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MS Workstation

MS Data Handling User's Guide

QUALITY SYSTEM ISO 9001 CERTIFIED

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Contents

Data Handling Overview

Data Handling encompasses the procedures and tasks performed after the Mass Spectrum has been collected. Often it is easier to develop data handling methods after acquiring the data.

There are four processes in developing Data Handling methods: 1. Acquiring data, 2. Displaying data, 3. Building a Compound List, and 4. Building a Data Handling Method.

Acquiring Data

After the chromatographic and mass spectrometric methods are developed, prepare a series of standard solutions of the compounds of interest, at up to ten levels covering the range of concentrations for which the method will be used. If you are using one or more internal standards, be sure that the concentration of the internal standard(s) is the same as that in the midpoint of the expected sample concentration range.

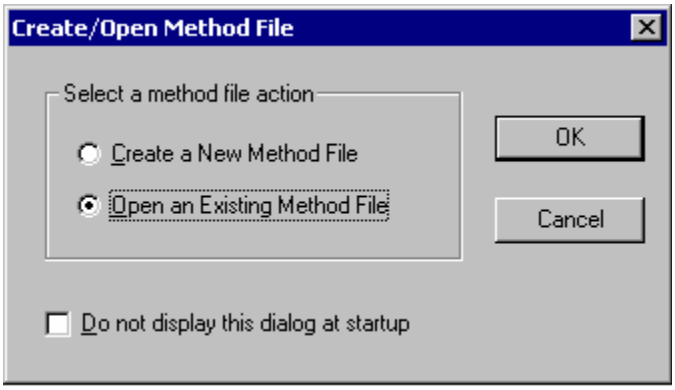
Next, make at least two injections of your standards: a mid-level standard which is used to identify the component peaks, and a low- level standard is used to optimize the peak detection and identification parameters. Of course, you may inject all levels of the standards at this time and recalculate the calibration data after the data handling method is developed.

Building a Data Handling Method

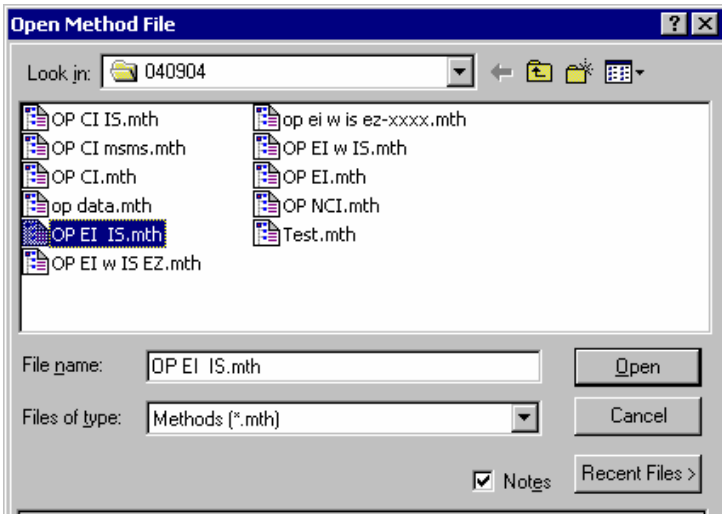
Displaying data, building a Compound List, and building a Data Handling Method are the focus of this Users Guide. Follow the example.

Open the Method Builder application by clicking on the  icon in the MSWS toolbar.

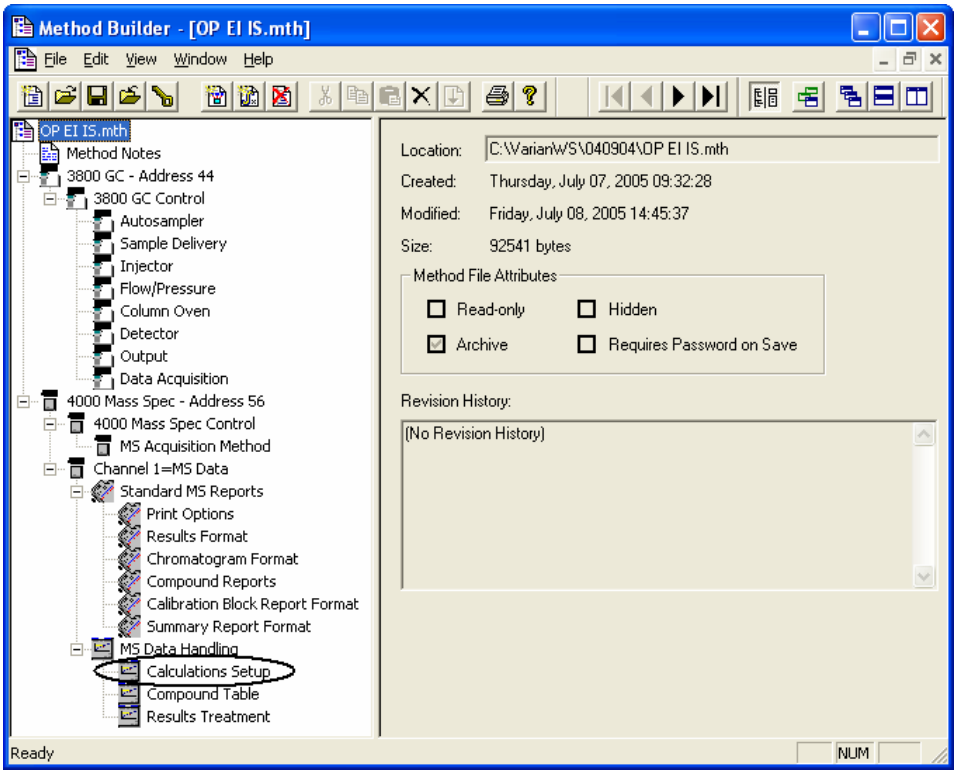
Click **Open an Existing Method File** and **OK**.



Find the method file add a Data Handling method to (usually this will be the acquisition method) and open it.



The Method Builder displays the method.



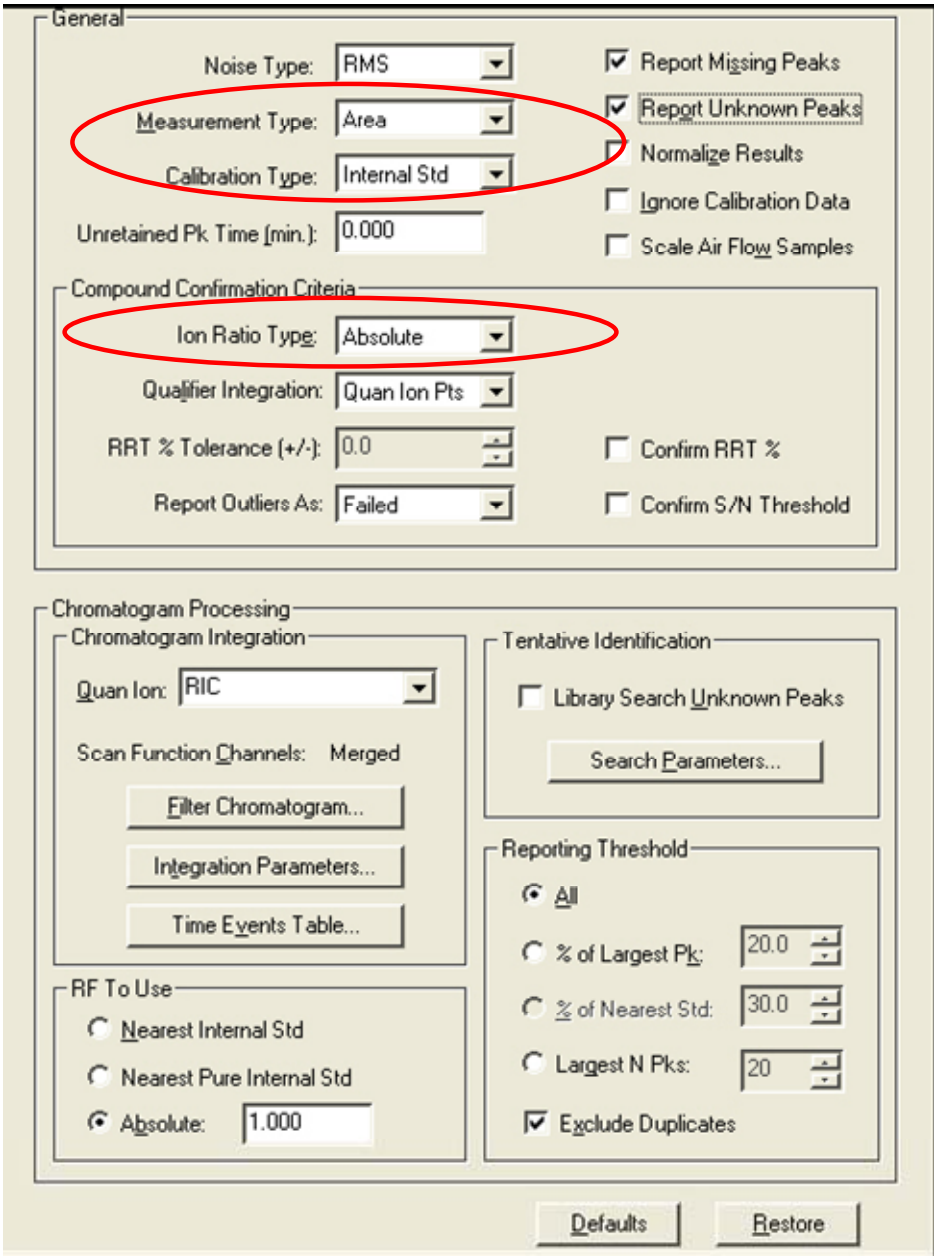
From the pane on the left, select **Calculations Setup** in the MS Data Handling section.

For this exercise, the following was selected in the General section.

Measurement Type - **Area**

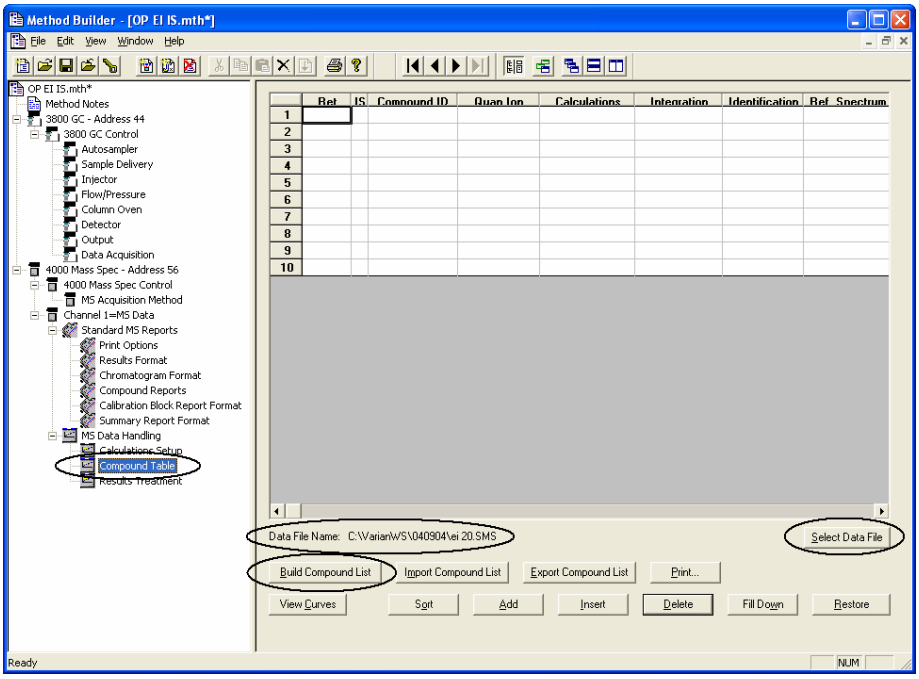
Calibration Type - **Internal Std**

Ion Ratio Type - **Absolute**



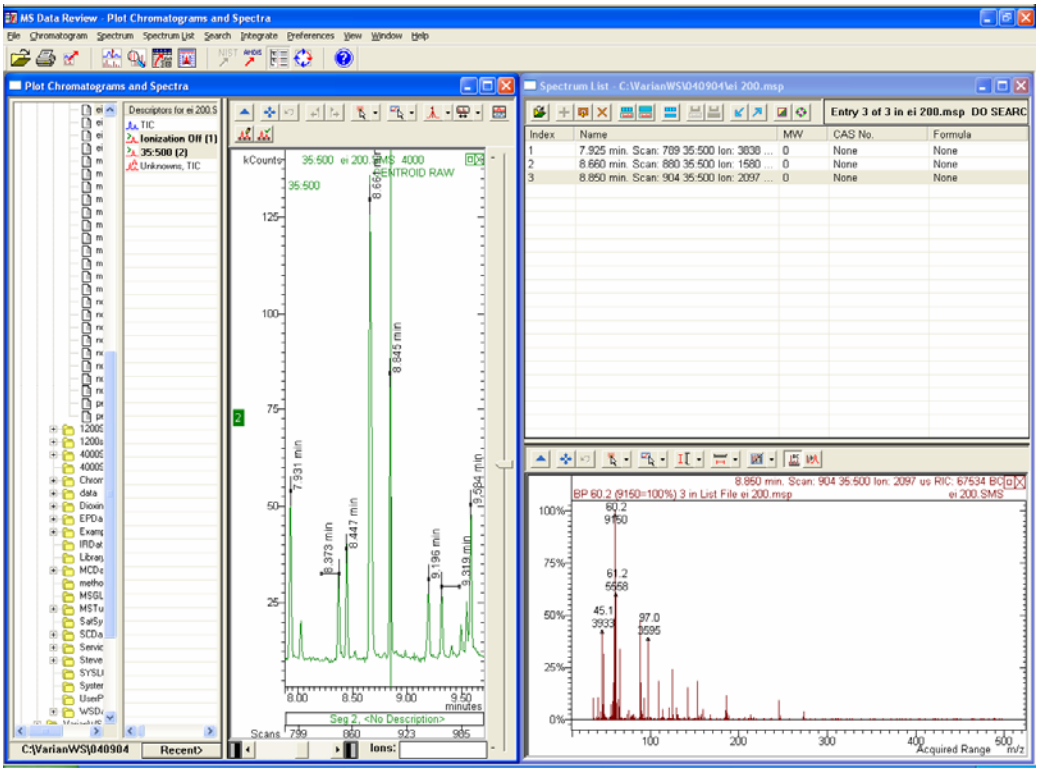
From the left side click **Compound Table** in the MS Data Handling section.

Click **Select Data File** and the middle level standard file are selected and the name of the file appears. Because the names of the peaks correspond to the standard compounds are know **Build Compound List** is clicked.



Click **Build Compound List** to open MS Data Review.

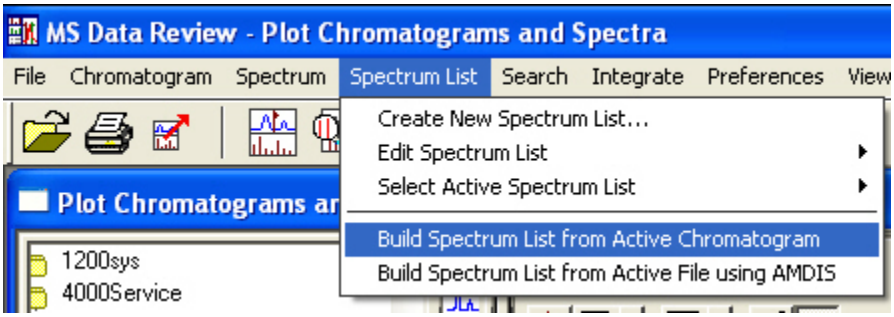
Import the compounds from this spectrum list into the compound table of the method.



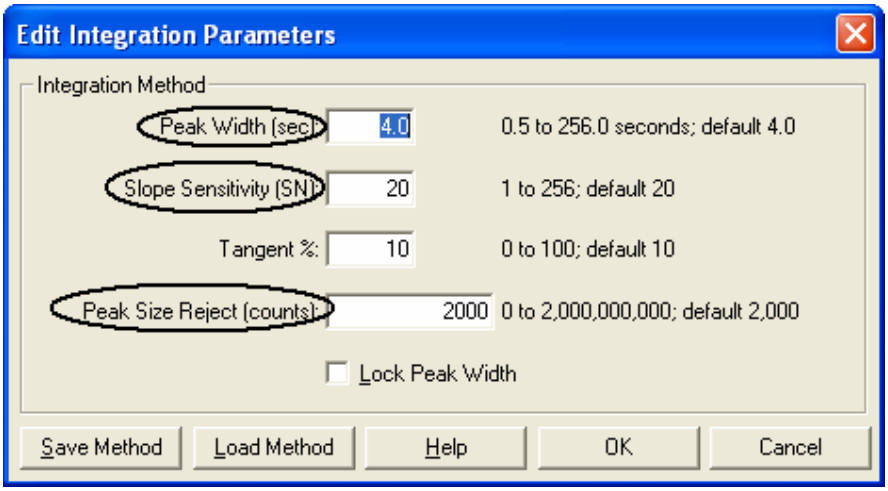
Building a Spectrum List

Click the apex of the peaks to display the corresponding spectrum. Also the spectrum is added to a Spectrum List with the same name as the data file such as, 200.msp.

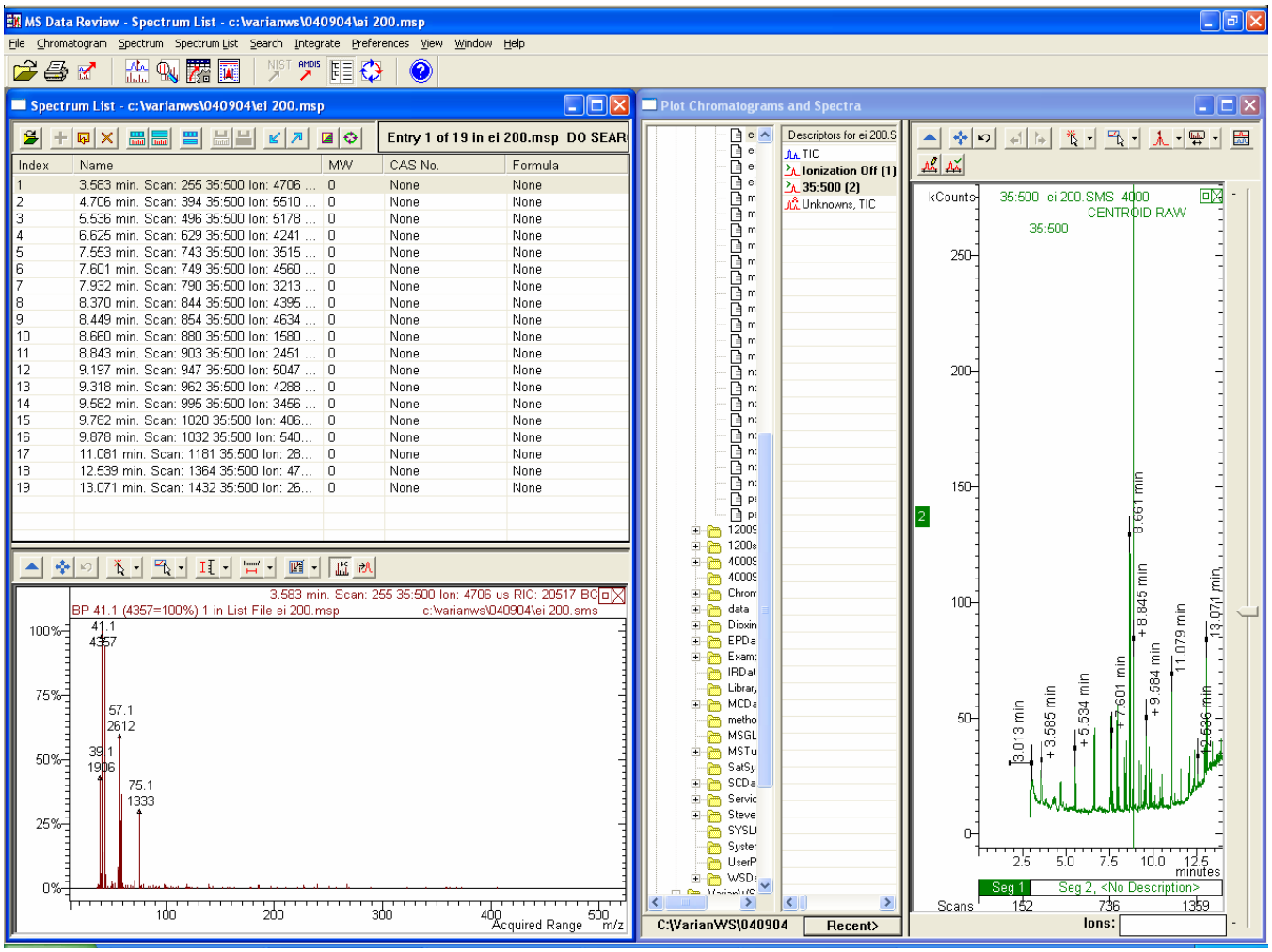
Click **Build Spectrum List from Active Chromatogram** from the Spectrum List menu in MS Data to build the Spectrum List automatically.



Adjust the **Peak Width**, **Slope Sensitivity**, and **Peak Size Reject** parameters so that your compounds of interest are integrated and most of the extraneous peaks are rejected. Click **OK** to integrate the chromatogram and generate the Spectrum List.



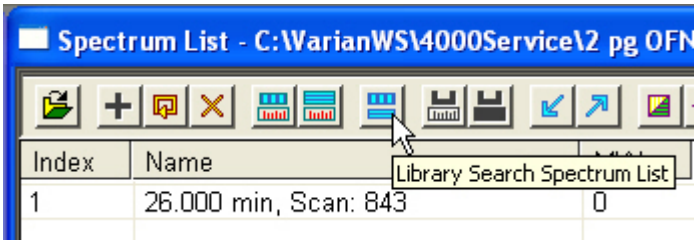
You should have a spectrum list with the same name as your data file, with an entry for each integrated peak using your parameters.

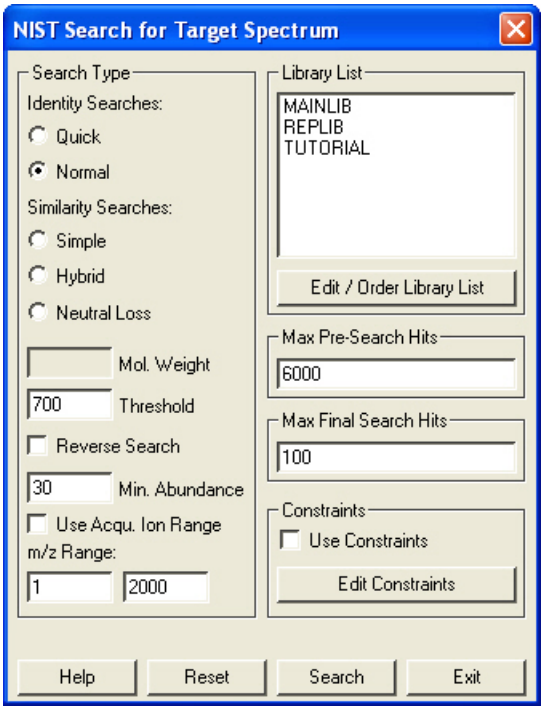


Identifying a Peak

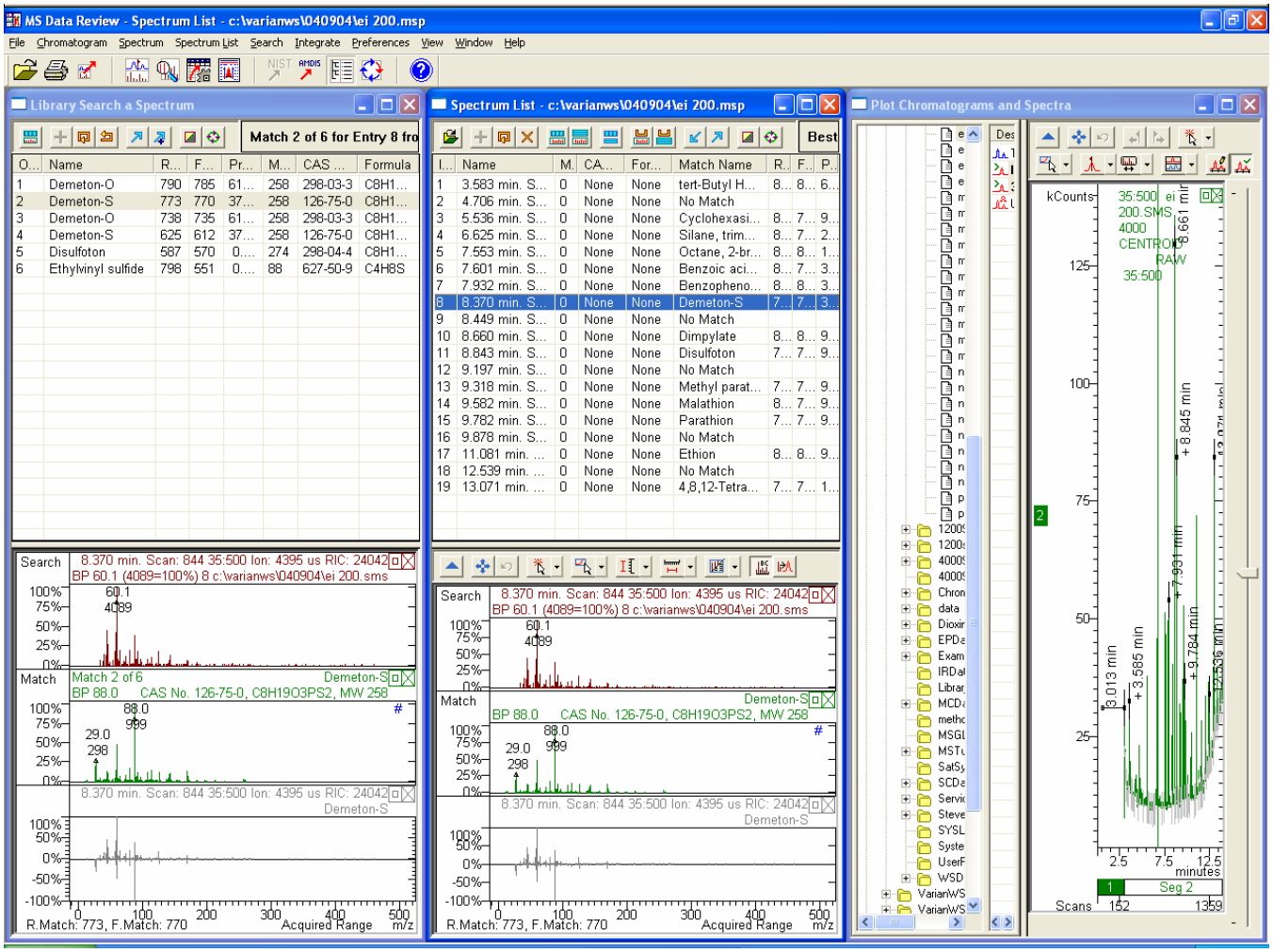
Identify peaks, such as, your standard compounds by doing a library search on the Spectrum List.

Click **Library Search SpectrumList** in the Spectrum List toolbar, to open a window to edit the library search parameters.





After editing, click **Search**. The spectrum list is updated with the name of the best match for each peak found in the library, along with the match quality values. Some of the peaks may be due to matrix or column bleed, delete these by selecting them one at a time, and clicking the  icon. Sometimes, the best match may not represent the actual compound in the standard. For example, the pesticide Demeton-O gives the best match, but we know that the isomer Demeton-S was actually included in the standard.



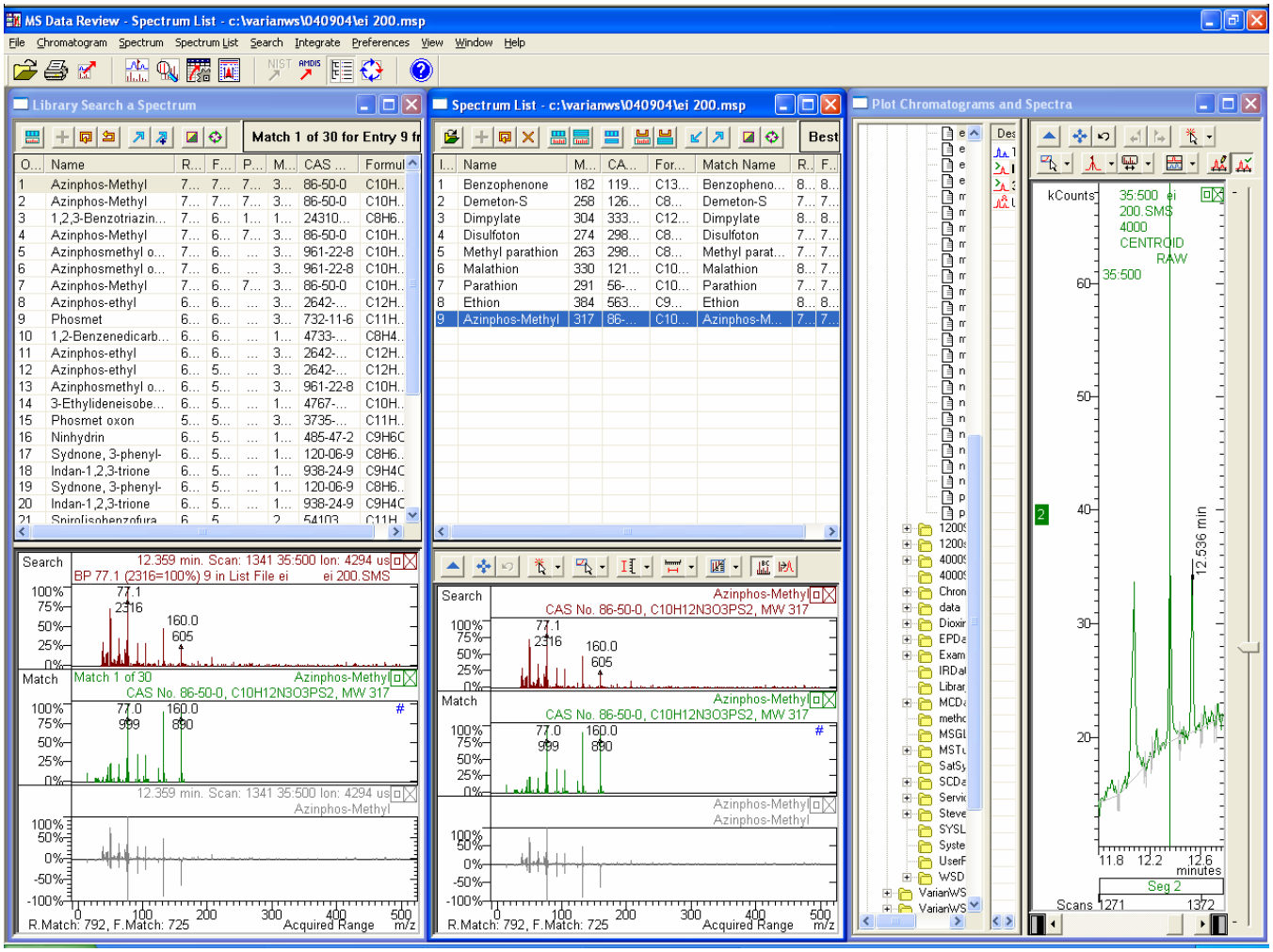
Select the **Demeton-S** line.

Click on the **Library Search Spectrum** icon to display the complete list of matches for the selected peak.

Click the second line in the list, which has the correct name.

Click **Replace Match in Spectrum List Window** icon to update the Spectrum List.

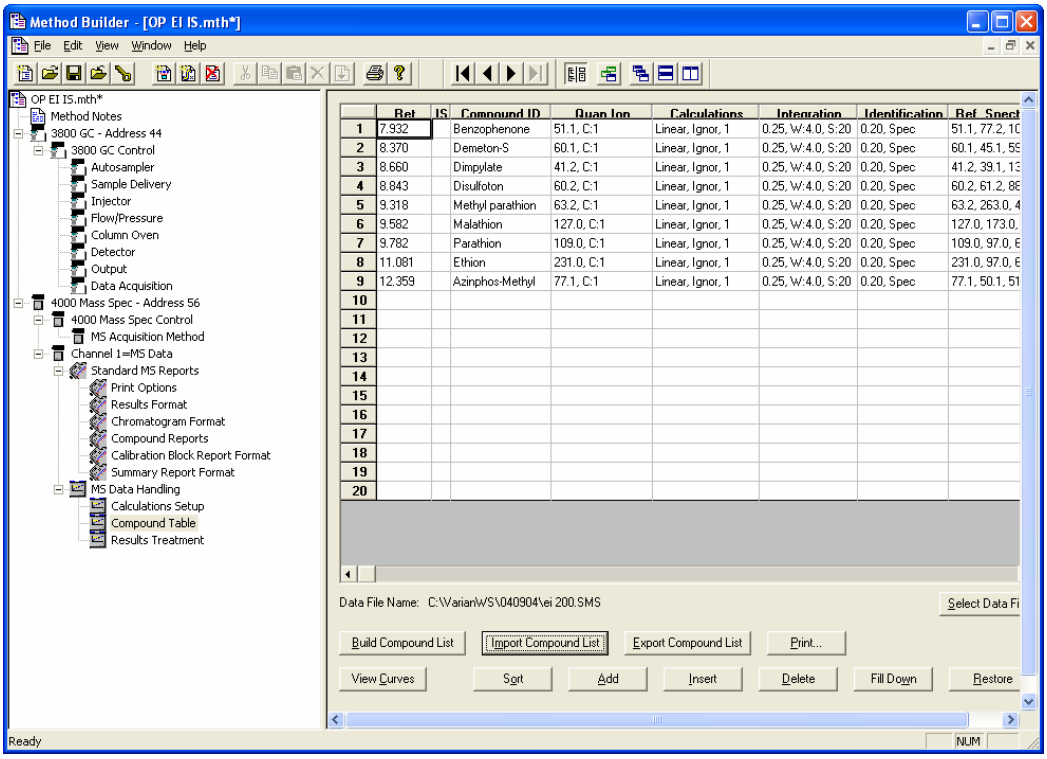
Continue editing the Spectrum List until you have correctly identified all the compounds in your standard, and click the **Update All Searches with Matches** icon to complete the SpectrumList.


MS Data Review - Spectrum List - c:\varianws\040904\ei 200.msp
File Chromatogram Spectrum Spectrum List Search Integrate Preferences View Window Help
Library Search a Spectrum
Match 1 of 30 for Entry 9 fr
Spectrum List - c:\varianws\040904\ei 200.msp
Plot Chromatograms and Spectra
Search
Match
12.359 min. Scan: 1341 35:500 Ion: 4294 us
BP 77.1 (2316=100%) 9 in List File ei
ei 200.SMS
Match 1 of 30
Azinphos-Methyl
CAS No. 86-50-0, C10H12N3O3PS2, MW 317
R.Match: 792, F.Match: 725
Acquired Range
m/z
kCounts
35:500 ei
200.SMS
4000
CENTROID
RAW
35:500
12.536 min
minutes
Seg 2
Scans 1271 1372

Importing a Compound List

Close MS Data Review and return to the Method Builder application.

Click **Import Compound List** and select the .msp file you just completed.



All the identified peaks are in the Compound Table with default values for the data handling parameters.

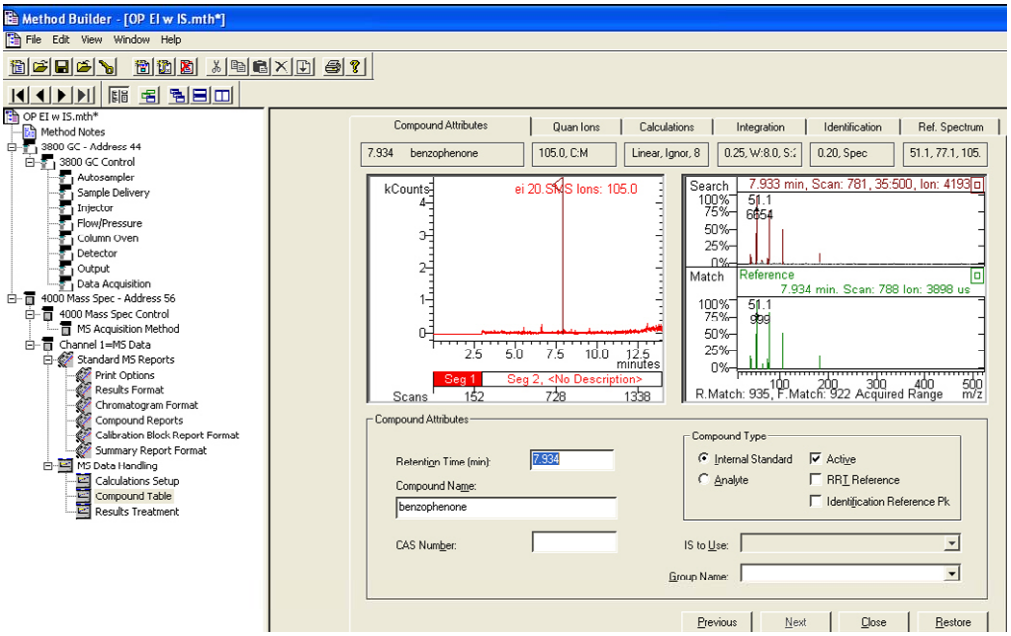
Identifying Internal Standards

Double-click the line in the compound table containing the IS information, (in this example, the benzophenone peak).

Select **Internal Standard** in the Compound Type section of the Compound Attributes tab.

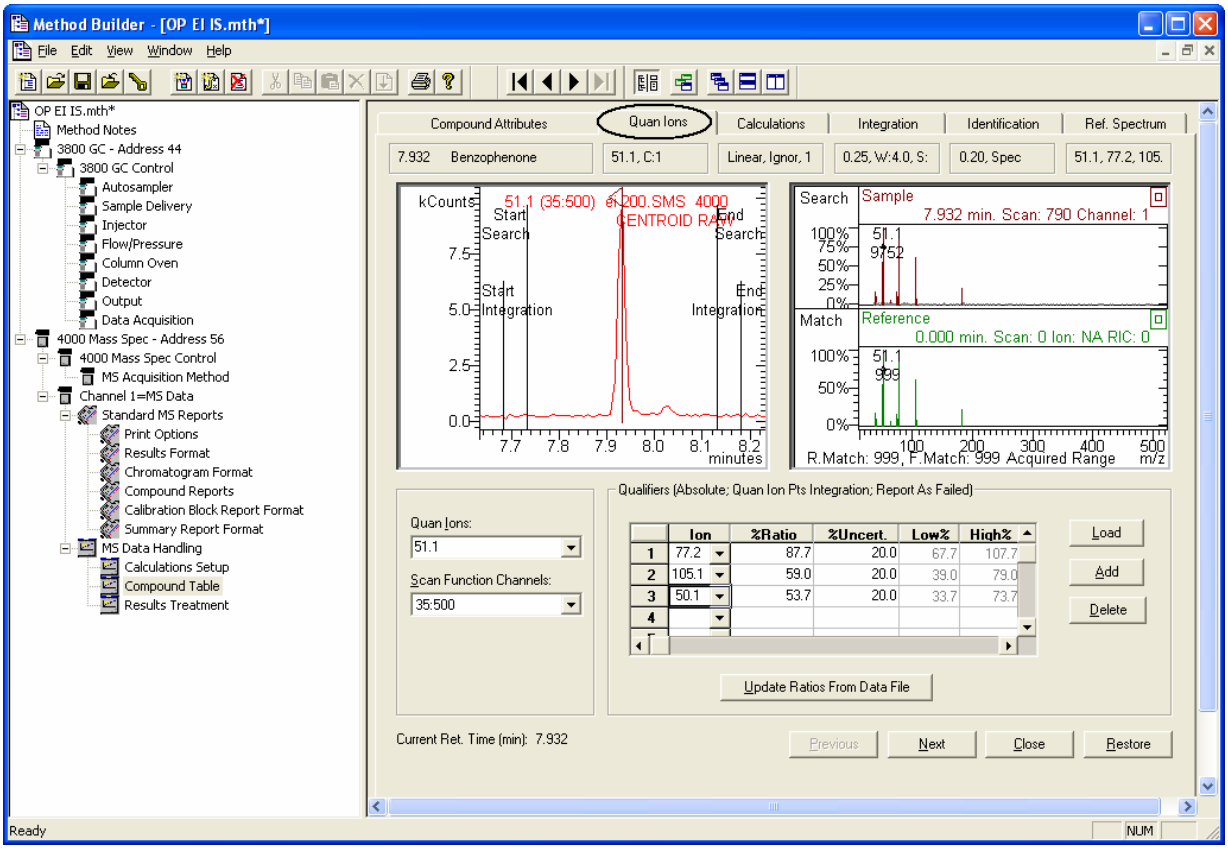
Designate the internal standard peak as a Reference peak and/or a RRT Reference to help identify analyte peaks. Use only Internal Standard peaks as Reference or RRT Reference peaks, because IS and Reference peaks must be present and easily identifiable in every chromatogram.

Note: Using a Reference Peak to identify a compound may cause the wrong peak to be identified, especially if peak retention times are very close together. Using a Reference Peak temporarily shifts the Expected Retention Times and Search Windows. If the method is searching for the peak “Nearest” to an expected Retention Time, the wrong peak may be identified.



Examining Spectra

Click **Quan Ions** and examine the sample and reference spectra.



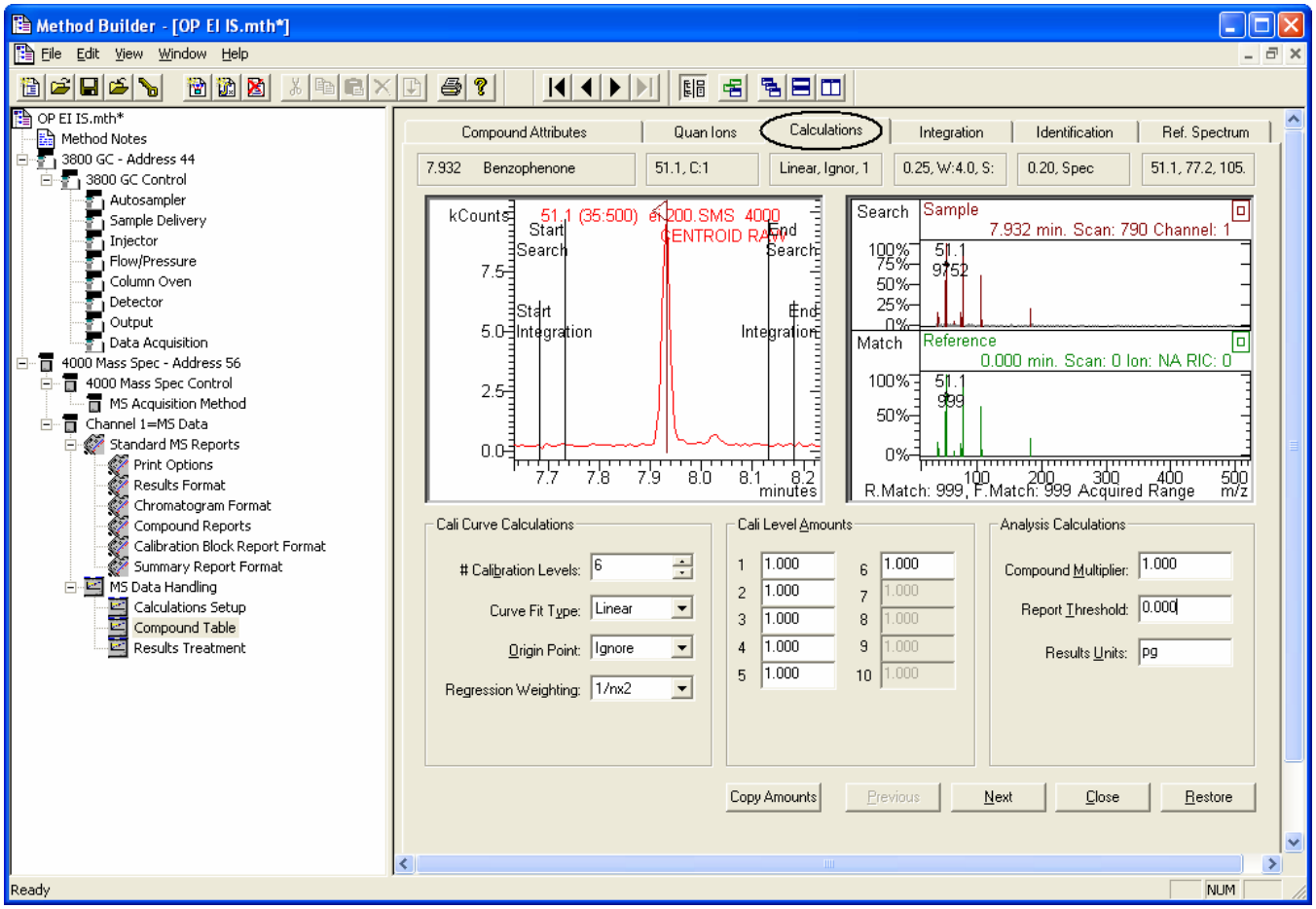
The default Quan ion is the most intense ion in the sample spectrum. You can select another ion if you know of matrix interference with the default ion. If you wish to use qualifier ions, you can add them by clicking on the **Load** button in the Qualifiers section. This loads the most intense non-quan ions into the table and calculates their abundance relative to the selected quan ion.

Select the method of calculating relative abundance limits for the qualifier ions (Relative or Absolute) in the Calculations Setup section of the method.

Enter the percentage limits for each ion on in this table.

Add or delete ions to get a list of ions, of the compound of interest. For instance, you may want to select a less intense, higher m/z ion in preference to a more intense lighter ion that might have matrix interference.

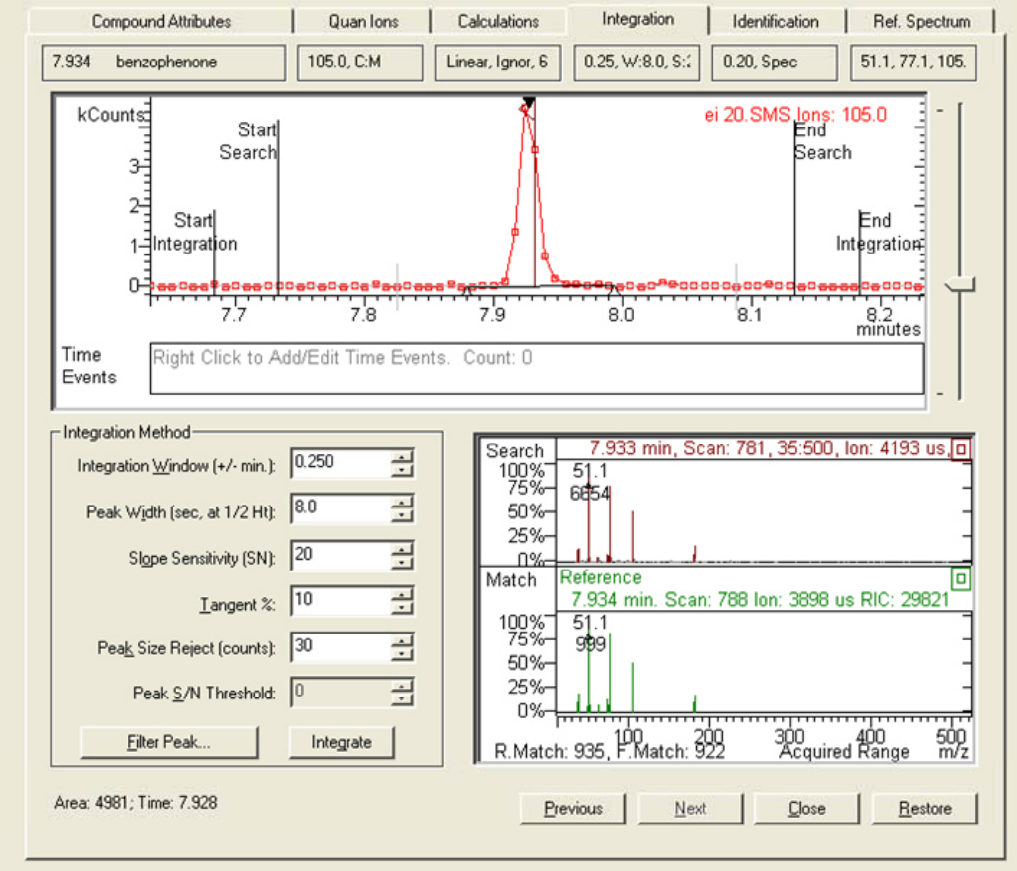
Select the Calculations tab and enter the # **Calibration Levels**, six in this example, and the **Curve Fit Type**, the **Origin treatment**, and the **Regression** weighting formula.



Because of the Internal Standard peak, 1.000 is entered for the Cali Level Amounts for each level.

Adjusting Integration Parameters

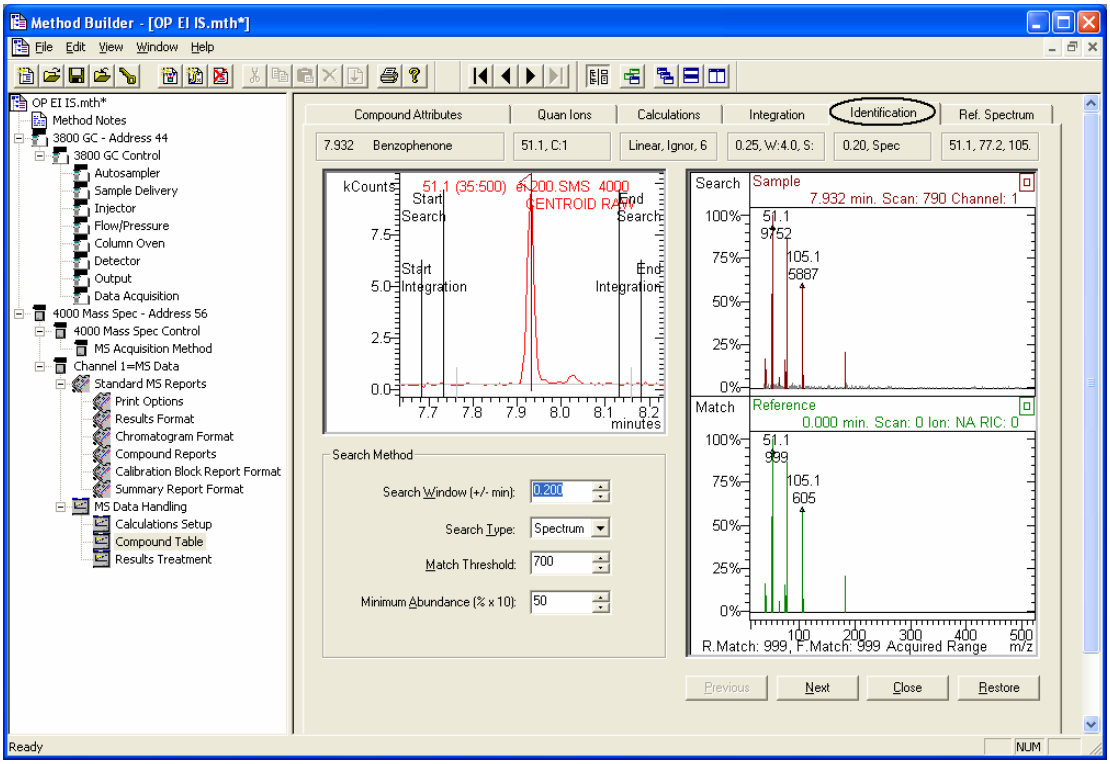
Select the Integration tab and adjust the integration parameters, if necessary.



Because the Internal Standard is generally present in easily detected quantities in every chromatogram, it is probable that the default values can be used. If desired, you can invoke filtering of the peak data by clicking on the **Filter Peak…** button and setting the filter parameters as you like. You can test the effects of any changes you make in the integration tab by clicking on the **Integrate** button and observing the effect on the Peak Area and Integrated Peak display.

Setting Up Search Parameters for Target Peak Identification

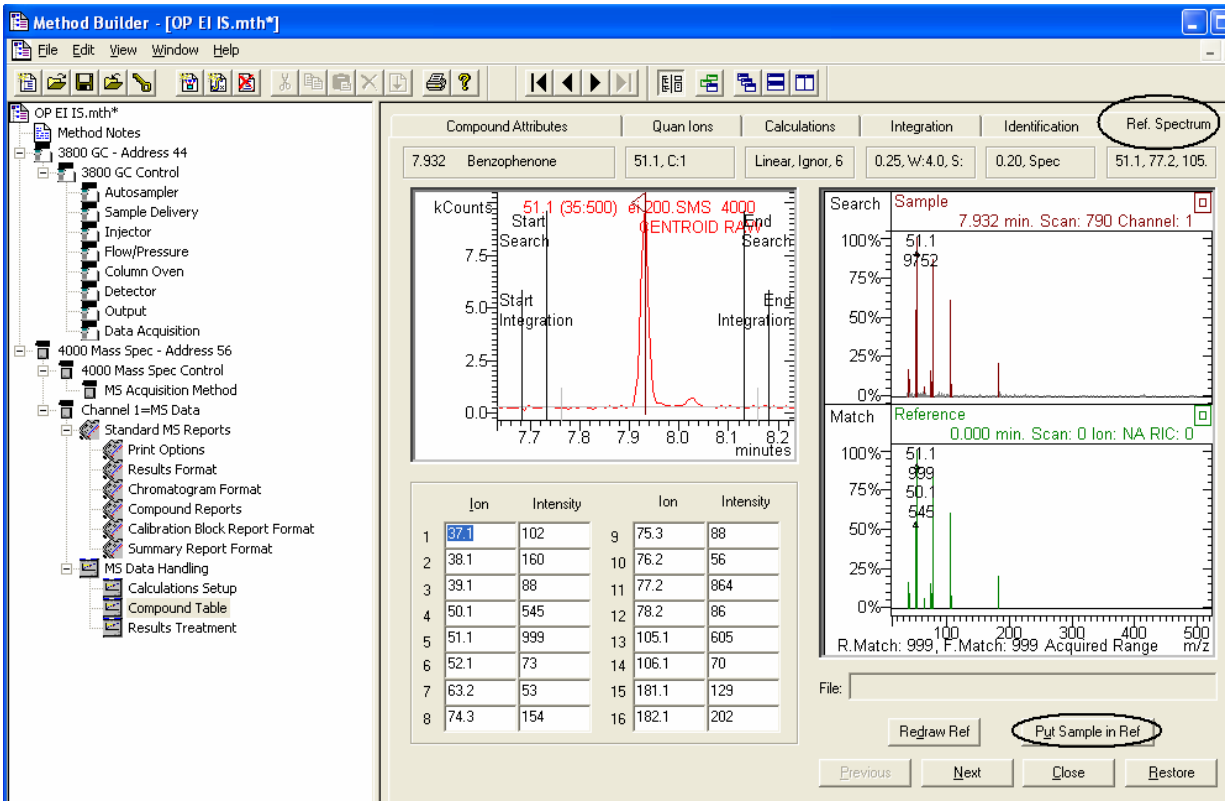
The Identification tab is used to set up the search parameters for target peak identification.



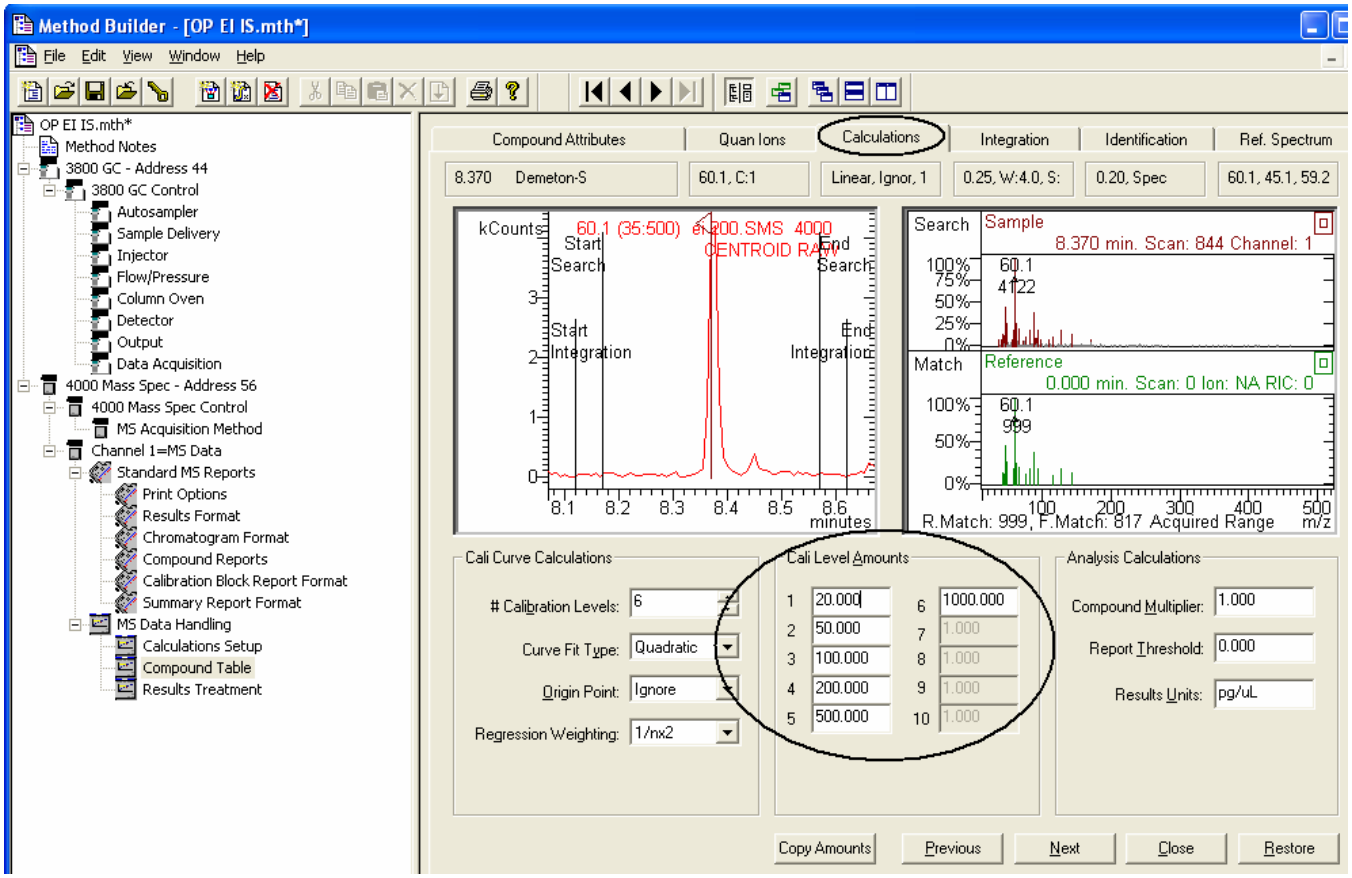
You can specify the **Search Window** time range within which the target must be found, the **Search Type** (Spectrum or Retention time), the minimum required **Match Threshold**, and the **Minimum Abundance** to be considered in calculating a match.

Editing Reference Spectrum

In the Ref. Spectrum tab, you can edit the reference spectrum to improve the match reliability, and, if necessary, use a sample spectrum as the reference, for those cases where the compound of interest is not found in the library, or where the MS conditions do not allow matching to a standard library.

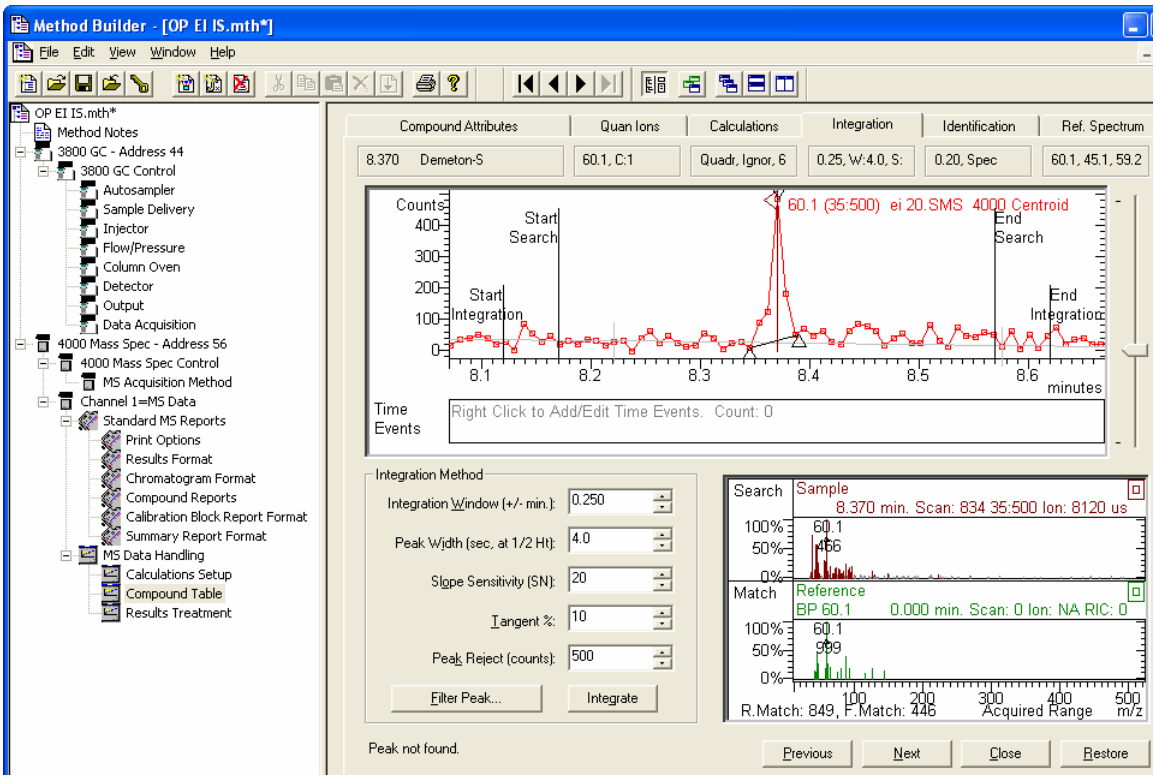


For target compounds, the editing of the Compound Table is similar to that for the Internal Standard, except of course the compound is identified as an Analyte in the Compound Attributes tab, and the appropriate Internal Standard is selected. In the Calculations tab, the Calibration Curve Calculations are changed to match the IS, and the Calibration Level Amounts are changed to reflect the levels found in the calibration standards.

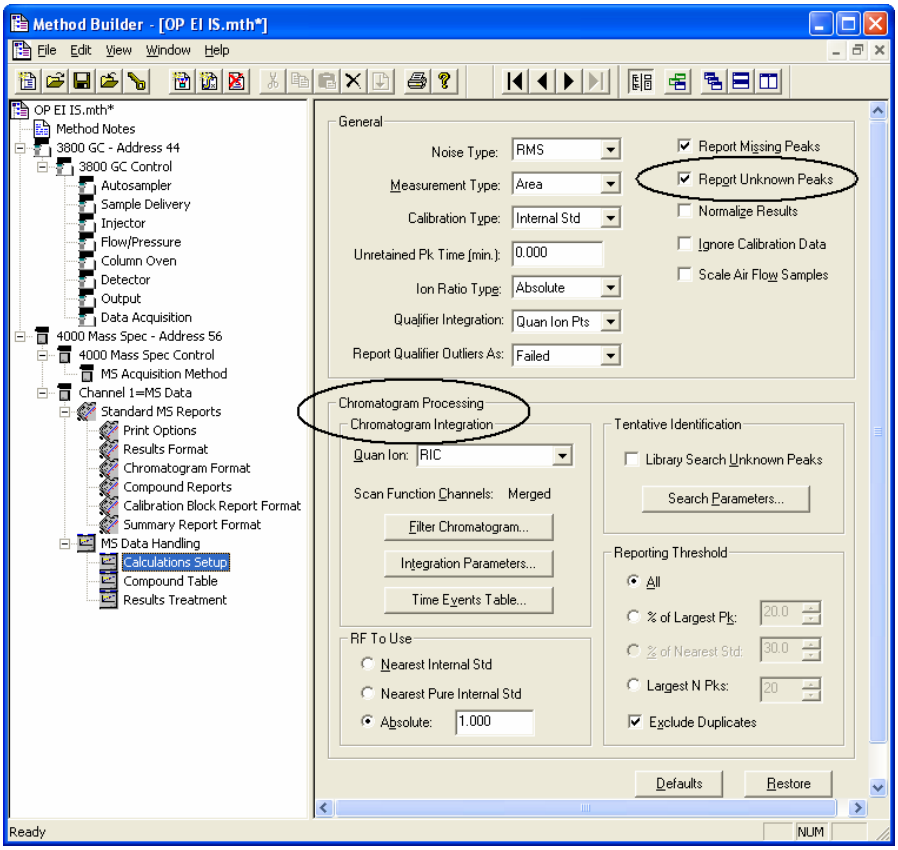


Fine Tuning Integration Parameters

Finally, to fine tune the Integration parameters, close the target compound dialog, click on **Select Data File**, and select the file for the lowest level calibration standard. Double-click on the first target compound in the Compound List and select the Integration tab.



Examine the integrated peak display and adjust the integration method until the peak is processed as desired when you press the Integrate button. You may need to use Timed Events so that the software can perform the appropriate integration. Repeat the process for each of the remaining target compounds. Once you have found integration parameters that work for most or all of the compounds, you may wish to use these same parameters to integrate unknown peaks. If so, select the Calculations Setup section of the method, check the Report Unknown Peaks box, and modify the parameters in the Chromatogram Processing section as required.


Method Builder - [OP EI IS.mth*]
File Edit View Window Help
OP EI IS.mth*
Method Notes
3800 GC - Address 44
3800 GC Control
Autosampler
Sample Delivery
Injector
Flow/Pressure
Column Oven
Detector
Output
Data Acquisition
4000 Mass Spec - Address 56
4000 Mass Spec Control
MS Acquisition Method
Channel 1=MS Data
Standard MS Reports
Print Options
Results Format
Chromatogram Format
Compound Reports
Calibration Block Report Format
Summary Report Format
MS Data Handling
Calculations Setup
Compound Table
Results Treatment
General
Noise Type: RMS
Measurement Type: Area
Calibration Type: Internal Std
Unretained Pk Time (min.): 0.000
Ion Ratio Type: Absolute
Qualifier Integration: Quan Ion Pts
Report Qualifier Outliers As: Failed
Report Missing Peaks
Report Unknown Peaks
Normalize Results
Ignore Calibration Data
Scale Air Flow Samples
Chromatogram Processing
Chromatogram Integration
Quan Ion: RIC
Scan Function Channels: Merged
Filter Chromatogram...
Integration Parameters...
Time Events Table...
Tentative Identification
Library Search Unknown Peaks
Search Parameters...
Reporting Threshold
All
% of Largest Pk: 20.0
% of Nearest Std: 30.0
Largest N Pks: 20
Exclude Duplicates
RF To Use
Nearest Internal Std
Nearest Pure Internal Std
Absolute: 1.000
Defaults
Restore
Ready
NUM

Generating Calibration Curves

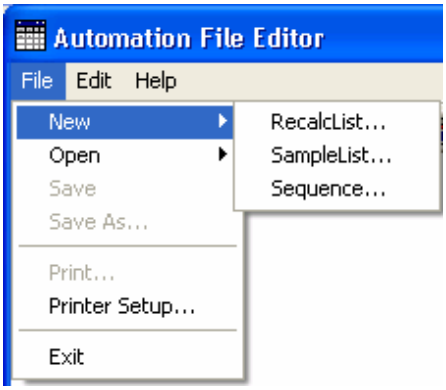
Once all the target compounds have been properly identified and the integration parameters have been optimized, calibration curves for all the compounds of interest may be generated. If the data files for all the replicates of all the calibration levels were generated by execution of a SampleList, a RecalcList probably already exists which can be used to generate the calibration curves.

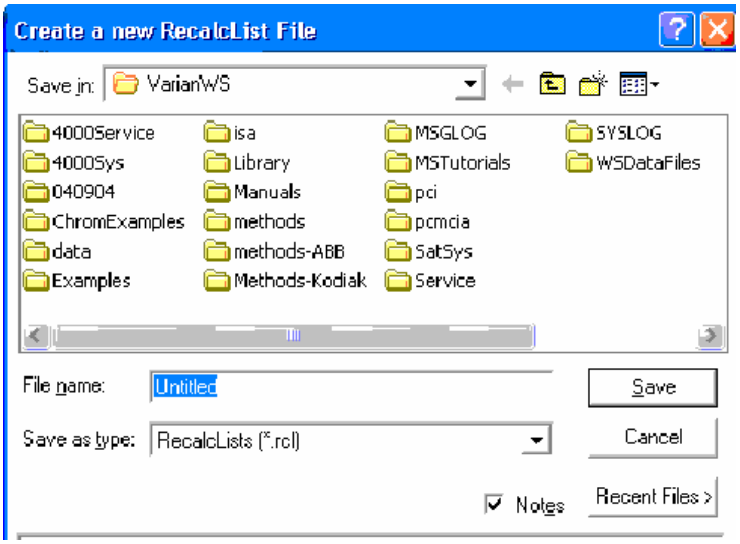
Building a RecalcList

You will need to build a RecalcList if:

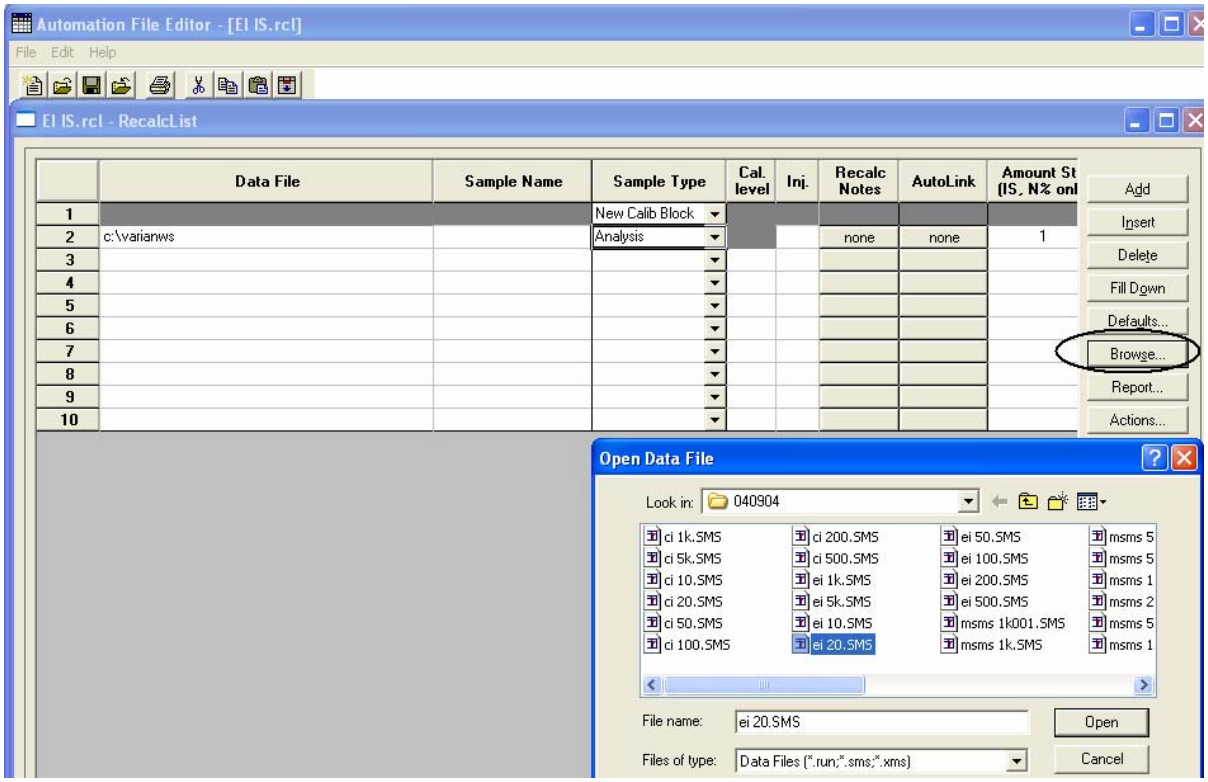
- The injections were made individually.
- The injections were made from a number of different SampleLists.
- You chose not to automatically generate a RecalcList.

Click on the “Edit Automation Files” icon in the MS Workstation Toolbar and select either **File→New** or **File→Open** and then ***RecalcList…*** to access either a new RecalcList or an existing RecalcList. If you are generating a new RecalcList, select the folder in which the calibration files currently reside and give the RecalcList a meaningful File name.



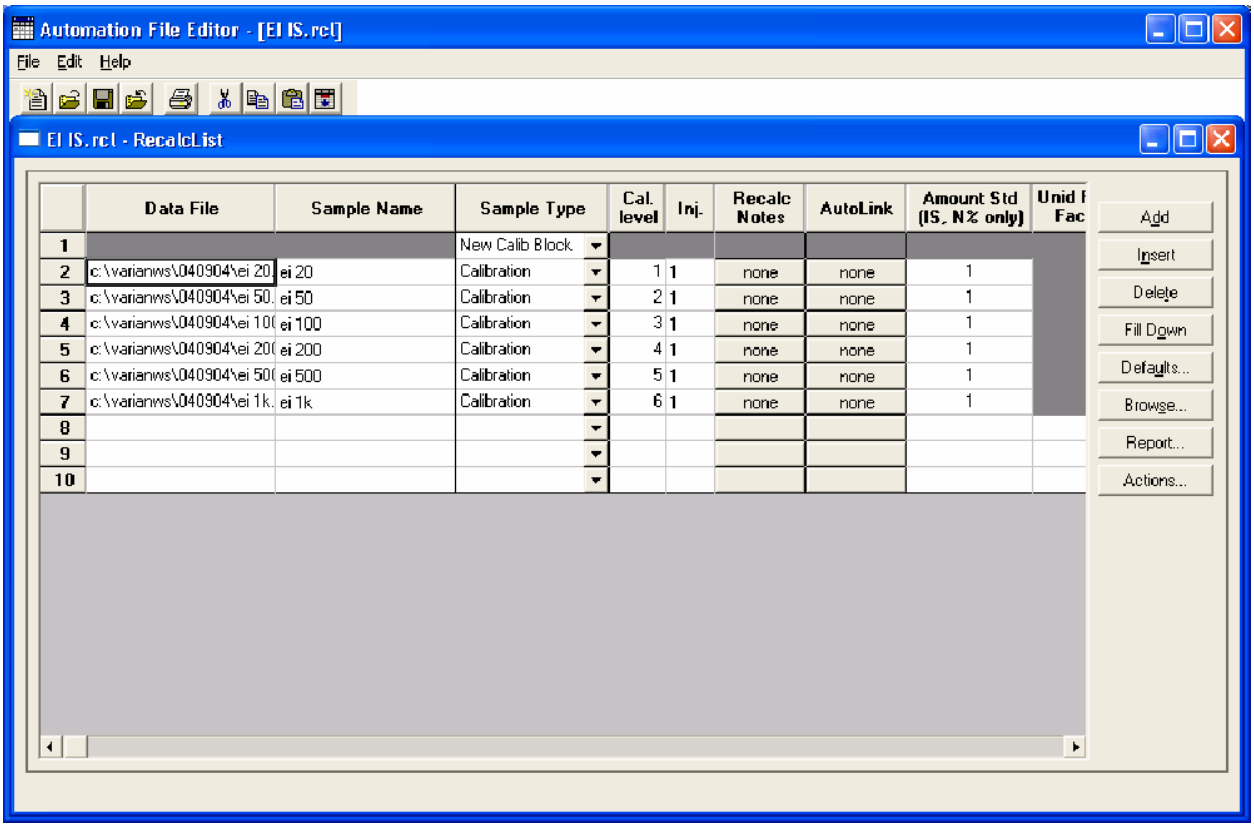


When the new RecalcList appears, begin by adding a line and selecting **New Calibration Block** as the **Sample Type**. This will ensure that any recalculation is independent of previous actions. Next add another line and click on the **Data File** field, then click on the **Browse…** button.



A list of data files will appear. Select the first replicate of the lowest level standard and click on **Open** to add the filename to the RecalcList. Change the **Sample Type** to Calibration and the **Cal Level** to whatever it is labeled in the Compound Table (you can use 1 as the lowest level standard or the highest level standard, as long as the RecalcList and the compound table agree). Add any notes and

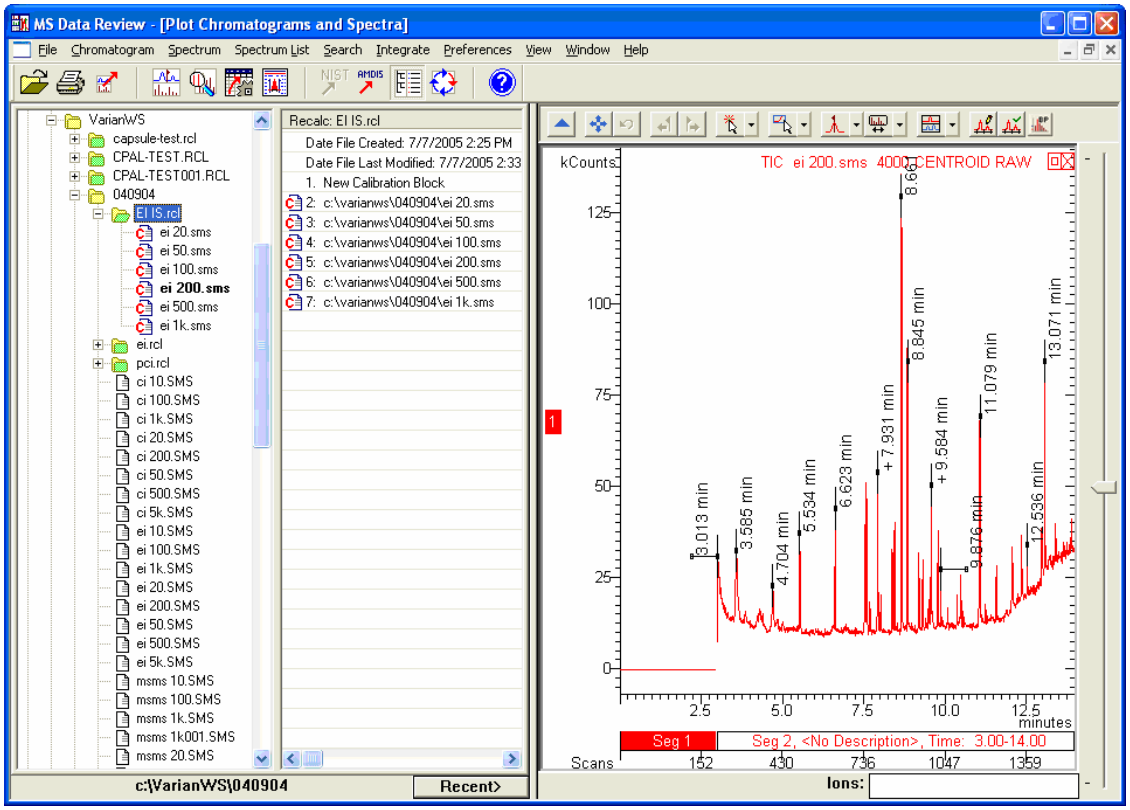
edit any of the other fields in the line as necessary. Add any replicates of this calibration level and then add the next level replicates.



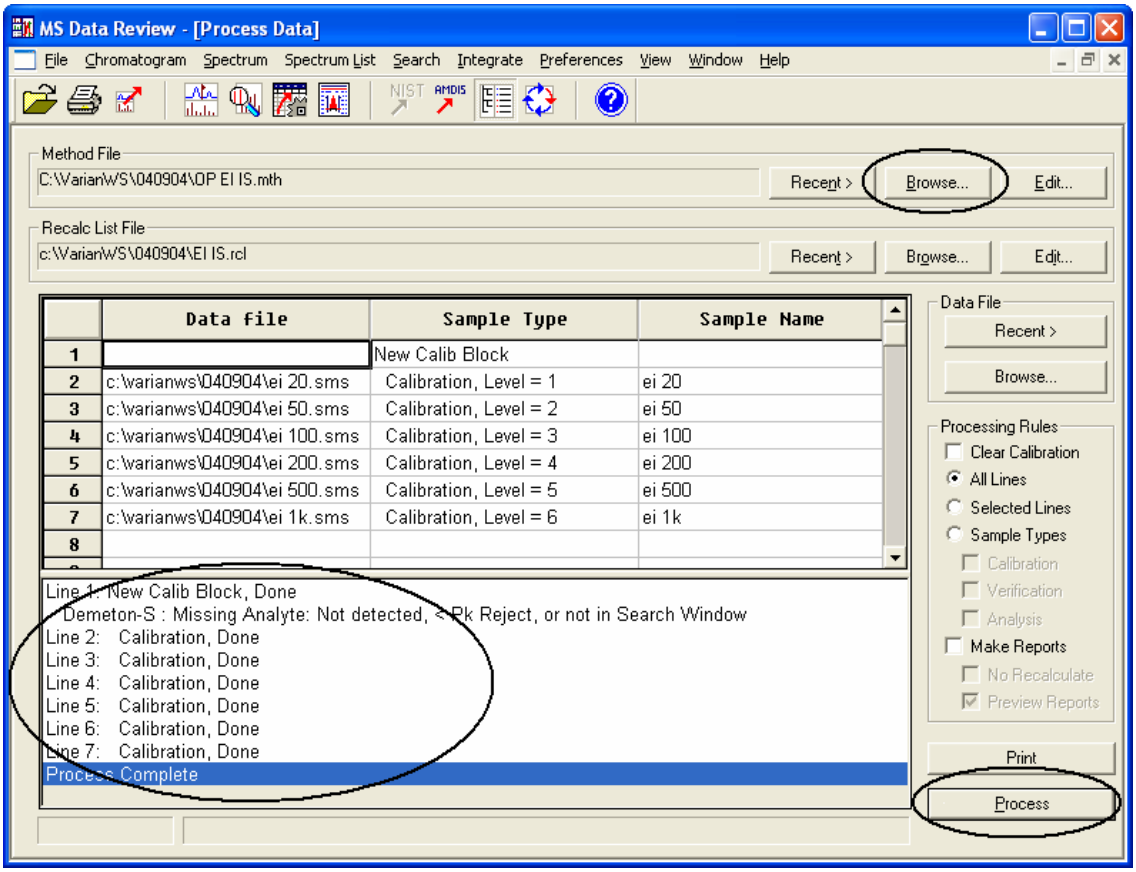
When all the calibration files have been entered, Save the RecalcList and close the Automation File Editor.

Process RecalcList

Click on the “MS Data Review” icon  in the Toolbar to open MS Data Review. From the **Plot Chromatograms and Spectra** screen, select the RecalcList you previously created from the Data Files pane.



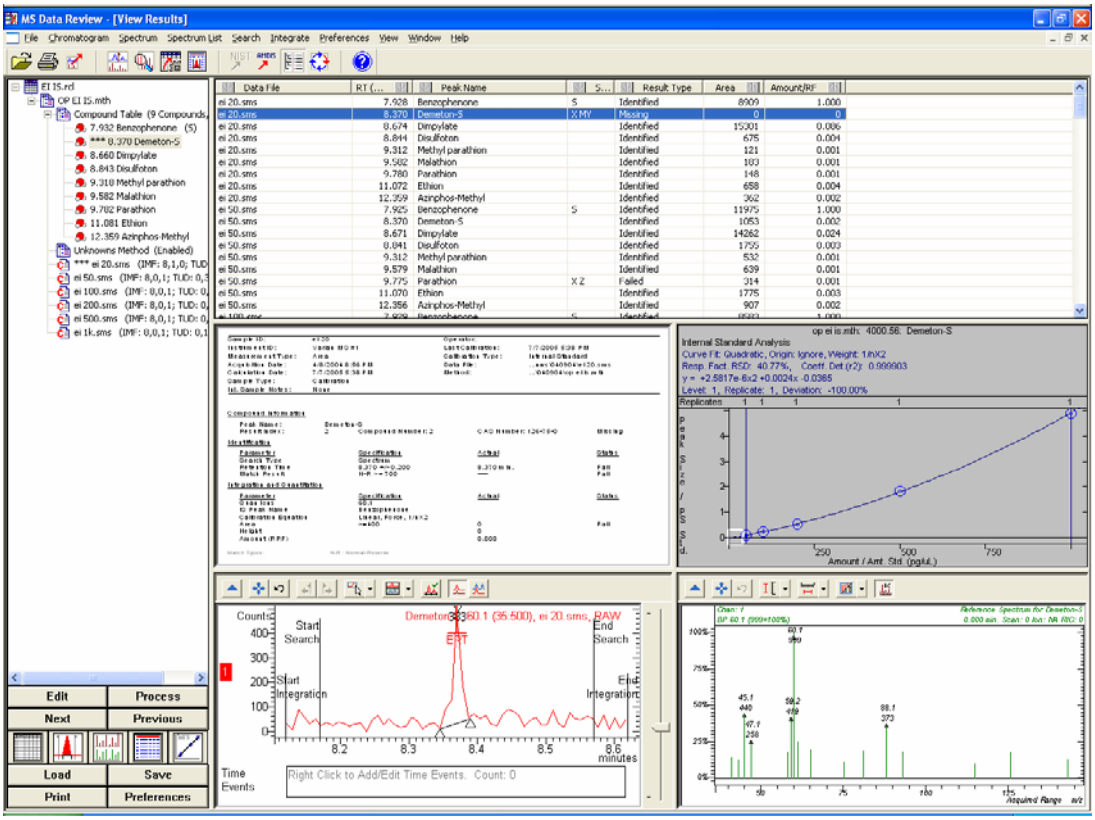
Click on the “Process Data” icon  to load the RecalcList in the Process Data screen.



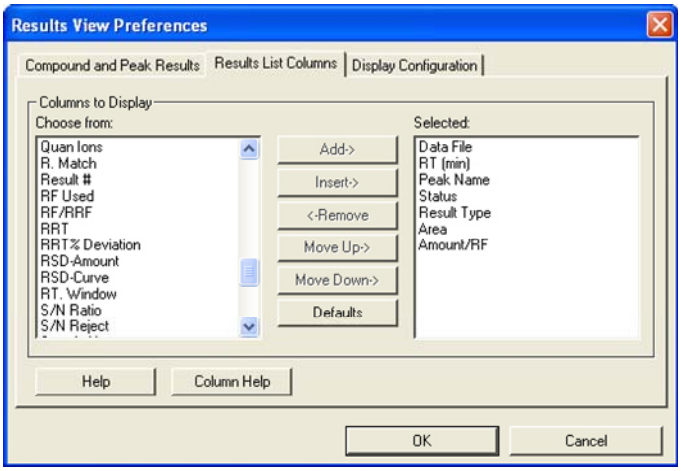
In the Method File line, Browse for the method file you wish to calibrate. Click on the **Process** button to execute the recalculation. As the recalculation proceeds, a line by line report of progress is generated, including any instances of missing or miscalculated compounds. When the recalculation is completed, you can proceed to view the results.

Viewing Results

Click on the “View Results” icon  to view and examine the results of the processed RecalcList.

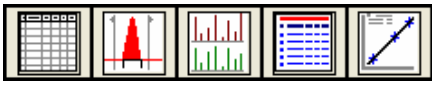


The previous screen shows the default panes. The current data file and compound in the data files pane along with the compound in the Results List. You can change the screen configuration from **Preferences**. The following is the Results View Preferences, You can add columns such as Multiplier, Divisor, Amt Std, and RF to the results list.



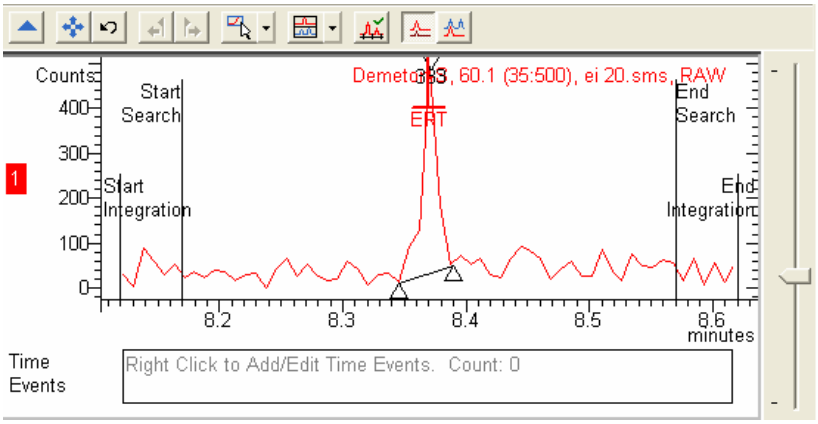
From the data files pane, clicking on a different compound or different data file will update the upper Results List and the other lower information panes. Conversely, clicking on a data file in the Results List will update the datafiles pane.

If some compounds were missed or miscalculated, click on the compound in the upper Results List pane. Then, check the Compound Report and the Integration Chromatogram panes to see what aspect of the peak quantitation failed. Each of the 5 main viewing panes can be viewed individually by clicking on the appropriate icon.



Clicking on the same icon again will restore all the panes.

The previous example shows that in data file, ei.20.sms, Demeton-S is reported as Missing with a status code X MY. The **M** indicates 'missing' and the **Y** indicates 'Peak not detected < Size Threshold or not in Peak Window'.



The Integration Chromatogram pane shows that the peak is in the peak window and was integrated.

Sample ID:	ei 20	Operator:	
Instrument ID:	Varian MS #1	Last Calibration:	7/7/2005 5:38 PM
Measurement Type:	Area	Calibration Type:	Internal Standard
Acquisition Date:	4/8/2004 8:56 PM	Data File:	...nws\040904\ei 20.sms
Calculation Date:	7/7/2005 5:38 PM	Method:	...\040904\op ei is.mth
Sample Type:	Calibration		
Inj. Sample Notes:	None		

Compound Information

Peak Name: Demeton-S
Result Index: 2 Compound Number: 2 CAS Number: 126-75-0 Missing

Identification

Parameter	Specification	Actual	Status
Search Type	Spectrum		
Retention Time	8.370 +/- 0.200	8.370 min.	Fail
Match Result	N-R >= 700	----	Fail

Integration and Quantitation

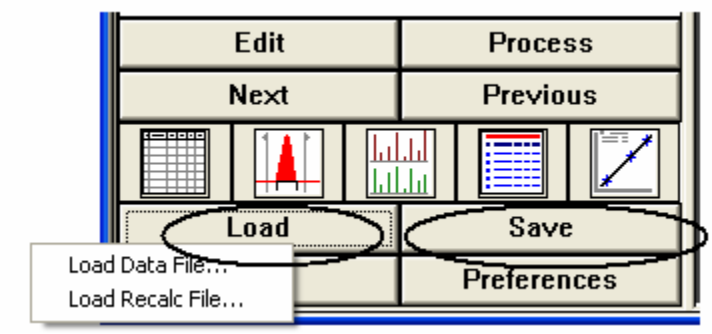
Parameter	Specification	Actual	Status
Quan Ions	60.1		
IS Peak Name	Benzophenone		
Calibration Equation	Linear, Force, 1/nX2		
Area	>=400	0	Fail
Height		0	
Amount (RRF)		0.000	

Match Types: N-R : Normal-Reverse

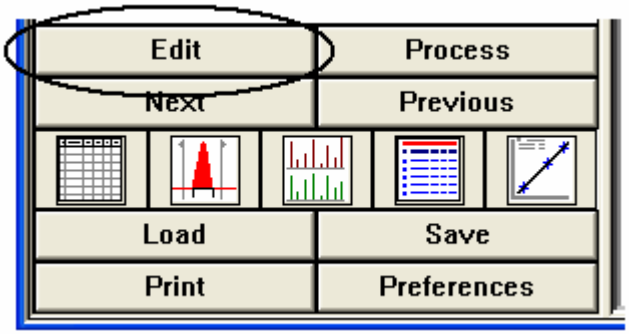
The Compound Report pane show that the area is below the peak threshold.

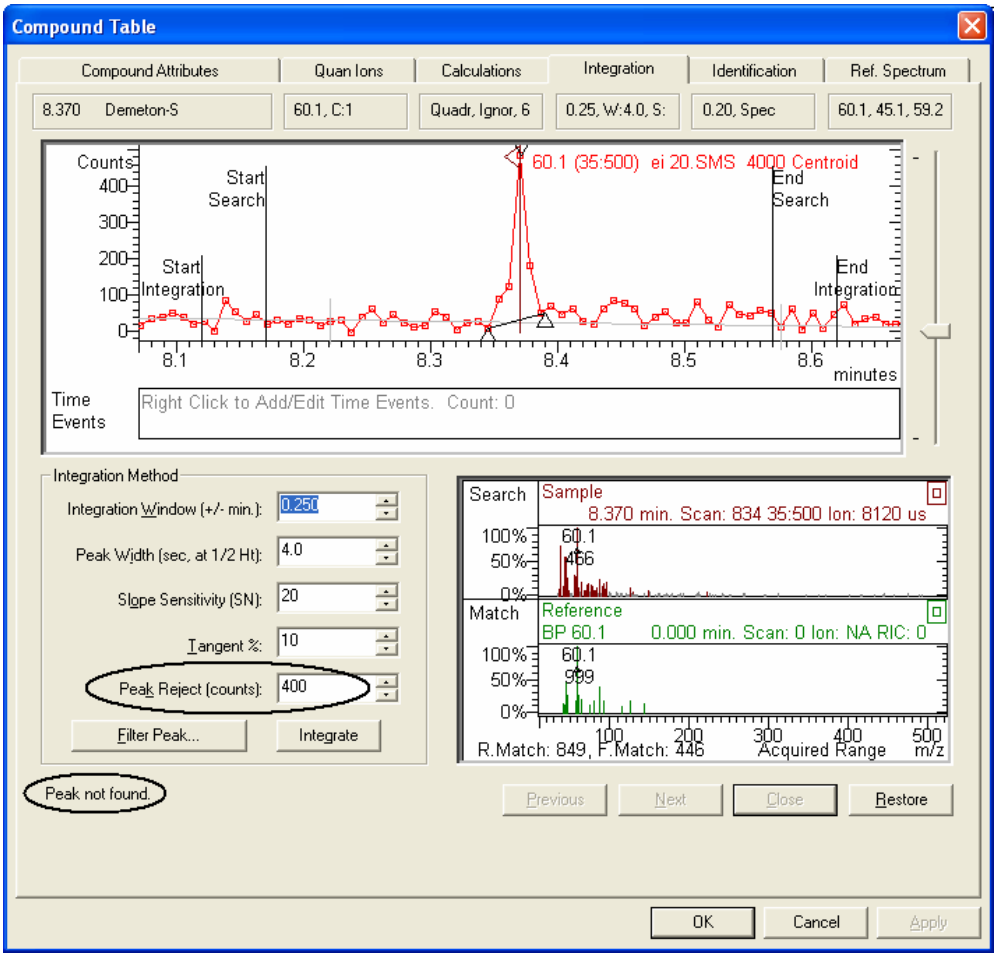
Modifying a Method

NOTE: All changes to the method and data file results in the Results View window are stored to temporary files. These changes will only be saved to the actual files when exiting the Results View window or by clicking on the **Save** button or by loading a new RecalcList or a new datafile. You will be prompted to save the changes, delete the changes or cancel the save operation.



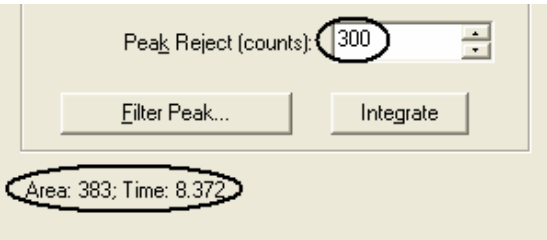
Click **Edit** to access the Compound Integration parameters to modify the method so that the peak is properly integrated.





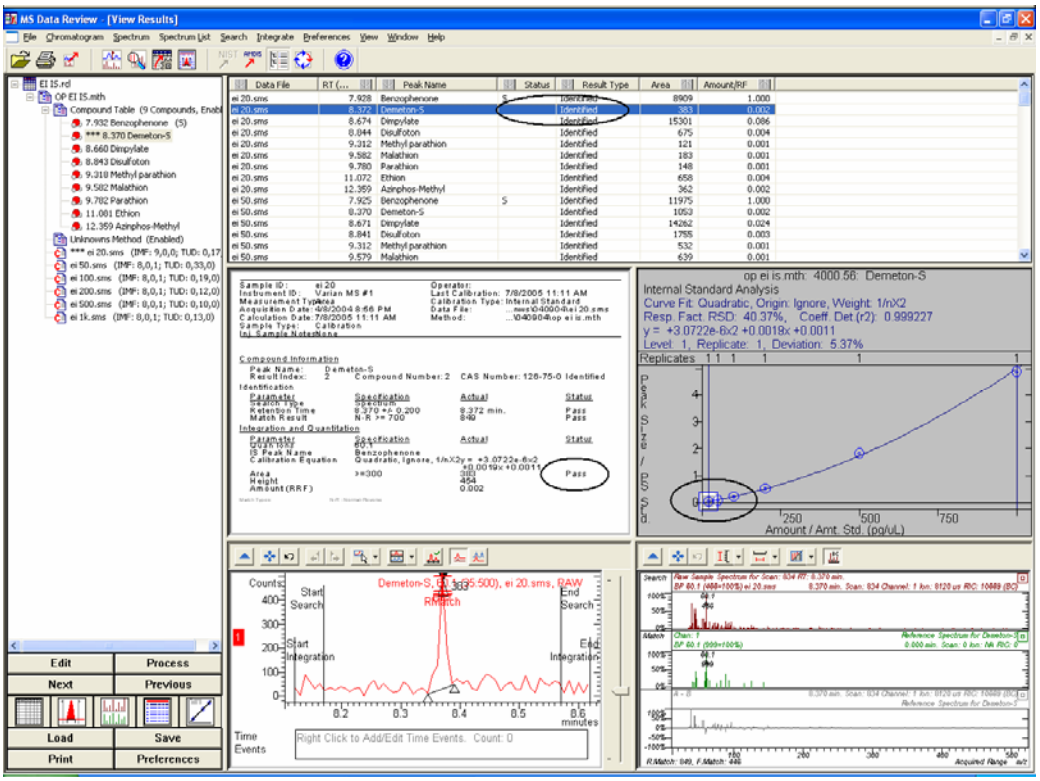
In this example, the Peak Reject needs to be lowered.

Click on the **Integrate** button to recalculate the results and note whether the compound is now properly processed.

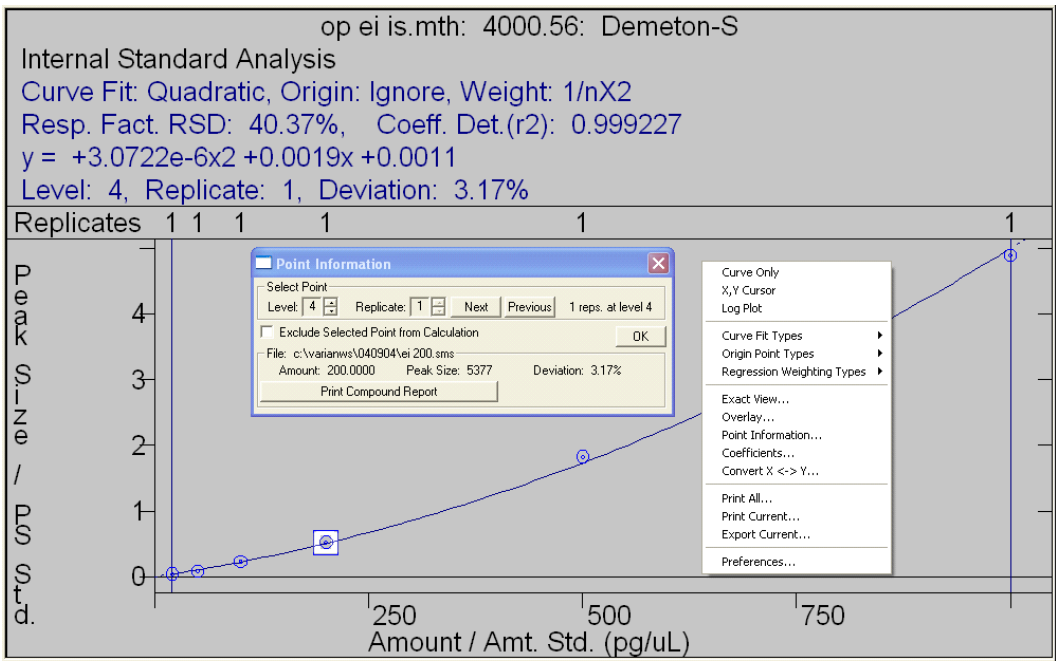


After clicking on the **OK** button to close the window, all the data files in the RecalcList that have that compound will be reintegrated and the new results will be updated in all the panes affected by the change.

NOTE: This example showed a change made to a calibration data file. This will cause all the calibration datafiles to be reintegrated to generate a new calibration curve and, in addition, any analysis data files will be reintegrated to generate updated results. If a change is made to an analysis data file, then only that data file will be reintegrated.



The results panes are now updated and the calibration curve pane has been updated with an additional level.

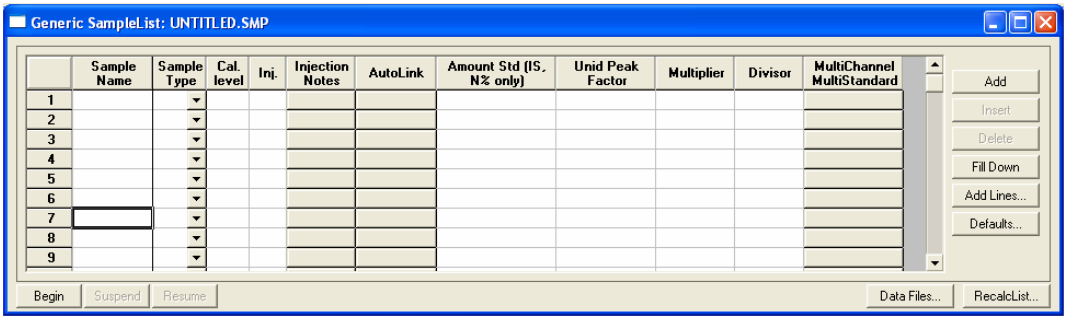


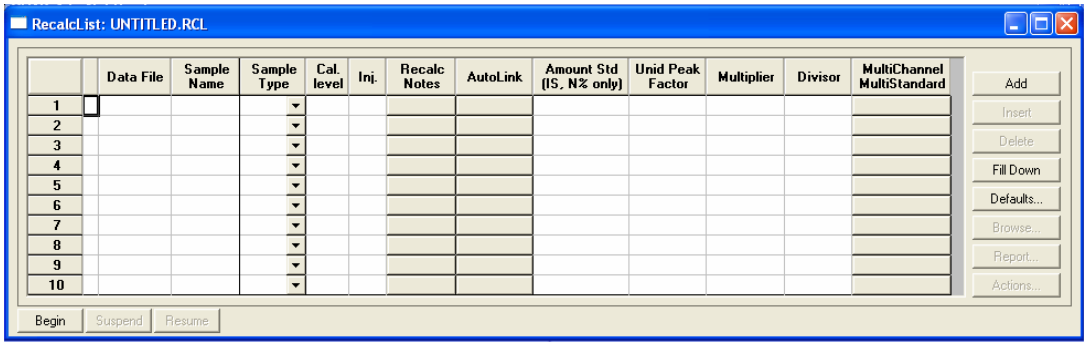
The calibration can be viewed and adjusted by right-clicking on the calibration curve pane and selecting an action. You may modify the **Curve Fit Type**, the **Origin Point Type**, and the **Regression Weighting Type** to optimize the fit of the data points to the calibration curve. You may also examine the individual replicate points and exclude a point from the curve from the **Point Information** selection.

When all the compounds at all calibration levels are properly detected, click on the **Save** button and save the changes to the method.

SampleList and RecalcList Fields

The following figures show that the SampleList and RecalcList have fields in common.





The following table describes the SampleList and RecalcList fields.

Item	Description
Data File	Select the path and file name of the data file in a RecalcList.
Sample Name	Up to 19 characters Sets the name of each sample in the SampleList or RecalcList.
Sample Type	Baseline, Analysis, Calibration, Verification, Print Calib, New Calib Block, AutoLink, Activate Method Sets the sample type, or automation action, of each line in the SampleList or RecalcList.

Item	Description
Cal. Level	1 to 10 Sets the calibration level of each calibration or verification sample in the SampleList RecalcList.
Inj	1 to 9 Sets the number of injections.
Injection Notes	Up to 180 characters Opens the Notes window for the selected sample to edit or create a note about the sample in a SampleList.
Recalc Notes	Up to 180 characters Opens the Notes window for the selected sample to edit or create a note about the sample in a RecalcList.
Unid Peak Factor	0 to 1,000,000.0 Sets a calibration factor for unidentified peaks. Not used by calibration samples.
Multiplier	0.000001 to 1,000,000.0 Sets a value for the multiplier. Results for the sample are multiplied by this value. Not used by calibration samples.
Divisor	0.000001 to 1,000,000.0 Sets a value for the divisor. Results for the sample are divided by this value. Not used by calibration samples.
Amount Standard	0.000001 to 1,000,000.0 GC Files: Sets the amount of the first internal standard. Used to calibrate results for Internal Standard and Normalized Percent calculations. Not used by calibration samples. MS files: Sets an ISFactor which is used by Analysis and Verification samples. It will be multiplied by the appropriate Compound Calibration Level Amount that is in the DH Method being used. Note that internal standards in Analysis and Verification samples always use the amount that is specified in Calibration Level 1.
MultiChannel MultiStandard	none, multiple, specific channel GC files: Opens the Data Handling Channels dialog box to specify the calibration parameters for up to four different Detector Channels. MS files: Not used by MS data handling. These GC detector channels are different from the scan function channels that may be specified in the MS method.
Add	Adds a line to the end of the SampleList or RecalcList.
Insert	Adds a new line before the highlighted line.
Delete	Deletes the highlighted line in the SampleList or RecalcList.
Fill Down	Causes the contents of the top cell in a series of highlighted cells to be copied to the cells below it. Used to edit all the cells in a column quickly.
Add Lines	Displays the Add Lines dialog box, allowing you to specify the number of lines to either insert or append to the spreadsheet, along with the values to use for each applicable field. Certain fields such as Sample ID and vial number can be automatically incremented.
Defaults	Displays the Set Defaults dialog box, allowing you to specify default values for each applicable field in the SampleList.

Item	Description
Data Files	Opens the Data File Generation dialog box to specify the naming scheme being used for Data Files generated from injections. Note that if the method contains both MS and standard GC DH method sections, then both sms and run files will be generated.
RecalcList	Opens the RecalcList Generation dialog box to specify the options for generating or updating RecalcLists after injections. Note that if the method used contains both MS and standard GC DH sections, then the generated Recalc List contains both sms and run files.