

Automated
Mass Spectral
Deconvolution &
Identification
System

AMDIS — USER GUIDE

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1 Introduction

1.1 *The AMDIS Program*

The **A**utomatic **M**ass Spectral **D**econvolution and **I**dentification **S**ystem allows you to automatically find any of a set of target compounds in a gas chromatography / mass spectral (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.

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 file, to use the retention index data along with the mass spectral data or to work without reference to the retention index data. In addition, AMDIS can also 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 is 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 Mass Spectral Database.

1.2 *How to Use this Manual*

There is a very brief introduction to the use of AMDIS in APPENDIX A. Please read Appendix A, following it step by step – even if you never read manuals. Most of this manual is also in the help system for the program.

The individual sections below give details about the options in the program as well as how to configure the display to fit your needs. APPENDIX B contains a set of questions that are frequently asked as well as some additional tips.

Almost everywhere in AMDIS the right mouse button will bring up options. These options will vary as you move around on the screen. In addition, the options change as you progress through an analysis. An option that is “grayed out” early in an analysis may be available later.

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

1.3.1 **Background**

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.

1.3.2 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.

1.3.3 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.

1.3.4 References

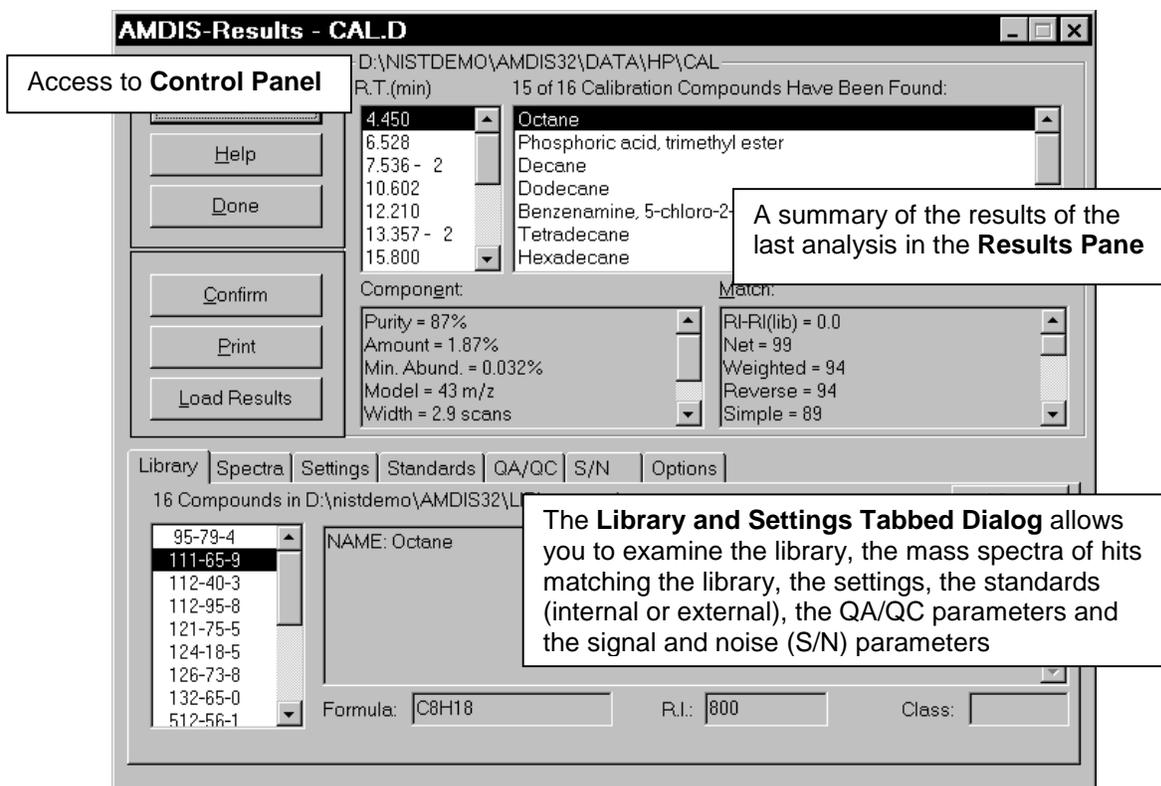
- [1] "Reconstructed Mass Spectra, A Novel Approach for the Utilization of Gas Chromatograph—Mass Spectrometer Data", Biller, J.E.; Biemann, K. *Anal. Lett.* **1974** 7 515-528.
- [2] "Spectral Deconvolution for Overlapping GC/MS Components" Colby, B. N. *J. Amer. Soc. Mass Spectrom.* **1992** 3 558-562.
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- [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 AMDIS Results Window

2.1 Introduction

For certain routine analysis, it may be sufficient to see only the results of the data evaluation. This is provided in a convenient form by the Results Window. Below is a view of the Results window. In the sections that follow, each of the three areas will be discussed (Sections 2.2 through 2.4). For a more detailed graphical view of the raw data and results, see the Confirm Window (Section 3).

The RESULTS Window provides:



NOTE: All settings, library files, standards, etc., shown in the tabbed dialog are for the analyzed data file. These may not be the current settings which will apply if a new analysis is undertaken. To check the current settings, select the Analyze button from the top button in the access to the Control Panel, then select the Settings button (see Section 4.5 and Appendix B.1.2).

The **Options** tab allows control over what is to be displayed and how it is displayed (See 2.4.7).

2.2 Control Panel

The buttons - **Analyze, Help, Done, Confirm, Print, Load Results** - control the program.

Analyze will bring up a window to allow you to select a type of analysis, the data file to be analyzed, the target library file, and the calibration data or library. The analysis is started from within this dialog box. For details, see Section 4.1.

The **Help** button displays on-line help.

The **Done** button finishes the session, and exits from the program.

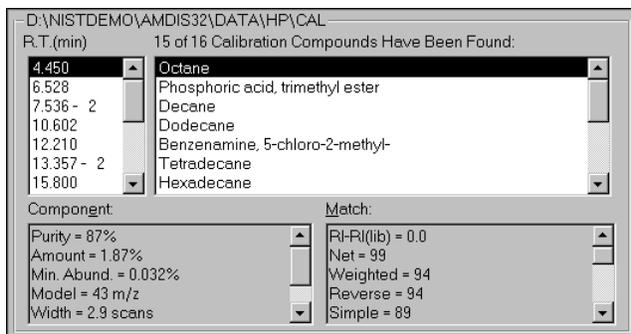
Confirm will take you to the Confirm Window (see section 3 below) which will show the data used by the program in making each of the identifications and allow you to study it in detail.

Print will bring up the option of printing an onsite or a laboratory report and controlling the material printed. See also 2.3.4 below.

Load Results will bring up a dialog box to select the results of a previous analysis to be displayed.

2.3 Results Pane

The four list boxes in the Results pane show the retention time for each chemical identified, name of the



compound found, the component parameters for the deconvoluted chromatographic peak, and the match factors between the mass spectrum of the component and that of the library. These list boxes are coupled, when you scroll either the retention time or the name list box, the other scrolls with it. In addition, the data in the component and match factor list boxes correspond to the highlighted item in the identification or retention time boxes, as do the library identification and the component and library spectra in the Tabbed Dialog.

2.3.1 Identifications

Within the Results pane the identifications are displayed in two coordinated scrolling lists, selecting an item in one list will select the corresponding item in the other list. The list on the left is the retention time of the component in minutes. The list on the right is the compound name from the library.

The program 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. AMDIS will always display the best match, but you are free to select whether to display other matches. *The number of compounds in the library matching the component is shown after the retention time.* You may select to display **all hits** or only the **best hit** in the compound identification window. By clicking on the right mouse button with the mouse in either identification list box, you may choose to print or view only the best hits, or all hits for each identified peak. For single items you can also toggle between the best hit and all hits by double clicking on the retention time or the name. If you choose all hits, all target chemicals which have spectra with match factors above your preset cut off will be listed in order of match factor.

The number of identifications printed will be a function of the **minimum acceptable match factor** you have specified (see Section 2.4.3). The default value for results to be reported is a match factor of 80 or more. If you change the value to less than 80, then the results for values of the match factor below 80 will be reported with leading question marks as follows:

?	75-79
??	70-74
???	<70

2.3.2 Component Parameters

The items shown and the order that they are shown can be set by either selecting the **right mouse button** when the mouse pointer is in the Component list box or by selecting the Options Tab in the tabbed dialog box in the lower half of the screen.

The program provides the following parameters for the component:

R.I.: retention index of the component – only calculated in some modes of analysis.

Scan: the scan number of the peak of the component.

Width: the full width at half maximum of the chromatographic component peak; the value is given in scans, not time. Typically quadrupole mass spectrometers scan about once a second.

Purity: percentage of the total ion signal at the component maximum scan belonging to the deconvoluted component. Note, the program extracts all of the ions associated with a component and adds them back together to find the total ion signal of the component.

Model: m/z value (or TIC, total ion chromatogram) used for determining component peak shape. If followed by one or two integers, neighboring components were deconvoluted using these m/z values as their model peaks. The model peak is generally the ion (or TIC) that rises and falls the fastest.

Min Abund: smallest observable mass spectral peak (as percent of base peak).

Amount: amount of the deconvoluted component relative to the integrated the total ion count for the entire chromatogram.

S/N: the signal to noise value of the peak.

Peak Tailing: The ratio of the area to the right (later time) of the center of the peak to area to on the left of the peak (earlier time). A well formed peak has a value near one. Excessive tailing will yield values much greater than 1.

2.3.3 Match Factors

The match factors shown and the order that they are shown can be set by either selecting the **right mouse button** when the mouse pointer is in the Match Factor list box or by selecting the **Options Tab** in the tabbed dialog box in the lower half of the screen.

The following values are always calculated:

Net: Final match quality value (100 = perfect match) for the match between the deconvoluted component and library spectra (minimum = 80 by default, may be reset in Settings 4.5.1). This value uses a number of factors, including the match factors below. It may be either higher or lower than any of these match factors. For example, a high purity value, will increase the net match factor.

Weighted: Scaled dot product of library and deconvoluted component using optimized weighting and scaling.

Simple: Simple dot product of library and deconvoluted component.

Reverse: Dot product in which deconvoluted component m/z peaks not present in library spectrum are ignored (i.e., treated as impurities).

In all cases the dot product is expressed as the cosine of the angle between the "vectors", i.e. it is normalized by the square of the library spectrum.

In addition, depending on the type of analysis run and the data available, additional matching parameters may be calculated:

Expec. R.T. : expected retention time, only shown if retention time is present in the library data file.

RI-RI(lib): Retention index minus the library value of the retention index. This is only shown if the retention index for the identified compound is in the library and the analysis type makes use of retention indices. This value is not shown for the "Simple" analysis type.

Corrections: There are a series of corrections applied to the weighted match factor to calculate the net match factor. Some of these 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 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. You do have control over one important correction – the amount of penalty assessed for retention mismatches between the observed peak and the library value.

RI Penalty: (Maximum penalty set by user) the amount of the penalty applied to the net match factor. The penalty is applied as a function of the difference between the measured retention index and the library retention index. In addition a penalty may be applied for library data with no retention index.

Reverse Logic: Usually a positive correction – the net match factor before corrections is a weighted sum of 70% of the weighted match factor and 30% reverse match factor. This promotes a match with strong peaks that match the target. Peaks due to impurities do not penalize the match.

Flagged/Uncertain 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 the flagged/uncertain peaks match the library component or do not match the library component. 1) If flagged/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 Peak Penalty** is applied based on the fraction of flagged/uncertain peaks that *do not match* the target compound. 2) If a match to a target compound is based predominantly on flagged peaks, a penalty is applied to reflect the lowered confidence in this match. The **Flagged Peaks Penalty** is based on the fraction of flagged/uncertain peaks that *do match* the target compound. Both of these corrections for flagged/uncertain peaks are only applied if there are uncertain deconvolutions in the data.

Few Peaks: In cases where the deconvoluted spectrum has only a few peaks, a factor is applied for small numbers of deconvoluted peaks. For deconvolutions with only one peak a match is not possible.

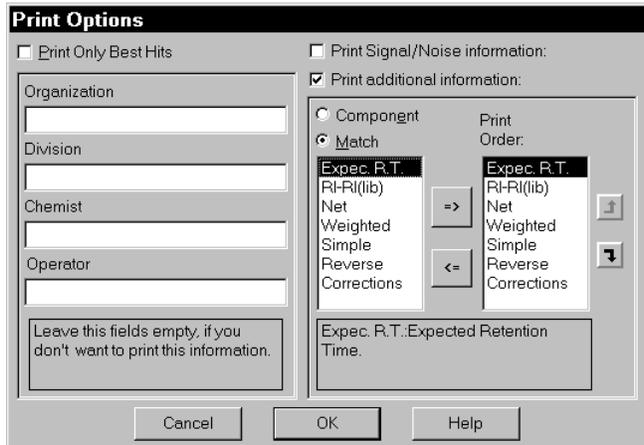
Purity: A small correction that allows for both additions and subtractions to the match factor to account for the purity of the component. Confidence in the answer is greater if all of the TIC is accounted for, but lower if there are other components mixed in with the TIC.

High Threshold: In cases where the threshold is set very high, 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 dominates the mass spectra, the peak intensities are rescaled in such a way that the relative intensities of the small peaks is unchanged, but ratio between the large and small peaks is reduced. The scaled component spectra is compared to the scaled target spectra. If the match is worse than in the unscaled case a penalty is applied.

Adjacent Peaks: If specific adjacent peak subtraction has been used it is assumed that the confidence in the resulting match is lowered. This correction reflects that lowered confidence.

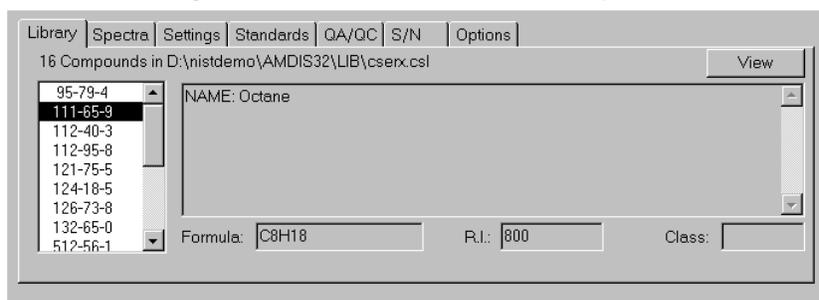
2.3.4 Print/View Options



By clicking on the right mouse button with the mouse in either the component or the match factor list box, you may select the option for the data printed or shown on the screen. You may choose to print or view various component parameters (2.3.2), match factors (2.3.3) and match factor corrections (2.3.3). The printed output may have the name of the organization, division, the chemist responsible for the data, and the operator of the instrument. You may leave any of these fields blank. You may also print **signal to noise** information and the additional information about the **component** and the **match**.

2.4 Library and Settings Tabbed Dialog

This tabbed dialog at the foot of the Results window provides access to a variety of information about the analysis. 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.



2.4.1 Library Tab

The **compound identification** data (chemical identification number and name) for the identified target library compound is tracked with the identification highlighted in the identifications list box. The compound name and synonyms are shown on the right. 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 the number can be assigned arbitrarily. The library compound shown is controlled by scrolling the list box of identification numbers or by changes in the results pane. The formula of the compound is given as well as the retention index and chemical class if available.

The **View** button allows you to see the details of the target library (see Section 6.8).

2.4.2 Spectra Tab

The deconvoluted spectrum is shown along with the associated library spectrum. The data shown track the selection in the **Identifications** list box above.

You have the option of showing either the library spectrum, the deconvoluted component spectrum or both. The selection of what is shown is via the 3 radio-buttons on the right hand side of the window. The data are shown both in tabulated form in a scrolling text box and in graphical form in the spectral window so that you can examine the results in more detail.

Peaks with question marks (?) next to them mean that the algorithm de-weighted these peaks since their deconvolution was uncertain. These are the flagged/uncertain peaks discussed in 2.3.3.

2.4.3 Settings Tab (Analysis & Instrument Parameters)

The settings tab brings up a display of the data recorded with the experimental run in a scrollable line of text on the left. Below this is the type of analysis performed and the Library files used in the analysis. On the right hand side of the window is a scrollable list of all user-controlled settings. **To change the settings**, select the **Analyze** button and within the dialog box that appears select the **Settings** button. See Section 4.5.

There are three types of settings for which values are given:

Identification Settings: Parameters used to define which identifications are reported.

Minimum Match Factor: The threshold net match factor value for an identification to be reported. Values at or above 80 are good matches, 70-79 are fair and less than 70 is poor. For most cases a match factor of 80 is the minimum that should be used if identification rather than detection is desired.

Multiple Identification: On/Off. If **On**, every time a compound is identified it will be reported. If **Off**, a compound will only be reported once and associated with the best identification.

Show Standards: On/Off. Will show/hide data on internal standards.

Use Retention Index: On/Off. Uses the retention index data for matching. The program will penalize a compound for being outside of the retention window that you define. The information on the match factor penalties due to mismatch of retention data is also shown.

Instrument Settings: Settings in the data file for the instrument or determined from the data file.

Low and High m/z: The low and high m/z values should correspond to the mass spectrometers settings. Values of m/z below the low value or above the high value are ignored in both the library and the data file during the deconvolution and in calculating the match factor. If there is a difference between the settings in the analysis and the data found in the data file, it will be reported in the **QA/QC** (see Section 2.4.5) tabbed dialog box.

Threshold: On/Off The minimum signal that is recorded by the instrument. Signals below the threshold are not recorded. If **On**, the value of the threshold will be shown.

Scan Direction: The scan direction – whether the instrument scans from high to low or low to high in m/z – is set automatically. This can be changed. For certain instruments and data formats, the scan direction is none. This means that no correction is applied to align the different masses. The instruments that are supported in their native file format are:

- Bruker
- Finnigan (GCQ, INCOS, and ITDS formats)
- HP Benchtop and MS Engines
- MassLynx NT Formats
- MicroMass
- Perkin-Elmer
- Shimadzu MS Files
- Varian Saturn Files
- Viking

In addition, the NetCDF format used by many manufacturers is supported.

Deconvolution Settings: In general the default settings are optimal.

Adjacent Peaks: Two/One/None; Default One. This sets the degree to which adjacent peaks, if present, are subtracted in the deconvolution. If **None** - no adjacent peaks are subtracted from the deconvoluted peak, this is faster and is useful for very clean data. The default (**One**) uses only the nearest adjacent peak (if present), from either side of the deconvoluted peak. **Two** will use peaks on either side of the deconvoluted peak.

Resolution: High/Medium/Low; Default Medium. As the level of resolution goes up, the program seeks peaks that are closer together.

Sensitivity: Very Low/Low/Medium/High/Very High; Default Medium. As the value for this parameter increases, increasingly noisy and broad peaks are sought at the expense of

analysis time and increased risk of false positives. An increase in the sensitivity will extract broader peaks, resulting in an increase in the extraction of smaller and noisier peaks.

Shape Requirement: High/Medium/Low; Default Medium. The shape requirement is a way of forcing all of the deconvoluted peaks to have the same shape. As the shape requirement increases, the shape of the individual ions must be more nearly the same.

Component Width: Maximum 20; Default 12. Equal to the number of scans across a well-resolved component peak at half-height. The value should be equal to or greater than the widest peak in the chromatogram. In the case of a chromatogram with very broad peaks, a larger value can be selected.

2.4.4 Standards Tab

There are two radio buttons:

External Standards: A report of the calibration standards used to establish the retention index calibration file. The retention time, the defined retention index and the compound name of the standard are given. These are the standards that are used to convert retention time data to retention index.

Internal Standards: A report of the internal standards used within the current run. Data include the retention time, the defined retention index, the compound name of the standard and the match factor found in the run.

2.4.5 QA/QC Tab (Quality Assurance / Quality Control)

A variety of information about the overall quality of the data.

The QA/QC tab shows two types of data selected by the radio buttons at the top of the dialog. These are **Results** and **Performance** as explained below.

Results

General Data Warnings: The first few lines contain various warnings such as which data were ignored. For example, if the data had been acquired with a lower limit of $m/z=40$, but the analysis had specified that the range start at $m/z=44$, the QA/QC window would report discarding the lower mass data. If the setting for the maximum mass in the analysis and the maximum mass found are different, the program will report the discrepancy. Very low noise levels which are often characteristic of high threshold settings are also reported.

Background: The background is displayed at low and high retention times. The data is displayed as the median at low RT and in terms of signal to noise (S/N) units at high RT. The median is the level such that the background is below this value 50% of the time. The low RT data is taken in the first half of the run, and the high RT data is taken in the last 10% of the run. For very dirty samples, one would expect to see high values in the late data. This would be confirmed by high values in the next run.

Solvent Tailing: The m/z value here should be the principal ion, or a unique ion of the solvent. The program shows the first value after the solvent delay in terms of S/N units. The reported values are given in 10,5,2,1 sequences, thus if the first point detected has the solvent peak at S/N of 20, then the time at which it falls below 20, 10, 5, 2, 1 will be reported. The fact that the S/N of the solvent fell below 50 before the run will also be noted. The solvent peak is a single mass peak. The definition of falling below a level requires that the signal be below that level for at least two successive scans.

Column Bleed: The m/z value should be that of an ion characteristic of the decomposition of the column. By default m/z 207 is used. The data reported are the average over the first half of the chromatogram and the average over the last 10%. The medians are reported in S/N units. Again, very high column bleed would be seen in high values of the median value for the high RT data.

Additional warnings of various sorts are included at the end of the text. High numbers of non-integral masses are reported with a note that the electron multiplier may be running at too high of

a voltage. Any missed internal standards are reported as well as how many of the internal standards were found.

Performance

Performance provides a history of the calibration / performance mixture runs.

A list of all of the data recorded in the calibration / performance mixture data runs is maintained. The compounds that are recorded are set by the user. (See Section 6.7).

2.4.6 S/N Tab: (Signal and Noise)

Signal

The **lowest mass** and the **highest mass** detected are recorded as is the **threshold** and the **number of distinct spectra** and **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. The total ion signal and the threshold are reported in the native units of the instrument.

Noise

Noise parameters, in some cases, suggest possible problems with the instrument.

The parameters are:

Noise constant: The proportionality constant between the square root of the signal and the noise for any signal. Larger values usually indicate that the electron multiplier is operating at an excessively high voltage.

Noise level: The median signal level for all ions - most of the signals are very small, mostly noise, so 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. Large numbers here indicate high noise levels.

Uncert. m/z: The fraction of peaks that are more than 0.25 units away from an integer m/z. Again, large values are usually associated with high noise levels and often with dirty ion sources or failing electron multipliers.

Doublet m/z: The fraction of unit m/z regions where more than one peak appears. Large values indicate high noise levels.

Spikes: Fraction of peaks where signal increases and falls in a single scan.

Saturated: Number of times a single ion peak had a signal level equal to the maximum the instrument can deliver.

Nulls scans: 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.

2.4.7 Options Tab

You may choose:

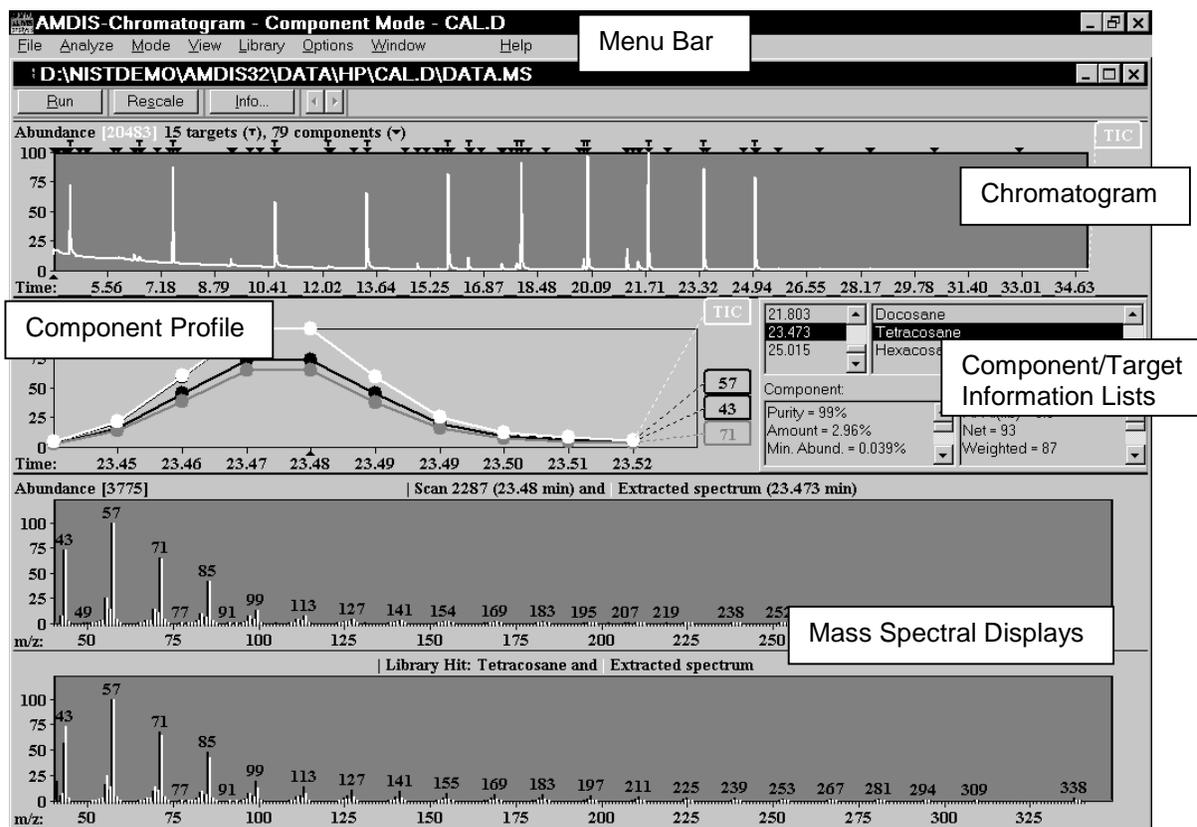
What results are displayed in the component and match list boxes - also selectable using the **right mouse button** when the mouse pointer is in the respective list windows.

Whether to show the best hit only or to show all hits - also selectable using the **right mouse button** from within the results list box.

3 AMDIS Confirm Window

3.1 Introduction

The CONFIRM Window allows you to see the data used in the identification in more detail than in the Results Window. In the Confirm Window, you can view either a single chromatogram or more than one chromatogram. All of the features discussed in this section are common to both of these modes.



The Confirm Window contains the menus File, Analyze, Mode, View, Library, Options, and Window as well as chromatographic and mass spectral displays. For the additional features available with multiple chromatograms, please refer to Section 3.5.

3.2 Menu Bar

The menu bar presents the following options:

3.2.1 File Menu

The file drop down menu bar item is used to open new files, save or print results, return to the analysis window or exit the program.

Open: Bring up a data file for examination or analysis.

Save Component MS: Save the current component into a .MSP file (see Section 6.9 for details on the format). The .MSP file can be used to search the NIST Mass Spectral Database.

File Settings: Opens a dialog box to allow you to either **Keep**, **Ask User** or **Delete** the results of an analysis. Since the results take up space on your hard-drive you may want to delete them after

each run - select **Delete Results**. If you often return to the results of an analysis and do not want to rerun the data select **Keep Results**.

Batch Job: Enables a series of data files to be selected for analysis. For more details see Section 7.

Generate Report: Generates a report of the currently active file in a tab delimited format suitable for import into data analysis programs. Options for contents can be set by selecting **Report Options**. The location of the report and whether to start a new file or add to an existing file (**Append to Report File**) can be specified. The field names are printed on the first line (also tab delimited) of the report file. In addition, if you change the options from the set used initially in the report, a new line of field names is printed.

Print Spectra: Prints a copy of the current chromatogram and spectra. You can choose any or all of **ion chromatogram, profile, scan, extracted spectrum, and library spectrum**.

Print Text Report: Prints a copy of the data on the compounds found in the analysis. **Onsite report** is a brief report that gives you most of the information you will need. To customize the report, select **Laboratory report** and set the options using the print options button.

Go to Results: Returns to the RESULTS window (see Section 2.2)

Exit: Exit the program.

Recently analyzed files: A list of the 6 (at most) most recently examined data files. Selecting one of these will bring the previously analyzed results into the program. If you have not saved the results (see File Settings above) the file must be reanalyzed.

3.2.2 Analyze Menu

Analyze GC/MS Data: Using the current settings to analyze the data (see Section 4.1). This is equivalent to the Analyze button in the Results Window. If an analysis has previously been run in this data file you will warned that the previous results will be overwritten. You must confirm that this is intended.

Settings: Access to all user controlled settings. For details see Section 4.5.

Post Analyze: Provides access to additional processing on the peaks. The post analysis will allow you to use tools that can classify components as to the probable functional groups within them without library spectra. Under this menu choice you can process the entire data file. For more details see Section 5.

Varmuza Classifiers: The classifiers developed by Prof. Varmuza's research group will displayed for the current component only. The name of the classifier and percent probability that this classifier is present (Y) or not present (N) in the component is listed. You can select or unselect a classifier by double clicking on it. The list can be sorted by name, value (%), or selection. To view the results for another component, click on the another component in the chromatogram. For more details see Section 5.2.

Add component to search list (N): Adds the currently highlighted component to a list of compounds to be searched using the NIST Mass Spectral library and search routines. N = the current number of compounds in the list. ***For this option, and the following search options, you must have installed the NIST MS search program and library.***

Go to the NIST MS Program: Using either the above **search list** (if it has been created) or the current component, calls the NIST Mass Spectral Search Program for Windows. The NIST MS program will automatically search the library if automation has been turned on. If the program does not automatically search, choose Search/User Spectrum from the pull down menu in the NIST MS program. Click on the No Automation button in the middle right side of the window. Check the Automatic Search On box. Choose OK to close the window, and OK again to save this change.

Use Uncertain Peaks: If checked, the uncertain peaks in the component will be included in the spectrum to be searched when added to the component search list. If unchecked these peaks will be omitted in the spectrum sent to the NIST program.

Search NIST Library: All of the deconvoluted spectra will be searched using the NIST MS program algorithms and the NIST MS library. The two match factors that are reported (weighted and reverse) are the same match factors reported in the NIST MS program. You will be shown a box to set search criteria for the NIST library search. This search does not use uncertain peaks.

Number Hits Reported: Three radio buttons control the number of hits reported for each target. Specify the maximum number of hits, the minimum match factor, or the minimum probability %.

Number Components Searched: Choose between searching a specified number of largest components or components that are larger than a specified percentage of the signal.

Search Mode: Choose between quick and normal. The quick search limits the size of the NIST MS library subset through an initial screening.

3.2.3 Mode Menu

Select how the data is to be displayed.

In all modes you may expand the vertical or horizontal scale by dragging - start at one corner of what you want to take up the full scale, hold down the left mouse button and drag the mouse to the diagonal corner and release the left mouse button. The right mouse button always has the options to unzoom, rescale, change between log and linear scales and display the exact mouse location. You can toggle between the four modes by clicking on the mode in the pull down menu. The mode that you are currently in is indicated by the "grayed out" choice. It is also identified in the top Bar above the menu bar.

Target Only: If target compounds are found in the data analysis, the target confirmation window will be displayed. In this mode there are 5 basic regions of the window. A **chromatogram display** at the top shows the entire chromatogram. Below the chromatogram is a **component display** which shows only that portion of the chromatogram that was directly used in the deconvolution. To the right are four **target data list boxes** which give the retention time of the identified component, the name of the target found, the component data, and data on the match between the library and the component spectra. Below the component data are two mass spectra. The upper is a display of the **raw and extracted spectra**, the lower is a display of the **extracted and library spectra**.

Component: If no compounds from the target library are found, the confirmation window will change to the standard component display window. In this mode there are 4 basic regions of the window. A **chromatogram display** at the top shows the entire chromatogram. In the middle of the window the **component display** shows only that portion of the chromatogram that was directly used in the deconvolution. Three **component data list boxes** on the right give the retention time for the component, the model peaks that were used to define the component (if more than one is determined), and the characteristics of the component. At the bottom of the window is a mass spectral display which displays both the **raw and the deconvoluted spectra**.

Note: In the chromatogram, the location of each deconvoluted chromatographic peak (component) is indicated by a small triangle (▼). The identified target compounds are indicated by a small **T** above the triangle. In the target mode, only **T** will appear at the top of the chromatogram. In the component mode both the components (▼) and targets (**T**, if any are found) will be shown. Within the component mode you can toggle between **component** and **target** data by clicking on either triangles or **T**'s at the top of the chromatogram.

No chromatogram: In this mode the total ion chromatogram panel is omitted. Only the portions of the chromatogram corresponding to the identified target compound peaks are shown in the component display. When you switch to the No Chromatogram Mode, only the targets are shown, any component not identified as a target is not shown.

Manual: This mode allows you to do your own background subtraction or to constrain the use of a particular peak as the model peak. For more details see Section 1.

3.2.4 View Menu

Options for changing the appearance of the screen:

Show Window: The screen contents may be customized by selecting some set of the following panels:

Ion Chromatogram – the total ion chromatogram window

Component Profile – component display and data list boxes

Scan – the raw and deconvoluted scan

Library – the deconvoluted scan and the associated library spectrum

If a selection is "grayed out" the program will not allow you to change that selection in the current mode (e.g. if you are in No Component mode the Show Window will not allow you to show an ion chromatogram). In the component mode the view will automatically shift from showing the library to showing only the scan as you select identified (target **T**) peaks or unidentified (component **▼**) peaks.

Undock: The set of scrollable data list boxes on the right hand side of the component may be separated from the component display. They can be undocked and moved around on the screen. The undocked list box will remain on the screen even if the component window is not being shown. This allows you to make the other windows larger and to move the list box to any position on the screen.

Show Mouse Position: Show or hide the abundance, time, scan and m/z values for the mouse position.

On Cursor: Show scan or m/z values on the cursor.

On Top: Show data at the top of the pane that the mouse is in (either scan, time and abundance, or m/z and abundance).

Off: Do not show mouse position data.

Changing the mouse parameters here applies to all graphs. You may also change the parameter for a single window by using the right mouse button in that window.

Thick Lines: If checked will show all lines in the graphs with a thick pen. Especially useful if you wish to use screen captures for presentations.

Color Selected Peaks: Default Off. When On (Checked), the peaks in a spectrum for which ion chromatograms are being shown in the component display will have the same color as the corresponding ion chromatogram. The selected peaks are controlled by the "active" window (chromatogram or component display). When Off, only a small colored triangle will be shown below the peak. To add individual m/z chromatograms to the total ion chromatogram window, click on the desired m/z in the mass spectrum. The m/z chromatograms can be removed from the total ion chromatogram by clicking on the m/z value in the box to the right of the chromatogram.

Auxiliary Information: This option provides access to the data shown in the Tabbed Dialog at the bottom of the Results Window (see Section 2.4).

Same Time Scale: This is only active if two chromatograms are displayed. The use of multiple chromatograms is discussed in Section 3.5. When checked, both chromatograms keep the same time scale, even when zoomed using the mouse.

3.2.5 Library Menu

You may edit libraries or add data to libraries from current experiments, from text files, or from files created using the NIST Mass Spectral Database Program. For details about building and editing libraries, please see Section 6.

Build One Library: Allows the construction of a library from the data file being analyzed. You may select any deconvoluted spectrum to add to the library.

Library Transfer: Allows for the addition of components from one library to another.

3.2.6 Options Menu

Options for changing the appearance of the screen:

Select m/z Values to be Displayed: Generally m/z values are selected by clicking on the appropriate spectral line in one of the displayed spectra. Use this dialog box to select the m/z values to be displayed in the ion chromatogram and in the component profile displays. The color of a displayed m/z value corresponds to the color of the m/z number on the left. You can also specify the number of peaks to be normally displayed in the component window. The TIC will always be displayed along with the largest peaks. In addition, you may specify whether to show all models of a particular component. If you select to display all models, and there are peaks that have been subtracted in the deconvolution of the component, they will be shown.

Display Threshold: The threshold limit is set by the percent of the total ion current associated with the reconstructed component. This threshold value **does not** affect the deconvolution parameters. It will reduce the number of peaks that are displayed on the screen, and is useful with very noisy data. To change the deconvolution parameters see Section 4.5.3.

Font: Change the display font including style, size, color, and script.

Color: Change the color of the background, m/z lines, etc. The lower left hand side of the color selection box provides a list of all of the screen elements that can be altered. Once a color has been applied it is saved. You can save a number of different color settings.

NOTE: Blue and red triangles (▼) are used to designate the components and **T** for the targets. The red designates the currently selected component or target. These colors can not be changed.

3.2.7 Window Menu

You may open more than one data file at a time, choose **File/Open/Open in New Window**. It is also possible to have two chromatograms in a single window (see Section 3.5). The program can keep up to 9 windows **open**. The open windows can be either active or minimized. The window is **active** if it is not minimized. If a window is minimized it is represented by an icon at the bottom of the main AMDIS window.

Cascade: Standard window control to arrange all **active** windows in a cascade so that all window titles can be read.

Tile: Standard window control to show all **active** windows in a tiled arrangement.

Arrange Icons: If a number of windows are open and have been minimized to icons, this command will neatly arrange all of the icons.

Close All: Will close all windows currently open.

List of open windows: A list of windows and current open file is given, as with most windows programs selecting one of these windows will change the data display to that for the file selected.

3.3 Data Displays

The program displays your data in a series of windows. You can change the display any time. In general, you may set the appearance of the display with the **right mouse button**. This will bring up a menu of options for you – the menu is specific to each window, although several features are common. Each of the specific data display windows is discussed in detail in Sections 3.3.1 through 3.3.5.

3.3.1 Chromatogram Display

In the upper pane, the ion chromatograms for each ion as well as the total ion chromatogram that can be displayed. Specific m/z chromatograms can be added by clicking on the appropriate mass in one of the mass spectral panes or by selecting the **Options menu/ Select m/z** (see Section 3.2.6).

The graph can be zoomed by dragging the mouse to set a rectangle around the area of interest (depress the left mouse button at one corner of the area to be expanded and move the mouse diagonally to the opposite corner, then release the mouse button).

A click on the **right mouse button** will bring up a menu, the first item is to restore the display to the prezoomed condition. If several zooms have been done in succession, the option to **Unzoom All** is also provided.

NOTE: AMDIS will always analyze the data shown in the chromatogram display, thus if a smaller area of the chromatogram is selected, the analysis is restricted to this region. Considerable processing time can thus be saved.

The small right and left arrows located above the chromatogram, to the immediate right of the **Info** Tab, enable scrolling through the zoomed chromatogram. The individual component peaks can be selected from the Component/Target Information List box (see Section 3.3.3). The peak will be brought into the display with the same resolution as set by the zooming.

3.3.2 Component Ion Chromatogram Display (Component Profile)

This smaller chromatographic pane shows the total ion current and the largest mass chromatogram peaks over the region actually used in the deconvolution of the component. The model peak, the peak showing the most rapid rise and fall, is always shown and is assumed to be the most characteristic peak for a particular component. The model peak is often the total ion current, but in complex mixtures with many coeluting peaks, the individual ion signals are far more useful. The number of peaks shown is set by selecting the **Options/ Select m/z** (see Section 3.2.6).

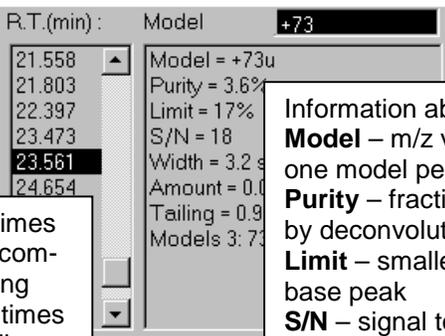
In most cases, the model peak and the largest deconvoluted peaks (total number selected by user – see Section 3.2.2) in the spectrum are shown. In cases where a specific peak has been subtracted, it will be shown even if that peak does not occur in deconvoluted component.

In general, the chromatogram display is active. As a result, when a peak is selected (by clicking on it with the mouse) the individual ion chromatogram will be shown in the chromatogram display. If you click the mouse in the Component Display window, it will become active and any m/z selected in the mass spectral displays will show up as a single ion chromatogram in this 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.

3.3.3 Component/Target Information List

Depending upon whether you are looking at a component or a target, the data shown will be in different formats. If you are in the **Target Mode** (see Section 3.2.3) the list boxes will contain target data. If you are in the **Component Mode**, you have the option of looking at either targets or components. If a **T** is red then you will see target information, if a **▼** triangle is red then you will see component information.

If you are looking at a **Component**, the List Boxes will show:



A list of retention times for each resolved component. Highlighting different retention times with the mouse will change the active spectra. The selected component is noted by the red ▼ triangle in the chromatogram.

Information about the component:
Model – m/z value of the model peak, more than one model peak may be listed
Purity – fraction of TIC at the peak accounted for by deconvoluted component
Limit – smallest m/z peak observable relative to base peak
S/N – signal to noise for the component
Width – full width at half maximum
Amount – fraction of total ion signal due to this component
Tailing – area after maximum divided by area before maximum
Models – a listing of all the model peaks considered

Details on these component parameters are given in Section 2.3.2.

If the data selected is a **Target**, then the following list boxes will be shown:

The screenshot shows a software interface with several data fields and callout boxes. The interface includes a list of retention times (23.473, 25.015) and a list of identified targets (Docosane, Tetracosane, Hexacosane). Below these are fields for Component (Purity = 99%, Amount = 2.96%) and Match (RI-RI(lib) = 0.0, Net = 93). Callout boxes provide detailed explanations for each section.

A list of the retention times for each identified target. A dash followed by a number indicates the number of the target compounds that has a sufficiently high match factor.

A list of the identified targets, this is linked to the list of retention times.

Data relating to the component, this list box contains the same information as shown in the right hand list box of the component data display. The only addition is the **RI** – retention index, which is shown only in modes where it is calculated.

Target Match factors
RT-RT(lib)- only calculated if library data is available
RI – retention index, only calculated in some modes
Net – net match factor
Weighted – weighted (by mass) match factor
Simple – unweighted match factor
Reverse – reverse match factor
Corrections – corrections applied to weighted match factor

These lists give the same data as in the RESULTS Window (see Section 2.3.3).

3.3.4 Component Mass Spectral Display (Scan Display)

This window shows the raw mass spectrum (scan) and the deconvoluted spectrum at a given retention time. The raw MS is shown as black vertical lines, the deconvoluted MS is shown as white lines. The lines for each m/z are directly adjacent to one another. You may expand the spectra by using the mouse to zoom any region. The right mouse button will also allow you to show only the raw or deconvoluted spectrum. The data are shown as normalized mass spectra. In order to determine the absolute values of the signals, multiply the value on the y-axis by the value given in the parenthesis following 'Abundance' at the upper right.

Clicking on a mass peak in the component display window, will cause the corresponding ion chromatogram to be toggled on or off in the chromatogram display. The color of the ion chromatogram will correspond to the color of the small triangle under the spectral line – you may set the display to change the entire line color rather than just add a colored triangle (**View/Color Selected Peaks**).

The right mouse button provides a number of options. These are discussed in Section 3.3.6.

3.3.5 Target Mass Spectral Display (Library Display)

Once the data have been analyzed, the mass spectra of the component and the library are shown. **The overall behavior of the window is similar to the Component Mass Spectral Display.** The component spectrum is white in both windows. In the Target Mass Spectral Display, the library spectrum is black.

3.3.6 “Right Mouse” Options in Data Displays

The right mouse button provides a number of options in all displays. Many of the features are the same in all of the windows. Most of the features accessible from the right mouse button can be accessed from other menus in the program.

Unzoom: If the spectrum or chromatogram has been zoomed to display more detail, it can be unzoomed. The unzoom takes you down one zoom at a time.

Unzoom All: If multiple zooms have been done, this will take you back to the original unzoomed scale.

Show Mouse Position: On Cursor, On Top, Off – Three choices for showing the location of the mouse. **On Cursor** gives brief information (m/z for spectra, scan number for chromatograms).

On Top gives more detailed information – m/z, signal strength for spectra; scan, time and signal strength for chromatograms. The data is shown in the upper right hand corner of the active display. **Off** turns the display of the mouse position off.

Log Scale for Chromatogram/Spectra: Toggles between linear and log scales for the abundances/intensities.

Freeze: m/z interval, Abundance – None, one or both may be selected. Normally the program will adjust both the intensity and mass range so that each spectrum fits on the screen. You may want to freeze a mass range (m/z interval), for example, to look at a narrow range of masses over a number of scans, or to look at the absolute intensity of a set of masses as you examine a number of scans. You may freeze either the mass range or the intensity range or both. They will freeze in the current state, but you may zoom them. Freezing the m/z interval locks both the scan and library x-axis to the same interval. Freezing the Abundance affect only the y-axis in the window where the right mouse was activated.

Show Spectra: Scan, Component, Both or Library, Component, Both. Normally both are shown. For some complex spectra or to prepare data for publication you may want to show only one or the other.

Show Uncertain Peaks: If checked (default) the program will show peaks that are uncertain in their deconvolution as dotted lines. The treatment of these peaks is discussed in Section 2.3.3.

Show Window: If selected this will bring up a check box that will allow you to select which of the windows discussed in Section 3.2.4 are shown. This is useful if you need to see data on a small screen or are trying to show multiple chromatograms.

NIST Library: Add Component to Search List (N), Search NIST Library – Allows you to make a list of components to send to the NIST Search Program or to search an individual spectrum immediately.

3.3.7 Additional Options in the Chromatogram Display

Many of the right mouse options discussed in Section 3.3.6 also apply to the chromatogram window. There are a couple options that are only available in the chromatogram window.

Rescale: You may cut off the top of a peak or the bandline when zooming in on data. Rescale adjusts the abundance axis to include all data. Note, there is one scale for the TIC and a different scale for the individual ion chromatograms. There is only one scale for all displayed individual ion chromatograms. You may need to omit a strong ion to see the variation of a small ion. Rescale will adjust the axis after such a deletion. There is also a **Rescale button** above the chromatogram display.

Autoscale: Applies only to the individual m/z ion chromatograms. If this is checked, the abundance axis will automatically adjust to the highest peak as m/z in chromatograms are added and removed. If it is not checked, the abundance axis will be determined by the first m/z ion chromatogram added to the display.

Show Component on Chromatogram: The program reconstructs the total ion chromatogram of the component from the deconvoluted ion chromatograms. It is often useful to see how much of the total signal is accounted for and how long a range the program used. Note – the range of

scans over which a component is determined is shown when the component is highlighted with a red line over the triangle. This can often only be seen with the chromatogram display zoomed.

Show only Current Chromatogram: Normally checked, if unchecked the program will show the reconstructed chromatograms of all of the components. This can get to be messy, but, at high magnification, which it does allow you to see the degree to which components overlap.

File: Open, Add, Close – **Open** will open a new file. **Add** will place an additional chromatogram in the same chromatogram display window. **Close** will close the currently active file. To display more than one chromatogram see Section 3.5.

3.3.8 Options in the Component/Target Information Lists

The **right mouse button** will bring up a number of options – in the component display mode, you may choose to **Search the NIST Library** or **Undock**. The Undock will allow the list boxes to float free so they can be moved around. This is useful in saving screen space since it is possible to not show the component window and allow the individual mass spectral window to be larger. Searching the NIST Library will allow you to either search the NIST library with the current component or to add that component to a list to be searched later (see Section 3.2.2). In the target mode the same choices are available, but only when the mouse is in the upper left hand list box. In the upper right hand list box, the list of targets identified, the right mouse button allows you to choose between **Best Hits Only** or **All Hits**. If Best Hits Only is chosen, only the target compound with the highest match factor is shown. If All Hits is chosen, all of the target library compounds with match factors greater than the minimum will be shown. When the mouse is over the lower two list boxes, the right mouse button will bring up the choice of **View Options** or **Print Options**. Selecting either one of these will open up a set of choices as to what you wish to have printed or displayed. You may select to omit some data either from the printed or displayed output.

3.4 Button Bar – Run, Rescale and Information.

Below the menu bar contains three buttons, Run, Rescale, and Info, as well as two arrow tabs.

Run: To run the analysis with the current settings on the portion of the data file shown in the chromatogram display window.

Rescale: Will readjust the vertical axis to make the largest peak in the ion-chromatogram region displayed at full height.

Info: Will bring up the Auxiliary Information tabbed dialog box similar to the tabbed Dialog Box (Section 2.4).

Arrow Tabs: A pair of arrows for moving the zoomed window.

3.5 Display of Multiple Chromatograms

There are many cases where it is useful to have two or more chromatograms open at once. In AMDIS there are two ways to have multiple chromatograms. You may display as many as 10 chromatograms as separate windows, each with its deconvoluted spectra. Or, you may have only 2 chromatograms, both will appear as TIC displays, but only one set of component/profile and mass spectral information will be displayed depending upon which of the chromatograms is selected.

3.5.1 Multiple Chromatograms in Independent Windows

In the case of multiple independent windows, each chromatogram has all of the attributes of the entire program as discussed in Section 3.3. All of the features work exactly as before with one exception. If you **Undock** in any of the windows it will be applied to all. If the identification list box has been undocked, it will show on the top of the window which of the chromatograms it is displaying.

To open a file in a separate window, select **File / Open** from the menu bar. After choosing the file, select the **Open in new window** button on the lower right of the dialog. The active window will be indicated by the active window color on the top bar. Note – when you print, only the active chromatogram and the spectra you select will be printed. You can select which displays to see independently in each of the windows.

If you have multiple chromatograms, you can force the time axis to be the same and to track with zooming. Select **View / Same Time Scale** from the menu bar. This applies to chromatograms in all windows.

3.5.2 Two Chromatograms in One TIC Window

In this mode you can only have two chromatograms at any one time. This mode has only one open window and can provide more screen space for data. Only the spectral data from the active chromatogram is present. It is not possible in this mode to show two spectra from different runs at the same time.

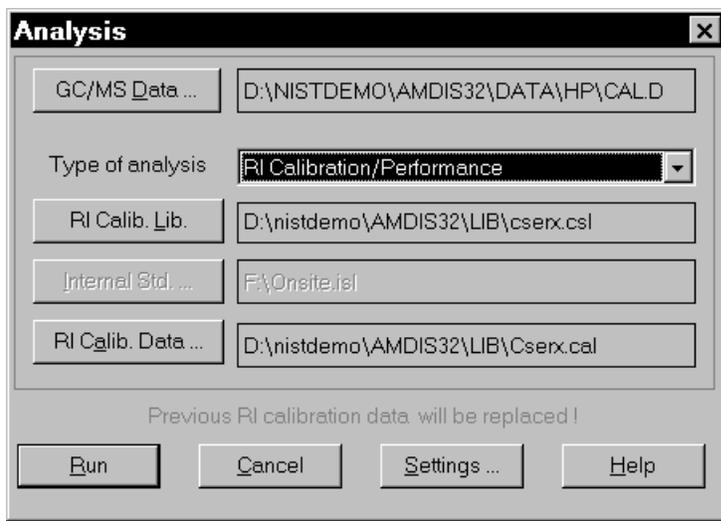
To open a file in the same chromatogram window, you must have the mouse in the TIC window and use the right mouse button. Go to the last item that appears when you hold down the right mouse button is File. Choose **File/Add** to select a file to be added to the current window.

If you have undocked the Identification list boxes, then the name on the top of the identification list box is the name of the active spectra.

4 Setting Analysis Parameters

4.1 Introduction

The analysis parameters can be set from either the **Results Window** or the **Confirmation Window**. In each case, you will see a tabbed dialog box that allows you to set all of the parameters of the analysis. In the **Results Window** select **Analyze** in the Control Panel. In the **Confirmation Window**, use the menu bar to select **Analyze/Analyze GC/MS Data**. In each case you will receive a window similar to the one below:



This box contains the following buttons:

GC/MS Data: Selecting this button will bring up a file selection menu. Use this menu to select the experimental data you wish to analyze.

Type of Analysis: This selection is a drop down list box that allows you to set the type of analysis, for details see Section 4.2.

Target Library, RI Calibration Library, Internal Standards: You may specify the target, calibration, or internal standard library. The library types are discussed in detail in Section 4.3.

RI Calibration Data: Choose the name of the file to contain the retention time/retention index information.

Run: Start the analysis.

Cancel: Close window and do not perform analysis.

Settings: To set or change the analysis parameters. As discussed in Section 4.5, this will bring up a tabbed dialog box within which you may specify all additional parameters.

Help: Bring up on-line help windows.

4.2 Type of Analysis

Simple: Uses only the mass spectral data for the individual target compounds in the library 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.

Use RI Calibration Data: This type of analysis uses an external calibration file. It assumes that on a periodic basis, an experiment is run to generate a correlation between retention time and retention index for a set of retention index standards. In addition to mass spectral data, if the identified compound is not within a specified retention window, the program will penalize the match factor by a specified amount. Retention indices will be used in the match factors **only** if the box **Use Retention Index** is checked in the **Settings/Identif.** Window (see Section 4.5.1). If this box is not checked, the retention indices will be reported but not used in the match factors. The calibration file must first be created by running a RI Calibration/Performance Analysis – see below.

Use Internal Standards: In this mode the calculation of retention indices is determined from a set of internal standards that are co-injected with the unknown mixture. AMDIS uses the same logic to find and analyze the internal standards that it uses to find target compounds. The found or missing internal stan-

dards will be reported in the QA/QC report that is created for each analysis (see Section 2.4.5). The program uses all detected internal standards to compute retention indices for all targets identified. The mass spectral and retention index data for the internal standards are in a separate internal standards library (.ISL). You can only specify an internal standards library when the program needs to use this library. This analysis mode is useful when the sample to be analyzed contains known compounds with known retention indices.

Use RI Calibr. Data + Internal Standards: In this mode the retention indices are calculated from the external calibration file as in **Use RI Calibration Data** type of analysis. The internal standards are used only to insure that the instrument functioning correctly or that the sample preparation has been performed correctly. The internal standards are not used to calculate retention indices. The penalty for mismatch of the retention data is set by the user, and must be chosen in the 4.5.1 Identification (Identif. Tab) window.

RI Calibration/Performance: Unlike all of the previous analysis modes, this is presumed to be run on a clean mixture of known composition. This analysis establishes the correlation between the retention time of a component and the retention index using the set of standards specified in the calibration library. Usually, the C-series or the normal hydrocarbons 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. When this analysis type is selected, the target library button changes to the calibration library. In this type of analysis you will need to choose both the current calibration standards library (.CSL) and the calibration (.CAL) file in which the calibration standard retention time/index data are stored. You have the option of selecting the name for the calibration data (.CAL) file. This file name might be selected to reflect the date or the column type. The name is retained for future use. It is possible to have multiple calibration files (with different names), although only the one specified is used. Creating a calibration file is described in Section 6.6.

Performance Check: This analysis type only checks the performance for compounds identified as performance compounds in the .CSL library. It does not perform a calibration. The results of the performance check appear in QA/QC Performance window from the Info button.

4.3 Library Types

AMDIS uses three different types of libraries – the target library (.MSL), the calibration library (.CSL) and the internal standard library (.ISL). Only the target library is designed to have multiple spectra per compound. The calibration libraries (.CSL) have flags in them to allow for the recording of data over long periods of time. The program can thus keep a record of the performance of the GC/MS (see Sections 4.5.7 and 6.7).

Selecting any of the libraries brings up the library portion of the settings tabbed dialog box. For more details see Section 4.5.4.

Target Library: Select and/or view the Target Library. If the Type of Analysis is RI Calibration/Performance, the label will change to **RI Calibr. Lib**. In each case the library selected will be the library used for the analysis.

Internal Std: Select the library of internal standards. This option is only active if the type of analysis is Use Internal Standards or Use RI Calibr. Data + Internal Standards.

Calibration Data: Select the file in which the results of the RI Calibration/Performance Analysis are stored. Once a calibration has been run, the results are stored in this .CAL file for future use. This .CAL file is not the library of calibration compounds (.CSL) selected in RI Calibr. Lib. It is a simple text file listing the retention time (RT) and retention index (RI) values for the standard compounds in the calibration library (.CSL). These values are used to convert the retention times to retention indices.

4.4 Run Button

When you have selected the data file, the type of analysis and the target libraries, the **Run** button will perform the analysis.

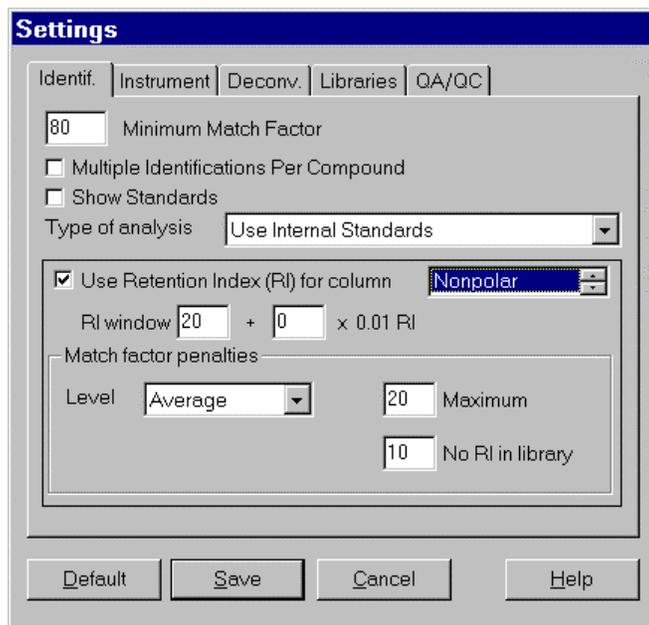
4.5 Settings Button

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 identification tab (Identif.) allows you to set limits as to what will be shown and whether or not to use retention index data. The instrument tab sets the type of instrument. The deconvolution tab (Deconv.) sets the parameters for deconvolution. The libraries tab allows you to set the target libraries used. The quality control (QA/QC) tab allows you to set parameters that are used in checking for the proper running of the instrument.

4.5.1 Identification (Identif. Tab)

Within this dialog, you can set the parameters for the basic identification.



Minimum Match factor: The lowest match factor that the program will accept to print out a target identification. The default value of 80 has been derived from extensive testing for false positive results. You may choose any value here, but all data below 80 will be reported with flags. These are in the form of question marks (?) in front of the name (see Section 2.3.1).

Multiple Identifications per Compound: Default is off. If on, the system will allow the same compound to be identified for more than one component. In the case of some isomers this is desirable. If off, a compound will be reported only once, associated with the best match.

Show Standards: Default is Off. If on, the system will report all internal

standards found in the Identification list. They are reported in retention order at the end of all of the other identifications, regardless of when they appear in the chromatogram.

Type of Analysis: This allows the same set of choices as noted in Section 4.2. It is here as a convenience.

Use Retention Index for Column: The default is off. This is used solely when an analysis type that uses retention index data is selected. If the box is checked, the analysis will include retention index information in the calculated match factors. Once this option is selected it will activate the other choices in the lower half of the tabbed dialog. In general the use of retention data can reduce false positive identifications. No points are added to a match factor for being within a retention window, but points can be subtracted from the match factor for being outside a specified retention window.

Column: A number of columns are predefined. The first two are nonpolar and polar, then a number of specific columns DB-1, SE-54, DB-35, OV-17, Rtx-1301, OV-1701, Carbowax, User-1 and User- 2. These do not represent recommendations by NIST. Data for a number of columns can be stored with each target compound.

RI Window: Default is 20. The window is defined as the value on each side of the library retention time within which **no** penalty is applied. Outside of the window, a penalty is applied that depends upon the maximum penalty and the level you set. You may also allow the retention window to increase linearly with retention index. The linear factor is set in the second window on this line. The default value is zero. You may choose any value greater than 0. Thus if 5 is chosen, the window would be 20 at RI=0, 25 at RI=100, 70 at RI=1000, etc.

Level: The level choices are weak, average, strong. The default is average. This sets how fast the maximum penalty is applied. At the strong level the maximum is applied within 1 window beyond the initial window. For average, the maximum is applied within 3 windows, for weak within 5 windows. The effect is linear. For example, with a strong level of RI penalty and the RI found is 1.5 RI windows away from the library value, a penalty of 0.5 of the maximum would be applied. (The first one window is penalty free, the strong level applied the maximum in one additional window, which we are half way into.) In the case of average, the penalty would have only been 0.1667 or one-sixth of the maximum.

Maximum: The maximum RI penalty that is to be applied, even for a complete miss. The default is 20, which is enough to make an almost perfect match fall below the 80 match factor considered an identification.

No RI in library: If there is no RI data in the target library, you have the option of applying a penalty. The default value is 10, which requires a very good match to be better than 80. Again, the value can be set by you.

4.5.2 Instrument Tab

Low and high m/z: (Default low = 50 and high = 400) These values should correspond to the values that are used by the instrument. If the **Auto** check box is selected, the program will use the data found in the file and automatically select the minimum and/or the maximum m/z value. If you have selected values that are very different from the experimental values, you can cause the program to improperly correct for the scan bias. If the values found in the file are different from the values that you enter, the difference will be reported in the QA/QC report generated by the program (see Section 2.4.5).

Threshold: (Default **Off**) The program can set a threshold different from the one given in the GC/MS data file. In especially noisy chromatograms, setting the threshold higher can reduce the effect of noise and may speed up processing.

Scan Direction: None, Low to High, High to Low. The scan direction is machine dependent and thus coupled to the data file format.

Data File Format: This is a list of all of the native file formats that AMDIS can read. These are:

- Bruker
- Finnigan (GCQ, INCOS, and ITDS formats)
- HP Benchtop and MS Engines
- MassLynx NT Formats
- MicroMass
- NetCDF
- Perkin-Elmer
- Shimadzu MS Files
- Varian Saturn Files
- Viking

Note that NetCDF is an industry standard exchange format not a specific instrument company file format. 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.

4.5.3 Deconvolution (Deconv. Tab)

Component Width: The width that a component is assumed to be. The parameter is in terms of mass spectral scans. The default is 12 scans. In general, larger settings will slightly slow down the program, but the effect is small. The program will adjust for wider peaks to some degree. If you are analyzing data with very wide peaks then you may want to increase this number, or if all of your data is very narrow, you may wish to decrease it slightly.

Adjacent Peaks Subtracted: Two/One/None; Default One: This sets the degree to which adjacent peaks, if present, are subtracted in the deconvolution. If None - no adjacent peaks are sub-

tracted from the deconvoluted peak, this is fastest and is useful for very clean data. The default (One) uses only the nearest adjacent peak (if present) from either side of the deconvoluted peak. Two peaks will use two peaks on either side of the deconvoluted peak.

Deconvolution Sensitivity: Very Low/Low/Medium/High/Very High; Default Medium: As the value for this parameter increases, increasingly noisy and broad peaks are sought at the expense of analysis time and increased risk of false positives. An increase in the sensitivity will extract broader peaks, hence smaller and noisier peaks. As the sensitivity is increased, smaller multiples of the noise factor are accepted as distinct peaks.

Deconvolution Resolution: High/Medium/Low; Default Medium: As the level of resolution goes up, the program separates peaks that are closer together.

Shape Requirement: High/Medium/Low; Default Medium: The shape requirement is a way of forcing all of the deconvoluted peaks to have the same shape. As the shape requirement increases, the shape of the individual ions must be more nearly the same.

4.5.4 Libraries

The Target Compound, Internal Standard, and Retention Index (RI) Calibration Libraries as well as the RI Calibration Data file can be set here. The view button will allow you to examine each library or data file. This dialog within the tabbed dialog will be brought up by the program each time you want to select a new library or calibration file.

The library **View** button allows the library or calibration file to be viewed. See Section 6.8 for details.

4.5.5 Quality Control (QA/QC Tab)

While many of the parameters that are determined for each component or target can be used for quality control, there are two areas for which specific QA/QC features. These concern the degree of solvent tailing and the amount of solvent bleed. Both of these parameters are assessed by the use of characteristic ions.

Solvent Tailing - the m/z value set here should be the principal ion, or a unique ion of the solvent. The program calculates the value of the solvent peak at the end of the solvent delay and then reports the time when the solvent peak drops below the next 10,5,2,1 sequence until either the peak goes to a S/N of 1 or the chromatogram ends. For example, if after the solvent delay the program finds the solvent peak at S/N of 200, the report would be: Solvent falls below S/N of 500 before the run begins, solvent falls below S/N = 200 at 0.5 min, below S/N=100 at 0.61 min, below S/N=50 at 0.72 min, below S/N=20 at 3.5 min, below S/N=10 at 12.2 min, below S/N = 5 never.

Column Bleed - the m/z value set here should be that of characteristic ion of the decomposition of the column. By default, 207 is used since this is a characteristic ion of many of the common GC columns.

4.5.6 Save – Default – Cancel – Help

Default: Return to the default settings.

Save: Select to save any changes you have made – Exit back to calling screen.

Cancel: Leaves the program in the state when you came into the tabbed dialog.

Help: Brings up a context sensitive help.

4.5.7 A Note on RI Calibration / Performance Analysis

There are a number of special features that are built into the RI Calibration / Performance Analysis. These are based on the assumption that you will use the normal paraffins (n-alkanes) as standards for the RI calibration and that you would like to keep a record of the results from one

performance mixture run to another. The additional assumption is that some standard performance evaluation mixture such as the Grob mixture is used for performance analysis.

In the case of the retention index calibration analysis, the calibration library has the provision to require a specific peak. Since the molecular ions of the paraffins are usually present, but small, requiring them to be present can better insure identification. In addition, the program requires that the compounds be identified in order. It is very easy in a noisy environment to mistake a C10 for a C12 normal paraffin. These same identification problems exist with other reference compounds (for example the methyl esters) and the same logic would be applied.

The results of the calibration are shown in the **Library and Settings Tabbed Dialog** under Standards (see Section 2.4.4) if the radio button External Calibration is selected.

Any compound selected for performance monitoring must be specified in the RI Calibration Library (.CSL) when editing a compound during library maintenance (see Section 6.7). The recent history of compounds selected for use in performance monitoring is shown under QA/QC (see Section 2.4.5) if the Performance radio button is selected. Data for retention time, retention index, height, width and tailing of the peaks as well as the match factor is shown. The date and file in which the data are found is also shown. This allows you to see if there are any changes in the performance of the instrument over time.

5 Post Analysis Classification Of Spectra

Under the menu choice Analyze there are two post analysis options, post analyze and Compound Classifier. Both of these options allow you to go beyond target analysis, and even beyond using the entire NIST database to analyze the data. These options allow classification of the individual components by chemical classes.

5.1 Postanalyze

This option will allow you to analyze the entire data file with three distinct classifiers, the Compound Classifier, the CW Classifier, or Spectral Similarity.

5.2 Compound Classifier

This is 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 referenced in Section 5.6. 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). Appendix C gives detailed descriptions and chemical structures for these groups. See also Section 3.2.2.

5.3 Chemical Weapons Related Classifiers (CW Classifier)

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.

5.4 Spectral Similarity

This 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. A pull down menu appears to the right of the buttons at the top of the screen. You can use this to view the components in each class of spectral similarity, noted by **T**'s at the top of the chromatogram.

5.5 Compare Data Files

The feature 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 (using File/Open In/Active Window for the second file). Then each file should be separately analyzed. At this point the compare option is active and may be selected from the Analyze/Post Analyze menu.

Since many very small components may be observed in two otherwise nearly identical files, there is an "minimum S/N" option to separately label such weak components. This should be adjusted to suit your needs. S/N is the total signal strength as measured by all ions in a component.

After a "comparison" is done, a selection box appears in the top window for the active data file (the active data file's name is in dark blue, the other name is grayed out). Click on any area of a data file to make it active. If any targets have been identified, they may be displayed using the **(nn) Targets** selection, where nn is the number of target compounds identified. The simply puts you in the mode without the post analysis comparison results being shown. Other selections show unique or common components of the two data files: **Match** shows all matching components, **Match/Larger** shows components that are 3 or more times larger in one file than the other, **Unique/Significant** shows components present only in the active data files whose signals exceed the selected "minimum S/N" threshold described above, **Unique/Trace** shows all unique components less than the specified minimum S/N values, and **All Classes** shows all components used in matching (not useful in this mode).

Miscellaneous:

- 1) Use **View/Same Time Scale** to conveniently compare data files.
- 2) Whether or not two components in different files match depends on both their spectral similarity and their relative retention times. Components are 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" (perfect match = 1) and RT1 and RT2 are retention times in seconds in the two data files.

- 3) 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.

5.6 Compound Classification References

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.

6 Library Development and Maintenance

6.1 Introduction to Libraries

The library editor can only be accessed from the **Confirm Window**. The Library menu item has two choices. The first, **Build One Library**, allows you to build a library from the components in an experimental data file. The second, **Library Transfer**, allows you to create a library out of other AMDIS libraries or out of data from the NIST Mass Spectral library.

You can view existing libraries from both the confirm and the results window, see Sections 3.4 and 2.4.1. However, spectra can be edited only from within Build One Library or Library Transfer.

6.2 Library Types

There are three different library types that are used by AMDIS plus one additional type of file that it accepts for input. The library editors and transfer programs are specific to different library types.

Target (*.MSL, *.CID) - Basic library, spectra and chemical identification information kept in separate files. The identification information is in a *.CID file with the same name as the *.MSL file. Thus a perfume library would have the spectra in perfume.msl and the compound names and formula in perfume.cid. The data is linked by a unique chemical identification number. For convenience the Chemical Abstracts Service Registry number is used when available, but any unique value can be used. Deconvoluted spectra taken from GC/MS data files are identified by a file name identifier followed by Nxxxx. Multiple spectra per compound are allowed. The spectra and identification information are edited separately.

Retention Index Calibration/Performance (*.CSL) - Retention index calibration library with optional GC/MS performance mixture data. Each compound is assumed to have only one spectrum, thus there is no distinct chemical identification file. Spectra and identification information are edited together. You may have multiple spectra with the same compound identification in the calibration library, but the library routine does not link the spectra through a .CID file as it does for target libraries. This 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 to be used for either RI Calibration or Performance Log or both, see Section 6.7. Additionally, you may specify that a particular m/z peak be identified for correct identification of a compound. This is particularly useful when identifying similar compounds (e.g. a homologous series such as hydrocarbons).

Internal Standards (*.ISL) - Similar to .CSL library but used only for co-injected standards or for any components considered to be such internal standards. This library does not contain the tags to track compounds for a performance log or to be used for retention index calibration. If you want to use internal standards for either RI calibration or performance tracking, those compounds must be included in the RI Calibration/Performance library (*.CSL file).

Simple Mass Spectral (*.MSP) - Set of files to be used to search NIST Mass Spectral Database. AMDIS can read .MSP files generated by the NIST Mass Spectral Database. A spectrum from AMDIS can be sent as an .MSP file to the NIST Mass Spectral Database to search the database for a match. Conversely, a spectrum in the database can be saved as a .MSP file and then added to an AMDIS library.

6.3 Library Editors

6.3.1 Build One Library

When this option is selected, a window appears to add the currently highlighted component to a library. The **title bar of the window** will show the library to which you are making the addition. The large button at the top left shows the component that will be added. Also, the delete and edit buttons are on the top line. On the left is a list box containing all of the spectra currently in the

destination library. At the bottom is a box containing information about each spectrum. The information corresponds to the highlighted spectrum (use a mouse click) in the list box. On the left are the **hide** and **sort** pull down menus and the **files**, **exit** and **help** buttons. Since many of the features are common between Build One Library and Library Transfer, they will be described in Section 6.4.

6.3.2 Library Transfer

This option allows you to copy compounds and spectra from one library to another. In this window data can be transferred from the source to the destination library. However, only the destination library data can be edited or deleted.

The title bar contains the names and types of the source (from) and destination (to) libraries. The list box on the left contains the source library spectra. The destination library spectra are listed in the box on the right. There is a Sort pull down menu for each list box. The **Add** button to add a deconvoluted spectrum from the GC/MS data is below the source list box. At the bottom is the box containing information about the highlighted spectrum (in either the source or destination library). In the middle are the transfer, edit, delete, files, exit and help buttons. Again, the details are explained in Section 6.4.

Source and Destination data can be singly or multiply selected using standard windows mouse operations. Mouse click for single selection, shift mouse click to select all from the currently selected item to the item under the mouse, drag - holding the mouse left button down while dragging the mouse selects all items, ctrl mouse click to select only those under the mouse.

6.4 Library Window Functions

6.4.1 Library Information and Display Options

The Spectral Data List Boxes contain lists of the data in a given library. These lists can be sorted by compound name, chemical i.d. number, or by retention index. You can double click on any spectrum in a list box to access the compound/spectra editors for that chemical. Data about each compound is listed in the box at the bottom of the screen. You can choose to not display extra data (hide), to display the numerical spectral data (text), or to have a graphical display of the data (spectrum).

6.4.2 Editing Options

Add...: Add the deconvoluted spectrum of the peak selected in the chromatogram (Confirm Window). While either Library editing window is open you may change the peak selected in the Confirm Window. In this way you can quickly select a number of compounds to be added to the library from a single chromatogram. If you are making this addition to a target library (.MSL) you will be given the choice of adding the spectrum as a new compound or linking it to an existing compound as a replicate spectrum. If you add a spectrum from a GC/MS data file the chemical ID contains the name of the specific data file so it can be tracked later.

Replicate Spectrum: Will add the replicate spectrum assigning it the identity of the currently highlighted spectrum in the destination library. The replicate spectrum will have the same .CID file as the highlighted spectrum in the destination library (the compound name will change to that of the highlighted compound). It will **not** replace the currently highlighted spectrum it will simply add another spectrum associated with the same compound name.

New Compound Spectrum: Will add the spectrum as a new compound, giving it the name shown on the Add button. The name will be the RI (if calculated) and the RT along with the data file name. For instruments that distinguish between the data by the directory name (for example Hewlett-Packard files) the subdirectory name will be used. Once the addition has been made, the editor highlights the new compound in the list of the spectra in the library.

NOTE: If you are building a calibration/performance or an internal standard library (*.csl or *.isl) you will only be allowed to add a **New Compound Spectrum**. You can add two spectra of the same compound, but they will not be linked by a .CID file. You can edit any spectrum by selecting it from the list, clicking on the Edit button and choosing either Spectrum editor or Compound editor.

Add all: Add all, or some portion of, component spectra to the library. A dialog box allows you to choose to add components from all components, from unidentified components, or from identified components. The destination library is listed with the choice to add spectra to or replace all spectra in the library. You can select to add some number of the largest components, all components above some percentage of the total signal, and whether to include/not include uncertain peaks in the spectra.

Transfer: Copies spectra from source library to destination library.

Edit: You may edit compound data, the mass spectrum or exit (cancel) from this editor. See Section 6.5 for details.

Delete: Delete spectra from the destination library. You do not have to save the library files, it is done automatically. If you inadvertently add a compound, you need only delete it again, however, if you delete a compound from the library it is gone. You will be asked each time if you want to delete a spectrum.

6.4.3 Other Options

Files: This button brings up a window that allows you to load both source and destination libraries, create a new destination library, save a destination library under a new name or switch between the Build One Library and Library Transfer editors.

Exit: Leave the library editor.

Help: Bring up on-line help windows.

6.5 Compound and Spectra Editors

6.5.1 Compound Editor

The compound editor is only used when you are working with Target Library files (.MSL). As noted in Section 6.2, the Target Library presumes that there may be more than one spectrum associated with each chemical compound. The compound editor allows you to enter the chemical ID number, formula, chemical class, retention index, retention index window, retention time, s/n reference mass, response factor (Resp. Factor, used to calculate concentration), name, and synonyms. The link between the compound and the spectrum is made exclusively by the chemical ID. Thus all spectra with the same chemical ID number share the same compound data.

If you have added a spectrum to a Target Library from the data file, it will be assigned an ID. Once you identify the compound, you may want to change the ID, to do this you must select **New** next to the chemical ID. This will bring up a box with the old and the new chemical ID, enter the new ID. Once you have done that AMDIS will correct the entry in the chemical identification file (*.cid) as well as the target library file (*.msl). A different Retention Index and Retention Window (how broad you want) can be added for each column. Note, the first element in the list of **Names and Synonyms** is the name used in the spectral display window. If you place the new chemical name on the line above the old name, the information as to the origin of the spectral data will not be lost. The old name containing the file data becomes a synonym for the compound.

In addition you may add **Notes** that are relevant to the compound.

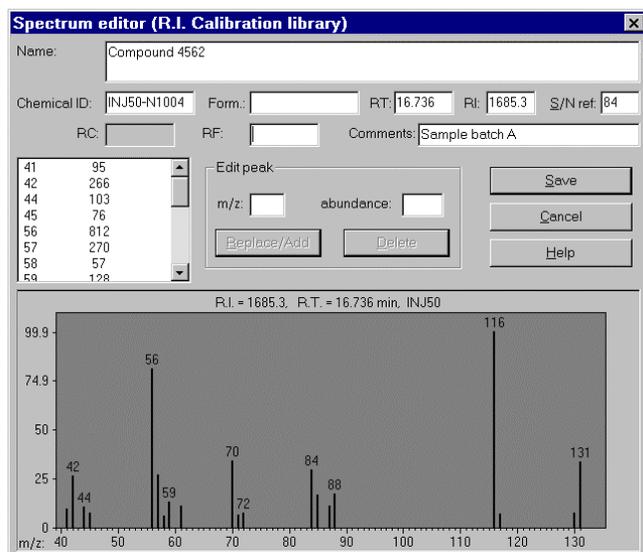
6.5.2 Spectrum Editor

Target Library Spectrum edit mode: 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. The mass spectrum is

shown in the bottom panel, the numerical form is in the left list box (m/z in the left column, abundance in the right column). To edit a peak, click on the m/z value in the list box, click on the peak in the spectrum, or enter the m/z value in the Edit peak box (center of window). You can delete peaks, add peaks, or change the abundance of an existing peak. Choose the add/replace or delete buttons to make the correction. As you edit the spectrum you will notice that the display of the spectrum at the bottom of the dialog box changes to reflect your edits. Choose Save to save these changes, Cancel will not save the changes.

In this mode you may not change the name, formula, RI or RT. You may change the chemical ID number (normally the Chemical Abstracts Registry Number if known, but any unique number can be used) if you determine that the spectrum actually belongs to another compound. If you change the ID to a chemical ID already in the library, that chemical information (.CID) file will be applied to this spectrum. This spectrum will become a replicate spectrum for the chosen id. If you change the ID to one for which there is no corresponding chemical ID you will be switched over to the compound editor to enter the needed information. Even if this spectrum was added as a replicate spectrum it will now have its own chemical identification file (.CID) file. It will no longer be a replicate of the original ID.

Calibration/Performance or Internal Standards Library edit mode: Since there is no separate



chemical information file for Calibration Libraries, you may edit both the compound information and the spectrum in this window. You may enter the name, a chemical ID, the formula, retention time (RT) and retention index (RI), mass to monitor s/n (s/n ref), real concentration (RC), and response factor (RF), as well as comment about the data. Within this window you may also delete the peak (for example, if it is an impurity peak), or edit either the mass or the intensity. Once you are satisfied, you must **Save** the results. If you cancel, all edits will be lost.

In the **Calibration Library mode**, when you Save the spectrum you will be presented with a box to allow you to choose if the spectrum should be used

in establishing the retention index calibration, (**Use for RI calibration**), used in tracking performance history (**Use in Performance Log**), and to specify that a specific peak must be present (**Require Peak**). If you select Require Peak, you need to specify the value of m/z that you require. You do not need to specify the minimum intensity, but you may. You must choose OK in this window to save changes to the spectrum made in the previous window.

To calculate a concentration, AMDIS requires the input of a response factor and real concentration for one compound in the Internal Standards library, a response factor for every compound in the Target library that you want a concentration for, and you must choose either an internal standards analysis or use RI and internal standards analysis.

When editing an .ISL library you can specify a response factor for a single compound to use for concentration determination. You can add RF for more than one compound, but AMDIS will use the RF for the first compound that it finds in the library.

6.6 Setting up Calibration Files for Use with AMDIS

In order to use retention indices as an aid in identifying compounds, AMDIS relies on a separate calibration run to establish the correspondence between the retention time and the retention index. While the data files used by AMDIS are simple text files, it is better to use the editors discussed in Section 6.5 to insure valid formats. There are two ways to set up a calibration or target file:

1. Use the gas chromatogram with its associated mass spectra - **Library from Data File**
2. Use the NIST Mass Spectral Database for Windows - **Library from NIST MS Database**

The process of creating the database for calibration is slightly different than creating the target database.

6.6.1 Create Calibration Library from a Data File

This discussion will use the straight chain hydrocarbons (C₆-C₃₀ - C-series, RI 600 - 3000), as an example, but any series of calibration compounds can be used. The library that is used by AMDIS for calibration has an extension .CSL - calibration spectral library. The calibration library should have only one spectrum per calibration compound, but this is not essential. Each of the spectra for the compounds must have a retention index and may optionally have a retention time. In addition, each entry has the name, formula and chemical identification number (Chemical Abstracts Service registry number if available, otherwise any unique designator).

Using a high quality chromatogram of the C-series, analyze the data with AMDIS selecting "simple" as the type of analysis. The target library is not important. Once the data file has been analyzed, go into to the **Confirm Window** if you were not already there. Under **Mode** from the menu bar, select **Component**. The top pane of the window will show the total ion chromatogram. Each of the C-series will have been deconvoluted. If the system is unusually noisy, you may see multiple deconvolutions of the same compound. Depending upon your target library, none of the components may have been identified.

Starting with the earliest eluting component, click on the small triangles above the peak. Alternatively you may use the list box showing the elution time directly below the chromatogram. With the first peak highlighted, select **Library/Build One Library** from the menu bar.

The dialog box that results will stay on the screen until you select Exit, it can be moved off of the main AMDIS window. The currently highlighted peak will be identified by a button:

"Add -> 6.222 min XXX"

Where the time represents the time of the peak and XXX a designator of the file name.

Assuming that you are building a file for the first time or that the library that is shown on the top of the window is not correct, select the **Files** button, and either select the **Create New Library** or **Load Library** button in the window that appears. Enter the name of the new file in the dialog box or select the library to be edited. Be sure to select a file with a *.csl extension. The behavior of the library editor depends upon which type of library you are working on.

Assuming that you are selecting a new library, when you have typed in the name and returned, you will see a blank list. Select the Add button noted above. Once the spectrum has been added, it can be edited. With the newly added spectrum highlighted, select the **Edit** button.

In the Spectrum Editor, change the name, enter the correct chemical ID, add the formula and enter the retention index. The last is crucial. If desired, also add comments. When finished select **Save**. If you are editing a calibration library, the program will bring up a dialog box with three check boxes:

Use for RI Calibration (followed by a value)

Use in Performance Log

Require Peak (followed by a m/z value and a minimum percent abundance value)

If you have entered a value for RI in the editor, the **Use for RI Calibration** box will be checked, if not you may enter a value now and check the box. **You must check this box for the spectrum and its associated RI to be used in the calibration.** If you wish the system to keep a history of the retention and mass spectral data for the compounds, you should also check the **Use in Performance Log** box. The **Require Peak** option enables the system to distinguish between the various hydrocarbons all of which have similar spectra. Typically the m/z value of the molecular ion is entered. The minimum percent abundance can be ignored. For each component that you want to include in the library you will need to save the spectra in the same way. The library is saved after each operation, no direct saving of the library is needed.

6.6.2 Create Library from NIST MS Database

The same method as discussed here can be used to create a library from any text file that conforms to the NIST file format, discussed briefly below.

The NIST MS database can be used to create an .MSP file. This file is created by exporting the desired spectra from the NIST MS database program using library tools. Files created by the NIST MS program with the .MSP extension can be read by AMDIS.

To convert from either an .MSP file or another library, use the **Library/Library Transfer** menu choice. If the correct libraries are not set as the source or the destination (the library to be built), use the **Files** button and select the libraries. The Files button will also provide the option of creating a new library.

Highlight the spectra that you wish to transfer and select **Add**. Once the spectra are in the new library you will need to specify the retention index data if it is not already present. If there is retention index data for a compound in the original library, it will automatically be set to **Use for RI Calibration** when transferred to a .CSL library.

6.7 Using the Performance Log Feature

In addition to the calibration compounds (for example the normal paraffins) the calibration library can contain performance mixture spectra. For example, a classic and widely used performance mixture is the "Grob Mixture", which can be purchased ready-made. The spectra for the components of the Grob mix are added to the .CSL library and then AMDIS will look for each of these compounds in the calibration / performance mixture runs.

The performance mix compounds can be used to track the performance of the instrument over time and to provide a quality control mechanism. The compounds which have the **Use in Performance Log** choice selected will be tracked in each calibration run. The compound name, the retention time, the peak height, the peak width (FWHM), the tailing (Area to right of maximum/area to left of maximum), match factors and the date and file for each analysis is reported. Changes in the relative or absolute values for these compounds can be tracked.

Note that each time the calibration / performance mixture is run, the calibration file is recalculated so that long term drifts do not affect the retention index calibration. The performance log records the retention time in order to observe these long term trends.

To add spectra for performance mix compounds from a data file it is best to first do a calibration run using the calibration library (*.CSL). This will create a calibration file (.CAL) then by using **Use RI Calibration Data** for the analysis type, the retention indices for all of the performance mix components will be calculated. You can follow the directions in Section 6.6.1. Make sure to check the **Use in Performance Log** box. In the Spectrum Editor you must choose Save to access the dialog to check Use in Performance Log.

6.8 Viewing the Library or Calibration

Any listing of the library will provide a **View** button. When selected a dialog display will be shown that gives you the option of scanning through the library which can be sorted by **ID number, Name, or Retention Index** (if present). The selection box on the right of the window gives these choices. Above this selection box is one that has choices of Hide, Spectra and Text. **Hide** will show only the name, ID number, formula and retention index if present. **Text** will show the entire record of the spectrum. The exact file will be shown with all of the internal headers. **Spectrum** will plot the mass spectrum in the normal manner.

For calibration, the View will show the calibration compounds, the corresponding times and retention indices. For calibration libraries (.CSL files) there is an indication in the name of the compound if it is to be used for calibration (c) or performance monitoring (p). The order is cp, if either or both is missing a # is printed, thus #p indicates a compound will be used for performance monitoring but not calibration.

6.9 Format of NIST .MSP files

The native format which the NIST MS Database exports files in is shown below. However, as you can see from looking at the other formats, the NIST MS Database 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 (i.e. not a field name followed by a colon) is the direct output of the NIST MS database. The fourth line in the NIST98 Mass Spectral Program example (.MSP) 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.

Examples

NIST 98	
<p>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;</p>	<p>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;</p>
<p>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)</p>	<p>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)</p>

7 Batch Jobs

7.1 *Create and Run a Batch Job*

Under **File / Batch Jobs** there are two selections, the first **Create and Run a Batch Job** will bring up a dialog box that will allow you to select chromatograms (data files) to be added to a list for running without direct supervision. Each chromatogram in the batch job can be analyzed in any of the modes allowed under AMDIS. For example, the first member of the set could be a **RI Calibration / Performance** run which would then generate the data needed for analyzing the rest of the runs with **Use RI Calibration Data**, or if internal standards were used, **Use RI Calibration Data + Internal Standards**. In all cases the Target, RI Calibration, and Internal standards libraries must be specified in the settings (see Section 4.5.4) before the batch job is started.

7.2 *Show Results of Last Job*

Under this option, the last batch job that was run will be brought up and you may select the data that you wish to see.

8 Manual Deconvolution

8.1 Overview of Manual Deconvolution

There are two operations that can be done in the Manual Deconvolution window. 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. You must be in Manual mode to do either of these things.

8.2 Background Subtraction

In the manual mode two buttons appear above the chromatogram (**Manual On/Off** and **Deconv.**). To use normal background subtraction, select **Manual On**. The **Deconv.** button will be grayed out. Using the right mouse button you may set the cursor to specify the **Signal**, the **Background** to be subtracted, or to **Clear** (erase) the specifications. The region specified to be taken as the peak, subtracted or erased is indicated **below** the axis by a short line of either red (the peak) or blue (the background). Within these regions the signal is averaged. The background is subtracted from the peak. The resulting spectrum is shown as a component in the lower window (note you may have turned the lower window off or you may have specified that it only show the scan and not the component). To zoom an area of the chromatogram, you must toggle **Manual-Off**, zoom the region of interest, the toggle **Manual-On** to choose peak and background areas.

The resulting spectrum can be searched using the NIST library or can be added to your own AMDIS library. Note that you may search the NIST library by using the right mouse button when the cursor is in the spectrum window.

8.3 Manual Deconvolution

Before you start the manual deconvolution process, it is best to use the mouse to zoom the peak or peak cluster of interest to the full screen. There is a limit on the manual deconvolution of 64 scans. To do a manual deconvolution set the Manual On/Off button to **Manual Off** and select the **Deconv.** button. A dialog box will appear.

The dialog shows the range of scans on the screen. Select Copy to make this the active region, or type in the range. In the lower half of the box there are two ways to specify the model peak shape over the region defined, draw the peak or specify a particular m/z peak.

To select the peak, first activate the peak selection box by clicking on + in the top line of the selection box in the bottom of the window. You may use the mouse to click the desired m/z value in the mass spectral window (a new cursor will appear to allow selection) or type the value of the peak in the m/z box provided. Once you have selected the model peak, select **Calculate**. You may also select peaks to be used as models to be subtracted from the first peak. This is done using the second and third lines of the box.

You may select two peaks and then try them out by selecting whether to use the peaks or not (the Use checkboxes).

If you select draw, the cursor will change into a pencil shape and you may select the shape of the curve. For the first point, put the mouse where you wish to start in the chromatogram. Each time you click the mouse a new point on the model curve will be defined. The resulting shape can then be used a model peak. The program will deconvolute the chromatogram based on this model peak. As a general rule, you will always do better letting the program select the model peaks.

A. Quick Start AMDIS

A.1 Introduction

This appendix will take about 15 minutes to go through. It is designed to use the three data files supplied with AMDIS for HP instruments and the one data file for a Finnigan GCQ. This introduction should be used with the very first opening of AMDIS. If you have already used AMDIS, a few of the initial windows will look different.

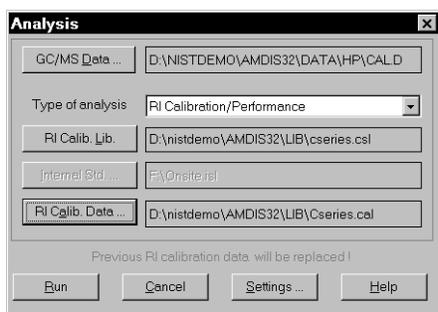
Within AMDIS there are three ways of analyzing a GC/MS file 1) use only mass spectral data for identification; 2) use mass spectral data and internal standards and 3) use mass spectral data and external standards. The first illustration will use external standards.

To view this help window and AMDIS at the same time, minimize all other applications and choose Tile Vertically from the right mouse click in the task bar.

A.2 HP File With External Calibration

Using external standards, the retention index correlation to retention time will be established by separate calibration runs, run once a day. The retention times in data files are converted to retention indices using a linear interpolation. To use retention indices in the analysis, they must first be determined from a calibration run (see Section 4.2).

When you start up AMDIS for the first time, you will be asked to confirm a file or instrument type. For the purpose of this demonstration, select **HP files**. The screen that will be shown next has a single menu choice - **File**, from which you should select **Open**. You should then select **Data** (a subdirectory) and **HP** (another subdirectory) and **CAL.D** (also a subdirectory). The program will bring up a chromatography file and the menu selections will change. Select **Analyze**, and then **Analyze GC/MS Data**. A dialog box (below - right) will come up. It will have the name of your file (CAL.D).



The second line in the box is the type of analysis. Using the arrow tab on the right side, bring down the selections and choose **RI Calibration/Performance**. The third line is now the RI Calibration Library. Select the button **RI Calib. Lib.** You will be shown a tabbed dialog box with RI Calibration Library highlighted. Select **Select New** and you will be given a file selection box. The Calibration Library you are looking for is in NISTDEMO\AMDIS32 (or AMDIS16) if you used the default install conditions. It is entitled **cseries.csl**. Once this is selected you are done. Press **Save** at the bottom of the tabbed dialog box. It will close. The data file which will contain the retention time/retention index

correlation data is named automatically (last line) with the same name as the calibration library, but with a different extension (.CAL instead of .CSL).

Now press **RUN** in the Analysis window. Depending on the speed of your computer you will get the results within 15 sec to 2 min.

Since this is a quick guide, we will not cover all the features of the program. You can see the results by selecting the various compounds identified using your mouse in the list box on the upper right side. To

RT	RI	Names
4.450	800.0	Octane
7.536	1000.0	Decane
10.602	1200.0	Dodecane
13.357	1400.0	Tetradecane
15.800	1600.0	Hexadecane
17.995	1800.0	Octadecane
19.987	2000.0	Eicosane
21.803	2200.0	Docosane

see what data was retained by the program for the calibration select the **Info** button on the button bar below the menu bar. In the tabbed dialog box that appears, select **Standards**. You will see the data (on the left) that will be used to convert retention times (RT) to retention indices (RI). When you are finished viewing the results press **Done**.

What you have done so far is to establish the

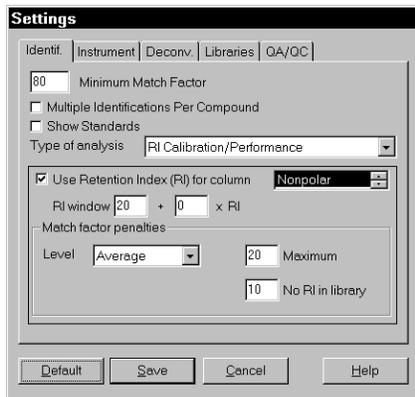
Retention Index calibration. Now you are ready to use that information to analyze data. Go back to the **Analyze** menu selection. Select **Analyze GC/MS Data** again.

In the first line select a new file - find the file **grob.d**. Select it. Press **Open**.

In the second line choose the analysis type as **Use RI Calibration Data**.

In the third line choose **grobx.msl** as the library.

Now select the **Settings** button.



In the tabbed dialog that comes up (on the left) select **Identif.** (Identification), which is the first tab. In the Identification dialog, check **Use Retention Index (RI)**. Click on **Save**. Keep the defaults for now.

You will be returned to a dialog similar to the first figure. Notice that the bottom line contains the same name you used in the previous run for the calibration data. Now Press **Run**.

You will be asked if you want to save the files from the last analysis unless you had specified that you were not to be asked after the last analysis.

Again the results will appear in about the same amount of time as the previous run. In the Match list box (right hand side of screen) there is data about the difference between the RI observed and the

RI in the library. If this number is too large there is a penalty applied to the match factor.

Even though a GC/MS is typically very stable for several days, it is normal good laboratory practice to do a quality control run each day. That same quality control run can be used to establish the calibration file for the day.

A.3 Simple Analysis and NIST Library Search

The next example is run under very different conditions.

Go to the **Analyze** menu selection. Again select **Analyze GC/MS Data**.

In the first line of the dialog box select the file **test.d**.

Say **Keep** in the next box that asks if you want to keep or delete the files that you just created. The default is to always ask if you want to keep or delete files. You can change this default by choosing options and setting the default to keep or delete.

In the second line choose the analysis select **Simple** - since the chromatographic conditions are different from the previous example, the same calibration file cannot be used.

Press **Run**. In a Simple analysis only the MS data is used for match factor. No account is taken of the retention time or index.

You will see most of the strong peaks identified. Identified peaks are indicated by a "T" mark above the peak. These are components that are identified from the target library. From 11 to 12 min there are two peaks that are not identified - these are indicated by small triangles. Note that the triangle will be over every peak, the T only over the identified peaks.

To expand the TIC graph so you can see the results better, put the mouse at the top of the TIC window at about 11 min. **While holding down the left mouse button**, drag the mouse to the bottom of the TIC window at about 12 min. You will see a box as you drag the mouse and when you let go of the left mouse button at 12 min the display will have been expanded. You may click the mouse on the small triangle above the large peak at 11.120 min or use the list box showing the times each peak eluted. Clicking on the 11.120 min entry will move the red highlighting to the correct peak. This peak was not identified from the target library Grobx. The small triangle should change from blue to red. You can search the full NIST database from AMDIS with this component.

To use the NIST library search go to **Analyze** on the menu bar again and select **Go to NIST MS Program**. The program will search using the deconvoluted spectrum. Then choose **Analyze/Go to NIST MS Program** to switch to the library. If the search does not automatically take place, double click on component at scan 901 in the clipboard to search the NIST library. The component will be identified as Acenaphthene-d10, a common internal standard. From the NIST Search Program use the ALT-TAB keys or

the task bar to get back to AMDIS. Select the peak at 11.737 and repeat the search of the NIST library - highlight the triangle pointing to the peak and search as above. This peak will be identified as Diethyl Phthalate.

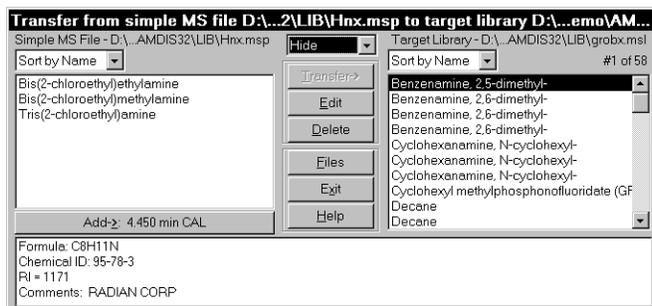
A.4 Finnigan GCQ File and Library Building

One last file, with two new ideas.

AMDIS accepts a number of native GC/MS instrument formats. The previous files were HP format, this next file is from a Finnigan instrument.

Again go to **Analyze** and select **Analyze GC/MS Data**. From the dialog box select **Settings**. Once the Settings Tabbed Dialog comes up select **Instrument**. Under Data File Format select **Finnigan GCQ Files**. **Save** your choice. You will see that a file selection dialog appears. Go to NIST-DEMO/AMDIS/DATA/FI_GCQ and select **GCG.MS**. Check to see if your run type is still **Simple** in the **Ident. Tab**. Now without changing anything else press **Run**.

You should not have gotten any identifications. The compounds in the GC are not in the target library. We will now add these compounds from another library. To do this select **Library** from the menu bar. Select **Library Transfer** from the menu. The program will show you a window with data from two libraries.



ies. On the left, the source library is a small three spectrum library of data taken from the full NIST/EPA/NIH Mass Spectral Database using the Library Export feature of the database. These spectra can be added to you GrobX library by highlighting them and pressing **Transfer**. Once this is done, **Exit** the library and press Run again. You will now find the three largest peaks identified.

NOTE: To see the targets, you may need to click on one of the small T symbols that indicate

the location of target peaks in the TIC. You can also switch to Target only in the **Mode** selection on the menu bar.

This is a quick start up. The help file and the rest of the manual will give aid in doing other tasks.

B. How Do I ... ?

B.1 System Setup

B.1.1 How Do I Change the Settings for An Analysis?

From the RESULTS Window select the button marked **Analyze**. Within the resulting dialog box select the **Settings** button.

The current settings are the settings of the data currently being displayed after analysis. They can be changed for subsequent analysis as follows:

To CHANGE the current settings	
In the RESULTS Window	In the CONFIRM Window
Select Analyze , then the Settings Button at the bottom of the window.	Select the Analyze from the menu bar, then select Settings from the pull down menu.

B.1.2 How Do I Find Out What Settings Were Used for the Current Analysis?

In the RESULTS WINDOW, the settings for the analysis of the **current file** are shown in the tabbed dialog box at the bottom of the window. In the CONFIRM Window, the same tabbed dialog box can be seen by selecting **Info** button below the menu bar or via the menu choice **View/Auxiliary Information**.

To VIEW the current settings	
In the RESULTS Window	In the CONFIRM Window
Use the Settings Tab in the Tabbed Dialog box at the bottom of the screen.	Select the INFO button at the top of the screen and then the Settings Tab of the Tabbed Dialog box that is displayed.

B.2 Data Analysis

B.2.1 Analysis Without Retention Index Data (simple analysis)

For a detailed example please see Appendix A.3.

B.2.2 Analysis With Retention Index Data

For a detailed example please see Appendix A.2.

B.2.3 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. In the CONFIRM Window, **File/Open** will allow you to bring in data that has been analyzed as well as new data. If the file has been analyzed, the program will find the associated analysis files.

B.2.4 How Do I Use Data for Calibration and Performance Monitoring

Only data in the calibration / performance mix library can be used. 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**. See Sections 0 and 6.7.

B.2.5 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, select **Analyze/Go to NIST MS Program**. You must have the NIST Mass Spectral Database for Windows installed on the computer. You may also select a series of components to be analyzed at once.

To do this select **Analyze/Add component to search list**. The number behind the menu item indicates the number of components in the search list. If there are any components in the search list, the **Analyze/Search NIST library** will act on the search list 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 library. Proceed with search by double clicking on component in the Clipboard.

B.3 Library Maintenance

B.3.1 How Do I Add Spectra to a Library?

To add a spectrum from the GC/MS data file, select that component peak, 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, select **Options/Build One Library** from the menu bar. The highlighted peak will be on a large button at the top of the dialog box. Selecting that button will add the spectra to the current library.

This can only be done from within the Confirm Window, for more details see Section 4.

B.3.2 How Do I Delete Spectra From a Library?

To delete a spectrum from the library, select **Options/Build One Library** from the menu bar in the CONFIRM Window. Use the **Files** button to select the library you want to edit. Highlight the spectrum (or spectra) you want to delete. Press the **Delete** button. Similar actions can be taken with the designation library in **Options/Library Transfer**.

For more details see Section 6.

B.3.3 How Do I Edit Spectra or Compound Information in a Library?

To edit a spectrum or compound information in the library, select **Library/Build One Library** (CONFIRM Window) highlight the spectrum you wish to edit and press Edit. You have the option of editing the spectrum or the compound information. If you edit the compound information, it will affect all replicate spectra with the same compound identification number. Similar actions can be taken with the target library under **Library/Library Transfer**.

B.4 Display Modification

B.4.1 How Do I Expand (Zoom) a Plot?

The chromatogram and mass spectra windows can be zoomed. Select an area by pressing down the **left mouse button** at one corner and holding it down while dragging to the opposite corner. This will expand the selected area to the full size of the plotting window. To unzoom press the **right mouse button** while the mouse pointer is in the plotting window and select **Unzoom** on the menu that appears. If you have done multiple zooms, you can step back one at a time or **Unzoom all** to go back to the full display.

B.4.2 How Do I Switch Between Log and Linear Axis?

With the mouse pointer in the plotting window that you want to change, press the **right mouse button** and select the log scale. If log scale is active, deselect the log scale.

B.4.3 How Do I Change Colors and Weight of Lines?

In the CONFIRM Window, the **View/Thick Lines** will toggle between thin and thick lines on all CONFIRM Window plots and **View/Color Selected Peaks** toggles their coloring. The **Options/Color** brings up a dialog box that allows all of the colors of the window to be set.

B.4.4 How Do I Find the Exact Position of a Peak?

With the mouse in a chromatogram, spectra or component window, select the **right mouse button**. Choose **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 the dialog above the plot is selected.

B.4.5 How Do I Show Components on a Plot?

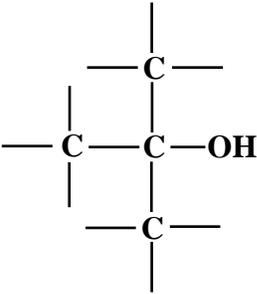
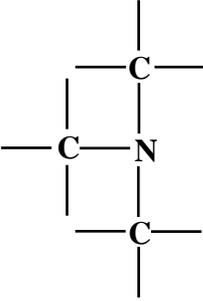
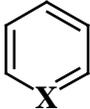
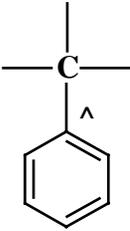
With the mouse pointer in the chromatographic plot or the component plot window, select the right mouse button. From the menu that is shown, select **Show component on chromatogram** for the chromatographic plot or **Show component** for the component window.

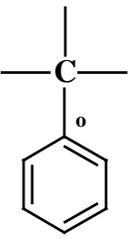
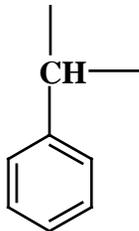
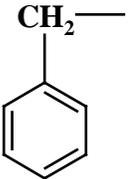
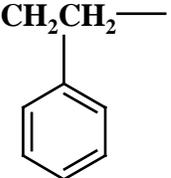
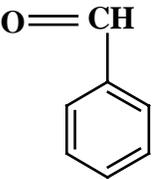
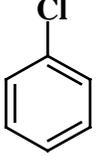
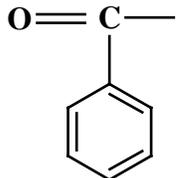
B.4.6 How Do I Change the Data that is Shown or Printed About a Component?

In the Results Window or the CONFIRM Window, place the mouse over the **Component** or **Match** list boxes. The **right mouse button** will bring up a selection of **View Options** and **Print Options**. Select the one you wish to change. From within the dialog box, select the items and order that you wish to see or print.

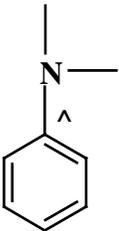
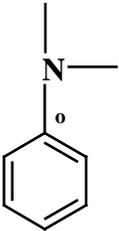
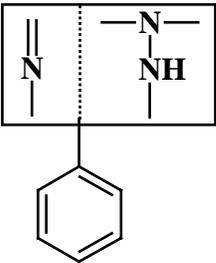
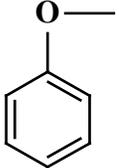
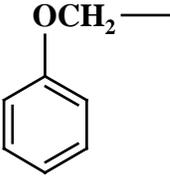
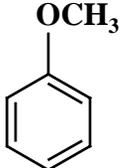
C. Compound Classification Analysis

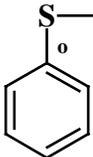
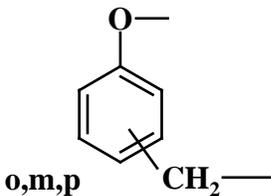
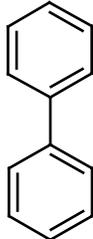
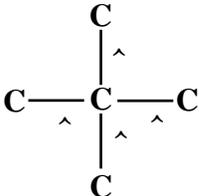
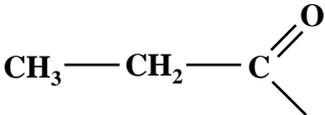
The brief name that is given in AMDIS is shown in the second column, the structure is shown in the first column and a description is given in the third column. Note that the description differentiates between those classifiers that define functional groups (func), specific elements (elem), aromatic (aromatic) groups, and alkyl (alkyl) groups. There are a few classifiers that describe bonds (bond).

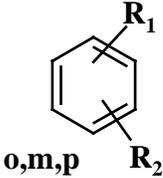
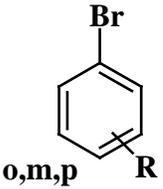
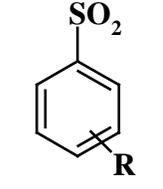
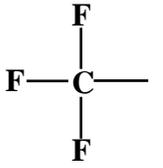
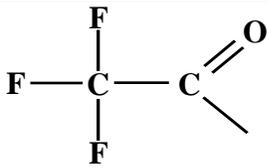
Structure	name	Description
	Alc tert	func: alcohol tertiary (no ester)
	Am tert	func: amine tertiary (no amide)
Condensed aromatic rings	ar cond	aromatic: con- densed rings
	ar het	aromatic: hetero- aromatic
More than one aromatic ring (any type)	ar poly	aromatic: more than 1 aromatic ring (any type)
	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

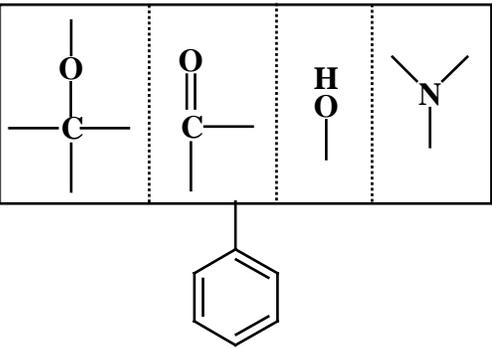
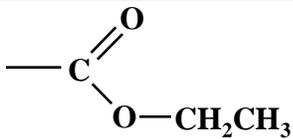
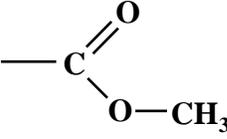
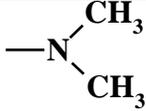
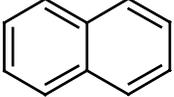
Structure	name	Description
	ar-CO,N2	aromatic: aryl - -C-O or -C=O or -N=N
	ar-CO-CH2	aromatic: aryl - CO-CH2
	ar-COO	aromatic: aryl - COO (benzoic acid/ester)
<p>o,m,p R</p>	ar-COOCH2*	aromatic: ester C6H4-COO-CH2- (subst. at o,m,or p)
	ar-F	aromatic: aryl - F
	ar-N	aromatic: aryl - N

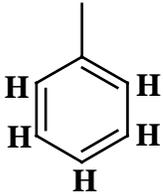
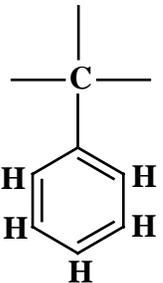
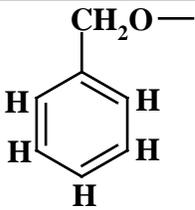
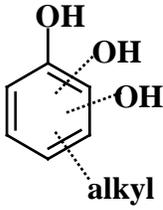
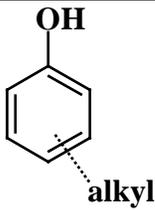
Structure	name	Description
	ar-N ch	aromatic: aryl - N (chain-bond)
	ar-N r	aromatic: aryl - N (ring-bond)
	ar-N,NHN	aromatic: aryl - -N= or -NH-N
	ar-O	aromatic: aryl - O
	ar-O-CH ₂	aromatic: aryl - O- CH ₂
	ar-O-CH ₃	aromatic: aryl - O- CH ₃ (methoxy)

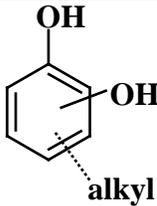
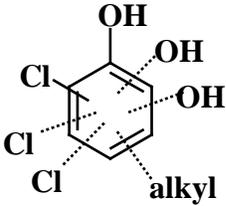
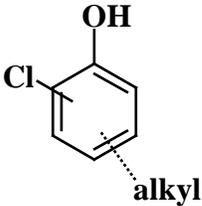
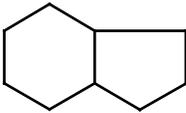
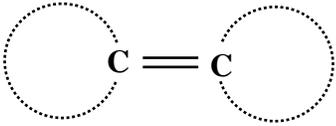
Structure	name	Description
	ar-S r	aromatic: aryl - S (S in ring)
B_x	B	elem: boron (any number)
	benz-O	aromatic: CH2 - C6H4 - O - (o,m,p)
	Biphenyl	aromatic: biphenyl
Br_x	Br	elem: bromine (any number)
	C quart ch	alkyl: C quarternary (4 chain-bonds to C- atoms)
$C_{10}H_{21}$ —	C10 H21	alkyl: C10 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

Structure	name	Description
$C_5H_{11}-$	C5 H11	alkyl: C05 H11
$C_nH_{2n-1}-$	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-)
 <p>o,m,p R₁ R₂</p>	C6H4 omp	aromatic: C6H4 di-substituted (o,m,p) benzene ring
 <p>Br o,m,p R</p>	C6H4-Br	aromatic: C6H4 - Br (o,m,p substituted)
 <p>SO₂ R</p>	C6H4-SO ₂	aromatic: C6H4 - SO ₂ (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
 <p>F F-C-F</p>	CF ₃	func: CF ₃ trifluoromethyl
 <p>F F-C-C=O F</p>	CF ₃ -CO	func: CF ₃ - CO

Structure	name	Description
$-(\text{CH}_2)_6-\text{C}(=\text{O})-$	(CH ₂) ₆ -CO	func: ketone (CH ₂) ₆ - CO
$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{C}=\text{C} \\ \diagup \\ \text{CH}_3 \end{array}$	(CH ₃) ₂ >C=C	bond: isopropylidene
$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3-\text{Si}- \\ \\ \text{CH}_3 \end{array}$	(CH ₃) ₃ Si	func: trimethylsilyl
$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3-\text{C}- \\ \\ \text{CH}_3 \end{array}$	(CH ₃) ₃ -C	alkyl: tertiary butyl
$\text{CH}_3-\text{C}(=\text{O})-$	CH ₃ -CO	func: acetyl CH ₃ -CO
$\text{CH}_3-\text{C}(=\text{O})-\text{O}-$	CH ₃ -COO	func: acetoxy CH ₃ -COO
$\text{CH}_3-\text{C}(=\text{O})-\text{O}-\text{CH}_2-$	CH ₃ -COOCH	func: ester of acetic acid CH ₃ COO-CH ₂
$\text{CH}_3-\text{O}-\text{CH}_2-$	CH ₃ -O-CH ₂	func: CH ₃ - O - CH ₂
Cl_x	Cl	elem: chlorine (any number)

Structure	name	Description
	CO-C ₆ H ₃ -O	aromatic: tri-subst. benzene ring: -C=O or -C-O or -O
	et-est	func: ester: ethyl
C _x H _y	hydr carb	alkyl: hydrocarbon
	me-est	func: ester: methyl
N _x	N	elem: nitrogen (any number)
N ₂	N 2	elem: nitrogen: 2 atoms
	N(CH ₃) ₂	func: dimethyl-amine -N(CH ₃) ₂
CH ₃ —(CH ₂) ₃ —O—	n-C ₄ H ₉ -O	func: butyl-oxy n-C ₄ H ₉ - O
	Naph	aromatic: naphthaline ring system
—CH ₂ CH ₂ —NH—	NH-CH ₂ -CH ₂	func: CH ₂ - CH ₂ - NH
—N=O	NO	func: nitrogen-oxygen bond (single or double)

Structure	name	Description
Non aromatic compound	non ar	aromatic: non aromatic
P_x	P	elem: phosphorous (any number)
	ph	aromatic: C6H5- (phenyl)
	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.

Structure	name	Description
	phen-2-OH	aromatic: phenol (2 OH), alkyl-subst.
	phen-Cl	aromatic: phenol - Cl (1-3 OH, 1-3 Cl), alkyl-subst.
	phen1-Cl1	aromatic: phenol - Cl (1 OH, 1 Cl), alkyl-subst.
	r 5+6	ring: 5-ring and 6-ring condensed
	r>C=C<r	bond: C=C between 2 rings
S_x	S	elem: sulphur (any number)
$\text{—S=CH}_2\text{—}$	S-CH ₂	func: S - CH ₂
Si_x	Si	elem: silicon (any number)
Si_1	Si 1	elem: silicon: 1 atom
$Si_{>=2}$	Si >=2	elem: silicon: >= 2 atoms

Glossary

Calibration – limited here to the process of determining the relationship between retention time and retention index.

Component - The word eluent or component 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.

Deconvolution - The term is used here in the broad sense of extracting one signal from a complex mixture. The treatment of noise, the correction for base line drift, and the extraction of closely coeluting peaks from one another is all part of the deconvolution process.

Model Peak - The peak that represents the most rapid rise and fall of a signal. It is assumed to be the most characteristic of the peaks for a particular component. It is often the total ion current, but in complex mixtures with many coeluting peaks, the individual ion signals are far more useful.

Retention Index - The retention index 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.

Retention Time - The time that the compound is held up on the GC column. Usually a retention index is used rather than a retention time.

Contacts

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