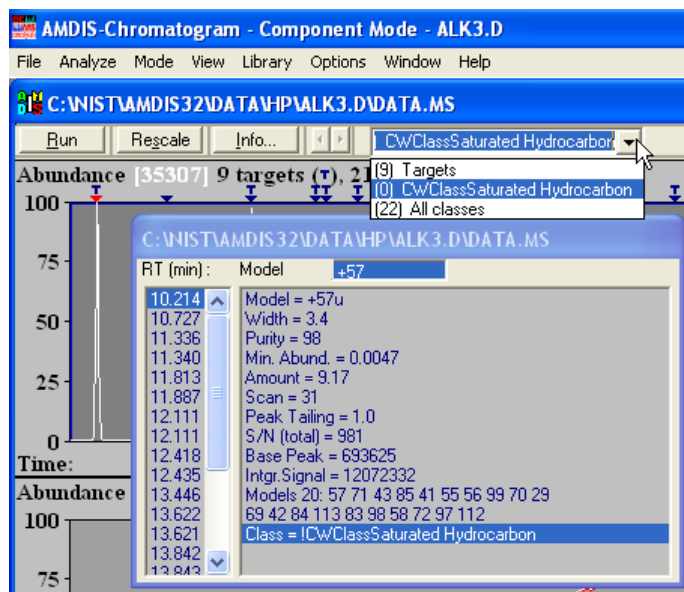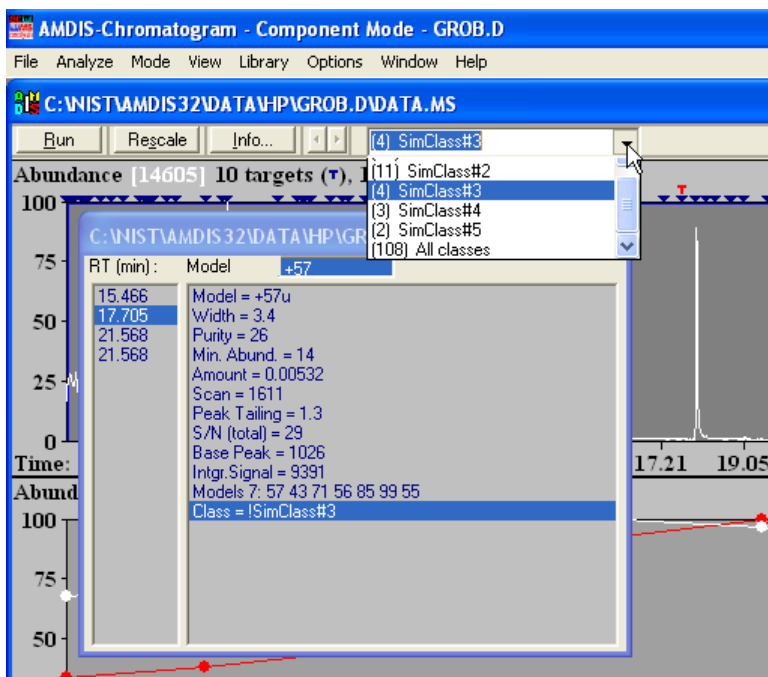
Figure 137 - CW classifers processing

You can also see a drop-down list box that has appeared to the right of the button bar at the top of the *Confirm* window.

For an explanation of the **Class** information and how you use the pull down selector to view the components in each class see **7.1.5 Viewing Classes**.

7.1.3   Spectral Similarity

The CW Classifier analysis technique is a classification of all sets of components within a GC/MS data file for which the extracted spectra are similar. The definition of similarity is that the match factors between the spectra within a GC/MS data file are sufficiently high. In most cases these compounds will be related structurally.

Having already done a standard AMDIS analysis, select **Spectral similarity** in the *Postprocess Spectra* dialog (**7.1 Postprocess**) and click **OK**.

When you now view components in the *Information Lists*, **Class** = information is shown at the bottom of the component's information in the right pane and shows the specific class type.
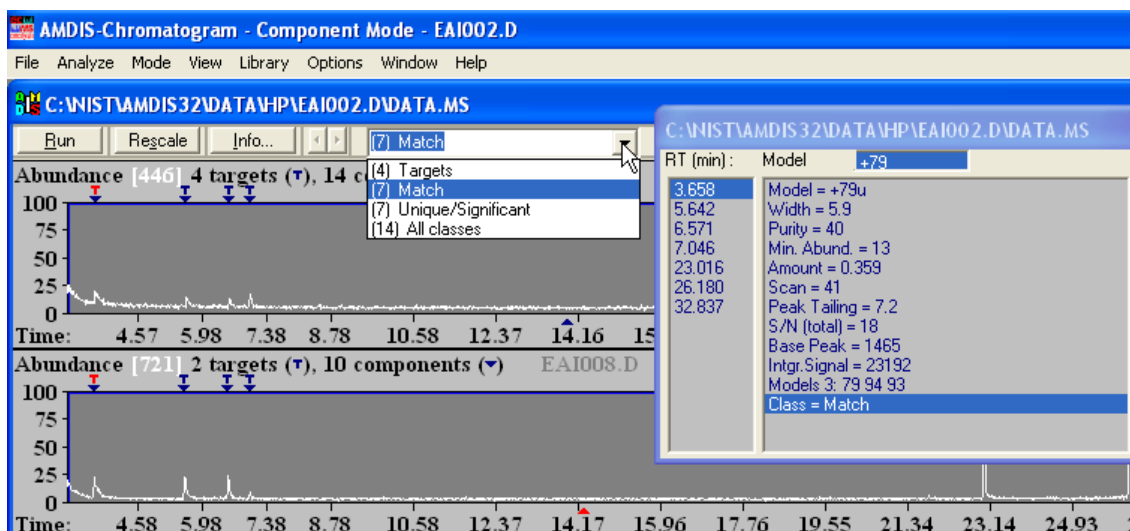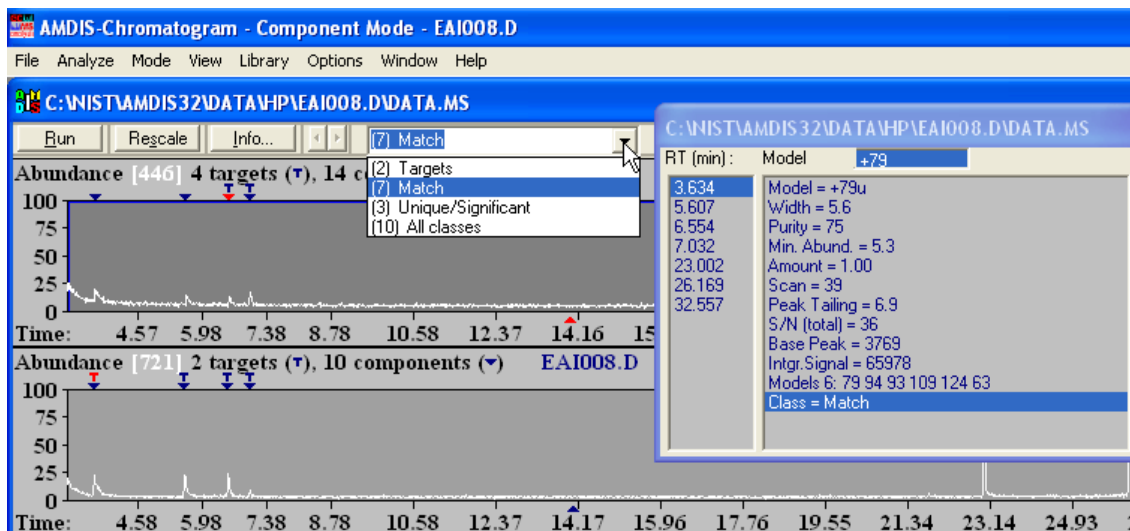

Figure 138 - Spectral similarity processing

You can also see a drop-down list box that has appeared to the right of the button bar at the top of the *Confirm* window.

For an explanation of the **Class** information and how you use the pull down selector to view the components in each class see **7.1.5 Viewing Classes**.

7.1.4   Compare Data Files

The Compare Data Files analysis technique is designed to compare two similar GC/MS data files. For this comparison to be active, it is first necessary to load two data files into a single window (for example, using **File | Open In | Active Window** for the second file). Then each file should be separately analyzed (for example, using **3.1.2.1 Analyze | Analyze GC/MS Data**).

At this point the **Compare data files** option is active in the *Postprocess Spectra* dialog (**7.1 Postprocess**). Select it, and note that **Minimum S/N** becomes enabled.

Since many very small, non-matching components may be observed in two otherwise nearly identical files, the **Minimum S/N** parameter is a threshold value, below which such weak components will be separately labeled (as **Unique/Trace**). Non-matching components with a **S/N (total)** value at or above this threshold will also be labeled distinctly as **Unique/Significant**. See **3.3.3.1 Component Mode Details** for **S/N (total)**.

Specify a **Minimum S/N** to suit your needs and then click **OK**

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

Whether or not two components in different files match depends on both their spectral similarity and their relative retention times. The same component is considered to be present in both files when the following expression is greater than 0.6

$$5 * MF / (5 + |RT1 - RT2|)$$

where MF is the spectral similarity "match factor" (expressed as 1 for a perfect match) and RT1 and RT2 are the retention times in seconds in the two data files. From this you can see that components with a perfect spectral match must be less than 3.33 seconds apart to be considered as a component class of **Match**; and need to be closer together as MF decreases (for example, less than 2.5 seconds when MF = 0.9).

When you now view components in the *Information Lists*, **Class** = information is shown at the bottom of the component's information in the right pane and and shows the specific class type.

Figure 139 - Compare data files processing - file 1

Figure 140 - Compare data files processing - file 2

You can also see a drop-down list box that has appeared to the right of the button bar at the top of the *Confirm* window. Each data file has its own set of class information and clicking anywhere within its specific display area will cause its values to be displayed.

> **Note:**   You can use **View | Same Time Scale** to conveniently compare data files.

For an explanation of the **Class** information and how you use the drop-down list box to view the components in each class see **7.1.5 Viewing Classes**.

7.1.5   Viewing Classes

After clicking **OK** in the *Postprocess Spectra* dialog (**7.1 Postprocess**) and when processing is complete, a drop-down list box appears to the right of the button bar at the top of the *Confirm* window.
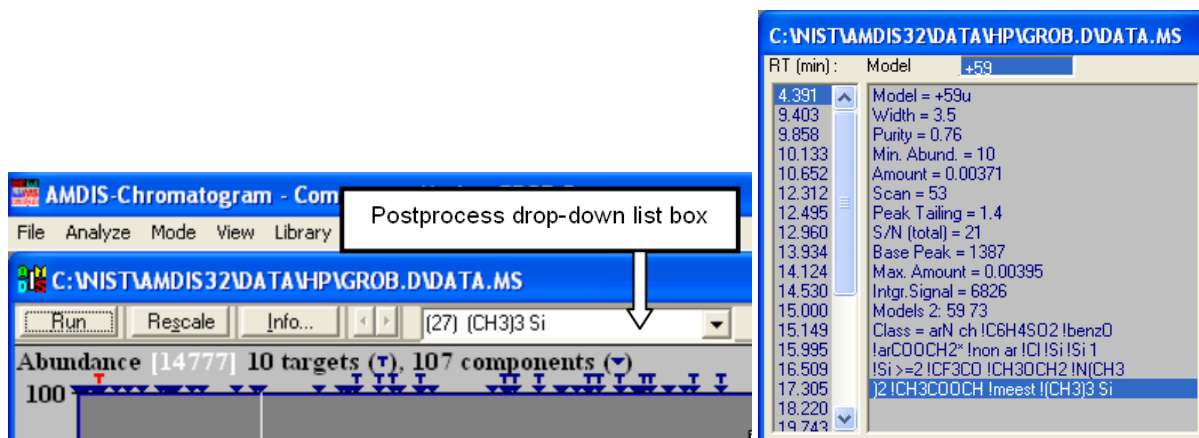

Figure 141 - Postprocess drop-down list box

If there are 2 data files in the *Chromatogram Display* area, the list box will be for the active data file (**3.4.2 Two Chromatograms in One Window**).

The type of contents of the list box will vary according to the actual Postprocess analysis type selected, but in all cases the are handled in the same manner. Each selection is of the general form:

(n) type

where:
> **type**   is a category of like compounds
> **(n)**   shows how many compounds of category **type** have resulted from the analysis

Irrespective of the particular selection made, the *Chromatogram Display* area's header always shows the number of targets and components resulting from the original analysis (for example, **10** and **107** respectively in **Figure 141 - Postprocess drop-down list box**). The number of component symbols (▼) is also the same (and so 107 here), but the number of **T** symbols changes to reflect the specific selection (and so there will be 27 in this case).

If you click on a particular **T** or ▼, the *Information Lists* area shows its details. However, unlike after a standard analysis the information shown for a **T** or ▼ is exactly the same and is the component's standard information with additional details of its **Class** (**Figure 141 - Postprocess drop-down list box**). If a component has multiple classes, each class type is separated by an exclamation mark (!).

In all cases the first listed type is always **(n) Targets** and is the number of targets identified by the standard analysis. If you select this and then click on a **T** symbol, the drop-down list box disappears and the *Information Lists* area shows target information in the normal manner. Simply click on a ▼ symbol to restore the drop-down list box.

In a similar manner the last in the list is always **(n) All classes** and is the total number of components detected by the standard analysis.

The other specific types of **type** depend on the Postprocess analysis type used:

> **Structural classifier**   see **7.1.5.1 Types for Structural Classifier**;
>
> **CW classifier**   see **7.1.5.2 Types for CW Classifier**;
>
> **Spectral similarity**   see **7.1.5.3 Types for Spectral Similarity**;
>
> **Compare data files**   see **7.1.5.4 Types for Compare Data Files**.

and are detailed in the following sections.

7.1.5.1   Types for Structural Classifier

The drop-down list box for the Structural Classifer analysis technique (**7.1.1 Structural Classifier**) shows all of the distinct Varmuza classifiers identified by considering all of the data file's components. If a particular classifier has not been identified, it is absent from the list.

7.1.5.2   Types for CW Classifier

The drop-down list box for the CW Classifer analysis technique (**7.1.2 CW Classifier (Chemical Weapons Related Classifiers)**) shows all of the distinct CW classifiers identified by considering all of the data file's components. If a particular classifier has not been identified, it is absent from the list.

7.1.5.3   Types for Spectral Similarity

The drop-down list box for the Spectral Similarity analysis technique (**7.1.3 Spectral Similarity**) shows all of the distinct spectral similarity groupings identified by considering all of the data file's components. Groupings are labeled **SimClass#n**; where **n** starts at **1** and increments by 1.

All of the components within a particular **SimClass#n** will have a common and distinct spectral pattern that distinguishes them from all other components' spectra. If you are analyzing, for example, a clean calibration mixture of normal hydrocarbons, you may well only get a single **SimClass#1**. However, a "dirty" environmental sample will have numerous **SimClass#n** groupings.

7.1.5.4   Types for Compare Data Files

The drop-down list box for the Compare Data Files analysis technique shows the results of comparing all of the components in the currently active data file with all components in the inactive data file and determining if they match, or if they are unique. There is a signal-to-noise parameter you can set to help control this and for a pair of components to be considered a match (one from each file), the two must fall within a relatively narrow retention time window; details of both of these are given in **7.1.3 Spectral Similarity**.

> **Note:**   The currently active *Chromatogram Display* is easily distinguishable by the filename in its title bar being bold, whereas the inactive filename is subdued.
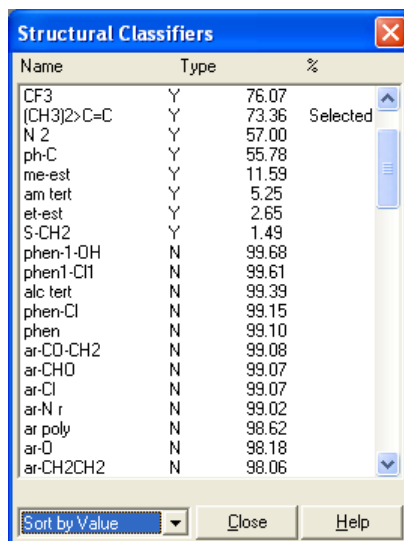
To get comparison results for both data files it is necessary to make each active in turn and perform the Compare Data Files analysis technique on each. Whenever you then make the other file active, the drop-down list box changes to reflect that file's values.

A component will be assigned to one of the following groupings:

Match/Larger   a pair of components match, but one of them is at least 3 times larger than the other;

Match   a pair of components match and neither is 3 times 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 described 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.

7.2 Structural Classifiers

Click **Analyze | Structural Classifiers** (**3.1.2.4 Analyze | Structural Classifiers**) to invoke the *Structural Classifiers* dialog to view the structural classifier values for the current component or target.

Figure 142 - Structural Classifiers dialog

The dialog lists the **Name** of each structural classifier and the percentage probability (**%**) that the classifier is present (**Type = Y**) or not present (**Type = N**) in the component being analyzed.

If you are interested in conveniently examining the values for specific classifiers, you can choose any number of them by double clicking on each in turn; at which point **Selected** appears to the right to show which have been selected. To cancel a selection, double-click on it again.

You can also highlight any number of entries using standard *Windows* multi-select techniques and then right-click to activate the context menu. When you click the single **Save Selection** command, the highlighted entries all show **Selected** and any **Selected** entries outside of this range are deselected.

To take full advantage of the **Selected** feature, you would now sort the list of classifiers to show selected ones at the top (see **Sort by Value** below).

The order in which the list is presented is controlled by the selection in the drop-down list box at the bottom left of the dialog. The list box has the following selections:

No Sort　　　　No specific order.

Sort by Name　　The classifiers are listed in alphabetical order.

Sort by Value　　The classifiers are listed with all **Type = Y** appearing first and then all **Type = N**. Within each **Type**, the classifiers are ordered from high to low **%**.

Sort by Selection　　The classifiers are listed with all **Selected** classifiers appearing first (the **Type = Y** ones preceding those of **Type = N**), all remaining **Type = Y** appearing next and finally all remaining **Type = N**. Each of these 4 classifier groupings is ordered from high to low **%**.

To view the results for another component, simply select it in the *Confirm* window.

Click **Close** to exit from the dialog.

The structural classifiers are a set of classification criteria developed by Prof. Kurt Varmuza and his students at the Technical University Vienna. The details of the method are given in a series of papers (**7.3 Varmuza Classification References**). In brief this set of classifiers looks at peak patterns that are characteristic of specific functional groups (or in some cases small sets of related functional groups).

Chapter **12 Compound Classification Analysis** gives detailed descriptions and chemical structures for these groups.
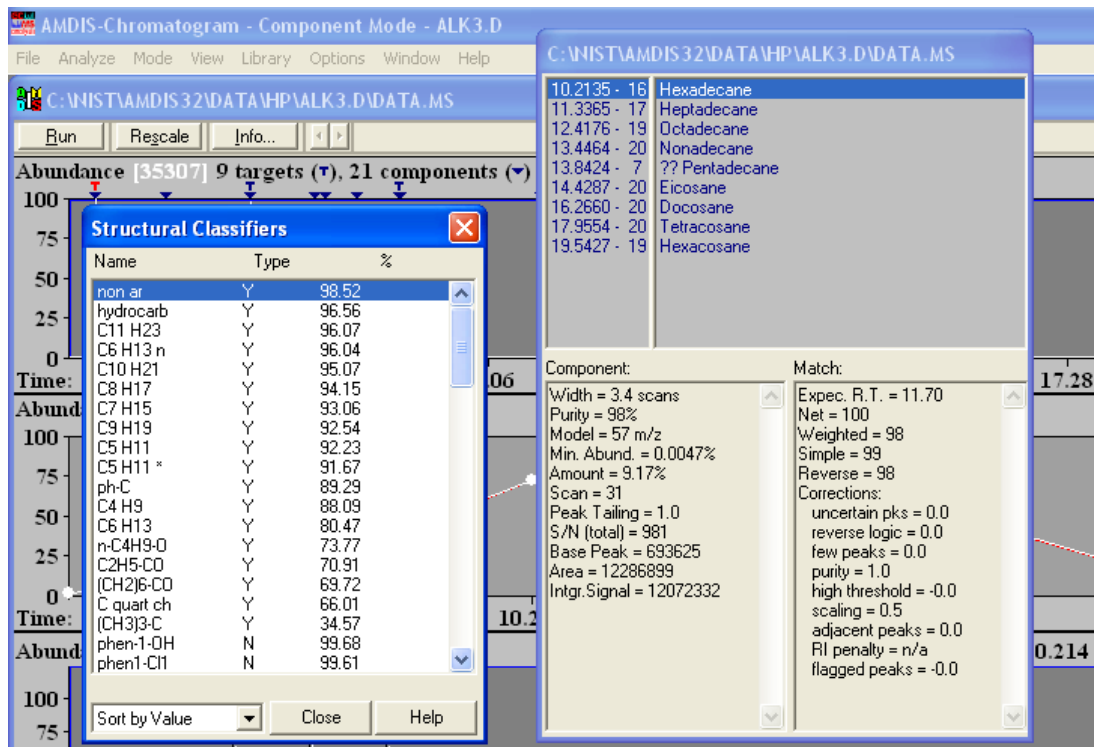
As an example, consider:

Figure 143 - Structural classifiers by value

The classifiers have been sorted by value in the *Structural Classifiers* dialog and the results for the target at 10.2135 minutes, **Hexadecane**, are being shown. You can see that there is a 98.52% chance that this target is **non ar** (non aromatic), 96.56% chance of being **hydrocarb** (a hydrocarbon) and 96.07% chance of containing **C11 H23** (alkyl $C_{11}H_{23}$). Correspondingly, it is definitely not a phenol type compound as it has a 99.68% chance of not being **phen-1-OH** and a 99.61% chance of not being **phen1-Cl1**.

7.3   Varmuza Classification References

Varmuza K., Werther W.: "Mass spectral classifiers for supporting systematic structure elucidation," *J. Chem. Inf. Comput. Sci.*, **36**, 323-333 (1996).

W. Werther, H. Lohninger, F. Tancl and K. Varmuza, Classification of Mass Spectra: A comparison of yes/no classification methods for the recognition of simple structural properties. Chemometrics and Intelligent Laboratory Systems, 22 (1994), 64-76.

H. Scsibrany and K. Varmuza, Common substructures in groups of compounds exhibiting similar mass spectra, Fresenius J. Anal. Chem, 344 (1992), 220-222.

K. Varmuza, W. Werther, D. Henneberg and B. Weimann, Computer-aided Interpretation of Mass Spectra by a Combination of Library Search with Principal Component Analysis. Rapid Communications in Mass Spectrometry, 4 (1990), 159-162.

8   Libraries

AMDIS supports a variety of library types (**8.1 Library Types**) that are developed and maintained using either of two distinct, but similar, library editors (**8.2 Library Editors**). These editors can only be accessed from the **Library** menu in the *Confirm* window.

The first is accessed using **Build One Library** (**8.2.1 Build One Library Editor**) and is designed for building a library from the components in an experimental data file.

The second is accessed using **Library Transfer** (**8.2.2 Library Transfer Editor**) and is primarily designed for building a library from data in other AMDIS libraries, or out of data created from libraries utilized by the NIST *MS Search* program.

> **Note:**   Although the content of AMDIS libraries and of the libraries utilized by the NIST *MS Search* program (ie, the NIST mass spectral database and NIST user libraries) are quite similar, nevertheless they have distinct formats and are not directly interchangeable.

There are other ways in which libraries can be simply viewed, but not edited (**8.6 Viewing a Library**).

If you wish to use retention indices as an additional aid in identifying targets, AMDIS relies on a separate calibration run to create an *RI Calibration Data* file and this utilizes a *Calibration & Standards Library* (**8.3 Using Libraries for AMDIS Calibration Purposes**).

You can use AMDIS to perform a basic concentration calculation by utilizing a suitably prepared *Internal Standards Library* and by selecting the *Use Internal Standards for RI* or *Use RI Calibr. Data + Internal Std.* analysis type (**8.4 Using a Library for Concentration Calculations**).

The Performance Log file is a cumulative record over time of pertinent information that helps you to track the performance of your instrument and so provides some level of quality control. To do this it utilises "performance check compounds" in a *Calibration & Standards Library* (**8.5 Using the Performance Log to Track Instrument Performance**).

8.1   Library Types

There are three different varieties of library, a retention index calibration file and one additional type of file generated by the NIST *MS Search* program, that AMDIS can utilize for an analysis. For the purpose of this documentation they are all considered to be AMDIS "library types":

Target Compounds Library	(MSL)	see **8.1.1 Target Compounds Library (MSL)**;
Calibration & Standards Library	(CSL)	see **8.1.2 Calibration & Standards Library (CSL)**;
Internal Standards Library	(ISL)	see **8.1.3 Internal Standards Library (ISL)**;
RI Calibration Data File	(CAL)	see **8.1.4 RI Calibration Data File (CAL)**;
Mass Spectral Transfer File	(MSP)	see **8.1.5 Mass Spectral Transfer File (MSP)**.

Although each of these five types has many similarities, nevertheless there are differences and these are reflected in the slightly different editor dialogs called from the main library editors to edit individual library entries.

> **Note:**   Library editing facilities are only available in the *Confirm* window.

According to the type of analysis being performed, from one to three different library types are used as input to the analysis process; as shown in the table. In one case only, a library is also created.

	Library Type			
Analysis Type	**MSL / MSP ***	**CSL**	**ISL**	**CAL**
Simple	Yes	-	-	-
RI Calibration/Performance	-	Yes	-	Yes (created)
Use Retention Index Data	Yes	-	-	Yes
Use Internal Standards for RI	Yes	-	Yes	-
Use RI Calibr. Data + Internal Stds.	Yes	-	Yes	Yes
Performance Check for RI	-	Yes	-	Yes

Table 1 - Library types used for an analysis

* Either an MSL library, or an MSP file, can be selected.

All of these library files are actually text files with a straightforward format and are described in Chapter **14 Formats**.

8.1.1   Target Compounds Library (MSL)

A *Target Compounds Library* is utilized in several AMDIS analysis types and is what deconvoluted spectra are compared against to see if there is a match (a "hit"). From this point of view, it is interchangeable with a *Mass Spectral Transfer* file; but the two do have significant differences.

A *Target Compounds Library* holds a list of target compounds' information and associated spectra. Unlike the other libraries, which only have one spectrum per compound entry, this library may have multiple spectra per compound entry. It is also unique in having the ability to store retention index information for multiple column types.

As such, a *Target Compounds Library* is actually stored as two separate files. These have the same "root" name, but different extensions; one being MSL and the other CID. The unique spectral information is held in the MSL file and the common compound information is in the CID file. For example, a perfume library named *PERFUME* would have the spectra in *PERFUME.MSL* and compound information in *PERFUME.CID*.

> **Note:**   You do not really need to know about the different files and their extensions, since AMDIS automatically handles the two files. For convenience, whenever a *Target Compounds Library* is being selected or displayed within AMDIS, it is only the MSL file extension that is shown.

The related spectral and compound information are linked in the two files by a unique chemical identification number (**Chemical ID**). For convenience the Chemical Abstracts Service Registry number (CAS number) is used when available, but any unique value can be used. For example, when deconvoluted spectra are added to a library from a GC/MS data file, they are initially identified by a file name identifier followed by -Nxxxx; for example GROB-N1003.

Structuring the library in this manner ensures that a target compound in the library will only be identified once and, additionally, the spectrum presented for it will be the best spectrum match of all the spectra stored for it; subject of course to it satisfying the standard **Minimum match factor** threshold and any other match criteria.

The spectral and compound information are edited separately. The editors are described in **8.2.5 Spectrum Editor** and **8.2.4 Compound Information Editor**, respectively.
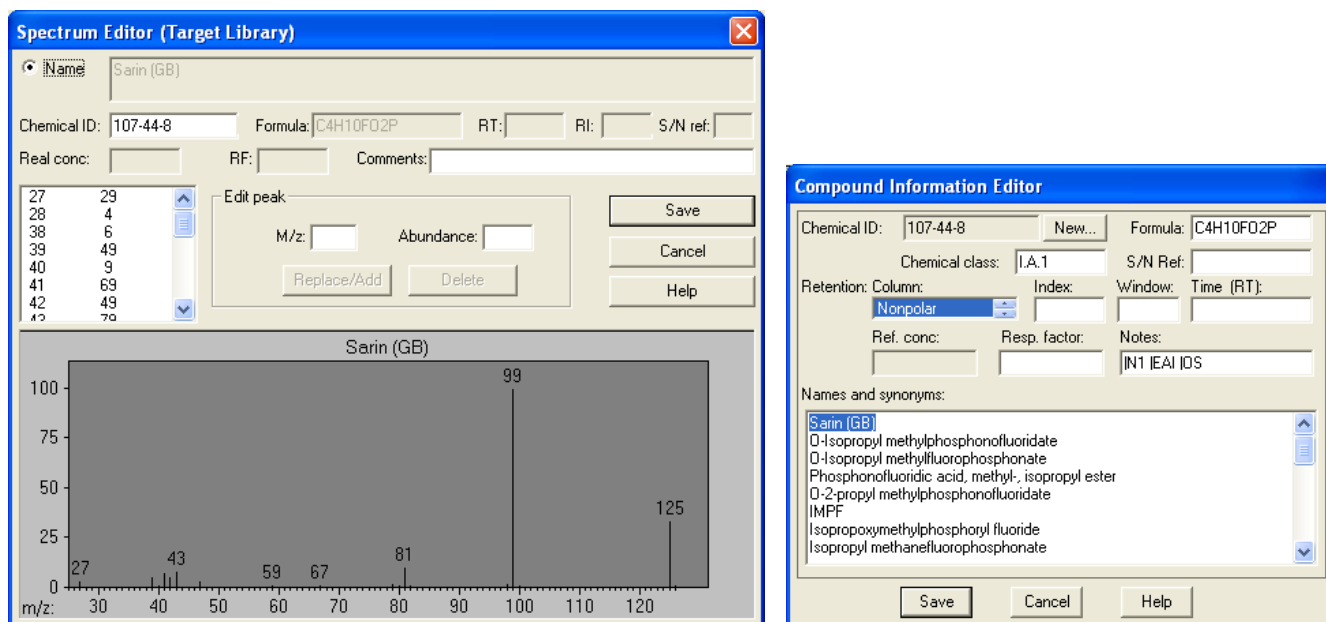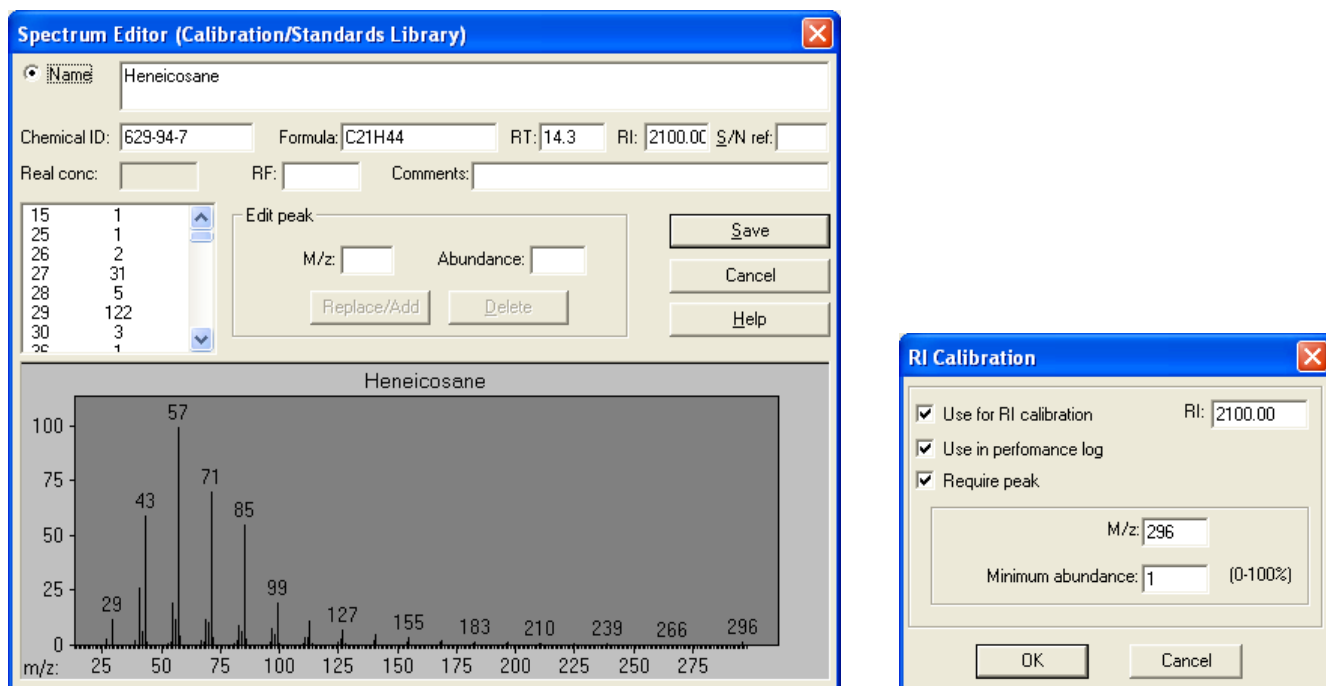
Figure 144 - The spectrum and information editors for an MSL library

8.1.2   Calibration & Standards Library (CSL)

A *Calibration & Standards Library* is used to identify calibration and performance compounds when creating an *RI Calibration Data* file, or when doing a performance analysis.

A *Calibration & Standards Library* is a list of calibration/performance compounds' information and associated spectra; one per compound entry and with all information held in a single file with a CSL extension. To be of practical use, the compound information must contain retention index values.

In a *Calibration & Standards Library* a compound is generally intended to have only one spectrum.

However, you can, should you wish, have multiple spectra for the same compound by specifying multiple entries with the same **Name**, but a different chemical identification (**Chemical ID**); for example, by adding an incremental suffix to the actual value. However, unlike with a *Target Compounds Library*, a compound with multiple entries may be identified, and so reported, multiple times and this may not be desirable. Additionally, any change made to the compound information of one entry is not automatically copied to the compound information of the other entries of the same **Name** and so may subsequently cause confusion.

> **Note:**   Adding different spectra with the same **Chemical ID**, but a different **Name**, is a variation of the above and has the same implications.

> **Note:**   Adding different spectra with the same compound **Name** and with the same **Chemical ID** is actually possible, but is not recommended. If you do choose to do this, the entry from these having the best spectral match will be reported as the single hit; subject to it satisfying the standard **Minimum match factor** threshold and any other match criteria.

The spectral and identification information are edited in a single dialog.

Figure 145 - The editor and Save dialog for a CSL library

Since a *Calibration & Standards Library* contains both the compounds to be used for retention index calibration and the compounds to be tracked for the Performance Log, you have the option of tagging each compound as **Use for RI calibration** or **Use in performance log**, or both. Additionally, you may specify that a particular m/z peak (**Require peak**) must be found for correct identification of a compound. This is particularly useful when identifying similar compounds (eg, a homologous series such as hydrocarbons).

These features and the editor are described in **8.2.6 Combination Editors**.

To learn more about setting up a *Calibration & Standards Library* see **8.3 Using Libraries for AMDIS Calibration Purposes**.

8.1.3 Internal Standards Library (ISL)

An *Internal Standards Library* is used to identify internal standards for those analyses where internal standard compounds have been co-injected with the sample; or where certain components within a sample are to be treated as internal standards.

An *Internal Standards Library* is a list of internal standard compounds' information and associated spectra; one per compound and with all information held in a single file with an ISL extension.

In an *Internal Standards Library* a compound is generally intended to have only one spectrum.

However, you can, should you wish, have multiple spectra for the same compound by specifying multiple entries with the same **Name**, but a different chemical identification (**Chemical ID**); for example, by adding an incremental suffix to the actual value. However, unlike with a *Target Compounds Library*, a compound with multiple entries may be identified, and so reported, multiple times and this may not be desirable. Additionally, any change made to the compound information of one entry is not automatically copied to the compound information of the other entries of the same **Name** and so may subsequently cause confusion.

> **Note:** Adding different spectra with the same **Chemical ID**, but a different **Name**, is a variation of the above and has the same implications.

> **Note:** Adding different spectra with the same compound **Name** and with the same **Chemical ID** is actually possible, but is not recommended. If you do choose to do this, the entry from these having the best spectral match will be reported as the single hit; subject to it satisfying the standard **Minimum match factor** threshold and any other match criteria.

The spectral and identification information are edited in a single dialog and the editor is described in **8.2.6 Combination Editors**.
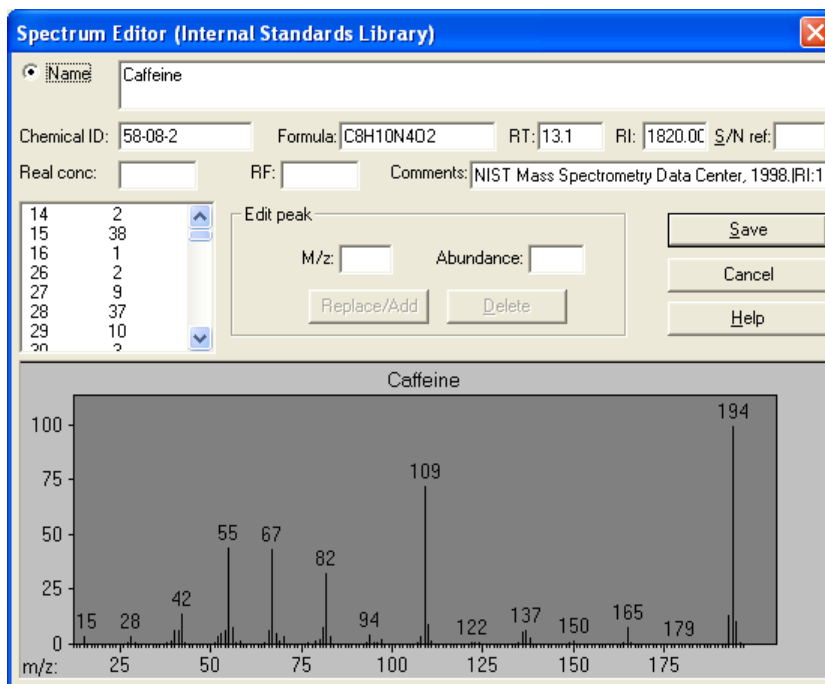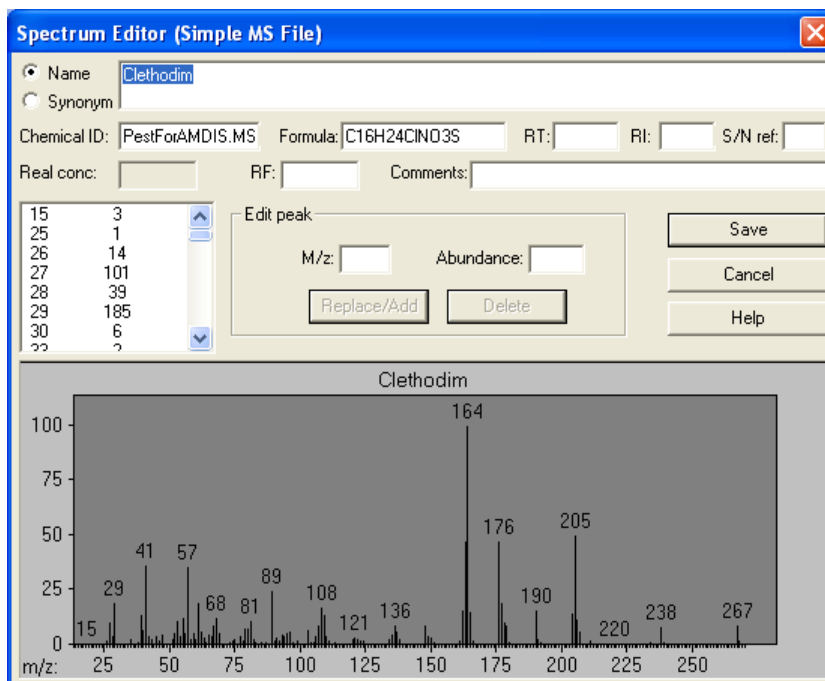

Figure 146 - The editor for an ISL library

8.1.4   RI Calibration Data File (CAL)

An *RI Calibration Data* file is used to compute a retention index (RI) from the retention time (RT) of a found target compound in those analysis types where an RI value is being utilized.

An *RI Calibration Data* file is essentially a paired list of retention time and retention index values; which are held in a file with a CAL extension.

> **Note:**   Details of the *RI Calibration Data* file format are given in **14.1 RI Calibration Data File (CAL) Format**.

Like the other library types, an *RI Calibration Data* file can be used as input for certain analysis types. However, unlike the other types, which are only creatable using a library editor, an *RI Calibration Data* file is created by the *RI Calibration/Performance* analysis type and does not have an editor in AMDIS.

The values held in an *RI Calibration Data* file and used/created during an analysis can be seen in the **RI calib** section of the Auxiliary Information's **Standards** tab (**5.4 Standards Tab**).

8.1.5   Mass Spectral Transfer File (MSP)

A *Mass Spectral Transfer* file is utilized in several AMDIS analysis types and is what deconvoluted spectra are compared against to see if there is a match (a "hit"). In this sense it is interchangeable with a *Target Compounds Library* file; but the two do have significant differences.

A *Mass Spectral Transfer* file holds a list of target compounds' information and associated spectra; one per compound entry and with all information held in a single file with an MSP extension.

In a *Mass Spectral Transfer* file a compound is generally intended to have only one spectrum.

However, you can, should you wish, have multiple spectra for the same compound by specifying multiple entries with the same **Name**, but a different chemical identification (**Chemical ID**); for example, by adding an incremental suffix to the actual value. However, unlike with a *Target Compounds Library*, a compound with multiple entries may be identified, and so reported, multiple times and this may not be desirable. Additionally, any change made to the compound information of one entry is not automatically copied to the compound information of the other entries of the same **Name** and so may subsequently cause confusion.

Note: Adding different spectra with the same **Chemical ID**, but a different **Name**, is a variation of the above and has the same implications.

Note: Adding different spectra with the same compound **Name** and with the same **Chemical ID** is actually possible, but is not recommended. If you do choose to do this, the entry from these having the best spectral match will be reported as the single hit; subject to it satisfying the standard **Minimum match factor** threshold and any other criteria.

A *Mass Spectral Transfer File* is created by the NIST *MS Search* program.

Note: The *Mass Spectral Transfer* file format is also adopted as the mechanism to pass a spectrum from AMDIS for searching with the NIST *MS Search* program (**3.1.2.6 Analyze | Go to NIST MS Program** and **3.1.2.8 Analyze | Search NIST Library**).

The spectral and identification information are edited in a single dialog. The editor is described in **8.2.6 Combination Editors**.

Figure 147 - The editor for an MSP file

Note: Details of the *Mass Spectral Transfer* file format are given in **14.2 Mass Spectral Transfer File (MSP) File Format**.

8.2 Library Editors

AMDIS has two distinct, but similar, library editors. They are only available in the *Confirm* window and are accessed from the **Library** menu.

The first is accessed using **Build One Library** (**8.2.1 Build One Library Editor**) and is designed for building a library from the components in a data file.

The second is accessed using **Library Transfer** (**8.2.2 Library Transfer Editor**) and is primarily designed for building a library from data in other AMDIS libraries, or out of data created from libraries utilized by the NIST *MS Search* program.

Both library editors have many common or similar features and these are described in **8.2.3 Library Editor Features**.

A library entry holds mass spectral peak information and compound information. The *Target Compounds Library* has separate spectrum and compound information editors (**8.2.5 Spectrum Editor** and **8.2.4 Compound Information Editor**). However, the other library types (excluding the *Mass Spectral Transfer* file) have a

combination spectrum and compound information editor. The combination editors for each library type are very similar, but do have minor differences (**8.2.6 Combination Editors**). The *Mass Spectral Transfer* file does not have an editor in AMDIS.

8.2.1   Build One Library Editor

The *Build One Library* editor is designed for building and maintaining a library from the components found when a data file is analyzed (**3.1.2.1 Analyze | Analyze GC/MS Data**). However, it can also be used when no analysis has been performed; by utilizing a manually generated spectrum (**10 Manual Background Subtraction and Manual Deconvolution**).

In both cases, the *Build One Library* editor is invoked by clicking **Library | Build One Library**.

The following is an example showing the *Build One Library* editor when invoked for an analyzed data file. However, the only difference in the editor for analyzed and unanalyzed files is to do with the **Add** and **Add All** buttons (**8.2.3.2.2 Add Button** and **8.2.3.2.3 Add All Button**).
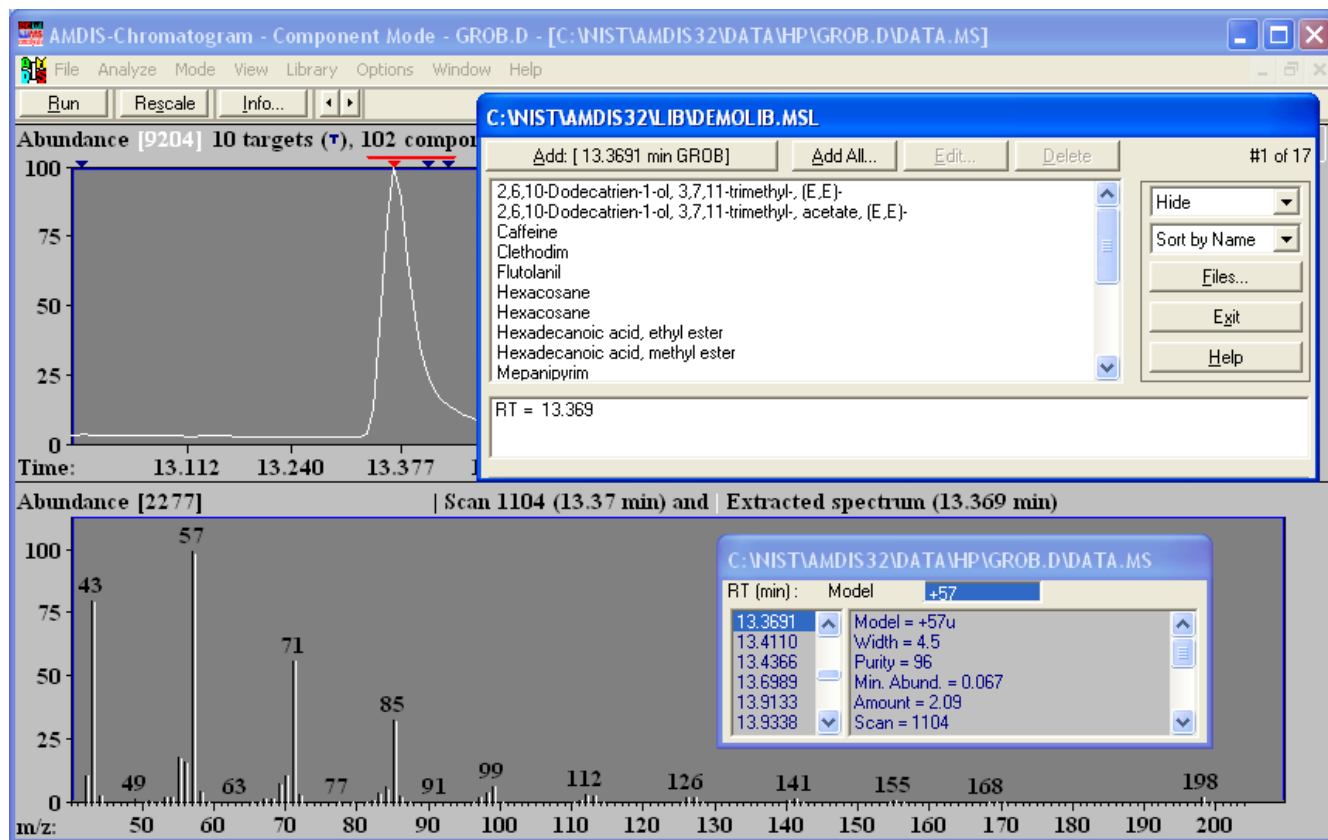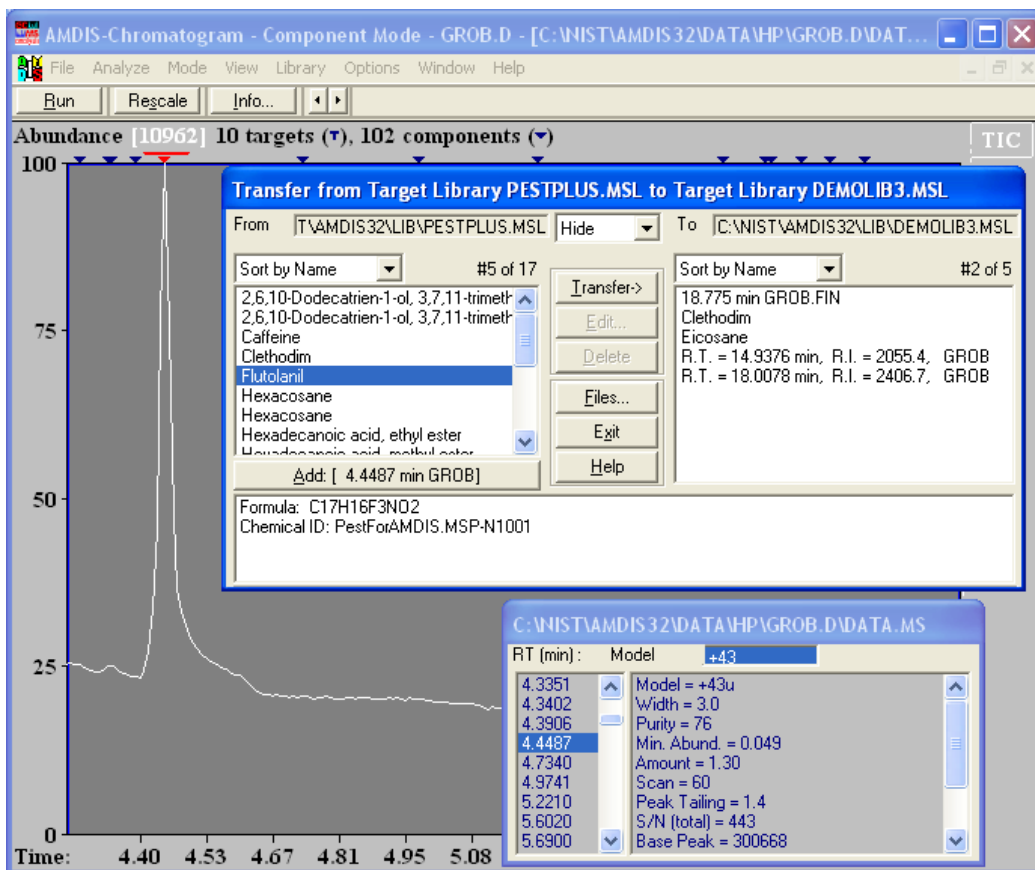

Figure 148 - Build One Library editor and an analyzed data file

Since most operations are common for the *Build One Library* and *Library Transfer* editors, the operations are described in **8.2.3 Library Editor Features**.

The title bar of the *Build One Library* editor shows the location and name (including type) of the current library being edited.

The *Build One Library* editor is commonly used as follows:

a)   Using the **Files** button, select the library to be modified; by type and name. If you are creating a new library, simply specify a type and new name of your choice.

b)   Analyze a data file to yield components; alternatively do a manual analysis to create one or more component spectra.

c) Select a component and click the **Add** button to append it to the destination library. Alternatively, you can use **Add All** to add multiple components from an automatically analyzed file. A component is added with its spectrum and a few of its compound information values are given default values.

d) Typically you may now want to examine or modify the details of an individual library entry that you have just added; or perhaps an existing entry. To do this, first highlight the entry and then click **Edit**.

    1) If editing a *Target Compounds Library*, click **Spectrum** to edit spectrum information, or **Compound** to edit compound information.

    2) As desired, modify one or more of the entry's compound information values.

    3) If appropriate, the entry's spectrum can be modified (m/z peaks can be added or deleted and peak intensities can be modified).

    4) Click **Save** to exit from the dialog. In the case of a *Calibration & Standards Library*, an additional dialog appears. As desired, modify its information and click **OK**.

e) To delete one or more entries from the library, highlight them and click **Delete**.

f) As appropriate, repeat any of (b) - (e) for the selected library.

8.2.2   Library Transfer Editor

The *Library Transfer* editor is primarily designed for building a library from data in other AMDIS libraries, or out of data created from libraries utilized by the NIST *MS Search* program.

However, in common with the *Build One Library* editor, it can also utilize components found when a data file is analyzed (**3.1.2.1 Analyze | Analyze GC/MS Data**), or equally when no analysis has been performed; by utilizing a manually generated spectrum (**10 Manual Background Subtraction and Manual Deconvolution**).

The *Library Transfer* editor is invoked by clicking **Library | Library Transfer**.

The following is an example showing the *Library Transfer* editor when invoked with a source and destination *Target Compounds Library* and an analyzed data file.

    Note: The only difference in the editor for analyzed and unanalyzed files is to do with the **Add** and **Add All** buttons (**8.2.3.2.2 Add Button** and **8.2.3.2.3 Add All Button**).

Figure 149 - Library Transfer editor editor and an analyzed data file

Since most operations are common for the *Build One Library* and *Library Transfer* editors, the operations are described in **8.2.3 Library Editor Features**.

The title bar of the *Library Transfer* editor shows the location, name and type of the current source (**From**) and destination (**To**) libraries. In the editor window the source library is in the left list box and the destination library in the right list box.

The *Library Transfer* editor is commonly used as follows:

a)   Using the **Files** button, select the (destination) library to be modified; by type and name. If you are creating a new library, simply specify a type and new name of your choice.

b)   Using the **Files** button, select the source library (type and name) from which you want to copy one or more entries to the destination library.

> **Note:** The source and destination library types do not have to be the same; in fact any combination is possible.

c)   Highlight one or more source library entries and click **Transfer** to copy them to the destination library; this copies spectral and compound information values.

d)   If you want to examine or modify the details of an individual destination library entry, first highlight it and then click **Edit**.

1)   If editing a *Target Compounds Library*, click **Spectrum** to edit spectrum information, or **Compound** to edit compound information.

2)   As desired, modify one or more of the entry's compound information values.

3)   If appropriate, the entry's spectrum can be modified (m/z peaks can be added or deleted and peak intensities can be modified).

4) Click **Save** to exit from the dialog. In the case of a *Calibration & Standards Library*, an additional dialog appears. As desired, modify its information and click **OK**.

e) To delete one or more entries from the <u>destination</u> library, highlight them and click **Delete**.

f) You can also select an analysis component, or create a component manually, and add it to the destination library using the **Add** button. In this case you will often want to then use the **Edit** button; step (d).

g) As appropriate, repeat any of (b) - (f) for the selected library.

8.2.3   Library Editor Features

The *Build One Library* editor (**8.2.1 Build One Library Editor**) and the *Library Transfer* editor (**8.2.2 Library Transfer Editor**) have very similar features; and these are described below (**8.2.3.1 Display Options** and **8.2.3.2 Editing Options**).

8.2.3.1   Display Options
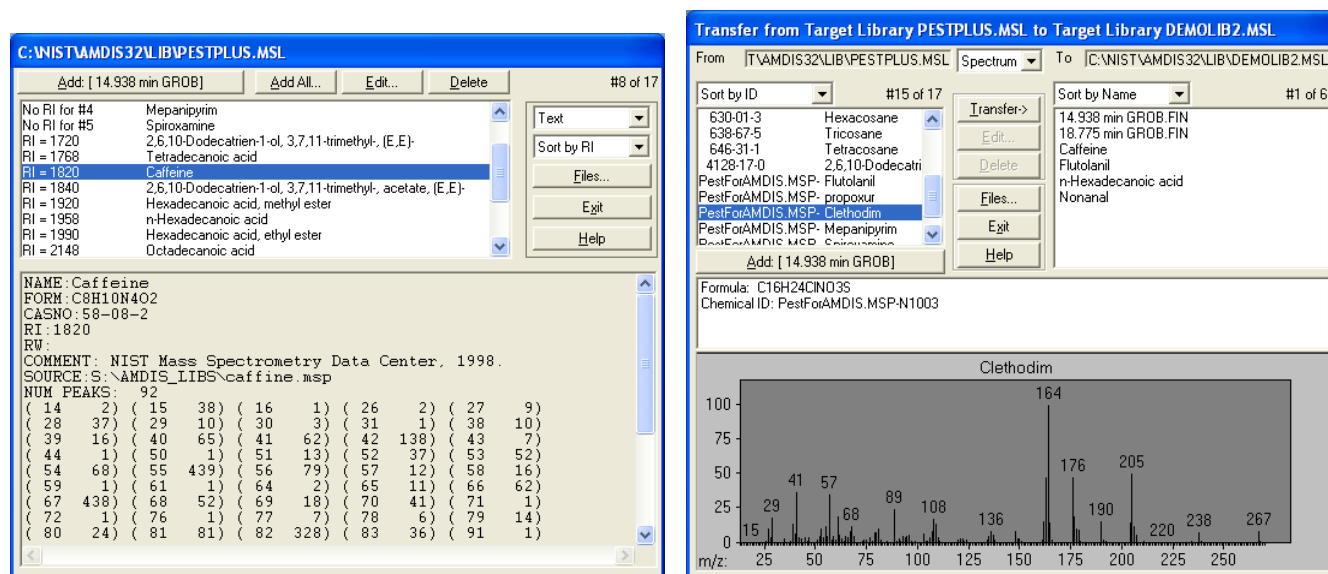
Figure 150 - Display features

The *Build One Library* editor and the *Library Transfer* editor have very similar display features and options as follows:

a) A title bar showing the library name(s) and type(s) being displayed. The *Library Transfer* editor also has non-editable **From** and **To** fields showing the same; but with additional location information.

b) One or two list boxes showing the entries in the library; one entry per line. To the top right of an area is shown which entry is currently selected and how many entries there are in the library (eg, **#8 of 17**).

   A single entry is selected (highlighted) by clicking on it. As appropriate, multiple entries are selected (and so highlighted) using standard *Windows* multi-select techniques.

c) A selector determining how the entries in each list box are ordered (sorted). The selection also determines the type of information shown for an entry.

   If **Sort by Name** is selected, the entries are listed in increasing alphanumeric order of compound name; which is the only information shown for an entry.

   If **Sort by ID** is selected, the entries are listed in increasing alphanumeric **Chemical ID** order. The compound name is also shown for an entry.

   If **Sort by RI** is selected, the entries are listed in increasing alphanumeric **RI** order; entries with no RI value are shown as **No RI for #n** and listed first (**#n** is the entry number). The compound name is also shown for an entry.

The identical operation to that described for this selector is also available from the library editor's context menu (**8.2.3.2.7 Context Menu**).

d) A selector determining what information is shown in the lower portion of the window for a highlighted entry.

If **Spectrum** is selected, a center panel shows some of the entry's compound information (eg, **Formula**) and its spectrum is presented graphically below.

If **Text** is selected, a textual representation of the spectrum is shown as integer, mass-intensity pairs (intensities are normalized to 1000). There is also a textual header showing some of the entry's compound and spectrum source information, and a peak count (**NUM PEAKS**).

If **None** is selected, the center panel only of the **Spectrum** display is shown.

The identical operation to that described for this selector is also available from the library editor's context menu (**8.2.3.2.7 Context Menu**).

e) Various command buttons supporting editing operations (**8.2.3.2 Editing Options**).

f) The **Exit** button to close the editor window.

The identical operation to that described for this button is also available from the library editor's context menu (**8.2.3.2.7 Context Menu**).

g) The **Help** button to invoke the help system; directly showing help for the editor.

8.2.3.2 Editing Options

The *Build One Library* editor and the *Library Transfer* editor both have command buttons to permit you to:

a) Select an existing library to be edited, or create a new empty library. Additionally with the *Library Transfer* editor you can select a source library from which entries can be copied (**8.2.3.2.1 Files Button**).

b) Add new entries to the selected library from an automatically analyzed, or manually deconvoluted, data file (**8.2.3.2.2 Add Button** and and **8.2.3.2.3 Add All Button**).

Additionally with the *Library Transfer* editor you can add entries from the source library (**8.2.3.2.4 Transfer Button**).

c) Edit an entry of the selected library (**8.2.3.2.5 Edit Button**).

d) Delete one or more entries of the selected library (**8.2.3.2.6 Delete Button**).

You can also right-click anywhere within a library editor list box to invoke a command menu that permits you to perform various library operations (**8.2.3.2.7 Context Menu**).

In some cases you may wish to make a backup copy of a library before making changes to it. If so, use the **Save Library As** button in the *Files* dialog (**8.2.3.2.1 Files Button**).

8.2.3.2.1 Files Button

The **Files** button invokes the *Files* dialog (**Figure 151 - Files dialog**) from which you can:

→ select an existing library to be used or edited (**8.2.3.2.1.1 Load Library Button**);

→ create a new empty library (**8.2.3.2.1.2 Create New Library Button**);

→ save a copy of the library being modified (**8.2.3.2.1.3 Save Library As Button**);

→ exit from the dialog (**8.2.3.2.1.4 Cancel and Close Buttons**);

→ switch to the other editor (**8.2.3.2.1.5 Switch To Library Transfer and Switch To One Library Buttons**).
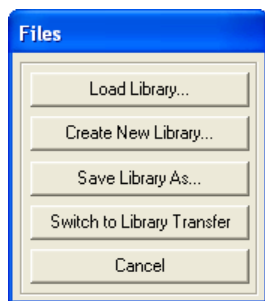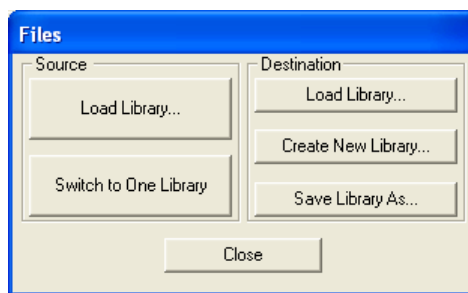
Build One Library editor   *Library Transfer* editor

Figure 151 - Files dialog

8.2.3.2.1.1   Load Library Button

The **Load Library** button invokes a standard *Windows* file browser dialog to enable you to select a library **File name**; ensure that **Files of type** is set to the type of library you wish to select (**8.1 Library Types**).

For the *Build One Library* editor there is only one **Load Library** button to select the library to be edited.

However, the *Library Transfer* editor has two buttons. Here the **Destination** library is the one that is edited and the **Source** library is used to select entries to be transferred (copied) to the **Destination** library.

> **Note:**   It is completely acceptable for the **Source** and **Destination** libraries to be of different types. In this case as much information as is compatible is transferred. If the **Source** is a *Target Compounds Library* having entries with multiple spectra, each spectrum is created as a separate entry if the **Destination** library is not a *Target Compounds Library*.

The identical operation to that described for the **Load Library** button is also available from the library editor's context menu (**8.2.3.2.7 Context Menu**).

8.2.3.2.1.2   Create New Library Button

The **Create New Library** button invokes a standard *Windows* file browser dialog to enable you to specify the **File name** of a new library to be created; ensure that **Save file as type** is set to the type of library you wish to create (**8.1 Library Types**).

When you click **OK** to exit the dialog, the specified library is created, but it is "empty"; it has no entries.

> **Note:**   If you have specified the name of an existing library, you are asked to confirm if you wish to proceed and replace (overwrite) it.

> **Note:**   It is acceptable for the current and specified libraries to be of different types. In this case as much information as is compatible will be copied when subsequently transferring entries (**8.2.3.2.4 Transfer Button**).

The identical operation to that described for the **Create New Library** button is also available from the library editor's context menu (**8.2.3.2.7 Context Menu**).

8.2.3.2.1.3   Save Library As Button

The **Save Library As** button invokes a standard *Windows* file browser dialog to enable you to specify the **File name** of the new library to be created as a copy of the current library; ensure that **Save file as type** is set to the type of library you wish to create (**8.1 Library Types**).

When you click **OK** to exit the dialog, the specified library is created as a copy of the current library.

> **Note:**   If you have specified the name of an existing library, you are asked to confirm if you wish to proceed and replace (overwrite) it.

> **Note:**   It is acceptable for the current and specified libraries to be of different types. In this case as much information as is compatible is copied. If the **Source** is a *Target Compounds Library* having entries with multiple spectra, each spectrum is created as a separate entry if the **Destination** library is not a *Target Compounds Library*.

The identical operation to that described for the **Save Library As** button is also available from the library editor's context menu (**8.2.3.2.7 Context Menu**).

8.2.3.2.1.4   Cancel and Close Buttons

The **Cancel** button (*Build One Library* editor) and the **Close** button (*Library Transfer* editor) exit from the *Files* dialog; returning to the respective editor.

8.2.3.2.1.5   Switch To Library Transfer and Switch To One Library Buttons

The **Switch To Library Transfer** button and the **Switch To One Library** button cause a swap from the current editor to the other editor.

8.2.3.2.2   Add Button

The **Add** button is used to add new entries to the selected library from either an automatically analyzed data file (**8.2.3.2.2.1 Automatic Deconvolution**) or a manually deconvoluted data file (**8.2.3.2.2.2 Manual Deconvolution**). Further, it is possible to add entries using library spectra from the results of a library search (**8.2.3.2.2.3 NIST Library Search**). If none of these operations have been performed for the currently open data file, the **Add** button is not available for selection.

> **Note:**   The exact text of the **Add** button will vary as it shows the chosen spectrum's retention time and will either give the data file name for an automatic analysis, or show that it is a manual deconvolution. If available, retention index information will also be shown. If the data file name is greater than 8 characters, it is expressed in the MS-DOS 8-character format, which makes each name unique, but is not always very representative of its actual name. Since this representation is only used for the button text and not name information stored within a library entry, it is not a practical problem.

Whilst a library editor window is open you can keep selecting new spectra to be added. This is particularly convenient if you wish to add multiple spectra in one editing session.

The newly created library entry will contain a copy of the added spectrum, except that any uncertain peaks in analyzed data will be excluded (for more about uncertain peaks, see **3.1.2.7 Analyze | Use Uncertain Peaks**).

If a spectrum is added from a target, the assigned **Chemical ID** is simply a copy of the target's value.

However, when a component's spectrum is added from a data file the automatically assigned **Chemical ID** contains the name of the data file so it can be easily tracked later. The format is NAME-Nnnnn, where nnnn increments for the same data file (NAME). The equivalent format is adopted for manually created data, except that NAME will always be "DATA".

In all cases **Comments** will contain basic information about the target/component. This will be the RT and data file name; and also RI if available for analyzed data. For manually created data, **Manual** precedes the data file name.

Once a spectrum has been added, its library entry can be examined and modified by clicking the **Edit** button (**8.2.3.2.5 Edit Button**). The **Delete** button is also available to delete an entry (**8.2.3.2.6 Delete Button**).

8.2.3.2.2.1   Automatic Deconvolution

When a data file has been automatically analyzed; for example, by selecting **Analyze | Analyze GC/MS Data** (**3.1.2.1 Analyze | Analyze GC/MS Data**), you can click on any component or target in the *Information Lists* area and the **Add** button's label changes to reflect the selection.

For example, **Add: [15.813 min GROB]** shows that the component (or target) is at 15.813 minutes in data file *GROB*. Note that, the component's (or target's) spectrum can also be displayed in the editor (**8.2.3.1 Display Options**).
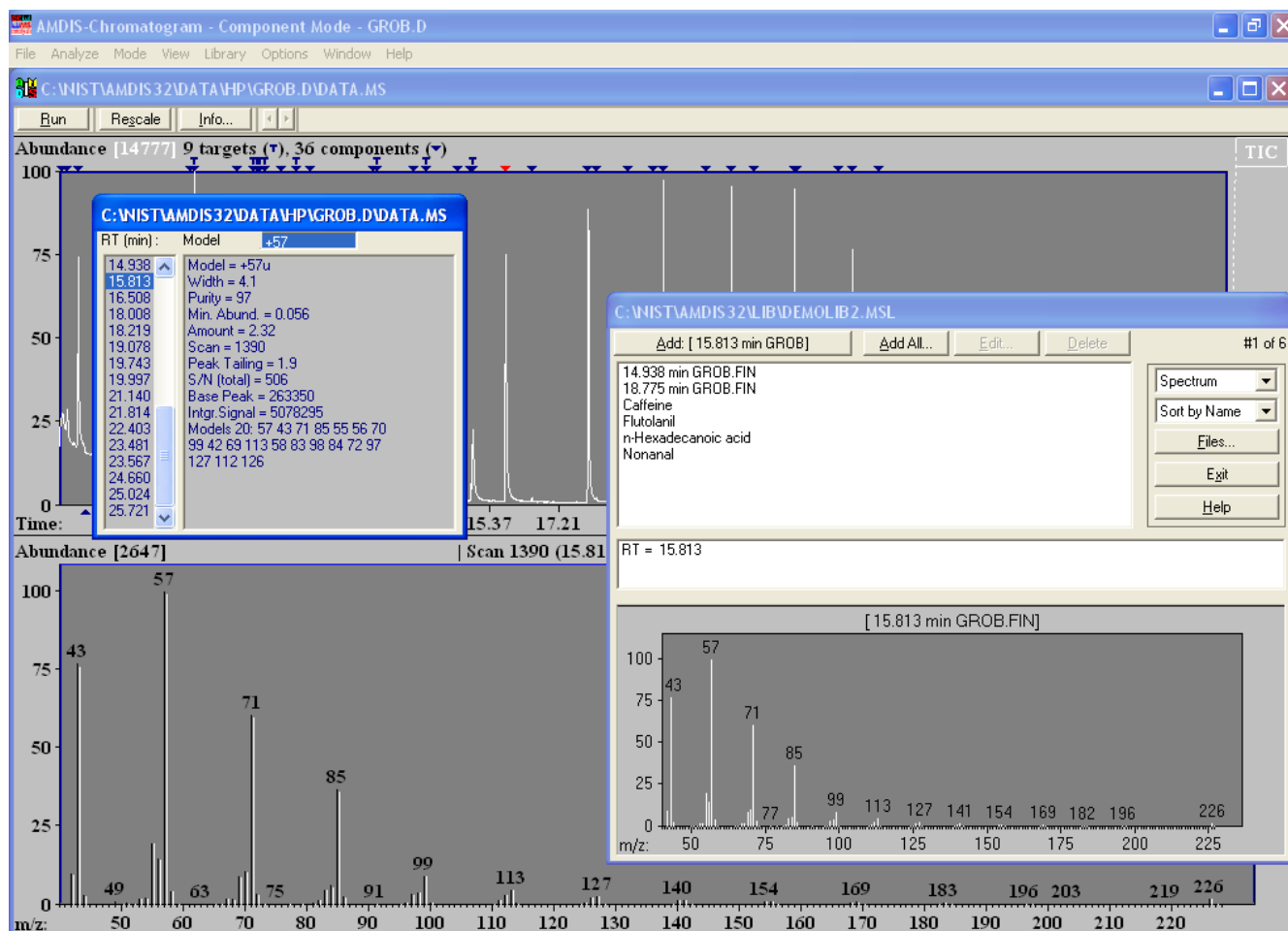
Figure 152 - Automatic mode Add

If retention index (RI) information has been computed during the analysis, the **Add** button's label shows its value. For example, **Add: [R.I.   1434.1   15.813 min GROB]** in the following:
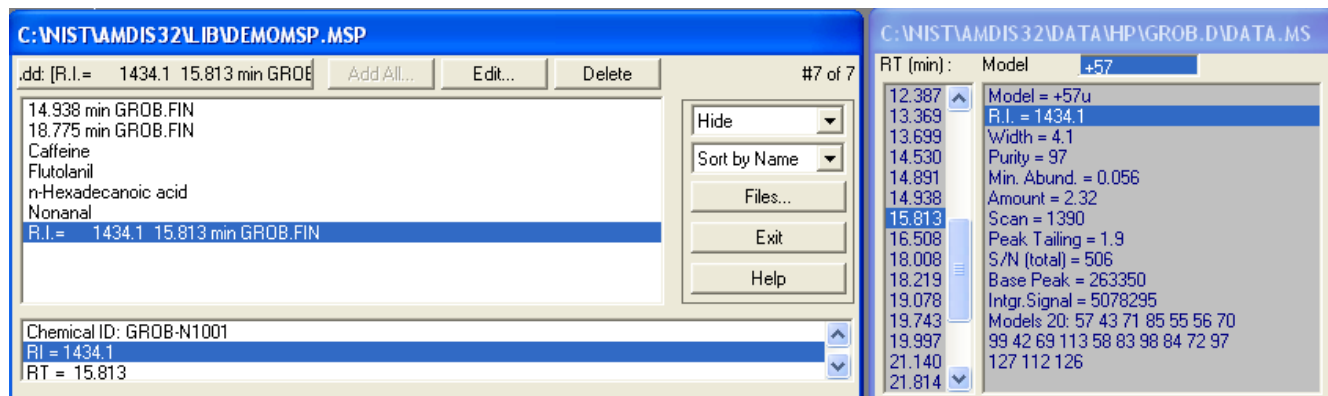

Figure 153 - RI information shown

Exactly what happens when you click the **Add** button will depend on the type of library:

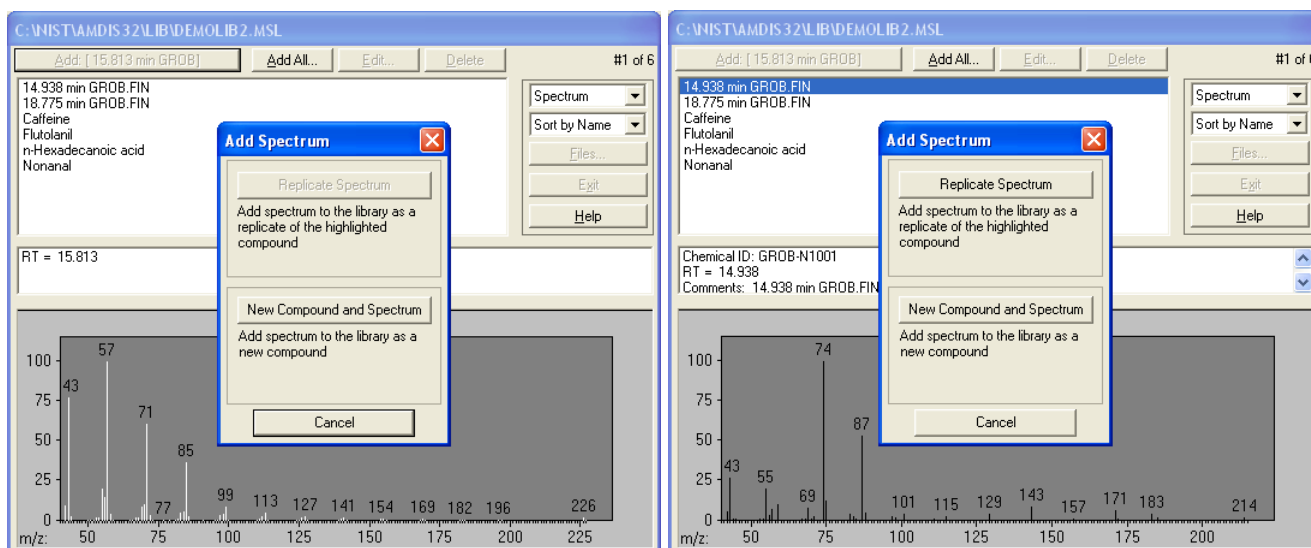a)   For a *Target Compounds Library* the *Add Spectrum* dialog appears.

Figure 154 - Adding to an MSL library

This dialog has the **Replicate Spectrum** and **New Compound and Spectrum** buttons.

If there was no highlighted current library entry when the dialog was invoked, only the **New Compound and Spectrum** button is available. Click this to add the new spectrum as a new entry representing a new compound; with its own **Chemical ID** and other compound information.

However, if an entry was already highlighted (for example, **14.938 min GROB.FIN** in **Figure 154 - Adding to an MSL library**), the **Replicate Spectrum** button is also available to allow you to add the new spectrum as an additional spectrum for the current compound. In this case the original compound information remains unchanged.

If you click **New Compound and Spectrum** and an entry with the same **Chemical ID** already exists, the *Library already has spectrum with the same ID* dialog appears.
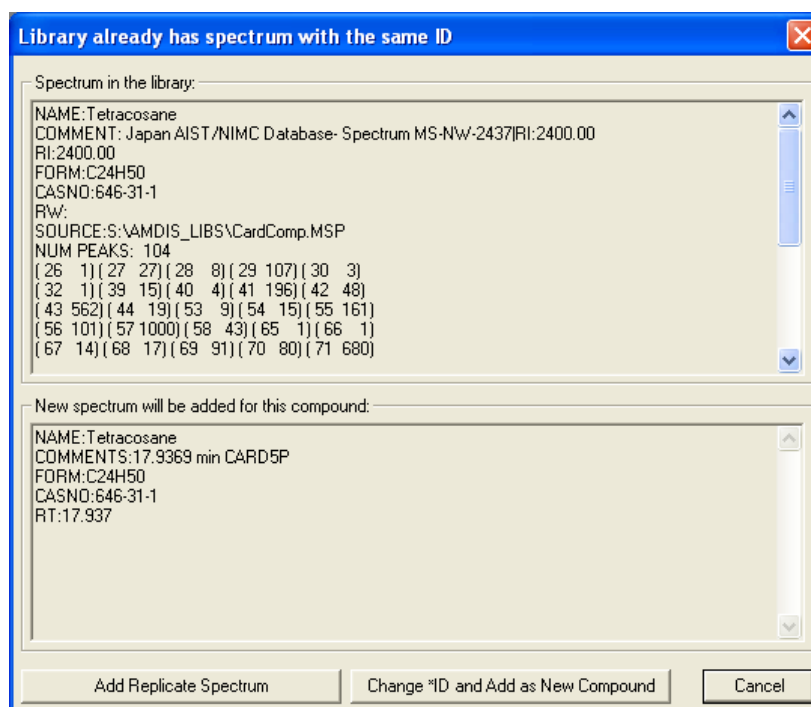
Figure 155 - Duplicate ID dialog

Click **Add Replicate Spectrum** to add the new spectrum as an additional spectrum for the current compound. In this case the original compound information remains unchanged and this is identical in operation to the **Replicate Spectrum** button of the previous *Add Spectrum* dialog.

Alternatively, click **Change *ID and Add as New Compound** button to invoked the *Set New Chemical ID* dialog.
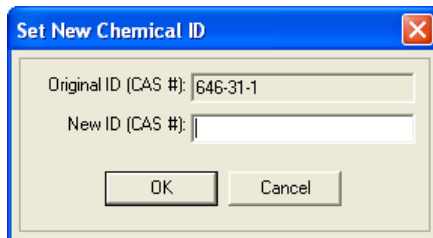
Figure 156 - Set new chemical ID dialog

Enter a **New ID (CAS #)** and click **OK**.

b)	For all library types other than a *Target Compounds Library*, the **Add** button does not invoke a dialog, but immediately adds the spectrum as a new compound entry and highlights it.
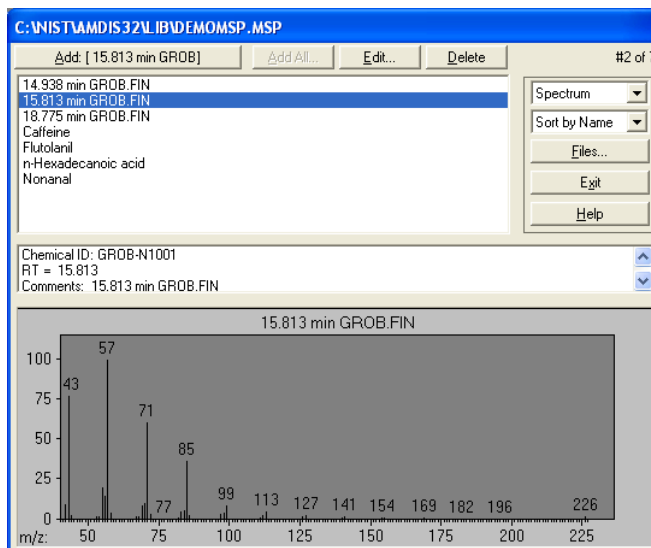
Figure 157 - Adding to a non-MSL library

#### 8.2.3.2.2.2	Manual Deconvolution

When a data file is being manually analyzed (**10 Manual Background Subtraction and Manual Deconvolution**), the **Add** button's label changes to reflect the manually constructed spectrum (component).

For example, **Add: [10.6602 min Manual DATA]** shows that the spectrum is centered at 10.66 minutes and is manually created data.
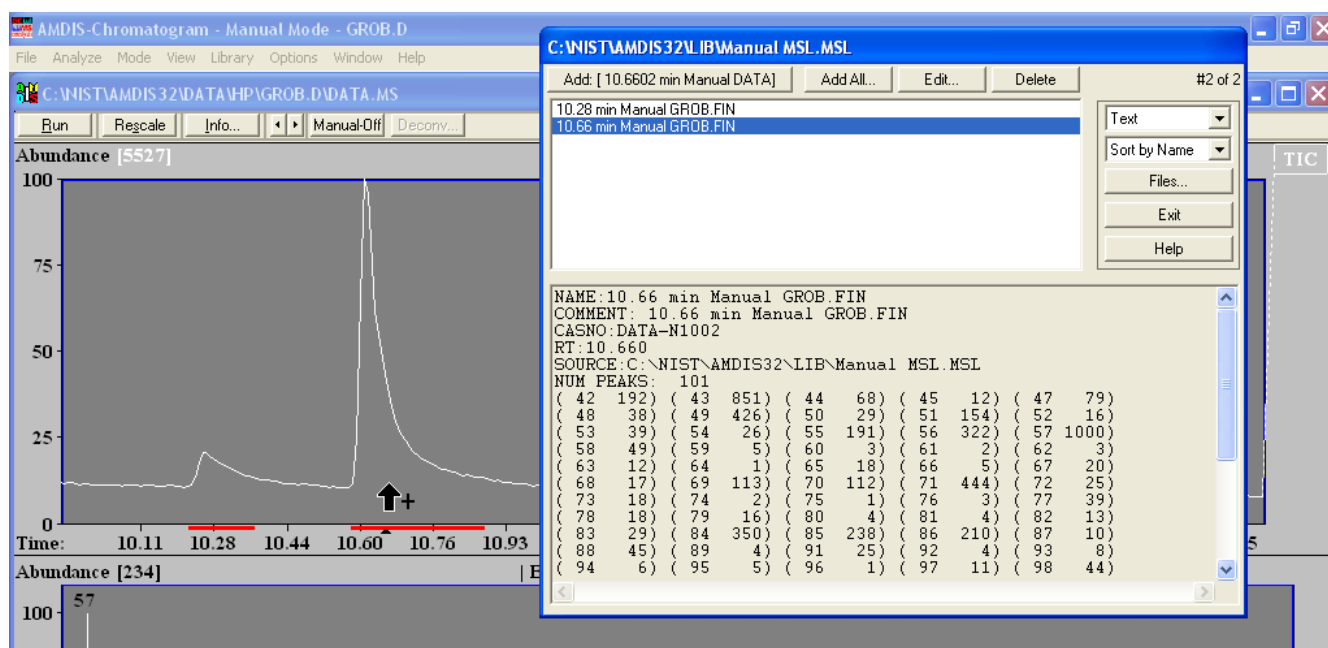
Figure 158 - Manual mode Add

If background areas have been subtracted, the label simply says **Add: [Manual DATA]**.

Note that, the component's spectrum can also be displayed in the editor (**8.2.3.1 Display Options**). The way in which the **Add** button works and all other respects of manual mode operation are identical to how components are handled in automatic mode and are described there (**8.2.3.2.2.1 Automatic Deconvolution**).

8.2.3.2.2.3 NIST Library Search

After a NIST library search operation (**3.1.2.8 Analyze | Search NIST Library**), you would normally view results from the search; either alone (**3.1.4.9 View | Show NIST Search**) or in combination (**3.1.4.10 View | Show Merged Results**).

In either case, to add a search result spectrum, first highlight a hit in the *Information Lists* that is from the NIST search and then click the **Add** button. If you are editing a library other than a *Target Compounds Library*, the *What spectrum to save* dialog immediately appears. If you are editing a *Target Compounds Library* the *Add Spectrum* dialog initially appears and the *What spectrum to save* dialog will appear after clicking the **New Compound and Spectrum** button (but not if you click the **Replicate Spectrum** button).
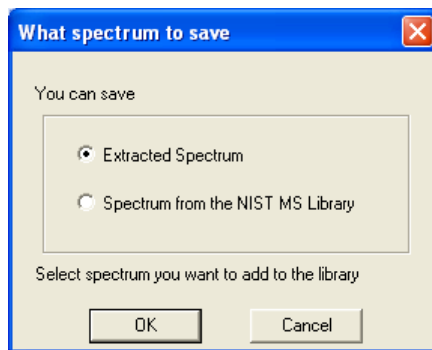
Figure 159 - What spectrum to save dialog

Click **Spectrum from the NIST MS Library** if you want to save a copy of the spectrum identified in the library search process. Otherwise, click **Extracted Spectrum** to save the extracted spectrum created by the original deconvolution process (analysis). Click **OK** to complete the process.

The entry's **Comments** will be suffixed with **Library Spectrum** if you chose the **Spectrum from the NIST MS Library** option.

Note: In general it is a good idea to add high quality spectra from your own instrument to the library. However, if you got a weak identification, it may be because the spectrum is not of high quality. In this case you should add the NIST library spectrum.

8.2.3.2.3   Add All Button

The **Add All** button is used to add new entries to the selected library from an automatically analyzed data file (**8.2.3.2.2.1 Automatic Deconvolution**).

Note: The **Add All** button is only available for a *Target Compounds Library*.

Although **Add All** is also available after a NIST library search operation (**3.1.2.8 Analyze | Search NIST Library**), it will always add extracted spectra, rather than spectra identified in the library search process. If you wish to do the latter (one at a time), use the **Add** button instead (**8.2.3.2.2 Add Button**).

Click the **Add All** button to invoke the *Add All Spectra* dialog.
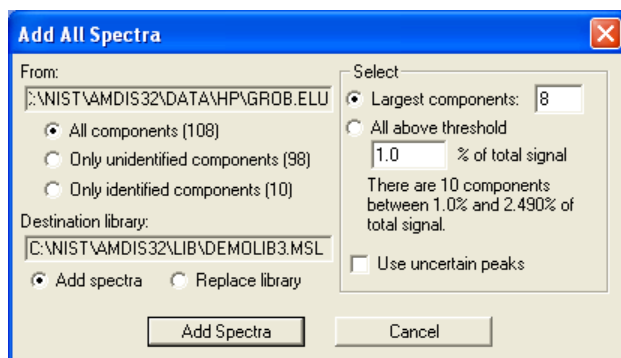
Figure 160 - Add All Spectra

There are various parameters in the dialog that together determine which type of component is considered, then which of that type are subsequently selected and finally whether the added spectra replace or add to the current library's spectra.

The **From** parameter has a read-only text box to show you the current result file's name and location. This is followed by a radio button selection to allow you to select which type of component is to be searched.

Click **All components (n)** to select all of the components identified by AMDIS; where **(n)** tells you how many of them there are. To select only those components that AMDIS did not identify in its library, click **Only unidentified components (n)**. Otherwise, click **Only identified components (n)** to select only the targets found by AMDIS.

Having selected the component group that you want to add spectra from, you can now further limit how many of those components will be added using the **Select** parameter group and choosing one of two magnitude thresholds. In both cases it is the component's "amount" value that is being utilized (**3.3.3.1 Component Mode Details**).

Click **Largest components** and enter how many components are required of the **From** group's type (**8** from **All components (108)** in **Figure 160 - Add All Spectra**). This imposes an absolute maximum on the number of components that will be added. The selection process starts with the component having the largest amount value and works its way down in amount until the specified number has been reached, or there are no more components remaining; based on the **From** group's selection.

Alternatively click **All above threshold** and enter a percentage of total signal amount (**1.0** in **Figure 160 - Add All Spectra**). This is an absolute amount threshold above which all selected components will be added. The text below the threshold value shows you how many components will be selected by the specified value and as you enter a new value this text will change, as appropriate.

The **Use uncertain peaks** checkbox allows you to determine whether or not uncertain peaks are included in the spectra that are added. Select it to use them; otherwise clear it. Uncertain peaks are discussed in **3.1.2.7 Analyze | Use Uncertain Peaks**.

The **Destination library** parameter has a read-only text box to show you the current library file's name and location. This is followed by a radio button selection. Click **Add spectra** to add the selected spectra to the library using the **Add Spectra** button. Alternatively, click **Replace library** to specify that the selected spectra will

completely replace the current library's entries. In that case the **Add Spectra** button's text changes to **Replace Library**.

When a spectrum is added to the library, the automatically assigned Chemical ID contains the name of the data file so it can be easily tracked later. The format is NAME-Nnnnn, where nnnn increments for the same data file (NAME).

Similarly, Comments will contain basic information about the target/component. This will be the RT and data file name; and also RI if available for analyzed data.

Once a spectrum has been added, its library entry can be examined and modified by clicking the **Edit** button (**8.2.3.2.5 Edit Button**). The **Delete** button is also available to delete an entry (**8.2.3.2.6 Delete Button**).

8.2.3.2.4   Transfer Button

The **Transfer** button only exists in the *Library Transfer* editor (**Figure 149 - Library Transfer editor and an analyzed data file**) and is used to copy entries from the source library to the destination library.

The **Transfer** button is only available for selection when at least one entry of the source library is highlighted. Multiple entries can be highlighted using standard *Windows* multi-select techniques.

Click **Transfer** to make a copy of all highlighted source library entries in the destination library.

> **Note:**   The source library entries that have been copied still exist in the source library after the copy.

If the source and destination library types are of the same type (for example, both MSLs), an exact copy of each entry is made.

However, it is possible and completely acceptable for the source and destination libraries to be of different types (for example, an MSL and a CSL). In this case the spectra and as much compound information as is compatible are transferred.

The identical operation to that described for the **Transfer** button is also available from the library editor's context menu (**Transfer Spectra** command, **8.2.3.2.7 Context Menu**).

8.2.3.2.5   Edit Button

The **Edit** button is used to edit both the compound and the spectral information of a library entry.

The **Edit** button is only available for selection when an entry of the library is highlighted. For the *Library Transfer* editor, the edit operation relates to the destination library only.

> **Note:**   If you happen to have multiple entries highlighted, the **Edit** button is still available and will enter the editor for the most recently highlighted entry.

> **Note:**   The **Edit** button has an analogous **Edit Spectrum** command on the library editor's context menu (**8.2.3.2.7 Context Menu**).

Although the basic editing capabilities are very similar for all library types, a *Target Compounds Library* has separate compound and spectrum editors, whereas the other types have a single, combined functionality editor.

As such, when you click **Edit** for a *Target Compounds Library*, an intermediate *Edit* dialog appears, whereas with other library types you go straight to the appropriate combination editor (**8.2.6 Combination Editors**).
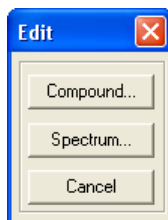

Figure 161 - Intermediate Edit dialog for a Target Compounds Library

Click **Compound** to enter the *Compound Information Editor* for a *Target Compounds Library* (**8.2.4 Compound Information Editor**).

Click **Spectrum** to enter the *Spectrum Editor* for a *Target Compounds Library* (**8.2.5 Spectrum Editor**).

The identical operation to that described for the **Edit** button is also available from the library editor's context menu (**8.2.3.2.7 Context Menu**).

8.2.3.2.6 Delete Button

The **Delete** button is used to delete library entries.

The **Delete** button is only available for selection when at least one entry of the library is highlighted. Multiple entries can be highlighted using standard *Windows* multi-select techniques. For the *Library Transfer* editor, the delete operation relates to the destination library only.

Click **Delete** to delete all highlighted library entries. A warning message appears asking you to confirm the delete operation. Click **Yes** to confirm it, or **No** to cancel it.

> **Important Note:** The delete operation is immediate (once **Yes** has been clicked) and irreversible. If you accidentally delete an entry, you can only restore it by creating a new entry and reentering all of its information again from scratch.

> **Caveat:** If it is a *Target Compounds Library*, which supports multiple spectra for the same compound (ie, Chemical ID), you will still have the compound information available unless you have deleted all spectra for that compound.

The identical operation to that described for the **Delete** button is also available from the library editor's context menu (**Delete Spectra** command, **8.2.3.2.7 Context Menu**).

8.2.3.2.7 Context Menu

If you right-click anywhere within a library editor list box, a command menu appears (the "context-sensitive" menu).
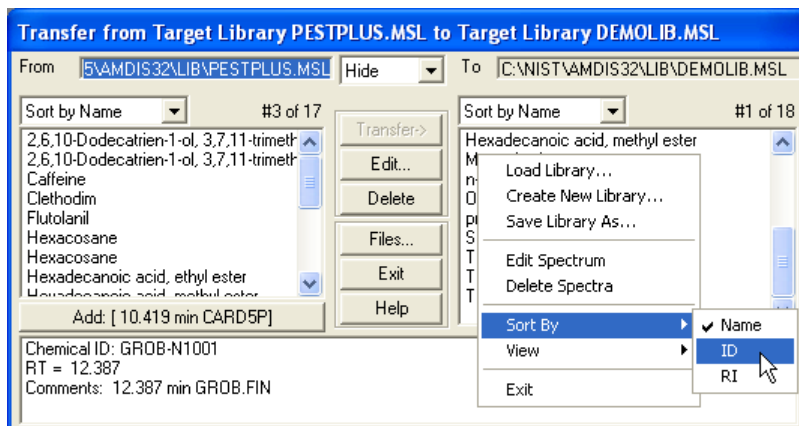
Figure 162 - Library editor context menu

The context menu in the *Build One Library* editor is the same as that invoked for the destination library of the *Library Transfer* editor. However the *Library Transfer* editor's source library list box invokes a similar, but less extensive, context menu.

The context-sensitive menu is used to:

→ load an existing library or create a new library

Load Library	see **8.2.3.2.7.1 Load Library**
Create New Library	see **8.2.3.2.7.2 Create New Library**
Save Library As	see **8.2.3.2.7.3 Save Library As**

→ manipulate library entries

Edit Spectrum	see **8.2.3.2.7.4 Edit Spectrum**
Delete Spectra	see **8.2.3.2.7.5 Delete Spectra**

| **Transfer Spectra** | see **8.2.3.2.7.6 Transfer Spectra** |

→ control aspects of the library editor display

| **Sort By** | see **8.2.3.2.7.7 Sort By** |
| **View** | see **8.2.3.2.7.8 View** |

→ exit from the library editor

| **Exit** | see **8.2.3.2.7.9 Exit** |

Since this menu is context sensitive, not all commands will always be available.

8.2.3.2.7.1 Load Library

The **Load Library** command is always present and available. It invokes a standard *Windows* file browser dialog to enable you to select the type and name of a new library.

Its operation is identical to that described for the **Load Library** button (**8.2.3.2.1.1 Load Library Button**).

8.2.3.2.7.2 Create New Library

The **Create New Library** command is present and available, except for the *Library Transfer* editor's source library list box; in which case it is not present. It invokes a standard *Windows* file browser dialog to enable you to specify the type and name of a library you wish to create.

Its operation is identical to that described for the **Create New Library** button (**8.2.3.2.1.2 Create New Library Button**).

8.2.3.2.7.3 Save Library As

The **Save Library As** command is present and available, except for the *Library Transfer* editor's source library list box; in which case it is not present. It invokes a standard *Windows* file browser dialog to enable you to specify the type and name of the library to be created as a "copy" of the current library.

Its operation is identical to that described for the **Save Library As** button (**8.2.3.2.1.3 Save Library As Button**).

8.2.3.2.7.4 Edit Spectrum

The **Edit Spectrum** command is present, except for the *Library Transfer* editor's source library list box. It is only available for selection when a library entry has been highlighted. It is used to edit both the compound and the spectral information of a library entry.

Its operation is identical to that described for the **Edit** button (**8.2.3.2.5 Edit Button**).

8.2.3.2.7.5 Delete Spectra

The **Delete Spectra** command is present, except for the *Library Transfer* editor's source library list box. It is only available for selection when one or more library entries have been highlighted. It is used to delete library entries.

Its operation is identical to that described for the **Delete** button (**8.2.3.2.6 Delete Button**).

8.2.3.2.7.6 Transfer Spectra

The **Transfer Spectra** command is only present for the *Library Transfer* editor's source library list box. It is only available for selection when one or more library entries have been highlighted. It is used to copy entries from the source library to the destination library.

Its operation is identical to that described for the **Transfer** button (**8.2.3.2.4 Transfer Button**).

8.2.3.2.7.7 Sort By

The **Sort By** command is always present and available. It has a sub-menu containing the **Name**, **ID** and **RI** commands, which determine how the entries in that particular list box are ordered (sorted). The currently active sub-menu command is shown with a check mark to its left.

The operation of each command is identical to the similarly named options described in **8.2.3.1 Display Options**.

8.2.3.2.7.8 View

The **View** command is always present and available. It has a sub-menu containing the **Hide All**, **Show Spectrum** and **Show Text** commands, which determine what information is shown in the lower portion of the window for a highlighted entry. The currently active sub-menu command is shown with a check mark to its left.

> **Note:** Although the command exists in both list boxes of the *Library Transfer* editor, a change in either list box applies to both list boxes.

The operation of each command is identical to the similarly named options described in **8.2.3.1 Display Options**.

8.2.3.2.7.9 Exit

The **Exit** command is always present and available. It is used to close the editor window.

The operation of this command is identical to the **Exit** button described in **8.2.3.1 Display Options**.

8.2.4 Compound Information Editor

The *Compound Information Editor* is used to edit the compound information for a *Target Compounds Library* only; as discussed in **8.1.1 Target Compounds Library (MSL)**. An entry's corresponding spectral information is edited using the *Spectrum Editor* (**8.2.5 Spectrum Editor**).

> **Note:** All other library types utilize a combined spectral and information editor (**8.2.6 Combination Editors**).

Also, unlike the other libraries which only have one spectrum per compound entry, a *Target Compounds Library* may have multiple spectra per compound entry and is unique in having the ability to store retention index information for multiple column types.

As such, a *Target Compounds Library* is actually stored as two separate files, which have the same "root" name, but different extensions (MSL and CID) and it is in the CID file that the compound information is held. An entry's related spectral information in the MSL file is linked to it by a unique chemical identification number (**Chemical ID**). Hence all spectra with the same **Chemical ID** share the same compound data.

> **Note:** You do not really need to know about the different files and their extensions, since AMDIS automatically handles the two files. For convenience, whenever a *Target Compounds Library* is being selected or displayed within AMDIS, it is only the MSL file extension that is shown.

In the library editor, click the **Edit** button, followed by **Compound**, to invoke the *Compound Information Editor*.

Figure 163 - Compound Information Editor

The *Compound Information Editor* enables you to view and/or edit all of a compound's non mass spectral data. Click **Save** to preserve any edits and exit from the editor; otherwise click **Cancel** to exit without saving any changes.

Chemical ID

The **Chemical ID** is a unique identifier for a compound entry of the library. For convenience the Chemical Abstracts Service Registry number (CAS number) is normally used when available, but any unique value can be utilized.

Indeed, when library entries are added from a data file (**8.2.3.2.2 Add Button** and **8.2.3.2.3 Add All Button**) the **Chemical ID** is automatically assigned and contains the name of the data file so it can be easily tracked later.

Since the **Chemical ID** is the crucial value used to identify entries within a library and in particular is the key value linking any multiple spectra of a single compound, it cannot be changed directly. Instead click **New** to invoke the *Set New Chemical ID* dialog.
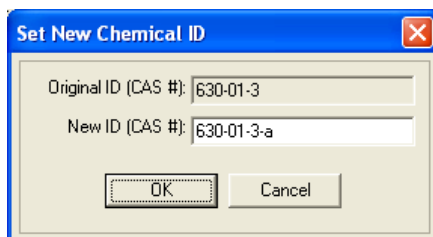
Figure 164 - Set New Chemical ID dialog

Enter the required **New ID (CAS #)** and click **OK**. A warning message informs you that all spectral entries that were assigned to the original **Chemical ID** will now be assigned to its new value.

> **Note:** This message is automatic and so does not necessarily mean that there are multiple entries with the original **Chemical ID**. Neither does it check for the existence of the new **Chemical ID**.
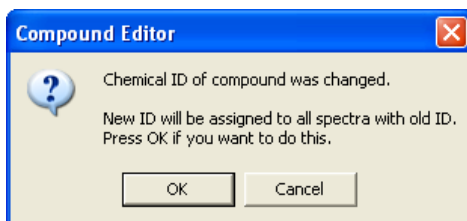
Figure 165 - Chemical ID change warning message

Click **OK** to proceed and return to the *Compound Information Editor*. If the new **Chemical ID** already exists in the library, the *Compound Information Editor* will now completely show values for that pre-existing entry.

> **Important Note:** Actually, this change (and any others in the editor) only become final when you click **Save** to subsequently exit from the *Compound Information Editor*. However, once you have saved the changes they cannot be undone and so be very careful not to change to a **Chemical ID** that already exists, unless you specifically mean to do that. If you recognize in time that you have made a mistake, click **Cancel** to exit from the editor.

Formula

The chemical **Formula** is used for display purposes only. There is no specific syntax for it and no checks are made on what you enter.

Chemical class

The **Chemical class** is used for display purposes only. There is no specific syntax for it and no checks are made on what you enter.

Ref. Ion

The signal-to-noise reference mass (**Ref. Ion**) is an integer m/z value. When an analysis is performed, the signal-to-noise value for **Ref. Ion** is computed for a found target and displayed as **S/N (m/z n)** in the *Information Lists* (**3.3.3.2 Target Mode Details**).

Retention...

When samples are acquired using a number of different gc columns and retention index data are being used, it is convenient to be able to create a single library of target compounds and store multiple, column-specific retention index values. This is supported for a *Target Compounds Library* only using the three **Retention** parameters (**Column**, **Index** and **Window**).

> **Note:** If activated, a particular column's values are used during an analysis (**Use retention index (RI) for column** parameter, **6.3.1 Identif. (Identification) Tab**).

...Index

The retention **Index** value for the compound; which can be expressed to a few decimal places.

...Window

The **Window** is unused.

...Time (RT)

The expected retention **Time (RT)** of the target in decimal minutes.

This value is never used to help locate targets during an analysis, but is simply used for reporting purposes as a QA/QC-type aid. For example, it can be presented as **Expec. R.T.** in the *Information Lists* **Match** values (**3.3.3.2 Target Mode Details**). Another example is in the **Results** section of the **QA/QC** tab of the *AMDIS Results* dialog where it is presented as **RT(expected)** (Additional Warnings, **5.5.1 Results**).

Ref. conc

Ref. conc is not applicable for a *Target Compounds Library* and so is not available to edit.

Resp. factor

In certain circumstances AMDIS will perform a basic concentration calculation for a found target (**8.4.1 Calculating a Concentration**). In that case, a target's response factor (**Resp. factor**) is used as a multiplier of the known concentration of the relevant internal standard.

Notes

Notes are available for your own use for any purpose and accept any text. They can only be viewed from within the *Compound Information Editor*.

Names and synonyms

The **Names and synonyms** accept any text and have one entry per line. The first line is the name by which the entry will be generally known and represented throughout AMDIS and all subsequent lines are synonyms.

> **Note:** Since you can edit anywhere within this area, you can easily modify or replace the first line to give the entry a new name.

Synonyms are also presented on the **Library** tab of the *AMDIS Results* dialog (**5.1 Library Tab**), but are otherwise unused within AMDIS.

8.2.5 Spectrum Editor

The *Spectrum Editor* is used to edit the spectral information for a *Target Compounds Library* only; as discussed in **8.1.1 Target Compounds Library (MSL)**. An entry's corresponding compound information is edited using the *Compound Information Editor* (**8.2.4 Compound Information Editor**).

> **Note:** All other library types utilize a combined spectral and information editor (**8.2.6 Combination Editors**).

Also, unlike the other libraries which only have one spectrum per compound entry, a *Target Compounds Library* may have multiple spectra per compound entry.
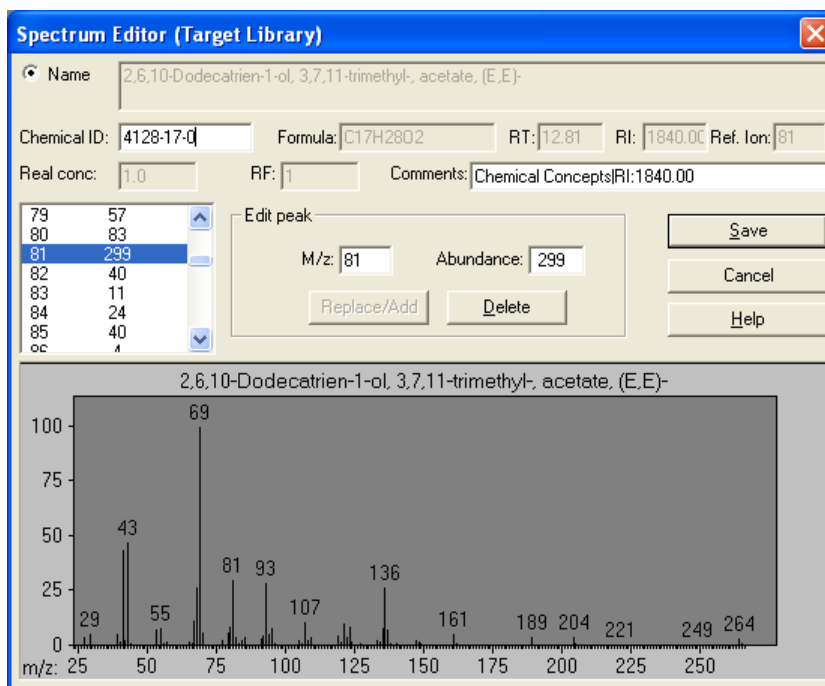
Figure 166 - Spectrum Editor

Although the primary reason for editing spectra is to correct chemical identification numbers when previously unknown species are identified and to add comments, the *Spectrum Editor* also allows you to edit a mass spectrum and to view the compound information entered using the *Compound Information Editor*.

Click **Save** to preserve any edits and exit from the editor; otherwise click **Cancel** to exit without saving any changes.

Name

The **Name** by which the compound will be generally known and represented throughout AMDIS. It can only be viewed here and is edited using the *Compound Information Editor*.

Chemical ID

The **Chemical ID** is a unique identifier for a compound entry of the library. For convenience the Chemical Abstracts Service Registry number (CAS number) is normally used when available, but any unique value can be utilized.

When an entry is first added to a library (other than by using the *Library Transfer* editor), it is assigned a generic name based on the data file it was taken from (**8.2.3.2.2 Add Button** and **8.2.3.2.3 Add All Button**). Typically you will want to modify this to something more meaningful, once known.

Since the **Chemical ID** is the crucial value used to identify entries within a library and in particular is the key value linking any multiple spectra of a single compound, care should be taken when assigning it a new value. <u>**Note in particular**</u> that if you assign it to a pre-existing value and click **Save**, there is no warning and the current spectrum becomes a replicate spectrum of the other compound. As such, any specific non-mass-spectral compound values (for example, RT, RI, Formula) you may have already entered for it are lost; since it inherits the other compound's values.

When you finally click **Save** to save any changes to the entry, if the **Chemical ID** has been modified to a new unique value a dialog appears asking you if you want to go directly to the *Compound Information Editor*. Enter **Yes** to do it now, or **No** if you wish to do it later.
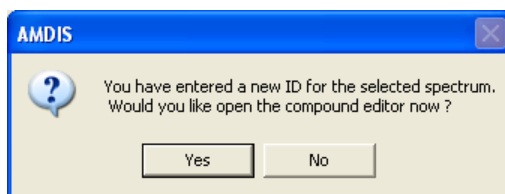
Figure 167 - Enter compound editor dialog

Note: If this spectrum was originally a replicate and you assigned it a new unique **Chemical ID**, it now has its own distinct compound identity. Although initially its compound values will be set as copies of the original compound's values, it is no longer a replicate of the original compound.

Formula

The chemical **Formula** of the compound. It can only be viewed here and is edited using the *Compound Information Editor*.

RT

The expected retention time (**RT**) of the compound. It can only be viewed here and is edited using the *Compound Information Editor*.

RI

The retention index (**RI**) of the compound. It can only be viewed here and is edited using the *Compound Information Editor*.

Ref. Ion

The signal-to-noise reference mass (**Ref. Ion**) of the compound. It can only be viewed here and is edited using the *Compound Information Editor*.

Real conc

Real conc is not applicable for a *Target Compounds Library* and so is not available to edit.

RF

The response factor (**RF**) of the compound. It can only be viewed here and is edited using the *Compound Information Editor*.

Comments

Comments are available for your own use for any purpose and accept any text. However, when an entry is first added to a library (other than by using the *Library Transfer* editor), it is assigned generic comments based on the data file it was taken from (**8.2.3.2.2 Add Button** and **8.2.3.2.3 Add All Button**).

M/z and **Abundance**

The **Edit peak** parameter group enables you to **Add**, **Replace** or **Delete** any m/z peak for the stored mass spectrum.

When you first enter the editor, the **Delete** button is unavailable for selection. The left button is labelled **Replace/Add** and is also unavailable. This button's label will subsequently change to read **Add** or **Replace**, according to what is appropriate based on your current input to **M/z** and **Abundance**.

If you want to delete a peak or modify its abundance you can simply select (highlight) it in the list of peaks to the left of the **Edit peak** parameter group; scrolling the list as necessary. This will place the selected values into the **M/z** and **Abundance** fields and the **Delete** button will be enabled for you to delete it. To modify its abundance simply enter a new **Abundance** value. At this point the **Delete** button will be disabled and the left button will be enabled and labelled **Replace**; click it to change the peak's abundance to the new value.

To add a peak that does not already exist, simply enter its **M/z** and provide its **Abundance**. You will notice that as you progressively enter the **M/z** value, the text of the left button will immediately reflect whether or not the currently shown **M/z** value already exists. If the value does not already exist, it will be **Add**; otherwise it will be **Replace**. Click **Add** when appropriate.

As you edit the spectrum you will notice that the graphical display of the spectrum changes to reflect your edits.

8.2.6   Combination Editors

Whereas a *Target Compounds Library* has separate editors for spectral and compound information (**8.2.5 Spectrum Editor** and **8.2.4 Compound Information Editor**) all other library types utilize a single combination editor.
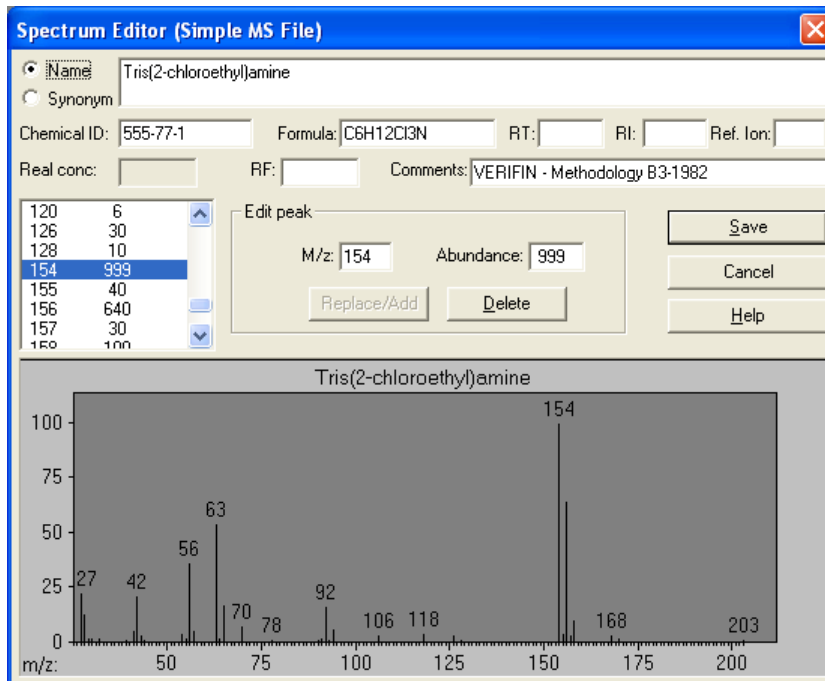
Figure 168 - Combination library editor

In the editor, all features are common to all library types; with the exception of **Synonym** and **Real conc** which are separately specific to a particular library type (as described below).

The exact effect of clicking the **Save** button is different for a *Calibration & Standards Library* (as described in **8.2.6.1 Saving Changes**), but in general you click it to preserve any edits and exit from the editor; otherwise click **Cancel** to exit without saving any changes.

Name

When you click on the **Name** radio button, the compound's name is presented in the text box to its right. This will accept any text and is the name by which the compound will be generally known and represented throughout AMDIS.

Synonym

The **Synonym** radio button is only seen in the editor for a *Mass Spectral Transfer* file. When you click on it, the compound's synonyms are presented in the text box to its right. It will accept any text and you can have multiple synonyms by inputting them one per line (using the Enter key).

Synonyms are also presented on the **Library** tab of the *AMDIS Results* dialog (**5.1 Library Tab**), but are otherwise unused within AMDIS.

Chemical ID

The **Chemical ID** is a unique identifier for a compound entry of the library. For convenience the Chemical Abstracts Service Registry number (CAS number) is normally used when available, but any unique value can be utilized.

When an entry is first added to a library (other than by using the *Library Transfer* editor), it is assigned a generic name based on the data file it was taken from (**8.2.3.2.2 Add Button** and **8.2.3.2.3 Add All Button**). Typically you will want to modify this to something more meaningful, once known.

Since the **Chemical ID** is the crucial value used to identify entries within a library, <u>care should be taken</u> when assigning it a new value. Since the value you enter is not checked, it is possible to set it the same as that of another entry in the library. Whereas this might be what you want (if the entries are replicate spectra for the same compound, for example), there is no automatic alignment of compound information (as with a *Target Compounds Library*).

> **Note:** If you were to subsequently transfer entries with the same **Chemical ID** to a *Target Compounds Library* (using the *Library Transfer* editor, for example), <u>only one set of compound information would be preserved</u> and applied to all replicate spectra.

Formula

The chemical **Formula** is used for display purposes only. There is no specific syntax for it and no checks are made on what you enter.

RT

The expected **RT** (retention time) of the target in decimal minutes.

This value is never used to help locate targets during an analysis, but is simply used for reporting purposes as a QA/QC-type aid. For example, it can be presented as **Expec. R.T.** in the *Information Lists* **Match** values (**3.3.3.2 Target Mode Details**). Another example is in the **Results** section of the **QA/QC** tab of the *AMDIS Results* dialog where it is presented as **RT(expected)** (Additional Warnings, **5.5.1 Results**).

RI

The retention index (**RI**) value for the compound; which can be expressed to a few decimal places.

Ref. Ion

The signal-to-noise reference mass (**Ref. Ion**) is an integer m/z value. When an analysis is performed, the signal-to-noise value for **Ref. Ion** is computed for a found target and displayed as **S/N (m/z n)** in the *Information Lists* (**3.3.3.2 Target Mode Details**).

Real Conc

Real conc is only available to edit for an *Internal Standards Library* as it only has significance for an internal standard compound and is the actual (real) concentration of that compound. It can be expressed to a few decimal places.

In certain circumstances AMDIS will perform a basic concentration calculation for a found target. In that case, the **Real conc** value of one internal standard is used as the basis for calculating all other concentrations. Details are given in **8.4 Using a Library for Concentration Calculations**.

RF

In certain circumstances AMDIS will perform a basic concentration calculation for a found target. In that case, a target's response factor (**RF**) is used as a multiplier of the known concentration (**Real conc**) of the relevant internal standard; which is itself adjusted by its own **RF**. Details are given in **8.4 Using a Library for Concentration Calculations**.

Comments

Comments are available for your own use for any purpose and accept any text. However, when an entry is first added to a library (other than by using the *Library Transfer* editor), it is assigned generic comments based on **the** data file it was taken from (**8.2.3.2.2 Add Button** and **8.2.3.2.3 Add All Button**).

M/z and **Abundance**

The **Edit peak** parameter group enables you to **Add**, **Replace** or **Delete** any m/z peak for the stored mass spectrum.

When you first enter the editor, the **Delete** button is unavailable for selection. The left button is labelled **Replace/Add** and is also unavailable. This button's label will subsequently change to read **Add** or **Replace**, according to what is appropriate based on your current input to **M/z** and **Abundance**.

If you want to delete a peak (for example, because it is an impurity) or modify its abundance, you can simply select (highlight) it in the list of peaks to the left of the **Edit peak** parameter group; scrolling the list as necessary. This will place the selected values into the **M/z** and **Abundance** fields and the **Delete** button will be enabled for you to delete it. To modify its abundance simply enter a new **Abundance** value. At this point the **Delete** button will be disabled and the left button will be enabled and labelled **Replace**; click it to change the peak's abundance to the new value.

To add a peak that does not already exist, simply enter its **M/z** and provide its **Abundance**. You will notice that as you progressively enter the **M/z** value, the text of the left button will immediately reflect whether or not the currently shown **M/z** value already exists. If the value does not already exist, it will be **Add**; otherwise it will be **Replace**. Click **Add** when appropriate.

As you edit the spectrum you will notice that the graphical display of the spectrum changes to reflect your edits.

8.2.6.1   Saving Changes

Except for a *Calibration & Standards Library*, when you click **Save** any changes you have made immediately become permanent (click **Cancel** if you want to exit without saving any changes).

However, for a *Calibration & Standards Library*, the *RI Calibration* dialog appears for you to set some additional values that are specific to compounds in this type of library.
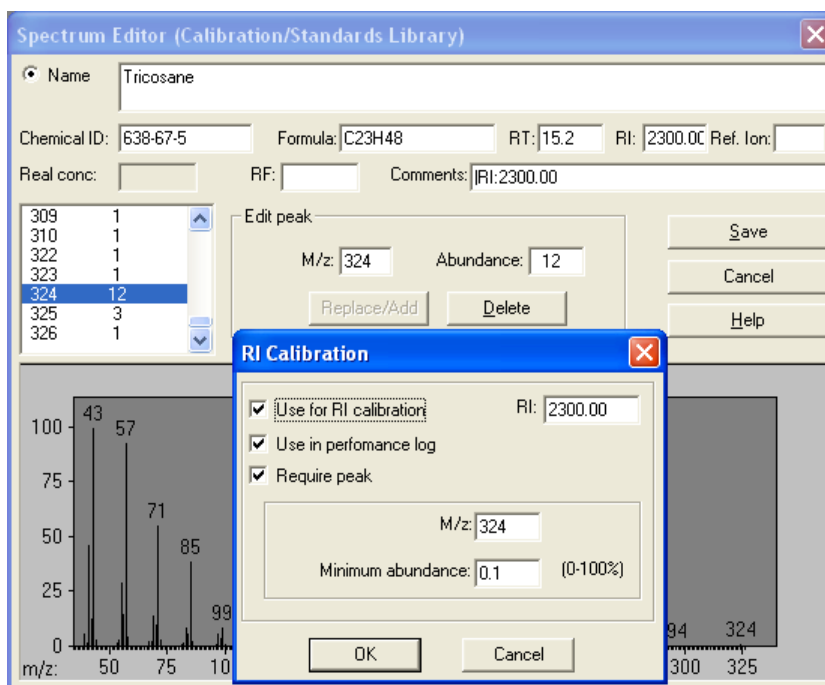
Figure 169 - RI Calibration dialog

If you want this compound to be used as a calibration compound in the RI calibration process, select the **Use for RI calibration** checkbox. Its use is described in **6.1.3 RI Calibration/Performance**. The retention index value (**RI**) is the same as shown in the main dialog and may be changed here also.

> **Note:**   If **RI** is set to zero or blank, **Use for RI calibration** is disabled. However, as soon as you enter a non-zero value, it is enabled again.

> **Note:**   If **RI** is non-zero, and you clear **Use for RI calibration**, the compound is not used for calibration purposes in a subsequent analysis. However, if you re-enter this dialog in the future (with **RI** still non-zero), it will revert to being set; the assumption here being that you probably want to use it because there is a non-zero value

If you want this compound to be used in tracking performance history, select the **Use in performance log** checkbox. Its use is described in **6.1.3 RI Calibration/Performance**.

Compounds in a *Calibration & Standards Library* may often be from an homologous series and so have very similar spectra. As an aid to correct identification of each you can select **Require peak** for an entry and specify that a particular **M/z** peak must be present (typically the molecular ion); and optionally above a **Minimum abundance** percentage. As a reminder, the **Abundance** value shown in the main editor dialog is an absolute value scaled to 1000.

Finally, to save any changes here and those in the main editor dialog, click **OK**. However, if you click **Cancel**, control is returned to the main editor dialog and only any changes in the *RI Calibration* dialog are cancelled. You would then click **Cancel** in the main editor dialog to cancel changes made in it (to the current entry only).

8.3   Using Libraries for AMDIS Calibration Purposes

If you wish to use retention indices as an additional aid in identifying targets, AMDIS relies on a separate calibration run to create an *RI Calibration Data* file; which establishes the correspondence between retention time and retention index. The compounds used when creating an *RI Calibration Data* file are held in a *Calibration & Standards Library* and are referred to as "retention index standards".

> **Note:**   The analysis types employing an *RI Calibration Data* file are shown in **Table 1 - Library types used for an analysis**.

There are two basic ways to set up a *Calibration & Standards Library*:

→   Use mass spectra of an acquired data file (**8.3.1 Creating a Calibration & Standards Library from a Data File**)

→   Use mass spectra from the NIST mass spectral database (**8.3.2 Creating a Calibration & Standards Library from the NIST Mass Spectral Database**)

8.3.1   Creating a Calibration & Standards Library from a Data File

A *Calibration & Standards Library* can be created from an acquired data file.

Generally the data file will be of the straight chain hydrocarbons (the "C series") in the range C6 to C30, or thereabouts; and so RI's in the range 600 to 3000, or thereabouts. The "C series" is particularly favored, since it provides a sequence of conveniently and regularly spaced chromatographic peaks. In fact, you may use any data file, but ideally it should contain a "series" of evenly spaced and distinctive calibration compounds; covering the RI range that you are interested in.

> **Note:**   A *Calibration & Standards Library* should have only one (ideally high quality) spectrum per calibration compound; but this is not enforced.

Proceed as follows:

1)   Acquire a high quality data file of the calibration compounds you wish to use.

2)   In the *Confirm* window, analyze the data file with **Type of analysis** set to **Simple** (**3.1.2.1 Analyze | Analyze GC/MS Data**).

> **Note:**   You can select any *Target Compounds Library* as it is irrelevant whether or not any targets are found.

3)   Select the **Mode | Component** command (**3.1.3.2 Mode | Component**). The top pane of the window will show the total ion chromatogram. Each of the acquired calibration compounds should have been deconvoluted and so represented as components. If the acquisition system was unusually noisy, you may see multiple deconvolutions of the same compound; which is not desirable. In the latter case you should ideally adjust your instrument conditions and acquire a less noisy file.

As mentioned above, it is not necessary for any components to have been identified as targets.

4)   Starting with the earliest eluting component of interest, click on the small triangle above its peak; alternatively, highlight its retention time in the *Information Lists* (**3.3.3.1 Component Mode Details**).

5)   With the first component highlighted, select the **Library | Build One Library** command to invoke the *Build One Library* editor.

Assuming that you are building a library for the first time, click the editor's **Files** button, followed by the **Create New Library** button. In the *Create New Library* dialog, set **Save file as type** to **Calibr. & Stds Library** (***.CSL**) and set **File name** to the name you wish to use for the new *Calibration & Standards Library*. Click **OK** to return to the *Build One Library* editor.

If you are modifying an existing library and it isn't the one currently shown in the editor, click the **Files** button, followed by the **Load Library** button and select it.

> **Note:** The *Build One Library* editor will stay on the screen until you choose to close it by clicking its **Exit** button. Of course you can move it around the screen in the normal *Windows* manner.

6) The label of the **Add** button identifies the retention time and data file name of the currently selected component (**8.2.3.2.2.1 Automatic Deconvolution**). Click the **Add** button to add that component to the library.

7) Highlight the newly added entry and click the **Edit** button to invoke the *Spectrum Editor (Calibration/Standards)* dialog (**8.2.6 Combination Editors**). In the editor enter appropriate values for the component. To be of subsequent use, the component's **RI** <u>must</u> be entered. Although not essential, it is a very good idea at this time to enter the component's **Name**, **Chemical ID** and **Formula**. Also add **Comments** if you wish.

8) Click the **Save** button to invoke the *RI Calibration* dialog (**8.2.6.1 Saving Changes**). The **Use for RI calibration** checkbox will be selected unless you didn't specify an **RI** value in the previous dialog. If an **RI** value was not previously specified, you must do that now; else the compound will not be used subsequently for RI calibration purposes.

For homologous series components especially (since they are so very similar), it is very useful to specify a certain characteristic (usually high mass) m/z peak that must be present in a component spectrum to subsequently identify a component as being that compound. To do this select **Require peak** and specify an **M/z** value (typically the molecular ion) and optionally a **Minimum abundance** percentage value. Only if a component has the requested **M/z** with an abundance greater than the **Minimum abundance**, will it be identified as a match.

> **Note:** Care should be taken in specifying the **Minimum abundance** value. Bearing in mind the fluctuation of intensity between acquisitions, do not set it too high such that it could erroneously exclude a real match.

If you also want the compound to be used for performance logging purposes, select the **Use in performance log** checkbox (**8.5 Using the Performance Log to Track Instrument Performance**).

Click **OK** to save any changes and exit back to the *Build One Library* editor.

9) Select the next component of interest and repeat steps (6) to (8) until you have saved all of the calibration compounds to the library.

10) Click **Exit** when you are ready to exit from the *Build One Library* editor.

8.3.2 Creating a Calibration & Standards Library from the NIST Mass Spectral Database

In general it is a good idea to use high quality mass spectra from your own instrument when adding compounds to any library. However, there may be situations where this is not possible or desirable. In that case, you can add one or more spectra from the NIST mass spectral database; or any other library supported by the NIST *MS Search* program.

> **Note:** A similar method to that discussed here can be used to create or modify a library from any text file that conforms to the NIST *Mass Spectral Transfer* file's format; discussed briefly in **14.2 Mass Spectral Transfer File (MSP) File Format**.

When specific details are given in the procedure below for the NIST *MS Search* program, it is for version 2.0. If you have a different version, the same principles still apply. If you are unfamiliar with the specific program facilities being utilized here, you may wish to use the program's help system to locate information on "export".

Proceed as follows:

1) In the NIST *MS Search* program locate one or more NIST mass spectral database entries that you wish to have in your *Calibration & Standards Library*.

> **Note:** For version 2.0 you could utilize the **Names** sub-tab of the **Lib. Search**, **Other Search**, **Names** or **Librarian** main tabs.

2) Use standard *Windows* selection techniques to select (highlight) the one (or more as appropriate) compounds that you wish to have in your library.

   Now use the NIST *MS Search* program's "export" command to write the selection to a *Mass Spectral Transfer* file in a location and with a name of your choosing. You will be warned if the selected name already exists and given a chance to avoid overwriting it.

   > **Note:** For version 2.0 you right-click on a highlighted entry to access the export command.

3) Repeat steps (1) and (2), as required, until you have a number of *Mass Spectral Transfer* files representing the spectra to be included in an AMDIS library.

4) In AMDIS, select the **Library | Transfer Library** command to invoke the *Library Transfer* editor.

5) Assuming that you are building a library for the first time, click the editor's **Files** button, followed by the **Create New Library** button. In the *Create New Library* dialog, set **Save file as type** to **Calibr. & Stds Library (*.CSL)** and set **File name** to the name you wish to use for the new *Calibration & Standards Library*. Click **OK** to return to the *Library Transfer* editor.

   If you wish to modify an existing library and it isn't the one currently shown as the "destination" (**To** value) in the editor, click the Files button, followed by the **Destination** group's Load Library button and select it.

   > **Note:** The *Library Transfer* editor will stay on the screen until you choose to close it by clicking its **Exit** button. Of course you can move it around the screen in the normal *Windows* manner.

6) Now click the editor's **Files** button again, followed by the **Source** group's **Load Library** button. In the *Select Source Library* dialog, ensure that **Files of type** is set to **Simple MS File (*.MSP)** and observe that the files you created in step 2 are all listed.

7) Set **File name** to one of the names you wish to use for the copying of its entries to the new *Calibration & Standards Library* and click **Open**. Now click **Close** to return to the *Library Transfer* editor.

8) Use the standard functionality of the editor to transfer all of the entries from the source library to the destination library (**8.2.3.2.4 Transfer Button**).

9) Highlight a transferred entry in the destination library and click the **Edit** button to invoke the *Spectrum Editor (Calibration/Standards)* dialog (**8.2.6 Combination Editors**). In the editor enter appropriate values for the component. To be of subsequent use, the component's **RI** <u>must</u> be entered. Although not essential, it is a very good idea at this time to enter the component's **Name**, **Chemical ID** and **Formula**. Also add **Comments** if you wish.

10) Click the **Save** button to invoke the *RI Calibration* dialog (**8.2.6.1 Saving Changes**). The **Use for RI calibration** checkbox will be selected unless you didn't specify an **RI** value in the previous dialog. If an **RI** value was not previously specified, you must do that now; else the compound will not be used subsequently for RI calibration purposes.

   If you re adding compounds that are very similar (for example, from an homologous series), it is very useful to specify a certain characteristic (usually high mass) m/z peak that must be present in a component spectrum to subsequently identify a component as being that compound. To do this select **Require peak** and specify an **M/z** value (typically the molecular ion) and optionally a **Minimum abundance** percentage value. Only if a component has the requested **M/z** with an abundance greater than the **Minimum abundance**, will it be identified as a match.

   > **Note:** Care should be taken in specifying the **Minimum abundance** value. Bearing in mind the fluctuation of intensity between acquisitions, do not set it too high such that it could erroneously exclude a real match.

   If you also want the compound to be used for performance logging purposes, select the **Use in performance log** checkbox (**8.5 Using the Performance Log to Track Instrument Performance**).

   Click **OK** to save any changes and exit back to the *Library Transfer* editor.

11) Repeat steps (6) thru (10) if you have more *Mass Spectral Transfer* files to transfer entries from.

12) Click **Exit** when you are ready to exit from the *Library Transfer* editor.

8.4 Using a Library for Concentration Calculations

You can use AMDIS to perform a basic concentration calculation by utilizing a suitably prepared *Internal Standards Library* and by selecting the *Use Internal Standards for RI* or *Use RI Calibr. Data + Internal Std.* analysis type (**Table 1 - Library types used for an analysis**).

> **Note:** If this basic concentration calculation is inadequate for your purposes, you may be able to take advantage of the **File | Generate Report** command (**3.1.1.8 File | Generate Report**) to create a simple tab-delimited format, text file of results which you can subsequently import into a data analysis program for processing.

To calculate a concentration, AMDIS requires <u>one compound only</u> in the *Internal Standards Library* to have a real concentration <u>and</u> response factor. Further, each compound for which you want a concentration calculating must have a response factor value in the target library (*Target Compounds Library* or *Mass Spectral Transfer* file).

> **Note:** It is possible for more than one internal standard to have a real concentration and response factor. However, in that case only one will be used and that will be the one found at the lowest retention time.

Computational details are given in **8.4.1 Calculating a Concentration**.

The resultant concentrations of targets are presented as **Conc.** in the *Information Lists* **Match** values (**3.3.3.2 Target Mode Details**).

To prepare the *Internal Standards Library* for use in calculating concentrations proceed as follows:

1) In the normal manner use either of the library editors to create an *Internal Standards Library* containing the internal standard compounds you wish to use (**8.2 Library Editors** and **8.1.3 Internal Standards Library (ISL)**).

2) In the library editor, locate the entry you will be using for concentration calculations and use the **Edit** button to enter the combination editor for it (**8.2.6 Combination Editors**).

3) Set **Real conc** to the actual concentration of the internal standard that will be spiked into the sample(s) you will subsequently analyze.

4) You must now set **RF** to a suitable value. Often you will set it to **1.0** for easy comparison with the **RF** values you set in the target library. However, this is not mandatory and you may set any positive, non-zero value.

5) If you wish to prepare more than one internal standard compound for possible use, repeat steps (2) to (4), but remember that <u>only the earliest eluting compound</u> that is located will be used.

To prepare the *Target Compounds Library* or *Mass Spectral Transfer* file for having concentrations calculated for its compounds, proceed as follows:

1) In the normal manner use either of the library editors to create a *Target Compounds Library* or *Mass Spectral Transfer* file containing the target compounds you wish to use (**8.2 Library Editors** , **8.1.1 Target Compounds Library (MSL)** and **8.1.5 Mass Spectral Transfer File (MSP)**).

2) In the library editor, locate a compound whose concentration you wish to have calculated during an analysis and use the **Edit** button to enter the appropriate editor for it (**8.2.4 Compound Information Editor** or **8.2.6 Combination Editors**).

3) Set **Resp. factor** (*Target Compounds Library*) or **RF** (*Mass Spectral Transfer* file) to the response factor of this compound relative to the response of the internal standard compound set up for concentration calculations in the *Internal Standards Library*.

4) Repeat steps (2) and (3) for any further compounds.

8.4.1 Calculating a Concentration

Target compound concentrations will be calculated by AMDIS during an analysis when all of the following conditions are met:

1) An *Internal Standards Library* has been prepared with normally just one compound having a specified concentration (**Real conc**) and response factor (**RF**) that are both greater than zero. If more than one compound is set up in this way, the values for the earliest eluting internal standard compound that is found during the analysis will be used.

2) A *Target Compounds Library* or *Mass Spectral Transfer* file has been prepared with response factor values (**Resp. factor** or **RF**, respectively) greater than zero for all compounds whose concentrations you want computing.

3) The analysis type is *Use Internal Standards for RI* or *Use RI Calibr. Data + Internal Std.* and the chosen libraries satisfy the conditions of (a) and (b) above.

4) When the analysis is run, an internal standard compound must be found that has **Real conc** and **RF** values that are both greater than zero. Target compound concentrations will then be calculated for found targets having response factor values greater than zero.

A target compound's concentration is computed as follows:

$\text{Conc}_{target} = (\text{Conc}_{standard} * \text{RF}_{standard} / \text{RF}_{target}) * (\text{Area}_{target} / \text{Area}_{standard})$

The computed concentrations are presented as **Conc.** in the *Information Lists* **Match** values (**3.3.3.2 Target Mode Details**).

Information relating to processed internal standards can be seen in:

a) The **Standards** tab of the *AMDIS Results* dialog (**5.4.2 Internal Standard Mode**).

b) The **QA/QC** tab of the *AMDIS Results* dialog (**5.5.1 Results**).

c) In the *Information Lists* by selecting **Show standards** in **Identif.** tab of the *Analysis Settings* dialog (**6.3.1 Identif. (Identification) Tab**).

8.5   Using the Performance Log to Track Instrument Performance

The Performance Log file is a cumulative record over time of pertinent information that helps you to track the performance of your instrument and so provides some level of quality control.

It records information for those compounds in a *Calibration & Standards Library* that are flagged as "performance check compounds" and are found as targets when the library is used for an *RI Calibration/Performance* or *Performance Check for RI* analysis.

The information that is stored for each found compound is:

a) its observed retention time;

b) its computed retention index;

c) its model peak's height, width and tailing values;

d) its match factor;

e) the acquisition date and time and the name of the data file used for the analysis.

This information can be viewed at any time on the **QA/QC** tab of the *AMDIS Results* dialog (**5.5.1.3 Scan Sets Information**).

The practical steps involved in performance monitoring will be similar to the following:

1) Initially acquire a clean mixture of known composition containing one or more compounds that you wish to use for performance check purposes. For example, a classic and widely used performance mixture is the "Grob Mixture"; which can be purchased ready-made.

2) If you don't already have a *Calibration & Standards Library* that has been set up with "calibration check compounds", create one (**8.3 Using Libraries for AMDIS Calibration Purposes**).

3) Manually identify a "performance check compound" in the data file. For this you may wish to utilize the **Analyze | Search NIST Library** (**3.1.2.8 Analyze | Search NIST Library**) or **Analyze | Go to NIST MS Program** (**3.1.2.6 Analyze | Go to NIST MS Program**) commands.

4) In the *Build One Library* editor, open the *Calibration & Standards Library* that you are planning to use and add the identified compound to the *Calibration & Standards Library*; typically by using the **Add** button (**8.2.3.2.2 Add Button**).

5) Highlight the newly added entry and click the **Edit** button to invoke the *Spectrum Editor (Calibration/Standards)* dialog (**8.2.6 Combination Editors**). In the editor enter appropriate values for the compound. Although not essential, it is a very good idea at this time to enter the compound's Name, Chemical ID and Formula. Also add Comments and the compound's RI if you wish.

6) Click the **Save** button to invoke the *RI Calibration* dialog (**8.2.6.1 Saving Changes**).

Select the Use in performance log checkbox to flag this as a "performance check compound".

If required, select Require peak and specify an M/z value (typically the molecular ion) and optionally a Minimum abundance percentage value. Only if a component has the requested M/z with an abundance greater than the Minimum abundance, will it be identified as a match.

> **Note:** Care should be taken in specifying the Minimum abundance value. Bearing in mind the fluctuation of intensity between acquisitions, do not set it too high such that it could erroneously exclude a real match.

Click **OK** to save any changes and exit back to the *Build One Library* editor.

7) Repeat steps (3) to (6) until you have saved all of the "performance check compounds" to the library.

8) Click **Exit** when you are ready to exit from the *Build One Library* editor.

9) Whenever you are ready to record performance data, prepare a sample with all (or some) of the performance check compounds in it and then do a *Performance Check for RI* analysis (**6.1.7 Performance Check for RI**).

Alternatively you can do the performance check at the same time as creating an *RI Calibration Data* file; using the *RI Calibration/Performance* analysis (**6.1.3 RI Calibration/Performance**).

8.6 Viewing a Library

Of the five library types supported in AMDIS (**8.1 Library Types**), all but the *RI Calibration Data* file can be viewed and edited using either of the library editors (**8.2 Library Editors**).

However, the **View** button of the *Analysis Settings* dialog's **Libr.** tab can be used to view (but not edit) any of the five library types (**6.3.4 Libr. (Libraries) Tab**).

Once an AMDIS analysis has been performed, you can view library information relating to its results by using the **Library** tab (**5.1 Library Tab**) in either the *Confirm* window's or *Results* window's auxiliary information area (**5 Auxiliary Information**). The *Target Compounds Library* or *Mass Spectral Transfer* file used for the most recent analysis can also be viewed (but not edited) using the tab's **View** button.

9   Batch Jobs

A batch Job file is essentially a list of data files, where each data file has its associated analysis instructions. Job files are created, modified and initiated to run in an automated manner using the **File | Batch Job | Create and Run Job** command (**9.1 Create and Run Job**). During processing a report can be automatically created. The results of the last run Job file can be examined using the **File | Batch Job | Show Results of Last Job** command (**9.2 Show Results of Last Job**).

>   **Note:**   Job files are only available for the *Confirm* window.

When a Job file is run, its list of data files is analyzed (processed) one at a time and in sequence. Each data file has its own associated analysis type, but all files share a single set of analysis settings and these are the analysis values current at the time the Job file is run. A shared option allows you to specify whether or not a cumulative report (text file) is to be generated and, if so, whether all hits or only a specified number per analysis are to be included in it.

9.1   Create and Run Job

The **Create and Run Job** command is used to create new Job files, modify existing Job files and to run a Job file. An option exists to sequentially write the analysis results of the processed files to a tab-delimited format, text file; suitable for import into data analysis programs.

Click **File | Batch Job | Create and Run Job** to invoke the *Build Job File* dialog.
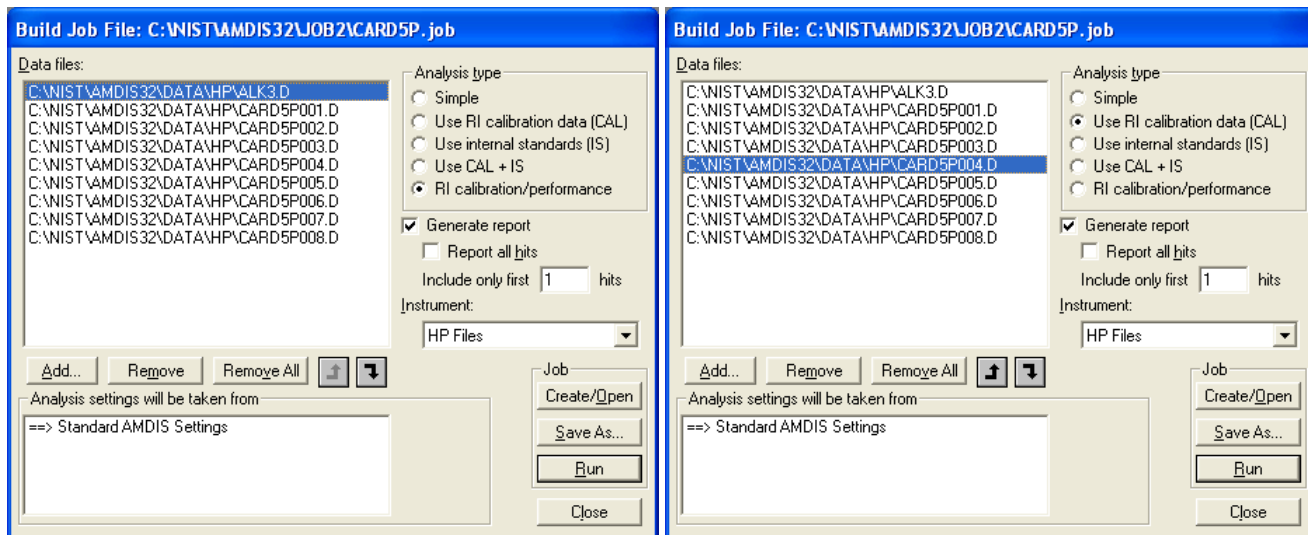
Figure 170 - Build Job File dialog

On entry to the *Build Job File* dialog the **Data files** list box will show the files of the most recently accessed Job file; whose name is displayed in the title bar of the dialog (**CARD5P.job** in the figure).

You can now choose to:

a)   create a new Job file (**9.1.1 Creating a New Job File**);

b)   modify an existing Job file (**9.1.2 Modifying an Existing Job File**);

c)   run a Job file (**9.1.3 Running a Job File**).

9.1.1   Creating a New Job File

You can either create a new Job file from scratch, or by copying an existing one.

To create a new Job file from scratch, proceed as follows:

a)   Click the **Create/Open** button in the *Build Job File* dialog (**Figure 170 - Build Job File dialog**) to invoke the *Open* dialog.

b)   In the *Open* dialog, ensure that **Files of type** is set to **Job Files (*.job)**. As necessary, set **Look in** to the folder where you wish the Job file to be stored.

c)   Enter a new name of your choice as **File name**.

d)   Click **Open** to exit from the *Open* dialog.

e)   The *Build Job File* dialog redisplays with the **Data files** list box empty.

f)   Now use the dialog's **Add** button to add the first data file and continue using the dialog's editing capabilities (**9.1.2.1 Editing Options**).

g)   When you have finished editing, simply click **Save As** to invoke the *Save As* dialog and immediately click **Save** to complete the creation of the Job file and to return to the *Build Job File* dialog.

To create a new Job file by copying an existing one, proceed by first opening the existing one as described in **9.1.2 Modifying an Existing Job File**. Now click **Save As** to invoke the *Save As* dialog, specify the **File name** of the new file and click **Save** to complete the creation of the Job file and to return to the *Build Job File* dialog

9.1.2   Modifying an Existing Job File

To modify an existing Job file, proceed as follows:

a)   Click the **Create/Open** button in the *Build Job File* dialog (**Figure 170 - Build Job File dialog**) to invoke the *Open* dialog.

b)   In the *Open* dialog, ensure that **Files of type** is set to **Job Files (*.job)**. As necessary, set **Look in** to the folder where the Job file to be modified is stored and set **File name** to its name.

c)   Click **Open** to exit from the *Open* dialog.

d)   The *Build Job File* dialog redisplays with the **Data files** list reflecting the selected Job file.

e)   Now use the dialog's editing capabilities to modify the file (**9.1.2.1 Editing Options**).

f)   When you have finished editing, simply click **Save As** to invoke the *Save As* dialog and immediately click **Save** to complete the modification of the Job file and to return to the *Build Job File* dialog.

9.1.2.1   Editing Options

Having created a new Job file (**9.1.1 Creating a New Job File**), or opened an existing one (**9.1.2 Modifying an Existing Job File**), the basic editing process in the *Build Job File* dialog (**Figure 170 - Build Job File dialog**) is similar to the following:

a)   Select the **Instrument** used to acquire your data files and adjust the **Data files** list using the **Add**, **Remove** or **Remove All** buttons. Ensure **Analysis type** is set appropriately after each file is added (**9.1.2.1.1 Manipulating the Data Files List**).

b)   Select the **Generate report** checkbox if you want a textual report file created and then specify how many hits are to be stored (**9.1.2.1.2 Report Generation**).

c)   Click **Save As** to save changes and then click **Run** to initiate the processing and creation of the result file and, if requested, report file (**9.1.3 Running a Job File**). Otherwise, click **Close** to exit from the dialog without processing.

> **Note:**   If you have made changes to a Job file and then click **Close** or **Run** without having first saved the changes, you are warned and given the opportunity to save or discard the changes before proceeding; or to return to the *Build Job File* dialog.

9.1.2.1.1   Manipulating the Data Files List

To add a data file to the **Data files** list in the *Build Job File* dialog (**Figure 170 - Build Job File dialog**), click **Add**. The features of the invoked *Select Data File* dialog are almost identical to those of the **File | Open** command's dialog and are described in detail for it (**3.1.1.1 File | Open**). One difference is that this dialog permits multiple file selection (using standard *Windows* techniques). When you click **Open** to exit from the *Select Data File* dialog, all selected files are added to the end of the **Data files** list.

As you add files to the **Data files** list, each is added with the **Analysis type** indicated by the current radio button selection. You can change the **Analysis type** of a data file at any time. Simply highlight one or more files in the

Data files list (using standard *Windows* multi-selection techniques) and click the appropriate radio button to assign that **Analysis type** to all selected files.

> **Note:** As you highlight a data file, the **Analysis type** radio button selection will change, as necessary, to reflect the value currently assigned to the file.

When a job file is run, the data files are processed in sequence and so their order may be important. For example, the first file might be used to create an *RI Calibration Data* file, which the others may then utilize with a *Use Retention Index Data* or *Use RI Calibr. Data + Internal Std.* analysis.

To reorder files, two arrow-head type buttons are used; up-arrow ⬆ and down-arrow ⬇. First of all highlight the file you want to reposition. One or both of these buttons will be enabled; according to which direction it is possible to move the file. Click the appropriate button to move it one position up or down in the list and repeat as necessary for this file and then for any others.

To remove one or more data files from the **Data files** list, highlight the file(s) and click **Remove**. If you wish to remove every data file in the list, simply click **Remove All** at any time.

9.1.2.1.2 Report Generation

When a Job file is run, it can write the analysis results of the processed files to a tab-delimited format, text file; suitable for import into data analysis programs.

In the *Build Job File* dialog (**Figure 170 - Build Job File dialog**), select **Generate report** to activate this.

You can now choose to report all hits for each target by selecting **Report all hits**. If instead, you clear its checkbox, **Include only first <n> hits** is enabled for you to select how many of each target's top hits you want reporting.

As each data file is analyzed, its results are appended to the report file.

> **Note:** If no targets are found for a particular data file, no information is written to the report file for that data file.

The report file is created in the same folder as the Job file and has the same root name as it, but with a ".txt" extension. For example, the report file for *CARD5P.job* would be named *CARD5P.TXT*.

> **Note:** If a report file of that name already exists, it will be overwritten without warning.

9.1.3 Running a Job File

A Job file is run by clicking **Run** in the *Build Job File* dialog (**Figure 170 - Build Job File dialog**).

> **Note:** If you have made changes to a Job file and then click **Run** without having first saved the changes, you are warned and given the opportunity to save or discard the changes before proceeding.

Processing starts with the first data file in the **Data files** list and continues in sequence until all files have been analyzed. The analysis is identical to that of the **Run** button's operation (**3.2.1 Run**) and will use the current analysis settings, as set up using the full facilities of the *Analyze GC/MS Data* dialog (**3.1.2.1 Analyze | Analyze GC/MS Data**), but with the exception that the type of analysis is determined by the **Analysis type** stored in the Job file for each data file.

Depending on the chosen analysis type and the complexity and amount of the GC/MS data, an analysis could take several seconds. In that case you would see the progress dialogs, which include a **Cancel** button.

If you click **Cancel**, the analysis will stop for the current data file and no further data files from the Job file will be processed. However, the results for all of its prior data files will be preserved, as will the current state of any requested report file.

When the run has finished, the *Results of Last Batch Job* dialog appears and lists the processed files. It is the same dialog as that invoked with the **File | Batch Job | Show Results of Last Job** command and is described in **9.2 Show Results of Last Job**.

9.2 Show Results of Last Job

The **Show Results of Last Job** command displays the data files that were processed by the most recent Job file run (**9.1.3 Running a Job File**). By clicking on an individual file name, the results of its analysis are

immediately displayed in the normal manner in the *Confirm* window as though the analysis run had just completed its processing. As such, the results may be manipulated fully using standard functionality.

Click **File | Batch Job | Show Results of Last Job** to invoke the *Results of Last Batch Job* dialog.
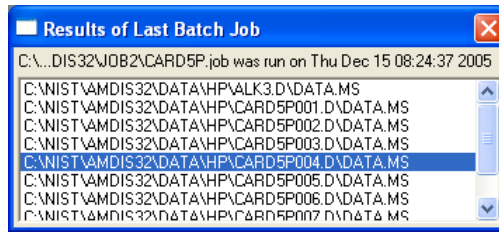
Figure 171 - Results of Last Batch Job dialog

To view the analysis results for a particular file, simply click on its name in the dialog and the AMDIS display will change to show the results.

If the Job file run was cancelled, the *Results of Last Batch Job* dialog will show an error code.
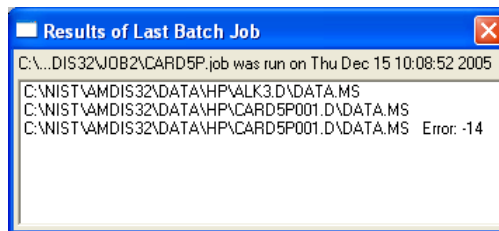
Figure 172 - Results display for cancelled Job run

All files listed prior to the cancelled file can still be selected as normal to view their results.

10 Manual Background Subtraction and Manual Deconvolution

Manual background subtraction and manual deconvolution are techniques that enable you to semi-manually create a spectrum from a data file.

These techniques apply to the *Confirm* window only and are only available when results do not already exist for the currently displayed data file. In this case, the **Manual-On** and **Deconv** buttons appear in the button bar.
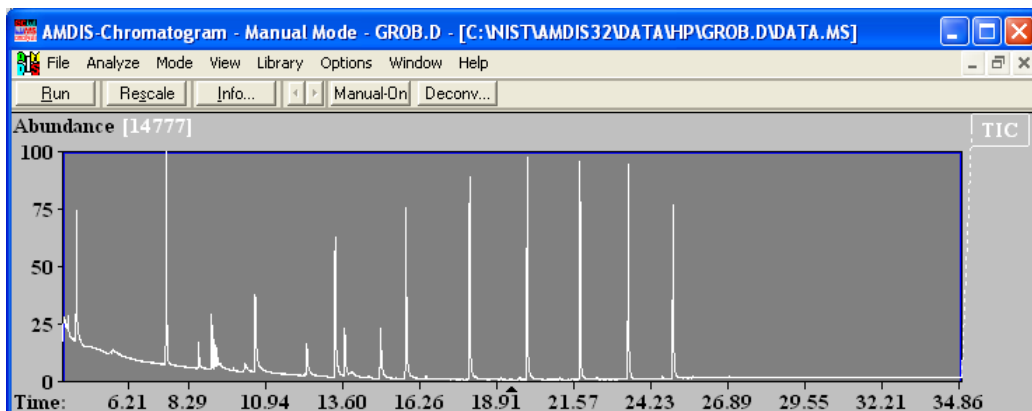
Figure 173 - Manual mode available

Click **Manual-On** to activate manual background subtraction (**10.1 Manual Background Subtraction**) or click **Deconv** for manual deconvolution (**10.2 Manual Deconvolution**).

Having generated a spectrum manually, typically you might then perform an immediate library search against the NIST mass spectral database (**3.1.2.6 Analyze | Go to NIST MS Program**) and perhaps add the spectrum to one of the various AMDIS library types (**8.2.3.2.2 Add Button**).

10.1 Manual Background Subtraction

The manual background subtraction functionality is provided for those situations where you wish to attempt to manually create a good quality spectrum from a total ion chromatogram. For example, where the automatic deconvolution analysis has generated an unknown component spectrum that you wish to identify, but the spectrum does not give good results when searched against the NIST mass spectral database. Alternatively, you may wish to simply create a high quality spectrum for addition to an AMDIS library.

If results do not already exist for the currently displayed data file, the *Confirm* window's button bar has the **Manual-On** button available to enable the semi-manual creation of a spectrum. Click **Manual-On** and note that the button label changes to **Manual-Off**. Simply click the button at any time to alternate between having manual subtraction mode off and manual subtraction mode on.
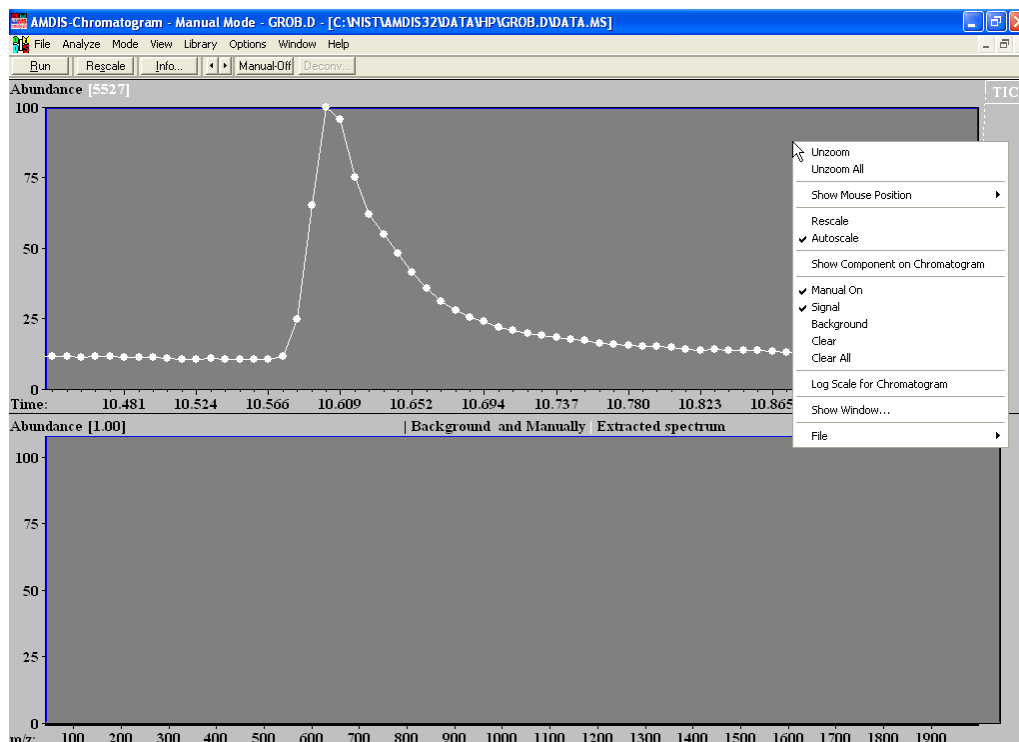
Figure 174 - Manual subtraction mode active; showing context menu

With manual subtraction activated, you can right-click in the *Chromatogram Display* to show the context menu. This is fully described in **3.3.1.1 Context Menu**. For manual subtraction we are only concerned about the group of commands **Manual On**, **Signal**, **Background**, **Clear** and **Clear All**.

The **Manual On** command has a check mark next to it when it is active and no check mark when it is not. Its setting reflects the state of the button bar's button and clicking on either alters the setting for both.

When **Manual On** is not checked, the other 4 commands are shown as being unavailable for selection; conversely when it is checked, all 4 are available.

> **Note:** When one of the **Signal**, **Background** or **Clear** commands is clicked, a check mark appears to its left. However, since these 3 menu commands are mutually exclusive, only one can be checked at any one time and so the check mark next to the previously checked command is removed.

The basic process for performing background subtraction is similar to the following:

a) Open a data file that does not already have results and expand the display about the chromatographic peak of interest.

> **Note:** If the data file already has results when you open it, you will need to delete them. To do this, first ensure that the *AMDIS Options* dialog is either set to automatically delete files, or to ask you about that (**3.1.1.6 File | Options**). Open a different data file and, if asked, choose to delete the results for the current file. Now reopen your chosen file.

b) Click the button bar's **Manual-On** button; or right-click in the *Chromatogram Display* and click the **Manual On** command. The mouse pointer will change appearance to reflect the current selection of **Signal**, **Background** or **Clear** in the context menu.

Signal	Background	Clear
(Peak top range selector)	(Peak background range selector)	(Peak range clearer)
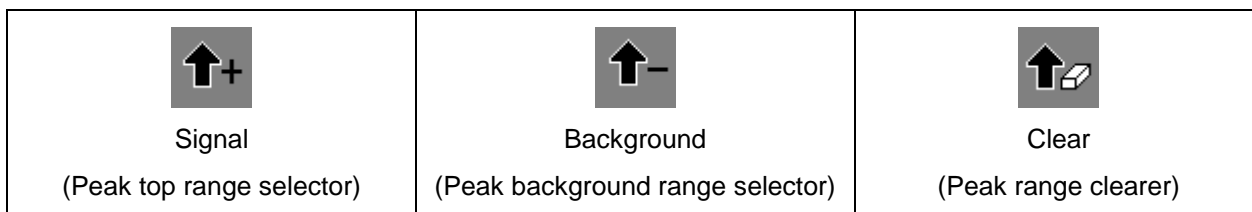
Figure 175 - Mouse pointers for manual subtraction ranges

c) If **Signal** does not already have a check mark next to it, click it and note that the mouse pointer appears as a thick black up-arrow with a black plus symbol (**+**) to its right (**Figure 175 - Mouse pointers for manual subtraction ranges**).

d) Position the mouse pointer to one edge of the range of scans you wish to average to represent the peak top. Select the range of scans by doing click-drag-release with the left mouse button and note that a red line appears just below the x-axis to reflect the chosen range and there is a small black triangle that shows the position of the averaged spectrum. The spectrum itself is displayed in the *Mass Spectral Display* as white peaks.

> **Note:** If you do not see the *Mass Spectral Display* area, it is because it is not selected for viewing. Use the *Show Window* dialog to select it (**3.1.4.1 View | Show Window**).
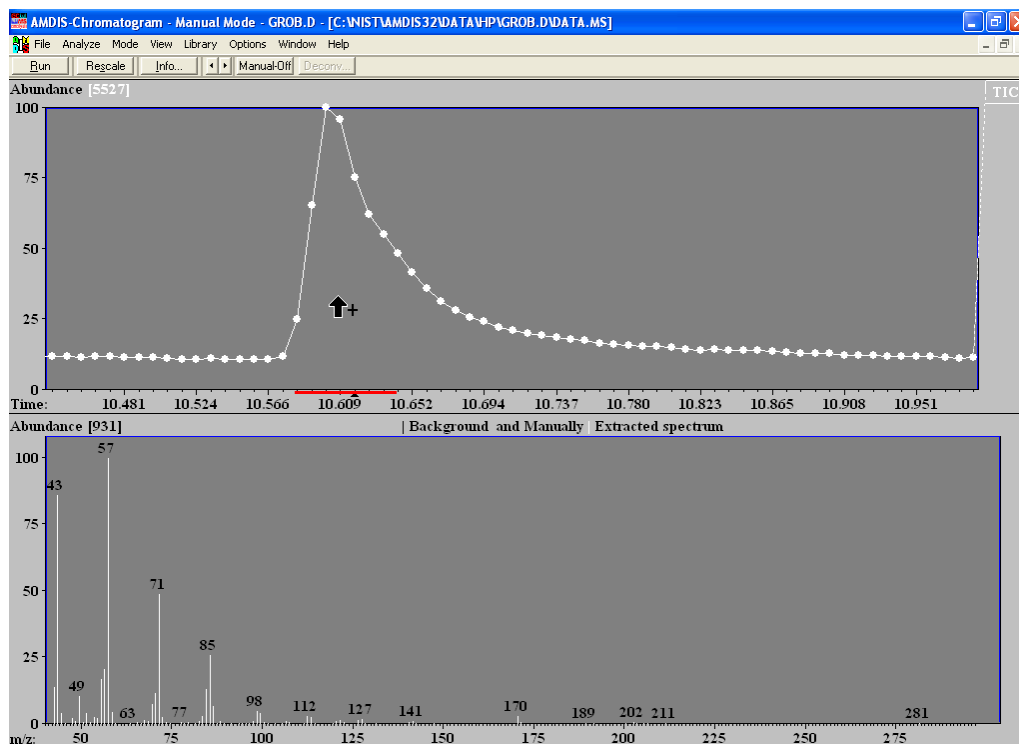
Figure 176 - Peak top range selected

e) Right click in the *Chromatogram Display* to activate the context menu and click **Background**. Note that the mouse pointer now appears as a thick black up-arrow with a black minus (-) symbol to its right (**Figure 175 - Mouse pointers for manual subtraction ranges**).

f) Position the mouse pointer to one edge of a range of scans you wish to average to represent the peak background. Select the range of scans by doing click-drag-release with the left mouse button and note that a blue line appears just below the x-axis to reflect the chosen range. The background subtraction process is automatic and immediately subtracts the averaged background spectrum from the averaged peak top spectrum and the resultant spectrum is displayed in the *Mass Spectral Display* as black peaks.

Now you may typically choose another background range to the other side of the peak (in fact you can choose several ranges, if you wish). Each time you choose an additional range the subtraction is

automatic and uses the average of <u>all</u> background scans in however many background ranges are selected.
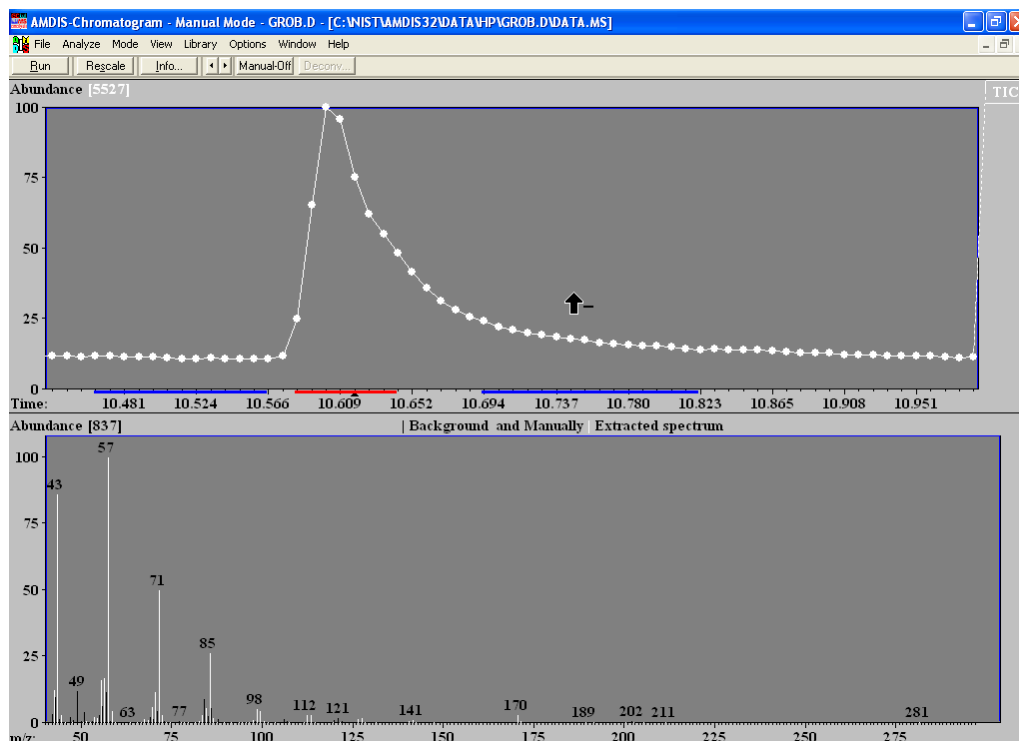
Figure 177 - Two background ranges selected

g) If you want to modify any of the selected ranges (peak top or background), right click in the *Chromatogram Display* to activate the context menu and click **Clear**. Note that the mouse pointer now appears as a thick black up-arrow with a white eraser icon to its right (**Figure 175 - Mouse pointers for manual subtraction ranges**).

h) Position the mouse pointer to one edge of a range of scans you wish to remove from the average process. Select the range of scans by doing click-drag-release with the left mouse button and note that the corresponding portion of red or blue line below the x-axis disappears. The background subtraction process is again automatic and adjusts for the new range of scans.

i) If you want to completely and instantly clear all range selections, you can conveniently do that by right clicking in the *Chromatogram Display* to activate the context menu and clicking **Clear All**. Note that, since there are now no scans to average, this will also clear the *Mass Spectral Display*.

Having generated a spectrum manually, typically you might then perform an immediate library search against the NIST mass spectral database (**3.1.2.6 Analyze | Go to NIST MS Program** or from the context menu of the *Mass Spectral Display* **3.3.3.3.1 NIST Library**). You may choose to add the spectrum to one of the various AMDIS library types (**8.2.3.2.2 Add Button**).

If you wish to select a different area of the chromatogram to work on, it is necessary to first click the **Manual-Off** button. You are then able to unzoom the *Chromatogram Display*, and then zoom in on a new peak. Finally click **Manual-On** to return to manual subtraction mode.

10.2 Manual Deconvolution

The manual deconvolution functionality is provided for those situations where you wish to manually perform a deconvolution using a model peak of your choice. The model peak shape can either be determined from the chromatogram trace of a single m/z you choose, or it can be a shape drawn by you. You can optionally select one or two m/z's or draw peak shapes (in any combination) to form the basis of subtraction for adjacent peaks.

The resultant extracted spectrum can be searched against the NIST mass spectral database, or alternatively added to an AMDIS library.

> **Note:** As a general rule you will do better by letting AMDIS select the model peaks during its automatic deconvolution process (**3.2.1 Run**).

If results do not already exist for the currently displayed data file, the *Confirm* window's button bar holds the **Manual-On/Manual-Off** button and the **Deconv** button. If **Manual-Off** is showing, click it so that it becomes **Manual-On**; otherwise **Deconv** will not be available for selection.

Ensure that the *Chromatogram Display* and *Mass Spectral Display* areas are both selected for viewing (**3.1.4.1 View | Show Window**).

Click **Deconv** to invoke the *Deconvolution* dialog.
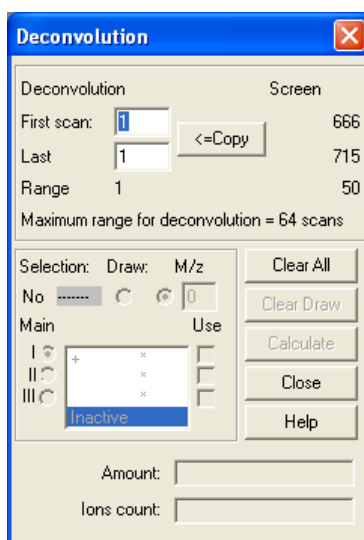
Figure 178 - Blank Deconvolution dialog

The top section of the dialog is where you select the range of scans to be used for the deconvolution. In order for you to easily see your deconvoluted component shape and also since there is a limit of 64 scans, it is advisable to zoom the display to the area of interest prior to entering the dialog.

The start and end scan numbers of the display on entry to the dialog are shown under **Screen**, as is the scan range these represent. If this range is 64 or less, the **Copy** button is available for selection. If you click **Copy**, it sets up the **First scan**, **Last** and **Range** values for the **Deconvolution** to reflect the **Screen** values. At any time you can manually enter values for **First scan** and **Last**. A horizontal red line beneath the chromatogram axis indicates the currently chosen range.

In the center section of the dialog the **Selection** controls allow you to define the exact model to be used in the deconvolution process. Under **Main** are the three model selections you can make (**I**, **II** and **III**) to form the model peak; where the two whose radio buttons are not selected are subtracted from the one whose radio button is selected. Each model selection can be flagged as being drawn manually, or given an m/z value. To do this, first click in the boxed area to the right of the **I**, **II** and **III** radio button that you want to set up, such that a highlight bar is shown.

> **Note:** You will not be able to do this if the settings of **First scan** and **Last** do not have a **Range** value of 64 or less.

If you want to use an m/z, click on the radio button under **M/z** and either enter a value directly for it, or click on a peak in the *Mass Spectral Display* area (**10.2.1 Selecting the Model Peak from the Spectrum**).

If instead you want to draw the model peak, click the **Draw** radio button and then draw it manually in the *Chromatogram Display* area (**10.2.2 Drawing a Model Shape**).

As **I**, **II** and **III** are defined, their corresponding **Use** checkbox is automatically selected. You cannot alter the checkbox selection for the one who radio button is selected, but you can for the other two and you would do this

if you want to experiment with different combinations. Similarly, by clicking on the radio button of one selection, it becomes the model from which the other two are subtracted.

Note: It is not possible to use a drawn model as the model from which others are subtracted.

The boxed area summarises the selections by showing the appropriate m/z for each, or **Draw**, if it is drawn. On the left is a plus (+) or minus (-) symbol to show how the subtraction will be performed.

Note: If a drawn peak is not yet drawn, an asterisk (*) will be there instead of **Draw**.

Finally, the color that is used in the *Chromatogram Display* for the highlighted selection is shown underneath **Selection** as the color of the dashed line shown there (for example, selection **III** is yellow in the figure).
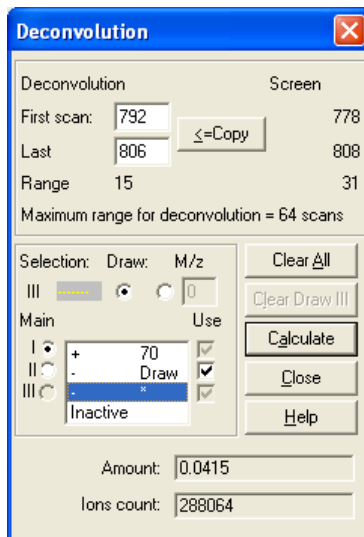
Figure 179 - Deconvolution dialog showing model selections

You can click **Clear All** at any time to cancel all model selections and their values so you can start again.

If you have drawn a model, you can highlight it and then click the **Clear Draw n** button; where n is one of **I**, **II** or **III**, according to which model you selected. This reverts the drawn model to its default state along the retention time axis.

The **Calcuate** button is available for selection whenever there is a valid model selection. When clicked, it takes account of the model selections in **Use** and the subtraction indicators (+ and -), computes a component using the selected scan **Range** and displays the chromatogram and spectral results (**10.2.3 Interpreting the Component Display**).

The computed **Amount** and **Ions count** are shown; where **Amount** is the area of the component (**Ions count**) relative to the total ion count for the entire chromatogram, expressed as a percentage.

Click **Close** at any time to exit the dialog.

10.2.1 Selecting the Model Peak from the Spectrum

To provide an m/z value for a model selection in the *Deconvolution* dialog, you can either directly enter its value in the **M/z** field, or you can position the mouse cursor in the *Mass Spectral Display* and click it at the required m/z value. The *Chromatogram Display* also shows the chromatogram trace of the selected m/z.

Note: To remove an m/z trace, click on its colored numeric identifier to the right of the trace display area.

As the cursor moves into the *Mass Spectral Display* area it changes shape and also indicates which selection is being made by showing a I, II or III; for example, in the figure it shows II and the selection of m/z 43.

Note: The m/z selected is the closest integer value to exactly where the mouse cursor is positioned, rather than the nearest mass spectral peak to its left. As such, you may wish to first zoom the display range one or more times by doing a click-drag-release type operation below the m/z axis.
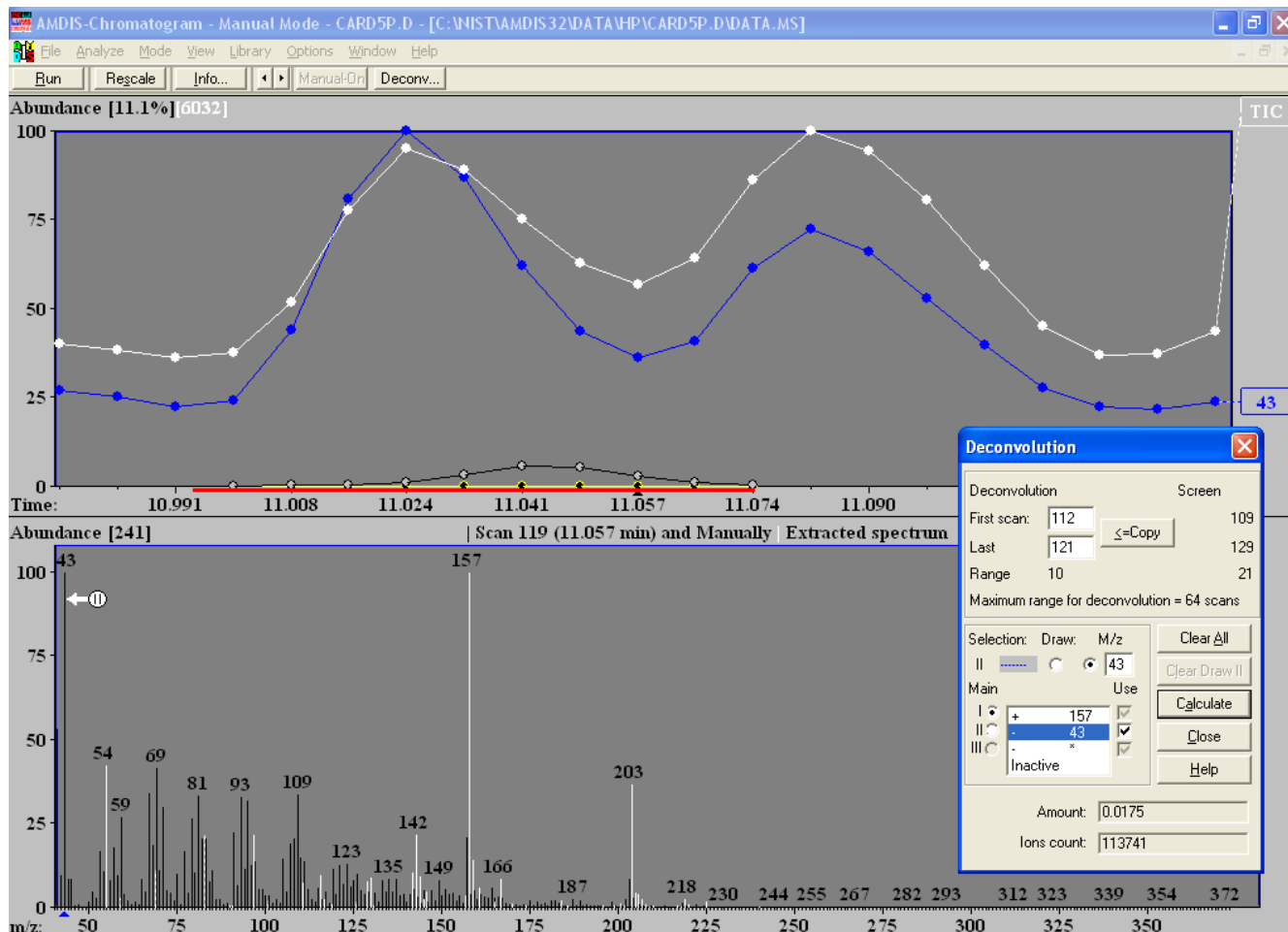
Figure 180 - Selecting an m/z value from a spectrum

If you make a mistake or change your mind, simply reposition the mouse cursor and click again.

To select the m/z for another model selection, first highlight it in the *Deconvolution* dialog and then click your chosen m/z peak in the *Mass Spectral Display*.

To learn more about what is being displayed see **10.2.3 Interpreting the Component Display**.

10.2.2   Drawing a Model Shape

If you have chosen to manually draw a model shape, the line representing the shape starts off as a horizontal line on the retention time axis and is the width of the deconvolution **Range**. The line is actually divided into segments between each scan; where each scan is represented as a large dot in the same way as all other chromatograms (but only if the displayed range is small enough).

As the cursor moves into the deconvolution range in the *Chromatogram Display* area it changes to a pencil shape and also indicates which selection you are drawing by showing a I, II or III; for example, in the figure it shows III.

To modify a model shape, simply click in the display at the point you wish to select for the contribution of the scan to the cursor's right and that scan's dot is positioned there and the two adjacent line segments adjust accordingly. Continue this process until the shape you require has been drawn.

The figure shows model selection **III** drawn as a yellow line. In this case it represents (models) the contribution of the larger peak on the right to the small, but distinct, component (centered near to 11.041 minutes) that is obscured by the larger peak on the left.
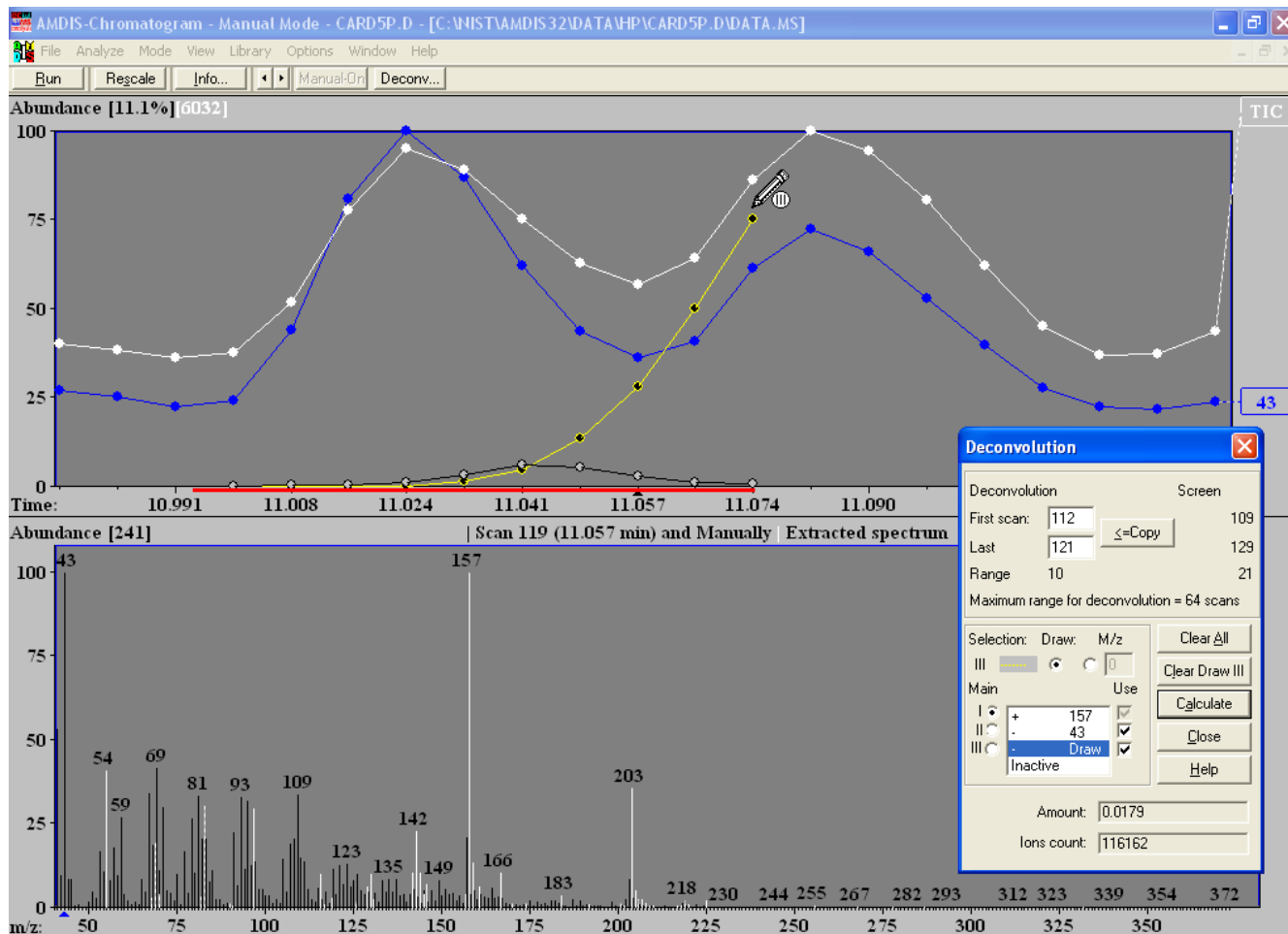
Figure 181 - Drawing a model shape

A quick way of starting with a fresh line drawing is to click the **Clear Draw n** button in the *Deconvolution* dialog; where n is one of **I**, **II** or **III**, according to which model you selected. This resets the model shape to the default flat line along the axis.

To learn more about what is being displayed see **10.2.3 Interpreting the Component Display**.

10.2.3 Interpreting the Component Display
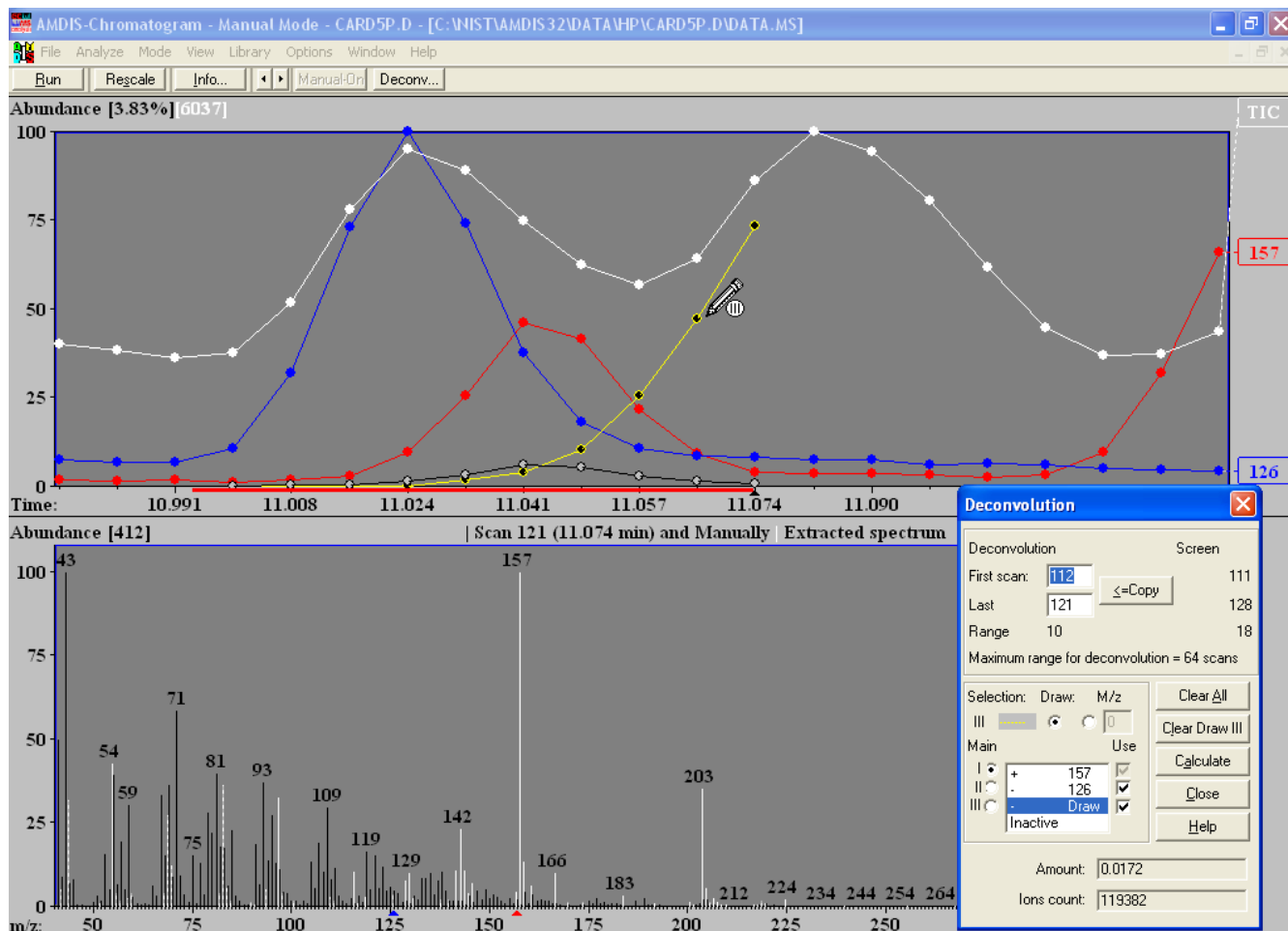
Figure 182 - Component calculation using all 3 model selections

For manual deconvolution, you need to be able to see the *Chromatogram Display* area and the *Mass Spectral Display* area. If you cannot see both, use the **View | Show Window** command to select them (**3.1.4.1 View | Show Window**).

The *Chromatogram Display* area typically shows:

a)   The TIC trace in white. Normally this will be scaled to 100% on the y-axis.

b)   The single mass chromatograms of model selection m/z's. These are colored the same as shown for their **Selection** in the *Deconvolution* dialog.

c)   The trace of any drawn model selections. These are colored the same as shown for their **Selection** in the *Deconvolution* dialog. However, you can visibly differentiate them from m/z selections since drawn traces show the large "dots" with a black centre.

d)   The component trace. This is always shown as a black line with light-centered large "dots" and is initially drawn horizontally along the chromatogram axis. Each time you click **Calculate** in the *Deconvolution* dialog, it will be redrawn to show the component's shape.

e)   A horizontal red line beneath the chromatogram axis to indicate the currently chosen deconvolution range.

For futher information about the *Chromatogram Display* in general, see **3.3.1 Chromatogram Display**.

The *Mass Spectral Display* area shows:

a)   The original spectrum as black m/z peaks.

b)   The extracted spectrum as white m/z peaks; with "uncertain" peaks drawn as a dashed line. Each time you click **Calculate** in the *Deconvolution* dialog, it will be recomputed and redrawn.

Uncertain peaks are those ions in a mass spectrum that AMDIS finds impossible to assign to a specific component due to the uncertain deconvolution of the mass spectral data. For futher information about uncertain peaks and about the *Mass Spectral Display* in general, see **3.3.4 Mass Spectral Display**.

Each time you click **Calculate** in the *Deconvolution* dialog, the *Chromatogram Display* and *Mass Spectral Display* areas change to reflect the new component. Similarly, the dialog itself shows the **Amount** and **Ions count** values for the component; where **Amount** is the area of the component (**Ions count**) relative to the total ion count for the entire chromatogram, expressed as a percentage.

Note: **Amount** and **Ions count** are the same as **Amount** and **Area**, respectively, described in **3.3.3.1 Component Mode Details**. If both are displayed as zeroes, this is probably because you have selected a drawn model in the *Deconvolution* dialog as the **Main** model (**10.2 Manual Deconvolution**).

11 How Do I?

This chapter attempts to answer some of the common questions that you might have about AMDIS and is divided into topic sections for easier reference; as follows:

System Setup   see **11.1 System Setup**

Data Analysis   see **11.2 Data Analysis**

Library Maintenance   see **11.3 Library Maintenance**

Display Modification see **11.4 Display Modification**

11.1 System Setup

This section answers some common questions relating to System Setup. See:

11.1.1 How Do I Change the Settings for An Analysis?

11.1.2 How Do I Find Out What Settings Were Used for the Current Analysis?

11.1.1 How Do I Change the Settings for An Analysis?

The analysis parameters can be set from both the *Results* window and the *Confirmation* window.

In the *Results* window first select the **Analyze** button in the *Control Panel* (**4.1 Control Panel**).

In the *Confirmation* window first select the **Analyze | Analyze GC/MS Data** command (**3.1.2.1 Analyze | Analyze GC/MS Data**).

In either case, the *Analyze GC/MS Data* dialog is invoked to allow you to set all of the parameters of the analysis.

To achieve this click the **Settings** button to invoke the *Analysis Settings* dialog, which is introduced in **3.1.2.2 Analyze | Settings**, but whose parameters are described in detail in **6.3 Analysis Settings**.

After making any changes, click **Save** to exit from the dialog and the new analysis parameter values will be saved.

See also **6 Analysis**.

11.1.2 How Do I Find Out What Settings Were Used for the Current Analysis?

The analysis parameters relating to the analysis results currently being examined can be viewed in both the *Results* window and the *Confirmation* window.

The *Results* window has a dedicated information area (**4.3 Information Tabs**).

The *Confirm* window has two ways of getting access to this information (**3.1.4.6 View | Auxiliary Information** and **3.2.3 Info**), both of which present the *AMDIS Results* dialog.

In either case, click the **Settings** tab to see the settings that were used.

See also **5 Auxiliary Information**.

11.2 Data Analysis

This section answers some common questions relating to Data Analysis. See:

11.2.1 Analysis Without Retention Index or Retention Time Data (Simple Analysis)

11.2.2 Analysis With Retention Index Data

11.2.3 Analysis With Retention Time Data

11.2.4 How Do I Examine an Old Analysis?

11.2.5 How Do I Use Data for Calibration and Performance Monitoring?

11.2.1   Analysis Without Retention Index or Retention Time Data (Simple Analysis)

For a detailed example of an analysis that does not utilize retention index or retention time data, see **2.7 Analysis Type - Simple**.

11.2.2   Analysis With Retention Index Data

For a detailed example of an analysis using retention index data, see **2.9 Analysis Type - RI Calibration/Performance** and **2.10 Analysis Type - Use Retention Index Data**.

11.2.3   Analysis With Retention Time Data

For a detailed example of an analysis using retention time data, see **2.8 Analysis Type - Use Retention Time**.

11.2.4   How Do I Examine an Old Analysis?

From the *Results* window, the **Load Results** button will bring up a dialog box to allow you to select the previous results (**4.1.6 Load Results**).

In the *Confirm* window, **File | Open** will allow you to bring in data that have been analyzed as well as new data (**3.1.1.1 File | Open**). If the file has been analyzed, the program will automatically find the associated analysis files.

11.2.5   How Do I Use Data for Calibration and Performance Monitoring?

Only data in the *Calibration & Standards Library* can be used for calibration and performance monitoring.

To make the data available for use you must mark the spectrum for Calibration (if used to establish retention index calibration) and/or Use in Performance Log (if used for the Performance Log file).

See **8.3 Using Libraries for AMDIS Calibration Purposes** and **8.5 Using the Performance Log to Track Instrument Performance**.

See also **8.1.2 Calibration & Standards Library (CSL)**.

11.2.6   How Do I Search the NIST Database with a Component?

Once the component is selected either by selecting the small triangle above the TIC peak or by selecting the retention time in the retention time list, click **Analyze | Go to NIST MS Program** (**3.1.2.6 Analyze | Go to NIST MS Program**). You must have the NIST *MS Search* program and NIST mass spectral database installed on the computer.

You may also select a series of components to be analyzed at once. To do this select a component and then click **Analyze | Add Component/Scan to Search List (n)**. The number in parenthesis behind the menu item **(n)** indicates the number of components already in the search list. Repeat this for each component of interest (**3.1.2.5 Analyze | Add Component/Scan to Search List (n)**).

If there are any components in the search list, the **Analyze | Go to NIST MS Program** command will now act on the search list and not on the current component.

When all of the desired components have been added to the search list, **Go to NIST MS Program** will switch to the NIST *MS Search* program. Proceed with search by double clicking on component in the Clipboard.

11.3   Library Maintenance

This section answers some common questions relating to Library Maintenance (available only in the *Confirm* window). See:

11.3.1 How Do I Add Spectra to a Library?

The ability to add a spectrum from a GC/MS data file to a library can only be done from within the *Confirm* window.

First select the component peak you wish to add to the library; either by clicking on the small triangle above the peak, or by selecting it from the list of retention times. Once the peak is highlighted, click **Library | Build One Library** from the menu bar (**3.1.5.1 Library | Build One Library**).

The highlighted peak's details will be displayed on a large **Add** button at the top left of the dialog box. Clicking that button will add the spectrum to the current library (**8.2.3.2.2 Add Button**). Alternatively, you can first select a different library by clicking the **Files** button (**8.2.3.2.1 Files Button**).

Similar actions can be taken with the destination library when using the **Library | Library Transfer** command (**3.1.5.2 Library | Library Transfer**).

For more details about library operations see **8.2.1 Build One Library Editor** and **8.2.2 Library Transfer Editor**.

11.3.2 How Do I Delete Spectra From a Library?

The ability to delete a spectrum from a library can only be done from within the *Confirm* window.

First click **Library | Build One Library** from the menu bar (**3.1.5.1 Library | Build One Library**).

Use the **Files** button to select the library you want to edit (**8.2.3.2.1 Files Button**).

Highlight the spectrum (or spectra) you want to delete and then click the **Delete** button (**8.2.3.2.6 Delete Button**).

Similar actions can be taken with the destination library when using the **Library | Library Transfer** command (**3.1.5.2 Library | Library Transfer**).

For more details about library operations see **8.2.1 Build One Library Editor** and **8.2.2 Library Transfer Editor**.

11.3.3 How Do I Edit Spectra or Compound Information in a Library?

The ability to edit a spectrum or compound information in a library can only be done from within the *Confirm* window.

To edit a spectrum or compound information in the library, first click **Library | Build One Library** from the menu bar (**3.1.5.1 Library | Build One Library**).

Use the **Files** button to select the library you want to edit (**8.2.3.2.1 Files Button**).

Although the basic editing capabilities are very similar for all library types, a *Target Compounds Library* has separate compound and spectrum editors, whereas the other types have a single, combined functionality editor.

Highlight the spectrum you want to edit and then click the **Edit** button (**8.2.3.2.5 Edit Button**).

For library types other than a *Target Compounds Library* you go straight to the appropriate combination editor (**8.2.6 Combination Editors**).

However, for a *Target Compounds Library* an intermediate *Edit* dialog appears. To edit spectrum information click **Spectrum** to enter the *Spectrum Editor* for a *Target Compounds Library* (**8.2.5 Spectrum Editor**). To edit compound information click **Compound** to enter the *Compound Information Editor* for a *Target Compounds Library* (**8.2.4 Compound Information Editor**) and note that any edits to compound information will affect all replicate spectra with the same compound identification number.

Similar actions can be taken with the destination library when using the **Library | Library Transfer** command (**3.1.5.2 Library | Library Transfer**).

For more details about library operations see **8.2.1 Build One Library Editor** and **8.2.2 Library Transfer Editor**.

11.4   Display Modification

This section answers some common questions relating to Display Modification. See:

11.4.1   How Do I Magnify (Zoom) a Plot?

This applies only to the *Confirm* window.

Often the *Chromatogram Display* will be very "crowded" with target and component symbols overlapping. In order to see the detail of the chromatogram(s) and to easily choose (by clicking on it) a specific target or component symbol, it is necessary to first "zoom" (magnify) the display. Similarly, you may wish to zoom the *Mass Spectral Display* to see greater detail of the mass spectral peaks.

By using the "click-drag-release" technique, the display can be zoomed individually in either the x-direction or the y-direction, or in both directions simultaneously.

"Click-drag-release" is as follows. First click and hold the left mouse button at one end of the desired linear range, or one corner of the desired rectangular area. Now drag the mouse pointer to the opposite end or corner of the desired selection and finally release the button expand the selected area to the full size of the plotting area.

To unzoom click the right mouse button while the mouse pointer is in the plotting area and select **Unzoom** on the menu that appears (**3.3.1.1.1 Unzoom**). If you have done multiple zooms, you can step back one at a time using **Unzoom**, or use **Unzoom All** to go back to the full display (**3.3.1.1.2 Unzoom All**).

See also **2.4.5 Changing the Display Range**.

11.4.2   How Do I Switch Between Log and Linear Abundance Axis?

This applies only to the *Confirm* window.

In the *Chromatogram Display*, *Profile Display* and *Mass Spectral Display* you can choose to display the abundance axis with a log or linear scale.

With the mouse pointer in the display area that you want to change, click the right mouse button to display the "context sensitive" command menu.

In the *Chromatogram Display* select the **Log Scale for Chromatogram** command (**3.3.1.1.5 Log Scale for Chromatogram**).

In the *Profile Display* select the **Log Scale for Component** command (**3.3.2.1.2 Log Scale for Component**).

In the *Mass Spectral Display* select the **Log Scale for Spectrum** command (**3.3.4.1.2 Log Scale for Spectra**).

If the log scale is already active for a display area, the command will have a check mark next to it. In this case selecting the command will remove the check mark and redraw the abundance axis with a linear scale. Otherwise, selecting the command will place a check mark next to it and redraw the abundance axis with a log scale.

11.4.3   How Do I Change Colors and Weight of Lines?

This applies only to the *Confirm* window.

The **View | Thick Lines** command will toggle between thin and thick lines in all display areas (**3.1.4.4 View | Thick Lines**).

For the *Mass Spectral Display* area the **View | Color Selected Peaks** determines how mass spectral peaks are colored (**3.1.4.5 View | Color Selected Peaks**).

The **Options | Color** command invokes the *Color* dialog that allows all of the colors of the AMDIS window to be set (**3.1.6.3 Options | Color**).

11.4.4   How Do I Find the Exact Position of a Peak?

This applies only to the *Confirm* window.

With the mouse pointer in a *Chromatogram Display*, *Profile Display* or *Mass Spectral Display* area, click the right mouse button. From the command menu that is shown, click **Show Mouse Position** to select to show the location of the mouse on a small box attached to the mouse (**On Cursor**), in a dialog above the plot (**On Top**) or not at all (**Off**). The most information is given when **On Top** is selected.

See **3.1.4.3 View | Show Mouse Position**.

11.4.5   How Do I Show Components on a Plot?

This applies only to the *Confirm* window.

With the mouse pointer in the *Chromatogram Display* or *Profile Display* area, click the right mouse button. From the command menu that is shown, select **Show Component on Chromatogram** for the *Chromatogram Display* area (**3.3.1.1.7 Show Component on Chromatogram**) or **Show Component** for the *Profile Display* area (**3.3.2.1.4 Show Component**).

11.4.6   How Do I Change the Data that are Shown or Printed About a Component?

In the *Results* window or the *Confirm* window, place the mouse over the **Component** or **Match** areas of the *Information Lists* area. Click the right mouse button to bring up a selection of **View Options** and **Print Options**.
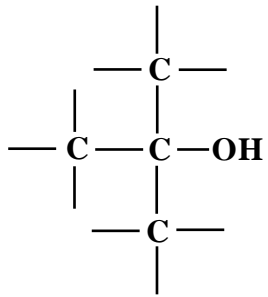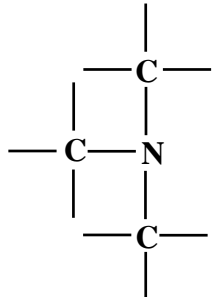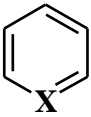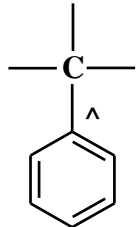
Highlight and click the one you wish to change. From within the dialog box that is invoked, select the items and the order in which you wish them to be presented.
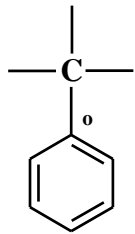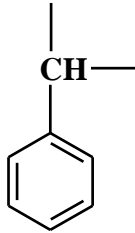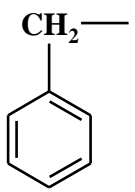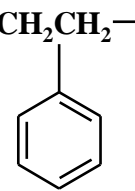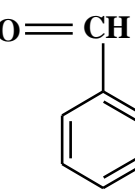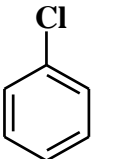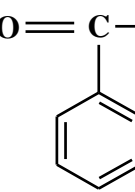
See **3.3.3.3.4 View Options** and **3.3.3.3.5 Print Options**.

12   Compound Classification Analysis
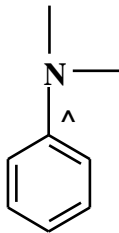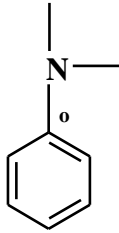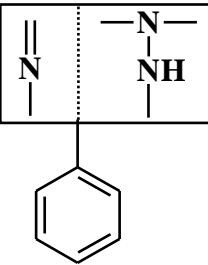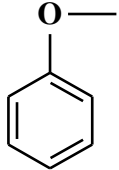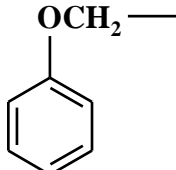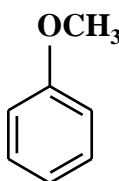
The brief name that is given in AMDIS is shown in the second column, the structure is shown in the first column and a description is given in the third column. Note that the description differentiates between those classifiers that define functional groups (func), specific elements (elem), aromatic (aromatic) groups, and alkyl (alkyl) groups. There are a few classifiers that describe bonds (bond).
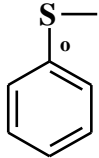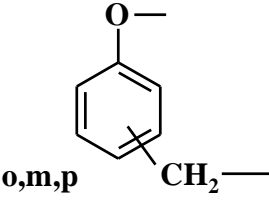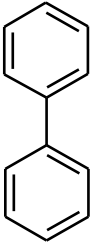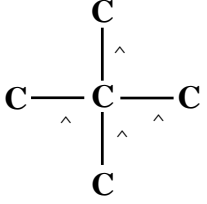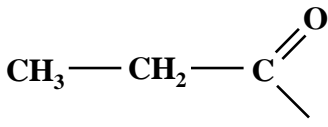
These classifications are based on work done at The Technical University of Vienna; see: Varmuza K., Werther W.: "Mass spectral classifiers for supporting systematic structure elucidation," *J. Chem. Inf. Comput. Sci.*, **36**, 323-333 (1996).
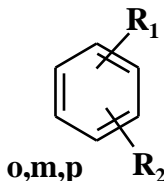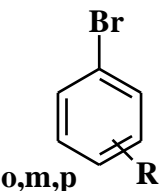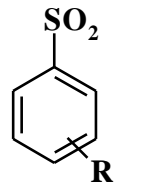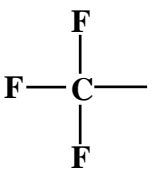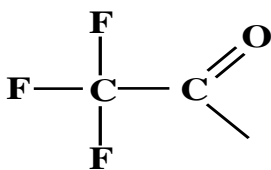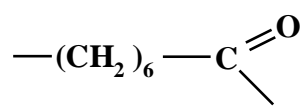
Structure	Name	Description
	Alc tert	func: alcohol tertiary (no ester)
	Am tert	func: amine tertiary (no amide)
Condensed aromatic rings	ar cond	aromatic: condensed rings
	ar het	aromatic: hetero-aromatic
More than one aromatic ring (any type)	ar poly	aromatic: more than 1 aromatic ring (any type)
	ar-C ch	aromatic: aryl - C (chain-bond)
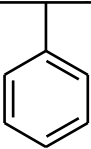
Structure	Name	Description
	ar-C r	aromatic: aryl - C (ring bond)
	ar-CH	aromatic: aryl - CH
	ar-CH2	aromatic: aryl - -CH2 or -CH3
	ar-CH2CH2	aromatic: aryl - CH2-CH2
	ar-CHO	aromatic: aldehyde aryl-CH=O
	ar-Cl	aromatic: aryl - Cl
	ar-CO	aromatic: aryl - C=O

Structure	Name	Description
O-C, O=C, N=N (aryl attached)	ar-CO,N2	aromatic: aryl - -C-O or -C=O or -N=N
$O=C-CH_2-$ (aryl)	ar-CO-CH2	aromatic: aryl - CO-CH2
$O=C-O-$ (aryl)	ar-COO	aromatic: aryl - COO (benzoic acid/ester)
$O=C-O-CH_2-$ (aryl, o,m,p R)	ar-COOCH2*	aromatic: ester C6H4-COO-CH2- (subst. at o,m,or p)
F (aryl)	ar-F	aromatic: aryl - F
N (aryl)	ar-N	aromatic: aryl - N
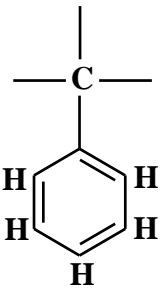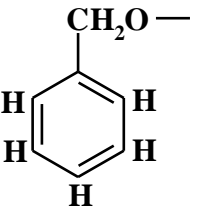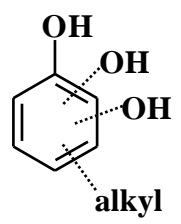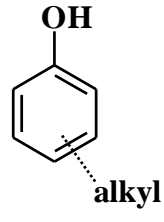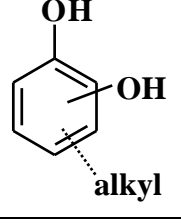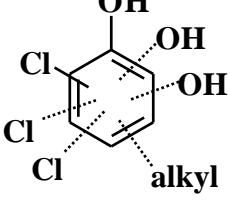
Structure	Name	Description
N, with aromatic ring, ^	ar-N ch	aromatic: aryl - N (chain-bond)
N, with aromatic ring, o	ar-N r	aromatic: aryl - N (ring-bond)
N=, NH-N, with phenyl ring	ar-N,NHN	aromatic: aryl - -N= or -NH-N
O—, with aromatic ring	ar-O	aromatic: aryl - O
OCH₂—, with aromatic ring	ar-O-CH2	aromatic: aryl - O-CH2
OCH₃, with aromatic ring	ar-O-CH3	aromatic: aryl - O-CH3 (methoxy)

Structure	Name	Description
	ar-S r	aromatic: aryl - S (S in ring)
B_x	B	elem: boron (any number)
	benz-O	aromatic: CH2 - C6H4 - O - (o,m,p)
	Biphenyl	aromatic: biphenyl
Br_x	Br	elem: bromine (any number)
	C quart ch	alkyl: C quarternary (4 chain-bonds to C-atoms)
$C_{10}H_{21}$—	C10 H21	alkyl: C10 H21
$C_{11}H_{23}$—	C11 H23	alkyl: C11 H23
	C2H5-CO	func: C2H5 - CO
CH_3— CH_2— O—	C2H5-O	func: ethoxy
C_4H_9—	C4 H9	alkyl: C04 H09
C_5H_{11}—	C5 H11	alkyl: C05 H11

Structure	Name	Description
C_nH_{2n-1}—	C5 H11 *	alkyl: C05 H11 or other alkyl
C_6H_{13}—	C6 H13	alkyl: C06 H13
n—C_6H_{13}—	C6 H13 n	alkyl: C06 H13 (n-)
	C6H4 omp	aromatic: C6H4 di-substituted (o,m,p) benzene ring
	C6H4-Br	aromatic: C6H4 - Br (o,m,p substituted)
	C6H4-SO2	aromatic: C6H4 - SO2 (o,m,p substituted)
C_7H_{15}—	C7 H15	alkyl: C07 H15
C_8H_{17}—	C8 H17	alkyl: C08 H17
C_9H_{19}—	C9 H19	alkyl: C09 H19
	CF3	func: CF3 trifluoromethyl
	CF3-CO	func: CF3 - CO
	(CH2)6-CO	func: ketone (CH2)6 - CO

Structure	Name	Description
CH_3, CH_3 >C=C	(CH3)2>C=C	bond: isopropylidene
CH_3—Si— with CH_3 above and CH_3 below	(CH3)3 Si	func: trimethylsilyl
CH_3—C— with CH_3 above and CH_3 below	(CH3)3-C	alkyl: tertiary butyl
CH_3—C with =O	CH3-CO	func: acetyl CH3-CO
CH_3—C with =O and O—	CH3-COO	func: acetoxy CH3-COO
CH_3—C with =O and O—CH	CH3-COOCH	func: ester of acetic acid CH3COO-CH2
CH_3—O—CH_2—	CH3-O-CH2	func: CH3 - O - CH2
Cl_x	Cl	elem: chlorine (any number)
(structure diagram)	CO-C6H3-O	aromatic: tri-subst. benzene ring: -C=O or -C-O or -O

Structure	Name	Description
$-C$ with $=O$ and $O-CH_2CH_3$	et-est	func: ester: ethyl
$C_x H_y$	hydrocarb	alkyl: hydrocarbon
$-C$ with $=O$ and $O-CH_3$	me-est	func: ester: methyl
N_x	N	elem: nitrogen (any number)
N_2	N 2	elem: nitrogen: 2 atoms
$-N$ with CH_3 and CH_3	N(CH3)2	func: dimethyl-amine - N(CH3)2
$CH_3-(CH_2)_3-O-$	n-C4H9-O	func: butyl-oxy n-C4H9 - O
naphthalene ring structure	Naph	aromatic: naphthaline ring system
$-CH_2CH_2-NH-$	NH-CH2-CH2	func: CH2 - CH2 - NH
$-N=O$	NO	func: nitrogen-oxygen bond (single or double)
Non aromatic compound	non ar	aromatic: non aromatic
P_x	P	elem: phosphorous (any number)
phenyl ring structure with H atoms	ph	aromatic: C6H5- (phenyl)

Structure	Name	Description
	ph-C	aromatic: C6H5 - C
	ph-CH2-O	aromatic: C6H5 - CH2 - O
	phen	aromatic: phenol (1-3 OH), alkyl-subst.
	phen-1-OH	aromatic: phenol (1 OH), alkyl-subst.
	phen-2-OH	aromatic: phenol (2 OH), alkyl-subst.
	phen-Cl	aromatic: phenol - Cl (1-3 OH, 1-3 Cl), alkyl-subst.

Structure	Name	Description
OH, Cl, alkyl (aromatic phenol structure)	phen1-Cl1	aromatic: phenol - Cl (1 OH, 1 Cl), alkyl-subst.
(5-ring and 6-ring condensed structure)	r 5+6	ring: 5-ring and 6-ring condensed
$C=C$ (between 2 rings)	r>C=C<r	bond: C=C between 2 rings
S_x	S	elem: sulphur (any number)
$-S=CH_2-$	S-CH2	func: S - CH2
Si_x	Si	elem: silicon (any number)
Si_1	Si 1	elem: silicon: 1 atom
$Si_{>=2}$	Si >=2	elem: silicon: >= 2 atoms

13   Glossary

All definitions below apply to the meaning of a word or term in the context of AMDIS and may have alternative or more extensive definitions elsewhere.

Analysis An <u>analysis</u> is the <u>deconvolution</u> and subsequent processing that AMDIS performs on a <u>data file</u> to ultimately yield <u>component</u> and <u>target</u> results. There are several different <u>analysis types</u>. For further information see, for example, **6 Analysis**.

Analysis Type AMDIS supports several different <u>analysis types</u> for determining whether or not <u>deconvoluted spectra</u> from <u>samples</u> match any of the <u>target compounds</u> (<u>targets</u>) stored in a referenced <u>target library</u>. The analysis type you choose will depend to a large extent on the type of sample data that you are working with. For further information see, for example, **6.1 Analysis Types**

Calibration <u>Calibration</u> is the process of determining the relationship between <u>retention time</u> and <u>retention index</u>. For further information see, for example, **6.1.3 RI Calibration/Performance**.

Component The word <u>component</u> (also known as eluent) is used in chromatography to designate the chromatographic peak that elutes from the column. Ideally, the peak represents a single compound eluting from the column. In the older literature, the word solute is also used. For further information see, for example, **1.3 An Overview of Automated GC/MS Identification by Steve Stein**.

Context Menu In many situations in AMDIS you can right-click with the mouse and invoke a menu of commands. By keeping the right mouse button held down, you can then move the mouse to highlight a particular command and release the button to activate that command. The commands presented for selection will vary according to the functional area of AMDIS you are currently in and then will often vary again according to the specific section of the display. Because these commands depend on where you are and what you are doing, the menu is known as the <u>context menu</u>. For further information see, for example, **1.1 How to Use This User Guide**.

Data File An experimental <u>data file</u> (also known as a raw data file) is acquired by a mass spectrometer from a <u>sample</u>. For further information see, for example, **1 The AMDIS Program**.

Deconvoluted Spectrum
A deconvoluted spectrum is the result of the AMDIS <u>deconvolution</u> process (this spectrum is also known as the extracted spectrum). For further information see, for example, **1.3 An Overview of Automated GC/MS Identification by Steve Stein** and **2.4.4 Mass Spectral Display**.

Deconvolution In the broad sense deconvolution is the process of extracting one signal from a complex mixture of signals. The treatment of noise, the correction for base line drift and the extraction of closely coeluting components from one another is all part of the deconvolution process, which yields a <u>deconvoluted spectrum</u>. For further information see, for example, **1.3 An Overview of Automated GC/MS Identification by Steve Stein** and **6.3.3 Deconv. (Deconvolution) Tab**.

Deskew Since chromatography is a continuous process, certain acquisition modes of certain mass spectrometers will acquire a mass spectrum in such a way that the ions acquired at one end of the scan will have different intensity charactieristics to those obtained at the other end. In general, this effect is more pronounced for longer scans times and is also impacted by how broad a chromatographic peak is relative to the duration of a scan. This practical issue "skews" the time-to-ion positions within a scan and so AMDIS offers a <u>deskew</u> process to correct for this as much as possible. For further information see, for example, **3.3.4 Mass Spectral Display** and **Scan direction**, **6.3.2 Instr. (Instrument) Tab**.

Extracted Spectrum
See Deconvoluted Spectrum.

Hit See Library Hit.

Internal Standard

An <u>internal standard</u> is a known compound that is co-injected with a <u>sample</u> as a form of marker compound. Following acquisition an internal standard should be easy to detect and when found can be used, for example, to compute a <u>retention index</u> for other <u>targets</u>. For further information see, for example, **6.1.5 Use Internal Standards for RI**, **6.1.6 Use RI Calibr. Data + Internal Std.** and **8.1.3 Internal Standards Library (ISL)**.

Library Hit

When a <u>deconvoluted spectrum</u> is compared against the spectra in a <u>target library</u> and a suitable match is found, the matching library compound is called a <u>library hit</u>. For further information see, for example, **1 The AMDIS Program**.

Model Peak

A <u>model peak</u> may be a single m/z value, an m/z value combination or the <u>TIC</u>. It is the peak that represents the most rapid rise and fall of a chromatographic signal when <u>components</u> are being sought as part of the <u>deconvolution</u> process. It is assumed to be the most characteristic of the signal peaks for a particular component. It is often the TIC, but in complex mixtures with many coeluting peaks, the individual ion signals are far more useful. For further information see, for example, **1.3 An Overview of Automated GC/MS Identification by Steve Stein** and **10.2 Manual Deconvolution**.

Multi-Select

There are standard *Windows* techniques to <u>multi-select</u> more than one item at a time. First of all you would left-click for a single selection. Now you can:
-use shift-left-click to select all from the currently selected item to the item now under the mouse;
- drag-select a range by holding the mouse left button down and then dragging the mouse to the the end of the selection you want;
- hold the ctrl key down and do mouse left-click to select individual additional entries.

Raw Data File   See Data File.

Retention Index   The <u>retention index</u> compares the time a compound is on the column to the times a set of compounds are on the column. The most common set of compounds used for retention indices are the straight chain hydrocarbons typically from C5 to C30. For further information see, for example, **6.1.3 RI Calibration/Performance** and **6.1.4 Use Retention Index Data**.

Retention Time   The time that the compound is held up on the GC column. Usually a <u>retention index</u> is used rather than a <u>retention time</u>. For further information see, for example, **6.1.2 Use Retention Time**.

RI   See Retention Index.

RT   See Retention Time.

Sample   <u>Sample</u> is a generic term for compound or mixture of compounds that are acquired by a mass spectrometer to create a <u>data file</u>.

Target   See Target Compound.

Target Compound

A <u>target compound</u> is a compound that is stored in a <u>target library</u>. There is various compound information held in the library, including the <u>target spectrum</u>. When AMDIS performs its <u>deconvolution</u> analysis process, it produces cleaned-up sample spectra that it then compares with the target compounds stored in a chosen library to find the best <u>library hit</u> for the <u>deconvoluted spectrum</u>. For further information see, for example, **1 The AMDIS Program**, **2.4.1 Chromatogram Display** and **8.1.1 Target Compounds Library (MSL)**.

Target Library   A <u>target library</u> holds one or more <u>target compounds</u>. For further information see, for example, **8.1.1 Target Compounds Library (MSL)**.

Target Spectrum   A <u>target spectrum</u> is simply the spectrum of a <u>target compound</u>. For further information see, for example, **1 The AMDIS Program** and **2.4.4 Mass Spectral Display**.

TIC   Total ion current (<u>TIC</u>) is a type of chromatogram that represents the sum total of all ions eluting at a particular time (scan) during the acquisition of a <u>sample</u>; where the ions are

stored as a mass spectrum of the sample. For further information see, for example, **2.4.1 Chromatogram Display** and **2.4.2 Profile Display**.

Zooming
<u>Zooming</u> is the process whereby the various graphical displays can either be zoomed (magnified) individually in the x-direction or the y-direction, or perhaps in both directions simultaneously. To zoom an area, do a click-drag-release operation with the left mouse button to "rubber band" (mark out) the area to be magnified. For further information see, for example, **2.4.5 Changing the Display Range**.

14 Formats

AMDIS utilizes several files that are in ASCII format. As such, these can be examined and in appropriate cases can even modified using a standard text editor; for example Notepad.

However, it is gernally unlikely that you will need ot do this and so <u>it is generally not recommended that you edit these files outside of AMDIS</u>. An inappropriate edit can cause unpredictable problems when AMDIS subsequently attempts to read the edited file.

The following file formats are described:

14.1 RI Calibration Data File (CAL) Format

Pk RT RI Net S/N Name

6.001 900.0 100 311 Nonane (128)

7.250 1000.0 100 413 Decane (142)

8.217 1100.0 100 491 Undecane (156)

9.042 1200.0 100 468 Dodecane (170)

9.781 1300.0 98 488 Tridecane (184)

10.468 1400.0 100 490 Tetradecane (198)

11.107 1500.0 100 591 Pentadecane (212)

11.708 1600.0 100 566 Hexadecane (226)

12.281 1700.0 100 653 Heptadecane (240)

12.825 1800.0 100 836 Octadecane (254)

13.835 2000.0 97 626 Eicosane (282)

14.757 2200.0 93 701 Docosane (310)

15.194 2300.0 97 964 Tricosane (324)

15.603 2400.0 98 547 Tetracosane (338)

Pk RT - the actual RT of the deconvoluted peak matched to the CSL entry "Name"

RI - the RI of "Name" in the CSL

Net - The computed Net value

S/N - the computed "S/N (total)" value

Name - the name of the compound in the CSL file matchde to this peak

14.2 Mass Spectral Transfer File (MSP) File Format

The native format which the NIST *MS Search* program exports files in is shown below. However, as you can see from looking at the other formats, the NIST *MS Search* program and AMDIS accept a number of formats. The important point is that the Name, Formula, and CAS number should be present and the Number of Peaks needs to be specified. In general, the program will ignore fields it does not understand.

The only unusual field stored in the file (ie, it is not a field name followed by a colon) is the direct output of the NIST *MS Search* program. The fourth line in the NIST98 example below is a list of other (mostly regulatory) databases in which the compound appears. The only requirement beyond this is that there must be 5 peaks on each line except the last.

Example:

NIST 98

Name: Phosphoric acid, trimethyl ester
Comments: ACCUSTANDARD Chemicals
Formula: C3H9O4P
Contributor: NIST MS Data Center.
RTECS, EPA, HODOC, EINECS
MW: 140
CAS#: 512-56-1; EPA#: 125515
DB#: 51394
Num Peaks: 43
13 2; 14 12; 15 126; 16 1; 17 1;
18 1; 28 27; 29 86; 30 17; 31 58;
32 9; 33 3; 40 1; 44 1; 45 11;
47 110; 48 15; 49 13; 50 2; 51 1;
61 2; 62 3; 63 10; 64 7; 65 51;
66 4; 77 13; 79 346; 80 279; 81 10;
82 2; 93 34; 95 251; 96 6; 97 1;
109 326; 110 999; 111 27; 112 7; 125 3;
140 138; 141 6; 142 1;:

Name: 2,6-Dimethylphenol
Formula: C8H10O
Contributor: VERIFINN
ID: 2
CASNO: 576-26-1
T(Source): 260C
EV: 70
Instrument: Finnigan MAT Triple quadrupole TSQ 45A
Inlet: GC
Num Peaks: 37
27 262;38 71;39 355;40 48;41 96;
43 76;50 87;51 199;52 77;53 131;
60 50;62 39;63 90;65 125;66 32;
74 20;77 409;78 194;79 242;80 22;
89 21;91 275;92 31;93 47;94 33;
102 20;103 140;104 91;105 46;107 948;
108 73;119 5;120 7;121 384;122 999;
123 85;124 5;

NAME: Sarin (GB)
FORM: C4H10FO2P
CASNBR: 107-44-8
NUM PEAKS: 33
(41, 74) (42, 55) (43, 103) (44, 7) (45, 8)
(47, 42) (48, 1) (50, 5) (51, 2) (52, 2)
(53, 1) (56, 2) (57, 3) (59, 14) (61, 1)
(63, 3) (65, 3) (66, 2) (67, 17) (78, 4)
(79, 40) (80, 12) (81, 118) (82, 9) (83, 9)
(97, 1) (99, 999) (100, 13) (101, 4) (125, 277)
(126, 9) (127, 1) (139, 3)

NAME: 1,2-Dimethylpropyl methylphosphonofluoridate
FORM: C6 H14 F O2 P
CASNO: 6154-51-4
NUM PEAKS: 44
(26 19) (27 164) (29 131) (31 14) (38 21)
(39 197) (40 28) (41 262) (42 139) (43 262)
(44 15) (45 68) (47 66) (50 16) (51 22)
(52 9) (53 66) (54 21) (55 614) (56 35)
(65 8) (66 21) (67 66) (68 9) (69 43)
(70 563) (71 121) (78 13) (79 28) (80 32)
(81 166) (82 457) (83 87) (98 107) (99 1000)
(100 16) (101 6) (110 6) (111 10) (124 75)
(125 791) (126 459) (127 46) (153 42)

15 Text Report Details

Reports are generated using the **File | Print Text Report** command (**3.1.1.9.1 Layout Details**).

There are two basic report types:

Laboratory report   see **15.1 Laboratory Report**

Onsite report   see **15.2 Onsite Report**

15.1 Laboratory Report

The Laboratory report is chosen by selecting **Print Laboratory report** in the *Print Text Report* dialog.

In the *Print Options* dialog the simplest (shortest) report you can generate is by selecting **Print only best hits** and clearing **Print signal/noise information** and **Print additional information** (**Figure 183 - Simplest Laboratory report**).

The most informative (longest) report is generated by clearing **Print only best hits** and selecting **Print signal/noise information** and **Print additional information** (**Figure 184 - Most informative Laboratory report**).

Refer to **Figure 183 - Simplest Laboratory report**.

The report's page header shows you the name of the result file being presented (**CARD5P.FIN**) and the page footer provides the date and time stamp of when the report was generated. Both show the page number. At the end of the report's data, the total page count is presented.

The report header shows the **Organization** and **Division** values specified in the *Print Options* dialog.

The report footer shows the **Chemist** and **Operator** values specified in the *Print Options* dialog.

The data header reports the data file name (**CARD5P.D**), the library used for targeting (**PESTPLUS.MSL**) and the number of targets identified (**16**).

The report body has 2 columns. The first (**RT(min)**) shows the retention time in minutes and the second (**Chemical Name**) shows the name of the best hit for each.

Refer to **Figure 184 - Most informative Laboratory report**.

When full information is being presented the report shows everything the simplest report does plus additional information as follows.

The data header now includes **Analysis Information**. This is a combination of the user-settable values used in the deconvolution process and some values determined during the deconvolution process.

The user-settable values are those of the *Analysis Settings* dialog; taken mainly from the **Instrument** and **Deconv.** tabs (**6.3 Analysis Settings**).

The computed values are a subset of those presented on the **S/N** tab of the *AMDIS Results* dialog (**5.6 S/N Tab**).

When there are two or more hits for a compound, the best hit is presented first and the other hits are presented below that and indented. For the best hit, full additional information is presented (component and match). However, only match information is presented for other hits since the component information is the same.

After all hits are presented, QA/QC signal/noise information is provided and is predominantly those values presented when the **Results** radio button is selected on the **QA/QC** tab of the *AMDIS Results* dialog (**5.5.1 Results**).

Print Text Report

- ○ Print Onsite report
- ● Print Laboratory report   [Print Options...]

[Print]   [Cancel]

Print Options

☑ Print only best hits   ☐ Print signal/noise information
                                ☐ Print additional information

Organization:
PDBS

Division:
Test Laboratory

Chemist:
Jane Doe

Operator:
Fred Bloggs

Empty fields will not be printed

○ Component
○ Match

Print order:

R.I.
Width
Purity
Model
Min. Abund.
Amount
Scan
Peak Tailing

R.I.
Width
Purity
Model
Min. Abund.
Amount
Scan
Peak Tailing

R.I.: Retention index (from observed retention time and prior RI calibration results).

[OK]   [Cancel]   [Help]

Figure 183 - Simplest Laboratory report

Figure 184 - Most informative Laboratory report

15.2   Onsite Report

The Onsite report is chosen by selecting **Print Onsite report** in the *Print Text Report* dialog.

In the *Onsite Report Print Options* dialog you can, for example, see basic information for all hits by clearing both **Print only best hits** and **Print additional information** (**Figure 185 - Onsite report (all hits, no additional)**).

As another example, to see detailed information for the best hits only, select **Print only best hits** and clear **Print additional information** (**Figure 186 - Onsite report (best hits, all additional)**).

Refer to **Figure 185 - Onsite report (all hits, no additional)**.

The report's page header shows you the name of the result file being presented (**CARD5P.FIN**) and the page footer provides the date and time stamp of when the report was generated. Both show the page number. At the end of the report's data, the total page count is presented.

The report header shows site information, including the date of the ONSITE library.

The report footer shows the **Inspector** and **Host** values specified.

The data header reports the data file name (**CARD5P.D**) and the number of targets identified (**16**).

The report body has 2 columns. The first (**RT(min)**) shows the retention time in minutes and the second (**Chemical Name**) shows the name of the hit(s) for each. When there are two or more hits for a compound, the best hit is presented first and the other hits are presented below that. Further, the best hit is preceded by the count of the total number of hits for that target (eg, 3 for the target at 17.1122 minutes).

Refer to **Figure 186 - Onsite report (best hits, all additional)**.

When additional information is being presented the report shows everything the simplest report does plus additional information as follows.

For the best hit, full additional information is presented (component and match).

> **Note:**   When there are two or more hits for a compound (not illustrated here), the best hit is presented first and the other hits are presented below that. However, only match information is presented for other hits since the component information is the same.

After all hits are presented, QA/QC signal/noise information is provided and is predominantly those values presented when the **Results** radio button is selected on the **QA/QC** tab of the *AMDIS Results* dialog (**5.5.1 Results**).
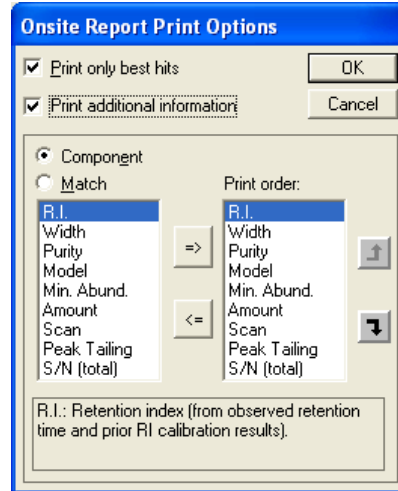
Figure 185 - Onsite report (all hits, no additional)

Figure 186 - Onsite report (best hits, all additional)

16   Contact

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