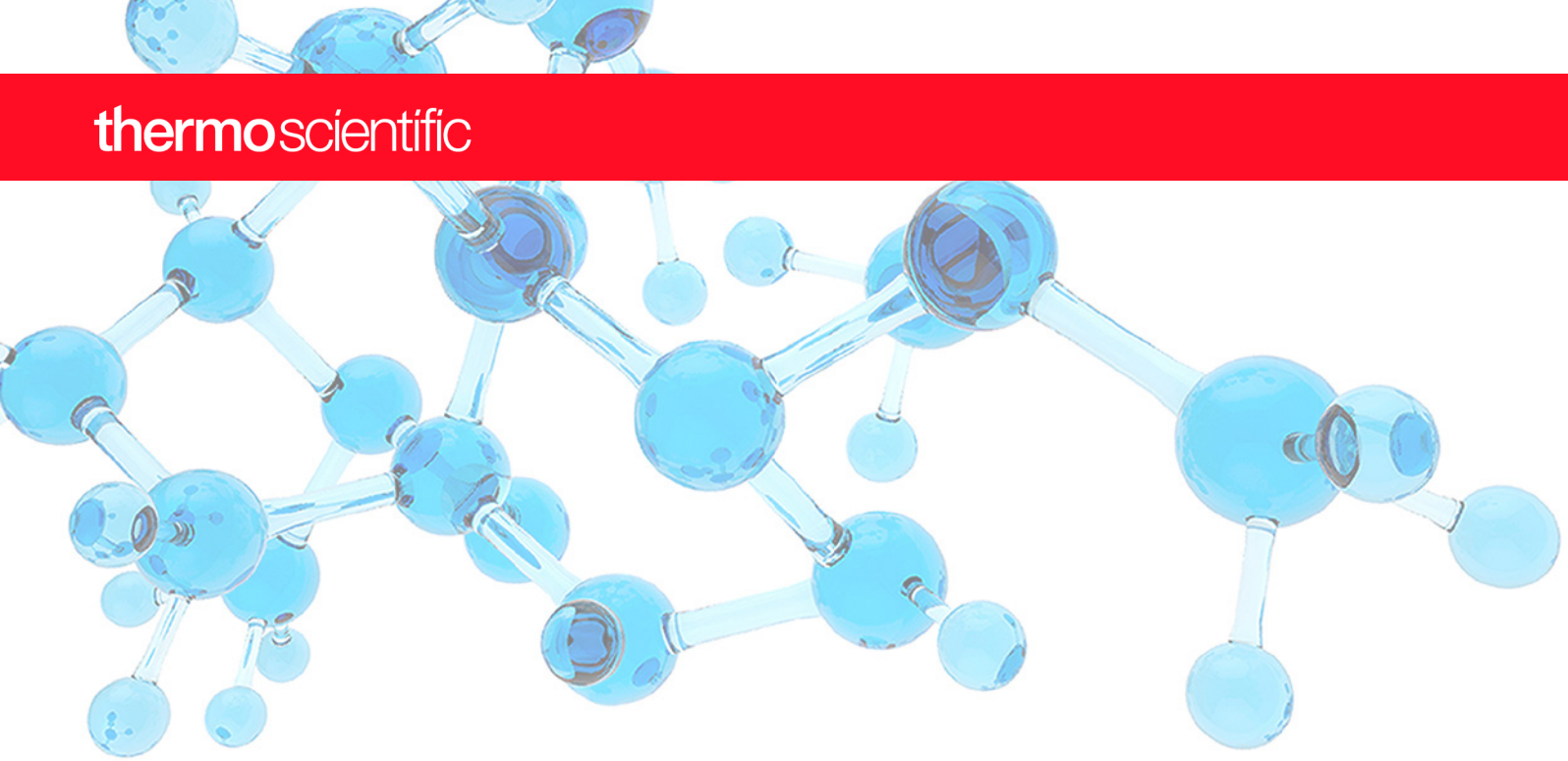
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TraceFinder

Lab Director User Guide

Software Version 5.0
Optimized for Environmental and Food Safety

XCALI-97859 Revision A • April 2019

DOCUMENTATION SURVEY

ThermoFisher SCIENTIFIC

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Contents

Preface . . . . . **vii**
Accessing Documentation vii
License Activation and Deactivation viii
Special Notices ix
Contacting Us x

Chapter 1 Using the Configuration Console **1**
Specifying Method Defaults 2
Specifying Acquisition Defaults 3
General Settings 3
Acquisition Submission Options 4
Multi-Channel LC 4
Intelligent Sequencing 5
Specifying Peak Detection Defaults 5
Specifying mzVault and NIST Libraries 21
Screening Libraries in a Quantitation Method 22
Screening Libraries in a Target Screening Method 22
Screening Libraries in an Unknown Screening Method 22
Specifying Adducts 22
Specifying Optional Settings 25
Delay Calibration 25
User Peak Detection Settings 25
EPA Tune Criteria 26
Qualitative Results 26
Qualitative Explorer 27
Processing Options 27
Compound Database Options 28
Configurability Access 28
Specifying the Report Selections 29
Specifying Workflow Selections 30
Specifying Application Views 30
Creating Custom Columns 31
Creating Custom Flags 32

Chapter 2 Using Compound Databases in the Method Development Mode 37
Using the Compound Database View for Small Molecules 38
Tree View Pane 39
Peak View Pane 42
Compound Details Pane for Small Molecules 46
Using the Compound Database View for Peptides 47
Tree View Pane 48
Peak View Pane 51
Compound Details Pane 56
Linking Internal Standards 58
Using the Peptide Predictor Wizard 59
Using the Peptide Modifications Editor 76
Importing and Exporting 81
Data Columns with Default Values 90

Chapter 3 Using Instrument Methods in the Method Development Mode 93

Chapter 4 Using the Method Development Mode for Quantitation Methods 97
Configuring Method Views 99
Creating a Master Method Workflow Template 100
Opening a Quantitation Method 101
Starting a New Quantitation Master Method 102
Selecting a Workflow Template 103
Starting a New Method with Method Forge 104
Importing an Xcalibur Master Method 113
Starting a Blank Method 115
Starting a Method Using the Compound Database 119
Editing a Quantitation Method 124
Modifying Retention Times 124
Editing the Acquisition Page 126
Editing the Processing Page 130
Editing the Compounds Page 137
Editing the QAQC Page 224
Editing the Groups Page 242
Editing the Intelligent Sequencing Page 244
Editing the Reports Page 248
Saving a Quantitation Method to a New Name 255
Creating a Method Template 256
Exporting Mass Data 271

Chapter 5 Using the Method Development Mode for Target Screening Methods 273
Opening a Target Screening Method 275
Starting a New Target Screening Method 277

Editing a Target Screening Method 279
Editing the Acquisition Page 279
Editing the Processing Page 282
Editing the Peak Detection Page 307
Editing the Reports Page 317
Saving a Target Screening Method to a New Name 318

Chapter 6 Using the Method Development Mode for Unknown Screening Methods . . . 321
Opening an Unknown Screening Method 323
Starting a New Unknown Screening Method 324
Editing an Unknown Screening Method 328
Editing the Acquisition Page 328
Editing the Processing Pages 332
Editing the Peak Detection Settings Page 356
Editing the Reports Page 367
Saving an Unknown Method to a New Name 369

Appendix A Isotopic Pattern Details 371
Isotopic Distribution in Exact Mass Spectra 371
Isotopic Pattern Score Calculations 375
Data Set Example 375
Calculating Mass and Intensity Deviations 377
Calculating Isotopic Pattern Score 378
Finding the Noise Value 383

Preface

This guide describes the configuration and method development tasks in the Thermo TraceFinder™ 5.0 application for a user with LabDirector or Supervisor permissions.

Contents

- Accessing Documentation
- License Activation and Deactivation
- Special Notices
- Contacting Us

❖ **To suggest changes to the documentation or to the Help**

Complete a brief survey about this document by clicking the button below. Thank you in advance for your help.



Accessing Documentation

The TraceFinder application includes complete documentation. For system requirements, refer to the Release Notes on the software DVD.

❖ **To view the TraceFinder manuals**

- From the Microsoft™ Windows™ taskbar, choose **Start > All Programs > Thermo TraceFinder > Manuals**.

  –or–

- From the application, choose **Help > Manuals**.

❖ **To view user documentation from the Thermo Fisher Scientific website**

1. Go to thermofisher.com.
2. Click the **Services & Support** tab.
3. On the right, click **Manuals & Protocols**.
4. In the Refine Your Search box, search by the product name.
5. From the results list, click the title to open the document in your web browser, save it, or print it.

   To return to the document list, click the browser **Back** button.

❖ **To view TraceFinder Help**

Open the TraceFinder application and choose **Help > TraceFinder Help**.

- To find a particular topic, use the Contents or Search panes.
- To create your own bookmarks, use the Favorites pane.

License Activation and Deactivation

Use the Thermo Scientific Product Licensing wizard to activate or deactivate the license for the TraceFinder application. To activate the license, you must have an activation code from Thermo Fisher Scientific. Before you transfer a license to another computer, deactivate the license.

When you first start the TraceFinder application, a dialog box displays the number of days remaining in your 120-day free evaluation license. If your evaluation license has expired, the License Activation wizard opens.

Note You can open the License Activation wizard at any time during your evaluation period by choosing Help > About TraceFinder and Licensing from the TraceFinder menu and then clicking Activate. If you already have a permanent license, a message tells you that your product is fully licensed.

Two types of licenses are available:

- 120-Day Evaluation Version (free of charge)
- Full Version License

The evaluation version is full-featured and automatically expires 120 days after activation. Any attempt to set back the system date automatically terminates this version. You can purchase and then activate the full version of the TraceFinder application during or after the free evaluation without reinstalling the software.

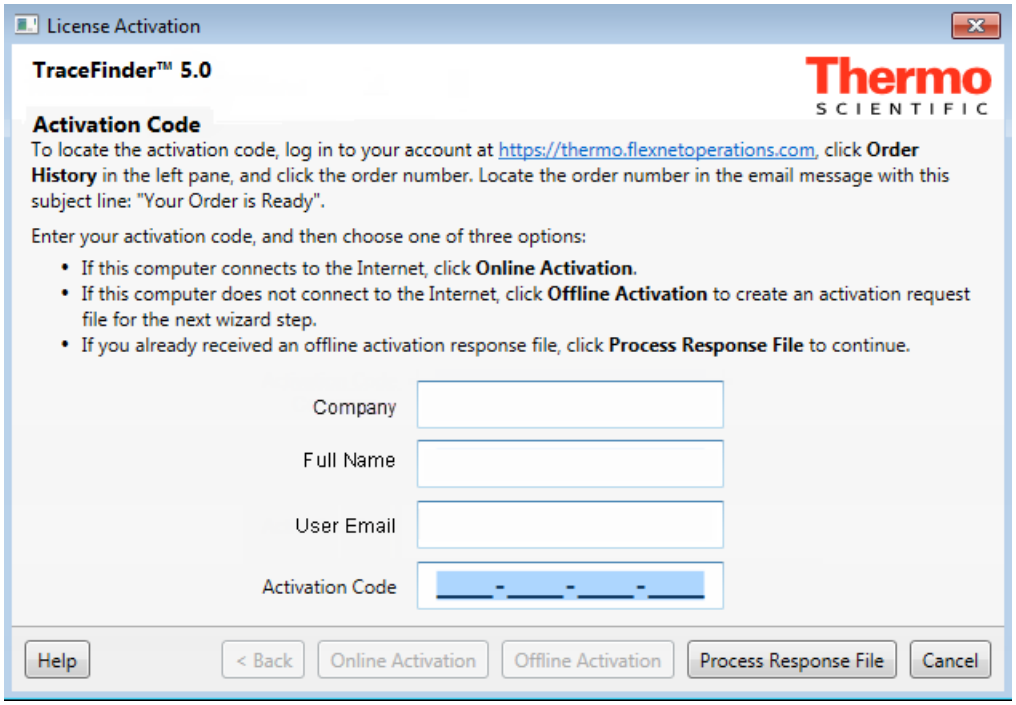
Each activation key is valid for two licenses. Any additional installation generates a different license and requires a different activation key. For software download and licensing questions, contact support at ThermoMSLicensing@thermo.com.

Use the License Activation wizard to activate or deactivate the license for the TraceFinder application. To activate the license, you must have an activation code from Thermo Fisher Scientific. You must deactivate the license before you transfer it to another computer.

❖ To start the license activation or deactivation process

1. Open the application.
2. Choose **Help > About TraceFinder and Licensing** to display the License Activation wizard.
3. Click **Activate (Deactivate)** to start the activation or deactivation process, as applicable.

   The License Activation wizard opens.



4. Follow the instructions in the License Activation wizard.

   For additional instructions, click **Help** in the wizard.

Special Notices

Make sure you follow the special notices presented in this guide. Special notices appear in boxes; those concerning safety or possible system damage also have corresponding caution symbols.

IMPORTANT Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.

Note Highlights information of general interest.

Tip Highlights helpful information that can make a task easier.

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[a] You can use your smartphone to scan a QR Code, which opens your email application or browser.

1

Using the Configuration Console

Use the features of the Configuration console to do any of the following:

- Activate features, such as multiplexing, intelligent sequencing, qualitative browsers, screening libraries, and compound database creation for small molecules and peptides.
- Select the reports that are available to users, the detector types, and the algorithms used for peak detection.
- Customize adduct definitions, additional sample grid columns, and flags.



Tip (Animation) To view "Configuration Console," choose **Help > Animations**.

Contents

- Specifying Method Defaults
- Specifying Acquisition Defaults
- Specifying Peak Detection Defaults
- Specifying mzVault and NIST Libraries
- Specifying Adducts
- Specifying Optional Settings
- Specifying the Report Selections
- Specifying Workflow Selections
- Specifying Application Views
- Creating Custom Columns
- Creating Custom Flags

Specifying Method Defaults

Use the Application – Method Defaults view of the Configuration console to specify the default laboratory and instrument names and the displayed mass precision.

Follow these procedures:

- To specify a default laboratory name and instrument name
- To specify the default mass precision

❖ To specify a default laboratory name and instrument name

1. Type the name of your laboratory in the Lab Name box.

   When you create a method, the application uses this default laboratory name for the Laboratory Name value on the Processing page of the Method View. The application uses this laboratory name in the report headings.

   The application does not apply this default laboratory name to previously created methods. By default, the laboratory name is Default Laboratory.

2. Type the name of your instrument in the Instrument Name box.

   When you create a batch, the application uses this default instrument name for the Instrument Name value. The application uses this instrument name in the report headings.

3. To save your changes, click **Apply.**

   The application does not apply this default instrument name to previously created batches. By default, the instrument name is Thermo Scientific Instrument.

❖ To specify the default mass precision

1. In the Display Mass Precision box, set the decimal value for the mass precision to an integer from **2** through **6**.

   The application uses this mass precision value to display mass values in the following locations:

   - Reports:
     - Blank Report
     - Confirmation Report (data spectra, library spectra, quantitation ion display, and qualitative ion display)
     - All High Density reports (*m/z* values)
     - Ion Ratio Failure Report (quantitation ion and qualitative ion)
     - Manual Integration Report (*m/z* value)
     - Quantitation Report (QIon)
   - All peaks on the Detection pages in the Method Development mode
   - The spectrum display in Data Review in the Analysis mode
   - The spectrum display in the Method Forge dialog box

IMPORTANT When you create a method using a raw data file, the application uses the filter precision value from the raw data file to create scan filters; however, it uses the Display Mass Precision value to show masses that are not embedded within filter strings and masses that are displayed on spectral plots.

2. To save your changes, click **Apply.**

Specifying Acquisition Defaults

Use the Application – Acquisition Defaults view to activate the following features:

- General Settings
- Acquisition Submission Options
- Multi-Channel LC
- Intelligent Sequencing

General Settings

Use the general settings to activate quick acquisition or automatic tray configuration for the autosampler.

Quick Acquisition

The quick acquisition option activates the Quick Acquisition feature in the Acquisition, Analysis, or Method Development mode.

❖ **To activate quick acquisition**

1. Select the **Quick Acquisition Allowed** check box.
2. To immediately apply this change, click **Apply**.

For a description of the Quick Acquisition features, refer to Appendix A, "Using Quick Acquisition" in the *TraceFinder User Guide*.

Automatic Autosampler Configuration

By default in the TraceFinder application, the autosampler automatically determines the tray configuration. When you use a Waters™ ACQUITY™ system, however, you must make this feature unavailable and manually specify the tray configuration when you create a batch.

❖ **To disallow automatic tray configuration**

1. Clear the **Allow Auto Sampler to Automatically Determine** … check box.
2. To immediately apply this feature change, click **Apply**.

Acquisition Submission Options

To control acquisitions, you can activate either submission option: full-sequence or single-sample. When you submit batches in Acquisition mode or submit Quick Acquisition batches in any mode, they run in first-in-last-out order—that is, the last batch submitted is the first batch to run. The only exception is when you submit a priority batch in Acquisition mode.

- For a Full Sequence Submission, priority batches always run immediately after the currently acquiring *batch* is completed.
- For a Single Sample Submission, priority batches always run immediately after the currently acquiring *sample* is completed.

❖ To specify acquisition submission features

1. Select either the **Full Sequence Submission** or the **Single Sample Submission** option:
   - Full Sequence Submission: Supports look-ahead features of the autosampler. When the instrument method specifies the look-ahead feature, the application functions like a multiplex driver and feeds the autosampler the next vial position.

     When you submit a batch, the autosampler begins preparing for all sample injections when the pre-run condition begins. All samples in the batch must be completed before other batches (even priority batches) can begin.

     Note The Full Sequence Submission feature is not available when you activate Intelligent Sequencing.

   - Single Sample Submission: Supports intelligent-sequencing features. When you submit a batch, the autosampler begins preparing for one sample injection at a time. Priority batches can interrupt the sample sequence in the currently acquiring batch.
2. To save your changes, click **Apply**.

   You must restart the application to apply this feature change.

Multi-Channel LC

The application uses multiplexing features in Acquisition mode when you specify channels for a sample in a batch. Refer to Chapter 3, “Using the Acquisition Mode” in the *TraceFinder User Guide*.

Note The Multi-Channel LC feature works only with LC/MS analytical instruments.

❖ To specify multiplexing features

1. Select the **Multi-Channel LC** check box.

   Note Multiplexing is not available when you activate Intelligent Sequencing.
2. Select the check box for each channel that you want to use for acquisition.

3. To immediately apply this feature change, click **Apply**.

Note When you activate multiplexing, the optional Single Sample Submission feature is not available.

Intelligent Sequencing

Use Intelligent Sequencing for single-sample submission. When you submit a batch, the autosampler begins preparing for one sample injection at a time. Higher priority batches can interrupt the sample sequence in the currently acquiring batch.

IMPORTANT The TraceFinder application does not perform Intelligent Sequencing during network acquisition.

❖ To activate the intelligent sequencing feature

1. Select the **Intelligent Sequencing** check box.

   Note Intelligent Sequencing is not available when you activate Processing Options.

   The Acquisition Submission Options default to Single Sample Submission. The Full Sequence Submission option is not available when you select the Intelligent Sequencing option.

2. To immediately apply this feature change, click **Apply**.

Specifying Peak Detection Defaults

Use the Application – Peak Detection Defaults view to specify a peak detection algorithm and its options and to determine the area under a curve. These parameters are available for quantitation methods only.

❖ To specify common detection parameters

1. In the Detector Type list, select a detector type.

   For detailed descriptions of the available detector types, see Common Peak Detection Parameters.

2. In the Mass Tolerance area, do the following:
   a. Select the unit of measure that you want to use (**MMU** or **PPM**).
   b. In the Value box, specify the number of millimass units or parts per million to use as the upper limit.

   The application applies this mass tolerance to the extracted chromatograms. The default is 500 MMU.

   Note For the Q Exactive™ mass spectrometer, set the Mass Tolerance to 5 PPM.

3. In the Retention Time area, do the following:
   a. In the Window box, specify the width of the window (in seconds) to indicate how far around the expected retention time the system will look for a peak apex.
   b. In the View Width box, specify the viewable size (in minutes) of the ion chromatogram display.
4. In the Ion Ratio Parameters area, do the following:
   a. In the Window Type list, select **Absolute** or **Relative** as the calculation approach for determining the acceptable ion ratio range.
   b. In the Window box, select the acceptable ion ratio range.
   c. In the Ion Coelution box, select the maximum difference in retention time between a confirming ion peak and the quantification ion peak.
5. In the Peak Detection Parameters area, select one of the detection algorithms: **Genesis**, **ICIS**, or **Avalon**.
6. Specify the parameters for the selected detection algorithm.

   For detailed parameter descriptions, see one of the following:

   - Auto Detection Method
   - Genesis Detection Method
   - ICIS Detection Method Configuration
   - Avalon Detection Method Configuration

Common Peak Detection Parameters

All of the detection algorithms use these parameters: Detector Type, Mass Tolerance, Retention Time, and Ion Ratio. When you create a new method from a compound database, the application inherits the peak detection parameters from the database.

Figure 1. Common peak detection areas

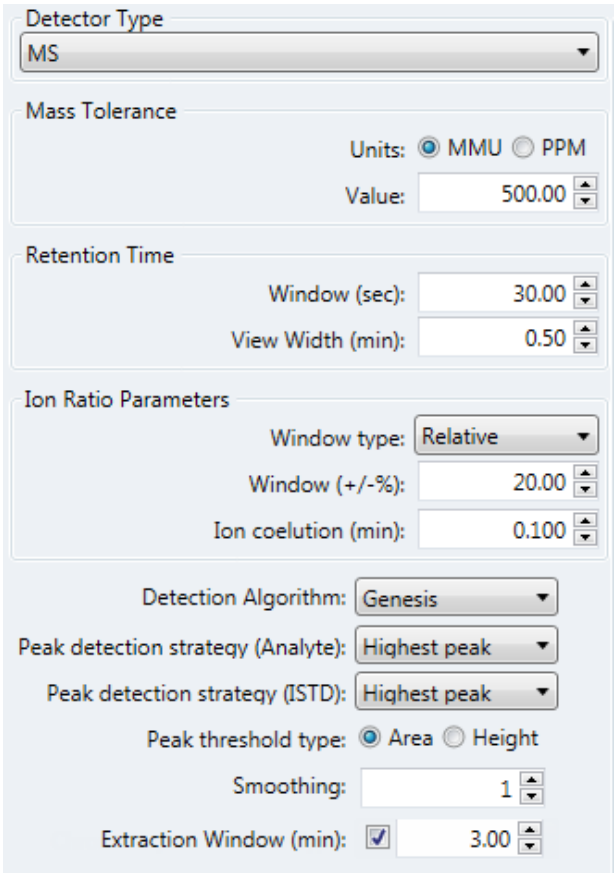


Table 1. Common peak detection parameters (Sheet 1 of 2)

Parameter	Description
Detector Type	MS: Mass spectrometer that ionizes sample molecules and then separates the ions according to their mass-to-charge ratio (*m/z*). PDA: Photodiode array detector providing a linear array of discrete photodiodes on an integrated circuit chip. It is placed at the image plane of a spectrometer so that a range of wavelengths can be simultaneously detected. Analog: Supplemental detectors (for example, FID, ECD). When you select this detector, any reports that display a QIon value show the value as **Analog** and any reports that display spectra show the spectra as **Not Available**. A/D card: If your detector is not under data system control, you can capture the analog signal and convert it to digital using an interface box (for example, SS420X) for storage in the raw data file. UV: A UV spectrophotometer (for variable-wavelength detection) or photometer (for single-wavelength detection) equipped with a low-volume flow cell. This detector detects analytes that readily absorb light at a selected wavelength.
Mass Tolerance	
Units	• (Default) MMU (millimass units) MMU is a static calculation to the extracted mass. • PPM (parts per million) PPM is a variable calculation dependent on the actual mass. The smaller the mass, the narrower the tolerance range. The larger the mass, the wider the tolerance range.
Value	Upper limit of MMU or PPM. Valid range: 0.1 through 50 000 Default: 500
Retention Time	
Window (sec)	Width of the window (in seconds) to indicate how far around the expected retention time the system will look for a peak apex.
View Width (min)	Viewable size (in minutes) of the ion chromatogram display. Changing the view width does not affect the process of peak detection; the application uses it only for graphical display.

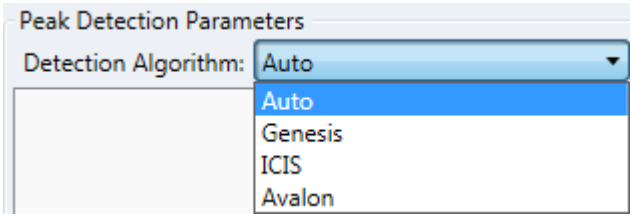
Table 1. Common peak detection parameters (Sheet 2 of 2)

Parameter	Description
Ion Ratio Parameters	
Window Type	Specifies the absolute or relative calculation approach for determining the acceptable ion ratio range.
Window (+/-%)	Specifies the acceptable ion ratio range.
Ion Coelution (min)	Specifies the maximum difference in retention time between a confirming ion peak and the quantification ion peak.
Detection Algorithm	Specifies the default peak detection algorithm. Valid values: Genesis, ICIS, Avalon
Peak Detection Strategy (Analyte)	Specifies the peak detection method used for analyte compounds. Highest Peak: Uses the highest peak in the chromatogram for component identification. Nearest RT: Uses the peak with the nearest retention time in the chromatogram for component identification.
Peak Detection Strategy (ISTD)	Specifies the peak detection method used for internal standard compounds. Highest Peak: Uses the highest peak in the chromatogram for component identification. Nearest RT: Uses the peak with the nearest retention time in the chromatogram for component identification.
Peak Threshold Type	Specifies whether the application identifies peaks by height or area.
Smoothing	Determines the degree of data smoothing to be performed on the active component peak prior to peak detection and integration. The ICIS peak detection algorithm uses this value. Valid values: Any odd integer from 1 through 15 points Default: 1
Extraction Window (min)	Specifies a window that limits how much of the entire trace the application processes. When cleared, the application processes the entire trace, which slows processing. Default: 3.00

Auto Detection Method

The application provides an automatic peak detection algorithm that uses hardcoded detection parameters, similar to an ICIS algorithm. Use the Auto detection algorithm to specify strategies for processing analyte and internal standard peaks. The method uses the default ICIS parameter settings with the following exceptions: the Smoothing parameter is set to 3, the Noise Method parameter is set to Repetitive, and the RMS option is selected. See ICIS Detection Method Configuration.

Figure 2. Auto peak detection



Genesis Detection Method

The application provides the Genesis peak detection algorithm for backward compatibility with Xcalibur™ 1.0 studies.

Figure 3. Genesis peak detection

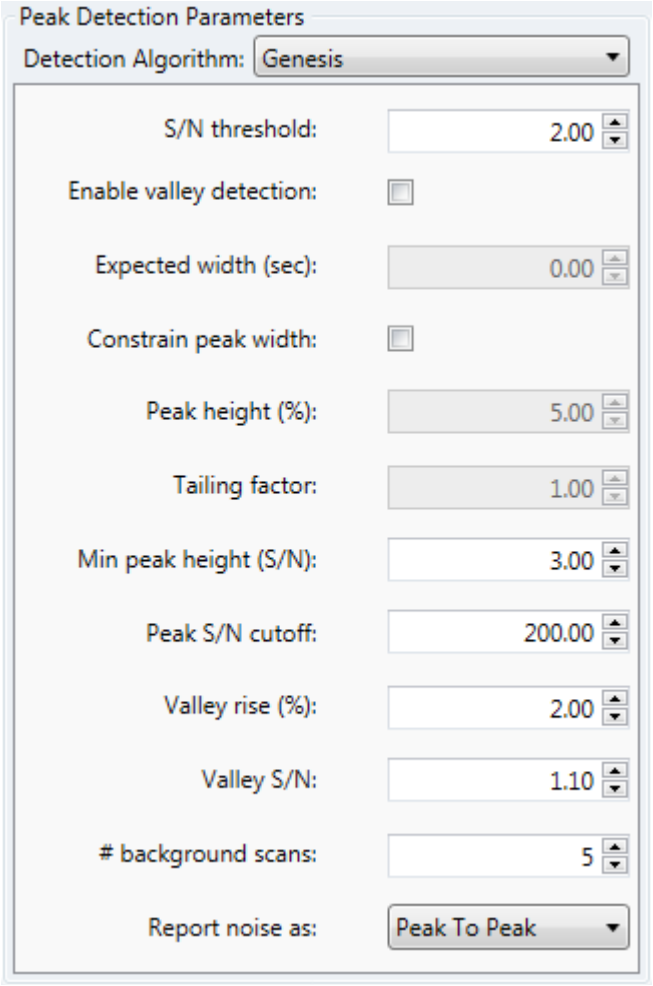


Table 2. Genesis peak detection parameters (Sheet 1 of 3)

Parameter	Description
Detection Algorithm	Specifies the Genesis peak detection algorithm.
S/N Threshold	Specifies the current signal-to-noise threshold for peak integration. Peaks with signal-to-noise values less than this value are not integrated. Peaks with signal-to-noise values greater than this value are integrated. Valid range: 0.0 through 999.0

Table 2. Genesis peak detection parameters (Sheet 2 of 3)

Parameter	Description
Enable Valley Detection	Uses the valley detection approximation method to detect unresolved peaks. This method drops a vertical line from the apex of the valley between unresolved peaks to the baseline. The intersection of the vertical line and the baseline defines the end of the first peak and the beginning of the second peak.
Expected Width (sec)	Specifies the expected peak width parameter (in seconds). This parameter controls the minimum width that a peak is expected to have if valley detection is enabled. With valley detection enabled, any valley points nearer than the *expected width*/2 to the top of the peak are ignored. If a valley point is found outside the expected peak width, the application terminates the peak at that point. The application always terminates a peak when the signal reaches the baseline, independent of the value set for the expected peak width. Valid range: 0.0 through 999.0
Constrain Peak Width	Constrains the peak width of a component during peak integration of a chromatogram. You can then set values that control when peak integration is turned on and off by specifying a threshold and a tailing factor. Selecting the Constrain Peak Width check box activates the Peak Height (%) and Tailing Factor options.
Peak Height (%)	A signal must be above the baseline percentage of the total peak height (100%) before integration is turned on or off. This text box is active only when you select the Constrain Peak Width check box. Valid range: 0.0 through 100.0%
Tailing Factor	Specifies the tailing factor that controls how the application integrates the tail of a peak. This factor is the maximum ratio of the trailing edge to the leading side of a constrained peak. This text box is active only when you select the Constrain the Peak Width check box. Valid range: 0.5 through 9.0
Peak S/N Cutoff	Sets the peak edge to values below this signal-to-noise ratio. This test assumes it has found an edge of a peak when the baseline adjusted height of the edge is less than the ratio of the baseline adjusted apex height and the peak S/N cutoff ratio. When the S/N at the apex is 500 and the peak S/N cutoff value is 200, the application defines the right and left edges of the peak when the S/N reaches a value less than 200. Valid range: 50.0 through 10000.0

Table 2. Genesis peak detection parameters (Sheet 3 of 3)

Parameter	Description
Valley Rise (%)	Specifies that the peak trace can rise above the baseline by this percentage after passing through a minimum (before or after the peak). This criteria is useful for integrating peaks with long tails. This method drops a vertical line from the apex of the valley between unresolved peaks to the baseline. The intersection of the vertical line and the baseline defines the end of the first peak and the beginning of the second peak. When the trace exceeds rise percentage, the application applies valley detection peak integration criteria. This test is applied to both the left and right edges of the peak. Valid range: 0.1 through 500.0
Valley S/N	Specifies a value to evaluate the valley bottom. Using this parameter ensures that the surrounding measurements are higher. Valid range: 1.0 through 100.0 Default: 2.0
# Background Scans	Specifies the number of background scans performed by the application.
Report Noise As	Determines if the noise used in calculating S/N values is calculated using an RMS calculation or peak-to-peak resolution threshold. Options are RMS or Peak To Peak.

ICIS Detection Method Configuration

The ICIS peak detection algorithm is designed for MS data and has superior peak detection efficiency at low MS signal levels.

Figure 4. ICIS peak detection

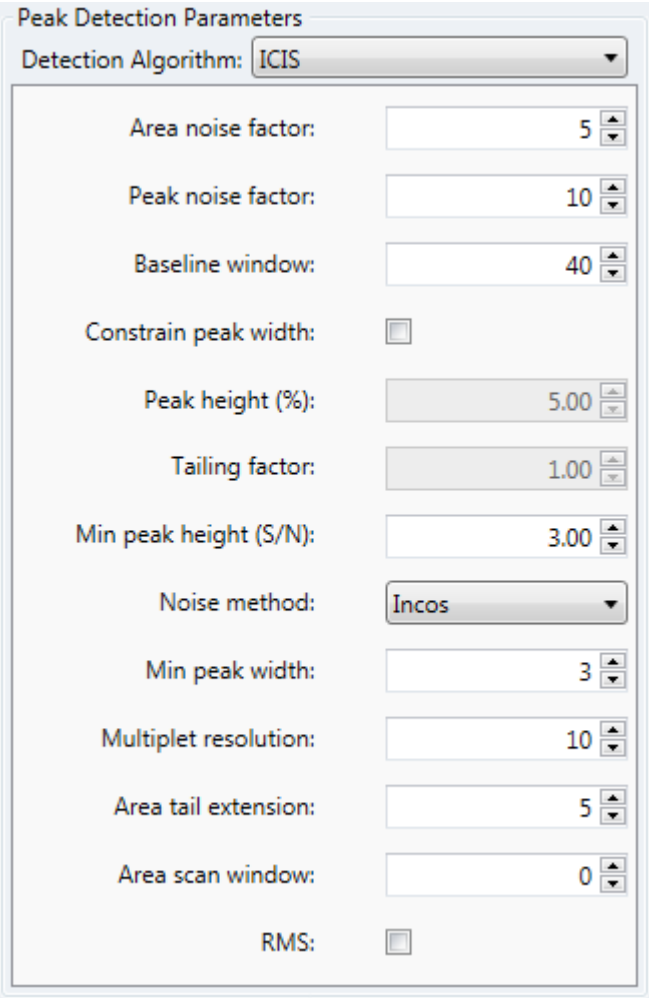


Table 3. ICIS peak detection parameters (Sheet 1 of 3)

Parameter	Description
Detection Algorithm	Specifies the ICIS peak detection algorithm.
Area Noise Factor	Specifies the noise level multiplier used to determine the peak edge after the location of the possible peak. The ICIS peak detection algorithm uses this value. Valid range: 1 through 500 Default: 5

Table 3. ICIS peak detection parameters (Sheet 2 of 3)

Parameter	Description
Peak Noise Factor	Specifies the noise level multiplier used to determine the potential peak signal threshold. The ICIS peak detection algorithm uses this value. Valid range: 1 through 1000 Default: 10
Baseline Window	Specifies that the application looks for a local minima over this number of scans. The ICIS peak detection algorithm uses this value. Valid range: 1 through 500 Default: 40
Constrain Peak Width	Constrains the peak width of a component during peak integration of a chromatogram. You can then set values that control when peak integration is turned on and off by specifying a peak height threshold and a tailing factor. Selecting the Constrain Peak Width check box activates the Peak Height (%) and Tailing Factor options.
Peak Height (%)	Specifies that the signal must be above the baseline percentage of the total peak height (100%) before integration is turned on or off. This text box is active only when you select the Constrain Peak Width check box. Valid range: 0.0 through 100.0%
Tailing Factor	Specifies the tailing factor that controls how the application integrates the tail of a peak. This factor is the maximum ratio of the trailing edge to the leading side of a constrained peak. This text box is active only when you select the Constrain the Peak Width check box. Valid range: 0.5 through 9.0
Min Peak Height (S/N)	Specifies the minimum peak height measured as a signal-to-noise ratio. Valid range: 0.00 through 999.00 Default: 3.0
Noise Method	Specifies the noise method as INCOS or Repetitive. INCOS: Uses a single pass algorithm to determine the noise level. The ICIS peak detection algorithm uses this value. Repetitive: Uses a multiple pass algorithm to determine the noise level. The ICIS peak detection algorithm uses this value. In general, this algorithm is more accurate in analyzing the noise than the INCOS Noise algorithm, but the analysis takes longer.
Min Peak Width	Specifies the minimum number of scans required in a peak. The ICIS peak detection algorithm uses this value. Valid range: 0 through 100 scans Default: 3

Table 3. ICIS peak detection parameters (Sheet 3 of 3)

Parameter	Description
Multiplet Resolution	Specifies the minimum separation in scans between the apexes of two potential peaks. This is a criteria to determine if two peaks are resolved. The ICIS peak detection algorithm uses this value. Valid range: 1 through 500 scans Default: 10
Area Tail Extension	Specifies the number of scans past the peak endpoint to use in averaging the intensity. The ICIS peak detection algorithm uses this value. Valid range: 0 through 100 scans Default: 5
Area Scan Window	Specifies the number of allowable scans on each side of the peak apex. A zero value defines all scans (peak-start to peak-end) to be included in the area integration. Valid range: 0 through 100 scans Default: 0
RMS	Specifies that the application calculates noise as RMS. By default, the application uses Peak To Peak for the noise calculation. RMS is automatically selected if you manually determine the noise region.

Avalon Detection Method Configuration

The Avalon peak detection algorithm is designed for UV data. The Avalon peak detection algorithm also supports negative peaks. You can edit the Event values from the Avalon Event List.

Figure 5. Avalon peak detection

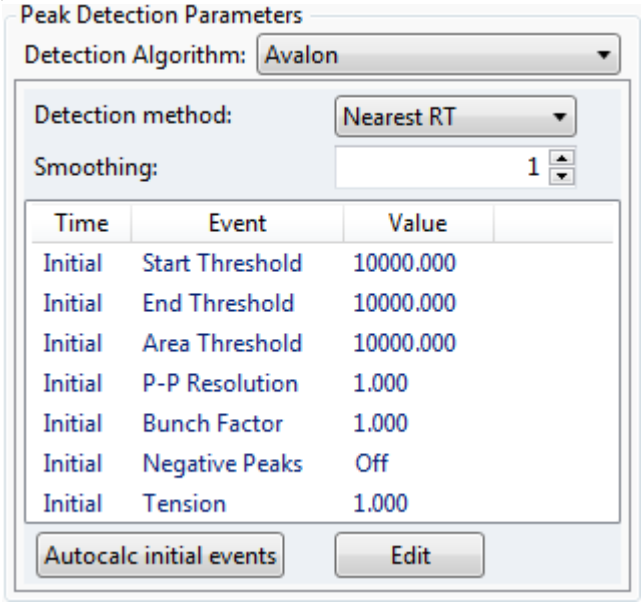


Table 4. Avalon peak detection parameters

Parameter	Description
Detection Algorithm	Specifies the Avalon peak detection algorithm.
Time/Event/Value	Displays the events specified in the Avalon Event List dialog box. Initially displays only the default events that cannot be edited or deleted.
Autocalc Initial Events	Automatically calculates the events in the Event list.
Edit	Opens the Avalon Event List dialog box where you can edit the Time/Event/Value parameters.

Avalon Event List

The event list includes both user-defined and noneditable default events. The application displays the default events when you choose Avalon sensitivity. You cannot delete these events or change their time or values. For a detailed list of events and value ranges, see Event types.

Figure 6. Avalon Event List dialog box

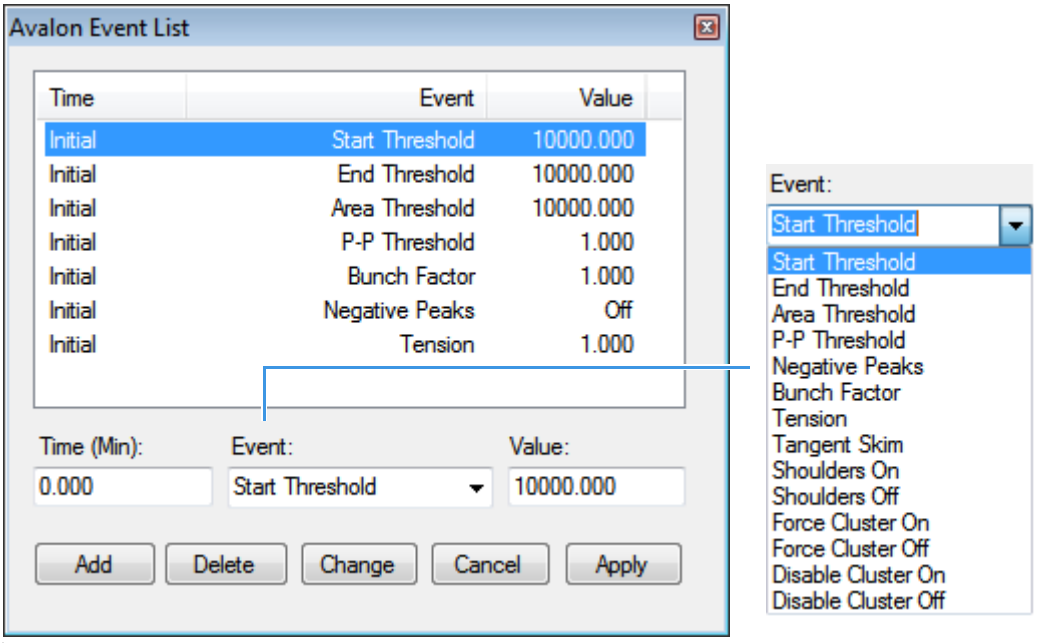


Table 5. Avalon Event List dialog box parameters

Parameter	Description
Time (Min)	Specifies the start time of the event.
Event	Specifies the type of event. For a detailed list of events and value ranges, see Event types.
Value	Specifies the value of the event.
Add	Adds a new event to the list with the current Time/Event/Value parameters.
Delete	Removes the selected Time/Event/Value parameter from the event list.
Change	Applies the current parameter values.
Cancel	Closes the dialog box without making any changes. Any additions, deletions, or changes revert to their original state.
Apply	Closes the dialog box.

Figure 7. Event types

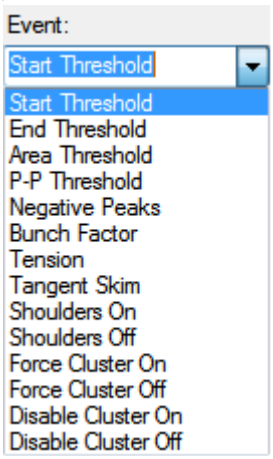


Table 6. Event type descriptions (Sheet 1 of 2)

Event type	Description
Start Threshold	Specifies the threshold at the start of a peak. The Start Threshold is directly related to the RMS noise in the chromatogram. Valid range: 0 through 999 999 999
End Threshold	Specifies the threshold at the end of a peak. The End Threshold is directly related to the RMS noise in the chromatogram. Valid range: 0 through 999 999 999
Area Threshold	Controls the area cutoff. Any peaks with a final area less than the area threshold will not be detected. This control is in units of area for the data. Valid range: 0 through 999 999 999
P-P Threshold	Specifies the peak-to-peak resolution threshold controls how much peak overlap must be present before two or more adjacent peaks create a peak cluster. Peak clusters have a baseline drop instead of valley-to-valley baselines. Specified as a percent of peak height overlap. Valid range: 0.1 through 99.99
Negative Peaks	Permits detection of a negative going peak. Automatically resets after finding a negative peak. Valid values: On or Off

Table 6. Event type descriptions (Sheet 2 of 2)

Event type	Description
Bunch Factor	Specifies the number of points grouped together during peak detection. This event controls the bunching of chromatographic points during integration and does not affect the final area calculation of the peak. A high bunch factor groups peaks into clusters. Valid range: 0 through 999
Tension	Controls how closely the baseline should follow the overall shape of the chromatogram. A lower tension traces the baseline to more closely follow changes in the chromatogram. A high baseline tension follows the baseline less closely, over longer time intervals. Valid range: 0 through 999.99 minutes
Tangent Skim	Specifies that you can tangent skim any peak clusters. By default, it chooses the tallest peak in a cluster as the parent. You can also identify which peak in the cluster is the parent. Tangent skim peaks are detected on either side (or both sides) of the parent peak. Tangent skim automatically resets at the end of the peak cluster. Valid range: 0 through 1
Shoulders On	Allows peak shoulders to be detected (peaks which are separated by an inflection rather than a valley) Sets a threshold for the derivative.
Shoulders Off	Disables peak shoulder detection. Valid range: 0 through 50
Force Cluster On	Forces the following peaks to be treated as a cluster (single peak).
Force Cluster Off	Ends the forced clustering of peaks.
Disable Cluster On	Prevents any peaks from being clustered.
Disable Cluster Off	Permits clusters to occur again.

Specifying mzVault and NIST Libraries

Use the specified screening libraries for both quantitation methods and target screening methods. For more information about the two ways you can use screening libraries, see Screening Libraries in a Quantitation Method and Screening Libraries in a Target Screening Method.

For a target screening method, when you specify the Library Search Type on the Processing page, select from these search types: mzVault™, NIST™, or NIST High Resolution. See Editing the Acquisition Page.

- mzVault search type: For a quantitation or target screening method, the application uses the mzVault library file (.db) specified in the Configuration console. You can search only one spectral library when you process a sample.
- NIST or NIST High Resolution search type: For the method, the application uses the NIST libraries specified in the Configuration console. You can choose to search multiple NIST libraries when you process a sample.

❖ To specify an mzVault screening library

1. Click **Browse** and locate the library that you want to use for screening.

   Note You can use only one search type when you process a sample. When you select NIST or NIST High Resolution as the library search type in your method, the application does not use the screening library that you specify here.

2. To immediately apply this feature change, click **Apply**.

❖ To specify NIST screening libraries

1. Click **Select**.

   The Select NIST Libraries dialog box opens, listing the libraries you installed for the application.

2. Select the check box for each NIST library that you want to use for screening and click **OK**.

   Note You can use only one search type when you process a sample. When you select mzVault as the library search type in your method, the application does not use the NIST libraries that you specify here.

3. To immediately apply this feature change, click **Apply**.

The application searches the specified screening library to identify or confirm a sample compound, matches the fragment ion spectrum in the library to the compound's ion spectrum, and returns the highest score (best match).

The application performs either a forward library search or a reverse library search. A forward search compares the mass spectrum of an unknown compound to a mass spectral library entry, whereas a reverse search compares a library entry to an unknown compound.

Screening Libraries in a Quantitation Method

In a quantitation method, you can enable library matching and set a score threshold to minimize poor matches. See the mzVault page. To match a compound, the resulting score from a library search must be higher than the specified threshold value.

Screening Libraries in a Target Screening Method

In a target screening method, you can specify the library search to either identify or confirm library matches and set a score threshold to minimize poor matches. See Editing the Processing Page.

- Identify or Confirm: The application identifies or confirms the sample compound. It searches the specified search library and returns the highest score (as a percentage value) for the fragment ion spectrum in that library that matches the compound's ion spectrum.
- Score Threshold: To identify or confirm the presence of a compound, the resulting score from a library search must be higher than the specified threshold value.

IMPORTANT To use a library search for identification or confirmation, these conditions must be met:

- The raw data file must contain higher energy collision-induced dissociation (HCD), source collision-induced dissociation (CID), or all ions fragmentation (AIF) ion spectra.
- The spectra must exist at a time point within the compound's elution time range.

Screening Libraries in an Unknown Screening Method

Unknown screening methods do not use screening libraries.

Specifying Adducts

An adduct ion is formed from a precursor ion and contains all of the constituent atoms of that ion and additional atoms or molecules. Adduct ions are often formed in the mass spectrometer ion source. Adducts can be either positive or negative.

Use the Application – Adducts view to specify the adducts that will be available for you to use in method development.

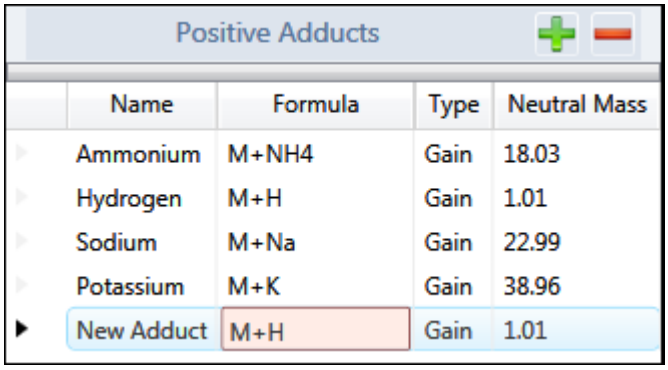
Follow these procedures:

- To add an adduct
- To remove an adduct

❖ To add an adduct

1. In the Positive Adducts or Negative Adducts pane, click the **Add New Adduct** icon,  .

   The application adds a new, editable row at the bottom of the Adducts list.



2. Type the formula for the new adduct ion.

   The formula syntax is alphanumeric and case sensitive. It can include parentheses and brackets.

   The formula specifies the difference between the neutral molecule and the charged ion that you expect to see in the results.

   For example, a sodium adduct has [M+Na]+ as the expected charged ion (where M is the neutral molecule), so you would type "Na" for the formula. A water adduct has [M+H+H2O]+ as the expected charged ion, so you would type "H3O" for the formula.

   IMPORTANT When you create an adduct formula, you can type both uppercase and lowercase letters; however, the application interprets all uppercase input as single-letter elements and all lowercase input as two-letter elements.

   For example, it interprets the string "inau" as In Au and "COSI" as C O S I.

   The application displays a type and neutral mass for the adduct formula you entered.

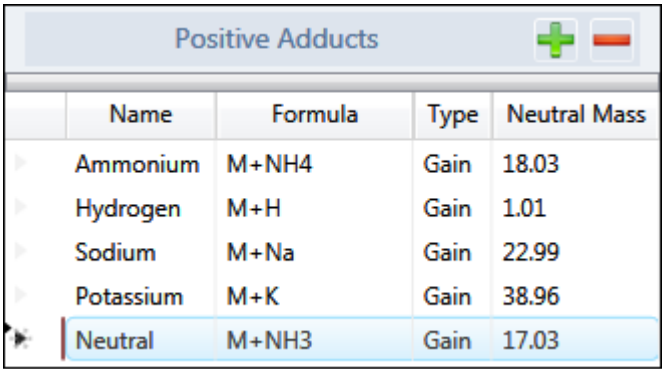


3. Select the default name (New Adduct) and type a name for the adduct.

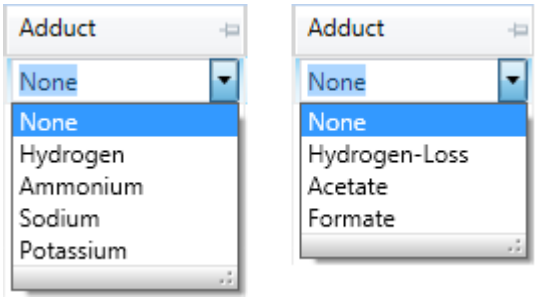
   You cannot change the type or neutral mass, but the application will correctly calculate these values later.

4. Press ENTER.

   The application adds the adduct to the adducts list and calculates the correct type (Gain or Loss) and the neutral mass.



   These adducts are available for you to select in the Compound Database view of the Method Development mode when you specify parameter values for compounds.



5. To immediately apply this feature change, click **Apply.**

❖ To remove an adduct

1. In the Positive Adducts or Negative Adducts pane, select the adduct that you want to remove.
2. Click the **Delete Adduct** icon,  .

   You can delete only adducts that you added to the adducts list. You cannot delete default adducts defined by the TraceFinder installation.

3. To immediately apply this feature change, click **Apply.**

Specifying Optional Settings

Use the Application – Optional Settings view to activate the following features:

- Delay Calibration
- User Peak Detection Settings
- EPA Tune Criteria
- Qualitative Results
- Qualitative Explorer
- Processing Options
- Compound Database Options
- Configurability Access

Delay Calibration

Use the Delay Calibration option to determine when the application calculates the calibration curve. Processing is faster but less responsive with this option. The application waits until processing of the last sample in a batch is completed (instead of after each sample) before it recalibrates the curve.

❖ To delay calculation of a calibration curve

1. Select the **Delay Calibration** check box.
2. To immediately apply this feature change, click **Apply**.

User Peak Detection Settings

Use the User Peak Detection Settings Allowed option to modify the method integration settings for specific compounds in Data Review.

❖ To enable the modify peak detection settings

1. Select the **User Peak Detection Settings Allowed** check box.
2. To immediately apply this feature change, click **Apply**.

EPA Tune Criteria

When you select the EPA Tune Criteria option, the application supports Tune, Breakdown, and Tune/Breakdown sample types and MSTune and Breakdown compound types. This option changes the Breakdown method to a Tune/Breakdown method on the Acquisition page of the Method View and adds the Tune tab to the QAQC page of the Method View.

❖ To enable the tune criteria

1. Make sure the **EPA Tune Criteria** check box is selected.

   The EPA Tune Criteria option is set as the default.

2. Click **Apply** and at the prompt, click **OK.**

For a description of the Tune/Breakdown method on the Acquisition page of the Method View, do one of the following:

- For quantitation methods, see Editing a Quantitation Method (Chapter 5).
- For target screening methods, see Editing a Target Screening Method (Chapter 6).
- For unknown screening methods, see Editing an Unknown Screening Method (Chapter 7).

For a description of the Tune page on the QAQC page of the Method View for a quantitation method, see Editing the QAQC Page.

Qualitative Results

When you select the Enable Qualitative Results Display and Processing option, the application displays the following qualitative processing features for quantitation methods and batches:

- Method Template Editor

  To specify qualitative settings in a method template, see To specify qualitative peak processing.

- Master method

  To specify qualitative settings on the Processing page for a method, see To specify qualitative peak processing parameters.

- Batch View

  To enable qualitative processing for a batch in Data Review, refer to Chapter 4, “Using the Analysis Mode for Quantitation Batches” in the *TraceFinder User Guide*.

- Data Review

  For descriptions of the Qualitative View features in Data Review, refer to Chapter 4, "Using the Analysis Mode for Quantitation Batches" in the *TraceFinder User Guide*.

- Acquisition wizard

  To enable qualitative processing for a batch in the acquisition wizard, refer to Chapter 3, "Using the Acquisition Mode" in the *TraceFinder User Guide*.

For GC configurations, the qualitative processing option is enabled by default.

For LC configurations, the qualitative processing option is not enabled by default.

Qualitative Explorer

You can use either the FreeStyle™ application or Qual Browser to display chromatograms and spectra, detect chromatogram peaks, search libraries, simulate spectra, subtract background spectra, apply filters, add text and graphics, create and save layouts, and view instrument parameters as they changed during the acquisition.

❖ To specify a qualitative explorer

Select either the **Thermo FreeStyle** or the **Thermo Xcalibur Qual Browser** option.

Note You can access the explorer by choosing Tools > Launch Qual Explorer in the main TraceFinder menu bar. Refer to Chapter 2, "Getting Started" in the *TraceFinder User Guide*.

Processing Options

Use these options to enable automatic batch processing or to allow unknown screening.

❖ To allow auto-reprocessing

1. Select the **Auto Reprocess** check box.

   The application automatically reprocesses a batch and displays the updated results in Data Review when you change the method or change the manual integration.

   When you clear this option, the application does not automatically reprocess batches or update the results in Data Review when you change the method or change the manual integration. It simply stores the changes to the method or the manual integration. It updates the Data Review results only when you manually reprocess the batch.

2. To immediately apply this feature change, click **Apply**.

Note Using auto-reprocessing can slow batch processing.

❖ **To allow unknown screening**

1. Select the **Allow Unknown Screening** check box.

   The application displays Unknown Screening features.

   When you clear this option, Unknown Screening features are not displayed in the application.

2. To immediately apply this feature change, click **Apply**.

Compound Database Options

Use these options to enable peptide compound databases or small molecule compound databases.

❖ **To allow users to create peptide compound databases**

1. Select the **Allow Peptide Compound Database Creation** check box.

   The New Peptide Compound Database command is enabled in the File menu of the Compound Database view in Method Development mode.

   When you clear this option, the New Peptide Compound Database command is not available in the File menu of the Compound Database view in Method Development mode; however, in the Compound Database menu, you can still access the Peptide Prediction Tool.

2. To immediately apply this feature change, click **Apply**.

❖ **To allow users to create small molecule compound databases**

1. Select the **Allow Small Molecule Compound Database Creation** check box.

   The New Small Molecule Compound Database command is enabled in the File menu of the Compound Database view in Method Development mode.

   When you clear this option, the New Small Molecule Compound Database command is not available in the File menu of the Compound Database view in Method Development mode.

2. To immediately apply this feature change, click **Apply**.

Configurability Access

Use this option to enable the commands so that you can configure the display of views in the application.

❖ **To allow users to configure the visibility of views**

1. Select the **Ability to Configure Visibility of Method and Application Views** check box.

2. To immediately apply this feature change, click **Apply**.

   The application adds the following:

   - Configuration icons ( ) to the Compounds and QAQC views for quantitation methods
   - A Configuration icon ( ) to the Reports views for all method types
   - The following commands to the Tools menu:
     - Configure Application Views—in all modes for all method types

       You can use this tool to control the views that are displayed in the application. Refer to Chapter 2, "Getting Started" in the *TraceFinder User Guide*.
     - Configure Method Views—in the Method Development mode for quantitation methods

       You can use this tool to control the pages of data that are displayed in the Method View. See Chapter 4, "Using the Method Development Mode for Quantitation Methods."

Specifying the Report Selections

❖ **To specify which reports are available**

Note The application displays the Excel template files in the following folder: …\TraceFinderData\Templates\ReportTemplates

Each template has an XLS file and a metadata file to support report generation.

Select the check box for each report that you want to make available.

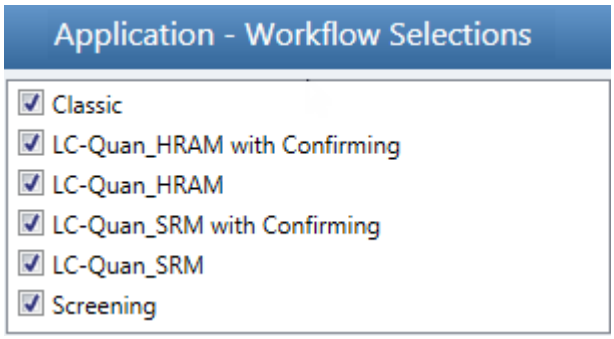
- To return the report selections to their original state (when you first opened this view), click **Undo**.
- To save your changes, click **Apply.**

The application makes your report settings immediately available.

Specifying Workflow Selections

The Workflow Selections list includes the default workflows provided with the TraceFinder installation and any additional workflows you have created. See Creating a Master Method Workflow Template.

The workflows selected here are available in Method Development mode from the Create Master Method dialog box. See Starting a New Quantitation Master Method, Starting a New Target Screening Method, or Starting a New Unknown Screening Method.



Specifying Application Views

Use the Application Views page to choose which modes (Acquisition, Analysis, or Method Development), or views within the modes, are displayed in the TraceFinder application.

❖ To specify which modes or views are available

1. Select the check box for each mode or view that you want to display.
2. Click **Apply**.

   The application immediately applies the view configurations you specified.

   Note Ensure that at least one mode and one view in that mode are available before you apply your changes.

3. When you finish specifying the modes and views that you want to display, click **Close**.

Creating Custom Columns

Use the Custom Columns page to add six additional columns to the samples list in batches. The application treats these custom columns the same as other columns when you export data to a Microsoft Excel™ spreadsheet or to a CSV file.

You can use the Modify Columns dialog box to display and change the order of these columns in the sample list (refer to the Column Display topic in the appropriate Analysis chapter in the *TraceFinder User Guide* for your quantitation, target screening, or unknown screening batch).

You can use the Field Chooser to display and change the order of these columns in the Data Review Samples pane (refer to the Samples Pane topic in the appropriate Analysis chapter in the *TraceFinder User Guide* for your quantitation, target screening, or unknown screening batch).

You can use the information in these columns, for example, for temperature control when you use Aria™ MX for multiplexing. You can use the column information for injector and multiple column module ports when you use the TurboFlow™ method with the Prelude™ or TLX data systems.

Follow these procedures:

- To add custom columns to new batches
- To create new batches without custom columns
- To control the display of custom columns

❖ To add custom columns to new batches

1. Select the **Enable Custom Columns** check box.

   The application adds six additional columns to the samples list in all new batches that you create.

   The Enable Custom Columns check box controls both the creation of custom columns on new batches and the display of custom columns in the Modify Columns dialog box in the Batch View and the Field Chooser in the Data Review Samples pane.

2. For each custom column, select the default column name and type your custom name.
3. Click **Apply**.

   The application adds the six custom columns to all new batches that you create.

Note Only new batches include these custom columns. The application does not add custom columns to previously created batches.

Note If you return to this page and change the custom column names, the application uses the new names only for future batches.

❖ **To create new batches without custom columns**

Clear the **Enable Custom Columns** check box and click **Apply**.

When you create new batches, they will not include custom columns, and the application hides the display of the custom columns for any previous batches that you created with custom columns enabled.

❖ **To control the display of custom columns**

- To make custom columns available for all batches, select the **Enable Custom Columns** check box and click **Apply**.
- To make custom columns not available for batches, clear the **Enable Custom Columns** check box and click **Apply.**

Creating Custom Flags

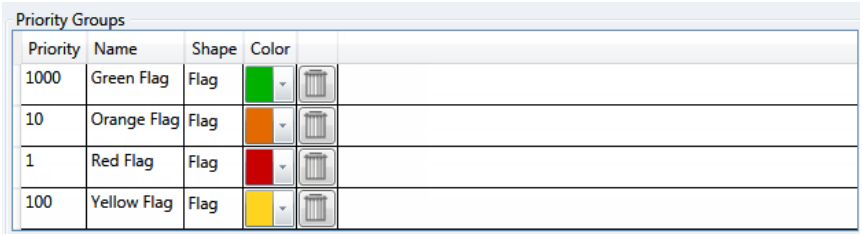
Use the Flag Customization view to customize error flags and conditions that indicate compound errors in Data Review for quantitation batches. You can edit the priority assigned to an error condition (flag rule) and the shape and color of the icon used to indicate the error. You can also delete an error condition or create a new one.

Follow these procedures:

- To edit priority groups
- To create a new priority group
- To edit flag rules
- To create a new flag rule
- To remove all customization

In the Priority Groups area, you can edit the priority, shape, or color of a flag. You can also delete a flag or create a new one. You cannot change the name of a flag.

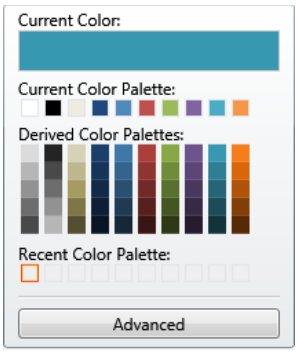
❖ **To edit priority groups**



1. Do any of the following:
   - Select the default Priority value and type a new value.

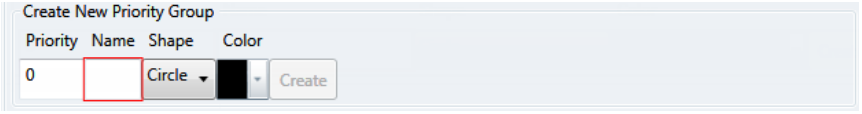
     A priority of 1 is the highest priority. The higher the Priority number, the lower the priority.

- Double-click the Shape value and select a new shape from the list.
- Open the Color list and select a new color from the color palette.



2. When you complete all your changes, click **Apply**.

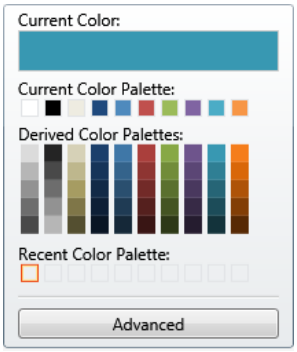
❖ To create a new priority group



1. In the Priority box, type a value.

   You can enter positive or negative numbers. The lower the number, the higher the priority.

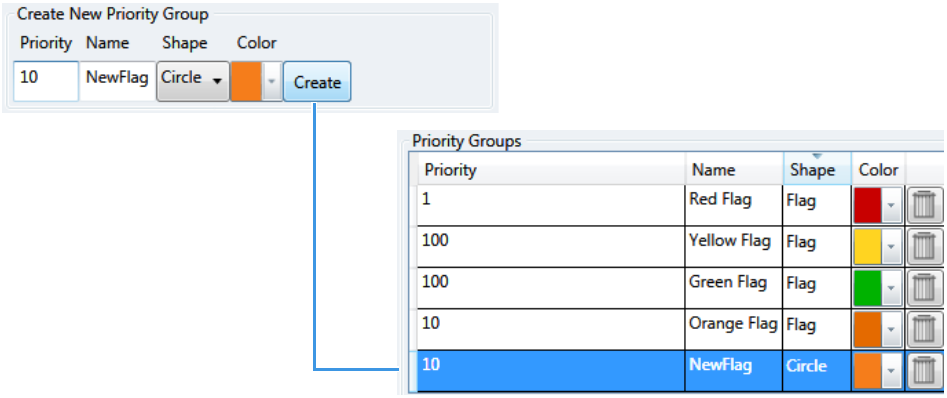
2. In the Name box, type a name for the new priority group.
3. Select a flag shape from the Shape list: **Circle**, **Square**, or **Flag**.
4. Open the Color list and select a color from the color palette.



5. (Optional) Click **Advanced** and select a color based on RGB, HSL, or CMYK color palettes.

6. Click **Create**.

   The application adds the new flag to the Priority Groups list, as in this example:



❖ To edit flag rules

Note You can edit the description and priority group for a flag rule, and you can delete a rule. You cannot edit the name or flag type for a rule.

1. Do any of the following:

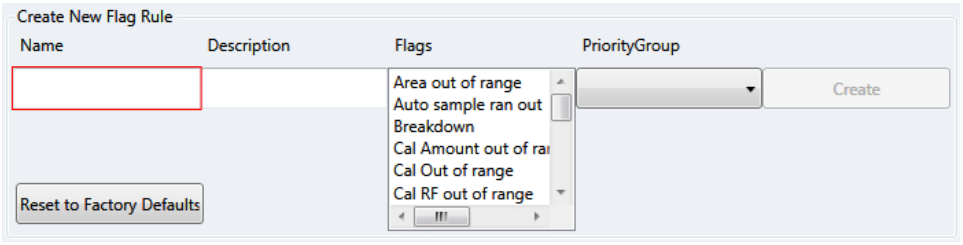
   - In the Description column, select the current text and type a new description.
   - Double-click the PriorityGroup value and select a new group from the list.
   - Click **Delete**.

     The application immediately removes the flag rule. To restore the deleted rule, click **Undo**.

     Note The Undo command removes all edits since the last Apply.

2. When you complete all your changes, click **Apply**.

❖ To create a new flag rule



1. In the Name box, type a name for the new rule.

   Keep the name short and make it intuitive.

2. In the Description box, type a description for the new flag rule.

   This description can be anything that you want and use as many characters as you want.

3. From the Flags list, select an error condition.
4. From the Priority Group list, select a priority group.

   This list includes both the default priority groups and any priority groups that you created. See To create a new priority group.
5. Click **Create**.

   The application adds your new flag rule to the end of the Flag Rules list.

❖ **To remove all customization**

Click **Reset to Factory Defaults**.

The application removes all new priority groups, new flag rules, and any edits to the groups or rules.

Advanced Dialog Box

Use the features in the Advanced dialog box to select custom colors for your flags, using RGB, HSL, or CMYK color standards.

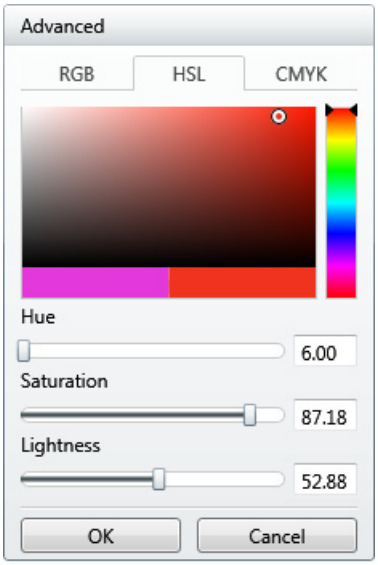
With the Red/Green/Blue (RGB) color palette, you can select a color with a specific RGB value, as displayed on a computer monitor.

Figure 8. Advanced RGB colors



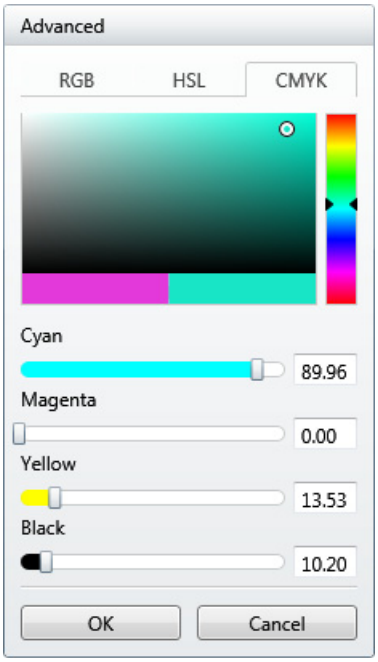
With the Hue/Saturation/Lightness (HSL) color palette, you can select a color with a specific HSL value, as is commonly used in computer graphics.

Figure 9. Advanced HSL colors



With the Cyan/Magenta/Yellow/Key (CMYK) color palette, you can select a color with a specific CMYK value, as you might specify in a color printer. The key (K) color used on printers is always black.

Figure 10. Advanced CMYK colors



2

Using Compound Databases in the Method Development Mode

The Compound Database view is available for quantitation, target screening, and unknown screening methods. The TraceFinder application supports both small molecule and peptide databases.

You can manage compound definitions for the current database in the Compound Database view. You can export compounds to a CSV file or mass list, or you can import compounds from an XML, a CSV, or a CDB file.

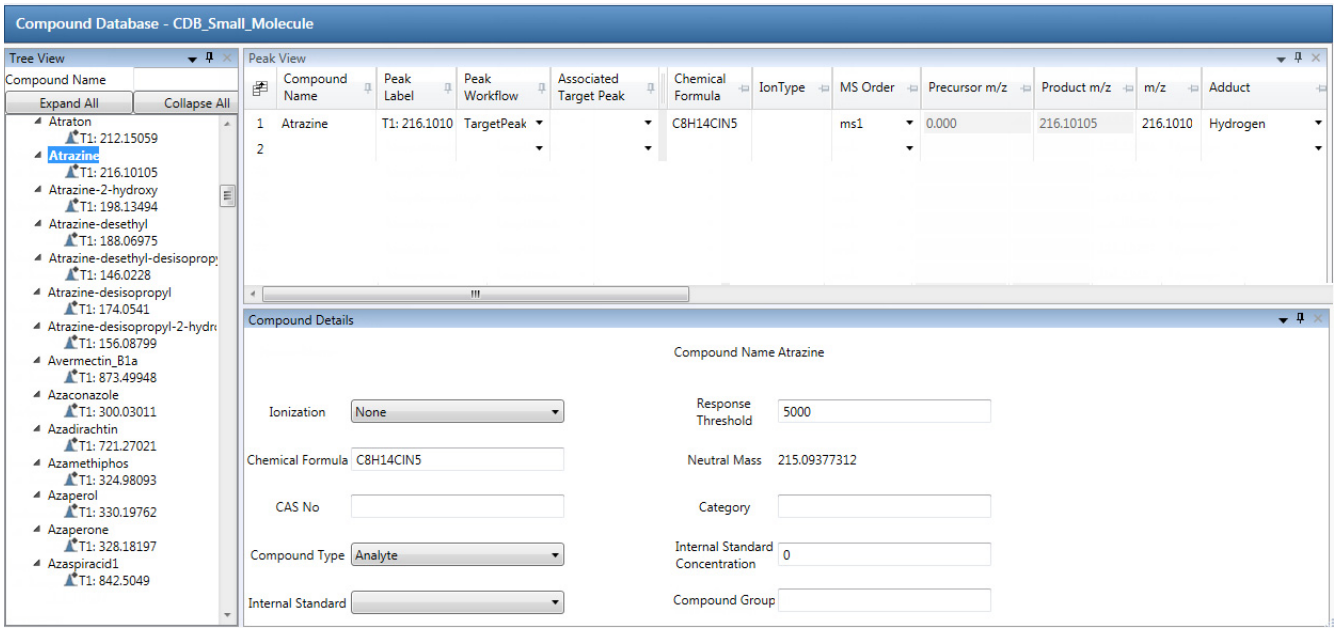
Contents

- Using the Compound Database View for Small Molecules
- Using the Compound Database View for Peptides
- Linking Internal Standards
- Using the Peptide Predictor Wizard
- Using the Peptide Modifications Editor
- Importing and Exporting

Using the Compound Database View for Small Molecules

The Compound Database view displays the small molecule data in the compound database. From this page, you can import small molecule compounds into the database, export compounds to a CSV file or mass list, add or remove compounds, and add or remove target peaks, confirming peaks, or fragments. You can also create new compound databases or save the current database to a new name.

Figure 11. Compound Database view for small molecules

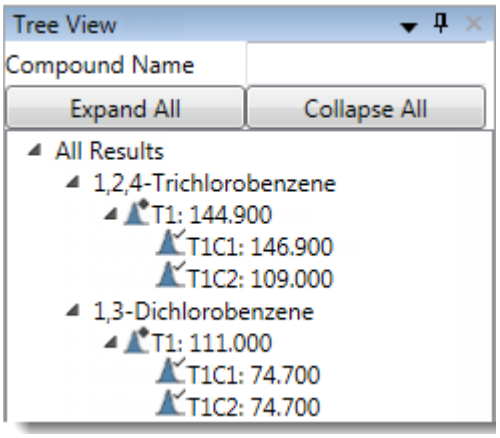


The Compound Database view includes the following features:

- Tree View Pane
- Peak View Pane
- Compound Details Pane for Small Molecules

Tree View Pane

The tree view for small molecule databases displays all compounds in the compound database, their target peaks, confirming peaks, and fragments.



Follow these procedures:

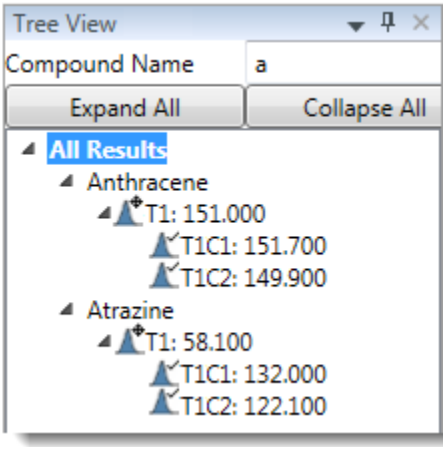
- To display only specific compounds
- To expand the names of all compounds in the database
- To collapse and reopen the names of all compounds in the database
- To read the tree view syntax
- To display the peak parameters and compound details for a compound

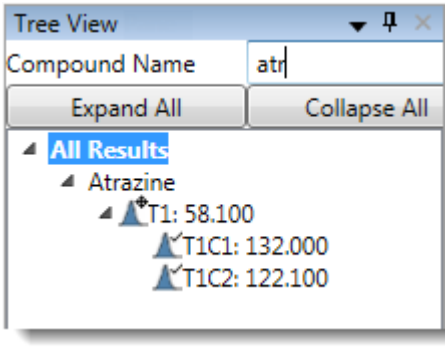
Tip (Animation) To view "Using the Tree View Pane," choose **Help > Animations**.

❖ To display only specific compounds

In the Compound Name box, type the name of the compound that you want to locate in the list. The Tree View list displays only compound names that contain the text you typed. This search feature is not case-sensitive.

In the following examples, as you type the letter **a**, the application displays all compounds that contain "a". As you continue to type **tr**, the application displays only compounds that contain the letters "atr".





Note When you click Expand All after typing text in the Compound Name box, the application expands only the compounds that contain the entered text.

❖ To expand the names of all compounds in the database

In the Tree View pane, click **Expand All**.

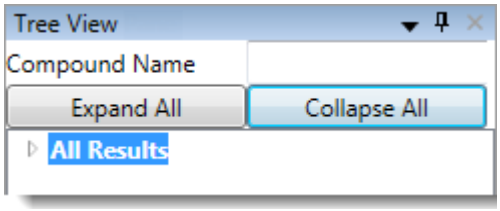
The application expands the target peaks, confirming peaks, and fragments for all compounds in the tree view.

Note When the Compound Name box contains text, the application displays only the compounds that contain the entered text.

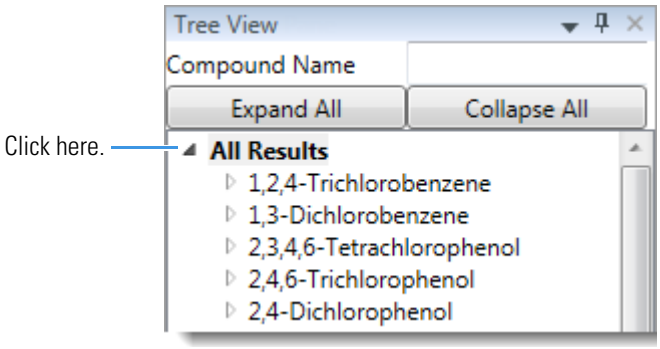
❖ To collapse and reopen the names of all compounds in the database

1. In the Tree View pane, click **Collapse All**.

The application collapses the target peaks and associated peaks for all compounds in the compound list and displays only the highest level or "All Results".



2. To display the names of all collapsed compounds in the compound list, expand **All Results**.



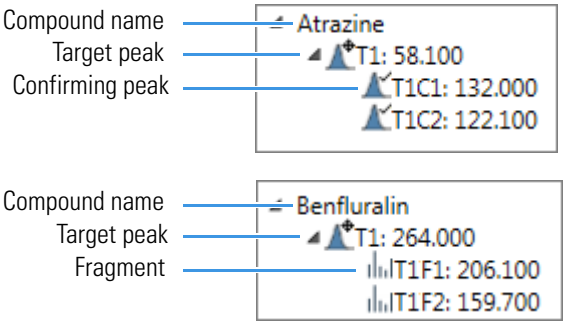
Note When the Compound Name box contains text, the application displays only the compounds that contain the entered text.

❖ To read the tree view syntax

Read the compound name, followed by the *m/z* for the target peaks, and then any confirming peaks or fragments and their *m/z*.

Identifiers for the peak types are as follows:

- T: Target peak
- C: Confirming peak
- F: Fragment
- P: Protein



❖ To display the peak parameters and compound details for a compound

In the Tree View pane, click the compound name.

The Peak View Pane displays the peak parameters for the selected compound and the associated confirming peaks and fragments.

The Compound Details Pane displays parameters that are common to all target peaks, confirming peaks, and fragments in the selected compound.

Peak View Pane

Use the Peak View Pane to display parameters for the compound you selected in the Tree View pane (or all compounds when you select All Results).

Tip (Animation) To view "Using the Peak View Pane," choose **Help > Animations**.

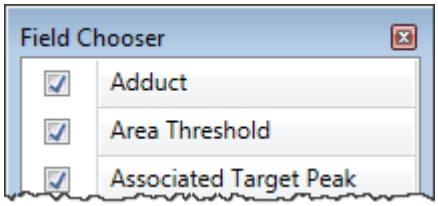
Follow these procedures:

- To hide or display columns in the Peak View pane
- To add a new compound
- To remove a compound
- To edit values in the compound database grid
- To specify a column as fixed

❖ To hide or display columns in the Peak View pane

1. Click the **Field Chooser** icon, , in the upper left corner of the pane.

   The Field Chooser displays all available columns of data for the Peak View pane.



2. Select the check box for each column that you want to display, or clear the check box for each column that you want to hide.

   The application displays or hides the columns in the Peak View pane.

3. When you are finished modifying the column display, click to close the Field Chooser.

❖ To add a new compound

1. Scroll to the bottom of the Peak View pane.

   There is always one blank row in the grid.

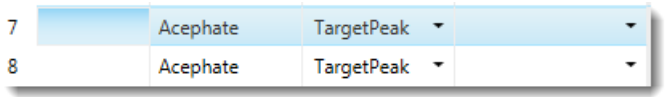
2. In the blank row, type or paste values into the columns.

   Note Some columns have dropdown lists from which you can select values.

3. Press ENTER to add another blank row to the grid.

❖ To remove a compound

1. Click anywhere in a row to select the compound.



2. Right-click and choose the **Delete Row**.

> **Note** Do not use CTRL+X to delete a compound. CTRL+X deletes only the parameter values in selected cells.

3. At the prompt to confirm this action, click **Yes**.

   The application removes the compound from the database grid.

❖ To edit values in the compound database grid

Do either of the following:

- Select the current column value and type a new value.

–or–

- Select single cells or entire columns whose values you want to copy, using a copy-and-paste operation.

  You can use this method to replicate entire columns. Click the column header to select the entire column, and then use CTRL+C and CTRL+V to replicate the column values.

When you change the charge state, adduct, or polarity for a compound, the application recalculates the precursor and product masses.

> **Note** Do not use CTRL+X to delete a compound. CTRL+X deletes only the parameter values in selected cells.

❖ To specify a column as fixed

In the column header, click the **Pin** icon,  .

The application moves the column to the left side of the grid.

When you use the scroll bar at the bottom of the Peak View pane, the pinned columns on the left remain fixed while the other columns scroll right and left.

Peak View Pane for Small Molecules

Use the Peak View pane to display parameters for the compound.

Figure 12. Peak View pane

Peak View Pane

	Compound Name	Peak Label	Peak Workflow	Associated Target Peak	Chemical Formula	MS Order	Precursor m/z	Product m/z	m/z
21	Atrazine	T1: 233.1276	TargetPeak		C8H14ClN5	ms1	0.000	233.1276	233.1276
22	Atrazine	T1T1: 174.05	Fragment	T1: 233.1276	C8H14ClN5	ms1	0.000	174.054	174.054
23	Atrazine	T1T2: 132.03	Fragment	T1: 233.1276	C8H14ClN5	ms1	0.000	132.0322	132.0322
24	Atrazine	T1T3: 104.00	Fragment	T1: 233.1276	C8H14ClN5	ms1	0.000	104.0008	104.0008
25	Atrazine	T2: 216.1010	TargetPeak		C8H14ClN5	ms1	0.000	216.10105	216.1010
26	Azaconazole	T1: 317.0566	TargetPeak		C12H11Cl2N:	ms1	0.000	317.05666	317.0566
27	Azaconazole	T1T1: 158.97	Fragment	T1: 317.05666	C12H11Cl2N:	ms1	0.000	158.9762	158.9762
28	Azaconazole	T1T2: 230.99	Fragment	T1: 317.05666	C12H11Cl2N:	ms1	0.000	230.9974	230.9974
29	Azaconazole	T1T3: 172.95	Fragment	T1: 317.05666	C12H11Cl2N:	ms1	0.000	172.9555	172.9555

Table 7. Peak View pane parameters (Sheet 1 of 3)

Column	Description
Compound Name	Alphanumeric name assigned to the compound.
Peak Label	User-specified label displayed in Analysis plots.
Peak Workflow	Specifies the compound as TargetPeak, Confirming, or Fragment.
Associated Target Peak	Specifies the target peak associated with a confirming peak or fragment.
Chemical Formula	Specifies the chemical formula that the application uses to calculate the *m/z* or precursor *m/z*. You cannot edit the chemical formula from the Peak View pane. To edit this value, use the Compound Details Pane for Small Molecules.
MS Order	Specifies that the confirming peaks come from the same scan (ms1) or are fragments from an adjacent scan (ms2).
Precursor *m/z*	The mass-to-charge ratio (*m/z*) of a target peak. The location of the center of a target precursor ion peak in mass-to-charge ratio units. In confirming peaks, the precursor mass is the same as the target peak precursor mass.
Product *m/z*	The mass-to-charge ratio of the quantitation ion. The location of the center of a target quan ion peak in mass-to-charge ratio (*m/z*) units.
m/z	Mass-to-charge ratio found in the spectra for the peak. Assumes that the charge is 1.

Table 7. Peak View pane parameters (Sheet 2 of 3)

Column	Description
Adduct	The adducts specified in the configuration file. To add or remove adducts from the default lists, see Specifying Adducts. Adducts affect the calculated amount of the extracted mass by adding to or subtracting from the neutral mass. Because adducts are polarity sensitive, select the Polarity parameter before selecting the Adduct value. Default positive values: None, Hydrogen, Ammonium, Sodium, Potassium Default negative values: None, Hydrogen-Loss, Acetate, Formate Default: None
Polarity	Positive or Negative
Charge State	The charge state of the ion (the *z* value in *m/z*). For example, a charge state of 2 with a negative polarity means that the compound has 2 more electrons than protons. Valid range: 1 through 10 Default: 1
Height Threshold	Controls the peak height cutoff. Any peaks with a height less than the height threshold will not be detected.
Area Threshold	Controls the area cutoff. Any peaks with a final area less than the area threshold will not be detected. This control is in units of area for the data. Valid range: 0 through 1 000 000 000
Collision Energy	The energy used when ions collide with the collision gas. Default: Read from the scan filter Valid range: –250.00 through +250.00
Retention Time	Specifies the time after injection when the compound elutes.
RT Window	Specifies the maximum viewable window around the identified chromatographic peak.
Lens	Valid range: –400 through +400
Energy Ramp	Valid range: 0 through 200
Target Ratio	Specifies the theoretical ratio of the confirming ion's response to the quantification ion's response as a percentage. Valid range: 1 through 100

Table 7. Peak View pane parameters (Sheet 3 of 3)

Column	Description
Integration Strategy	Specifies the order in which integration and summary are performed. • Individual: Performs integration and then sums the extracted ion current (XIC). • Summed: Sums the XIC and then integrates.
Group	User-defined alphanumeric names of groups to which the compound belongs. To create groups, see Editing the Groups Page.

Compound Details Pane for Small Molecules

This pane displays additional, editable parameters for the selected compound.

Figure 13. Compound Details pane

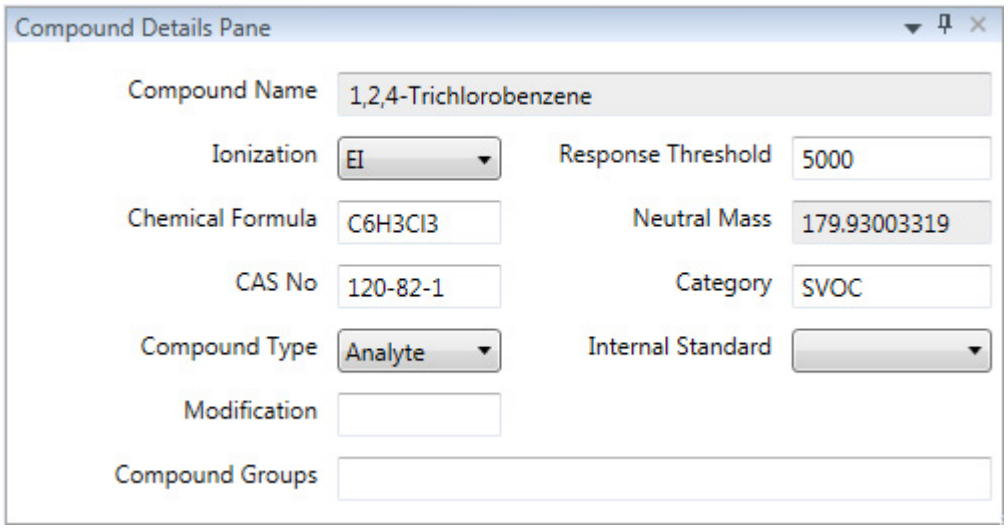


Table 8. Compound Details pane parameters (Sheet 1 of 2)

Parameter	Description
Compound Name	Specifies the alphanumeric name assigned to the compound.
Ionization	Specifies the alphanumeric identifier. Valid values: None, ESI, APCI, EI, CI, or APPI Default: None
Chemical Formula	Chemical formula for the compound. Used to calculate the neutral mass for the compound.
CAS No	The Chemical Abstract Service (CAS) number that the application matched with the compound.
Compound Type	Specifies the compound type as Analyte or Internal Standard.
Modification	Specifies any modification to the compound.
Compound Groups	User-specified groups to which the selected compound belongs.

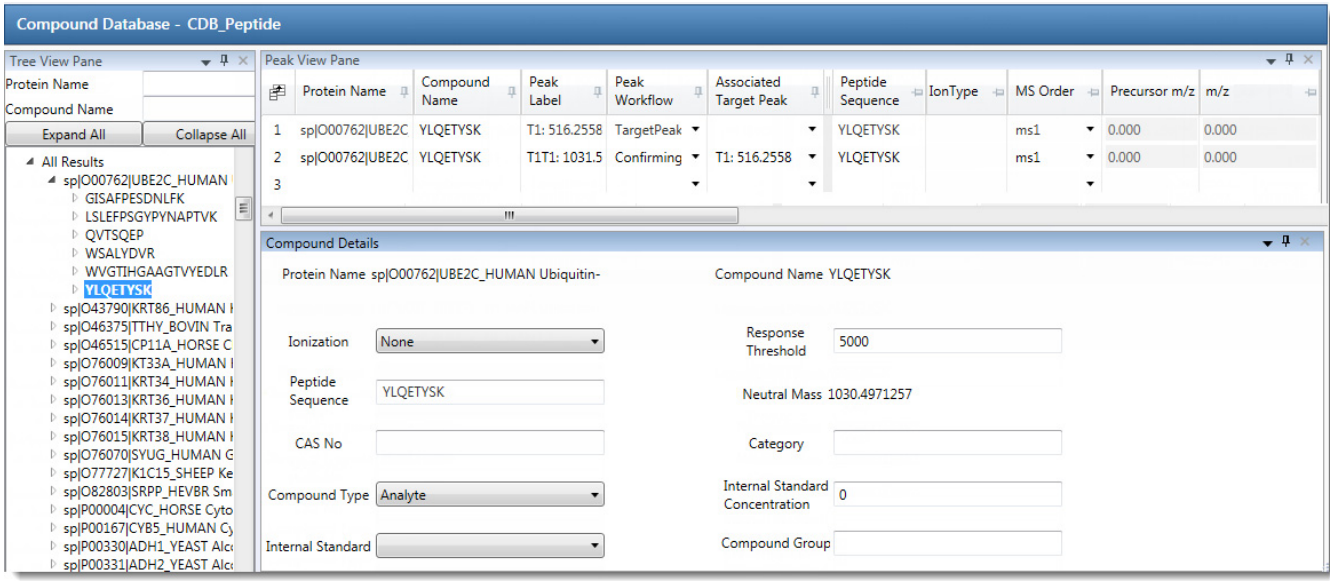
Table 8. Compound Details pane parameters (Sheet 2 of 2)

Parameter	Description
Response Threshold	Specifies the threshold used to integrate only peaks with a response greater than this value. A minimum response that must be met to allow peak confirmation. Used only for target screening methods and XIC experiments. Valid range: 1000 or greater Default: 5000
Neutral Mass	Specifies the mass calculated from the chemical formula. The sum of all AMU elements in the compound. This parameter is informational only; it is not used for peak detection.
Category	User-specified alphanumeric identifier, such as Class 1, Class 2, Opiates, and so forth.
Internal Standard	Specifies the internal standard (ISTD) for the selected compound. The list displays all compounds with the compound type of Internal Standard.

Using the Compound Database View for Peptides

The Compound Database view displays the peptide data in the compound database. From this page, you can import peptides into the database, export peptides to a CSV file or mass list, add or remove peptides, and add or remove target peaks, confirming peaks, or fragments. You can also create new compound databases or save the current database to a new name.

Figure 14. Compound Database view for peptides

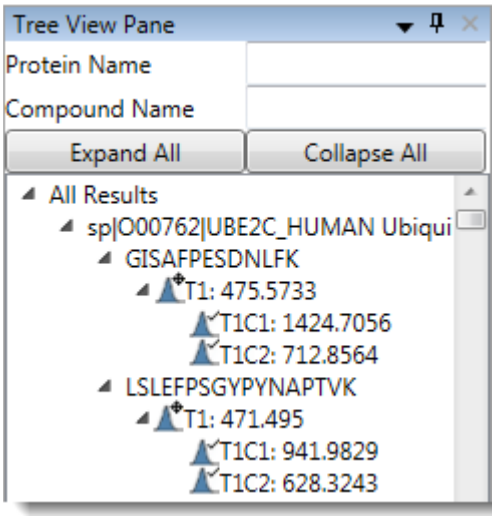


The Compound Database view includes the following features:

- Tree View Pane
- Peak View Pane
- Compound Details Pane

Tree View Pane

The tree view for peptide databases displays all proteins, compounds, and associated target peaks.



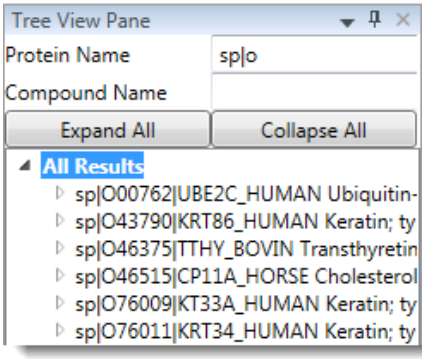
Follow these procedures:

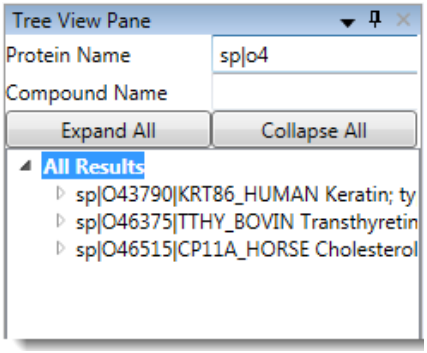
- To display only specific proteins
- To display only proteins that include specific compounds
- To expand the names of all compounds and proteins in the database
- To collapse and reopen the names of all compounds in the database
- To read the tree view syntax
- To display the peak parameters for a protein
- To display the compound details for a compound in a protein

❖ To display only specific proteins

In the Protein Name box, type the name of the protein that you want to locate in the list. The Tree View list displays only protein names that contain the text you typed.

In the following examples, as you type the letters **sp|o**, the application displays all proteins that contain "sp|o". As you continue to type **4**, the application displays only proteins that contain "sp|o4".





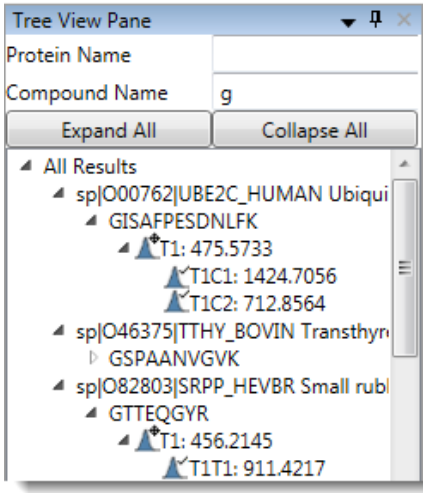
Note When the Protein Name box contains text, the application displays only the proteins that contain the entered text.

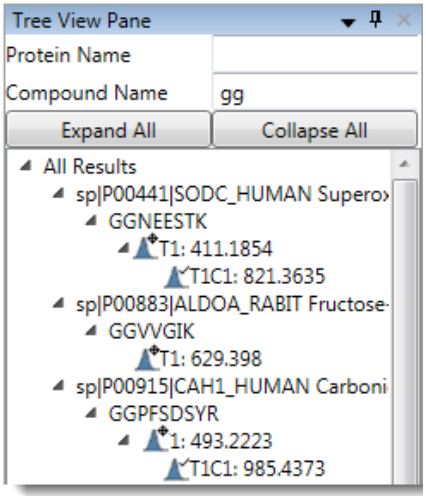
When both the Protein Name box and Compound Name box contain text, the tree view displays only items that meet both criteria.

❖ To display only proteins that include specific compounds

In the Compound Name box, type the name of the compound that you want to locate in the list. The Tree View list displays only the proteins that include compound names that contain the text you typed. This search feature is not case-sensitive.

In the following examples, as you type the letter **g**, the application displays all proteins that include compounds that contain "g". As you continue to type another letter **g**, the application displays only proteins that include compounds that contain the letters "gg".





Note When Compound Name box contains text, the application displays only the compounds that contain the entered text.

When both the Protein Name box and Compound Name box contain text, the tree view displays only items that meet both criteria.

❖ To expand the names of all compounds and proteins in the database

In the Tree View pane, click **Expand All**.

The application expands the proteins, compounds, and associated target peaks in the tree view.

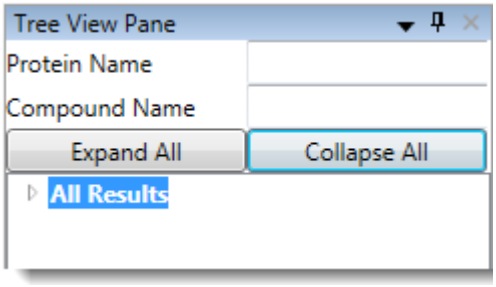
Note When the Protein Name box or Compound Name box contains text, the application displays only the proteins or compounds that contain the entered text.

When both the Protein Name box and Compound Name box contain text, the tree view displays only items that meet both criteria.

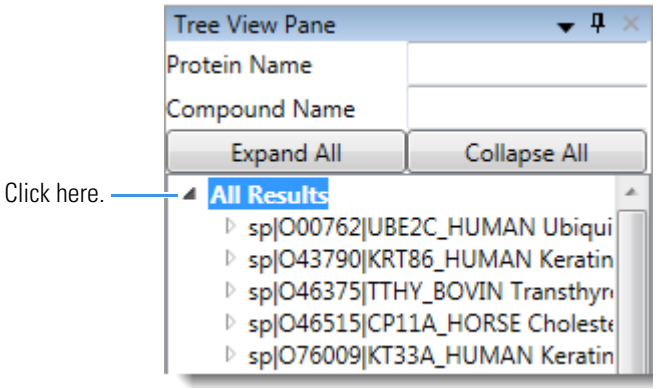
❖ To collapse and reopen the names of all compounds in the database

1. In the Tree View pane, click **Collapse All**.

   The application collapses the proteins, compounds, and associated target peaks for all proteins in the tree view and displays only the highest level or "All Results".



2. To display the names of all collapsed proteins in the tree view, expand **All Results**.



Note When there is text in the Protein Name box or Compound Name box, the application displays only the proteins or compounds that contain the entered text.

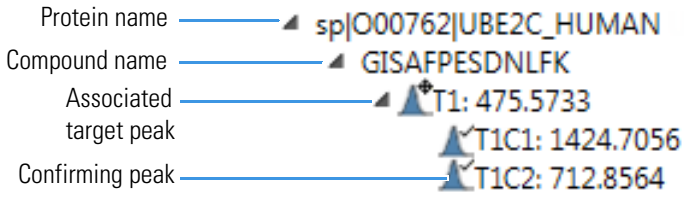
When both the Protein Name box and Compound Name box contain text, the tree view displays only items that meet both criteria.

❖ **To read the tree view syntax**

Read the compound name, followed by the *m/z* for target peaks, and then any confirming peaks or fragments and their *m/z*.

Identifiers for the peak types are as follows:

- T: Target peak
- C: Confirming peak
- F: Fragment
- P: Protein



❖ **To display the peak parameters for a protein**

In the Tree View pane, click the protein name.

The Peak View Pane displays the peak parameters for the selected protein, all included compounds, associated target peaks, and confirming peaks.

❖ **To display the compound details for a compound in a protein**

In the Tree View pane, click the compound name.

The Compound Details Pane displays parameters that are applied to associated target peaks and confirming peaks in the selected protein/compound combination.

Peak View Pane

Use the Peak View Pane to display parameters for the protein you selected in the Tree View pane (or all proteins when you select All Results).

Follow these procedures:

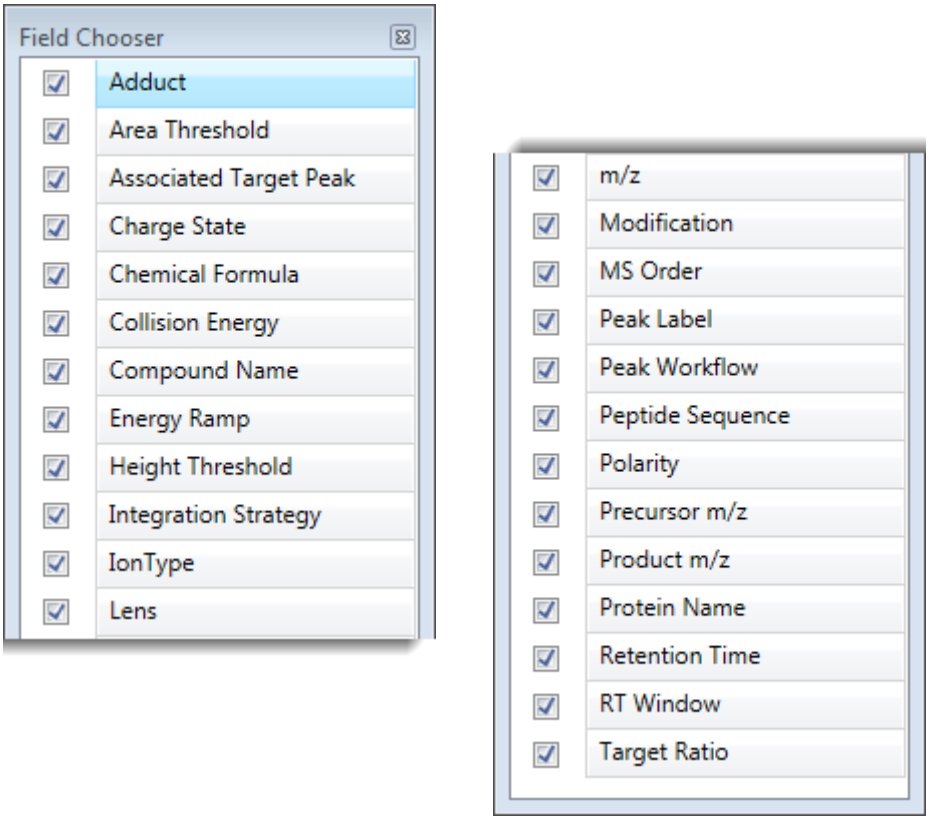
- To hide or display columns in the Peak View pane
- To add a new compound
- To remove a compound

- To edit values in the compound database grid
- To specify a column as fixed

❖ To hide or display columns in the Peak View pane

1. Click the **Field Chooser** icon, , in the upper left corner of the pane.

   The Field Chooser displays all available columns of data for the Peak View pane.



2. Select the check box for each column that you want to display, or clear the check box for each column that you want to hide.

   The application displays or hides the columns in the Peak View pane.

3. When you are finished modifying the column display, click to close the Field Chooser.

❖ To add a protein

1. Scroll to the bottom of the Peak View pane.

   There is always one blank row in the grid.

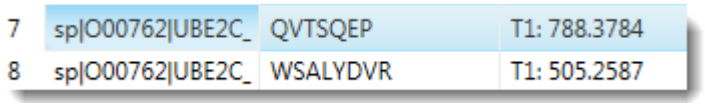
2. In the blank row, type or paste values into the columns.

   Note Some columns have dropdown lists from which you can select values.

3. Press ENTER to add another blank row to the grid.

❖ To remove a protein

1. Click the row number to select the protein.



2. Press DELETE.

Note Do not use CTRL+X to delete a protein. CTRL+X deletes only the parameter values in the selected cells.

3. At the prompt to confirm this action, click **Yes**.

   The application removes the protein from the database grid.

❖ To edit values in the compound database grid

Do either of the following:

- Select the current column value and type a new value.

–or–

- Select single cells or entire columns whose values you want to copy, using a copy-and-paste operation.

  You can use this method to replicate entire columns. Click the column header to select the entire column, and then use CTRL+C and CTRL+V to replicate the column values.

Note You cannot edit the values in the following columns: Chemical Formula, Peptide Sequence, Precursor *m/z*, Product *m/z*.

When you change the charge state, adduct, or polarity for a compound, the application recalculates the precursor and product masses.

❖ To specify a column as fixed

In the column header, click the **Pin** icon,  .

The application moves the column to the left side of the grid.

When you use the scroll bar at the bottom of the Peak View pane, the pinned columns on the left remain fixed while the other columns scroll right and left.

Peak View Pane

Use the features in the Peak View pane to display parameters for the protein you selected in the Tree View pane (or all proteins when you select All Results).

Figure 15. Peak View pane

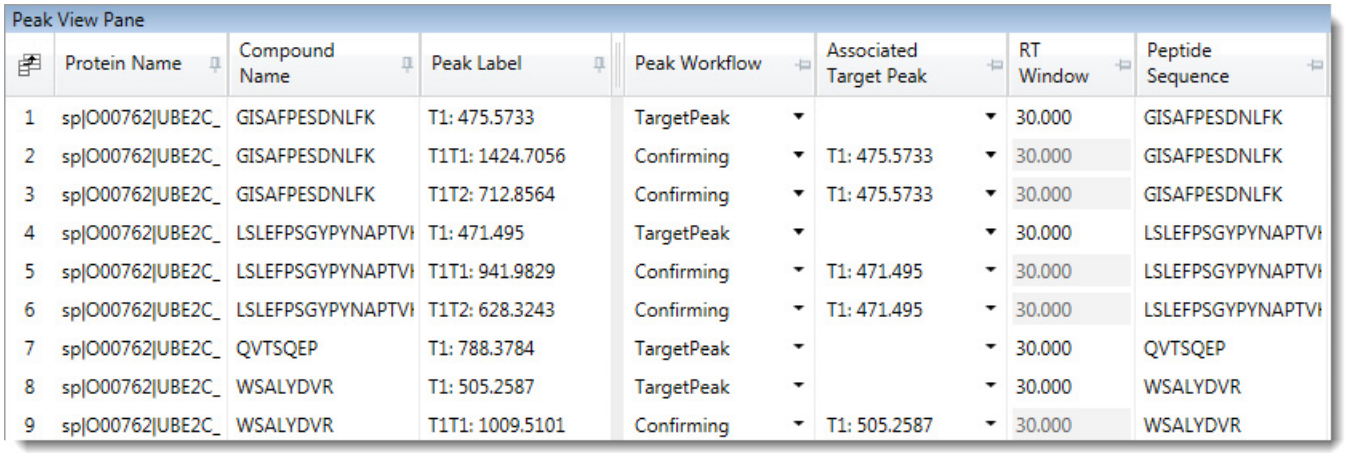


Table 9. Peak View pane parameters (Sheet 1 of 3)

Column	Description
Protein Name	Alphanumeric name assigned to the protein.
Compound Name	Alphanumeric name assigned to the compound. Default: the peptide sequence
Peak Label	User-specified label displayed in Analysis plots.
Peak Workflow	Specifies the compound as TargetPeak, Confirming, or Fragment.
Associated Target Peak	Specifies the target peak associated with a confirming peak or fragment.
Peptide Sequence	Specifies the peptide sequence that the application uses to calculate the *m/z* or precursor *m/z*. You cannot edit the peptide sequence from the Peak View pane. To edit this value, use the Compound Details Pane for Small Molecules. Available only for peptide databases.
Chemical Formula	Specifies the chemical formula that the application uses to calculate the product *m/z* or precursor *m/z*. You cannot edit the chemical formula from the Peak View pane. To edit this value, use the Compound Details Pane for Small Molecules.
MS Order	Specifies that the confirming peaks come from the same scan (**ms1**) or are fragments from an adjacent scan (**ms2**).
Precursor *m/z*	The mass-to-charge ratio (*m/z*) of a target peak. The location of the center of a target precursor ion peak in mass-to-charge ratio units. In confirming peaks, the precursor mass is the same as the target peak precursor mass.

Table 9. Peak View pane parameters (Sheet 2 of 3)

Column	Description
Product *m/z*	The mass-to-charge ratio of the quantitation ion. The location of the center of a target quan ion peak in mass-to-charge ratio (*m/z*) units.
m/z	Mass-to-charge ratio found in the spectra for the peak. Assumes that the charge is 1.
Adduct	The adducts specified in the configuration file. To add or remove adducts from the default lists, see Specifying Adducts. Adducts affect the calculated amount of the extracted mass by adding to or subtracting from the neutral mass. Because adducts are polarity sensitive, select the Polarity parameter before selecting the Adduct value. Default positive values: None, Hydrogen, Ammonium, Sodium, Potassium Default negative values: None, Hydrogen-Loss, Acetate, Formate Default: None
Modification	The application reads modifications from imported peptide files or from the Peptide Predictor wizard. Available only for peptide databases.
Polarity	Positive or Negative
Charge State	The charge state of the ion (the *z* value in *m/z*). For example, a charge state of 2 with a negative polarity means that the compound has 2 more electrons than protons. Valid range: 1 through 10 Default: 1
Height Threshold	Controls the peak height cutoff. Any peaks with a height less than the height threshold will not be detected.
Area Threshold	Controls the area cutoff. Any peaks with a final area less than the area threshold will not be detected. This control is in units of area for the data. Valid range: 0 through 1 000 000 000
Collision Energy	The energy used when ions collide with the collision gas. Default: Read from the scan filter Valid range: –250.00 through +250.00
Retention Time	Specifies the time after injection when the compound elutes.
RT Window	Specifies the maximum viewable window around the identified chromatographic peak.
Lens	Valid range: –400 through +400
Energy Ramp	Valid range: 0 through 200

Table 9. Peak View pane parameters (Sheet 3 of 3)

Column	Description
Target Ratio	Specifies the theoretical ratio of the confirming ion's response to the quantification ion's response as a percentage. Valid range: 1 through 100
Integration Strategy	Specifies the order in which integration and summary are performed. • Individual: Performs integration and then sums the XIC. • Summed: Sums the XIC and then integrates.
Ion Type	Specified as **b** or **y** with a numerical designation. Available only for peptide databases.
Group	Alphanumeric names of groups to which the compound belongs.

Compound Details Pane

This pane displays additional, editable parameters for the selected compound.

Figure 16. Compound Details pane

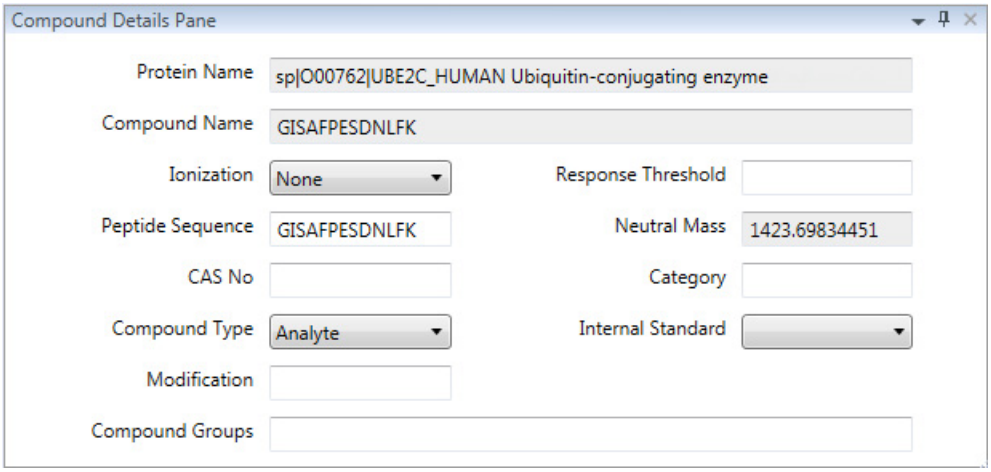


Table 10. Compound Details pane parameters (Sheet 1 of 2)

Parameter	Description
Protein Name	Displays the alphanumeric name assigned to the selected protein.
Compound Name	Displays the alphanumeric name assigned to the selected compound in the protein.
Ionization	Specifies the ionization process. Valid values: None, ESI, APCI, EI, CI, or APPI Default: None

Table 10. Compound Details pane parameters (Sheet 2 of 2)

Parameter	Description
Peptide Sequence	Specifies the order in which amino acid residues, connected by peptide bonds, lie in the chain in peptides and proteins. The sequence uses accepted Amino acid designations.
CAS No	Specifies the Chemical Abstract Service (CAS) number that the application matched with the selected compound.
Compound Type	Specifies the compound type as Analyte or Internal Standard.
Modification	Specifies any modification to the compound.
Compound Groups	Specifies comma-separated groups to which the selected compound belongs. Master methods created from this compound database display the compound groups on the Groups page in the Method View. See Editing the Groups Page.
Response Threshold	Specifies the threshold used to integrate only peaks with a response greater than this value. A minimum response that must be met to allow peak confirmation. Used only for target screening methods and XIC experiments. Valid range: 1000 or greater Default: 5000
Neutral Mass	Specifies the mass calculated from the peptide sequence. The sum of all AMU elements in the compound. This parameter is informational only; it is not used for peak detection.
Category	User-specified alphanumeric identifier, such as Class 1, Class 2, Opiates, and so forth.
Internal Standard	Specifies the internal standard (ISTD) for the selected compound. The list displays all compounds with the compound type of Internal Standard.

Table 11. Amino acid designations

Amino acid	Designation	Amino acid	Designation
Alanine	A	Leucine	L
Arginine	R	Lysine	K
Asparagine	N	Methionine	M
Aspartate	D	Phenylalanine	F
Cysteine	C	Proline	P
Histidine	H	Serine	S
Isoleucine	I	Threonine	T
Glutamine	Q	Tryptophan	W
Glutamate	E	Tyrosine	Y
Glycine	G	Valine	V

Note You can specify amino acid designations in either uppercase or lowercase letters.

Linking Internal Standards

In both small molecule and peptide compound databases, the TraceFinder application can link compounds to their internal standards when the compound name for the internal standard is the same as the target compound name appended with text and enclosed in brackets. For example, the application can link the internal standard *CompoundX[ISTD]* to the target compound *CompoundX*.



Tip (Animation) To view "Autolinking Internal Standards," choose **Help > Animations**.

❖ To specify a compound as an internal standard

1. In the Tree View pane, select the compound that you want to specify as an internal standard.
2. In the Peak View pane, select the compound name and append **[***]** to the name, for example: **Atrazine[ISTD]**.

   The application can automatically link this compound as the internal standard for the Atrazine compound.

Note You can also append [***] to the compound names in a CSV file or mass list before you import the compounds into the compound database.

❖ To link internal standards to target compounds

IMPORTANT Make sure you have designated internal standard compounds by appending [***] to their names.

Choose **Compound Database > Autolink Internal Standard and Target Compound** from the main menu.

The application does the following:

a. For each compound with a name that ends in **[***]**, sets the Compound Type to Internal Standard.

   Note The application does not overwrite a manually saved Compound Type of Analyte even when the compound name has **[***]** appended to its name.

b. For any *CompoundX* for which there is a *CompoundX[***],* sets *CompoundX[***]* as the internal standard for *CompoundX*.

   Note If the application does not find a target compound, *CompoundX,* to match a *CompoundX[***]*, the application performs no further action.

c. When there are multiple matching internal standard compounds, the application sets the first saved *CompoundX[***]* as the internal standard for the target compound.

   If the multiple internal standards were saved at the same time, the application displays a message that the target compound could not be linked to an internal standard because there are multiple internal standards.

   For example, for the target compound NGFILDGFPR, the application finds the internal standard compounds NGFILDGFPR[HeavyK] and NGFILDGFPR[LightR]. Because both [***] appends were added to the compound database at the same time, the application displays the error message.

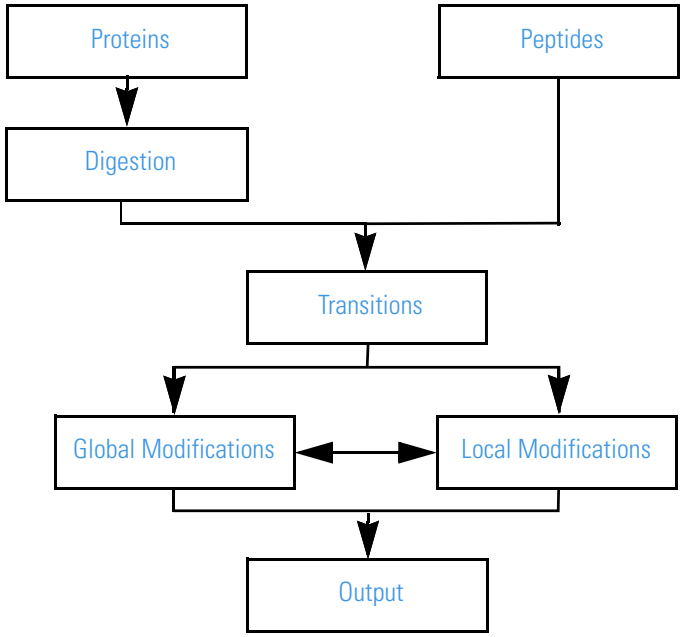
Using the Peptide Predictor Wizard

For protein data, use the Peptide Predictor wizard to predict the theoretical peptide fragments, modify any of the amino acids in the peptide, create a mass list of the peptides (unmodified, native peptides and modified peptides), and then either create a compound database with these masses or add these masses to an existing compound database.

For peptide data, use the Peptide Predictor wizard to modify any of the amino acids in the peptide, create a mass list from the peptides, and then either create a compound database with these masses or add these masses to an existing compound database.

You cannot specify both proteins and peptides in the Peptide Predictor wizard at the same time. However, you can use the Peptide Predictor wizard in two separate sessions to add proteins and peptides to the same compound database.

Figure 17. Peptide Predictor workflow



❖ To open the Peptide Predictor wizard

1. Choose **Compound Database > Peptide Prediction Tool > Peptide Prediction Tool** from the TraceFinder main menu.

   The Peptide Predictor wizard opens.

2. Begin your mass list with either protein data or peptide data.

   - See To begin a protein mass list.

   –or–

   - See To begin a peptide mass list.

❖ To begin a protein mass list

1. Set the Content Type to **Protein**.
2. Do one of the following:

   - Click **Browse**, locate a FASTA file, and click **Open**.

     The application displays the protein sequence in the FASTA file, overwriting any previous content in the pane.

     Note The maximum number of allowed proteins is 10. If you import a list with more than 10 proteins, the application imports only the first 10 protein sequences.

     Note You can manually edit the imported proteins.

   - Manually type in the protein sequences in FASTA file format.

     Follow these FASTA rules for specifying proteins (as demonstrated in the Example of an imported FASTA file showing human allergens):

     – Use the approved amino acid alphabet. See Amino Acids.

     – Begin the sequence with a single-line description. Use a greater-than (>) sign in the first column to distinguish the description from the sequence data. Or, follow the > with a sequence identifier and then the description. Use no space between the > and the first letter of the identifier, for example:

     ```
     >horsegi|418678|pir||MYHOZ myoglobin - common zebra [MASS=16950]
     ```

     – Follow the description with lines of sequence data, for example:

     ```
     GLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKASEDLKK
     HGTVVLTALGGILKKKG
     HHEAELKPLAQSHATKHKIPIKYLEFISDAIIHVLHSKHPGNFGADAQGAMTKALELFRNDIA
     AKYKELGFQG
     ```

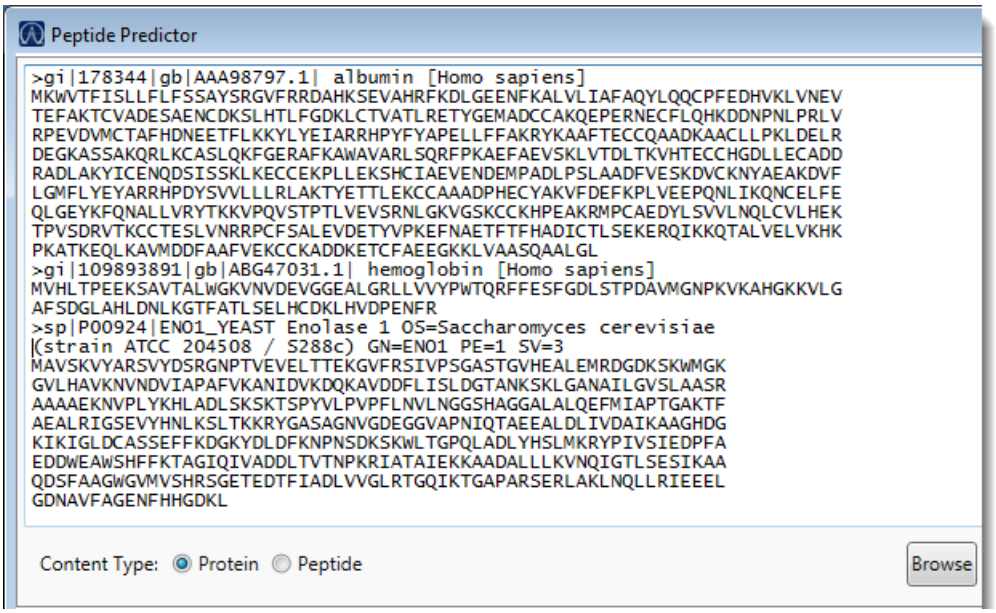
     – Keep all lines of text shorter than 80 characters.

     – Do not use any blank lines in the sequence.

– The sequence ends when another line starting with > appears, indicating the start of another sequence.

IMPORTANT The maximum number of allowed proteins is 10. If you create a list with more than 10 proteins and click Next, the application displays an error message.

Figure 18. Example of an imported FASTA file showing human allergens



The application displays the digestion parameters for proteins. See Digestion Page.

3. Select an enzyme.

   For a complete list of available enzymes, see Digestion Enzyme.

4. Specify the maximum number of internal cleavage sites within a peptide fragment that an enzyme can miss during the digestion process.

   The default is 0, meaning that the application considers the enzyme to have efficiently cleaved at all the possible cleavage sites in a protein with 100 percent specificity.

   The enzymatic digestion process does not always result in all the available cleavage sites in a protein being cleaved; therefore, it is important to specify the number of missed cleavage sites that can be present in a peptide fragment where the enzyme could have cleaved but did not.

5. Specify the number of starting amino acids in each sequence that the application ignores, for example:

6. Select to use either the monoisotopic mass or the average mass to calculate the mass of the ions.

7. Follow the instructions To specify transitions.

Digestion Page

Use the digestion page to specify the digestion parameters for proteins.

Figure 19. Digestion page for proteins

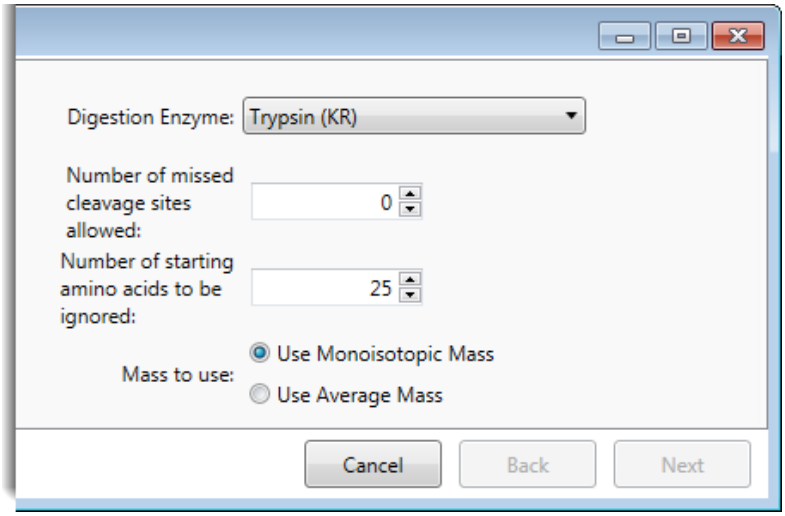


Table 12. Digestion page parameters (Sheet 1 of 2)

Parameter	Description
Digestion Enzyme	Specifies one of the following enzymes to digest the protein. • No Enzyme • Trypsin (KR) • Trypsin (KRLNH) • Trypsin (K) • Trypsin (R) • Chymotrypsin • Chymotrypsin (FWY) • Clostripain • Cyanogen Bromide • IodosoBenzoate • Proline Endopept • Staph Protease • GluC Bicarb • AspN • GluC • CNBR • ArgC • Elastase • Formic Acid • LysC • LysN

Table 12. Digestion page parameters (Sheet 2 of 2)

Parameter	Description
Number of Missed Cleavage Sites Allowed	Specifies the maximum number of missed cleavage sites that the application can allow per peptide fragment. Valid range: 0 through 10 Default: 0
Number of Starting Amino Acids to be Ignored	Specifies the number of amino acids at the beginning of each protein sequence that the application ignores. Valid range: 0 through 100 Default: 25
Mass to Use	Specifies the method used to calculate the mass. (Default) Use Monoisotopic Mass: The mass of the isotopic peak that is composed of the most abundant isotopes of those elements present in the molecular formula. Use Average Mass: The weighted average of the isotopic masses weighted by the respective isotopic abundances.

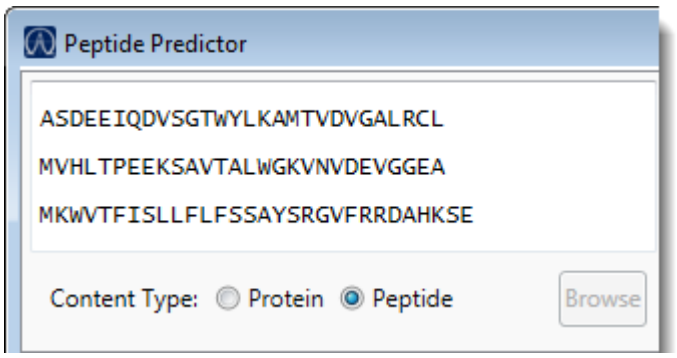
❖ To begin a peptide mass list

1. Set the Content Type to **Peptide**.
2. Manually type in the peptide sequences or paste the sequences from the Clipboard.

   Follow these rules for specifying peptides:

   - Use the approved amino acid alphabet. See Amino Acids.
   - Use a contiguous sequence of characters to define a single peptide.
   - Use any characters not in the alphabet as separators between multiple peptides. For example, the application parses the peptide sequence **ARNDCE/YVWTCEQ** into two peptides: ARNDCE and YVWTCEQ.

Figure 20. Example of a peptide file



3. Select to use either the monoisotopic mass or the average mass to calculate the mass of the ions. See Mass Page.
4. Follow the instructions To specify transitions.

Mass Page

Use the mass page to specify the method used to calculate the mass of the ions.

Figure 21. Mass page for peptides

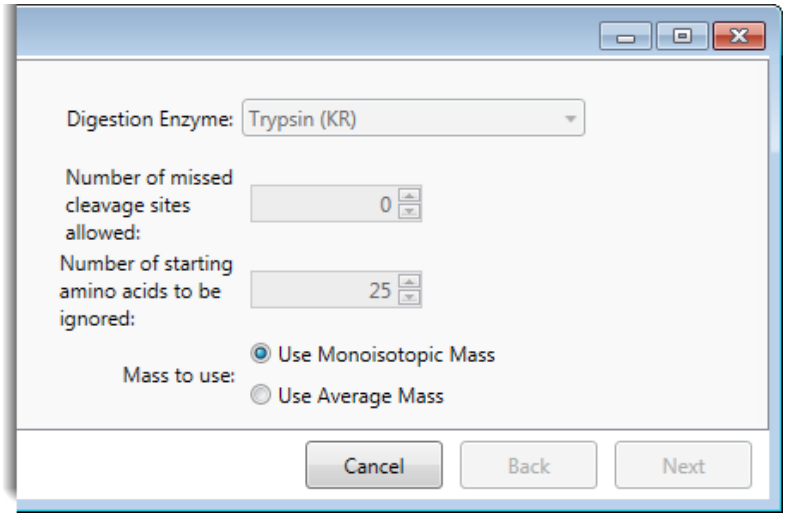


Table 13. Mass page parameter for peptides

Parameter	Description
Mass to Use	Specifies the method used to calculate the mass. (Default) Use Monoisotopic Mass: The mass of the isotopic peak that is composed of the most abundant isotopes of those elements present in the molecular formula. Use Average Mass: The weighted average of the isotopic masses weighted by the respective isotopic abundances.

❖ To specify transitions

1. Click **Next**.

   The Transitions Page opens where you can specify the output transition options that you want to use. Use the parameters on the transitions page to specify the mass range of the precursor and product ions to be processed.

   For proteins, the mass list on the transitions page shows the predicted list of peptides generated by the enzymatic cleavage process. See Example prediction of proteins that are broken at the cleavage sites.

For peptides, the mass list on the transitions page identifies all peptide sequences as user-created. See Example of user-created peptides.

Figure 22. Example prediction of proteins that are broken at the cleavage sites

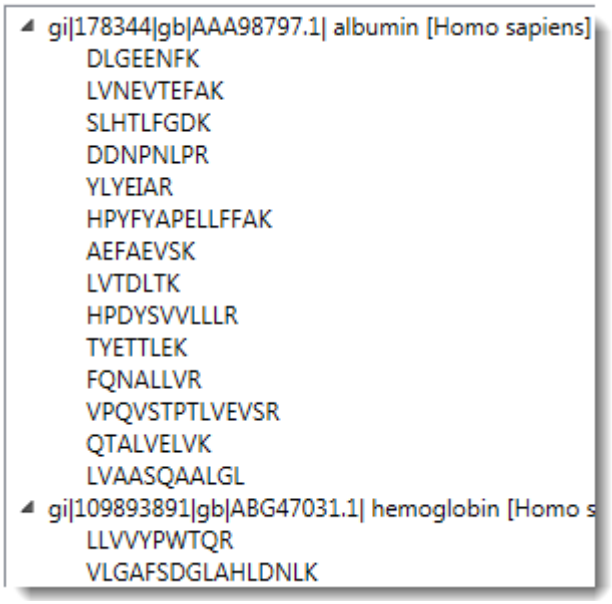
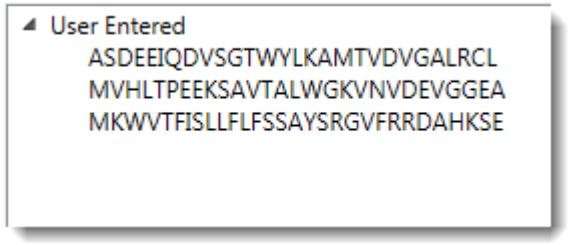


Figure 23. Example of user-created peptides



2. In the **Minimum m/z Allowed in the Instrument** box, enter a value for the minimum mass that your instrument can measure.
3. In the **Maximum m/z Allowed in the Instrument** box, enter a value for the maximum mass that your instrument can measure.
4. Choose a target mode: **All Transitions Within a Range** (MS2 order) or **Precursor Ion Options** (MS1 order).
   - When you select All Transitions Within a Range, do the following:
     i. Specify the From and To values for the ion type range.

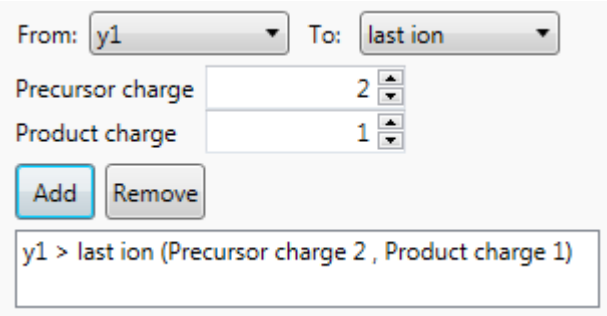
     For valid values, see All Transitions Within a Range.

     If none of the selected transitions are possible, the application does not create transitions.

     ii. Specify the values to use for the precursor charge and the product charge prediction.

iii. Click **Add**.

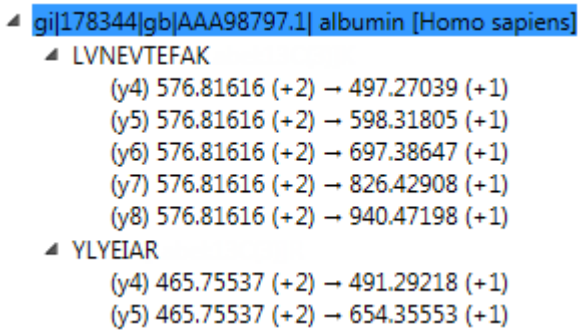
The application creates transitions from the specified transition range, precursor charge, and product charge.



When you click Next, the application applies these transitions to all peptides (as shown in Figure 24).

IMPORTANT You must add at least one transition before you can perform modifications on the amino acids.

Figure 24. Example of all transitions on the modifications page



- When you select Precursor Ion Options, do the following:

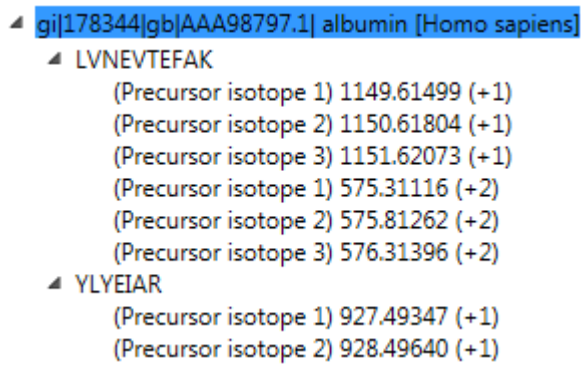
  i. Specify the minimum and maximum charge states to be used for the prediction.

  IMPORTANT You must specify a valid range before you can perform modifications on the amino acids.

  ii. Specify the number of isotopes that you want returned from the prediction.

  When you click Next, the application applies these precursor ion parameters to all peptides (see Example of precursor ion options on the modifications page).

Figure 25. Example of precursor ion options on the modifications page



5. Follow the instructions To specify local modifications or To specify global modifications.

Transitions Page

Use the transitions page to specify the output transition options that you want to use.

Figure 26. Transitions page

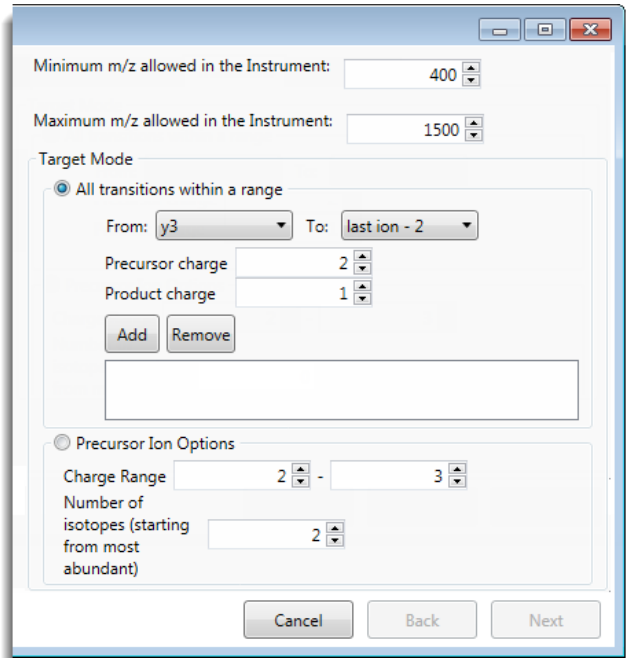


Table 14. Transitions page parameters (Sheet 1 of 2)

Parameter	Description
Minimum *m/z* Allowed in the Instrument	Specifies the minimum mass that your application can measure. Default: 400
Maximum *m/z* Allowed in the Instrument	Specifies the maximum mass that your instrument can measure. Default: 1500

Table 14. Transitions page parameters (Sheet 2 of 2)

Parameter	Description
Target Mode	
All Transitions Within a Range	
From	Valid values: • y1 • y2 • y3 • y4 • b1 • b2 • b3 • b4 • (mz higher than parent_mz) • (mz higher than parent_mz) –1 • (mz higher than parent_mz) –2 • (mz higher than parent_mz) +1 • (mz higher than parent_mz) +2 Default: y3
To	Valid values: • last ion • last ion – 1 • last ion – 2 • last ion – 3 • 3 ions from the start limit • 4 ions from the start limit • 5 ions from the start limit Default: last ion – 2
Precursor Charge	Valid range: 0 through 10 Default: 2
Product Charge	Valid range: 0 through 10 Default: 1
Add / Remove	Adds a transition based on the current parameter setting, or removes the selected transition definition.
Precursor Ion Options	
Charge Range	Specifies the beginning and ending values for the charge range. Valid range: 0 through 100 Default: 2 through 3
Number of Isotopes	Valid range: 0 through 10 Default: 0

❖ To specify local modifications

1. Click **Next**.

   The Modifications Page opens where you can specify point modifications for each amino acid or terminal.

2. In the left pane, select a peptide sequence.

   The right pane displays all amino acids in the sequence.

   Each amino acid has an amino and a carboxylic group. By convention, the peptides are written from the n-Terminal to the c-Terminal. The n-Terminal refers to the free amine group of the first amino acid. The peptide terminates with a free carboxylic group of the last amino acid, which is the c-Terminal.

   Note The defaults for the n-Terminal and the c-Terminal are H and OH, respectively. When you use the defaults, the application does not display the terminals in the mass list. If you modify the amino acid assigned to a terminal, the application displays the modified value in the mass list.

3. In the right pane, click each amino acid that you want to modify.

   A dropdown list displays all available modifications for each selected amino acid.

4. Select the modifications that you want to apply to each amino acid in the selected peptide.

5. Click **Apply** or click **Create Copy and Apply**.

   Apply: Applies the selected modifications to the respective amino acid in the selected peptide.

   Create Copy and Apply: Saves a copy of the original, unmodified file and then applies the selected modifications to the respective amino acid in the selected peptide.

   The application applies the modifications to each amino acid in the selected peptide.

   Note Local modifications overwrite any previous global modifications.

6. Repeat steps 2 through 5 for each peptide sequence that you want to modify.

7. Do one of the following:

   - To make global modifications, follow the instructions To specify global modifications, beginning with step 2.

     Note Global modifications do not overwrite previous local modifications.

   –or–

   - To save your mass list, follow the instructions To save the mass list.

❖ To specify global modifications

1. Click **Next**.

   The Modifications Page opens where you can specify global modifications for each amino acid or terminal.

   When you modify an amino acid in a top-level protein sequence, the application applies each modification to each instance of the amino acid in the mass list.

2. In the left pane, select a peptide sequence.

   The right pane displays all amino acids in the sequence.

   Each amino acid has an amino and a carboxylic group. By convention, the peptides are written from the n-Terminal to the c-Terminal. The n-Terminal refers to the free amine group of the first amino acid. The peptide terminates with a free carboxylic group of the last amino acid, which is the c-Terminal.

   Note The defaults for the n-Terminal and the c-Terminal are H and OH, respectively. When you use the defaults, the application does not display the terminals in the mass list. If you modify the amino acid assigned to a terminal, the application displays the modified value in the mass list.

3. In the right pane, click each amino acid that you want to modify.

   A dropdown list displays all available modifications for each amino acid.

4. Select the modifications that you want to apply to all instances of each amino acid.

5. Click **Apply** or click **Create Copy and Apply**.

   Apply: Applies the current modifications to all instances of the amino acids.

   Create Copy and Apply: Saves a copy of the unmodified file and then applies the current modifications to all instances of the amino acids.

   The application applies the modifications to all instances of the amino acids in the sequence file.

   Note Global modifications do not overwrite any previous local modifications.

   The following examples show global modifications to a single amino acid when the target mode is All Transitions Within a Range or Precursor Ion Options.

Figure 27. Example of All Transitions Within a Range

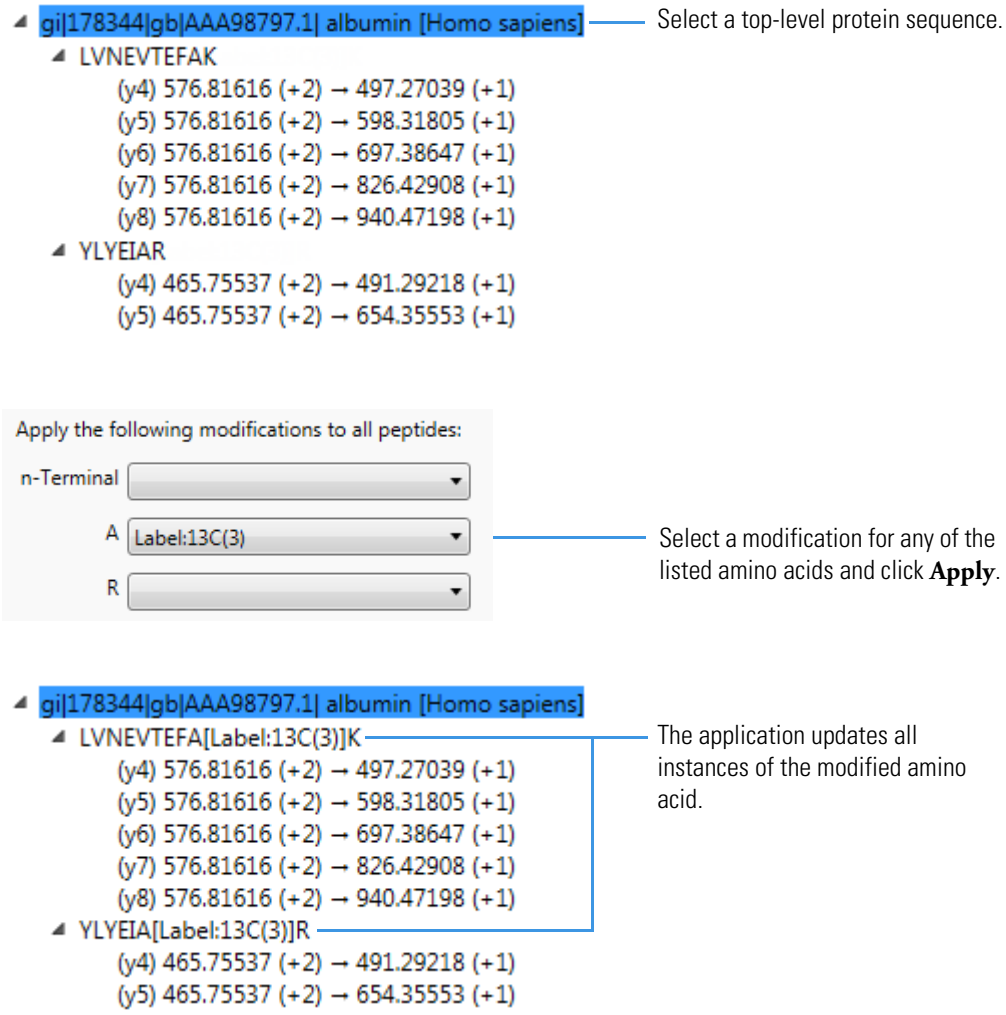
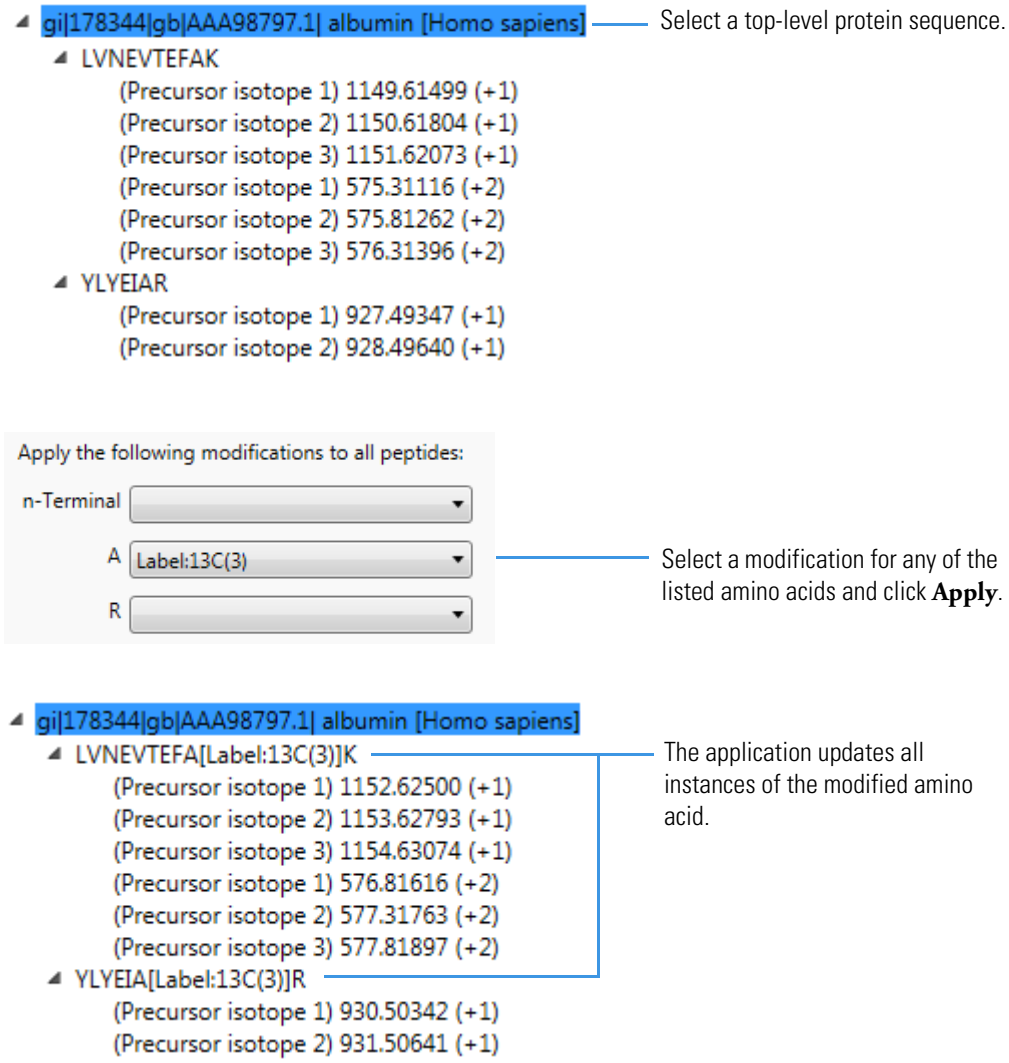


Figure 28. Example of Precursor Ion Options



Modifications Page

Use the modifications page to specify point modifications or global modifications for each amino acid or terminal.

Figure 29. Modifications page

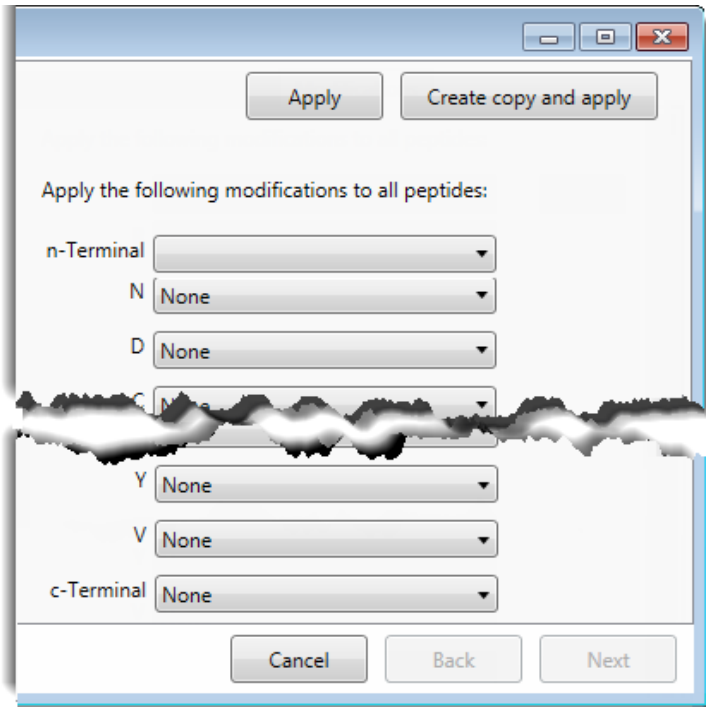


Table 15. Modifications page parameters (Sheet 1 of 2)

Parameter	Description		
Amino acids			
Single-letter codes for amino acids (lowercase characters are also allowed):			
A	alanine	L	leucine
R	arginine	K	lysine
N	asparagine	M	methionine
D	aspartic acid	F	phenylalanine
C	cysteine	P	proline
E	glutamic acid	S	serine
Q	glutamine	T	threonine
G	glycine	W	tryptophan
H	histidine	Y	tyrosine
I	isoleucine	V	valine

Table 15. Modifications page parameters (Sheet 2 of 2)

Parameter	Description
Modifications	
For each amino acid, the available modifications can include any one of the following:	
• Label:13C(3) • Label:13C(3)15N(1) • Label: 15N(1) • N15 Enriched • 13C Enriched • Carbamylation • Carboxymethylation • Methylation • di-Methylation • tri_Methylation • Heavy Argenine • Deamidation • Hydroxylation • Deglycosylation • Carboxyamidomethylation • Oxidation • Propionamide • ICAT-C • ICAT Heavy • Heavy Isoleucine • Heavy Leucine • Acetylation • Formylation • ITRAQ4plex Reagent 114 • ITRAQ4plex Reagent 115 • ITRAQ4plex Reagent 116 • ITRAQ4plex Reagent 117 • ITRAQ8plex Reagent 113 • ITRAQ8plex Reagent 114 • ITRAQ8plex Reagent 115	• ITRAQ8plex Reagent 116 • ITRAQ8plex Reagent 117 • ITRAQ8plex Reagent 118 • ITRAQ8plex Reagent 119 • ITRAQ8plex Reagent 121 • Heavy Lysine • TMT0_126 • TMT2_126 • TMT2_127 • TMT6_126 • TMT6_127 • TMT6_128 • TMT6_129 • TMT6_130 • TMT6_131 • Heavy Phenylalanine • Heavy Proline • Phosphorylation • Sulphation • Heavy Valine • OH • C-Amidation • N-Acetylation • N-Carbamylation • N-di-Methylation • N-Formylation • N-Methylation • H

❖ To save the mass list

1. Click **Next**.

   The Output Page opens where you can specify the output database and import options that you want to use.

2. To create a new compound database for the compounds, do the following:

   a. In the Output CDB Selection area, click **Create**.

      The New Compound Database dialog box opens.

b. In the New Compound Database dialog box, type a name for the new compound database and click **OK**.

3. To add these compounds to an existing compound database, do the following:

   a. In the Output CDB Selection area, click **Browse**.

      The Open Compound Database dialog box opens.

   b. In the Open Compound Database dialog box, select a compound database and click **Open**.

4. In the Import Options area, specify one of the following:

   - To identify all found peaks as target peaks, select the **All** option.
   - To identify only the first found peak as a target peak and identify all other peaks as confirming ions or fragments, select the **One** option and then select either the **Confirming Ion** or **Fragment** option.

5. Click **Finish**.

   The application add the identified peptides to the specified compound database, closes the Peptide Predictor wizard, and opens the compound database in the Compound Database view in the TraceFinder application.

Output Page

Use the output page to specify the output database and import options.

Figure 30. Output page

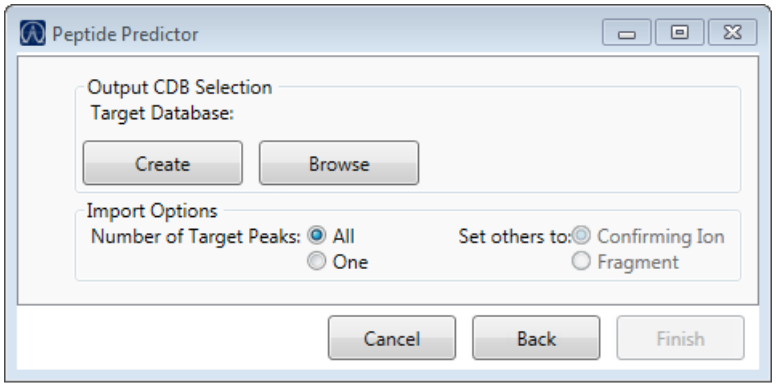


Table 16. Output page parameters (Sheet 1 of 2)

Parameter	Description
Output CDB Selection	
Create	Opens the New Compound Database dialog box where you can create a new compound database.
Browse	Opens the Open Compound Database dialog box where you can select a compound database to which you can add the peptide mass list.

Table 16. Output page parameters (Sheet 2 of 2)

Parameter	Description
Import Options	
Number of Target Peaks	Specifies the number of found peaks to identify as target peaks.
Set Others To	Specifies that other found peaks are identified as confirming ions or fragments.

Using the Peptide Modifications Editor

Use the Peptide Modifications Editor to define the modifications that are available in the Peptide Predictor wizard.

Follow these procedures:

- To open the Peptide Modifications Editor
- To edit the available default modifications
- To create custom modifications

❖ To open the Peptide Modifications Editor

Choose **Compound Database > Peptide Prediction Tool > Edit Peptide Modifications** from the TraceFinder main menu.

The Peptide Modifications Editor window opens.

❖ To edit the available default modifications

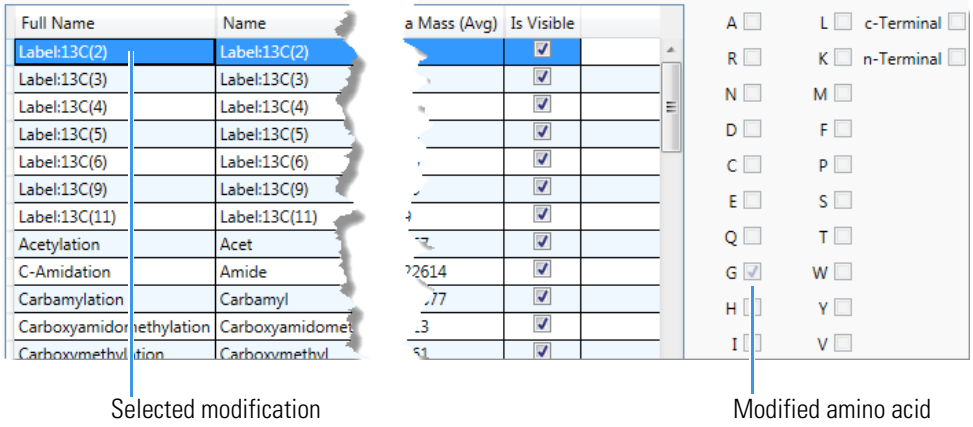
1. Click the **Default Modifications** tab.

   The Default Modifications Page displays the names and mass values for the application's default amino acid modifications. You cannot edit the values for a default modification; however, you can make a modification unavailable (not visible) in the Peptide Predictor wizard.

   By default, all modifications are visible in the Peptide Predictor wizard.

2. For each modification that you do not want displayed on the modifications page of the Peptide Predictor wizard, select the modification and clear the Is Visible check box.

When you select a modification, the application displays a check mark for each of the amino acids that this modification affects.



3. When you finish setting the visibility for the modifications, click **Save Visibility**.

   The application saves the visibility settings, and the Peptide Predictor wizard displays only the modifications that have the Is Visible option checked.

4. When you finish your changes to the default and custom modifications, click **Cancel**.

   The application discards any changes that you made after the last time you clicked **Save Visibility** on the Default Modifications page or after you clicked **Save Custom Modifications** on the Custom Modifications page and then closes the wizard.

Default Modifications Page

Use the Default Modifications page to select which modifications are visible in the Peptide Predictor wizard.

Figure 31. Default Modifications page

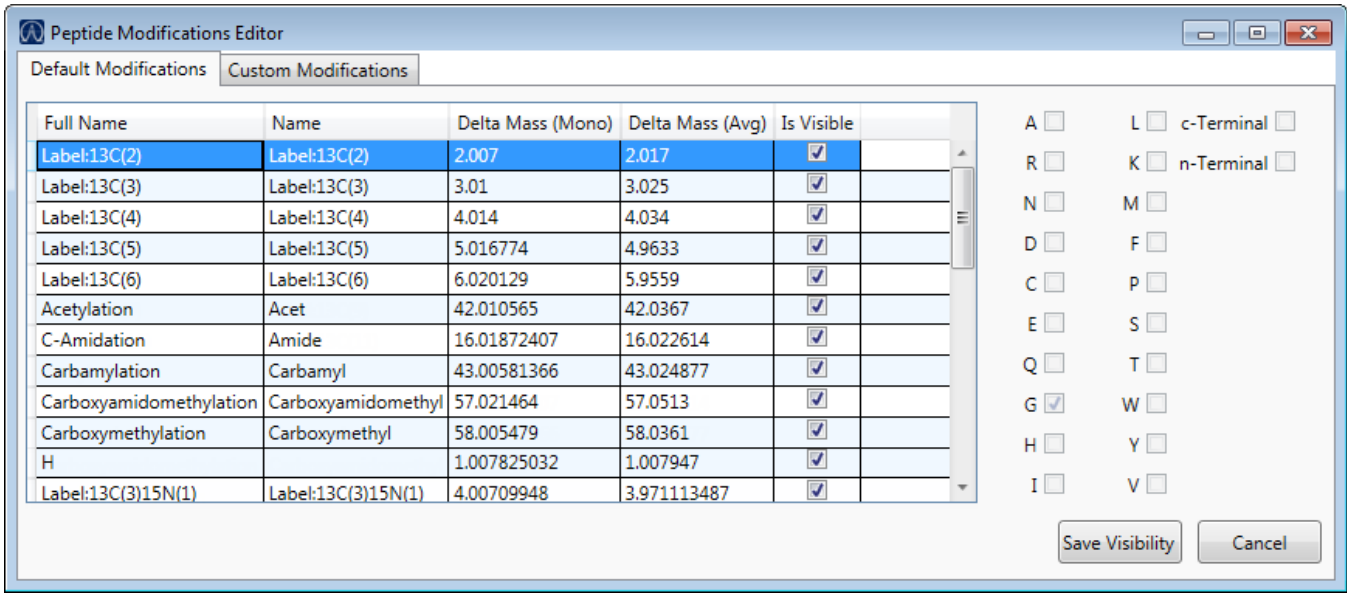


Table 17. Default Modifications page parameters

Parameter	Description
Modification List	
Full Name	Name of the modification.
Name	User-specified name for the modification.
Delta Mass (Mono)	Specifies the modification to the monoisotopic mass of the ion when it is calculated using the exact mass of the most abundant isotope of each element. The modification can be a positive or negative change.
Delta Mass (Avg)	Specifies the modification to the mass of the ion when it is calculated using the average atomic weight of each element. The modification can be a positive or negative change.
Is Visible	When selected, displays the modification on the modifications page of the Peptide Predictor wizard.
Amino Acids List	Specifies the amino acids associated with the selected modification. These values are not editable. For a list of amino acid abbreviations, see Amino Acids.
Function Buttons	
Save Visibility	Saves the visibility settings. The Peptide Predictor wizard displays only the modifications that have the Is Visible option checked and were saved.
Cancel	Discards any changes that you made after the last time you clicked **Save Visibility** and then closes the wizard.

❖ To create custom modifications

1. Click the **Custom Modifications** tab.

   The Custom Modifications Page displays the names and mass values for the user-created amino acid modifications.

2. Click **Add**.

   The application adds a new row where you can define a custom modification.

3. In the Full Name column, type a name for the modification.

4. In the Delta Mass (Mono) column, type the adjustment to the mass to use when you calculate the mass using the monoisotopic method.

   The value can be a positive or negative number.

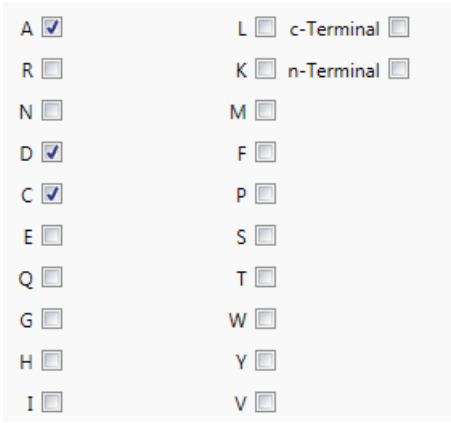
5. In the Delta Mass (Avg) column, type the adjustment to the mass to use when you calculate the mass using the average mass method.

   The value can be a positive or negative number.

6. To display the modification on the modifications page of the Peptide Predictor wizard, select the Is Visible check box.

By default, the Is Visible check box is not selected and the custom modification is not visible in the Peptide Predictor wizard.

7. Select the check box for each of the amino acids for which this modification is available.



8. Repeat steps 2 through 7 for each custom modification that you want to define.
9. Click **Save Custom Modifications**.
10. When you finish your changes to the default and custom modifications, click **Cancel**.

    The application discards any changes that you made after the last time you clicked **Save Visibility** on the Default Modifications page or after you clicked **Save Custom Modifications** on the Custom Modifications page and then closes the wizard.

Custom Modifications Page

Use the Custom Modifications page parameters to define custom modifications.

Figure 32. Custom Modifications page

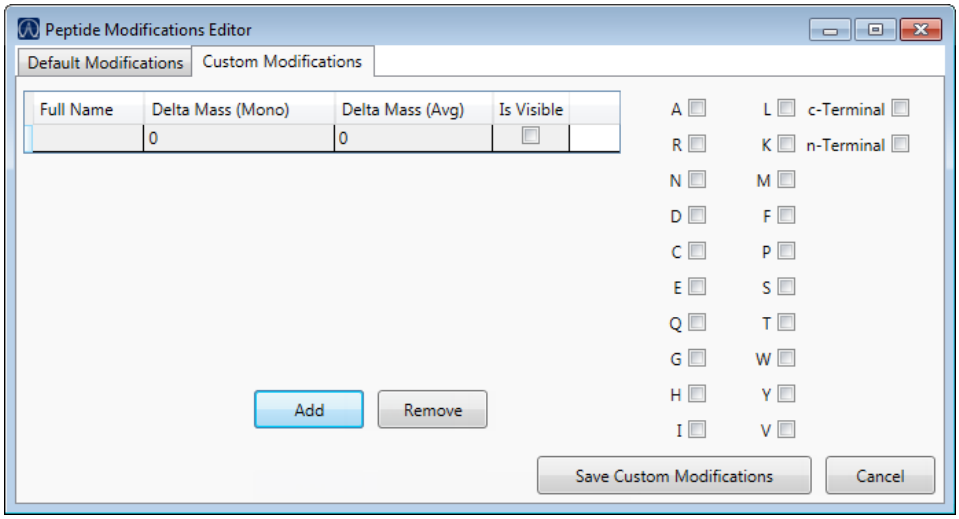


Table 18. Custom Modifications page parameters

Parameter	Description
Modification List	
Full Name	Name of the modification.
Delta Mass (Mono)	Specifies the modification to the monoisotopic mass of the ion when it is calculated using the exact mass of the most abundant isotope of each element. The modification can be a positive or negative change.
Delta Mass (Avg)	Specifies the modification to the mass of the ion when it is calculated using the average atomic weight of each element. The modification can be a positive or negative change.
Is Visible	When selected, displays the modification on the modifications page of the Peptide Predictor wizard.
Amino Acids List	Specifies the amino acids associated with the selected modification. For a list of amino acid abbreviations, see Amino Acids.
Function Buttons	
Add	Adds a new, empty row to the custom modifications list.
Remove	Deletes the selected modification.
Save Custom Modifications	Saves the custom modifications. The Peptide Predictor wizard displays only the modifications that have the Is Visible option checked and were saved.
Cancel	Discards any changes that you made after the last time you clicked **Save Custom Modifications** and then closes the wizard.

Importing and Exporting

To import and export compound data, follow these procedures:

- To import compounds from ToxID, ExactFinder, or legacy TraceFinder
- To import compounds from Skyline, PepFinder™, PinPoint, or mzVault
- To edit a mapping template
- To export all compounds to a CSV file
- To export selected compounds to a mass list

See also Data Columns with Default Values.



Tip **(Animation)** To view a demonstration of the import/export feature for compound databases, choose **Help > Animations** and select the applicable title:
- "Importing Compounds from a Compound Database"
- "Exporting Compounds from a Compound Database"
- "Importing Compounds from a Skyline Compound Database"

❖ To import compounds from ToxID, ExactFinder, or legacy TraceFinder

1. Choose **Compound Database > Import Compounds** from the main menu.

   Note If you have unsaved changes in your current compound database, a message prompts you to save the changes. Click **Yes** and continue with the import procedure.

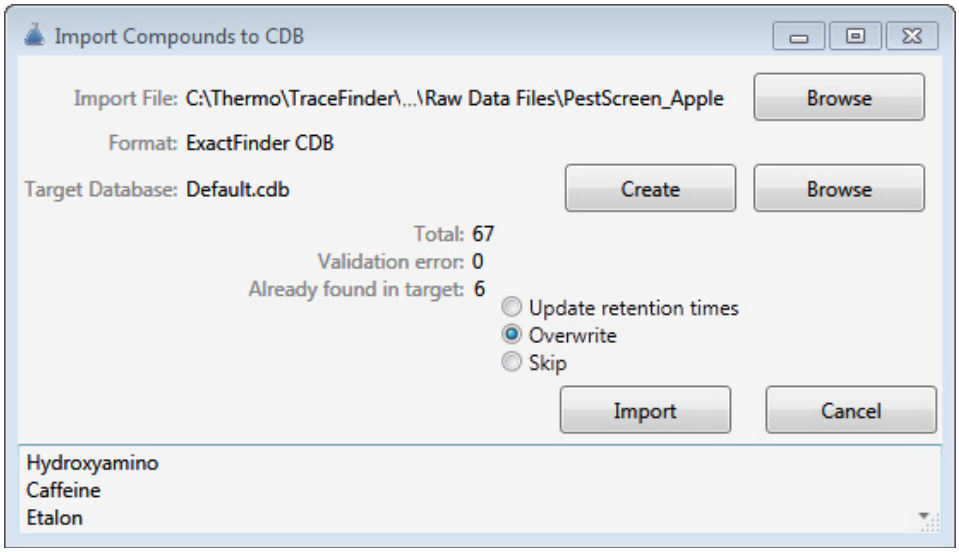
   The Select File to Import into the Current Compound Database dialog box opens.

   You can import compounds from the following file types:

   - ToxID™ Exactive™ CSV
   - ToxID MS2 CSV
   - TraceFinder CSV
   - TraceFinder Mass List XML
   - TraceFinder 4.0 Legacy CDB
   - ExactFinder™ 2.0 CDB
   - .include-masses

2. Locate the CDB, CSV, or XML compound database file that you want to import and click **Open**.

   The Import Compounds to CDB dialog box opens.



Note If the import file is missing required compound information, the application warns that it is unable to parse the file and identifies the missing columns.

3. To select a different compound database, click **Browse,** locate an XML, a CSV, or a CDB compounds file, and click **Open**.

4. To select a different target database, do one of the following:

   a. Click **Browse**.

   b. Locate an XML, a CSV, or a CDB compounds file.

   c. Click **Open**.

   –or–

   a. Click **Create**.

   b. Type a name for a new compound database.

   c. Click **OK**.

5. Confirm that the import file and the target database are correct.

   The dialog box reports the total number of compounds in the import file, the number of compounds with validation errors, and the number of compounds that already exist in the target database.

6. (Optional) Select one of these options:

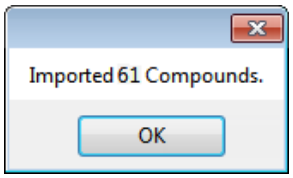
   - **Update Retention Times**: Updates only the retention times for compounds that already exist in the target database.

- **Skip**: Imports only those compounds that do not already exist in the target database.
- **Overwrite**: Replaces compounds that already exist in the target database with the imported compounds.

7. Click **Import**.

   The application imports the compounds from the imported file, adds them to any compounds already in the database, and alphabetically sorts them.

   The application reports the number of imported compounds.



8. Click **OK**.

❖ To import compounds from Skyline, PepFinder™, PinPoint, or mzVault

1. Choose **Compound Database > Import Compounds** from the main menu.

   Note If you have unsaved changes in your current compound database, a message prompts you to save the changes. Click **Yes** and continue with the import procedure.

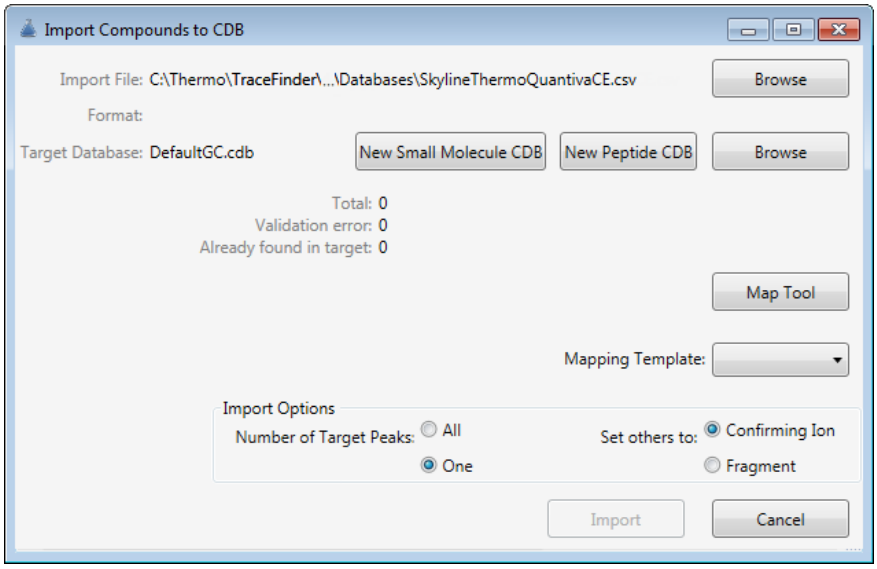
   The Select File to Import into the Current Compound Database dialog box opens.

   You can import compounds from the following file types:

   - Skyline
     - Thermo transition list (Thermo Template)
     - Thermo Quantiva transition list (Thermo Quantiva)
     - Thermo isolation list (isolation list Quantiva)
   - PepFinder 2.0 ion list
   - PinPoint
     - Orbitrap™/LTQ inclusion list
     - Q Exactive multiplexed list
     - Q Exactive multiplexed list with formula
     - Q Exactive/LTQ inclusion list - with charge state
     - Quantiva
     - TSQ™
   - mzVault Export for TF.csv

2. Locate the compound database file that you want to import and click **Open**.

The Import Compounds to CDB dialog box opens.



The application reports that a total of 0 compounds are available for importing. It notifies you that the CSV file is an unrecognized format and that you must use the Mapping Tool. To continue importing compounds from this CSV file to the current compound database, skip to step 5.

3. (Optional) To select a different import file, click **Browse,** locate an XML, a CSV, or a CDB compounds file, and click **Open**.

4. (Optional) To select a different target database, do one of the following:

   a. Click **Browse**.

   b. Locate an XML, a CSV, or a CDB compounds file.

   c. Click **Open**.

   –or–

   a. Click **New Small Molecule CDB** or **New Peptide CDB**.

   > **Note** The application displays the New... buttons only for the configured databases. See Specifying Optional Settings.

   b. Type a name for a new compound database.

   c. Click **OK**.

5. Select a Mapping Template.

   The template maps the unrecognized fields in the imported file to TraceFinder values and reports the number of compounds that it will import.

   In the lower pane, the application reports the values that are not recognized and cannot be imported.

6. (Optional) To edit the mapping template to ignore specific parameters when it imports the compounds, click **Map Tool**.

   The Mapping Tool dialog box opens. See To edit a mapping template.

   In the Mapping Tool, edit the mapped columns and then click **Save Template and Return**.

   The application returns to the Import Compounds to CDB dialog box.

7. (Optional) Specify one of the following Import Options:

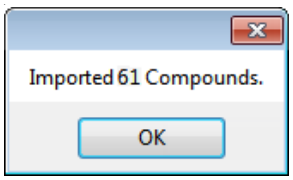
   - To identify all peaks as target peaks, select the **All** option.
   - To identify only the first peak as a target peak and identify all other peaks as confirming ions or fragments, select the **One** option and then select either the **Confirming Ion** or **Fragment** option.

   Note The application displays these options only when you are required to use a mapping template.

8. Click **Import**.

   The application imports the compounds from the imported file, adds them to any compounds already in the database, and alphabetically sorts them.

   The application reports the number of imported compounds.



   Click **OK**.

❖ To edit a mapping template

1. Click **Map Tool**.

   The Mapping Tool Dialog Box opens, displaying a preview of the database using the mapping template that you selected in the Import Compounds to CDB dialog box.

   The preview displays only 20 compound rows, starting with the row specified in the Data Starts on Row box.

Preview

CompoundName	RT	Window	Polarity	PrecursorMass	ProductMass	CollisionEnergy
LSSEAPALFQFDLK	15	30	Positive	787.42123	1157.594471	21.4
LSSEAPALFQFDLK	15	30	Positive	787.42123	1157.604471	22.4
LSSEAPALFQFDLK	15	30	Positive	787.42123	1157.614471	23.4
LSSEAPALFQFDLK	15	30	Positive	787.42123	1157.624471	24.4

2. (Optional) Specify the format that your template uses to display the columns of data:
   - Comma
   - Tab
   - Semicolon
   - Space
   - Other (specify the delimiter used in your template file)
3. (Optional) To change the starting row for the 20 compounds displayed in the preview, type a value in the Data Starts on Row box.

   The preview displays 20 compounds starting with the row that you specified.
4. For each parameter that you want the import operation to ignore, change the column header to **<Ignore Column>**.

   To return the header values to the original values, click **Refresh Preview**.
5. When you have completed your edits, click **Save Template and Return**.
6. When the application reports that the changes to the template were saved, click **OK**.

   The application returns you to the Import Compounds to the CDB dialog box. Continue with step 7 of the procedure To import compounds from Skyline, PepFinder™, PinPoint, or mzVault.

Mapping Tool Dialog Box

Figure 33. Mapping tool dialog box

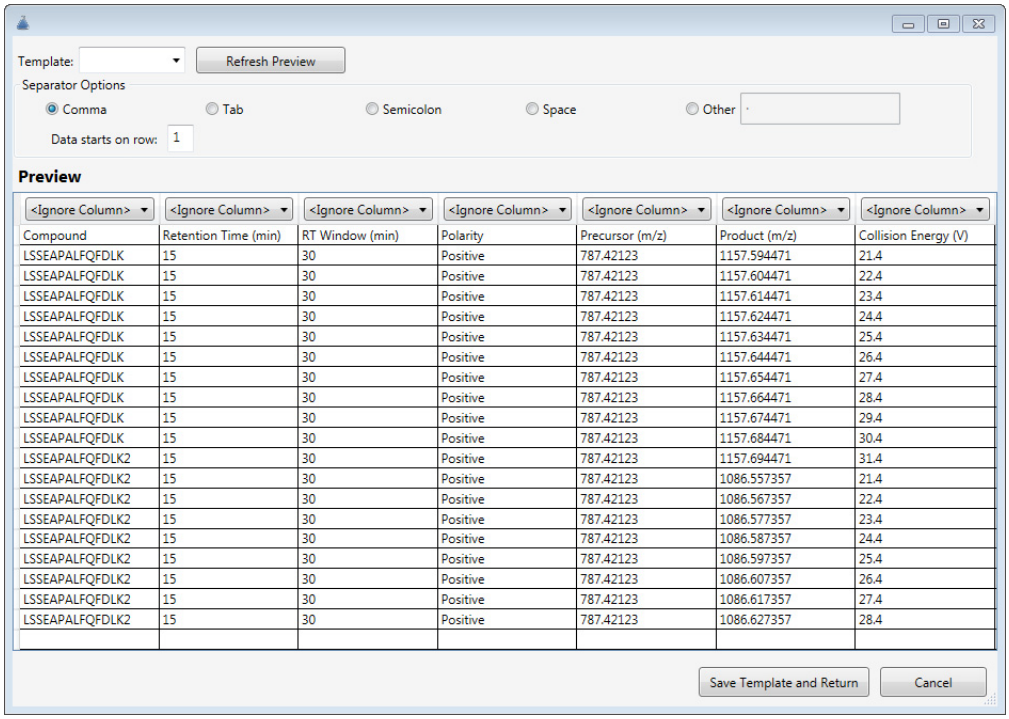


Table 19. Mapping Tool parameters

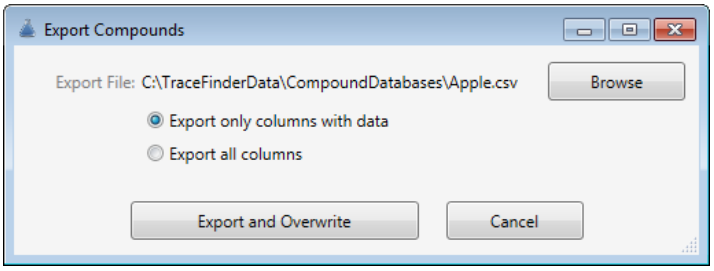
Parameter	Description
Template	Specifies the name of the current mapping template.
Refresh Preview	Returns the header values to the original template values.
Separator Options	Specifies that the import file uses one of the following to separate parameter values: Comma, Tab, Semicolon, Space, Other (user-specified delimiter)
Data Starts on Row	Specifies that the preview displays 20 compounds starting with this row.
Preview	Displays 20 compound rows from the import file.
Save Template and Return	Applies the current settings for importing the compounds and returns to the Import Compounds to CDB dialog box.
Cancel	Does not apply any changes and returns to the Import Compounds to CDB dialog box.

❖ To export all compounds to a CSV file

1. Choose **Compound Database > Export All Compounds to CSV File** from the main menu.

   Note If you have unsaved changes in your current compound database, a message prompts you to save the changes. Click **Yes** and continue with the export procedure.

   The Export Compounds dialog box opens.



2. (Optional) Click **Browse** and locate a different folder or file name where you want to write the exported compound database.

   Each parameter in the compound database editor is represented by a column of data in the spreadsheet. When you export compound data to a CSV file, each parameter is assigned to a column and each compound is assigned to a row.

3. Select one of these options:

   - **Export Only Columns with Data**: Writes only columns that contain nondefault data for at least one compound. This option does not export columns that contain only default data. See Data Columns with Default Values.
   - **Export All Columns**: Writes all columns to the CSV file, including columns that contain no data for any compound.

4. Click **Export**.

   The application stores the database as

   …\TraceFinderData\CompoundDatabases*databaseName*.csv.

An Excel window opens with the compound data in spreadsheet format.

Figure 34. Compound data in an Excel spreadsheet

	A	B	C	D
1	TraceFinder Compound Database Mass List Export			
2				
3	Compound Name	Workflow	Associated Target Peak	MS Order
4	1,2,4-Trichlorobenzene	TargetPeak		ms1
5	1,2,4-Trichlorobenzene	Confirming	1	ms1
6	1,2,4-Trichlorobenzene	Confirming	1	ms1
7	1,3-Dichlorobenzene	TargetPeak		ms1
8	1,3-Dichlorobenzene	Confirming	1	ms1
9	1,3-Dichlorobenzene	Confirming	1	ms1

You can use the tools in the spreadsheet to edit the data in the compound database and then import the data in the CSV file back into the TraceFinder application. If you delete a column from the spreadsheet and then import the CSV file, the application replaces the data in that column with default values. For a list of default values, see Data Columns with Default Values.

❖ To export selected compounds to a mass list

1. Select the compounds that you want to export.
2. Choose **Compound Database > Export Selected Compounds to Mass List** from the main menu.

   Note If you have unsaved changes in your current compound database, a message prompts you to save the changes. Click **Yes** and continue with the export procedure.

   Note If your configured instrument is other than a TSQ, an ISQ™, a Q Exactive, a TSQ Endura™, or a TSQ Quantiva™ mass spectrometer, a message prompts you for the export format: Triple Quadrupole, Q Exactive, TSQ Quantiva/Endura SIM, or TSQ Quantiva/Endura SRM. Select one of the formatting options and click **OK**.

   The Save As dialog box opens.
3. Locate the folder where you want to save the file, type a file name, and then click **Save**.

   The application writes the mass data for the selected compounds to the specified file, using one of the following formats that is compatible with your configured instrument.

   - Triple Quadrupole format

     The application writes the mass data to the specified .xml format file. The data in the exported file matches the TSQ XML data, which you can use in the instrument method editor of a TSQ application.

- Q Exactive format

  The application writes the mass data to the specified .xml.include-masses format file. The data in this file matches the Exactive XML data, which you can use in the instrument method editor of a Q Exactive application.

- TSQ Quantiva/Endura SIM format

  The application writes the mass data to the specified .xml format file. The data in this file matches the TSQ Endura, TSQ Quantiva, and Xcalibur XML data, which you can use in the instrument method editors of the associated applications. The application exports only the following compound parameters to the XML file:
  - Compound Name (as Name in the XML file)
  - Product Mass (as Mass in the XML file)
  - RT range (as StartTime and StopTime in the XML file)
  - Polarity
  - Lens (as TubeLens or S-Lens in the XML file)

- TSQ Quantiva/Endura SRM format

  The application writes the mass data to the specified .xml format file. The data in this file matches the TSQ Endura, TSQ Quantiva, and Xcalibur XML data, which you can use in the instrument method editors of the associated applications. The application exports only the following compound parameters to the XML file:
  - Compound Name (as Name in the XML file)
  - Precursor Mass
  - Product Mass
  - RT range (as StartTime and StopTime in the XML file)
  - Polarity
  - Lens (as TubeLens or S-Lens in the XML file)
  - Collision Energy

Data Columns with Default Values

When you export compounds to a CSV file with the Export Only Columns with Data option, the application writes only columns that contain nondefault data for at least one compound. This option does not export columns that contain only default data.

When you import compounds from a CSV data file that has missing columns, the application replaces the missing columns and uses default values for all compounds.

Table 20. Default values for compound parameters (Sheet 1 of 2)

CDB parameter	Default value
Compound Detail	
Formula	Blank
CAS	Blank

Table 20. Default values for compound parameters (Sheet 2 of 2)

CDB parameter	Default value
Category	Blank
Ionization	None
Response Threshold	5000
Target Peaks	
Precursor Mass	Blank
Collision Energy	0
Adduct	None
Lens	0
Energy Ramp	0
Confirming Peaks	
Precursor	0
Collision Energy	Blank

3

Using Instrument Methods in the Method Development Mode

You can also use the Instrument Method view for quantitation, target screening, and unknown screening methods. An instrument method is a set of experiment parameters that define the operating settings for an autosampler, mass spectrometer, and so on. Instrument methods are saved with a .meth file name extension.

IMPORTANT Do not open the Thermo Foundation Instrument Configuration window while the TraceFinder application is running.

Follow these procedures:

- To open the Instrument View
- To create a new instrument method
- To create a new multiplexing instrument method
- To open an instrument method

❖ To open the Instrument View

1. Click **Method Development** in the navigation pane.

   The Method Development navigation pane opens.

2. Click **Instrument View**.

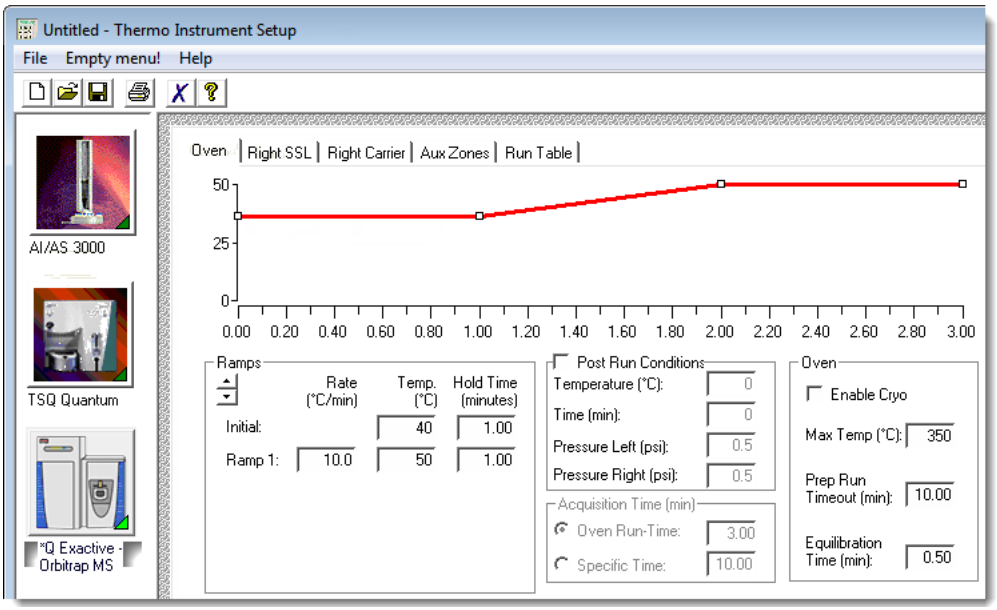
   The Instrument View opens.

❖ To create a new instrument method

1. Choose **File > New Instrument Method** from the main menu.

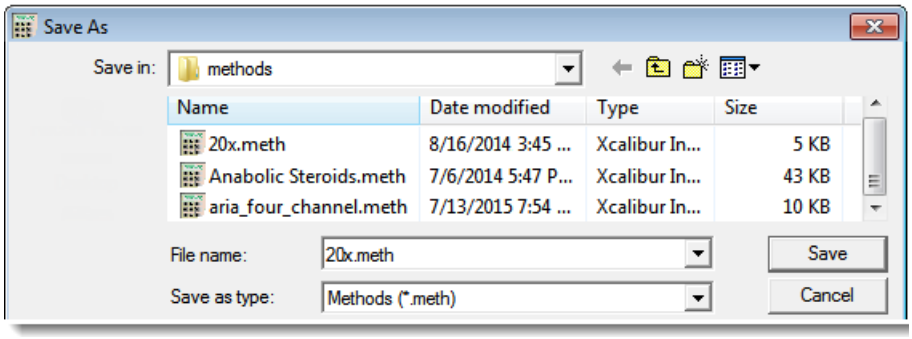
   The Thermo Instrument Setup window opens.

   Figure 35. Example instrument setup showing multiple configured instruments



2. Click the icon for the instrument that you want to use for the method.
3. Edit the values on the instrument page.
4. From the main menu in the Thermo Instrument Setup window, choose **File > Save As**.

   The Save As dialog box opens.

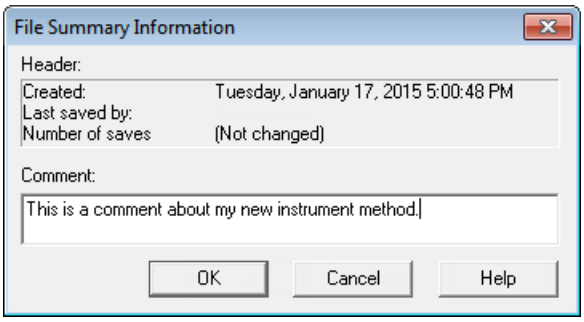


5. Locate a folder for the method.

IMPORTANT Only methods saved to the …\TraceFinderData\InstrumentMethods folder are available from the Instrument Method list on the Acquisition page in the Method View.

6. Select an instrument method name to overwrite, or type a new name for the instrument method, and click **Save**.

   The File Summary Information dialog box opens.



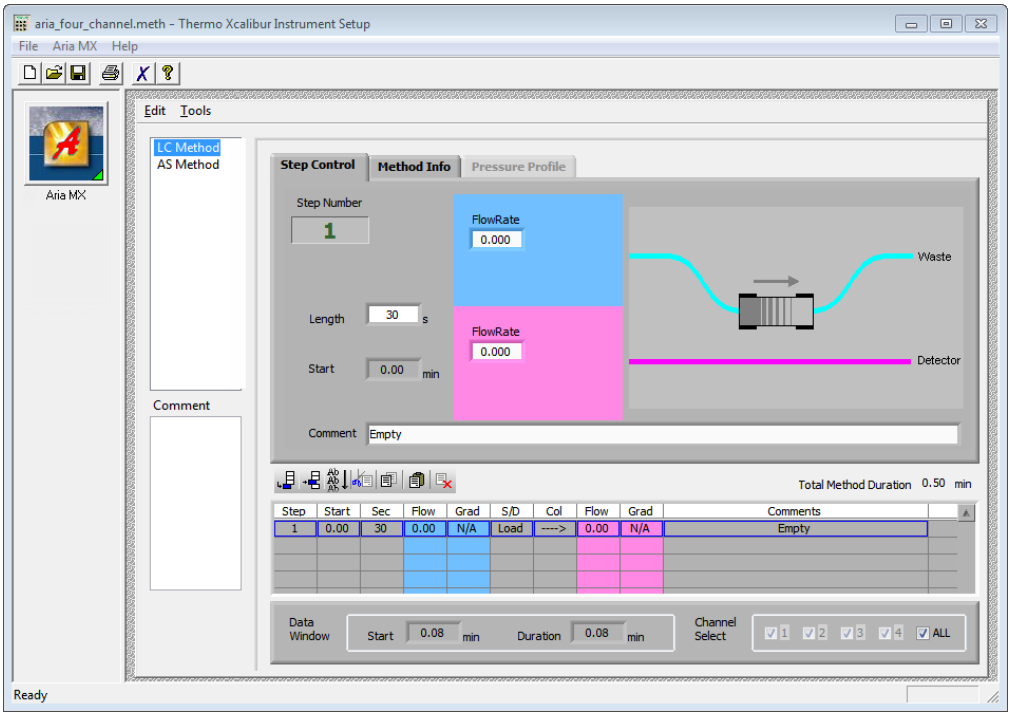
7. (Optional) Type a comment about the new instrument method.
8. Click **OK**.

❖ To create a new multiplexing instrument method

1. Choose **File > New Instrument Method** from the Thermo Instrument Setup main menu.

   The Thermo Xcalibur Instrument Setup window opens.

   Figure 36. Example instrument setup showing a configured multiplexed instrument



2. Click the icon for the instrument that you want to use for the method.

3. Edit the values for the instrument method.

   For information about specifying multiplexing values, refer to the documentation for your multiplexed instrument.

4. Specify the channels that you want to use for acquisition, as in this example:



5. From the main menu in the Thermo Xcalibur Instrument Setup window, choose **File > Save As**.

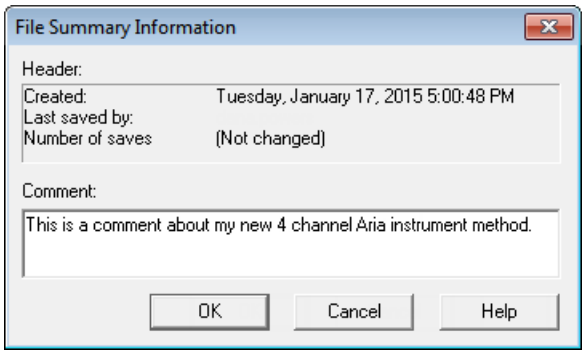
   The Save As dialog box opens.

6. Locate a folder for the method.

   IMPORTANT Only methods saved to the …\TraceFinderData\InstrumentMethods folder are available from the Instrument Method list on the Acquisition page in the Method View.

7. Select an instrument method name to overwrite or type a new name for the instrument method, and click **Save**.

   The File Summary Information dialog box opens.



8. (Optional) Type a comment about the new instrument method.
9. Click **OK**.

❖ To open an instrument method

1. Choose **File > Open Instrument Method**.

   An instrument method browser opens.

2. Select an instrument method from the list and click **Open**.

   The selected method opens in the Thermo Xcalibur Instrument Setup window. You can edit this method and save the changes, or you can save this method with another name.

   Note To open Help for any of your configured instruments, click **Help** on the instrument page.

4

Using the Method Development Mode for Quantitation Methods

Use the method development mode and its associated features for creating and editing a quantitation method. (See Method Development navigation pane.)

The TraceFinder application uses a method to specify the types of acquisition, processing, and reporting that occur with a batch of samples. You can create a method designed specifically to test for compounds in your assay. A quantitation method contains a list of compounds and the settings for detecting, processing, and reporting those compounds.

Contents

- Configuring Method Views
- Opening a Quantitation Method
- Starting a New Quantitation Master Method
- Editing a Quantitation Method
- Saving a Quantitation Method to a New Name
- Creating a Method Template
- Exporting Mass Data

The quantitation method that you create determines how the application works with a set of samples to provide a set of meaningful results. Part of this method, the instrument method, defines how the application acquires raw data. The rest of the method defines how to process the raw data, how the flag information displays the results, and how the reporting functionality defines the output for your data and results.

The application applies your method to a batch, which is a list of one or more samples. Together, the method and batch provide a workflow-oriented approach to the data processing and information reporting for batches of samples.

To speed up the creation of methods, you can create a method template. Using a method template helps you to develop methods faster because the application saves all of your commonly used method settings in a template, such as the number of confirming ions or the use of data-dependent scans.

Figure 37. Method Development navigation pane

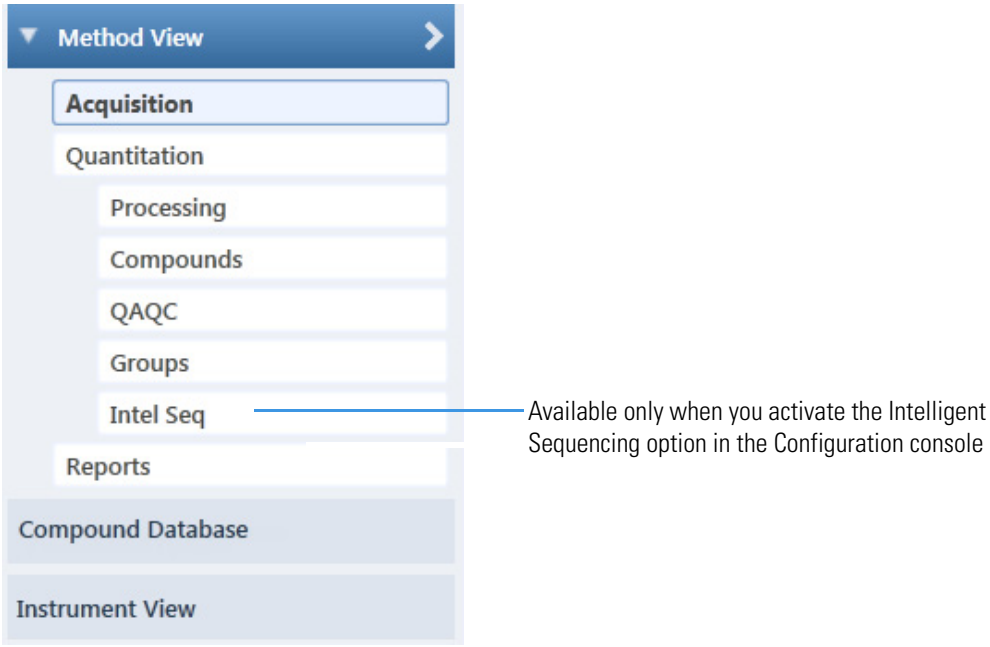


Table 21. Method Development navigation pane commands

Command	Description
Method View	Displays the Method View for the method.
Acquisition	Displays the Acquisition page. See Editing the Acquisition Page.
Quantitation	
Processing	Displays the Processing page. See Editing the Processing Page.
Compounds	Displays the Compounds page. See Editing the Compounds Page.
QAQC	Displays the QAQC page. See Editing the QAQC Page.
Groups	Displays the Groups page. See Editing the Groups Page.
Intel Seq	Displays the Intelligent Sequencing page. See Editing the Intelligent Sequencing Page. Available only when you activate the Intelligent Sequencing option in the Configuration console.
Reports	Displays the Reports page. See Editing the Reports Page.
Compound Database	See Chapter 2, "Using Compound Databases in the Method Development Mode."
Instrument View	See Chapter 3, "Using Instrument Methods in the Method Development Mode."

Configuring Method Views

You can choose which views and pages you want to display in the Method Development mode. The application then displays only the information from these views and pages in the Analysis mode. For example, when you clear the check box for Fragments in the Master Method Configuration dialog box, the Compound Details pane in the Analysis mode does not show a Fragments page.

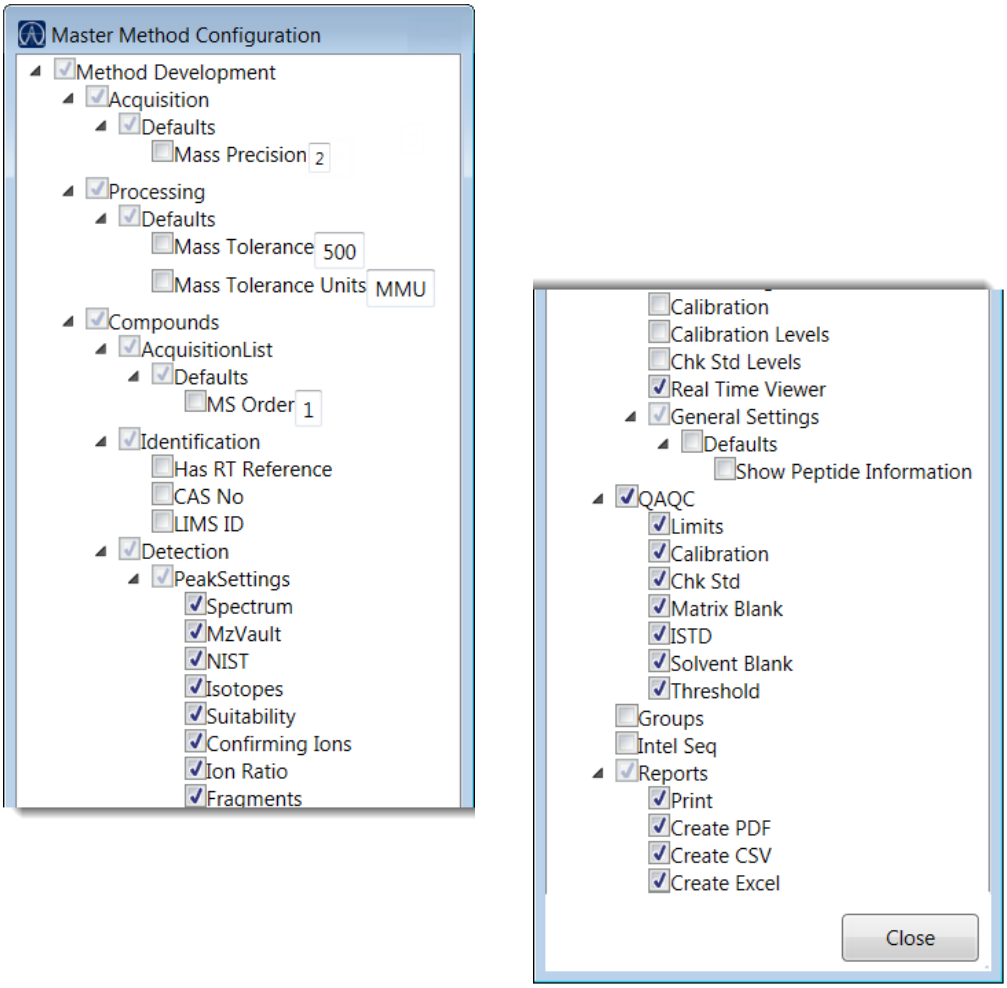
❖ To specify which views to display

1. Choose **Tools > Configure Method Views** from the main menu, or click the **Configure Method Views** icon, , in the toolbar.

   Note You must have an open method to see the Configure Method Views command on the Tools menu.

   The Master Method Configuration dialog box opens.

   Figure 38. Master Method Configuration dialog box



   All views and pages are selected by default.

2. Clear the check box for each view or page that you do not want to display.

   The application immediately applies the configurations you specified.

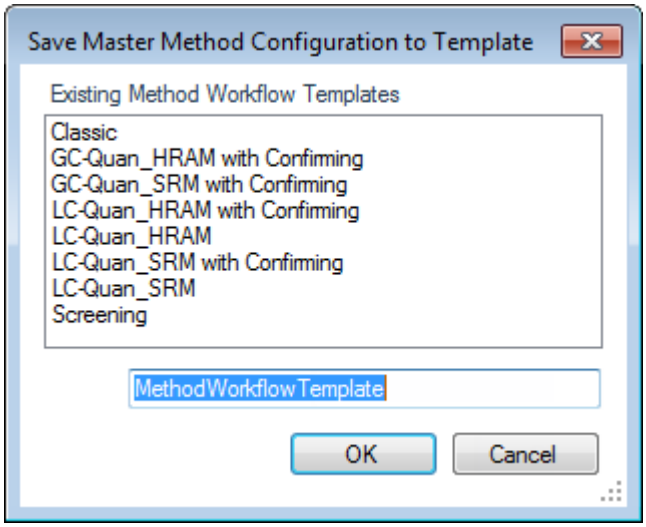
   Note A grayed-out check box indicates that the view or page name is required and cannot be cleared.

3. When you finish specifying the views and pages that you want to display, click **Close**.

Creating a Master Method Workflow Template

You can save any Method Development mode configuration as a workflow template.

1. Choose **Tools > Configure Method Views** to configure the Quantitation pages that you want to view.
2. Click the **Configuration** tab, , on the Compounds page to configure the lower-level Compounds pages that you want to view.
3. Choose **File > Save Master Method Configuration to Template** from the main menu.



4. Type the name of your workflow template, which overwrites the default name *MethodWorkflowTemplate*.
5. Click **OK**.

   When you create a new method, the Select Workflow Template list will include this new workflow template.

Opening a Quantitation Method

Use the TraceFinder application to open a method that was created and saved in the current application or converted from previous versions of TraceFinder. To convert legacy methods, refer to Chapter 2, "Getting Started" in the *TraceFinder User Guide*.

❖ To open a quantitation method in Method Development mode

1. Click **Method Development** in the navigation pane.

   For descriptions of all the features in the Method Development navigation pane, see Starting a New Quantitation Master Method.

2. Choose **File > Open > Master Method** from the main menu.

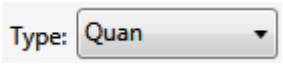
   Tip You can also open one of your most recently used method files. Choose **Files > Recent Files > *Method***.

   The Open Master Method Dialog Box opens.

3. (Optional) Select a different repository.

   The default repository is specified in the Administrator Console. Refer to the instructions in the *TraceFinder Administrator Console User Guide*.

4. Select **Quan** in the Type list.

   The method list displays all quantitation methods in the selected repository.

5. Select a quantitation method and click **Open**.

   The Acquisition Page for the selected method opens.

Open Master Method Dialog Box

Figure 39. Open Master Method dialog box

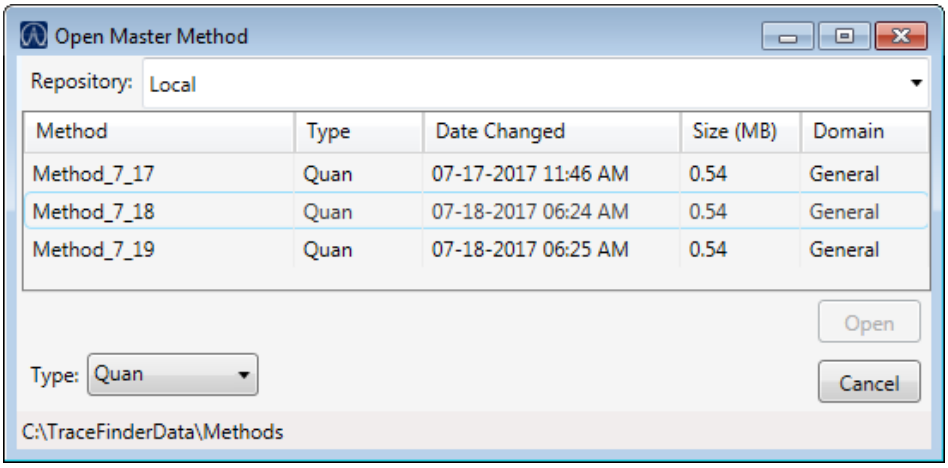


Table 22. Open Master Method dialog box parameters

Parameter	Description
Repository	The data repository where you want to store the master method.
Method	Name of the methods for the selected type.
Type	Type of method: Quan, Screening, or Unknown Only.
Date Changed	Date the method was last updated.
Size	Size of the method in megabytes.
Domain	TraceFinder domain for which the method was created.
Type	Type of method to display: Quan, Screening, Unknown Only, or Any.
Path	Path to the selected method in the …\TraceFinderData\Methods folder.

Starting a New Quantitation Master Method

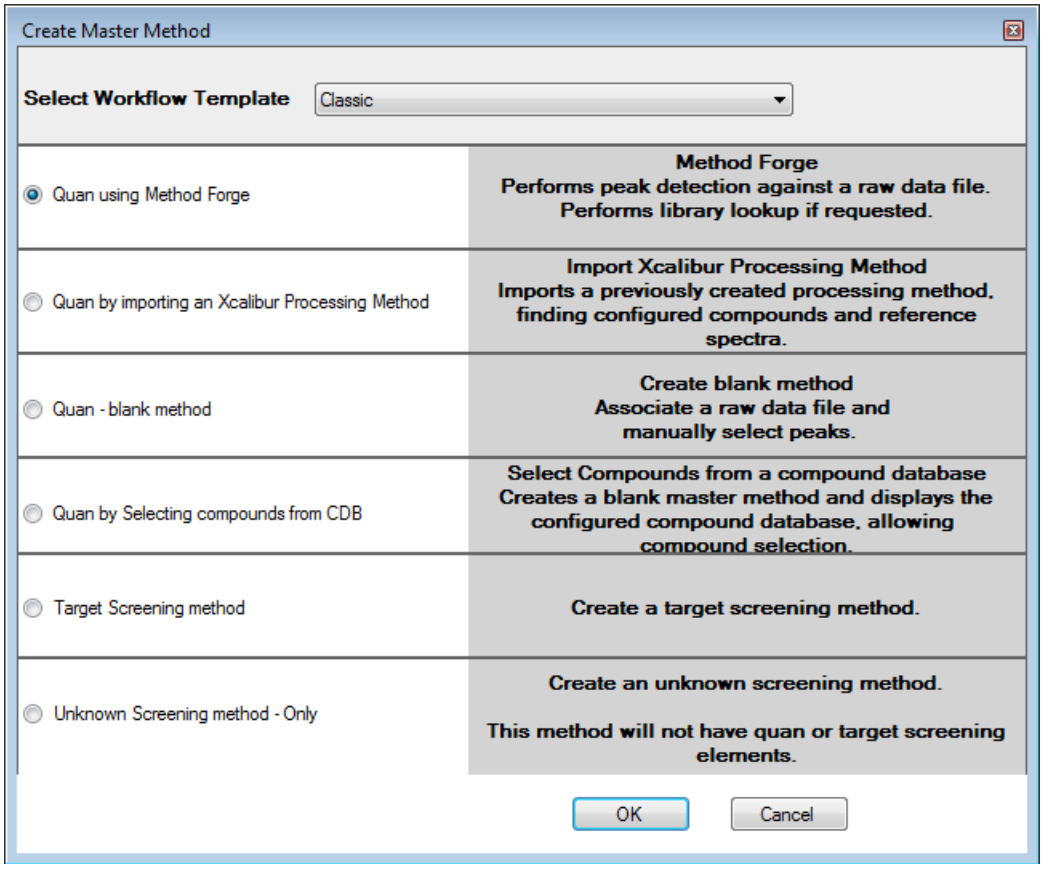
To create or start a specific method, select a workflow template and then select as applicable from one of the four different quantitation method procedures (or techniques) in the Create Master Method dialog box.

- Selecting a Workflow Template
- Starting a New Method with Method Forge
- Importing an Xcalibur Master Method
- Starting a Blank Method
- Starting a Method Using the Compound Database

Then, use the features of the Method View to complete and save the method.

To open the Create Master Method dialog box, choose **File > New > Master Method** from the main menu.

Figure 40. Create Master Method dialog box



IMPORTANT The Unknown Screening Method - Only technique is displayed only when you select the Allow Unknown Screening option in the application configuration console. See Processing Options.

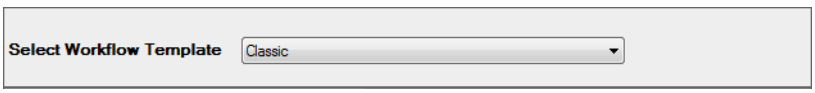
Selecting a Workflow Template

The Create Master Method dialog box displays the name of the current workflow template. You can choose any of the workflow templates that you previously saved to the following folder:

…\TraceFinderData\Templates\Workflows

❖ To select a workflow template

Select a workflow template from the list.



The Create Master Method dialog box displays only the method creation techniques that you can use with the selected workflow template. The Classic workflow makes all method creation techniques available.

Note Your laboratory might also have custom workflows that support specific types of methods.

Starting a New Method with Method Forge

With the Method Forge Dialog Box, you can manually create a new quantitation method by selecting peaks, selecting multiple compounds, renaming peaks, or comparing mass spectra from the library searches. You can also choose to let the application automatically create a method.

When the application automatically creates a method, it performs the following functions:

- Reviews your raw data file and identifies compounds that are present in your sample.
- Uses your mass spectral reference libraries to assign compound names and CAS numbers.
- Uses mass spectral information to select potential quantification and confirming peaks and a reference mass spectrum for the compound.

Note When the identified peak is from an analog trace, the application does not perform a library search and does not identify confirming peaks.



Tip (Animation) To view "Starting a New Method with Method Forge," choose **Help > Animations**.

Follow these procedures:

- To automatically select compounds to create a new method
- To manually select compounds to create a new method

❖ To automatically select compounds to create a new method

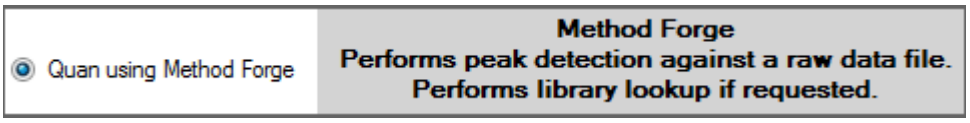
1. Choose **File > New > Master Method**.

   The Create Master Method dialog box opens.

2. Select a workflow template from the list.

   In addition to any custom workflows you created in your laboratory, the Classic workflow and any of the Quan workflows support the Quan Using Method Forge option.

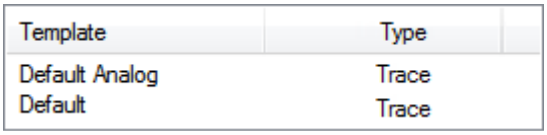
3. Select the **Quan Using Method Forge** option and click **OK**.



The Method Forge Dialog Box opens.

Use Method Forge to create a method from an existing raw data file or to create a new raw data file to use for the method.

Each method requires a processing method template. The application displays all saved method templates in the template list.



4. To select a processing method template, do one of the following:

   - Click **Open Method Template Editor** to create and save a new method template.

     See Creating a Method Template.

   - Select a method template from the template list, and click **Open Method Template Editor** to edit and save the method template.

   - Select a method template from the template list.

   The selected template is now selected by default each time you open the Method Forge dialog box until you restart the application.

5. Do one of the following:

   - To change only the method name, type a new name in the Method Name box.

     Note By default, the application names the method with the raw data file name.

   - To change only the repository, click **Select Master Method Location** and choose a different repository.

   - To change both the repository and the method name, click **Select Master Method Location,** choose a different repository, and either type a new method name or select an existing method name to overwrite.

6. Select the **Automatically Create the Master Method** check box.

7. Specify a raw data file by doing one of the following:

   a. In the Raw File Selection area, select the **Use an Existing Raw Data File** option.

   b. Browse to and select a raw data file to use for the method.

   c. Go to step 8.

   –or–

a. In the Raw File Selection area, select the **Acquire a New Raw Data File** option.

b. From the Instrument method list, select a method (.meth) file to use for acquiring the data.

c. In the Raw Filename box, type the name of the file where the application will write the raw data file.

d. In the Path box, type a path or browse to and select a folder where the application will save the raw data file.

e. (Optional) Type a comment about the acquired sample or the data file.

8. To acquire a new raw data file, do one of the following:

   - Select the **Manual Injection** option.

   –or–

   - Specify the autosampler settings:

     a. Select the **Use Autosampler** option.

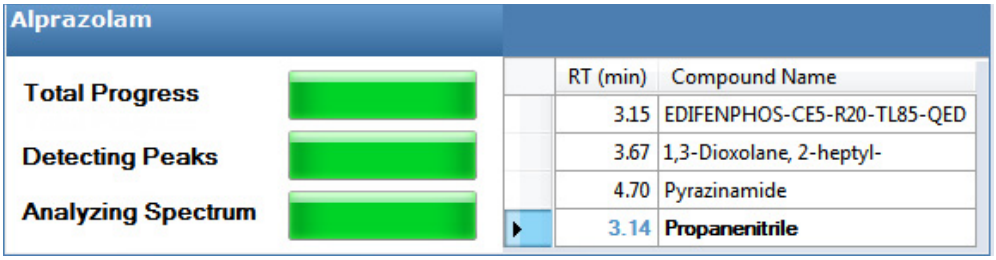
     b. In the Vial Position box, type a vial position.

     c. In the Injection Volume box, type an injection volume.

        Valid range: 0.1 through 5000 µL

9. To automatically create the method, click **OK** (or **Overwrite**).

   As the Method Forge creates the method, it displays the following status bars:



   - For analog peaks, the Method Forge displays the detected peak as *Peak@(RT)Analog*. The Method Forge does not perform a library search for peaks found in analog traces.
   - For mass spectral peaks, the Method Forge process searches the associated libraries and displays the identified compound names instead of the peak times.

   When you selected to acquire a new raw data file in step 6, the application completes the acquisition and then performs the following processes.

   Note If you selected a previously acquired raw data file in step 6, the application immediately begins these processes.

   - Method Forge performs peak detection, library searching (except for analog peaks), and identification of characteristic ion and reference spectra.

- Method Forge then loads this information into a new method and opens the Acquisition Page of the Method View.

When the application completes these processes, it returns to the Method View Acquisition page.

10. (Optional) Type a Lab Name and Assay Type.
11. From the Instrument Method list, select an instrument method.
12. From the Tune/Breakdown Method list, select a tune/breakdown method.

    By default, the tune/breakdown method is the same as the selected instrument method.
13. To save the new method, choose **File > Save** from the main menu.

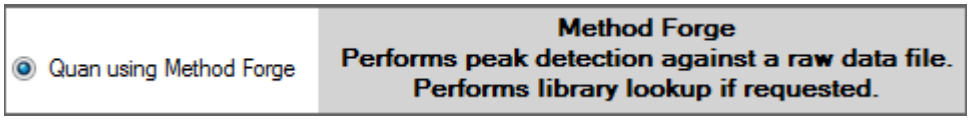
    For a detailed description of how to modify all the parameters in a method, see Editing a Quantitation Method.

❖ To manually select compounds to create a new method

1. Choose **File > New > Master Method**.

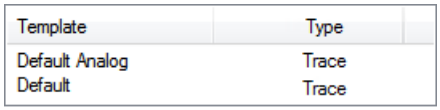
   The Create Master Method dialog box opens.
2. Select a workflow template from the list.

   In addition to any custom workflows you created in your laboratory, the Classic workflow and any of the Quan workflows support the Quan Using Method Forge option.
3. Select the **Quan Using Method Forge** option and click **OK**.



   The Method Forge dialog box opens. For detailed descriptions of all the features in the Method Forge dialog box, see Method Forge Dialog Box.

   Each method requires a qualitative peak processing template. The application displays all saved method templates in the template list.



4. To select a qualitative peak processing template, do one of the following:
   - Click **Open Method Template Editor** to create and save a new method template.

     See Creating a Method Template.
   - Select a method template from the template list, and click **Open Method Template Editor** to edit and save the method template. The application saves the methods to the following folder:

     …\TraceFinderData\Templates\Methods\EFS

- Select a method template from the template list.

The selected template is now selected by default each time you open the Method Forge dialog box until you restart the application.

5. Select the **Name the Master Method** check box and type a name for your method.

   You can enter a new method name, or you can enter an existing method name and overwrite it when you create the method. If you do not select this option, the method is named for the raw data file used to create the method.

   IMPORTANT When you select the Name the Master Method check box, you must enter a method name. To let the application name the method with the raw data file name, be sure to clear the Name the Master Method check box.

6. Ensure that the **Automatically Create the Master Method** check box is cleared.
7. To select a raw data file, browse to the file location.
8. To manually create the method, click **OK** (or **Overwrite**).

   The Method forge results view opens, listing all peaks found in the raw data file.

   For each peak listed in the RT column, the application displays a list of possible matches in the Libraries pane. The application selects the best match, displays the name in the Compound Name list, and displays the peak spectrum for that compound in the first Libraries pane.

Figure 41. Method forge results view

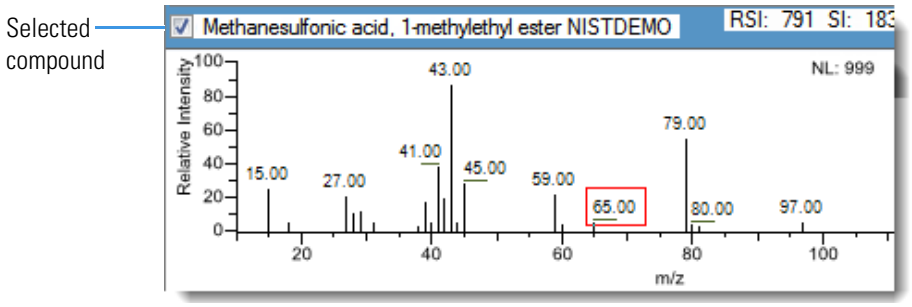


9. (Optional) To use a library compound other than the compound chosen by the application, do the following:

   a. Select the peak in the RT column.

   b. In the Libraries pane, scroll to the spectrum for the compound that you want to use.

c. Select the check box in the header of the spectrum pane.



d. Repeat these steps for each peak that you want to replace.

10. In the Compound Name list, use the CTRL or SHIFT keys to select each compound that you want to include in the method compound.

> **Note** When you select multiple compounds, the method forge results view does not display any spectrum panes.

11. (Optional) To exit the method forge results view without creating a method, click **Cancel**.

    The method forge results view closes and the application returns to the Method View without creating a method.

> **Note** To return to the method forge results view to create a method from the same results, choose **Method View > View Method Forge Results** from the main menu.

12. Click **Create**.

    The application uses all selected compounds to create the method and displays the Acquisition Page of the Method View.

13. From the Instrument Method list, select an instrument method.

14. From the Tune/Breakdown Method list, select a tune/breakdown method.

    By default, the tune/breakdown method is the same as the selected instrument method.

15. To save the new method, choose **File > Save** from the main menu.

    For a detailed description of how to modify a method, see Editing a Quantitation Method.

Method Forge Dialog Box

Use the Method Forge dialog box to create a new method.

Figure 42. Method Forge dialog box

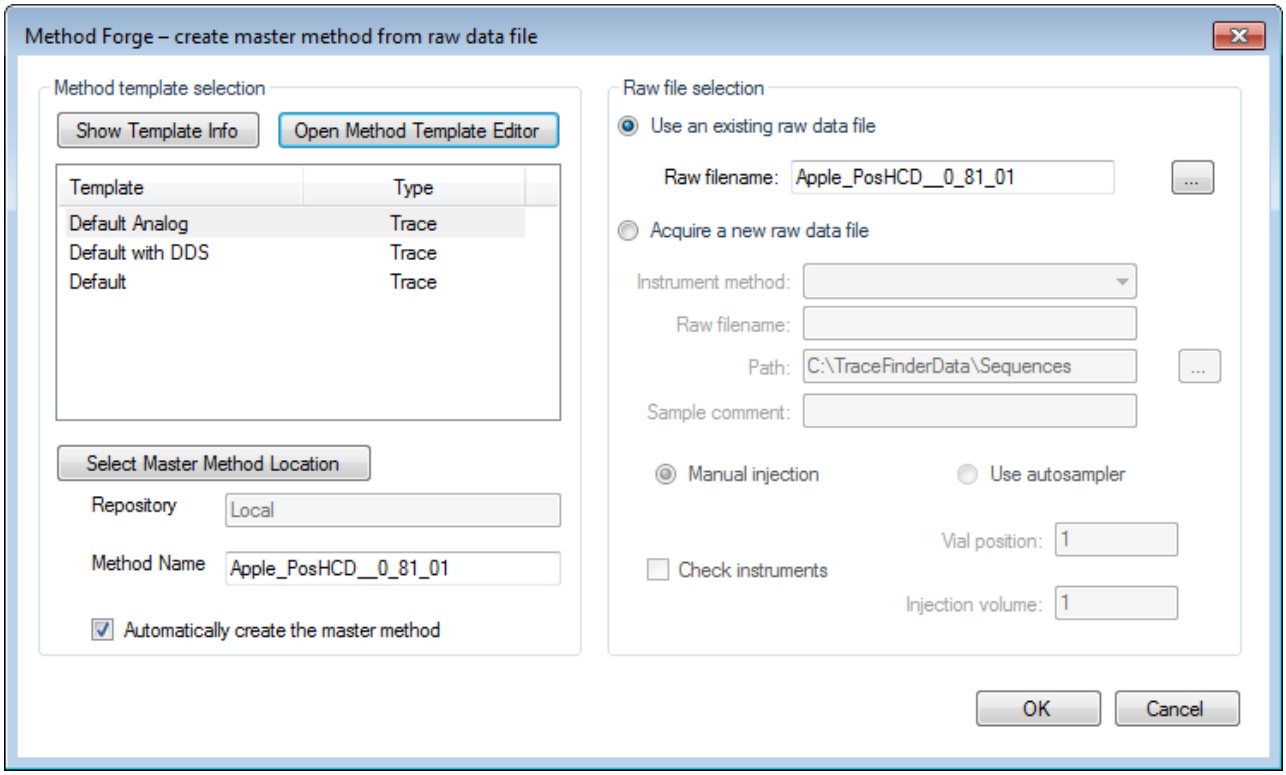


Table 23. Method Forge dialog box parameters (Sheet 1 of 2)

Parameter	Description
Method Template Selection	
Show Template Info	Displays the parameters used in the selected template.
Open Method Template Editor	Opens the Method Template Editor, where you can edit the currently selected method template. See Creating a Method Template.
Template	Specifies the method template to use to create this method. All methods require a method template.
Select Master Method Location	Opens the Save Master Method As dialog box where you can choose a repository and name for the method.
Repository	Displays the selected repository for saving the method.
Method Name	Specifies the name for the new method. If you do not specify a method name, the application uses the raw data file name for the method.
Automatically Create the Master Method	Specifies that when acquisition is completed, Method Forge performs peak detection, library searching, and identification of characteristic ion and reference spectra. This information is loaded into a new method. This process occurs immediately when you specify an existing raw data file.

Table 23. Method Forge dialog box parameters (Sheet 2 of 2)

Parameter	Description
Raw File Selection	
Use an Existing Raw Data File	Activates the Raw Filename box where you can select which raw data file to use to create the method.
Acquire a New Raw Data File	Activates functions to acquire data for the new raw data file used to create the method.
Instrument Method	Specifies the saved method (.meth) file used for acquiring the data.
Raw Filename	Specifies the file name where the application writes the raw data.
Path	Specifies the location where the application saves the raw data file.
Sample Comment	(Optional) Specifies a comment about the acquired sample or the data file.
Manual Injection	Performs a manual acquisition.
Use Autosampler	Performs an autosampler acquisition.
Vial Position	Specifies the tray vial number used for the autosampler acquisition.
Injection Amount	Specifies the volume (in milliliters) injected by the autosampler acquisition.
Check Instruments	Opens the Submit to Acquisition dialog box that prompts you to prepare the instrument before you acquire the sample used to create a method. Available only when you select the Acquire a New Raw Data File option.
Function Buttons	
Overwrite	Overwrites the specified method name. This function is activated only when the specified method name already exists.
OK	Creates a method using the data and parameters that you specified.
Cancel	Closes Method Forge and does not create a method.

Importing an Xcalibur Master Method

You can create a new method from an existing Xcalibur processing method.

Tip (Animation) To view "Importing an Xcalibur Master Method," choose **Help > Animations**.

❖ To import an Xcalibur method

1. Choose **File > New > Master Method** from the main menu.

   The Create Master Method dialog box opens.

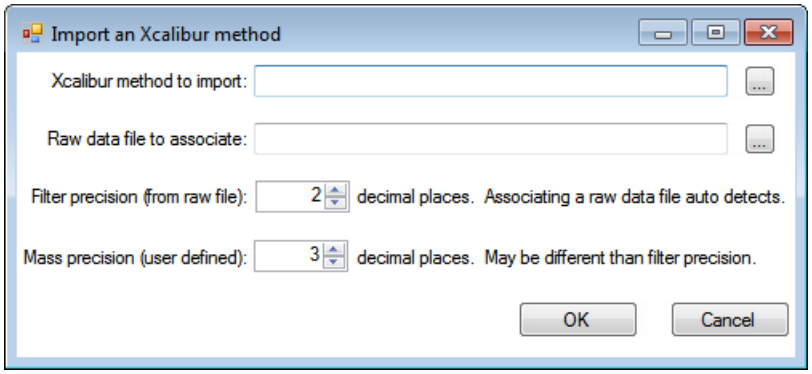
2. Select a workflow template from the list.

   In addition to any custom workflows you created in your laboratory, the Classic workflow and any of the Quan workflows support the option labeled Quan by Importing an Xcalibur Processing Method.

3. Select the **Quan by Importing an Xcalibur Processing Method** option and click **OK**.



   The Import an Xcalibur Method dialog box opens.



4. For the Xcalibur Method to Import box, browse to the location of the Xcalibur processing method file, and open the file.

   The application imports the compound information from the Xcalibur method file.

5. (Optional) For the Raw Data File to Associate box, browse to the location of a raw data file to associate with the method (or select from the list of previously associated raw data files), and open the file.

   To assure that this raw data file is always available to the method (for example, if you move the method to another system), the application saves the raw data file in the Methods folder:

   …\TraceFinderData\Methods*Methodname*

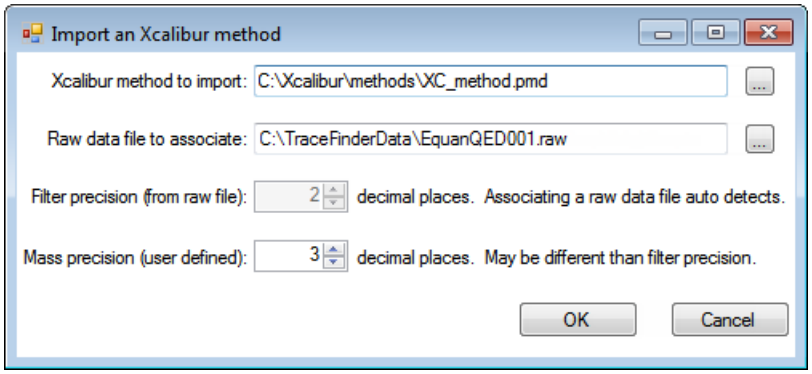
6. (Optional) Change the number of decimal places in the Filter Precision box.

   You can set the number of filter precision decimal places to any integer from 2 through 5.

   Note When you select a raw data file to associate, the application reads the filter precision from the file and this feature is not available.

7. (Optional) Change the number of decimal places in the Mass Precision box.

   You can set the number of mass precision decimal places to any integer from 2 through 6.



   Note When you associate a raw data file, the application reads the filter precision from the associated file so that you cannot change the Filter Precision value.

8. Click **OK**.

   The application adds all compounds found in the imported Xcalibur method and displays the Acquisition Page of the Method View.

9. From the Instrument Method list, select an instrument method.

10. From the Tune/Breakdown Method list, select a tune/breakdown method.

    By default, the tune/breakdown method is the same as the selected instrument method.

11. To save the new method, choose **File > Save** from the main menu.

    For a detailed description of how to modify a method, see Editing a Quantitation Method.

Starting a Blank Method

You can use the compounds in a previously acquired raw data file to create a new blank method.

Tip (Animation) To view "Starting a Blank Method," choose **Help > Animations**.

❖ To start a blank method

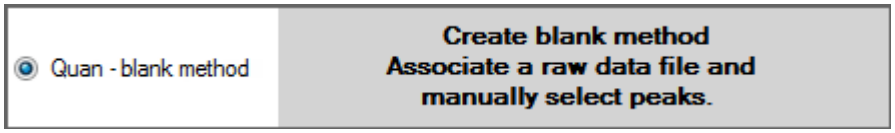
1. Choose **File > New > Master Method**.

   The Create Master Method dialog box opens.

2. Select a workflow template from the list.

   In addition to any custom workflows you created in your laboratory, the Classic workflow and any of the Quan workflows support the Quan – Blank Method option.

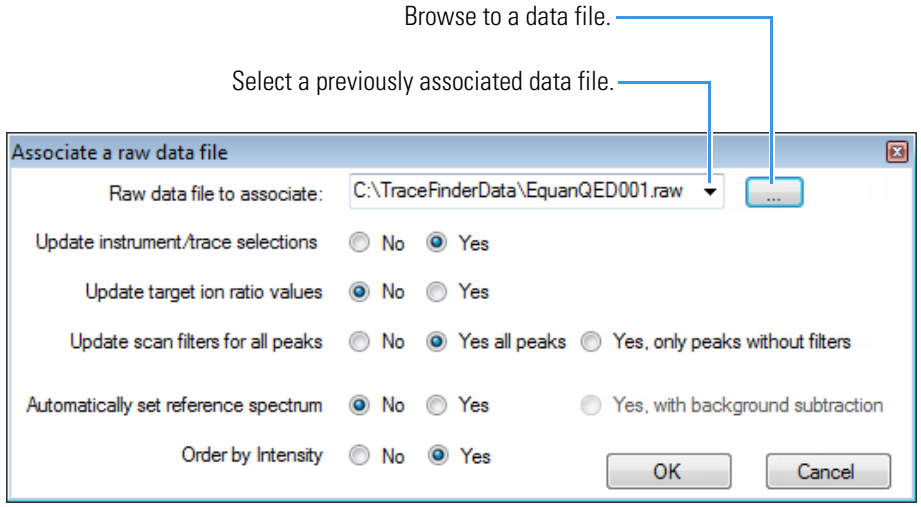
3. Select the **Quan – Blank Method** option and click **OK**.



   The Method View for a new, unnamed method opens. This method has no associated data. You can use the compounds in a previously acquired raw data file to create a new method.

4. From the Method View menu, choose **Associate a Raw Data File**.

   The Associate a Raw Data File dialog box opens.



5. Browse to a raw data file to associate with the method (or select from the list of previously associated raw data files) and open the file.

   To assure that this raw data file is always available to the method (for example, if you move the method to another system), the application saves the raw data file in the Methods folder:

   …\TraceFinderData\Methods*Methodname*

6. Select the update options to use for creating your method:

   - **Update Instrument/Trace Selections**: Reads the Detector and Trace options from the associated raw data file. On the Detection page, only detector types and traces that are defined in the raw data file are available. For detailed descriptions of the available Detector and Trace values, see Trace Selection.
   - **Update Target Ion Ratio Values**: Reads the ion ratio values from the associated raw data file.
   - **Update Scan Filters for All Peaks**:
     - **Yes, All Peaks** updates all peaks to use the scan filters from the associated raw data file.
     - **Yes, Only Peaks Without Filters** updates only peaks without scan filters to use the scan filters from the associated raw data file; it does not override any existing scan filter.
   - **Automatically Set Reference Spectrum**: Reads a reference spectrum from the associated raw data file.

     When you select Yes, with Background Subtraction, the application uses the background-subtracted reference spectrum during quantitative processing and reports the background-subtracted reference spectrum (indicated with BS in the scan heading) as the last scan for each compound in the Quantitation Report - 2 report.

     Note The background subtraction option is available only when you select a background subtraction method on the Acquisition page in the method. See Editing the Acquisition Page.

   - **Order by Intensity**: Reads intensity values from the associated raw data file and lists the peaks with the greatest intensity first. Intensity is based on either peak height or peak area, as specified for the method (see Detection Algorithm).
     - When a compound contains only target peaks, the most intense ion is listed first.
     - When a compound contains both target peaks and confirming peaks, the most intense target peak is listed first, followed by its ordered confirming peaks. The second most intense target peak is listed next followed by its confirming peaks, and so forth.
     - When you also select the Update Target Ion Ratio Values option, the application orders the ions by intensity first and then updates the target ion ratios.

Note You can use the Order by Intensity option with required confirmation criteria only when all peaks are target peaks. Otherwise, the application ignores this option. See To specify confirmation criteria for compounds.

Options that are set to No use the standard values in the method.

Note When you process a sample with this method and then manually integrate a peak, the application updates only the integration and does not change the displayed peak unless you use Associate a Raw Data File again.

7. Click **OK**.

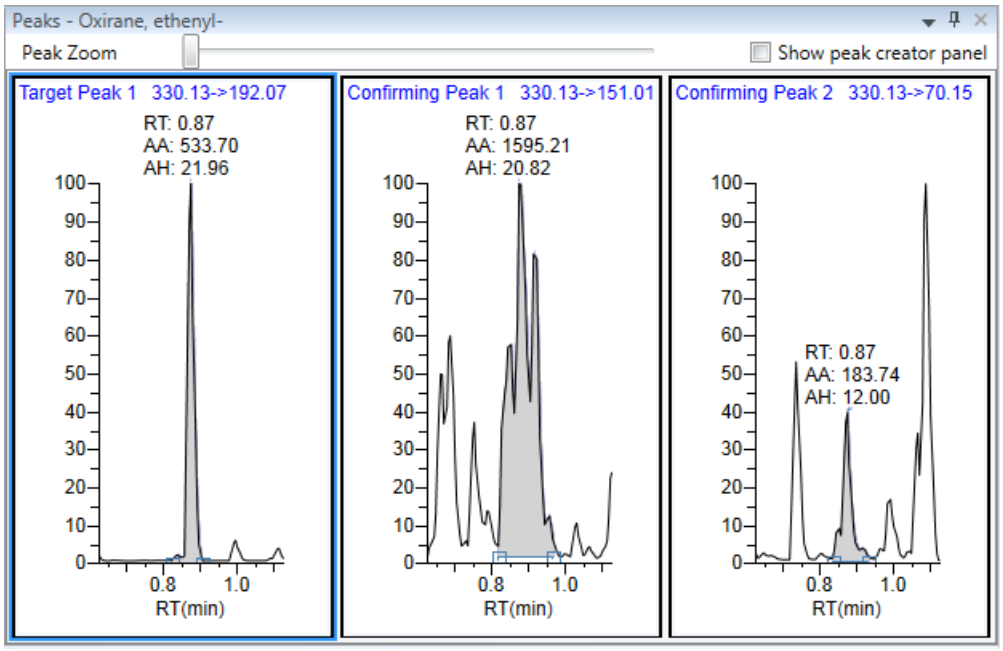
   The application displays the Acquisition page of the Method View.

8. Click **Compounds** in the navigation pane.

   The method must include at least one compound.

9. Click the **Detection** tab.

   The Detection page shows an empty Compounds pane. The Peaks pane displays the chromatographic data for the compounds in the raw data file.



10. Select the **Show Peak Creator Panel** check box.

    The Manual Peak Creator pane opens.

11. (Optional) Select a different trace.

12. (Optional) Select a filter from the Filter list.

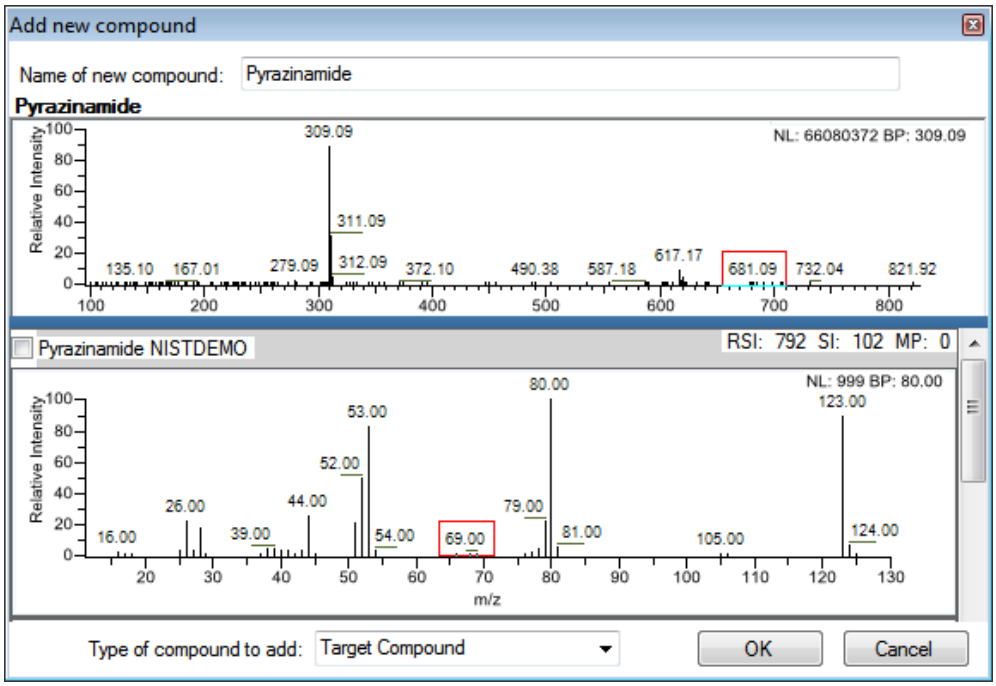
13. In the Peaks chromatogram, click to select the peak that represents the compound that you want to add to the method.

14. Right-click and choose **Add This Peak as New Compound**.

    The application performs a library search for the selected compound and uses the first match it finds as the compound name, the base peak of the mass spectrum as the target peak, and the second and third largest ions as the confirming peaks.

    - When the name of the first match does not exist in the method, the application adds this compound to the method and displays the name in the Compound list. You can now view and edit the parameters for this compound.
    - When the name of the first match is already in the method, the Add New Compound dialog box opens. You cannot overwrite a compound name in the method. If the selected peak already exists in the method, you must give it a new name to add it to the method. Or, you can select a different compound to add to the method, following step 15 through step 17.

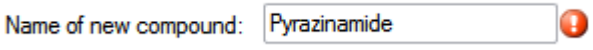
Figure 43. Add New Compound dialog box



15. Do one of the following:

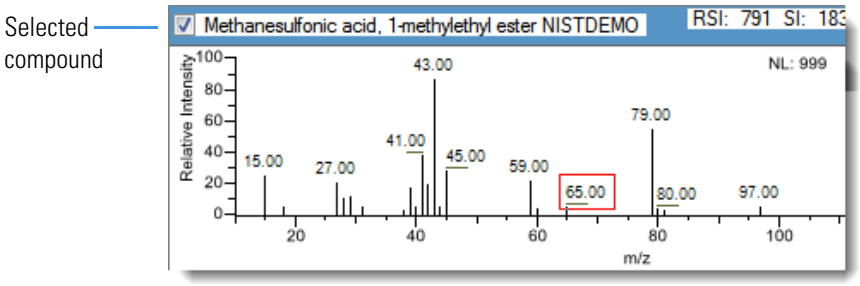
    - Type a unique name for the first matched compound.

      The application displays a red warning when the selected compound name already exists in the method. You cannot overwrite the compound name, and you cannot create a duplicate name in the method.

–or–

- To use a compound other than the first matched compound, scroll to the spectrum for that compound and select its corresponding check box in the title bar of the spectrum pane.



16. In the Type of Compound to Add list, select a compound type.
17. Click **OK**.
18. Repeat this procedure for each compound that you want to add to the method.

    For detailed descriptions of all the features on the Detection page, see Editing the Compounds Page.
19. Click **Acquisition** in the navigation pane.

    The Acquisition Page for the method opens.
20. From the Instrument Method list, select an instrument method.
21. From the Tune/Breakdown Method list, select a tune/breakdown method.

    By default, the tune/breakdown method is the same as the selected instrument method.
22. To save the new method, choose **File > Save** from the main menu and name the method.

    For a detailed description of how to modify the parameters in a method, see Editing a Quantitation Method.

Starting a Method Using the Compound Database

You can select compounds from a compound database to create a new method.

Tip (Animation) To view "Starting a Method Using the Compound Database," choose **Help > Animations**.

❖ To select compounds from the compound database

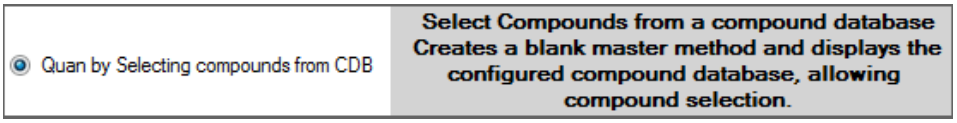
1. Choose **File > New > Master Method** from the main menu.

   The Create Master Method dialog box opens.

2. Select a workflow template from the list.

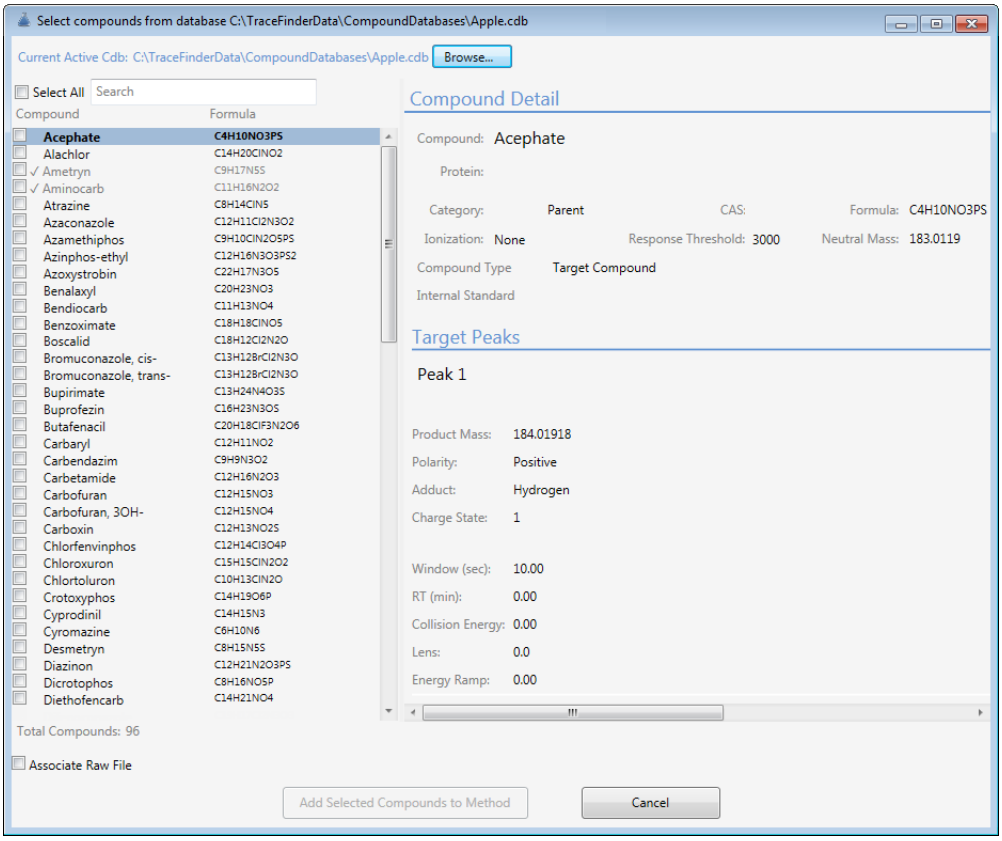
   In addition to any custom workflows you created in your laboratory, the Classic workflow and any of the Quan workflows support the option labeled Quan by Selecting Compounds from CDB.

3. Select the **Quan by Selecting Compounds from CDB** option and click **OK**.



   The Select Compounds from Database dialog box opens, listing all the compounds defined in the current compound database.

Figure 44. Select Compounds from Database dialog box



4. Click **Browse** and locate the compound database that you want to use.

5. Select the check box for each of the compounds that you want to add to the method.

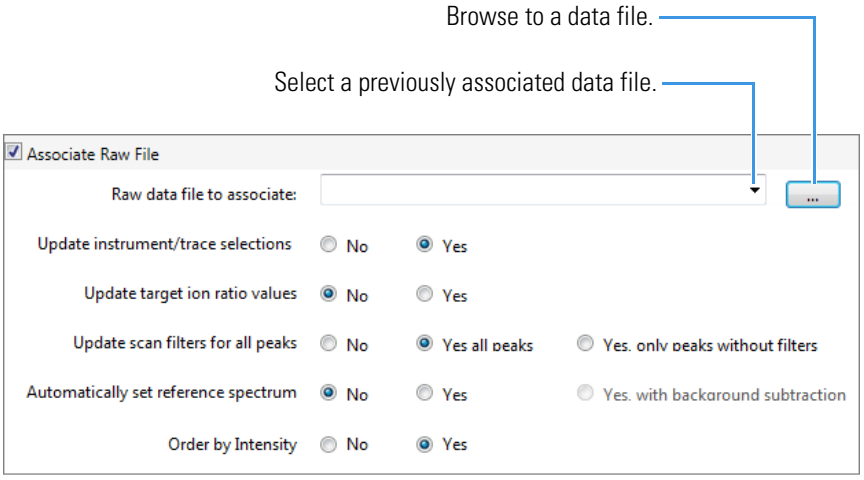
6. To select all compounds in the database, select the **Select All** check box at the top of the list.

7. (Optional) To associate a raw data file with the method, do the following:

   a. Select the **Associate Raw File** check box.

      The application adds the appropriate options to the Select Compounds from Database dialog box.

      Figure 45. Associate Raw File options in the Select Compounds … dialog box.



   b. Browse to a raw data file to associate with the method (or select from the list of previously associated raw data files) and open the file.

      To assure that this raw data file is always available to the method (for example, if you move the method to another system), the application saves the raw data file in the Methods folder:

      …\TraceFinderData\Methods*Methodname*

   c. Select the update options to use for creating your method:

      - **Update Instrument/Trace Selections**: Reads the Detector and Trace options from the associated raw data file. On the Detection page, only detector types and traces that are defined in the raw data file are available. For detailed descriptions of the available Detector and Trace values, see Trace Selection.
      - **Update Target Ion Ratio Values**: Reads the ion ratio values from the associated raw data file.
      - **Update Scan Filters for All Peaks**:
        – **Yes, All Peaks** updates all peaks to use the scan filters from the associated raw data file. These scan filters are available on the Trace Selection. See To specify an MS filter.
        – **Yes, Only Peaks Without Filters** updates only peaks without scan filters to use the scan filters from the associated raw data file; it does not override any existing scan filter.

- **Automatically Set Reference Spectrum**: Reads a reference spectrum from the associated raw data file.

  When you select Yes, with Background Subtraction, the application uses the background-subtracted reference spectrum during quantitative processing and reports the background-subtracted reference spectrum (indicated with BS in the scan heading) as the last scan for each compound in the Quantitation Report - 2.

  Note The background subtraction option is available only when you select a background subtraction method on the Acquisition page in the method. See Editing the Acquisition Page.

- **Order by Intensity**: Reads intensity values from the associated raw data file and lists the peaks with the greatest intensity first. Intensity is based on either peak height or peak area, as specified for the method (see Detection Algorithm).
  - When a compound contains only target peaks, the most intense ion is listed first.
  - When a compound contains both target peaks and confirming peaks, the most intense target peak is listed first, followed by its ordered confirming peaks. The second most intense target peak is listed next followed by its confirming peaks, and so forth.
  - When you also select the Update Target Ion Ratio Values option, the application orders the ions by intensity first and then updates the target ion ratios.

  Note You can use the Order by Intensity option with required confirmation criteria only when all peaks are target peaks. Otherwise, the application ignores this option. See To specify confirmation criteria for compounds.

Options that are set to No use the standard values in the method.

Note If you do not associate a raw data file, you might not see any peaks.

8. Click **Add Selected Compounds to Method** (or **Add Selected Compounds to Method and Associate Raw File)**.

   The application adds the selected compounds to the method and (optionally) associates the specified raw data file.

9. Click **Method View – Acquisition** in the navigation pane.

   The Acquisition Page for the method opens.

10. From the Instrument Method list, select an instrument method.

11. From the Tune/Breakdown Method list, select a tune/breakdown method.

    By default, the tune/breakdown method is the same as the selected instrument method.

12. To save the new method, choose **File > Save** from the main menu and name the method.

For a detailed description of how to modify a method, see Editing a Quantitation Method.

Editing a Quantitation Method

You can open a method to view or edit the compounds, method instructions, and reporting options.

See the instructions for the following tasks:

- Modifying Retention Times
- Editing the Acquisition Page
- Editing the Processing Page
- Editing the Compounds Page
- Editing the QAQC Page
- Editing the Groups Page
- Editing the Intelligent Sequencing Page
- Editing the Reports Page

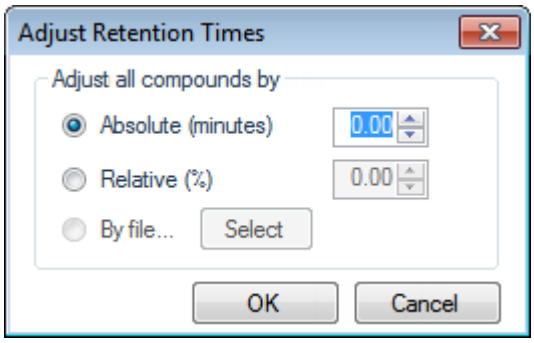
Modifying Retention Times

Use the Adjust Retention Times dialog box from any page in the Method View or Local Method view.

❖ To modify retention times in a method

1. Do one of the following:
   - Choose **Method View > Adjust Retention Times** from the main menu of the Method View in the Method Development mode.
   - Choose **Local Method > Adjust Retention Times** from the main menu of the Local Method view in the Analysis mode.

   The Adjust Retention Times dialog box opens.



2. Select the appropriate option (Absolute, Relative, or By File):
   a. Select the **Absolute** option.

b. Specify a positive or negative value to increase or decrease the expected retention times. (Valid range: –100.00 through +100.00)

c. Click **OK**.

The application increases or decreases the expected retention times for all compounds in the method by the specified number of minutes.

–or–

a. Select the **Relative** option.

b. Specify a positive or negative value to increase or decrease the expected retention times. (Valid range: –100.00 through +100.00)

c. Click **OK**.

The application increases or decreases the expected retention times for all compounds in the method by the specified percentage.

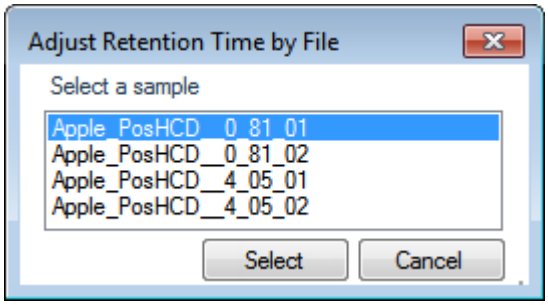
–or–

a. Select the **By File** option.

> **Note** This option is available only from the Local Method view after you have processed the samples in a batch.

b. Click **Select**.

The Adjust Retention Time by File dialog box opens, displaying all processed samples.



c. Select the sample whose retention times you want to use in the method.

d. Click **Select**.

e. Click **OK**.

For each detected peak in the selected, processed sample, the application overwrites the retention times for the matching compounds in the method. Any compounds not detected in the selected sample retain their retention times.

Editing the Acquisition Page

The Acquisition Page defines basic information about the method.

Follow these procedures:

- To specify acquisition information for a method
- To edit an instrument method
- To edit a tune/breakdown method

❖ To specify acquisition information for a method

1. In the Lab Name box, type the name to be displayed at the top of each printed, saved, or exported report.

   The default name is Default Laboratory.

2. In the Assay Type box, type the assay type to be targeted by the method.

3. From the Injection Volume box, select an injection volume (between **0.1** and **5000** µL) to be used for sample injection.

   Use the up/down arrows to change the volume in increments/decrements of 1 µL, or use the keyboard to enter non-integer injection volumes.

   IMPORTANT The application uses this injection volume in the method, not the injection volume from the instrument method.

4. From the Mass Precision box, select a precision value (**2** through **6**) as the number of decimal places to be used in reports and in peak and spectrum displays.

   The application uses this mass precision value to display mass values in the following locations:

   - Reports:
     - Blank Report
     - Confirmation Report (data spectra, library spectra, quantitation ion display, and qualitative ion display)
     - All High Density reports (*m/z* values)
     - Ion Ratio Failure Report (quantitation ion and qualitative ion)
     - Manual Integration Report (*m/z* value)
     - Quantitation Report (QIon)
   - All peaks on the Detection pages in the Method Development mode
   - The spectrum display in Data Review in the Analysis mode
   - The spectrum display in the Method Forge dialog box

IMPORTANT When you create a method using a raw data file, the application reads the filter precision value from the raw data file to create scan filters; however, the application uses the Display Mass Precision value when showing masses that are not embedded within filter strings and masses that are displayed on spectral plots.

❖ To edit an instrument method

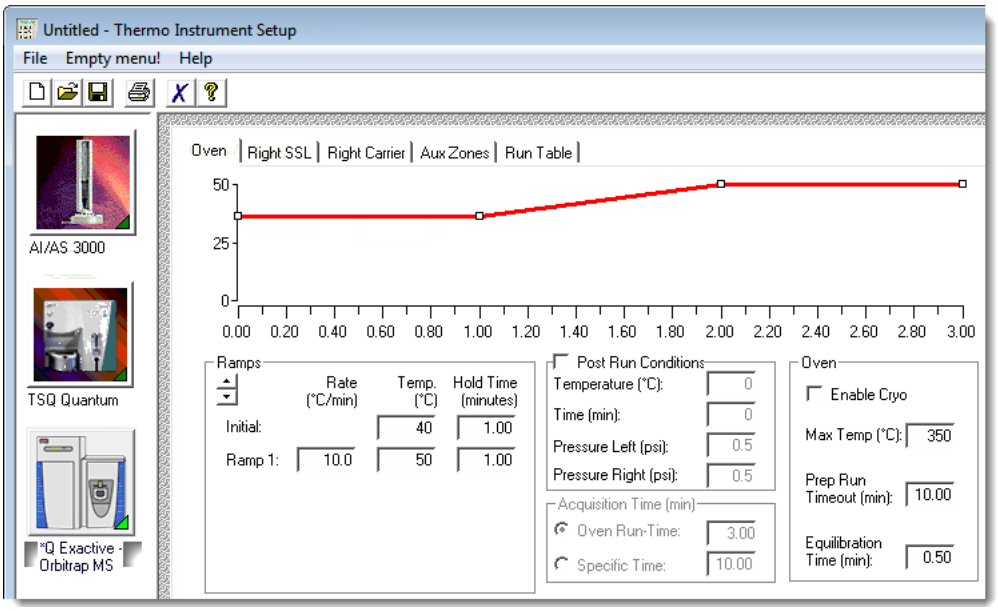
1. From the Instrument Method list on the Acquisition page, select an instrument method.



2. To edit the selected instrument method, click **Edit**.

   The Thermo Instrument Setup window opens. This example of an instrument setup shows multiple configured instruments.

Figure 46. Thermo Instrument Setup window

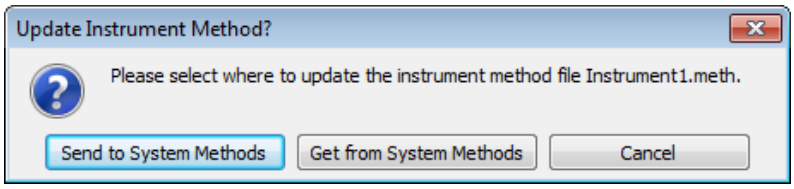


3. Edit the values on the instrument page for your instrument.
4. From the main menu in the Thermo Instrument Setup window, choose **File > Save** and then choose **File > Exit**.

   The application returns you to the Acquisition Page.

5. To update any changes to the instrument method after you created this quantitation method, click **Update**.

The Update Instrument Method dialog box opens.



6. Choose one of the following options:
   - **Send to System Methods**: Overwrites the instrument method in the …\TraceFinderData\InstrumentMethods folder with the current instrument method.
   - **Get from System Methods**: Overwrites the current instrument method with the instrument method in the …\TraceFinderData\Methods folder.
   - **Cancel**: Makes no changes to the instrument method in the current quantitation method.

❖ To edit a tune/breakdown method

From the Tune/Breakdown Method list, select a tune/breakdown method.



The instructions for editing and updating a tune/breakdown method are the same as for an instrument method. Follow the instructions To edit an instrument method.

Acquisition Page

Use the features on the Acquisition page to define basic method information for acquiring batch data.

Figure 47. Acquisition page for a quantitation method

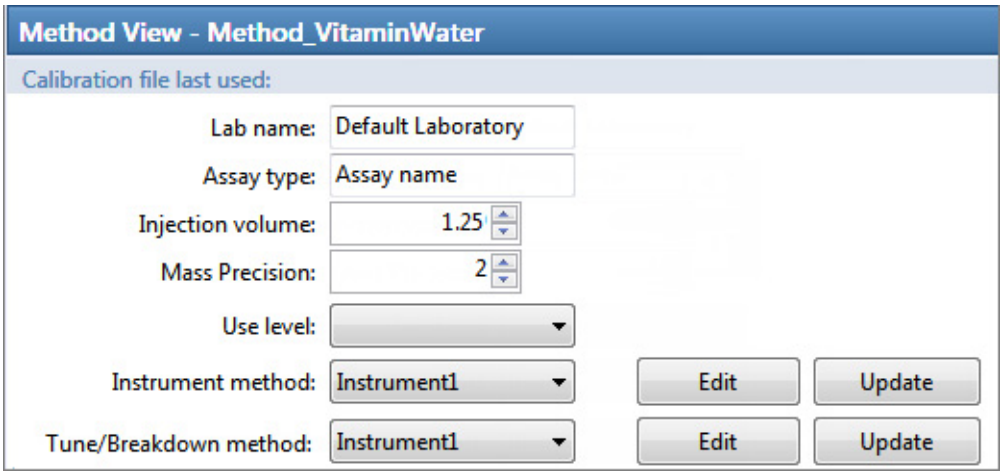


Table 24. Acquisition page parameters

Parameter	Description
Lab Name	Specifies the laboratory name to be displayed at the top of each printed, saved, or exported report. Default: Default Laboratory To specify this default laboratory name, see Specifying Method Defaults.
Assay Type	Specifies the name for the analysis type to be targeted by the method. The assay type associates the method with the analysis of a compound or specific class of compounds (for example, you might use an assay type of PAH for the analysis of Polynuclear Aromatic Hydrocarbons).
Injection Volume	Specifies the system uses the injection volume (in µL) for sample injection. For a more detailed explanation, refer to the documentation for the autosampler. The injection volume in the method overrides the injection volume in the instrument method. The injection volume in the batch overrides the injection volume in the method. Valid range: 0.1 through 5000 µL
Mass Precision	Specifies the number of decimal places used in reports and in peak and spectrum displays. Valid range: Integers from 2 through 6
Use Level	Specifies a calibration level.
Instrument Method	Specifies the instrument method used for acquiring samples.
Tune/Breakdown Method	Tune/Breakdown method used for processing samples.
Edit	Opens the Thermo Xcalibur Instrument Setup window where you can edit the instrument method or tune/breakdown method.
Update	Specifies one of the following: Send to System Methods: Overwrites the instrument method in the …\TraceFinderData\InstrumentMethods folder with the current instrument method or tune/breakdown method. Get from System Methods: Overwrites the current instrument method or tune/breakdown method with the instrument method in the …\TraceFinderData\Methods folder.

Editing the Processing Page

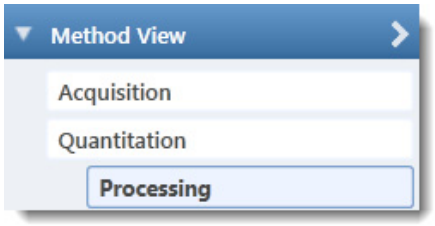
The Quantitation – Processing Page defines basic method information for processing batch data.

Follow these procedures:

- To open the Processing page
- To set automated background subtraction options
- To specify mass tolerance
- To specify a threshold override
- To specify qualitative peak processing parameters
- To specify library selection
- To add unknown screening to the method

❖ To open the Processing page

Click **Processing** in the Method View navigation pane. See Processing Page.



❖ To set automated background subtraction options

1. In the Background Subtraction Range Option list, select how you want the subtraction range determined from the following options:

   - **Before Peak**: Averages and subtracts a specified number of scans before the apex of the peak.
   - **After Peak**: Subtracts a specified number of scans after the apex of the peak.
   - **Both Sides of Peak**: Subtracts a specified number of scans from each side of the apex of the peak.

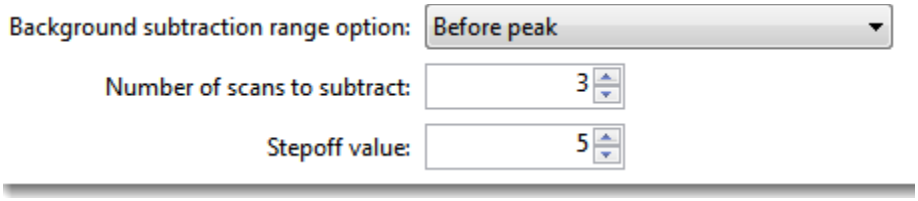
   When you create a reference spectrum with background subtraction, the application uses the selected method to conduct background subtraction of peak spectra during quantitative processing. The application then reports the background-subtracted reference spectrum (indicated with BS in the scan heading) as the last scan for each compound in the Quantitation Report - 2 report. It does not use background subtraction with qualitative processing.

2. In the Number of Scans to Subtract box, enter a number.

The application subtracts this number of scans from the background after averaging. When you select the Both Sides of Peak option, the application subtracts this number of scans from **each** side of the peak.

3. In the Stepoff Value box, enter a number.

   The application uses this offset value to average and subtract scans that are not adjacent to the apex of the peak, as in this example:



   Before Peak example:

   When the Number of Scans to Subtract equals 3 and the Stepoff Value equals 5, the application ignores the first 5 scans to the left of the peak and applies the averaging and subtraction to the 6th, 7th, and 8th scans to the left of the peak.

   After Peak example:

   When the Number of Scans to Subtract equals 3 and the Stepoff Value equals 5, the application ignores the first 5 scans to the right of the peak and applies the averaging and subtraction to the 6th, 7th, and 8th scans to the right of the peak.

   Both Sides of Peak example:

   When the Number of Scans to Subtract equals 3 and the Stepoff Value equals 5, the application ignores the first 5 scans to the left of the peak and the first 5 scans to the right of the peak. Then it applies the averaging and subtraction to both the 6th, 7th, and 8th scans to the left of the peak and the 6th, 7th, and 8th scans to the right of the peak.

❖ To specify mass tolerance

1. Select the units of measure that you want to use.
2. Specify the number of millimass units or parts per million to use as the *m/z* ± tolerance value.

   The application applies this mass tolerance to the extracted chromatograms.

❖ To specify a threshold override

To set a threshold override and include only peaks with areas above this designated threshold, do the following:

a. Select the **Threshold Override** check box.

b. In the associated box, type the threshold as an area value.

   This threshold overrides the Response Threshold value set in the compound database. The application ignores the peaks with areas below your specified threshold.

❖ To specify qualitative peak processing parameters

IMPORTANT The Qualitative Peak Processing options are available only when you select the Enable Qualitative Results Display and Processing option in the application configuration console. See Qualitative Results.

1. To indicate whether to select peaks by relative height or area and specify the percentage of the largest peak that results in compound selection, select the **Enable Peak Threshold** check box.

   To consider a peak for a processing method, the application uses the Enable Peak Threshold filter to determine which peaks meet the specified percentage of the height or area of the largest peak.

2. To display a specific number of the largest peaks by height or area, select the **Only Select Top Peaks** check box and enter the number of peaks to display.

3. To use the Genesis algorithm to match internal standards, select the **Use Genesis Algorithm for Qual Processing** check box and specify a value for internal standard matching.

   The application uses the Genesis algorithm to match internal standards in a range plus/minus the value that you specify. For additional information about the Genesis algorithm, see Genesis Detection Method.

4. Select or clear the **Exclude Matching Quan Peaks** check box and specify a value for the exclusion window.

   The application excludes target peaks in a range plus or minus the value that you specify.

5. To process samples that include data-dependent scans, select the **Use Data Dependent Scans** check box.

   When you process a sample using this feature, the application uses the TIC trace to find all data-dependent full scans, lists them, and performs a library search against the data-dependent MS/MS or MS^n scan.

   This option constrains the Data Review to only data-dependent scan spectra.

   In addition to the peak information, the TIC Report and TIC Summary Report display information about the data-dependent filtered data.

6. To indicate whether to select peaks above a minimum percentage of the nearest internal standard peak that results in compound selection, select the **Enable ISTD Threshold** check box and specify a minimum percentage.

   To consider a peak for a processing method, the application uses the Enable ISTD Threshold filter to determine which peaks meet the specified percentage of the height of the nearest internal standard peak.

   When you select the Enable ISTD Threshold check box, the method ignores values set for the Enable Peak Threshold parameter.

Note When you create a method with the Method Forge, the application ignores the parameters in the Qualitative Peak Processing area.

❖ To specify library selection

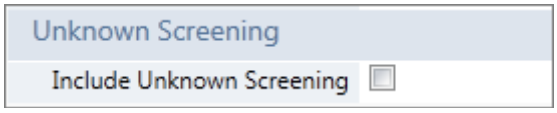
1. In the Library Selection area, select the check box for each library that you want to search.

   All libraries loaded on your instrument are displayed in the Library Selection area.

2. To limit the number of matches returned when the system searches a spectrum against the selected libraries, set a value in the Limit Library Hits box.

3. To specify how to sort the library searches, select a value from the Best Match Method list.

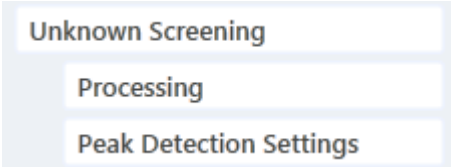
❖ To add unknown screening to the method



IMPORTANT The Include Unknown Screening option is available only when you select the Allow Unknown Screening option in the application configuration console. See Processing Options.

1. Select the **Include Unknown Screening** check box.

   The application adds the Unknown Screening features to the Method View navigation pane.



2. To specify unknown screening settings for peaks, libraries, databases, elements, and ChemSpider™ searches, click **Processing**.

   For further instructions, see Editing the Processing Pages.

3. To specify unknown screening peak detection settings, click **Peak Detection Settings**.

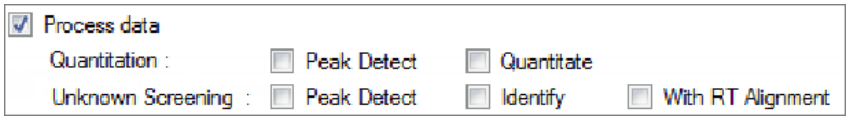
   For further instructions, see Editing the Peak Detection Settings Page.

   The application indicates that a quantitation method includes unknown screening features in the following locations:

   - In the Batch View in the Analysis mode, when you create a batch using a quantitation method, the title bar indicates that the method includes unknown screening features.



- In the Analysis navigation pane, the application displays access to local methods for both quantitation and unknown screening.
- In the Submit Options dialog box, when you submit a batch, the Process Data area shows all available process data for the batch.



You can select the detection options for each processing method that you want to use when you submit the batch.

Processing Page

Use the features on the Processing page to define basic information about the method.

Figure 48. Processing page for a quantitation method

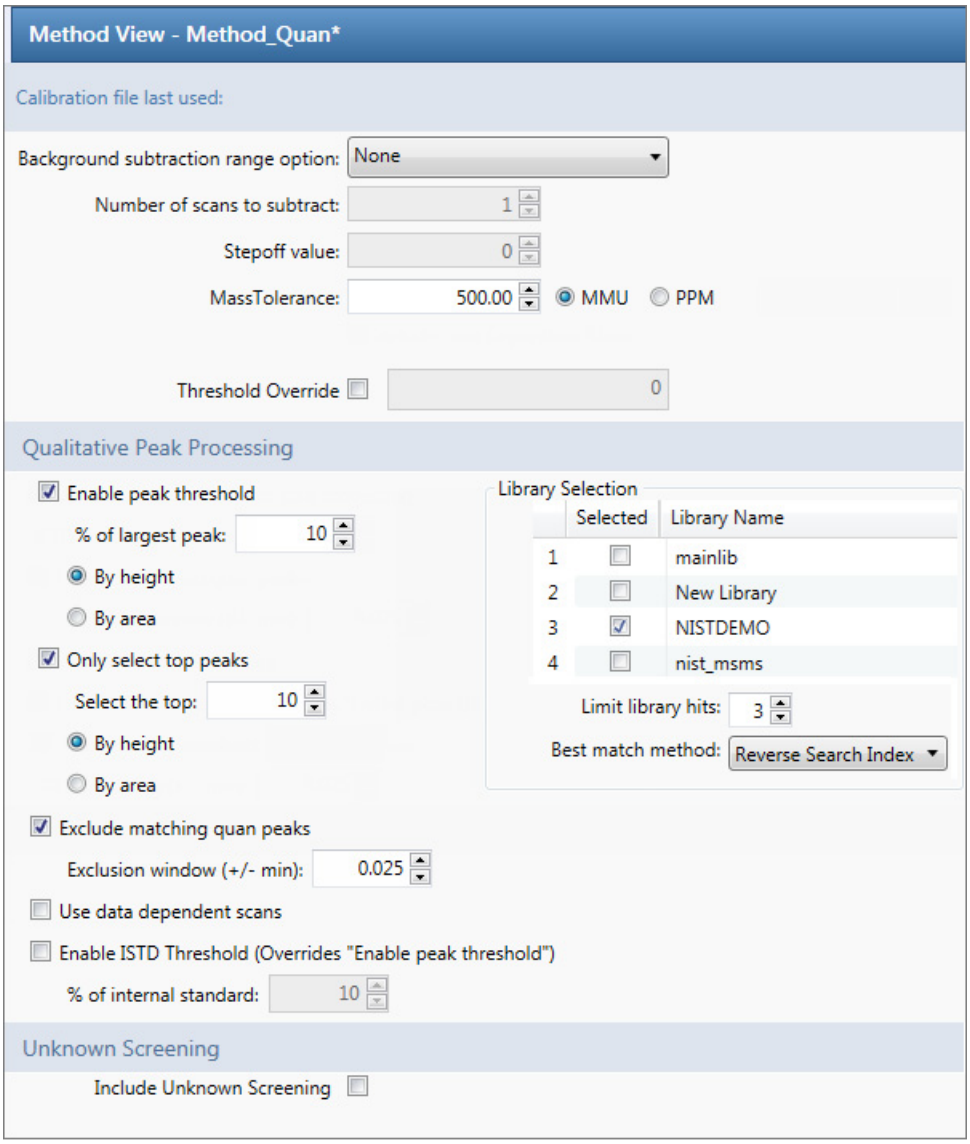


Table 25. Processing page parameters (Sheet 1 of 3)

Parameter	Description
Background Subtraction Range Option	Specifies the range used for background subtraction. Valid values: None, Before Peak, After Peak, Both Sides of Peak Default: None
Number of Scans to Subtract	Valid values: Even numbered integers Default: 0
Stepoff Value	Offset from the selected peak to the first subtracted peak.

Table 25. Processing page parameters (Sheet 2 of 3)

Parameter	Description
Mass Tolerance	Specifies the *m/z* ± tolerance value in MMU or PPM. Valid range: 0.1 through 50 000 • MMU (millimass units): MMU is a static calculation to the extracted mass. • PPM (parts per million): PPM is a variable calculation dependent on the actual mass. The smaller the mass, the narrower the tolerance range. The larger the mass, the wider the tolerance range. The default value and units are specified in the Configuration Console. See Specifying Peak Detection Defaults.
Include Data Dependent Filters	Specifies that the method includes data-dependent filters. Available only for quantitation methods.
Threshold Override	Overrides the Response Threshold value set in the compound database. The application ignores the peaks with areas below this specified threshold. Valid range: 1000 through 1 000 000 000 Default: 5000
Qualitative Peak Processing	
Enable Peak Threshold	Specifies whether to select peaks by relative height or area and the percentage of the highest peak that results in compound selection.
Only Select Top Peaks	Displays a specific number of the largest peaks by height or area.
Exclude Matching Quan Peaks	Compares the retention time of the internal standard in the method to the found retention time of the internal standard in the library search and excludes peaks outside the Exclusion Window range.
Exclusion Window	Defines a range plus/minus the Exclusion Window value that you specify.
Use Data Dependent Scans	Constrains the Data Review to only data-dependent scan spectra. In addition to the peak information, the TIC Report and TIC Summary Report display information about the data-dependent filtered data.
Enable ISTD Threshold	Specifies that, when identifying a peak, qualitative peak processing use the minimum threshold specified as a percentage of the nearest internal standard peak, rather than the threshold specified in the Enable Peak Threshold parameter in the method template.
% of Internal Standard	Percentage of the nearest internal standard peak to use as the minimum threshold for identifying a peak.
Library Selection	Specifies the libraries to use for qualitative peak processing.
Limit Library Hits	Specifies the number of matches returned when the system searches a spectrum against the selected libraries.

Table 25. Processing page parameters (Sheet 3 of 3)

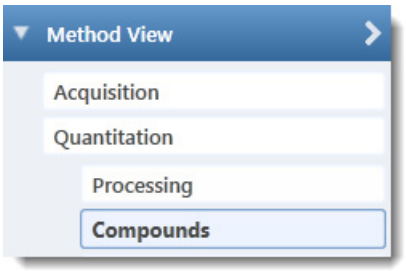
Parameter	Description
Best Match Method	Specifies how to sort the library searches. Valid values: Search Index, Reverse Search Index, Match Probability
Unknown Screening	
Include Unknown Screening	Adds unknown screening features to the quantitation method.

Editing the Compounds Page

Use the Compounds page to set all parameters for identifying, detecting, and quantifying the target compound list.

❖ To open the Compounds page

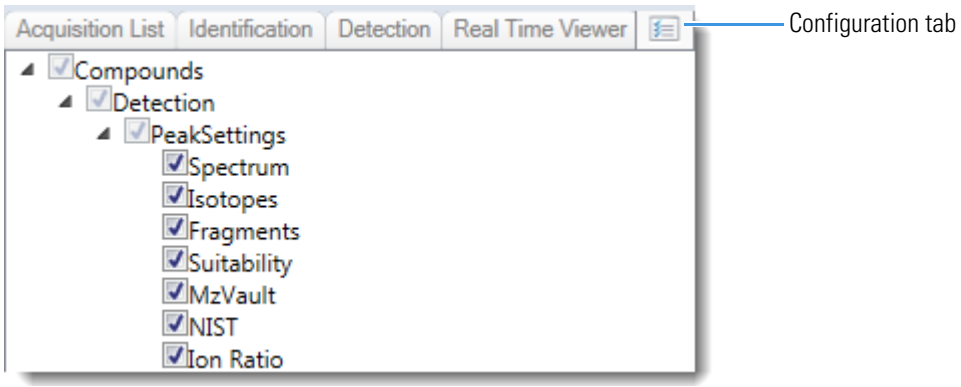
Click **Compounds** in the Method View navigation pane.



❖ To configure the visibility of pages on the Compounds page

1. Click the **Configuration** tab, (), in the Compounds page header.

   The configuration page opens and displays the configuration settings for the currently displayed pages—in this example, the Acquisition List, Identification, Detection, and Real Time Viewer pages.



   These pages are defined in the workflow template that was used to create the method. See Starting a New Quantitation Master Method.

Note Do not confuse these two tools:

- Configuration tab (eal Time Viewer): (Located with the page tabs) Modifies the current display.
- Configure Method Views icon ( ): (Located in the toolbar) Opens the Master Master Method Configuration dialog box where you can modify the display for all pages in the method (overriding the settings in the workflow template).

To change the pages that you want to display, use the **Configure Method Views** icon, , in the toolbar. See Configuring Method Views.

2. Select the check box for each lower-level page that you want to display in the view.

Note A grayed-out check box indicates that the page name is required and cannot be cleared.

The Compounds page immediately displays the tabs for only the selected pages.

Note Certain pages are required for this view and cannot be hidden.

From the Compounds page of the Method View, you can access the following pages:

- Acquisition List
- Identification
- Detection
- Calibration
- Calibration Levels
- Chk Std Levels
- Real Time Viewer

Of these pages, the Identification, Calibration, Calibration Levels, and Chk Std Levels pages use a right-click shortcut menu. See Using the Shortcut Menu Commands.

Acquisition List

The Acquisition List page displays all compounds defined for the current method in a display similar to the Compound Database view. From the Acquisition List Page, you can add additional compounds from the Compound Database or delete compounds from the method.

For detailed descriptions of all the features in the Compound Database views, see Using the Compound Database View for Small Molecules or Using the Compound Database View for Peptides.

Follow these procedures:

- To add a compound to the method
- To remove compounds from the method
- To specify a compound as an internal standard
- To link internal standards to target compounds

❖ To add a compound to the method

1. Click **Add Compound from Compound Database**.

   The Select Compounds from Database dialog box opens, listing all the compounds defined in the compound database. You cannot edit the compound data from here; you can only choose which compounds you want to include in your method.

2. Click **Browse** and locate the compound database that you want to use.

3. Select the check box for each of the compounds that you want to add to the method.

   Compounds that already exist in the method are grayed out. When you select one of these compounds, the application reminds you that it will overwrite the existing compound.

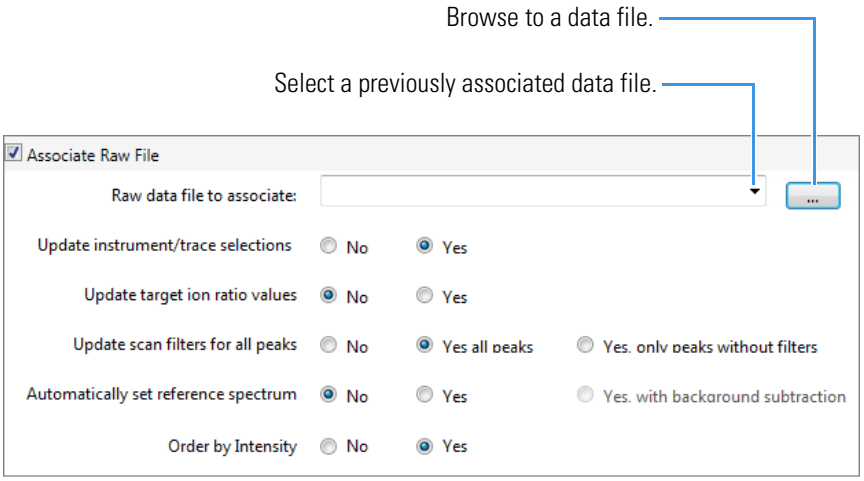
4. To select all compounds in the database, select the **Select All** check box at the top of the list.

5. (Optional) To associate a raw data file with the method, do the following:

   a. Select the **Associate Raw File** check box.

      The application adds the appropriate options to the Select Compounds from Database dialog box.

      Figure 49. Associate Raw File options in the Select Compounds … dialog box



b. Browse to a raw data file to associate with the method (or select from the list of previously associated raw data files) and open the file.

   To assure that this raw data file is always available to the method (for example, if you move the method to another system), the application saves the raw data file in the Methods folder:

   …\TraceFinderData\Methods*Methodname*

c. Select the update options to use for creating your method:

   - **Update Instrument/Trace Selections**: Reads the Detector and Trace options from the associated raw data file. On the Detection page, only detector types and traces that are defined in the raw data file are available. For detailed descriptions of the available Detector and Trace values, see Trace Selection.
   - **Update Target Ion Ratio Values**: Reads the ion ratio values from the associated raw data file.
   - **Update Scan Filters for All Peaks**:
     – **Yes, All Peaks** updates all peaks to use the scan filters from the associated raw data file.
     – **Yes, Only Peaks Without Filters** updates only peaks without scan filters to use the scan filters from the associated raw data file; it does not override any existing scan filter.
   - **Automatically Set Reference Spectrum**: Reads a reference spectrum from the associated raw data file.

     When you also select Yes, with Background Subtraction, the application uses the background-subtracted reference spectrum during quantitative processing and reports the background-subtracted reference spectrum (indicated with BS in the scan heading) as the last scan for each compound in the Quantitation Report - 2 report.

     Note The background subtraction option is available only when you select a background subtraction method on the Acquisition page in the method. See Editing the Acquisition Page.

   - **Order by Intensity**: Reads intensity values from the associated raw data file and lists the peaks with the greatest intensity first. Intensity is based on either peak height or peak area, as specified for the method (see Detection Algorithm).
     – When a compound contains only target peaks, the most intense ion is listed first.
     – When a compound contains both target peaks and confirming peaks, the most intense target peak is listed first, followed by its ordered confirming peaks. The second most intense target peak is listed next followed by its confirming peaks, and so forth.

– When you also select the Update Target Ion Ratio Values option, the application orders the ions by intensity first and then updates the target ion ratios.

Note You can use the Order by Intensity option with required confirmation criteria only when all peaks are target peaks. Otherwise, the application ignores this option. See To specify confirmation criteria for compounds.

Options that are set to No use the standard values in the method.

Note If you do not associate a raw data file, you might not see any peaks.

6. Click **Add Selected Compounds to Method** (or **Add Selected Compounds to Method and Associate Raw File**).

   The application adds the selected compounds to the method and (optionally) associates the specified raw data file.

❖ To remove compounds from the method

1. In the Tree View Pane, select the check box for each compound that you want to remove from the method.
2. Click **Remove Selected Compounds**.
3. To confirm this action, at the prompt, click **OK**.

   The application removes the selected compounds and all their peak information.

❖ To specify a compound as an internal standard

1. In the Tree View pane, select the compound that you want to specify as an internal standard.

   Compounds can link to their internal standards when the compound name for the internal standard is the same as the target compound name appended with text and enclosed in brackets. For example, the application can link the internal standard *CompoundX[ISTD]* to the target compound *CompoundX*.

2. In the Peak View pane, select the compound name and append [***] to the name, for example: **Atrazine[ISTD]**.

   The application can automatically link this compound as the internal standard for the Atrazine compound.

❖ To link internal standards to target compounds

IMPORTANT Make sure you have designated internal standard compounds by appending [***] to their names.

Click the shortcut menu icon, ☰, and choose **Autolink Internal Standard and Target Compound**.

The application does the following:

a. For each compound with a name that ends in [***], sets the Compound Type to Internal Standard.

Note The application does not overwrite a manually saved Compound Type of Analyte, even when the compound name has [***] appended to its name.

b. For any *CompoundX* for which there is a *CompoundX[***],* sets *CompoundX[***]* as the internal standard for *CompoundX*.

Note If the application does not find a target compound, *CompoundX,* to match a *CompoundX[***]*, the application performs no further action.

c. When there are multiple matching internal standard compounds, the application sets the first saved *CompoundX[***]* as the internal standard for the target compound.

If the multiple internal standards were saved at the same time, an error message informs you that the target compound could not be linked to an internal standard because there are multiple internal standards.

For example, for the target compound NGFILDGFPR, the application finds the internal standard compounds NGFILDGFPR[HeavyK] and NGFILDGFPR[LightR]. Because both [***] appends were added to the compound database at the same time, the application displays the error message.

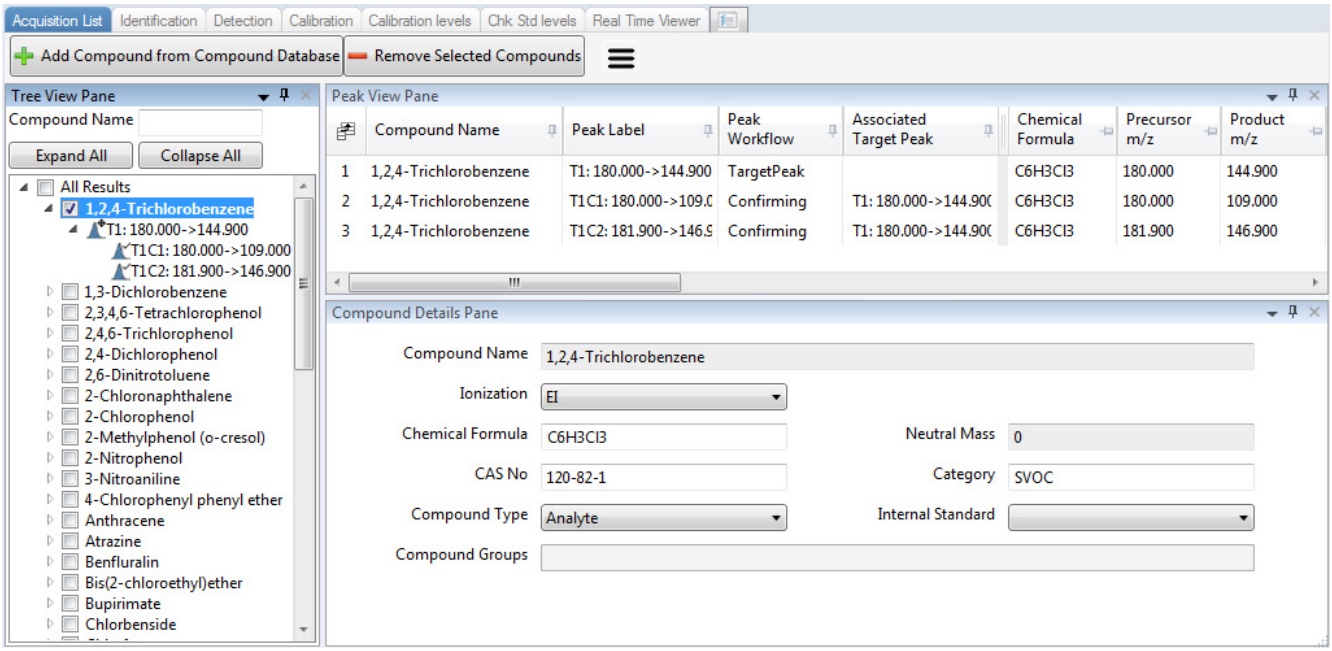


Tip (Animation) To view "Autolinking Internal Standards," choose **Help > Animations**.

Acquisition List Page

Use the features on the Acquisition List page to display all compounds defined for the method.

Figure 50. Acquisition List page

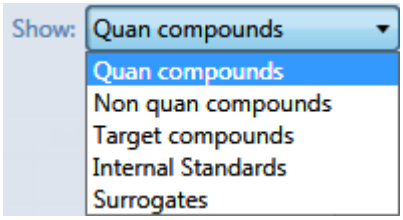


Identification

The Identification Page lists the compounds that are targeted for analysis, reporting, and other compound-specific values.

❖ To filter the displayed compounds

From the Show list, select the type of compounds that you want to display in the compounds list.



Compound type	Description
Quan Compounds	Displays only quantitation compounds, such as target compounds, internal standards, and surrogates.
Non Quan Compounds	Displays only non-quantitation compounds, such as native, breakdown, and tune compounds.
Target Compounds	Displays only target compounds.

Compound type	Description
Internal Standards	Displays only internal standard compounds.
Surrogates	Displays only surrogate compounds.

Identification Page

Use the features on the Identification page to display all compounds targeted by the method.

Figure 51. Identification page

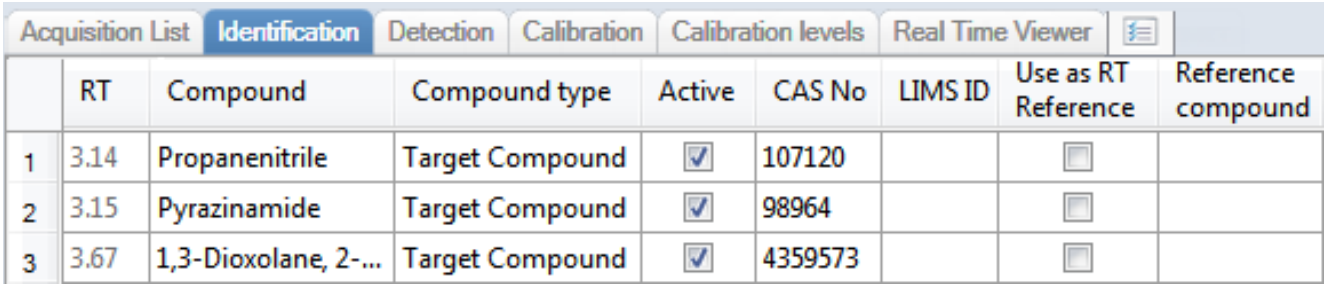


Table 26. Identification page parameters (Sheet 1 of 2)

Parameter	Description
RT	Retention time. The time after injection when the compound elutes. The total time that the compound is retained on the column. The application uses the RT and Window values to determine the start and stop time for the acquisition. Valid range: 0.00 through 999.00 Start time = RT – (Window/2) Stop time = RT + (Window/2) Start and stop range: 0.00 through 999.00
Compound	A list of identified compounds. To customize the compound names, click the cell and type a new name. To display a filtered list of compounds, use the Show list.
Compound Type	Displays the compound types as Target Compound, Internal Standard, Surrogate, MSTune, Native, or Breakdown. The application uses target compounds, internal standards, and surrogates in quantitative analysis.
Active	Identifies each compound to be included in data review and reporting. By default, all added compounds are set to active. This active or inactive setting populates the Batch View and Data Review view in the Analysis mode.
CAS No	The Chemical Abstract Service (CAS) number that the application matched with each compound. To change or add a number, click the CAS No cell and enter a new number.
LIMS ID	Laboratory Information Management System identification number.
Use as RT Reference	When performing peak detection with retention time standards, the application first identifies those compounds identified as retention time standards and then uses their observed retention times to adjust any associated target compound.

Table 26. Identification page parameters (Sheet 2 of 2)

Parameter	Description
Reference Compound	To be used for retention time adjustment for a compound. This list includes all compounds that are selected in the Use as RT Reference column.
Shortcut menu	The Identification page uses a right-click shortcut menu. See Using the Shortcut Menu Commands.

Detection

Use the Detection page to customize peak detection and integration for any ions that define peaks and compounds.

Figure 52. Detection page

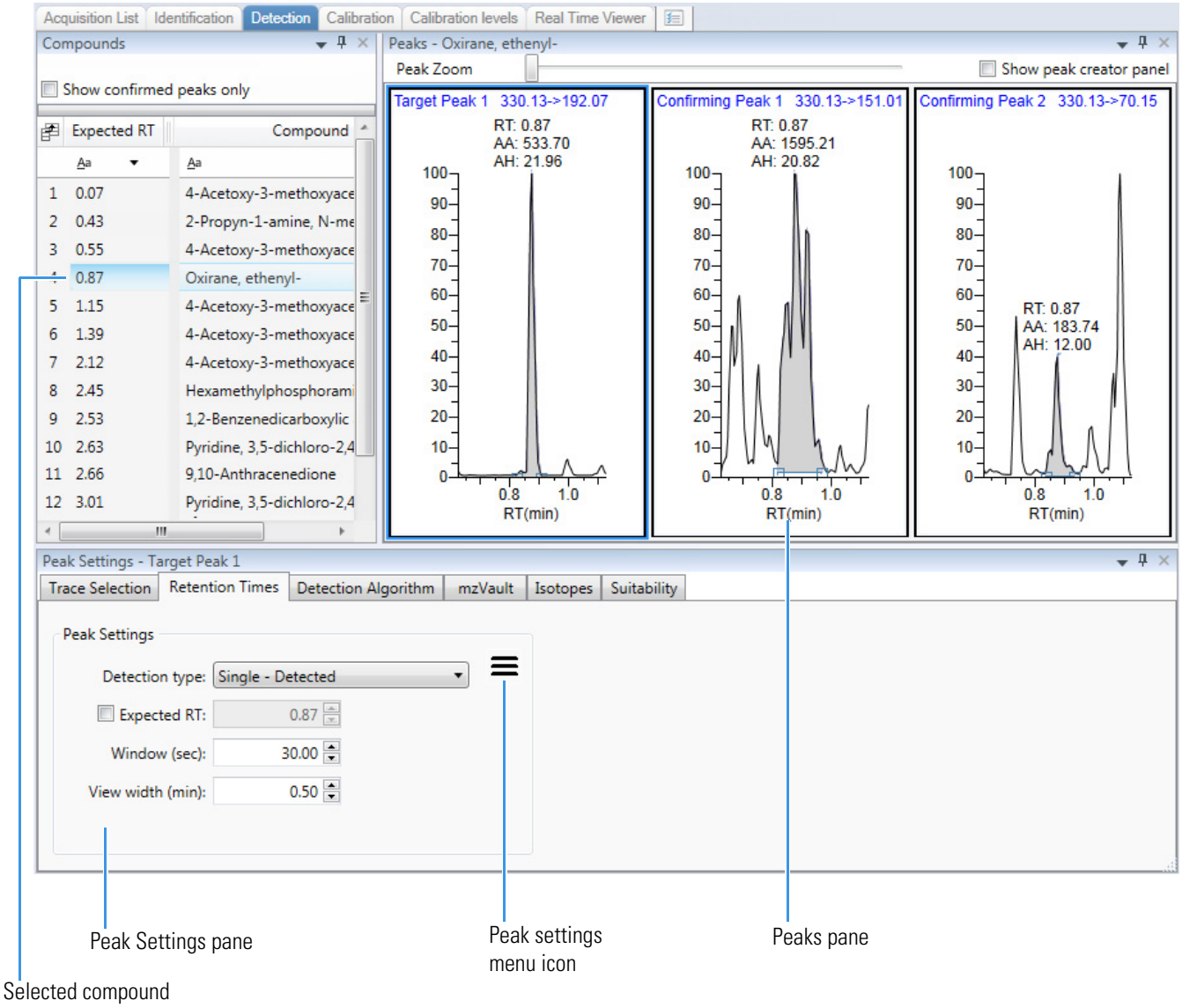


Table 27. Detection page panes (Sheet 1 of 2)

Pane	Description
Compounds	Lists all compounds in the method. The Compound list uses a right-click shortcut menu. See Using the Shortcut Menu Commands.
Peaks	Displays a chromatogram for the target peak and its confirming peaks.

Table 27. Detection page panes (Sheet 2 of 2)

Pane	Description
Peak Settings	The application displays the following additional pages when your method contains the relevant parameters: Retention Times, Trace Selection, Detection Algorithm, Suitability, Spectrum, NIST, mzVault, Isotopes, and Ratios.
Retention Times	Defines the retention time and window for a target peak.
Trace Selection	Displays a combination of the detector, trace, and filter values used for the compound.
Detection Algorithm	Defines the peak detection algorithm and its options.
Suitability	Determines if the column is degrading and identifies suspicious peaks eluting at the same time as the target compound.
Spectrum	Defines a reference mass spectrum for a target peak or compound.
NIST	Defines the criteria for NIST library matching.
mzVault	Defines the criteria for library matching.
Isotopes	Defines the criteria for identifying an isotope peak.
Ratios	Defines the criteria for evaluating, confirming, or qualifying ions.

On the Detection page, you can configure how the application detects and integrates characteristic ions for targeted compounds. You can also edit the list of characteristic ions for a specific compound. Refining these parameters in the method for each compound and its ions can reduce the degree of manual integration that would otherwise be required.

You can change the parameters used to identify a target peak, mass range, or confirming peak. The application automatically uses the first match it finds as the compound name, the base peak of the mass spectrum as the target peak, and the second and third largest ions as the confirming peaks.

Follow these procedures:

- To display only confirmed peaks
- To specify confirmation criteria for compounds
- To change the displayed information for detected peaks
- To add a new compound to the method
- To associate compounds from a raw data file
- To change the compound reference spectrum
- To replace a quantitation mass
- To add a mass to the existing quantitation mass ranges
- To add a target peak
- To add a spectral peak as a new compound

- To replace a target peak with a confirming peak
- To set a confirming peak as an additional target peak
- To add a trace to the Real Time Status pane
- To remove a confirming peak
- To save the new method

❖ To display only confirmed peaks

In the Compounds pane, select the **Show Confirmed Peaks Only** check box.

The Compounds pane displays only compounds that meet all required criteria.

❖ To specify confirmation criteria for compounds

Select the **Required** check box for any of the following Peak Settings pages:

- NIST
- mzVault
- Isotopes
- Suitability
- Ratios
- Trace Selection (for fragments only)

When peak detection finds a target peak at the specified location, it then confirms the peak by verifying these required criteria. If the target peak does not meet these criteria, then it is not confirmed.

To display only confirmed peaks, select the **Show Confirmed Peaks Only** check box in the Compounds pane.

Confirmed compounds are indicated in the Compound View in Data Review.

Note When you associate a raw data file, you can use the Order by Intensity option with required confirmation criteria only when all peaks are target peaks. Otherwise, the application ignores this option. See To specify confirmation criteria for compounds.

❖ To change the displayed information for detected peaks

1. Right-click the chromatogram plot for any of the target or confirming peaks and choose **Peak Display Settings**.

   The Chromatogram Plot Settings dialog box opens.

   Figure 53. Chromatogram Plot Settings dialog box

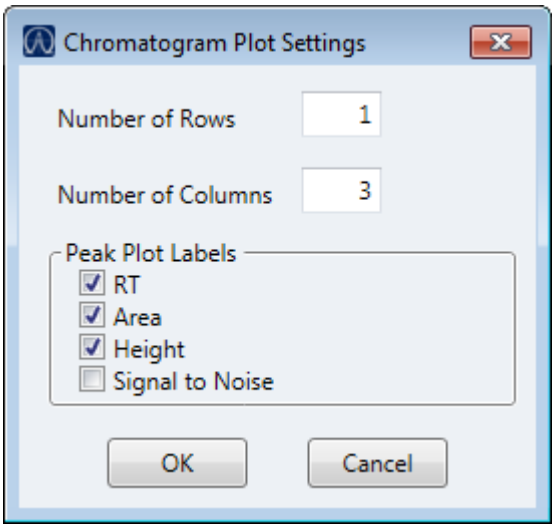


Table 28. Chromatogram Plot Settings dialog box parameters

Parameter	Description
Number of Rows	Specifies the number of rows visible in the Peaks pane. When Number of Rows equals 1, the application scales the height of all chromatograms to fill the Y dimension of the Peaks pane. Default: 1
Number of Columns	Specifies the number of columns visible in the Peaks pane. When Number of Columns equals 1, the application scales the width of all chromatograms to fill the X dimension of the Peaks pane. Default: 3
Peak Plot Labels	Displays or hides the following peak labels: • RT • Area • Height • Signal to Noise

2. To change the label display, do the following:

   a. Select the check box for the label that you want to display: peak retention time (**RT**), peak area (**Area**), peak height (**Height**), or signal-to-noise ratio (**Signal to Noise**).

   b. To remove a label, clear the label check box.

c. Click **OK**.

The application globally applies these label settings to all target peaks, confirming peaks, and internal standard peaks in the method.

3. To change how many rows and columns you want to view at one time in the display, do the following:

   a. Type a value for Number of Rows or Number of Columns.

   b. Click **OK**.

   c. Use the scroll bar to view peaks that are not visible in the Peaks pane.

   These values do not change the number of rows (compounds) and columns (peaks) that are displayed in the Peaks pane. The default is one row and three columns.

❖ To add a new compound to the method

1. Select the **Show Peak Creator Panel** check box.

   The Manual Peak Creator pane opens.

2. (Optional) Select a different Trace.

3. (Optional) Select a filter from the Filter list.

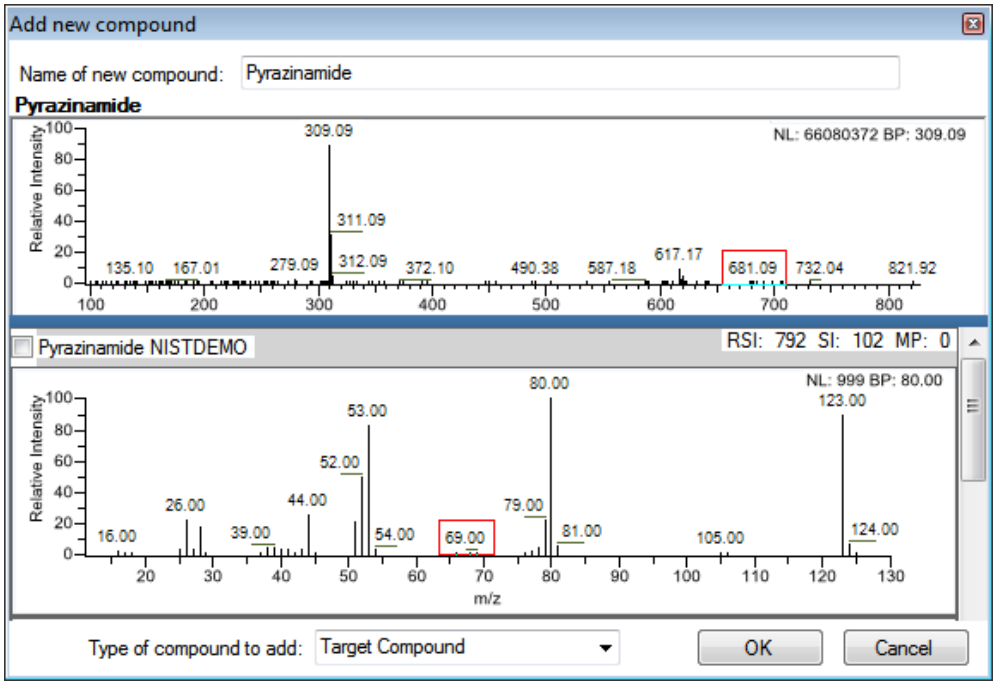
4. Click to select the peak in the chromatogram that represents the compound that you want to add to the method.

5. Right-click and choose **Add This Peak as New Compound**.

   The application performs a library search for the selected compound. The application uses the first match it finds as the compound name, the base peak of the mass spectrum as the target peak, and the second and third largest ions as the confirming peaks.

   - When the name of the first match does not exist in the method, the application adds this compound to the method and displays the name in the Compound list. You can now view and edit the parameters for this compound.
   - When the name of the first match is already in the method, the Add New Compound dialog box opens. You cannot overwrite a compound name in the method. If the selected peak already exists in the method, you must give it a new name to add it to the method. Or, you can select a different compound to add to the method, following step 6 through step 8.

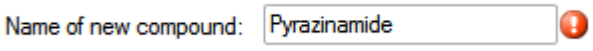
Figure 54. Add New Compound dialog box



6. Do one of the following:

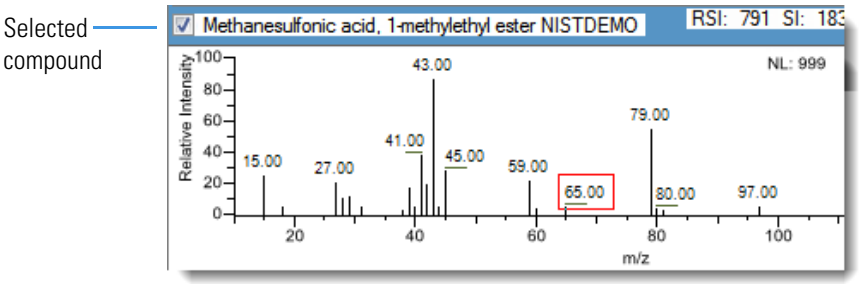
   - Type a new name for the first matched compound.

     The application displays a red warning when the selected compound name already exists in the method. You cannot overwrite the compound name, and you cannot create a duplicate name in the method. You must type a unique name.

   –or–

   - To use a compound other than the first matched compound, scroll to the spectrum for that compound and select its corresponding check box in the title bar of the spectrum pane.

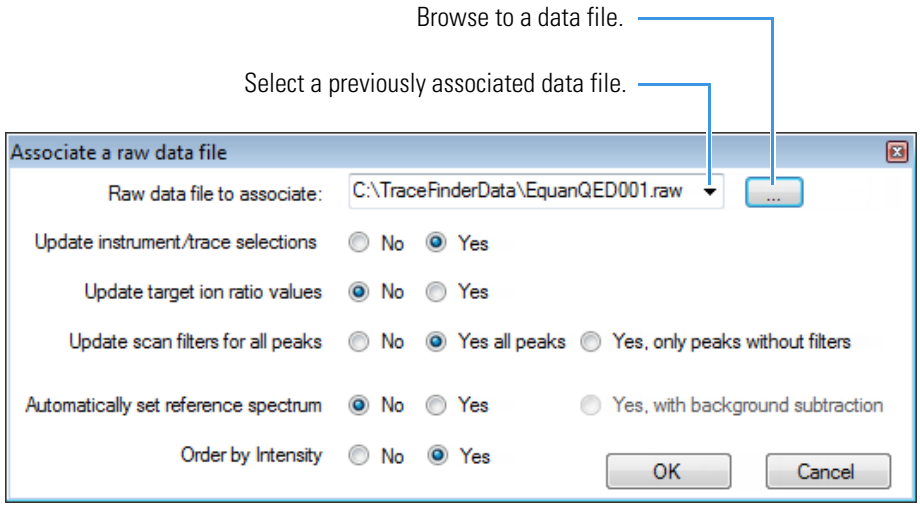
7. In the Type of Compound to Add list, select a compound type.
8. Click **OK**.

❖ To associate compounds from a raw data file

Tip You can add compounds from the current raw data file (begin at step 8), or you can associate another raw data file and add compounds from that file (begin at step 1).

1. From the main menu, choose **Method View > Associate a Raw Data File**.

   The Associate a Raw Data File dialog box opens.



2. Browse to a raw data file to associate with the method (or click the arrow and select from the list of previously associated raw data files) and open the file.

   To assure that this raw data file is always available to the method (for example, if you move the method to another system), the application saves the file in the Methods folder:

   …\TraceFinderData\Methods*Methodname*

3. To update the target ion ratio values when you associate this raw data file, select the **Yes** option.

4. To update the scan filters when you associate this raw data file, select the **Yes** option.

5. To set a reference spectrum, do one of the following:

   - Select the **Yes** option.

   –or–

   - Select the **Yes, with Background Subtraction** option.

   The application uses the background-subtracted reference spectrum during quantitative processing and reports the background-subtracted reference spectrum (indicated with BS in the scan heading) as the last scan for each compound in the Quantitation Report - 2 report.

Note This option is available only when you select a background subtraction method on the Acquisition page in the method. See Editing the Acquisition Page.

6. To read intensity values from the associated raw data file and order the peaks by intensity, select the **Yes** option.
7. Click **OK**.

   The application displays the chromatographic and spectrum data for the compounds in the associated raw data file.

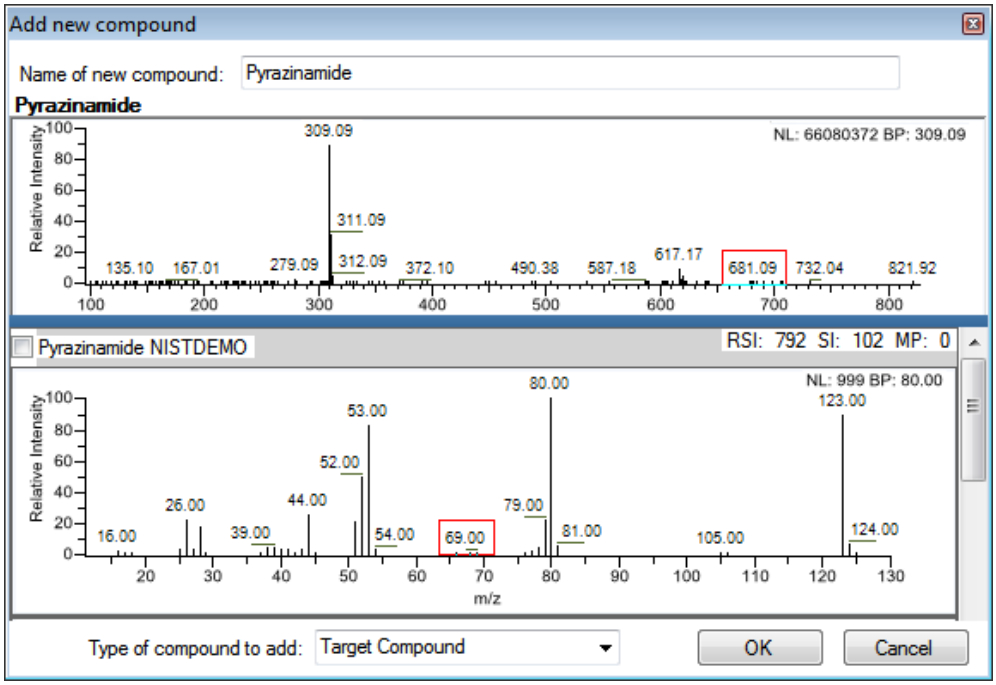
   IMPORTANT While the spectra pane displays the associated raw data file, you cannot display peak information for an original compound in the Compound list. You can display peak information only for compounds in the associated raw data file. To return the display functionality for all compounds in the method, save the method.

8. Select a filter from the Filter list.
9. Click to select the peak in the chromatogram that represents the compound that you want to add to the method.
10. Right-click and choose **Add This Peak as New Compound**.

    The application performs a library search for the selected compound. The application uses the first match it finds as the compound name, the base peak of the mass spectrum as the target peak, and the second and third largest ions as the confirming peaks.

    - When the name of the first match does not exist in the method, the application adds this compound to the method and displays the name in the Compound list. You can now view and edit the parameters for this compound.
    - When the name of the first match is already in the method, the Add New Compound dialog box opens. You cannot overwrite a compound name in the method. If the selected peak already exists in the method, you must give it a new name to add it to the method. Or, you can select a different compound to add to the method, following step 11 through step 12.

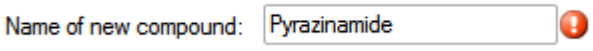
Figure 55. Add New Compound dialog box



11. Do one of the following:

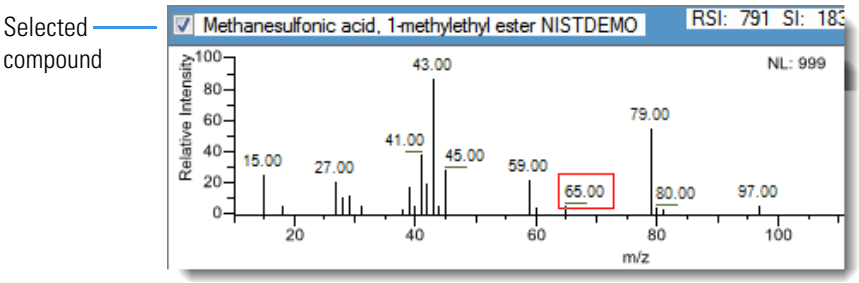
    - Type a new name for the first matched compound.

      The application displays a red warning when the selected compound name already exists in the method. You cannot overwrite the compound name, and you cannot create a duplicate name in the method. You must type a unique name.

    –or–

    - To use a compound other than the first matched compound, scroll to the spectrum for that compound and select its corresponding check box in the title bar of the spectrum pane.

12. In the Type of Compound to Add list, select a compound type.
13. Click **OK**.

❖ To change the compound reference spectrum

1. Select the **Show Peak Creator Panel** check box.

   The Manual Peak Creator pane opens.

2. In the chromatogram pane, click a peak.

   The application displays the spectrum for the selected peak in the spectrum pane.

3. In the spectrum pane, right-click and choose **Use This Spectrum for Compound Reference Spectrum**.

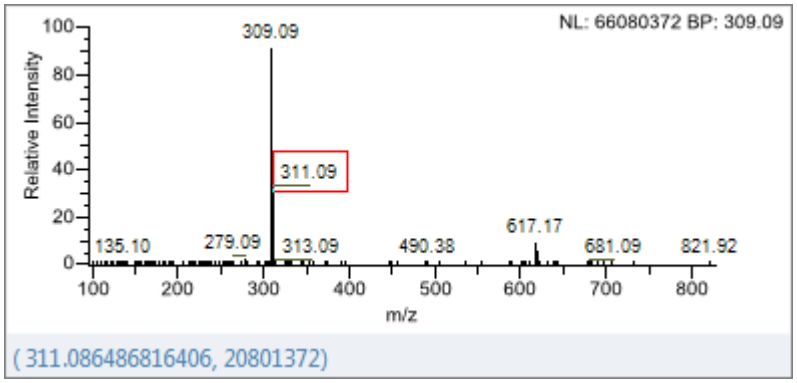
❖ To replace a quantitation mass

1. Select the **Show Peak Creator Panel** check box.

   The Manual Peak Creator pane opens.

2. Hold the cursor over a peak in the spectrum pane.

   The red box indicates the selected peak.



3. Right-click and choose **Set This Spectral Peak as Quan Value.**
4. Choose either **Don't Update Ion Ratios** or **Update Ion Ratios Using This Spectrum**.

   You can see the updated ion ratios on the Ratios page for the confirming peaks.

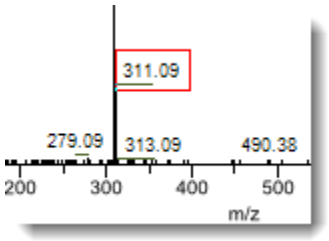
❖ To add a mass to the existing quantitation mass ranges

1. Select the **Show Peak Creator Panel** check box.

   The Manual Peak Creator pane opens.

2. In the spectrum pane, hold the cursor over a peak.

   The red box indicates the selected peak.



3. Right-click and choose **Add This Spectral Peak to Existing Quan Ranges.**
4. Choose either **Don't Update Ion Ratios** or **Update Ion Ratios Using This Spectrum**.

   The application adds the selected mass to the existing quantitation mass ranges to increase the signal.

   If you chose to update the ion ratios, you can see the updated ion ratios on the Ratios page for the confirming peaks.

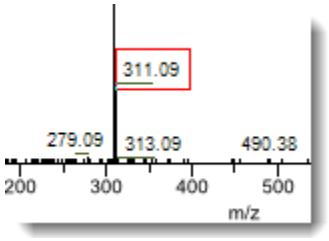
❖ To add a target peak

1. Select the **Show Peak Creator Panel** check box.

   The Manual Peak Creator pane opens.

2. In the spectrum pane, hold the cursor over a peak.

   The red box indicates the selected peak.



3. Right-click and choose **Add This Spectral Peak as New Quan Peak.**

   The application adds a new target peak to the compound.

4. To view the new target peak in the Peaks pane, use the vertical scroll bar or use the Chromatogram Plot Settings dialog box to add another row to the Peaks pane display.

   You can use the shortcut menu in the spectrum pane for this new target peak to perform any of the tasks that you would perform on the original target peak.

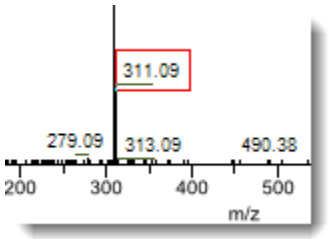
❖ To add a spectral peak as a new compound

1. Select the **Show Peak Creator Panel** check box.

   The Manual Peak Creator pane opens.

2. In the raw data file spectrum pane, hold the cursor over a peak.

   The red box indicates the selected peak.

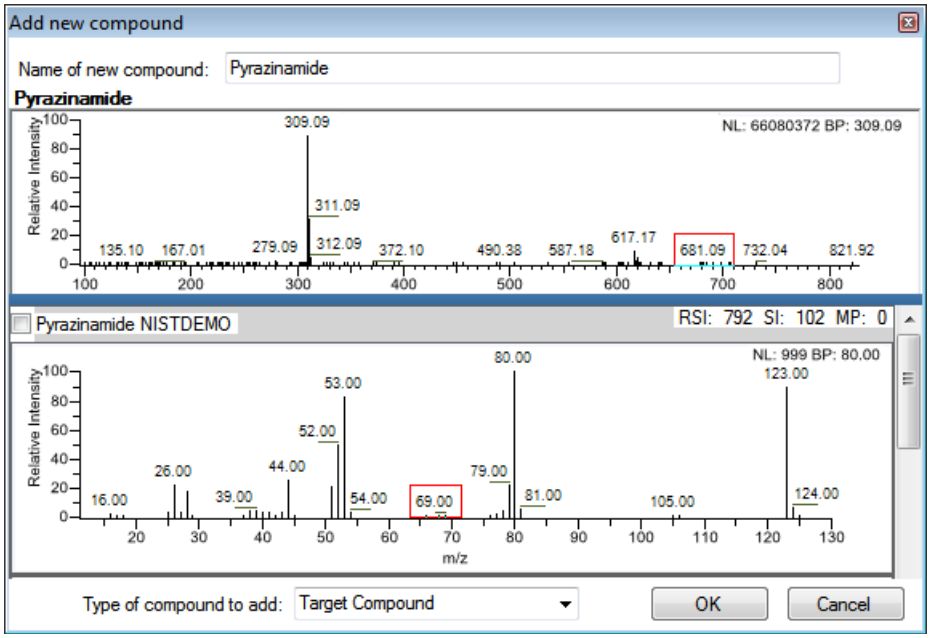


3. Right-click and choose **Add This Spectral Peak as New Compound.**

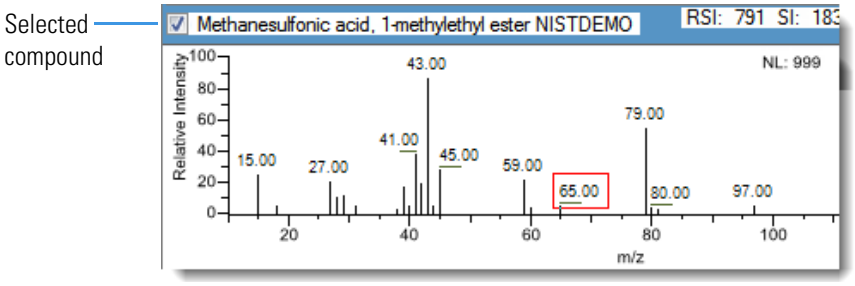
   The application performs a library search for the selected compound. The application uses the first match it finds as the compound name, the base peak of the mass spectrum as the target peak, and the second and third largest ions as the confirming peaks.

   When there are multiple matches, the Add New Compound dialog box opens. If the name of the first match is already in the library, the dialog box opens with the matching compound selected.

Figure 56. Add New Compound dialog box



4. (Optional) Make any of the following changes:
   - Change the name for the compound in the Name of New Compound box.
   - Use a compound other than the compound chosen by the application by scrolling to the spectrum for that compound and selecting the compound name in the title bar of the spectrum pane.



   - In the Type of Compound to Add list, select a compound type.
5. Click **OK**.

❖ To replace a target peak with a confirming peak

1. When you have multiple target peaks in the Peaks pane, select the target peak that you want to replace.
2. Right-click the confirming peak that you want to use as the target peak, and choose **Swap with Target Peak**.

   The application swaps the target peak and the confirming peak. The application replaces all information for the target peak with information for the confirming peak. This includes the expected retention time that the confirming peak inherited from the original target peak. The original target peak replaces the confirming peak. The application recalculates the ratios for all confirming peaks.

❖ To set a confirming peak as an additional target peak

Right-click the confirming peak that you want to use as the new target peak and choose **Promote to Separate Target Peak**.

The application creates a new target peak, using information from the confirming peak. This includes the expected retention time that the confirming peak inherited from the original target peak. The application removes all references to the confirming peak from the method.

❖ To add a trace to the Real Time Status pane

Right-click the target peak or confirming peak that you want to add to the Real Time Status pane and choose **Display in Real Time Viewer**.

The application moves the peak to the Traces To Display in Real Time Viewer pane on the Real Time Viewer page.

When you acquire samples with this method, the application displays the added trace in addition to the TIC in the Real Time Status pane.



Tip (Animation) To view "Specifying TIC Traces for the Real Time Status Pane," choose **Help > Animations**.

❖ To remove a confirming peak

Right-click the confirming peak that you want to remove and choose **Cut Confirming Peak.**

❖ To save the new method

1. Choose **File > Save**.

   The Save Master Method dialog box opens.

2. (Optional) Select a different repository.

   The default repository is specified in the Administrator Console. Refer to the instructions in the *TraceFinder Administrator Console User Guide*.

3. Type a new name for the method and click **Save**, or type the name of an existing method to overwrite and click **Overwrite**.

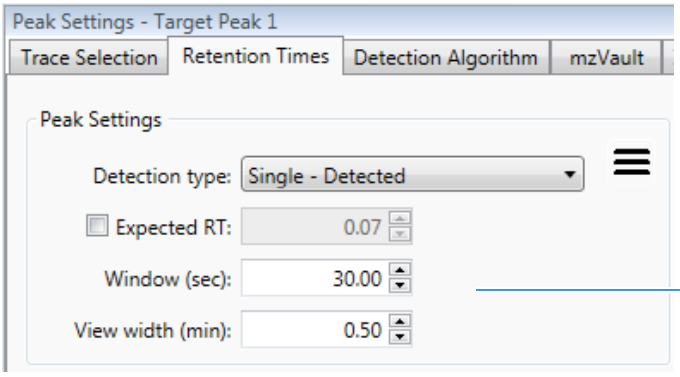
   The application saves the new method data in the following folder:

   …\TraceFinderData\Methods

Retention Times

Use the features on the Retention Times page to define the expected retention time or a retention time range for a selected target peak.

Figure 57. Retention Times page for a target peak



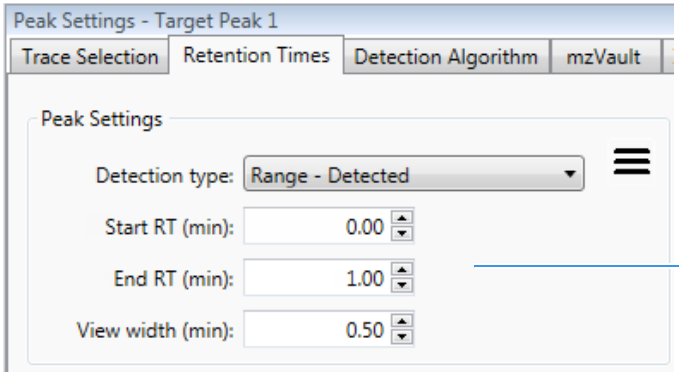


Table 29. Retention Times page parameters for target peaks (Sheet 1 of 2)

Parameter	Description
Detection Type	Single - Detected: (Default) Specified as a centered retention time window. The application integrates a distinct peak. In reports, the application displays the expected retention time and actual retention time values as Method RT and Detected RT, respectively. Range - Detected: Specified as a retention time start/end range. Range - Integrated: Specified as a retention time start/end range. The application integrates all peaks within the specified time range.

Table 29. Retention Times page parameters for target peaks (Sheet 2 of 2)

Parameter	Description
Expected RT	Specifies the expected retention time in minutes for a single peak. Available only for the Single - Detected detection type. When you select this parameter: • The specified retention time is required for confirming the peak. • The application does not process the full trace. This speeds the processing time. • The application also uses the Extraction Window parameter on the Detection Algorithm page.
Window (sec)	Width of the window (in seconds) to indicate how far around the expected retention time the system looks for a peak apex. Available only for the Single - Detected detection type.
Start/End RT (min)	Beginning and ending retention time window that can encompass multiple peaks. Available only for the Range - Detected and the Range - Integrated detection types. When you change from a Single - Detected detection type, these values default to the previous beginning and ending time calculated from the Expected RT and Window values.
View Width (min)	Viewable size of the ion chromatogram display. Changing the view width does not affect the peak detection process; the application uses it only for graphical display. When you select either Range - Detected or Range - Integrated as the detection type, you cannot select a View Width value less than the retention time range (*end time* minus *start time*).
Menu commands ☰	
Apply Current Peak Settings to All Peaks in the Compound	Copies the View Width and Window values to all peaks for the compound and updates the compound. Available only when a compound has multiple peaks.
Apply Current Peak Settings to All Peaks in the Method	Copies the View Width and Window values to all peaks for the method and updates the method.

Trace Selection

Use the Trace Selection Pages for Target, Confirming, and Fragment Peaks pages to define the detector and filters for each chromatogram trace.

Trace Selection page parameters are different for target and confirming peaks, fragments, and mass spectrometer and analog detectors. See the following examples:

- Trace Selection page for a target peak using a mass spectrometer detector
- Trace Selection page for a target peak using an analog detector
- Trace Selection page for a confirming peak using a mass spectrometer detector
- Trace Selection page for a confirming peak using an analog detector
- Trace Selection page for fragments using a mass spectrometer detector
- Trace Selection page for fragments using an analog detector

Follow these procedures:

- To specify ranges of masses for detection and integration of target peaks
- To specify masses for detection and integration of confirming peaks
- To specify ranges of masses for detection and integration of fragments
- To specify an XIC scan filter
- To specify an MS filter
- To specify the minimum number of fragments

❖ To specify ranges of masses for detection and integration of target peaks

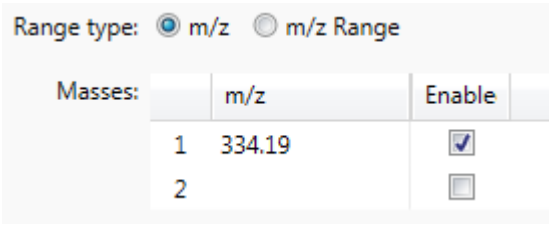
1. Select a target peak in the Peaks pane.
2. Select **MS** from the Detector list.
3. Select **Mass Range** from the Trace list.

> **Note** The parameters in the Mass Ranges area are available only when you set the Detector parameter to MS and the Trace parameter to Mass Range.

You can choose one of two methods for specifying the mass ranges. To specify a range around a center mass, follow step 4. To specify starting and ending values for mass ranges, follow step 5.

4. To specify a center mass range, do the following:
   a. Select the **m/z** option.

      The application displays a table of center mass values.



   b. (Optional) To create a new range, type a value in the *m/z* column of the last (empty) row and press ENTER.

      The application adds a new empty row.

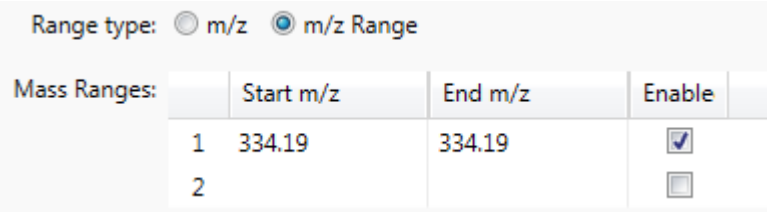
   c. (Optional) Add as many center mass values as you want.

   d. Select the **Enable** check box for each center mass that you want to use.

   When you process a batch with this method, the application uses a range of one amu that is centered on the *m/z* value for each enabled mass.

5. To create a new range of masses, do the following:
   a. Select the **m/z Range** option.

      The application displays a table of mass range values.



   b. (Optional) To create a new mass range, type values in the Start m/z and End m/z columns of the last (empty) row and press ENTER.

      The application adds a new empty row.

   c. (Optional) Add as many mass range values as you want.

   d. Select the **Enable** check box for each mass range that you want to use.

   When you process a batch with this method, the application sums the multiple ions specified by each enabled mass range.

❖ To specify masses for detection and integration of confirming peaks

1. Select a confirming peak in the Peaks pane.
2. Select **MS** from the Detector list.

3. Select **Mass Range** from the Trace list.

> **Note** The parameters in the Mass Ranges area are available only when you set the Detector parameter to MS and the Trace parameter to Mass Range.

4. To specify a center mass range, type or select a value in the m/z box.

   When you process a batch with this method, the application uses a range of one amu that is centered on the specified *m/z* value.

❖ To specify ranges of masses for detection and integration of fragments

1. Select a fragment in the Peaks pane.
2. Select **MS** from the Detector list.
3. Select **Mass Range** from the Trace list.

> **Note** The parameters in the Mass Ranges area are available only when you set the Detector parameter to MS and the Trace parameter to Mass Range.

4. (Optional) To create a new mass range, type values in the Start m/z and End m/z columns of the last (empty) row and press ENTER.
5. The application adds a new empty row.
6. (Optional) Add as many mass range values as you want.
7. In the Mass Ranges area, select the **Enable** check box for each mass range that you want to use.

   When you process a batch with this method, the application sums the multiple ions specified by each enabled mass ranges.

❖ To specify an XIC scan filter

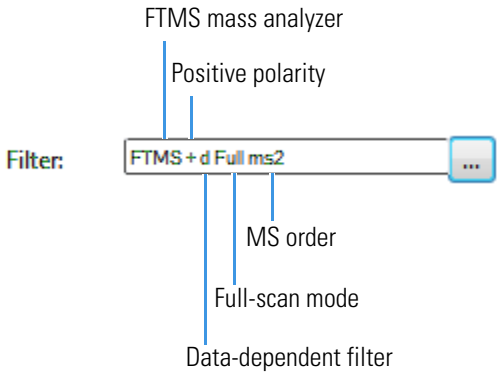
1. Click the Scan Filter browse button,  .

   The XIC Filter Dialog Box opens.

2. Specify your filter options.

3. Click **OK**.

   The application updates the chromatogram data using the specified XIC filter options, and the Scan Filter box indicates the parameters of the specified XIC filter, as in this example:



XIC Filter Dialog Box

Figure 58. XIC Filter dialog box

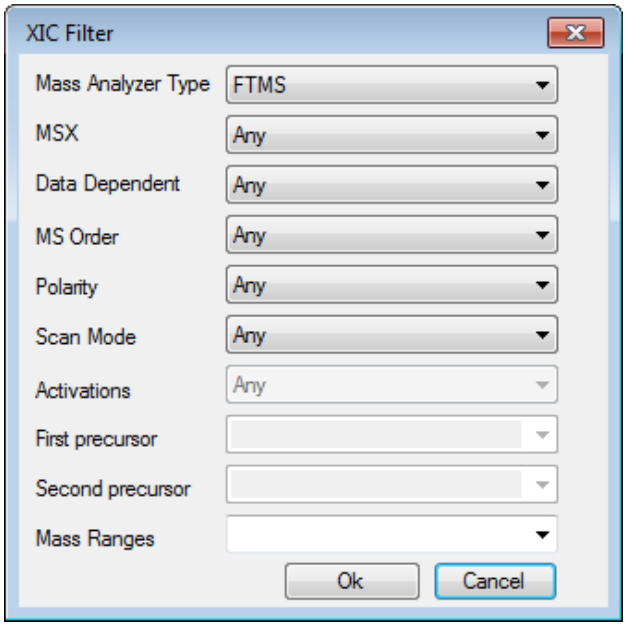


Table 30. XIC Filter dialog box parameters (Sheet 1 of 2)

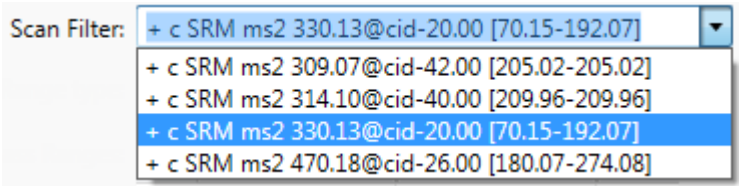
Parameter	Description
Mass Analyzer Type	Any: Any mass analyzer. FTMS: Fourier Transform Mass Spectrometer ITMS: Ion Trap Mass Spectrometer Sector: Static electric or magnetic sectors, or a combination of the two SQMS: Single Quad Mass Spectrometer TOFMS: Time-of-Flight Mass Spectrometer TQMS: Triple Quad Mass Spectrometer

Table 30. XIC Filter dialog box parameters (Sheet 2 of 2)

Parameter	Description
MSX	Any: For both MSX and non-MSX scans. On: For only MSX scans. Off: For only non-MSX scans.
Data Dependent	Any: For both data-dependent and non-data-dependent filters. On: For only data-dependent filters. Off: For only non-data-dependent filters.
MS Order	Any: For any MS order. MS: For only a single mass spec stage. MS2-MS3: For only multiple mass spec stages.
Polarity	Any: For both positive and negative scans. Positive: For only positive scans. Negative: For only negative scans.
Scan Mode	Any: For any scan mode. Full: Full-scan mode SIM: Selective ion monitoring SRM: Selective reaction monitoring CRM: Consecutive reaction monitoring Q1MS: MS using quadrupole 1 Q3MS: MS using quadrupole 3
Activations	Any: For any activation method. CID: Collision-induced dissociation MPD: Multiple photodissociation ECD: Electron capture dissociation PQD: Pulsed dissociation ETD: Electron transfer dissociation HCD: Higher energy collision-induced dissociation This parameter is available only when the MS Order is set to MS2 or MS3.
First Precursor	This parameter is available only when the MS Order is set to MS2 or MS3.
Second Precursor	This parameter is available only when the MS Order is set to MS3.
Mass Ranges	Specifies a mass range in [x-y] format. Select from the list or type a custom range.

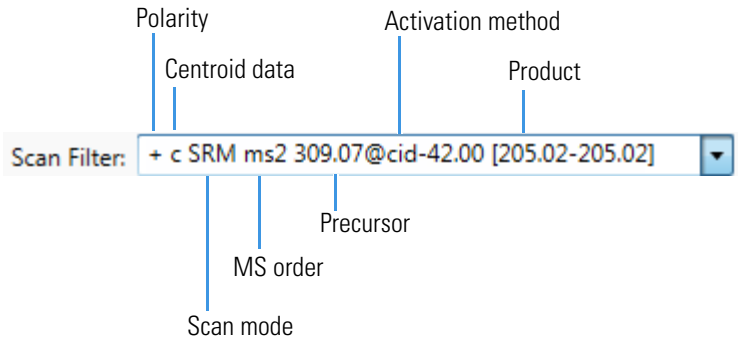
❖ To specify an MS filter

Select a filter from the Scan Filter list as shown in this example:



The Scan Filter list includes all available scan filters for the method.

The application updates the chromatogram data using the specified filter options, as in this example:



❖ To specify the minimum number of fragments

1. Select one of the following options on the Trace Selection page for fragments using a mass spectrometer detector:
   - **Global**: Applies the detection settings to all peaks in the compound.
   - **By Peak**: Applies the detection settings to only the selected fragment peak.
2. In the Minimum # of Fragments box, type the minimum number of fragments required to identify a compound.

   The value you specify cannot be greater than the number of fragments defined in the compound database.

To identify a compound, the application must find at least this minimum number of fragments when you process a sample for fragment ions.

Trace Selection Pages for Target, Confirming, and Fragment Peaks

Figure 59. Trace Selection page for a target peak using a mass spectrometer detector

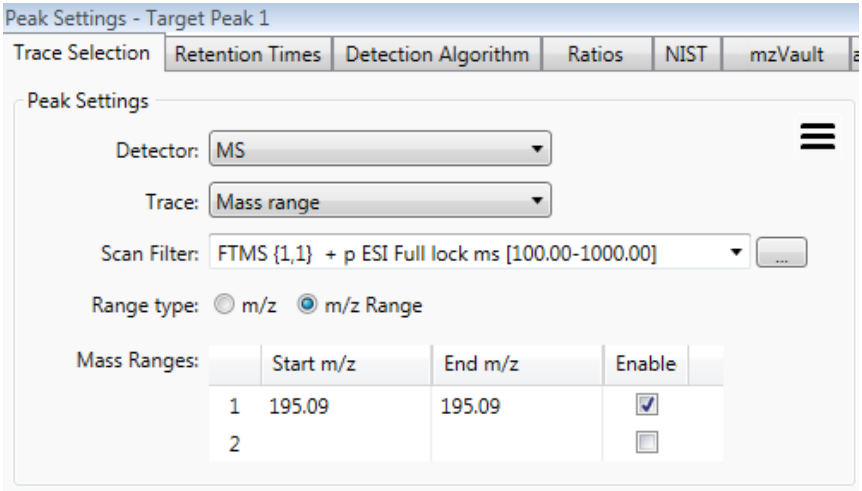


Figure 60. Trace Selection page for a target peak using an analog detector

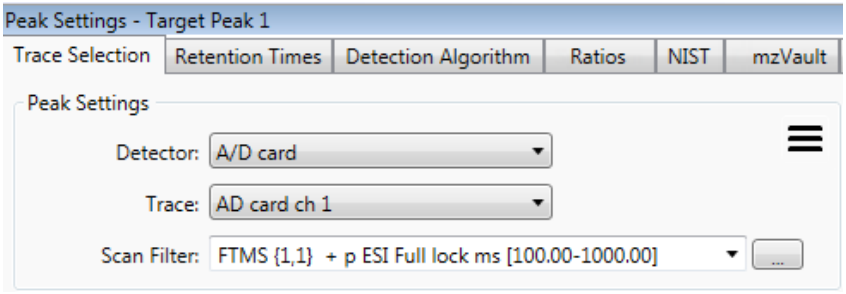


Figure 61. Trace Selection page for a confirming peak using a mass spectrometer detector

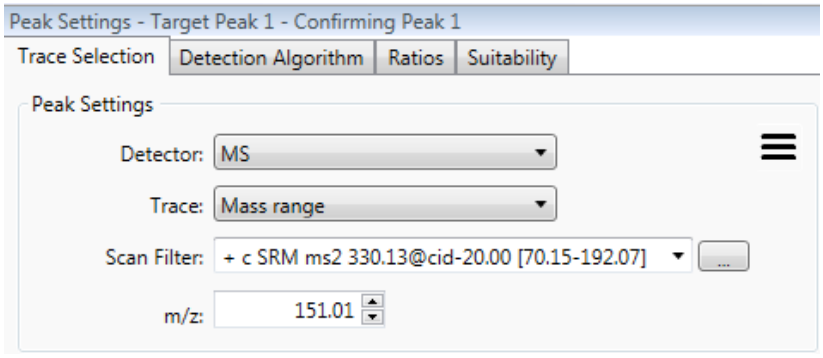


Figure 62. Trace Selection page for a confirming peak using an analog detector

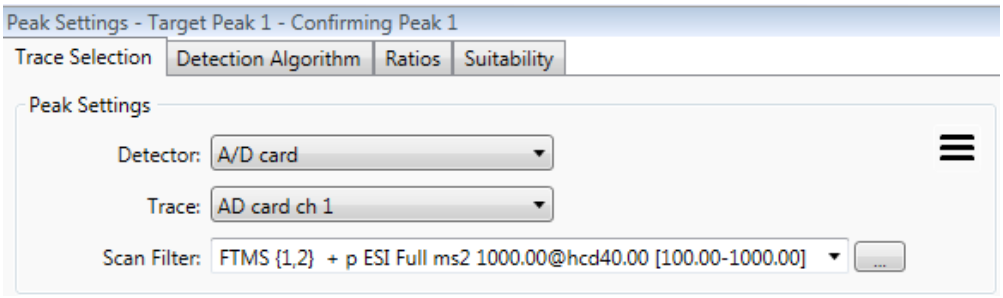


Figure 63. Trace Selection page for fragments using a mass spectrometer detector

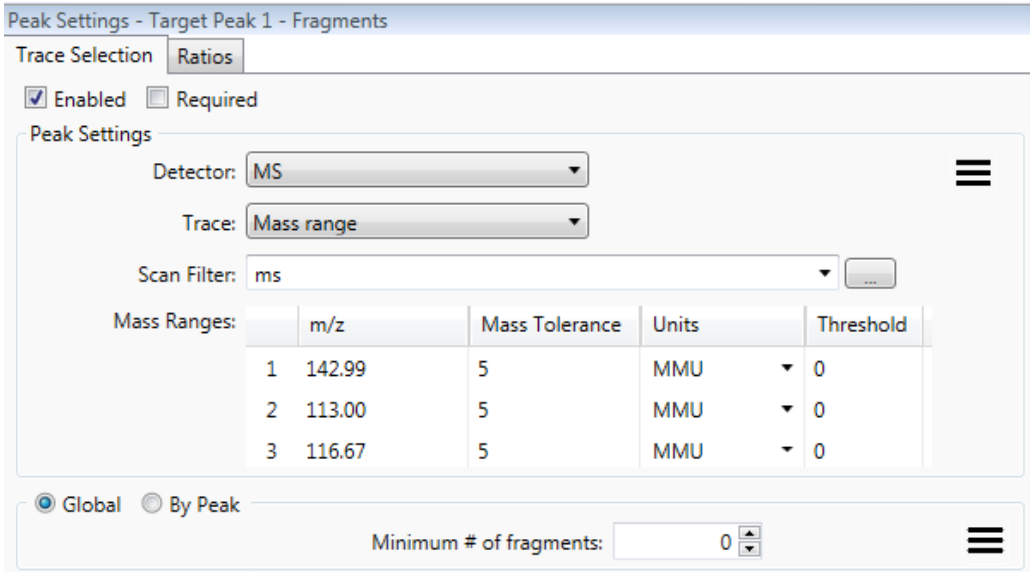


Figure 64. Trace Selection page for fragments using an analog detector

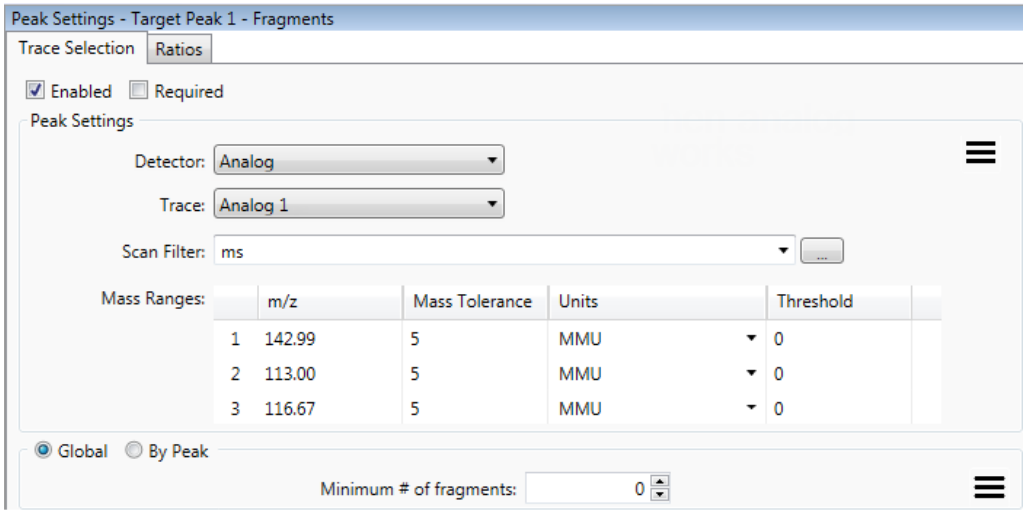


Table 31. Trace Selection page parameters (Sheet 1 of 2)

Parameter	Description
Detector	The available detector options depend on which detection options create the method. The method can use the standard options (all the listed options) or only the detection options used to acquire an associated raw data file. To specify the available detector options, see Specifying Peak Detection Defaults in Chapter 1, "Using the Configuration Console." MS: Mass spectrometer that ionizes sample molecules and then separates the ions according to their mass-to-charge ratio (*m/z*). PDA: Photodiode array detector providing a linear array of discrete photodiodes on an integrated circuit chip. It is placed at the image plane of a spectrometer to allow simultaneous detection of a range of wavelengths. Analog: Supplemental detectors (for example, FID, ECD). When you select this detector, any reports that display a QIon value show the value as Analog and any reports that display spectra show the spectra as Not Available. A/D card: If you have a detector not under data system control, you can capture the analog signal and convert it to digital using an interface box (for example, SS420X) for storage in the raw data file. UV: A UV spectrophotometer (for variable-wavelength detection) or photometer (for single-wavelength detection) equipped with a low-volume flow cell. This detector detects analytes that readily absorb light at a selected wavelength.
Trace	Represents a specific range of the data. The application uses the trace to identify the characteristic ions for a compound. MS detector options: Mass Range, TIC, or Base Peak. When you select Mass Range, you are prompted to enter the start and end *m/z* values for the ranges. PDA detector options: Spectrum Maximum, Wavelength Range, or Total Scan. Analog detector options: Analog 1, Analog 2, Analog 3, or Analog 4. You can configure these channel names in your instrument configuration. A/D Card detector options: AD Card ch1, AD Card ch2, AD Card ch3, or AD Card ch4. You can configure these channel names in your instrument configuration. UV detector options: Channel A, Channel B, Channel C, or Channel D. You can configure these channel names in your instrument configuration.
Scan Filter	Available only when you select the MS detector. Represents a particular data acquisition channel. For example, the filter option + *c Full ms [35.00-500.00]* represents a positive ion centroid signal acquired in single-stage, full-scan mode from *m/z* 35 to 500.

Table 31. Trace Selection page parameters (Sheet 2 of 2)

Parameter	Description
Range Type	Available only for target peaks when you select the Mass Range trace for an MS detector. m/z: Specifies a single *m/z* value for detection and integration. The application uses a range of one amu centered on this value. m/z Range: Specifies a range of *m/z* values for detection and integration. The application sums the multiple ions specified by these ranges.
Mass Ranges	Specifies ranges of masses that the application uses to sum the multiple ions specified by each enabled mass range.
Minimum # of Fragments (fragments only)	Specifies the minimum number of fragment masses that the application must identify when you process a sample for fragment ions. The value you specify for Minimum # of Fragments cannot be greater than the number of fragments defined in the compound database.
Menu commands ☰	
Apply Current Peak Settings to All Peaks in the Compound	Copies the peak settings to all peaks for the compound and updates the compound. Available only when a compound has multiple peaks.
Apply Current Peak Settings to All Peaks in the Method	Copies the peak settings to all peaks for the method and updates the method.

Detection Algorithm

Use the Detection Algorithm page to define the peak detection algorithm (sensitivity) and its options and to determine the area under a curve. There are four detection algorithms: Genesis, ICIS, Avalon, and Auto. Use the Genesis and Avalon detection algorithms for mass spectrometry detection. Use the ICIS detection algorithm primarily for analog detection. Use the Auto detection algorithm for a simplified analog detection.

On this page, you can specify how you want each mode to run. See the following for detailed descriptions of all the features on the Detection Algorithm page:

- For Genesis sensitivity, see Detection Algorithm Page for Genesis.
- For ICIS sensitivity, see Detection Algorithm Page for ICIS.
- For Avalon sensitivity, see Detection Algorithm Page for Avalon.
- For Auto sensitivity, see Detection Algorithm Page for Auto.

Detection Algorithm Page for Genesis

Use the Genesis detection algorithm for mass spectrometry detection. Specify applying the detection settings per peak or globally.

Figure 65. Detection Algorithm page for Genesis

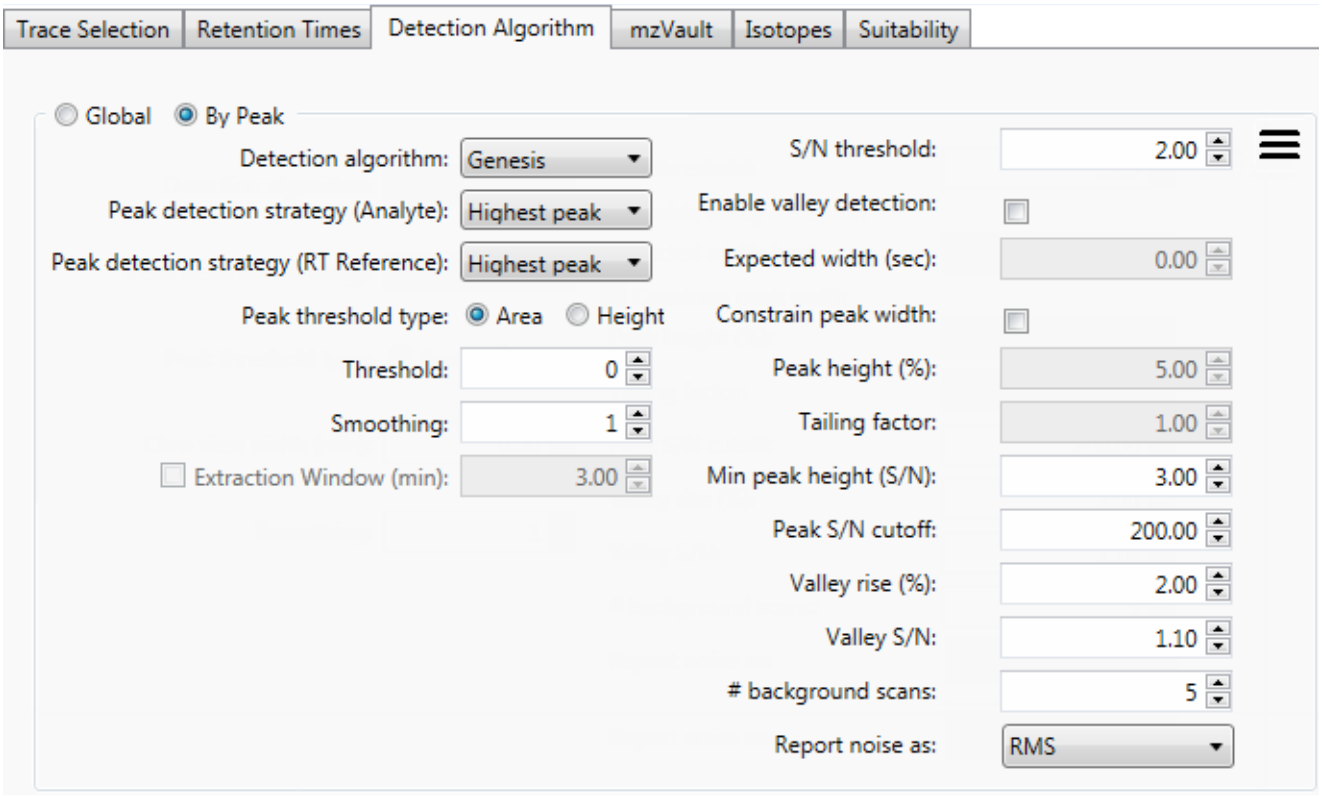


Table 32. Detection Algorithm page parameters for Genesis (Sheet 1 of 4)

Parameter	Description
Global/By Peak	Global: Applies the detection settings to all peaks in the compound. By Peak: Applies the detection settings to only the selected target or confirming peak.
Detection Algorithm	Specifies the Genesis peak detection algorithm.
Peak Detection Strategy (Analyte)	Highest Peak: Uses the highest peak in the chromatogram for component identification. Nearest RT: Uses the peak with the nearest retention time in the chromatogram for component identification.
Peak Detection Strategy (RT Reference)	Highest Peak: Uses the highest peak in the chromatogram for component identification. Nearest RT: Uses the peak with the nearest retention time in the chromatogram for component identification.
Peak Threshold Type	Specifies whether to select peaks by height or area. Default for Global: Height Default for By Peak: Area

Table 32. Detection Algorithm page parameters for Genesis (Sheet 2 of 4)

Parameter	Description
Threshold	Specifies the peak threshold for peak integration. Peaks with area or height values less than this value are not integrated. Peaks with values greater than this value are integrated.
Smoothing	Determines the degree of data smoothing to be performed on the active component peak prior to peak detection and integration. Valid values: Any odd integer from 1 through 15 points Default: 1f
Extraction Window (min) (Target peaks only)	Specifies a window that limits how much of the entire trace the application processes. When cleared, the application processes the entire trace, which can slow processing. Default: 3.00 **Note** The application applies this same setting to all associated confirming peaks.
S/N Threshold	Current signal-to-noise threshold for peak integration. Peaks with signal-to-noise values less than this value are not integrated. Peaks with signal-to-noise values greater than this value are integrated. Valid range: 0.0 through 999.0
Enable Valley Detection	Uses the valley detection approximation method to detect unresolved peaks. This method drops a vertical line from the apex of the valley between unresolved peaks to the baseline. The intersection of the vertical line and the baseline defines the end of the first peak and the beginning of the second peak.
Expected Width (sec)	The expected peak width parameter (in seconds). This parameter controls the minimum width that a peak is expected to have if valley detection is enabled. With valley detection enabled, any valley points nearer than the *expected width*/2 to the top of the peak are ignored. If a valley point is found outside the expected peak width, the application terminates the peak at that point. The application always terminates a peak when the signal reaches the baseline, independent of the value set for the expected peak width. Valid range: 0.0 through 999.0
Constrain Peak Width	Constrains the peak width of a component during peak integration of a chromatogram. You can then set values that control when peak integration is turned on and off by specifying a peak height threshold and a tailing factor. Selecting the Constrain Peak Width check box activates the Peak Height (%) and Tailing Factor options.
Peak Height (%)	A signal must be above the baseline percentage of the total peak height (100%) before integration is turned on or off. This text box is active only when you select the Constrain Peak Width check box. Valid range: 0.0 through 100.0%

Table 32. Detection Algorithm page parameters for Genesis (Sheet 3 of 4)

Parameter	Description
Tailing Factor	A factor that controls how the application integrates the tail of a peak. This factor is the maximum ratio of the trailing edge to the leading side of a constrained peak. This text box is active only when you select the Constrain the Peak Width check box. Valid range: 0.5 through 9.0
Min Peak Height (S/N)	Specifies the minimum peak height measured as a signal-to-noise ratio. Valid range: 0.00 through 999.00 Default: 3.00
Peak S/N Cutoff	The peak edge is set to values below this signal-to-noise ratio. This test identifies an edge of a peak when the baseline adjusted height of the edge is less than the ratio of the baseline adjusted apex height and the peak S/N cutoff ratio. When the S/N at the apex is 500 and the peak S/N cutoff value is 200, the application defines the right and left edges of the peak when the S/N reaches a value less than 200. Valid range: 50.0 through 10000.0
Valley Rise (%)	The peak trace can rise above the baseline by this percentage after passing through a minimum (before or after the peak). This method drops a vertical line from the apex of the valley between unresolved peaks to the baseline. The intersection of the vertical line and the baseline defines the end of the first peak and the beginning of the second peak. When the trace exceeds the rise percentage, the application applies valley detection peak integration criteria. The application applies this test to both the left and right edges of the peak. The rise percentage criterion is useful for integrating peaks with long tails. Valid range: 0.1 through 500.0
Valley S/N	Specifies a value to evaluate the valley bottom. Using this parameter ensures that the surrounding measurements are higher. Valid range: 1.0 through 100.0 Default: 2.0
# Background Scans	Number of background scans performed by the application.
Report Noise As	Determines if the noise used in calculating S/N values is calculated using an RMS calculation or a peak-to-peak resolution threshold. Options are RMS or Peak to Peak.

Table 32. Detection Algorithm page parameters for Genesis (Sheet 4 of 4)

Parameter	Description
By Peak menu commands ☰	
Apply Current Peak Settings to All Peaks in the Method	Updates all compounds in the method with the current settings on the Detection Algorithm page. These updates apply to both target and confirming peaks.
Apply Current Peak Settings to All Target Peaks in the Method	Updates all compounds in the method with the current settings on the Detection Algorithm page. These updates apply only to target peaks.
Apply Current Peak Settings to All Peaks with the Same Detection Algorithm	Uses the current settings on the Detection Algorithm page to update all compounds in the method that use the Genesis detection algorithm. These updates apply to both target and confirming peaks that use the Genesis detection algorithm.
Apply Current Peak Settings to All Peaks in the Compound	Updates all peaks in the current compound with the current settings on the Detection Algorithm page. These updates apply to both target and confirming peaks.
Apply Current Extraction Window Settings to All Peaks in the Method	Updates all peaks in the current method with the current Extraction Window value on the Detection Algorithm page. These updates apply to both target and confirming peaks.
Global menu command ☰	
Push Global Settings to All Peaks	Pushes the global settings to all peaks for the method and updates the method.

Detection Algorithm Page for ICIS

Use the ICIS detection algorithm primarily for analog detection.

Figure 66. Detection Algorithm for ICIS

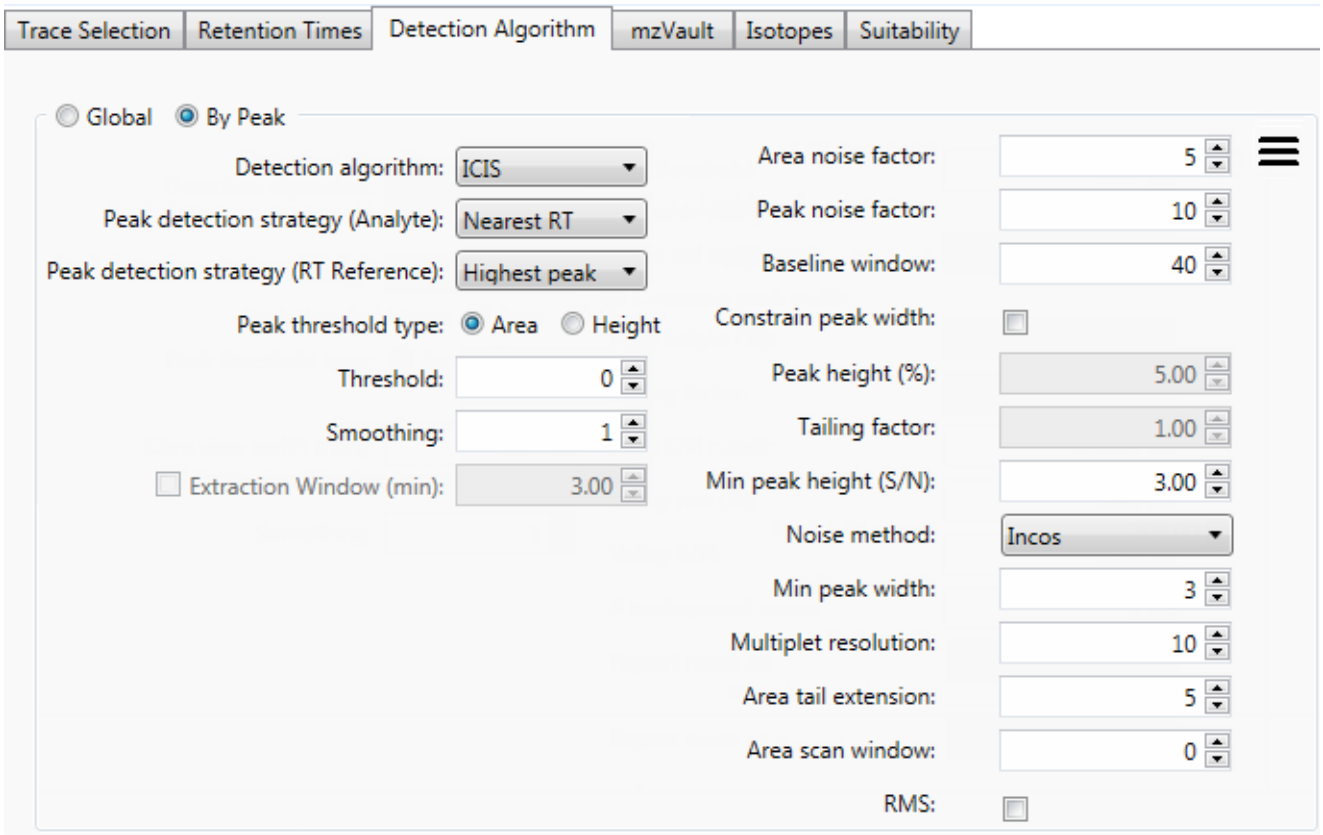


Table 33. Detection Algorithm page parameters for ICIS (Sheet 1 of 4)

Parameter	Description
Global/By Peak	Global: Applies the detection settings to all peaks in the compound. By Peak: Applies the detection settings to only the selected target or confirming peak.
Detection Algorithm	Specifies the ICIS peak detection algorithm, used primarily with analog detectors.
Peak Detection Strategy (Analyte)	Highest Peak: Uses the highest peak in the chromatogram for component identification. Nearest RT: Uses the peak with the nearest retention time in the chromatogram for component identification.
Peak Detection Strategy (RT Reference)	Highest Peak: Uses the highest peak in the chromatogram for component identification. Nearest RT: Uses the peak with the nearest retention time in the chromatogram for component identification.
Peak Threshold Type	Specifies whether to select peaks by height or area. Default for Global: Height Default for By Peak: Area

Table 33. Detection Algorithm page parameters for ICIS (Sheet 2 of 4)

Parameter	Description
Threshold	Specifies the peak threshold for peak integration. Peaks with area or height values less than this value are not integrated. Peaks with values greater than this value are integrated.
Smoothing	Determines the degree of data smoothing to be performed on the active component peak prior to peak detection and integration. Valid values: Any odd integer from 1 through 15 points Default: 1
Extraction Window (min) (Target peaks only)	Specifies a window that limits how much of the entire trace the application processes. When cleared, the application processes the entire trace, which can slow processing. Default: 3.00 **Note** The application applies this same setting to all associated confirming peaks.
The application also uses the default values for the following parameters for the Auto detection algorithm. The Auto detection page does not display these parameters. See Detection Algorithm Page for Auto.	
Area Noise Factor	The noise level multiplier used to determine the peak edge after the location of the possible peak. Valid range: 1 through 500 Default: 5
Peak Noise Factor	The noise level multiplier used to determine the potential peak signal threshold. Valid range: 1 through 1000 Default: 10
Baseline Window	The application looks for a local minima over this number of scans. Valid range: 1 through 500 Default: 40
Constrain Peak Width	Constrains the peak width of a component during peak integration of a chromatogram. You can then set values that control when peak integration is turned on and off by specifying a peak height threshold and a tailing factor. Selecting the Constrain Peak Width check box activates the Peak Height (%) and Tailing Factor options.
Peak Height (%)	A signal must be above the baseline percentage of the total peak height (100%) before integration is turned on or off. This text box is active only when you select the Constrain Peak Width check box. Valid range: 0.0 through 100.0%
Tailing Factor	A factor that controls how the application integrates the tail of a peak. This factor is the maximum ratio of the trailing edge to the leading side of a constrained peak. This text box is active only when you select the Constrain the Peak Width check box. Valid range: 0.5 through 9.0

Table 33. Detection Algorithm page parameters for ICIS (Sheet 3 of 4)

Parameter	Description
Min Peak Height (S/N)	Specifies the minimum peak height measured as a signal-to-noise ratio. Valid range: 0.00 through 999.00 Default: 3.00
Noise Method	The options are INCOS or Repetitive. INCOS: Uses a single pass algorithm to determine the noise level. Repetitive: Uses a multiple pass algorithm to determine the noise level. In general, this algorithm is more accurate in analyzing the noise than the INCOS Noise algorithm, but the analysis takes longer.
Min Peak Width	The minimum number of scans required in a peak. Valid range: 0 through 100 scans Default: 3
Multiplet Resolution	The minimum separation in scans between the apexes of two potential peaks. This is a filter to determine if two peaks are resolved. Valid range: 1 through 500 scans Default: 10
Area Tail Extension	The number of scans past the peak endpoint to use in averaging the intensity. Valid range: 0 through 100 scans Default: 5
Area Scan Window	The number of allowable scans on each side of the peak apex. A zero value defines all scans (peak-start to peak-end) to be included in the area integration. Valid range: 0 through 100 scans Default: 0
RMS	Specifies that the application calculates noise as RMS. By default, the application uses Peak To Peak for the noise calculation and automatically selects RMS if you manually determine the noise region.
By Peak menu commands ☰	
Apply Current Peak Settings to All Peaks in the Method	Updates all peaks in the current compound with the current settings on the Detection Algorithm page. These updates apply to both target and confirming peaks.
Apply Current Peak Settings to All Peaks with the Same Detection Algorithm	Uses the current settings on the Detection Algorithm page to update all compounds in the method that use the ICIS detection algorithm. These updates apply to both target and confirming peaks that use the ICIS detection algorithm.

Table 33. Detection Algorithm page parameters for ICIS (Sheet 4 of 4)

Parameter	Description
Apply Current Peak Settings to All Peaks in the Compound	Updates all compounds in the method with the current settings on the Detection Algorithm page. These updates apply to both target and confirming peaks.
Global menu command ☰	
Push Global Settings to All Peaks	Pushes the global settings to all peaks for the method and updates the method.

Detection Algorithm Page for Avalon

Use the Avalon detection algorithm for mass spectrometry detection.

Figure 67. Detection Algorithm page for Avalon

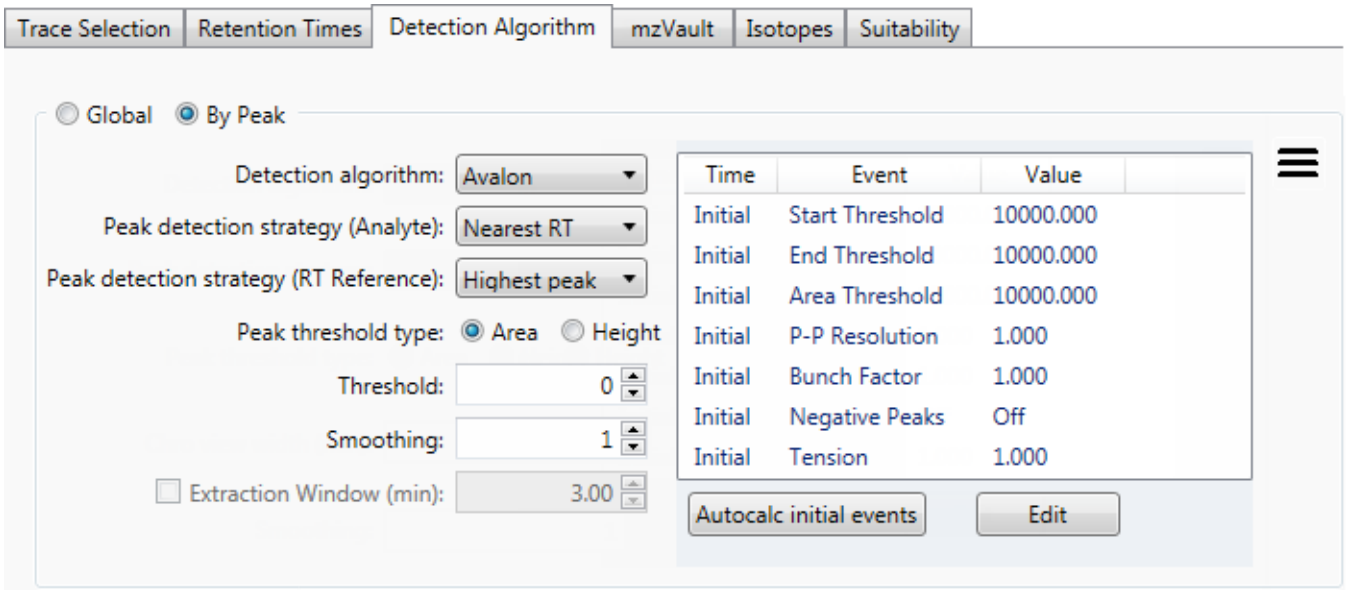


Table 34. Detection Algorithm page parameters for Avalon (Sheet 1 of 2)

Parameter	Description
Global/By Peak	Global: Applies the detection settings to all peaks in the compound. By Peak: Applies the detection settings to only the selected target or confirming peak.
Detection Algorithm	Specifies the Avalon peak detection algorithm.
Peak Detection Strategy (Analyte)	Highest Peak: Uses the highest peak in the chromatogram for component identification. Nearest RT: Uses the peak with the nearest retention time in the chromatogram for component identification.

Table 34. Detection Algorithm page parameters for Avalon (Sheet 2 of 2)

Parameter	Description
Peak Detection Strategy (RT Reference)	Highest Peak: Uses the highest peak in the chromatogram for component identification. Nearest RT: Uses the peak with the nearest retention time in the chromatogram for component identification.
Peak Threshold Type	Specifies whether to select peaks by height or area. Default for Global: Height Default for By Peak: Area
Threshold	Specifies the peak threshold for peak integration. Peaks with area or height values less than this value are not integrated. Peaks with values greater than this value are integrated.
Smoothing	Determines the degree of data smoothing to be performed on the active component peak prior to peak detection and integration. Valid values: Any odd integer from 1 through 15 points Default: 1
Extraction Window (min) (Target peaks only)	Specifies a window that limits how much of the entire trace the application processes. When cleared, the application processes the entire trace, which can slow processing. Default: 3.00 **Note** The application applies this same setting to all associated confirming peaks.
Autocalc Initial Events	Automatically calculates the events in the Event list.
Edit	Opens the Avalon Event List dialog box.
By Peak menu commands ☰	
Apply Current Peak Settings to All Peaks in the Method	Updates all peaks in the current compound with the current settings on the Detection Algorithm page. These updates apply to both target and confirming peaks.
Apply Current Peak Settings to All Peaks with the Same Detection Algorithm	Uses the current settings on the Detection Algorithm page to update all compounds in the method that use the ICIS detection algorithm. These updates apply to both target and confirming peaks that use the ICIS detection algorithm.
Apply Current Peak Settings to All Peaks in the Compound	Updates all compounds in the method with the current settings on the Detection Algorithm page. These updates apply to both target and confirming peaks.
Global menu command ☰	
Push Global Settings to All Peaks	Pushes the global settings to all peaks for the method and updates the method.

Avalon Event List

The event list includes both user-defined and noneditable default events. The application displays the default events when you choose Avalon sensitivity. You cannot delete these events or change their time or values. See Avalon Event List dialog box parameters. For a detailed list of events and value ranges, see Event types.

Figure 68. Avalon Event List dialog box

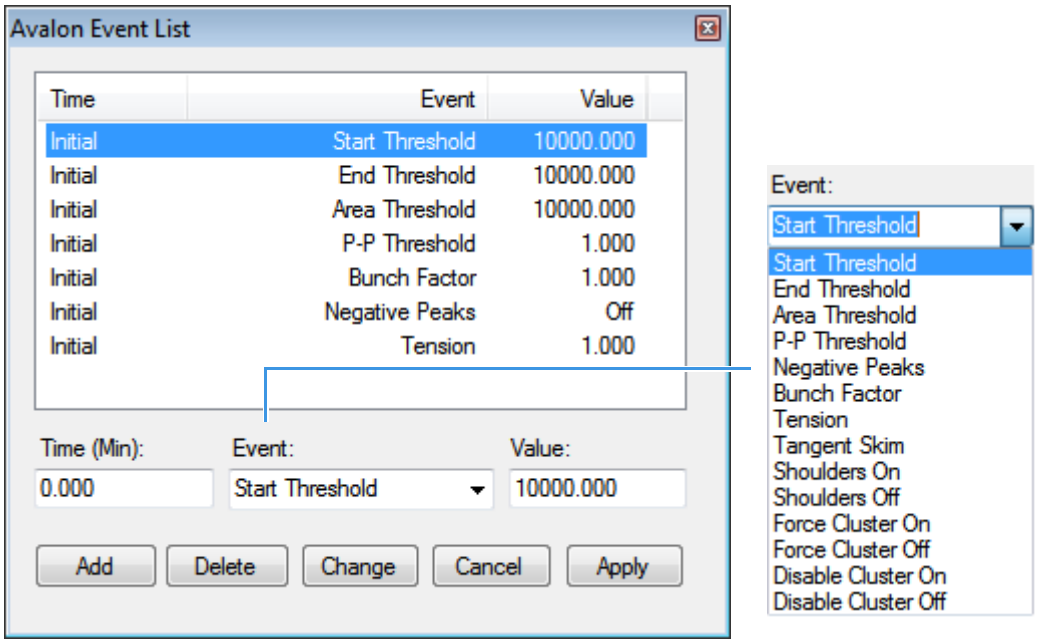


Table 35. Avalon Event List dialog box parameters

Parameter	Description
Time (Min)	Specifies the start time of the event.
Event	Specifies the type of event. For a detailed list of events and value ranges, see Event types.
Value	Specifies the value of the event.
Add	Adds a new event to the list with the current Time/Event/Value parameters.
Delete	Removes the selected Time/Event/Value parameter from the event list.
Change	Applies the current parameter values.
Cancel	Closes the dialog box without making any changes. Any additions, deletions, or changes revert to their original state.
Apply	Closes the dialog box.

Figure 69. Event types

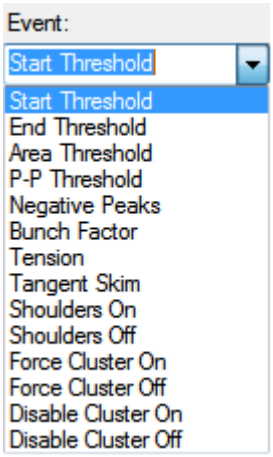


Table 36. Event type descriptions (Sheet 1 of 2)

Event type	Description
Start Threshold	Specifies the threshold at the start of a peak. The Start Threshold is directly related to the RMS noise in the chromatogram. Valid range: 0 through 999 999 999
End Threshold	Specifies the threshold at the end of a peak. The End Threshold is directly related to the RMS noise in the chromatogram. Valid range: 0 through 999 999 999
Area Threshold	Controls the area cutoff. Any peaks with a final area less than the area threshold will not be detected. This control is in units of area for the data. Valid range: 0 through 999 999 999
P-P Threshold	The peak-to-peak resolution threshold controls how much peak overlap must be present before two or more adjacent peaks create a peak cluster. Peak clusters have a baseline drop instead of valley-to-valley baselines. Specified as a percent of peak height overlap. Valid range: 0.1 through 99.99
Negative Peaks	Permits detection of a negative going peak. Automatically resets after finding a negative peak. Valid values: On or Off
Bunch Factor	Specifies the number of points grouped together during peak detection. This event controls the bunching of chromatographic points during integration and does not affect the final area calculation of the peak. A high bunch factor groups peaks into clusters. Valid range: 0 through 999

Table 36. Event type descriptions (Sheet 2 of 2)

Event type	Description
Tension	Controls how closely the baseline should follow the overall shape of the chromatogram. A lower tension traces the baseline to more closely follow changes in the chromatogram. A high baseline tension follows the baseline less closely, over longer time intervals. Valid range: 0 through 999.99 minutes
Tangent Skim	Using this event, you can tangent skim any peak clusters. By default, it chooses the tallest peak in a cluster as the parent. You can also identify which peak in the cluster is the parent. Tangent skim peaks are detected on either side (or both sides) of the parent peak. Tangent skim automatically resets at the end of the peak cluster. Valid range: 0 through 1
Shoulders On	Allows peak shoulders to be detected (peaks which are separated by an inflection rather than a valley) Sets a threshold for the derivative.
Shoulders Off	Disables peak shoulder detection. Valid range: 0 through 50
Force Cluster On	Force the following peaks to be treated as a cluster (single peak).
Force Cluster Off	End the forced clustering of peaks.
Disable Cluster On	Prevent any peaks from being clustered.
Disable Cluster Off	Permit clusters to occur again.

Detection Algorithm Page for Auto

Use the Auto detection algorithm primarily for analog detection.

Figure 70. Detection Algorithm for Auto

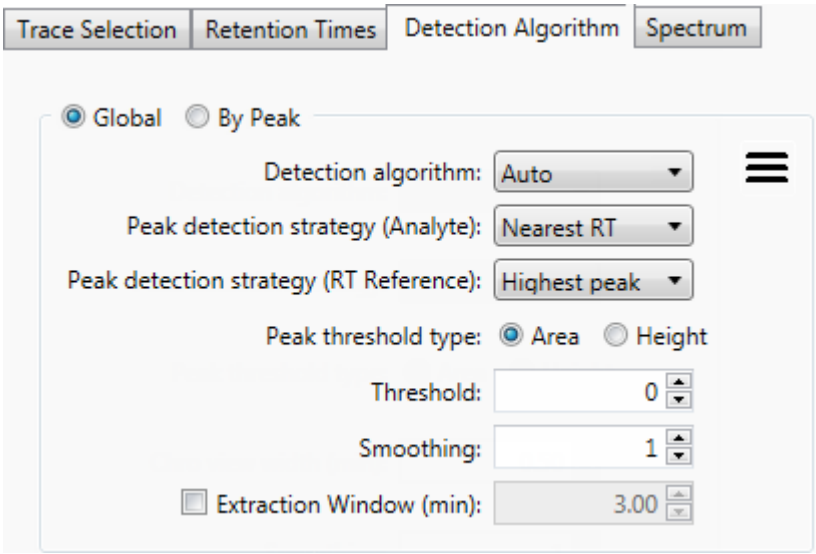


Table 37. Detection Algorithm page parameters for Auto (Sheet 1 of 2)

Parameter	Description
Global/By Peak	Global: Applies the detection settings to all peaks in the compound. By Peak: Applies the detection settings to only the selected target or confirming peak.
Detection Algorithm	Specifies the Auto peak detection algorithm that is used primarily with analog detectors. The Auto algorithm uses parameters that are identical to ICIS parameters; however, you cannot edit most of the parameters. These parameters use default values and are not displayed on the Auto detection page. See Detection Algorithm Page for ICIS.
Peak Detection Strategy (Analyte)	Highest Peak: Uses the highest peak in the chromatogram for component identification. Nearest RT: Uses the peak with the nearest retention time in the chromatogram for component identification.
Peak Detection Strategy (RT Reference)	Highest Peak: Uses the highest peak in the chromatogram for component identification. Nearest RT: Uses the peak with the nearest retention time in the chromatogram for component identification.

Table 37. Detection Algorithm page parameters for Auto (Sheet 2 of 2)

Parameter	Description
Peak Threshold Type	Specifies whether to select peaks by height or area. Default for Global: Height Default for By Peak: Area
Threshold	Specifies the peak threshold for peak integration. Peaks with area or height values less than this value are not integrated. Peaks with values greater than this value are integrated.
Smoothing	Determines the degree of data smoothing to be performed on the active component peak prior to peak detection and integration. Valid values: Any odd integer from 1 through 15 points Default: 1
Extraction Window (min) (Target peaks only)	Specifies a window that limits how much of the entire trace the application processes. When cleared, the application processes the entire trace, which can slow processing. Default: 3.00 **Note** The application applies this same setting to all associated confirming peaks.
By Peak menu commands ☰	
Apply Current Peak Settings to All Peaks in the Method	Updates all peaks in the current compound with the current settings on the Detection Algorithm page. These updates apply to both target and confirming peaks.
Apply Current Peak Settings to All Peaks with the Same Detection Algorithm	Uses the current settings on the Detection Algorithm page to update all compounds in the method that use the ICIS detection algorithm. These updates apply to both target and confirming peaks that use the ICIS detection algorithm.
Apply Current Peak Settings to All Peaks in the Compound	Updates all compounds in the method with the current settings on the Detection Algorithm page. These updates apply to both target and confirming peaks.
Global menu command ☰	
Push Global Settings to All Peaks	Pushes the global settings to all peaks for the method and updates the method.

Suitability

Use the Suitability page to determine if the column is degrading and to identify suspicious peaks eluting at the same time as the target compound. Suspicious peaks caused by highly retained compounds from a previous injection tend to have a broader than expected peak profile. Tailing peaks frequently indicate a degrading column. The Suitability page displays the parameter values so that you can check the suitability of chromatographic peaks during processing.

❖ To set system suitability parameters

1. Select one of the following check boxes:
   - **Enabled**: Specifies that the application applies the suitability criteria when identifying the target peak.
   - **Required**: When peak detection finds a target peak at the specified location, the application then confirms the peak by performing these suitability processes.
2. Select one of the following options:
   - **Global**: Applies the suitability settings to all peaks in the compound.
   - **By Peak**: Applies the suitability settings to only the selected fragment peak.
3. To perform symmetry testing, do the following:
   a. Select the **Symmetry Parameters** check box.
   b. Type a peak height for symmetry testing in the Peak Height box.
   c. Type a threshold for symmetry testing in the Symmetry Threshold box.
4. To carry out classification tests, do the following:
   a. Select the **Peak Classification Parameters** check box.
   b. To adjust Xcalibur peak width testing thresholds, type parameters in the Detect Peak Width area as follows:
      - To enter a peak height for the test, type a value in the Peak Height box.
      - To enter a minimum peak width threshold, type a value in the Min Peak Width box.
   c. To adjust the Xcalibur peak tailing test, type parameters in the Detect Tailing area:
      - To enter a peak height for the test, type a value in the Peak Height box.
      - To enter a threshold limit for peak tailing, type a value in the Failure Threshold box.
   d. To adjust the Xcalibur column overload test, type parameters in the Detect Column Overload area:
      - To enter a peak height for the test, type a value in the Peak Height box.
      - To enter a threshold limit for peak tailing, type a value in the Failure Threshold box.

Figure 71. Suitability page

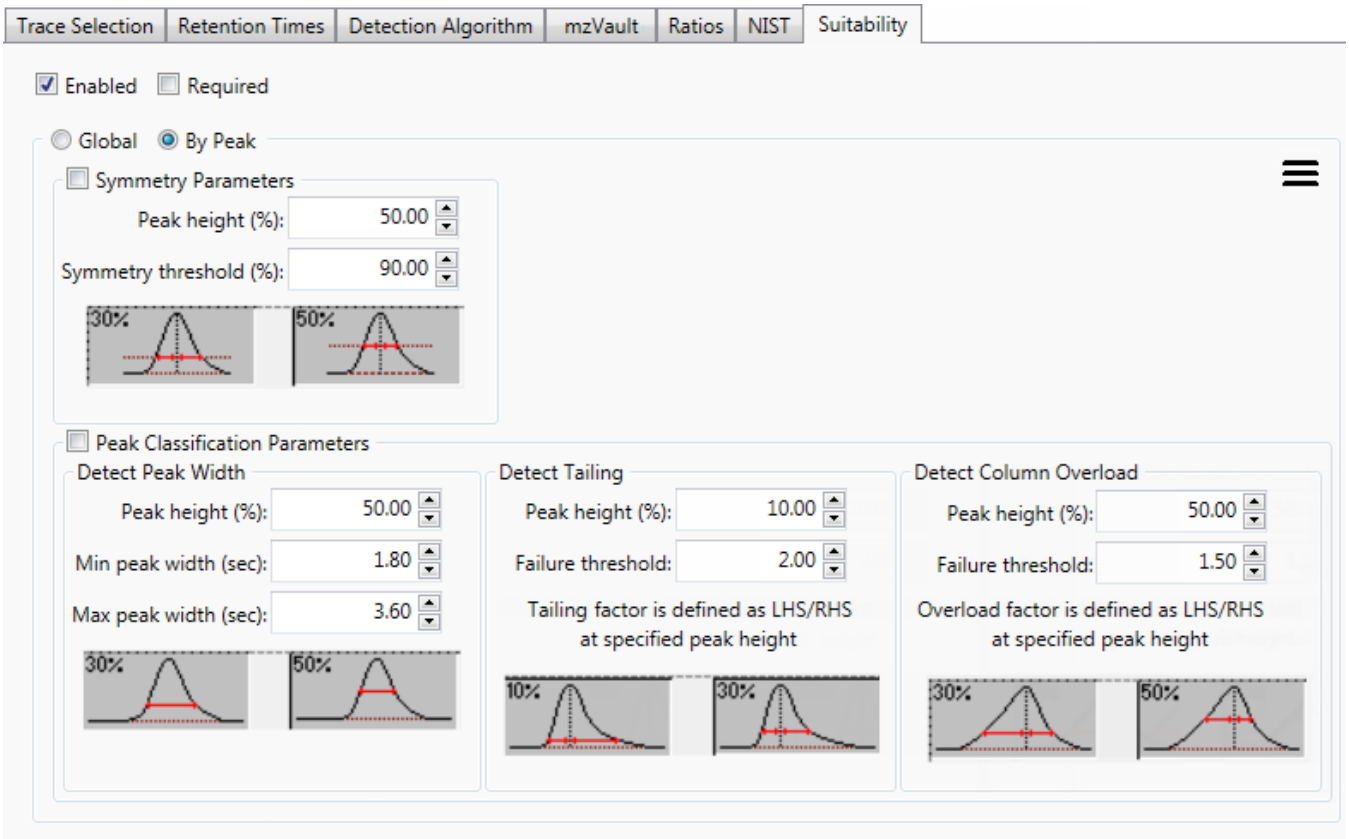
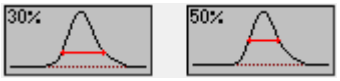
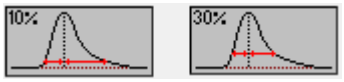
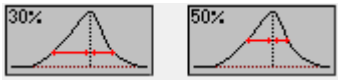


Table 38. Suitability dialog box parameters (Sheet 1 of 2)

Parameter	Description
Enabled/Required	Specifies one of the following: Enabled: Specifies that the application applies the current suitability criteria when identifying the target peak. The application considers a peak as "found" when peak detection finds the peak at the specified retention time using the specified scan filter. See Retention Times and Trace Selection. A "found" peak that fails the enabled suitability criteria is not considered confirmed. Required: When peak detection finds a target peak at the specified location, the application then confirms the peak by performing these suitability processes. If the target peak does not meet these suitability criteria, then it is not confirmed. Confirmed compounds are indicated in the Compound View in the Data Review. Refer to Chapter 4, "Using the Analysis Mode for Quantitation Batches" in the *TraceFinder User Guide*.
Global/By Peak	Global: Applies the suitability settings to all peaks in the compound. By Peak: Applies the suitability settings to only the selected target peak, confirming peak, or fragments.

Table 38. Suitability dialog box parameters (Sheet 2 of 2)

Parameter	Description
Symmetry Parameters	Determines the symmetry of the left and right sides of the detected peak.
Peak Height	The percentage of the peak height at which to compare the symmetry of the left and right peak widths.  Left and right widths measured at 30% and 50% of the peak height
Symmetry Threshold	The minimum percentage difference to be considered symmetrical and pass the suitability test.
Peak Classification Parameters	
Detect Peak Width	Determines the minimum width of each side of the peak measured at the specified percentage of the peak height.
Peak Height	The percentage of the peak height at which to measure the full peak width.  Full width measured at 30% and 50% of the peak height
Min Peak Width	Minimum peak width (measured at the specified percentage of the peak height) required to pass the suitability test.
Max Peak Width	Maximum allowed peak width (measured at the specified percentage of the peak height) to pass the suitability test.
Detect Tailing	The width of the right side of the peak divided by the width of the left side of the peak at the specified percentage of the peak height.
Peak Height	The percentage of the peak height at which to measure the left and right sides of the peak.  Left and right widths measured at 10% and 30% of the peak height
Failure Threshold	Minimum Detect Tailing value (RHS/LHS) required to pass the suitability test.
Detect Column Overload	The width of the left side of the peak divided by the width of the right side of the peak at the specified percentage of the peak height.
Peak Height	The percentage of the peak height at which to measure the left and right sides of the peak.  Left and right widths measured at 30% and 50% of the peak height
Failure Threshold	Minimum Detect Column Overload value (LHS/RHS) required to pass the suitability test.

Spectrum

Use the Spectrum page to display a mass spectrum for a target peak.

❖ To zoom in on the chromatogram or spectrum displays

1. Drag the cursor to delineate a rectangle.

   The display zooms in on the specified rectangle.

2. To return to the original display, right-click and choose **Reset Scaling**.

Spectrum Page

Use the shortcut menu commands on the Spectrum page to change the display or copy the graphic to the Clipboard.

Figure 72. Spectrum page

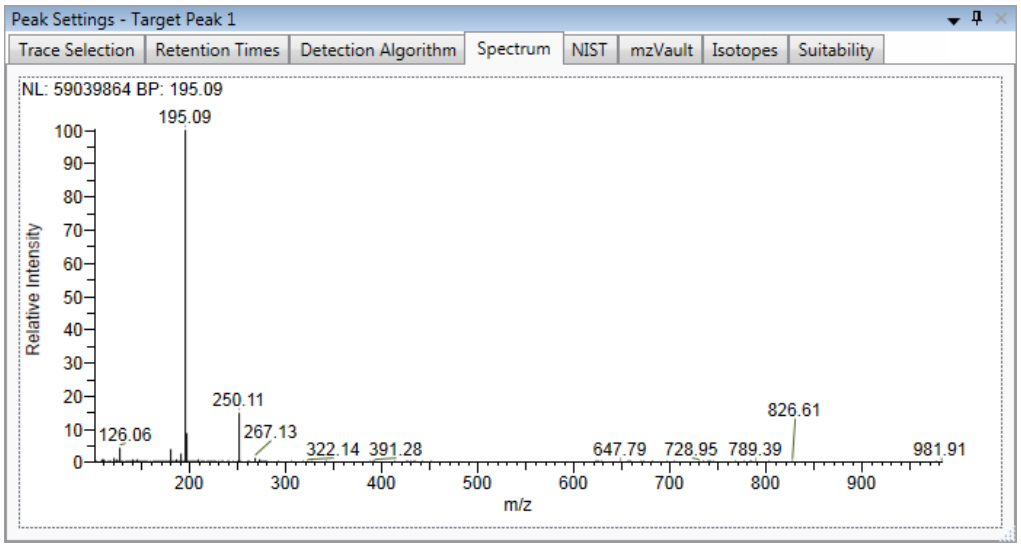


Table 39. Spectrum page shortcut menu commands

Command	Description
Reset Scaling	Returns the chromatogram or spectrum display to its original size after a zoom operation.
Copy to Clipboard	Copies the graphic display to the Clipboard.

NIST

Use the NIST Page to define the criteria for NIST library matching.

This page is available only for target peaks when you have specified a NIST screening library in the Configuration Console. See Specifying mzVault and NIST Libraries in Chapter 1, "Using the Configuration Console."

❖ To activate NIST library matching

1. Select one of the following options:
   - **Enabled**: Specifies that the application applies the NIST criteria when identifying the target peak.
   - **Required**: When peak detection finds a target peak at the specified location, the application then confirms the peak by performing these NIST processes.

   Note Because the NIST library is large, using this library can slow sample processing.

2. Select one of the following search types:
   - **Normal**: Uses a more extensive set of presearch screenings—a total of four different criteria.
   - **MS/MS**: Searches for an MS/MS spectrum in a library of MS/MS spectra.
   - **In-source HiRes**: Searches for an in-source/EI with an accurate ion *m/z* or an MS/MS spectrum in a library that contains in-source/EI accurate ion *m/z* or MS/MS spectra.
3. Select one of the following options:
   - **Global**: Applies the suitability settings to all peaks in the compound.
   - **By Peak**: Applies the suitability settings to only the selected target peak.
4. Specify the NIST parameters that you want to use for processing batches with this method.

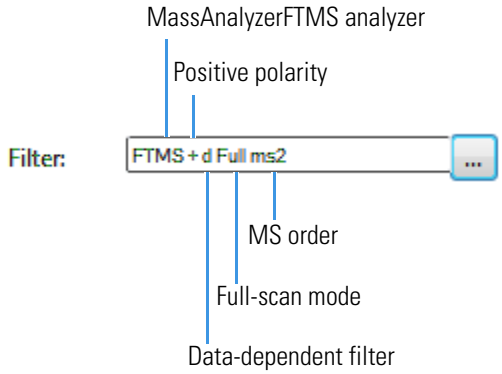
❖ To specify an XIC scan filter

1. Click the Scan Filter browse button,  .

   The XIC Filter Dialog Box opens.
2. Specify your filter options.

3. Click **OK**.

   The application updates the chromatogram data using the specified XIC filter options, and the Scan Filter box indicates the parameters of the specified XIC filter, as in this example:



XIC Filter Dialog Box

Figure 73. XIC Filter dialog box

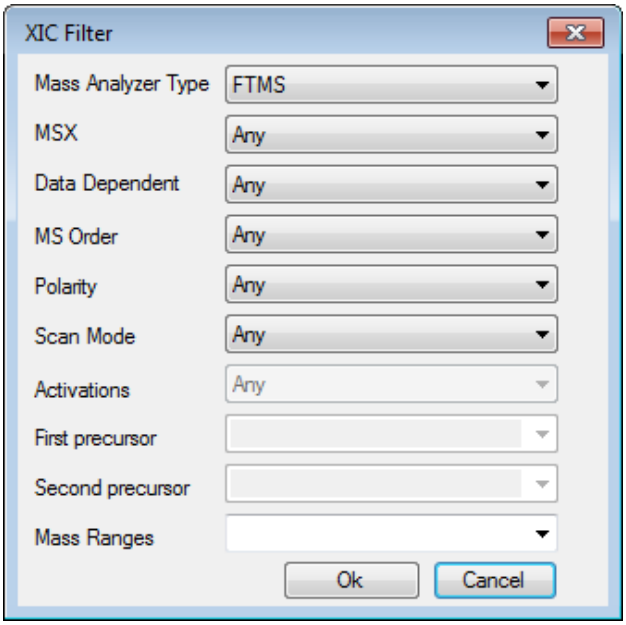


Table 40. XIC Filter dialog box parameters (Sheet 1 of 2)

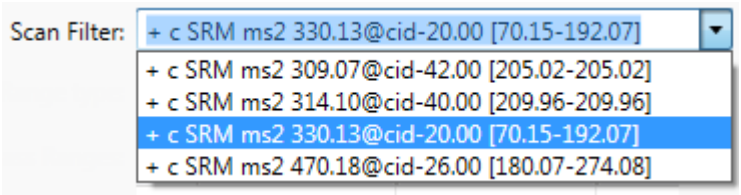
Parameter	Description
Mass Analyzer Type	Any: Any mass analyzer. FTMS: Fourier Transform Mass Spectrometer ITMS: Ion Trap Mass Spectrometer Sector: Static electric or magnetic sectors, or a combination of the two SQMS: Single Quad Mass Spectrometer TOFMS: Time-of-Flight Mass Spectrometer TQMS: Triple Quad Mass Spectrometer

Table 40. XIC Filter dialog box parameters (Sheet 2 of 2)

Parameter	Description
MSX	Any: For both MSX and non-MSX scans. On: For only MSX scans. Off: For only non-MSX scans.
Data Dependent	Any: For both data-dependent and non-data-dependent filters. On: For only data-dependent filters. Off: For only non-data-dependent filters.
MS Order	Any: For any MS order. MS: For only a single mass spec stage. MS2-MS3: For only multiple mass spec stages.
Polarity	Any: For both positive and negative scans. Positive: For only positive scans. Negative: For only negative scans.
Scan Mode	Any: For any scan mode. Full: Full-scan mode SIM: Selective ion monitoring SRM: Selective reaction monitoring CRM: Consecutive reaction monitoring Q1MS: MS using quadrupole 1 Q3MS: MS using quadrupole 3
Activations	Any: For any activation method. CID: Collision-induced dissociation MPD: Multiple photodissociation ECD: Electron capture dissociation PQD: Pulsed dissociation ETD: Electron transfer dissociation HCD: Higher energy collision-induced dissociation This parameter is available only when the MS Order is set to MS2 or MS3.
First Precursor	This parameter is available only when the MS Order is set to MS2 or MS3.
Second Precursor	This parameter is available only when the MS Order is set to MS3.
Mass Ranges	Specifies a mass range in [x-y] format. Select from the list or type a custom range.

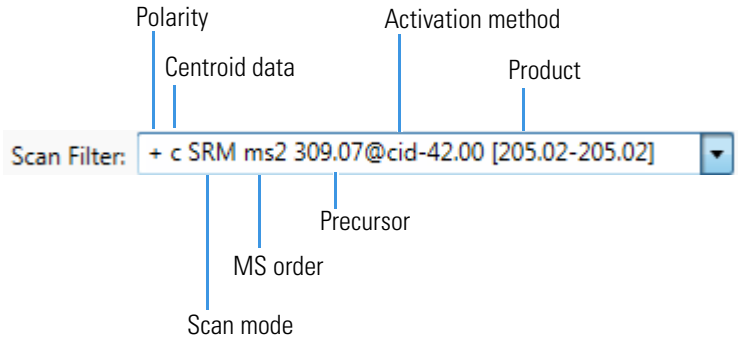
❖ To specify an MS filter

Select a filter from the Scan Filter list as shown in this example:



The Scan Filter list includes all available scan filters for the method.

The application updates the chromatogram data using the specified filter options, as in this example:



NIST Page

Use the NIST page to define the criteria for NIST library matching.

Figure 74. NIST page

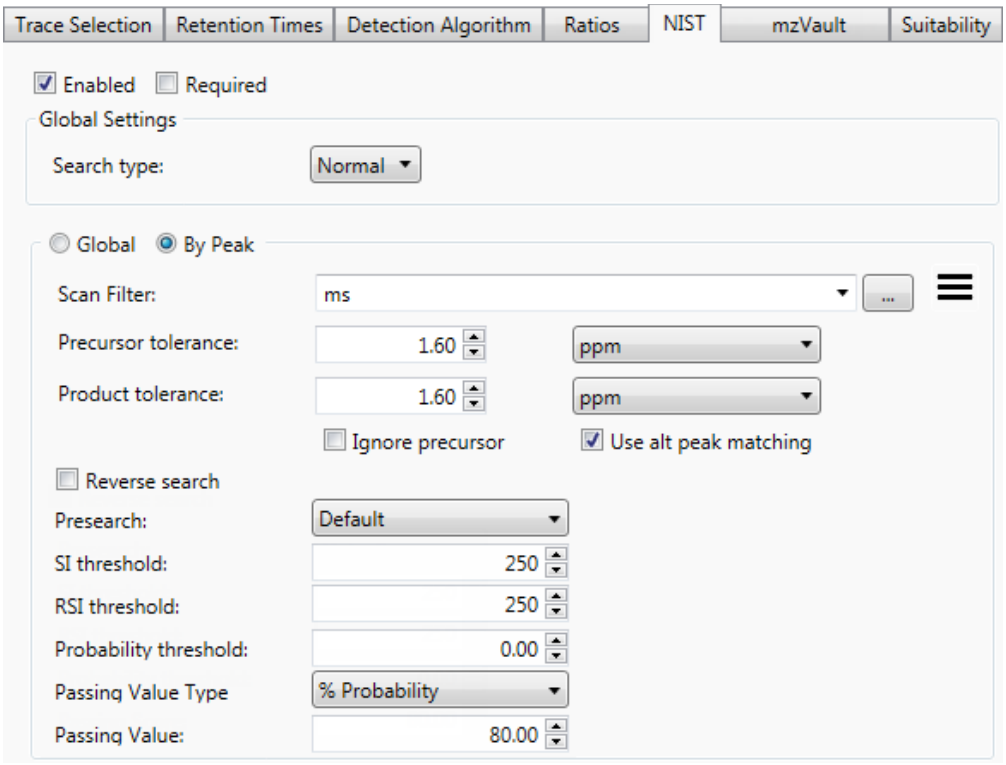


Table 41. NIST page parameters (Sheet 1 of 3)

Command	Description
Enabled/Required	Specifies one of the following: Enabled: Specifies that the application applies the NIST criteria when identifying the target peak. The application considers a peak as "found" when peak detection finds the peak at the specified retention time using the specified scan filter. See Retention Times and Trace Selection. A "found" peak that fails the enabled NIST criteria is not considered confirmed. Required: When peak detection finds a target peak at the specified location, the application then confirms the peak by performing these NIST processes. If the target peak does not meet these NIST criteria, then it is not confirmed. Confirmed compounds are indicated in the Compound View in the Data Review. Refer to Chapter 4, "Using the Analysis Mode for Quantitation Batches" in the *TraceFinder User Guide*.

Table 41. NIST page parameters (Sheet 2 of 3)

Command	Description
Search Type	Specifies the type of library search algorithm to use. Normal: Uses a more extensive set of presearch screenings—a total of four different criteria. MS/MS: Searches for an MS/MS spectrum in a library of MS/MS spectra. In-source HiRes: Searches for an in-source/EI with an accurate ion *m/z* or an MS/MS spectrum in a library that contains in-source/EI accurate ion *m/z* or MS/MS spectra.
Global/By Peak	Global: Applies the current NIST settings to all peaks in the compound. By Peak: Applies the current NIST settings to only the selected target peak.
Scan Filter	Represents a particular data acquisition channel. For example, the filter option + *c Full ms [35.00-500.00]* represents a positive ion centroid signal acquired in single-stage, full-scan mode from *m/z* 35 to 500.
Precursor Tolerance	Specifies the precursor *m/z* ± tolerance value as either amu or ppm. Valid range: 0.015 through 100 000 Default: 1.60 Available only when you select MS/MS or In-source HiRes search types.
Product Tolerance	Specifies the mass spectral peak *m/z* ± tolerance value as either amu or ppm. Valid range: 0.015 through 100 000 Default: 1.60 Available only when you select MS/MS or In-source HiRes search types.
Ignore Precursor	Ignores mass spectral peaks within the specified precursor tolerance range. Available only when you select MS/MS or In-source HiRes search types.
Use Alt Peak Matching	Specifies that the application uses alternate peak matching. Available only when you select MS/MS or In-source HiRes search types.
Reverse Search	Specifies that the NIST library search uses the reverse search method. A reverse search compares a library entry to an unknown compound (whereas a forward search compares the mass spectrum of an unknown compound to a mass spectral library entry).
Presearch	Specifies that the application uses presearch screening before calculating the spectrum-by-spectrum match factor.

Table 41. NIST page parameters (Sheet 3 of 3)

Command	Description
SI Threshold	The lower threshold value for the search index method that is used to search the NIST library. The application does not return results below this value. Valid values: 0 through 999 Default: 250
RSI Threshold	The lower threshold value for the reverse search index method that is used to search the NIST library. The application does not return results below this value. Valid values: 0 through 999 Default: 250
Probability Threshold	Threshold value for the match probability.
Passing Score	Valid values: 0.00 through 100.00 Default: 80.00
By Peak menu commands ☰	
Apply Current Peak Settings to All Peaks in the Method	Updates all peaks in the current compound with the current settings on the NIST page. These updates apply to both target and confirming peaks.
Apply Current Peak Settings to All Peaks in the Compound	Updates all compounds in the method with the current settings on the NIST page. These updates apply to both target and confirming peaks.
Global menu command ☰	
Push Global Settings to All Peaks	Pushes the global settings to all peaks for the method and updates the method.

mzVault

Use the mzVault page to define the criteria for library matching.

This page is available only for target peaks when you have specified an mzVault screening library in the Configuration Console. See Specifying mzVault and NIST Libraries.

❖ To activate library matching

1. Select one of the following options:
   - **Enabled**: Applies the current mzVault criteria when identifying the target peak.
   - **Required**: When peak detection finds a target peak at the specified location, the application then confirms the peak by performing the current mzVault processes.
2. From the Search Type list, select one of the following search types to use for matching: **Classic** or **HighChem**.

3. Select one of the following options:
   - **Global**: Applies the mzVault settings to all peaks in the compound.
   - **By Peak**: Applies the mzVault settings to only the selected fragment peak.
4. Specify the mzVault page parameters that you want to use for processing batches with this method.

   The application searches the library, matches the fragment ion spectrum in the library to the compound's ion spectrum, and returns the highest score (best match).

Figure 75. mzVault page

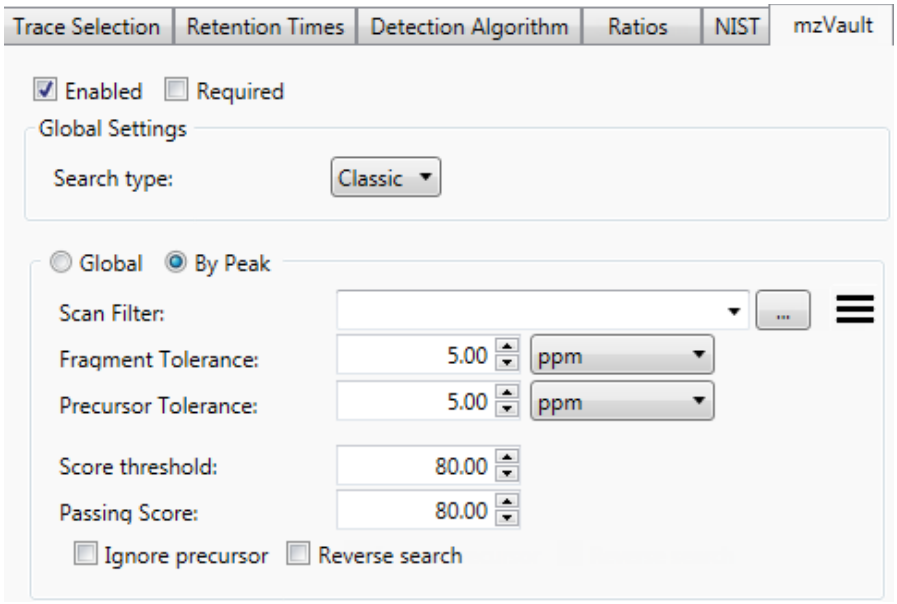


Table 42. mzVault page parameters (Sheet 1 of 3)

Command	Description
Enabled/Required	Specifies one of the following: Enabled: Specifies that the application applies the current mzVault criteria when identifying the target peak. The application considers a peak as "found" when peak detection finds the peak at the specified retention time using the specified scan filter. See Retention Times and Trace Selection. A "found" peak that fails the enabled mzVault criteria is not considered confirmed. Required: When peak detection finds a target peak at the specified location, the application then confirms the peak by performing these NIST processes. If the target peak does not meet these NIST criteria, then it is not confirmed. Confirmed compounds are indicated in the Compound View in the Data Review. Refer to Chapter 4, "Using the Analysis Mode for Quantitation Batches" in the *TraceFinder User Guide*.

Table 42. mzVault page parameters (Sheet 2 of 3)

Command	Description
Search Type	Specifies the type of library search algorithm to use. • Classic: Uses the standard library search algorithm. • HighChem: Uses the HighChem™ library search algorithm.
Global/By Peak	Global: Applies the detection settings to all peaks in the compound. By Peak: Applies the detection settings to only the selected target or confirming peak.
Scan Filter	Represents a particular data acquisition channel. For example, the filter option + *c Full ms [35.00-500.00]* represents a positive ion centroid signal acquired in single-stage, full-scan mode from *m/z* 35 to 500.
Fragment Tolerance	Specifies the fragment *m/z* ± tolerance value as either amu or ppm. Valid range: 0.1 through 1000.00 Default: 5.00
	Note This parameter might be identified as LibManagerSpectrumTolerance in audit log files.
Precursor Tolerance	Specifies the precursor *m/z* ± tolerance value as either amu or ppm. Available only when you select MS/MS or In-source HiRes search types. Valid range: 0.015 through 100 000 Default: 1.60
Score Threshold	Specifies the minimum height of a fragment ion peak. The peak of a fragment ion must be above this threshold for the application to find it. The application does not return results below this value. Valid range: 0 through 100 Default: 80.00
Passing Score	Valid range: 0 through 100 Default: 80.00
Ignore Precursor	Ignores mass spectral peaks within the specified precursor tolerance range. Available only when you select MS/MS or In-source HiRes search types.
Reverse Search	Specifies that the mzVault library search uses the reverse search method. A reverse search compares a library entry to an unknown compound (whereas a forward search compares the mass spectrum of an unknown compound to a mass spectral library entry).
	Note When you are using all ions fragmentation (AIF), data-independent (DIA), variable data-independent (vDIA), or source collision-induced dissociation (CID) data, the application automatically uses reverse search even if you do not select this option.

Table 42. mzVault page parameters (Sheet 3 of 3)

Command	Description
By Peak menu commands ☰	
Apply Current Peak Settings to All Peaks in the Method	Updates all peaks in the current compound with the current settings on the mzVault page. These updates apply to both target and confirming peaks.
Apply Current Peak Settings to All Peaks in the Compound	Updates all compounds in the method with the current settings on the mzVault page. These updates apply to both target and confirming peaks.
Global menu command ☰	
Push Global Settings to All Peaks	Pushes the global settings to all peaks for the method and updates the method.

Isotopes

Use the Isotopes page to define the criteria for identifying an isotope peak. To identify an isotopic pattern, the application must detect the compound for at least one of its defined adduct ions. The application identifies the elemental composition to match using the formula that is associated with the most intense adduct peak. The application then generates an isotopic pattern score (as a percentage value) for the match between the measured and expected isotopic patterns of the calculated elemental composition.

❖ To specify isotope criteria

1. Select either option:
   - **Enabled**: Specifies that the application applies the isotope criteria when identifying the target peak.
   - **Required**: When peak detection finds a target peak at the specified location, the application then confirms the peak by performing these isotope processes.
2. Select either option:
   - **Global**: Applies the isotope settings to all peaks in the compound.
   - **By Peak**: Applies the isotope settings to only the selected fragment peak.
3. In the Fit Threshold box, type the fit threshold percentage.

   The resulting score percentage from isotopic pattern matching must be higher than the specified fit threshold percentage.
4. In the Allowed Mass Deviation box, type the parts per million to use as the minimum deviation from the expected *m/z*.

   The isotopic pattern algorithm considers an isotope peak as found if its measured *m/z* is less than this amount away from its expected *m/z*. For best results, set this value to a number that causes up to 98 percent of all mass deviations to be smaller than the allowed mass deviation value.

5. In the Allowed Intensity Deviation box, type a value to specify the allowed intensity deviation of the mass spectrometer relative to the monoisotopic ion, as a percentage of the base peak height.

   The isotopic pattern algorithm considers an isotope peak as not found if its intensity, relative to the monoisotopic ion's intensity, is more than the deviation percentage from the theoretical relative intensity of the isotope ion. For best results, set this value to a number that causes up to 98 percent of all intensity deviations to be smaller than the allowed intensity deviation value.

Figure 76. Isotopes page

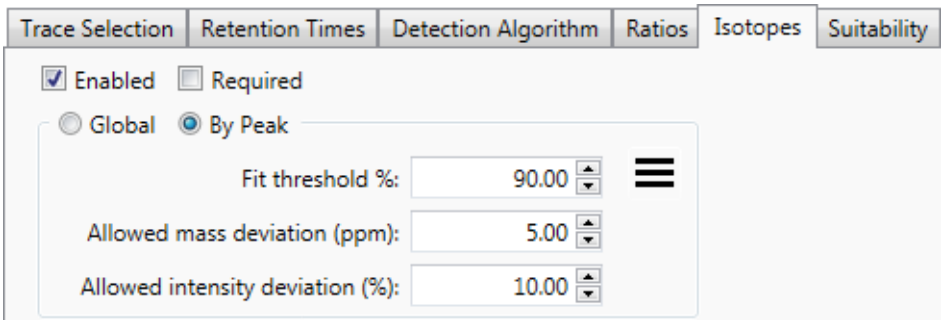


Table 43. Isotopes page parameters (Sheet 1 of 2)

Parameter	Description
Enabled/Required	Specifies one of the following: Enabled: Specifies that the application applies the current isotope criteria when identifying the target peak. The application considers a peak as "found" when peak detection finds the peak at the specified retention time using the specified scan filter. See Retention Times and Trace Selection. A "found" peak that fails the enabled isotope criteria is not considered confirmed. Required: When peak detection finds a target peak at the specified location, the application then confirms the peak by performing these isotope processes. If the target peak does not meet these isotope criteria, then it is not confirmed. Confirmed compounds are indicated in the Compound View in the Data Review. Refer to Chapter 4, "Using the Analysis Mode for Quantitation Batches" in the *TraceFinder User Guide*.
Global/By Peak	Global: Applies the detection settings to all peaks in the compound. By Peak: Applies the detection settings to only the selected target or confirming peak.
Fit Threshold %	To identify a compound, the resulting score percentage from isotopic pattern matching must be higher than the specified fit threshold percentage. Default: 90%

Table 43. Isotopes page parameters (Sheet 2 of 2)

Parameter	Description
Allowed Mass Deviation (ppm)	Specifies the allowed mass deviation in the spectrum data. The TraceFinder isotopic pattern algorithm considers an isotope peak as found if its measured *m/z* is less than this amount away from its expected *m/z*. For best results, set this value to a number that causes up to 98 percent of all mass deviations to be smaller than the allowed mass deviation value. Valid range: 3 through 100 ppm Default: 3 ppm
Allowed Intensity Deviation (%)	Specifies the allowed intensity deviation of the mass spectrometer, relative to the monoisotopic ion, as a percentage of the base peak height. The TraceFinder isotopic pattern algorithm considers an isotope peak as not found if its intensity relative to the monoisotopic ion's intensity is more than this deviation percentage from the theoretical relative intensity of the isotope ion. For best results, set this value to a number that causes up to 98% of all intensity deviations to be smaller than the allowed intensity deviation value. Default: 10%
Shortcut menu ☰	
Apply Current Peak Settings to All Peaks in the Method	Updates all compounds in the method with the current settings on the Isotopes page. These updates apply to both target and confirming peaks. When you use this command in the local method for a processed batch, the application prompts you to reprocess the batch to update the library settings.
Apply Current Peak Settings to All Peaks in the Compound	Updates all peaks in the current compound with the current settings on the Isotopes page. These updates apply to both target and confirming peaks. The application reprocesses all peaks in the compound and performs a new library search.

Ratios

Use the Ratios page to define the criteria for evaluating target peaks, confirming peaks, and fragments. The application detects compounds that have peaks outside their acceptable window and flags them in the Acquisition mode and in reports.

Ratio parameters are different for target peaks, confirming peaks, and fragments.

- Ratios for Target Peaks
- Ratios for Confirming Peaks
- Ratios for Fragments

Ratios for Target Peaks

Use the Ratios page to define the criteria for evaluating the selected target peak. The application detects compounds that have target peaks outside their acceptable window and flags them in the Acquisition mode and in reports. See Ratios page for target peaks.

❖ To specify ion ratio criteria

1. To specify that the application applies the current ratios criteria when identifying the target peak, select the **Enabled** check box.
2. In the Target Ion Ratio box, enter a value for the theoretical ratio of the confirming ion's response to the target ion's response.
3. From the Ion Range Calc Method list, select one of the following methods for calculating the ion ratio range windows:
   - **Manual** (default): Uses the manually entered target ion ratio window ranges.
   - **Average**: Uses the average ratio of the defined calibration levels.
   - **Level**: Displays an additional list where you can select a calibration level amount. To define these calibration levels on the Compounds page, see Calibration Levels.
   - **Weighted Average**: Uses the weighted average ratio of the defined calibration levels and assigns more weight to lower levels.
4. Select one of the following options:
   - **Global**: Applies the window type, response type, and window settings to all peaks in the compound.
   - **By Peak**: Applies the window type, response type, and window settings to only the selected target peak.
5. In the Window Type list, select **Absolute** or **Relative** as the calculation approach for determining the acceptable ion ratio range.
6. From the Response Type list, select **Area** or **Height**.
   - **Area**: Specifies that the application uses this area value in response calculations.
   - **Height**: Specifies that the application uses this height value in response calculations.
7. In the Window (+/-%) box, select the acceptable ion ratio range.
8. (Optional) Do one of the following:
   - To copy the Window Type, Response Type, and Window values to all target peaks for the compound, click the shortcut menu icon, ☰, and choose **Apply Current Peak Settings to All Peaks in the Compound**.
   - To copy the Window Type, Response Type, and Window values to all target peaks for the method and update the method, click the shortcut menu icon, ☰, and choose **Apply Current Peak Settings to All Peaks in the Method**.

Figure 77. Ratios page for target peaks

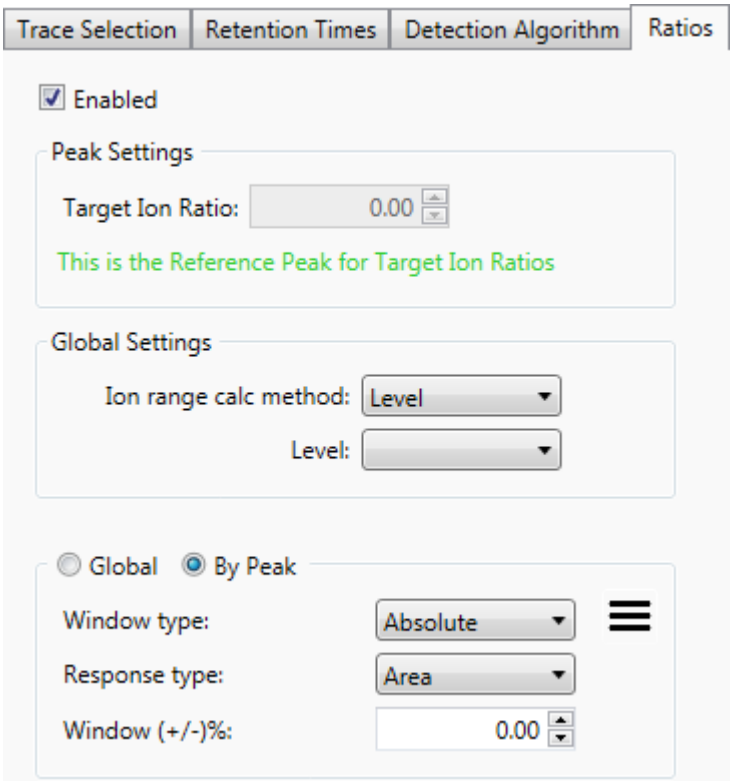


Table 44. Ratios page parameters for target peaks (Sheet 1 of 2)

Parameter	Description
Enabled	Specifies that the application applies the current ratio criteria when identifying the target peak. The application considers a peak as "found" when peak detection finds the peak at the specified retention time using the specified scan filter. See Retention Times and Trace Selection. A "found" peak that fails the enabled ratio criteria is not considered confirmed.
Target Ion Ratio	Specifies the theoretical ratio of the confirming ion's response to the target ion's response.

Table 44. Ratios page parameters for target peaks (Sheet 2 of 2)

Parameter	Description
Ion Range Calc Method	Specifies the selected ion range calc method used to calculate the ion ratio range windows: • Manual (default): Uses the manually entered target ion ratio window ranges. • Average: Uses the average ratio of the defined calibration levels. • Level: Displays an additional list where you can select a calibration level amount. To define these calibration levels on the Compounds page, see Calibration Levels. • Weighted Average: Uses the weighted average ratio of the defined calibration levels, and assigns more weight to lower levels.
Global/By Peak	Global: Applies the Window Type, Response Type, and Window values to all target peaks in the compound. By Peak: Applies the Window Type, Response Type, and Window values to only the selected target peak.
Window Type	Specifies the absolute or relative calculation approach for determining the acceptable ion ratio range.
Response Type	Specifies that the application uses height or area in response calculations.
Window (+/-%)	Specifies the acceptable ion ratio range.
Shortcut menu ☰	
Apply Current Peak Settings to All Peaks in the Method	Copies the Window Type, Response Type, and Window values to all target peaks for the method and updates the method.
Apply Current Peak Settings to All Peaks in the Compound	Copies the Window Type, Response Type, and Window values to all target peaks for the compound and updates the compound. Available only when a compound has multiple target peaks.

Ratios for Confirming Peaks

Use the Ratios page to define the criteria for evaluating the selected confirming peak. The application detects compounds that have confirming peaks outside their acceptable window and flags them in the Acquisition mode and in reports. See Ratios page for confirming peaks.

❖ To specify ion ratio criteria

1. To specify that the application applies the current ratio criteria when identifying the confirming peak, select the **Enabled** check box.

2. In the Target Ion Ratio box, enter a value for the theoretical ratio of the confirming ion's response to the target ion's response.
3. From the Ion Range Calc Method list, select one of the following methods for calculating the ion ratio range windows:
   - Manual (default)
   - Average
   - Level

     When you select Level, the application displays a Level list where you can choose a calibration level. To define the available calibration levels on the Compounds page, see Calibration Levels.
   - Weighted Average
4. Select one of the following options:
   - **Global**: Applies the coelution, window type, response type, and window settings to all peaks in the compound.
   - **By Peak**: Applies the coelution, window type, response type, and window settings to only the selected target peak.
5. To specify the maximum difference in retention time between a confirming peak and the quantification ion peak, set a value in the Coelution box.
6. In the Window Type list, select **Absolute** or **Relative** as the calculation approach for determining the acceptable ion ratio range.
7. From the Response Type list, select **Area** or **Height**.
   - **Area**: Specifies that the application uses this area value in response calculations.
   - **Height**: Specifies that the application uses this height value in response calculations.
8. In the Window (+/-%) box, select the acceptable ion ratio range.
9. (Optional) Do one of the following:
   - To copy the Coelution, Window Type, Response Type, and Window values to all target peaks for the compound, click the shortcut menu icon, ☰, and choose **Apply Current Peak Settings to All Peaks in the Compound**.
   - To copy the Coelution, Window Type, Response Type, and Window values to all target peaks for the method and then update the method, click the shortcut menu icon, ☰, and choose **Apply Current Peak Settings to All Peaks in the Method**.

Figure 78. Ratios page for confirming peaks

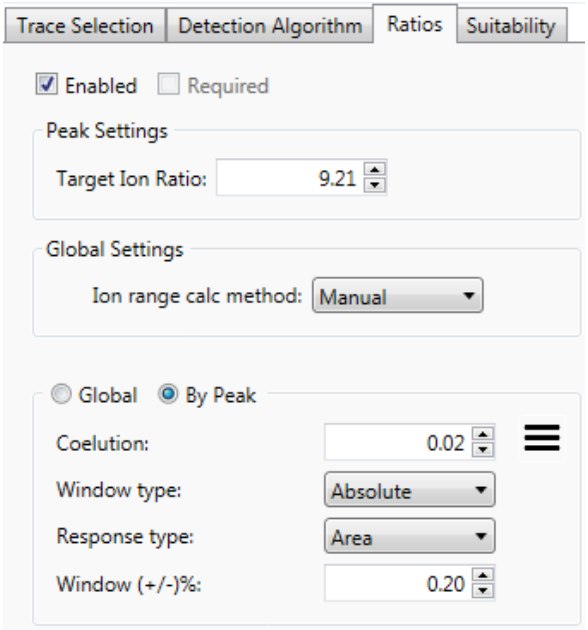


Table 45. Ratios page parameters for confirming peaks (Sheet 1 of 2)

Parameter	Description
Enabled/Required	Specifies one of the following: Enabled: Specifies that the application applies the current ratio criteria when identifying the confirming peak. The application considers a peak as "found" when peak detection finds the peak at the specified retention time using the specified scan filter. See Retention Times and Trace Selection. A "found" peak that fails the enabled ratio criteria is not considered confirmed. Required: When peak detection finds a confirming peak at the specified location, the application then confirms the peak by performing these ratio processes. If the confirming peak does not meet these ratio criteria, then it is not confirmed. Confirmed compounds are indicated in the Compound View in the Data Review. Refer to Chapter 4, "Using the Analysis Mode for Quantitation Batches" in the *TraceFinder User Guide*.
Target Ion Ratio	Specifies the theoretical ratio of the confirming ion's response to the target ion's response.

Table 45. Ratios page parameters for confirming peaks (Sheet 2 of 2)

Parameter	Description
Ion Range Calc Method	Specifies the selected ion range calc method used to calculate the ion ratio range windows: • Manual (default): Uses the manually entered target ion ratio window ranges. • Average: Uses the average ratio of the defined calibration levels. • Level: Displays an additional list where you can select a calibration level amount. To define these calibration levels on the Compounds page, see Calibration Levels. • Weighted Average: Uses the weighted average ratio of the defined calibration levels, and assigns more weight to lower levels.
Global/By Peak	Global: Applies the ratio settings to all confirming peaks in the compound. By Peak: Applies the ratio settings to only the selected confirming peak.
Coelution	Specifies the maximum difference in retention time between a confirming peak and the target peak. Valid range: 0.025 through 0.1
Window Type	Specifies the absolute or relative calculation approach for determining the acceptable ion ratio range.
Response Type	Specifies that the application uses height or area in response calculations.
Window (+/-%)	Specifies the acceptable ion ratio range.
Shortcut menu ☰	
Apply Current Peak Settings to All Peaks in the Method	Copies the Coelution, Window Type, Response Type, and Window values to all confirming peaks for the method and updates the method.
Apply Current Peak Settings to All Peaks in the Compound	Copies the Coelution, Window Type, Response Type, and Window values to all confirming peaks for the compound and then updates the compound. Available only when a compound has multiple confirming peaks.

Ratios for Fragments

Use the Ratios page to define the criteria for evaluating the selected fragment. The application detects compounds that have fragments outside their acceptable window and flags them in the Acquisition mode and in reports. See Ratios page for fragments and Ratios page parameters for fragments.

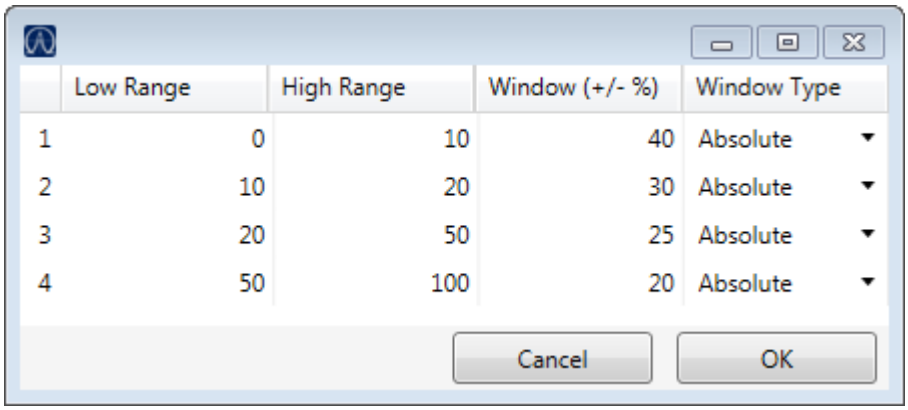
Follow these procedures:

- To specify ion ratio criteria
- To specify a reference fragment

❖ To specify ion ratio criteria

1. Select one of the following options:
   - **Enabled**: Specifies that the application applies the current ratio criteria when identifying the fragment.
   - **Required**: When peak detection finds a fragment peak at the specified location, the application then confirms the peak by performing these processes.
2. To edit the Target Ion Ratio table, do the following:
   a. Click **Edit**.

      The dialog box opens.

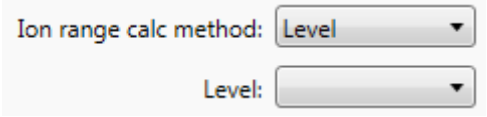
   b. For each range, you can type a new low or high value, change the Window(+/-%) value or change the Window Type.

      Ranges must be contiguous from 0 through 100. You cannot specify overlapping values or leave gaps between values.

   c. When you have completed your changes, click **OK**.
3. In the Window Type list, select **Absolute** or **Relative** as the calculation approach for determining the acceptable ion ratio range.

4. From the Ion Range Calc Method list, select a method for calculating the ion ratio range windows.

   When you select Level, the application displays a Use Level list where you can choose a calibration level. To define the available calibration levels on the Compounds page, see Calibration Levels.

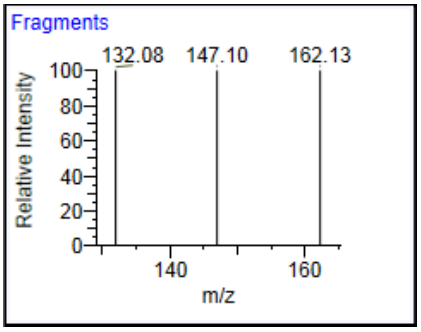


❖ To specify a reference fragment

1. In the mass table, select the **Reference** option for the appropriate mass.

   Each fragment mass in the Peaks pane is identified as a possible reference fragment.

Peaks pane



Ratios page

	Mass	Target Ion Ratio	Reference
1	162.13	0	◉
2	147.10	0	○
3	132.08	0	○

2. Enter a value for the Target Ion Ratio.

Figure 79. Ratios page for fragments

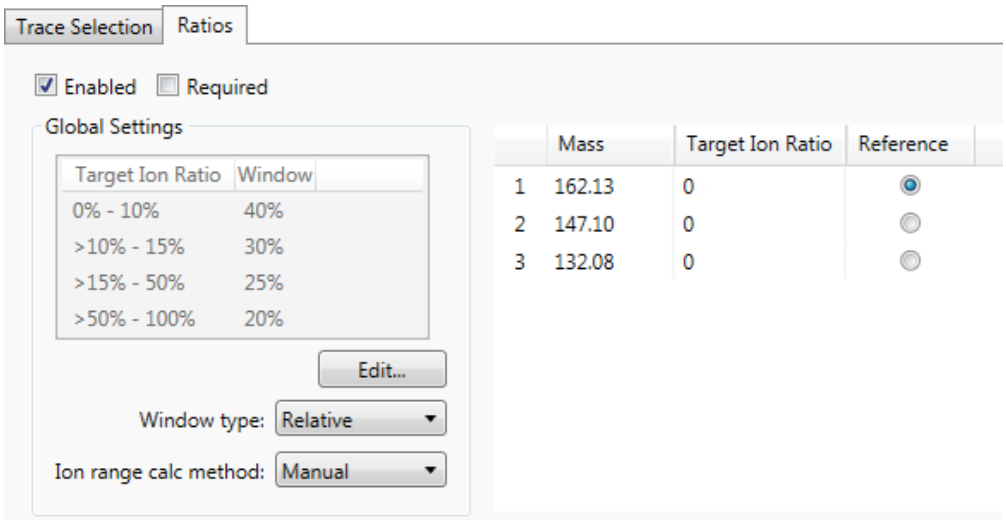


Table 46. Ratios page parameters for fragments

Parameter	Description
Enabled/Required	Specifies one of the following: Enabled: Specifies that the application applies the current ratio criteria when identifying the fragment. The application considers a fragment as "found" when peak detection finds the fragment at the specified retention time using the specified scan filter. See Retention Times and Trace Selection. A "found" fragment that fails the enabled ratios criteria is not considered confirmed. Required: When peak detection finds a fragment at the specified location, the application then confirms the fragment by performing these ratios processes. If the fragment does not meet these ratio criteria, then it is not confirmed. Confirmed compounds are indicated in the Compound View in the Data Review. Refer to Chapter 4, "Using the Analysis Mode for Quantitation Batches" in the *TraceFinder User Guide*.
Target Ion Ratio Table	Displays the ion ratio ranges and windows as specified in the dialog box. To open the dialog box and edit these values, click **Edit**.
Window Type	Specifies the absolute or relative calculation approach for determining the acceptable ion ratio range.
Ion Range Calc Method	Specifies the selected ion range calc method used to calculate the ion ratio range windows: • Manual (default): Uses the manually entered target ion ratio window ranges. • Average: Uses the average ratio of the defined calibration levels. • Level: Displays an additional list where you can select a calibration level amount. To define these calibration levels on the Compounds page, see Calibration Levels. • Weighted Average: Uses the weighted average ratio of the defined calibration levels, and assigns more weight to lower levels.

Calibration

Use the Calibration Page to set or edit the mathematical model used for preparing the initial calibration evaluation for one or more calibration standards.

Each target compound can have its own initial calibration settings, independent of the other compounds. You can modify the calibration approach on this page or in Acquisition mode when you view the results of an actual calibration batch.

Typically, general quantitation uses a measured response (area or height) to determine the amount of a compound contained in a sample. The application compares the response of an unknown, target compound to the response of a calibration sample that contains a known amount of the compound by building a calibration curve to interpolate the amount in the target compound.

To use a semi-quantitative process, you specify the compound's standard type as Estimated and then identify another compound as the linked compound. Instead of using the target compound to create a calibration curve, the application uses a calibration curve from the linked compound to calculate the amount in the target compound.

To use a real sample for the calibration procedure, you specify the compound's standard type as Std Addition. The autosampler divides the sample into multiple portions (one unspiked portion and at least two spiked portions). To maintain consistent conditions across all samples, the autosampler adds selected amounts of standard into the vials and adds a volume of a solvent calculated to maintain constancy in the total volume of liquid in each vial.

To exclude a compound when the application generates a calibration curve, you specify the compound's standard type as None.

Follow these procedures:

- To specify an internal standard type for a compound
- To specify an estimated standard type for a compound
- To specify a standard addition standard type for a compound
- To exclude a compound

❖ To specify an internal standard type for a compound

1. On the Identification page, specify at least one compound in the method as an internal standard compound type.
2. On the Calibration page, do the following:
   a. In the Standard Type column, select **Internal**.
   b. In the ISTD column, select the compound that you want to use as the internal standard for this compound.

      The application lists only compounds specified as internal standards on the Identification page.

Note To automatically link compounds to their internal standards, see Linking Internal Standards.

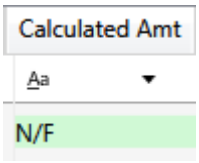
To view the internal standard peak in Data Review, refer to Chapter 4, "Using the Analysis Mode for Quantitation Batches" in the *TraceFinder User Guide*.

❖ To specify an estimated standard type for a compound

1. In the Standard Type column, select **Estimated**.
2. In the Linked Compound column, select any other compound in the method that you want to link to this compound.

   The Estimation Method value defaults to Ext Curve and is read-only.

   The Compound Results in Data Review display the Calculated Amt value as "N/F" (not found) highlighted in green.



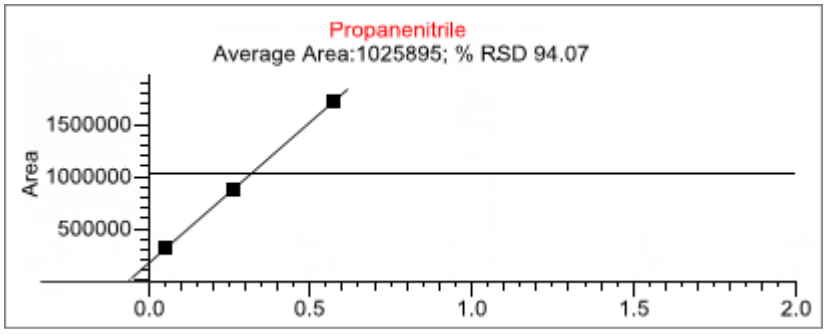
❖ To specify a standard addition standard type for a compound

In the Standard Type column, select **Std Addition**.

- The Curve Type column value defaults to Linear and is read-only.
- The Origin column value defaults to Ignore and is read-only.
- The Weighting column value defaults to Equal and is read-only.

When you process this sample, the application divides the sample into multiple portions: one portion is not spiked and at least two portions are spiked. The application calculates the analyte concentration as *intercept/slope*, where *intercept* is the *y*-intercept of the regression line and *slope* is the slope of the regression line.

When you use the Std Addition calibration, the *y*-intercept on the calibration curve might not be at 0, as shown in the following figure:



The Compound Results in Data Review display the following:

- The Calculated Amt value is the spiked amount from the calibration curve.
- The Theoretical Amt value is the level defined in the method.
- The Sample Amt value is the actual amount in the standard spike plus the spiked amount in each standard.

❖ To exclude a compound

In the Standard Type column, select **None**.

- When the application generates a calibration curve, this compound is excluded.
- All values for this compound are grayed out on the Calibration page.
- The application excludes this compound from the Limits page in the QAQC view.
- The application excludes this compound from the Calibration Levels page in the Compounds view.

Calibration Page

Use the features on the Calibration page to define the mathematical model used for preparing the initial calibration evaluation for one or more calibration standards.

Figure 80. Calibration page

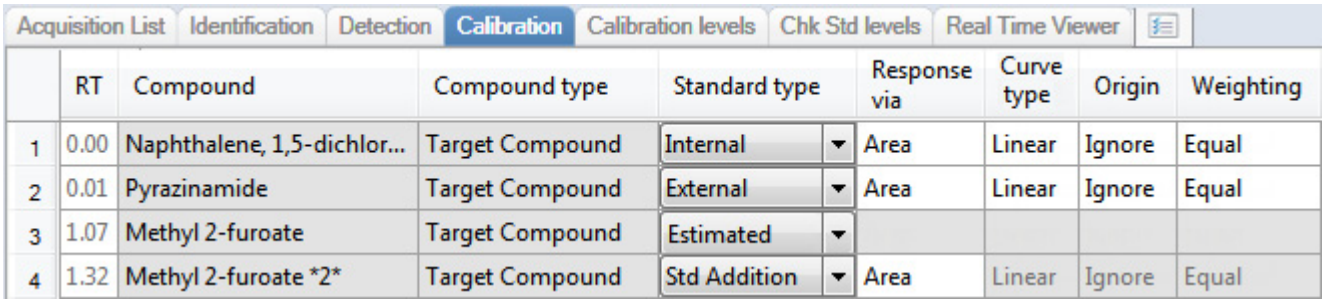


Table 47. Calibration page parameters (Sheet 1 of 2)

Parameter	Description
RT	Retention time. The time after injection when the compound elutes. The total time that the compound is retained on the column.
Compound	The compound name.
Compound Type	Displays the compound type as a Target Compound, Internal Standard, Surrogate, MSTune, Native, or Breakdown.
Standard Type	Specifies the standard type as Internal, External, Estimated, Std Addition, or None.
Response Via	Specifies the use of area or height. When you set the standard type to Estimated, this column is inactive.
Curve Type	Specifies Linear, Quadratic, or AverageRF curve types. When you set the standard type to Estimated, this column is inactive. When you set the standard type to Std Addition, this column value defaults to Linear and is read-only.
Origin	The origin treatment is Ignore, Include, or Force. The Origin and Weighting columns are available only when you use Linear or Quadratic curve types. When you set the standard type to Estimated, this column is inactive. When you set the standard type to Std Addition, this column value defaults to Ignore and is read-only.

Table 47. Calibration page parameters (Sheet 2 of 2)

Parameter	Description
Weighting	Specifies the weighting as Equal, 1/X, 1/X^2, 1/Y, or 1/Y^2. When you set the standard type to Estimated, this column is inactive. When you set the standard type to Std Addition, this column value defaults to Equal and is read-only.
Units	The units to be displayed with the calculated values.
ISTD	The internal standard (ISTD) for a target compound or surrogate. The list displays all compounds with the compound type of Internal Standard. This column is available only when you set the standard type to Internal.
Amount	The amount of the internal standard for ISTD compounds. When you set the standard type to Estimated, this column is inactive.
Linked Compound	This column is available only when the standard type is set to Estimated. The list of available compounds to be linked does not include any compounds whose standard type is set to Estimated.
Estimation Method	This column is unavailable for editing when the standard type is set to Estimated. • When the compound type for the associated linked compound is Target Compound, the estimation method is automatically set to Ext Curve. • When the compound type for the associated linked compound is Internal Standard, the estimation method is automatically set to Ratio.
Shortcut menu	The Calibration page uses a right-click shortcut menu. See Using the Shortcut Menu Commands.

Calibration Levels

On the Calibration Levels page for a method, you can define the standards for calibration. You can edit calibration levels and concentrations for master methods only. The contents of this page are read-only when you are editing a local method.

You can use the copy-and-paste functions in the shortcut menu to copy calibration levels from one column to another or from one method to another. For detailed instructions, see Copying and Pasting Column Values.

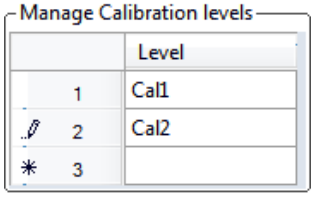
❖ To specify calibration levels and concentrations

1. Select the compound whose calibration levels and concentrations you want to define.

	RT	Compound			
1	3.15	Pyrazinamide			
2	3.67	1,3-Dioxolane, 2-heptyl-			
3	4.70	Pyrazinamide "2"			

2. In the Manage Calibration Levels area, type a value for the first calibration level.

   The application adds a new, empty calibration level row beneath the edited row.



3. Continue adding calibration levels.

   When you finish adding calibration levels, you can specify the concentrations for each compound at each level.

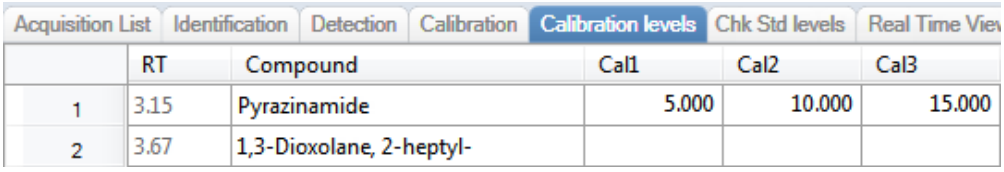
4. To enter the concentrations in the table, do the following:

   a. Select the first calibration level table cell.

   b. Click the cell again to make it editable.

   c. Type a concentration value.

5. Repeat step 4 for all calibration levels associated with the first compound.



6. To specify the same concentration values for all compounds, select the value that you want to copy, right-click, and choose **Copy Down**.

Figure 81. Calibration Levels page

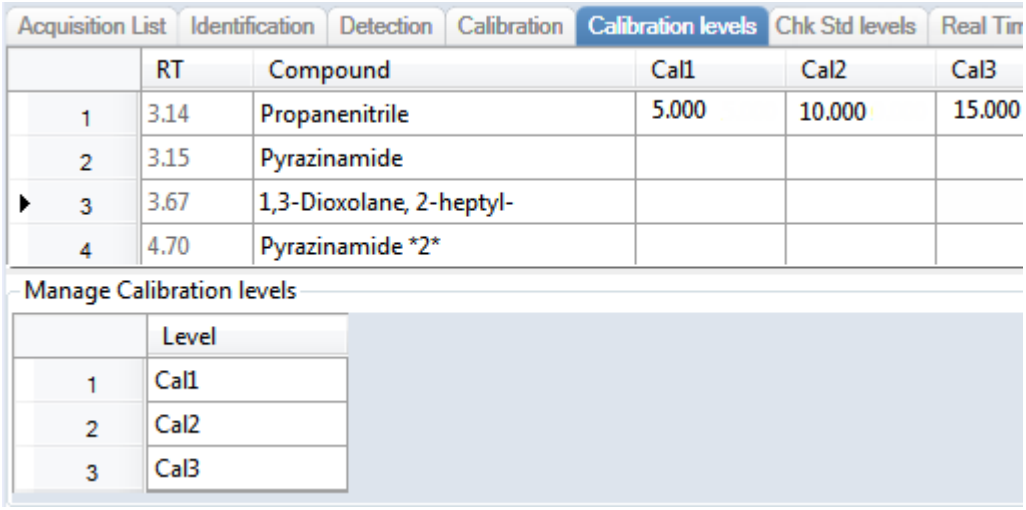


Table 48. Calibration Levels page parameters

Parameter	Description
RT	Retention time. The time after injection when the compound elutes. The total time that the compound is retained on the column.
Compound	The compound name.
CalLevel_1–CalLevel_n	User-defined calibration levels for the compound. The names you enter here become the column headers for the calibration levels.
Manage Calibration Levels	Defines values for each of the calibration level values for the selected compound.
Shortcut menu	The Calibration Levels page uses a right-click shortcut menu. See Using the Shortcut Menu Commands.

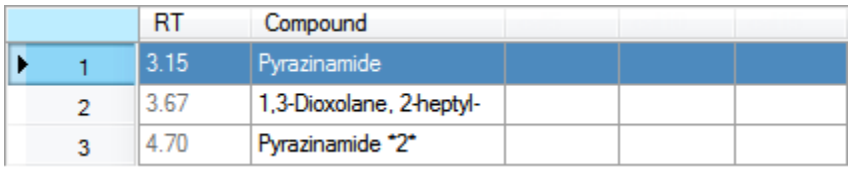
Chk Std Levels

Use the Chk Std Levels page for a method to define the standards for Chk Std levels. You can edit Chk Std levels for master methods only. The contents of this page are read-only when you are editing a local method.

You can use the copy-and-paste functions in the shortcut menu to copy Chk Std levels from one column to another or from one master method to another. For detailed instructions, see Copying and Pasting Column Values.

❖ To specify Chk Std levels and concentrations

1. Select the compound whose Chk Std levels, percentage test values, and concentrations you want to define.



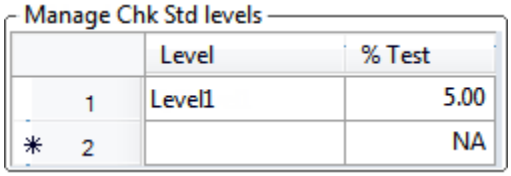
	RT	Compound			
1	3.15	Pyrazinamide			
2	3.67	1,3-Dioxolane, 2-heptyl-			
3	4.70	Pyrazinamide *2*			

2. In the Manage Chk Std Levels area, type a name for the first Chk Std level.

   The application adds a new, empty Chk Std level row below the edited row.

3. Type a value for the % Test.

   The % Test is the acceptable difference (as a percentage) between the known amount and the calculated (measured) amount of each Chk Std level.



4. Continue adding Chk Std levels and values for the percentage test.

Manage Chk Std levels

	Level	% Test
1	Level1	5.00
2	Level2	5.00
3	Level3	5.00

When you finish adding Chk Std levels, you can specify the concentrations for each level for each compound.

5. To enter the concentration values in the table, do the following:
   a. Select the first Chk Std level table cell.
   b. Click the cell again to make it editable.
   c. Type a concentration value.
6. Repeat step 5 for all Chk Std levels associated with the first compound.

Acquisition List | Identification | Detection | Calibration | Calibration levels | Chk Std levels | Real Time Vie

	RT	Compound	Level1	Level2	Level3
1	3.14	Propanenitrile	5.000	10.000	15.000
2	3.15	Pyrazinamide			

7. To specify the same concentration values for all compounds, select the value that you want to copy, right-click, and choose **Copy Down**.

Figure 82. Chk Std Levels page

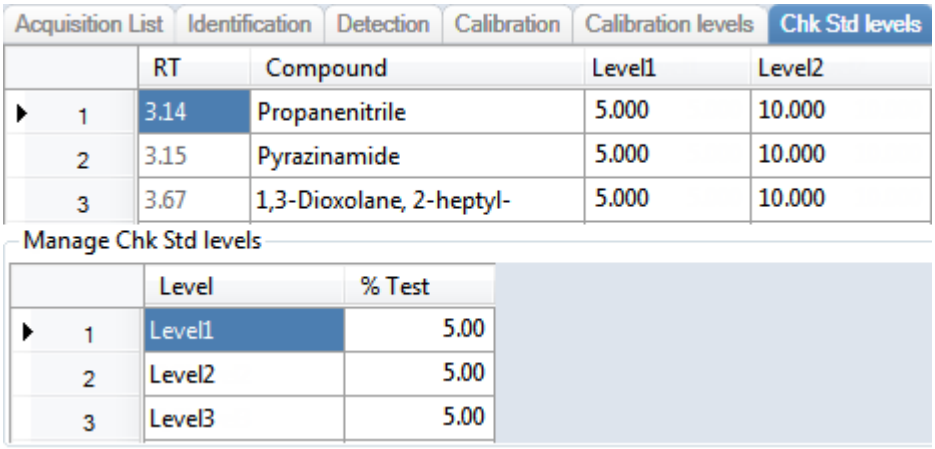


Table 49. Chk Std Levels page parameters (Sheet 1 of 2)

Parameter	Description
RT	Retention time. The time after injection when the compound elutes. The total time that the compound is retained on the column.
Compound	The compound name.

Table 49. Chk Std Levels page parameters (Sheet 2 of 2)

Parameter	Description
Level_1–Level_n	User-defined quality control levels for the compound.
Manage Levels	
Level	User-defined quality control level names. The names you enter here become the column headers for the levels.
% Test	A value for the acceptable difference (as a percentage) between the known amount and calculated (measured) amount of each level.
Shortcut menu	The Levels page uses a right-click shortcut menu. See Using the Shortcut Menu Commands.

Real Time Viewer

Use the Real Time Viewer page to specify which traces display in the Real Time Status pane or in the stand-alone Real Time Status window when you perform acquisition in Acquisition mode. Refer to Chapter 3, "Using the Acquisition Mode" in the *TraceFinder User Guide*.

Figure 83. Real Time Viewer page

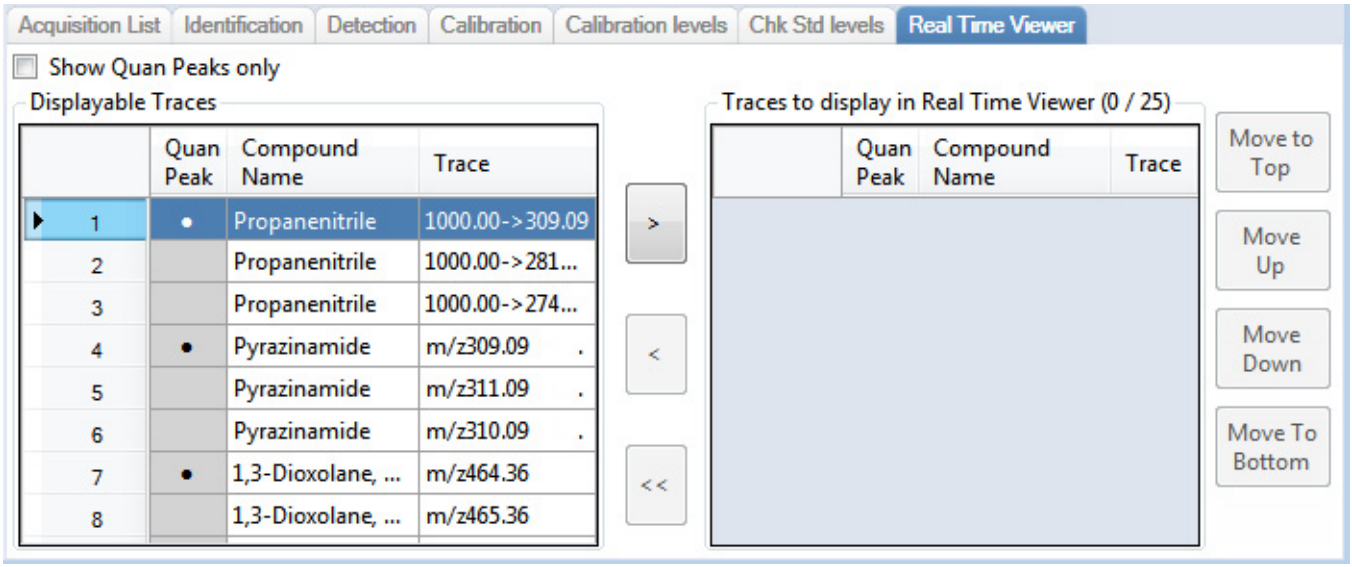
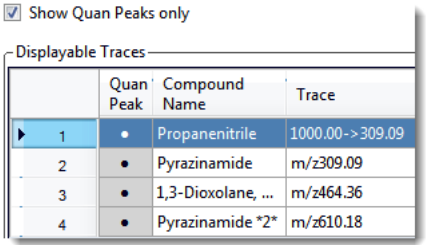


Table 50. Real Time Viewer page parameters

Parameter	Description
Show Quan Peaks Only	Displays only target peaks in the compounds list. Quantitative peaks are indicated with a black dot in the Quan Peak column. 
Displayable Traces	
Quan Peak	Dots indicate target peak traces. Unmarked traces indicate confirming peaks.
Compound Name	Names of all compounds in the method.
Trace	Lists the simple mass or precursor mass for all traces—both target peak and confirming peak—for each compound.
>	Moves the selected trace to the Traces to Display in Real Time Viewer pane.
<	Moves the selected trace to the Displayable Traces pane.
<<	Moves all traces to the Displayable Traces pane. To move multiple traces to the Traces to Display... pane, hold down the SHIFT key, select multiple traces, and then click >.
Traces to Display in Real Time Viewer (*n*/25)	List the traces to be displayed and the display order used in the real-time display in the Acquisition mode. Refer to "Real-Time Trace Display" in Chapter 3 of the *TraceFinder User Guide*. The maximum number of traces is 25.
Move to Top	Moves the selected trace to the top of the Traces to Display... list and the second position in the real-time display. The TIC is always the first position in the real-time display in the Acquisition mode.
Move Up	Moves the selected trace up one position in the list.
Move Down	Moves the selected trace down one position in the list.
Move to Bottom	Moves the selected trace to the bottom of the list.

Using the Shortcut Menu Commands

Each page on the Compounds page (except the Acquisition List, Detection, and Real Time Viewer pages) uses right-click, shortcut menu commands to display or hide the retention column, remove compounds from the method, copy data, or save the compound list to a CSV file.

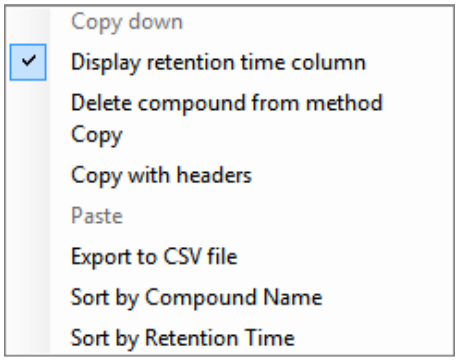


Table 51. Compounds page shortcut menu commands

Command	Description
Copy Down	Copies the value in the selected row to all rows below it. This command is available only when you have selected a value that can be copied down. Refer to Appendix C, "Using Copy Down and Fill Down" in the *TraceFinder User Guide*.
Display Retention Time Column	Displays or hides the RT column in the compound list.
Delete Compound From Method	Removes the selected compound from the current method.
Copy	Copies the data in the selected rows or columns to the Clipboard. Use this command to copy compound information to a text editor or spreadsheet application. You cannot paste this data back into the method development compound list.
Copy With Headers	Copies the data in the selected rows or columns and the associated column headers to the Clipboard. Use this command to copy sample information to a text editor or spreadsheet application. You cannot paste this data back into the method development compound list.
Paste	Pastes a single column of copied data from a text editor or spreadsheet application into the selected column. The pasted data must be valid data for the selected column.
Undo Last Paste	Removes the last pasted item in the method development compound list.
Export to CSV File	Opens the Save As dialog box where you can save the current compound list to a CSV file.
Sort by Compound Name	Sorts the compounds alphabetically from A to Z.
Sort by Retention Time	Sorts the compounds from shortest retention time to longest retention time.

Copying and Pasting Column Values

You can use the copy-and-paste functions in the shortcut menu to copy column values within a method or from one method to another. You can use these copy-and-paste techniques on any pages with grids of data in the Compounds and QAQC views.

After you copy grid values to the Clipboard, you can paste them into a text application such as Notepad, an email, a spreadsheet, other grid cells in the same method, or another method.

Tip When copying data into an application other than a TraceFinder method grid, use the Copy with Headers command, instead of the Copy command in the shortcut menu, to preserve the column headers.

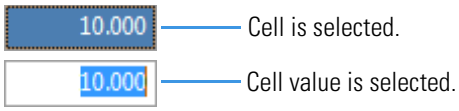
Follow these procedures:

- To copy a value from one cell to another cell
- To copy all values in a column to another column
- To copy multiple columns
- To copy an entire grid to another method

❖ To copy a value from one cell to another cell

1. Select a cell value to copy to the Clipboard.

   You can select either an entire table cell or just a cell value.

2. Right-click and choose **Copy**.

   The application copies either the selected cell value or the selected cell to the Clipboard.

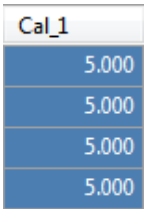
3. In this or another method, select the cell value or table cell that you want to overwrite.
   - If you copied a cell value, you can select the cell value or simply click in the cell.
   - If you copied an entire cell, you must select a table cell to overwrite.

4. Right-click and choose **Paste.**

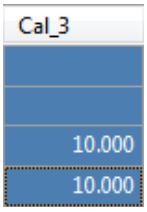
   The application replaces the selected value with the value copied to the Clipboard.

❖ To copy all values in a column to another column

1. Use the SHIFT key to select the column to copy.

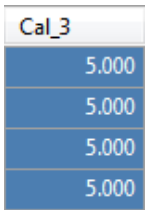
   

2. Right-click and choose **Copy**.
3. In this or another method, use the SHIFT key to select the column to overwrite.



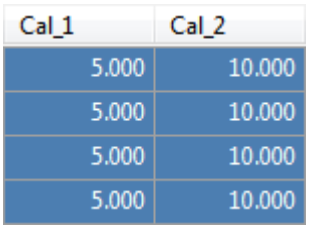
4. Right-click and choose **Paste.**

   The application overwrites the selected cells with the cells that you copied to the Clipboard.

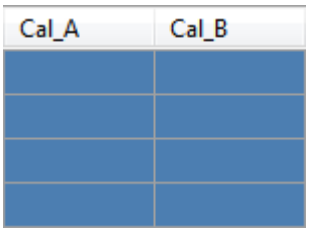


❖ To copy multiple columns

1. Use the SHIFT key to select the columns to copy.



2. Right-click and choose **Copy**.
3. In this or another method, use the SHIFT key to select the columns to overwrite.



4. Right-click and choose **Paste.**

   The application overwrites the selected cells with the cells that you copied to the Clipboard.

Cal_A	Cal_B
5.000	10.000
5.000	10.000
5.000	10.000
5.000	10.000

❖ To copy an entire grid to another method

1. Use the SHIFT key to select all the columns in the grid.

LOD (Detection limit)	LOQ (Quantitation limit)	LOR	ULOL (Linearity limit)	Carryover limit
10.000	20.000	30.000	0e0	0e0
10.000	20.000	30.000	0e0	0e0

2. Right-click and choose **Copy**.

3. In another method, use the SHIFT key to select the columns to overwrite.

LOD (Detection limit)	LOQ (Quantitation limit)	LOR	ULOL (Linearity limit)	Carryover limit
0.000	0.000	0.000	0e0	0e0
0.000	0.000	0.000	0e0	0e0

4. Right-click and choose **Paste.**

   The application overwrites the selected cells with the cells that you copied to the Clipboard.

   Note When the Clipboard contains more cells of data than there are selected target cells for the paste operation, the application overwrites the selected cells and reports that the Clipboard contents were truncated to fit the grid.

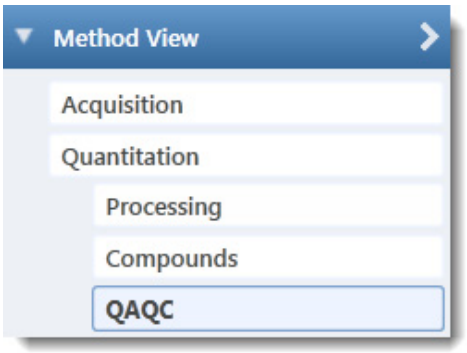
Editing the QAQC Page

Use the QAQC page to set limits and ranges so that the application can review the data and results as an aid to final approval.

On most QAQC pages, you can use the copy-and-paste functions in the shortcut menu to copy grid values from one column to another or from one method to another. For detailed instructions, see Copying and Pasting Column Values.

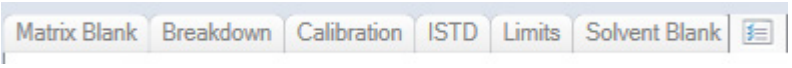
❖ To open the QAQC page

Click **QAQC** in the Method View navigation pane.

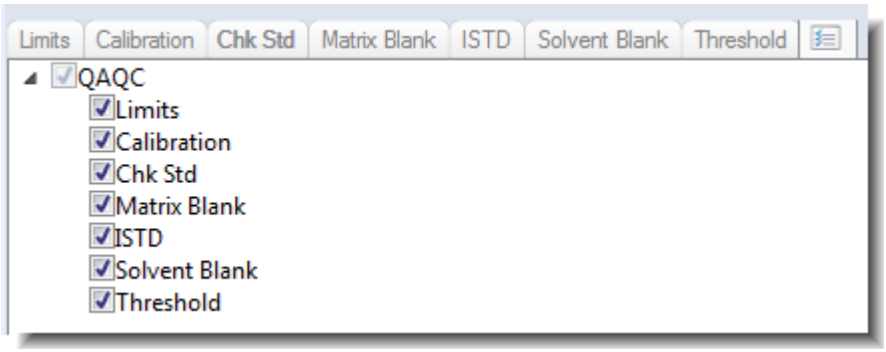


❖ To configure the visibility of pages on the QAQC page

1. Click the **Configuration** icon, , in the QAQC page header.



The configuration page opens.



2. Select the check box for each lower-level page that you want to display in the view.

> **Note** A grayed-out check box indicates that the page name is required and cannot be cleared.

The QAQC page immediately displays the tabs for only the selected pages.

From the QAQC page of the Method View, you can access these additional pages:

- Limits
- Calibration
- Chk Std
- Breakdown
- Matrix Blank
- ISTD
- Solvent Blank
- Threshold
- Lab Control
- Meth Val
- Matrix Spike
- Tune

Limits

Use the Limits page to define levels of review for quantified results. Quantified results appear in printed and electronic reports. You can also define when a quantified value is reported instead of reporting less than a particular limit.

Figure 84. Limits page

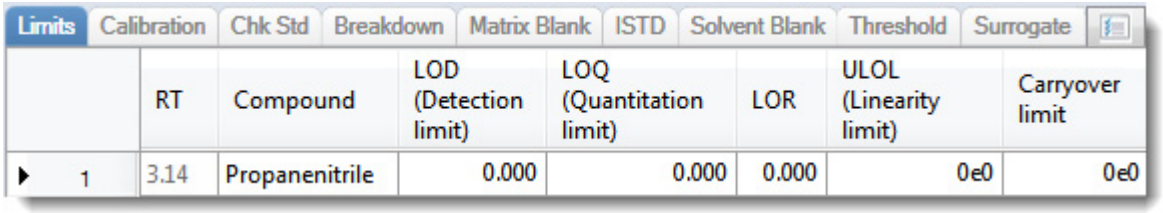


Table 52. Limits page parameters (Sheet 1 of 2)

Parameter	Description
RT	Retention time. The time after injection when the compound elutes. The total time that the compound is retained on the column.
Compound	The compound name.
LOD (Detection Limit)	Limit of detection. The lowest amount of a substance that can be detected. Usually derived from a method detection limit (mdl) study.
LOQ (Quantitation Limit)	Limit of quantitation. The lowest amount of a substance that can be confidently and accurately quantitated. This is usually the lowest calibration amount.
LOR	Limit of reporting. Also called cutoff in some industries. This is the lowest amount that can be reported, as determined by each laboratory's standard operating practices.

Table 52. Limits page parameters (Sheet 2 of 2)

Parameter	Description
ULOL (Linearity Limit)	Upper limit of linearity. This is usually the highest calibrator amount.
Carryover Limit	The highest amount of a substance that does not leave a residual amount in the instrument. If a substance has a carryover limit of 5, amounts higher than 5 usually dirty the instrument and leave residue behind, tainting the following sample. A carryover limit of less than 5 does not leave any residual amounts of the substance.

Calibration

Use the Calibration page to define acceptable criteria for initial calibration. The application compares the initial calibration results for each compound found in the sample to the values defined on this page.

In the Calibration report, the application flags the calculated values for internal standard compounds that exceed these limits.

Figure 85. Calibration page

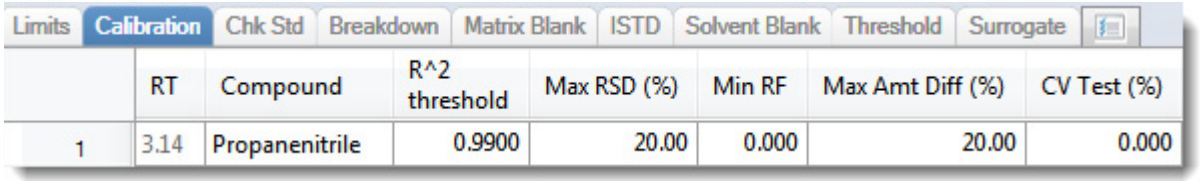


Table 53. Calibration page parameters (Sheet 1 of 2)

Parameter	Description
RT	Retention time. The time after injection when the compound elutes. The total time that the compound is retained on the column.
Compound	The compound name.
R^2 Threshold	The minimum correlation coefficient (r^2) for an acceptable calibration (when in linear or quadratic mode).
Max RSD (%)	The maximum relative standard deviation (RSD) for an acceptable calibration (when in average RF mode).
	Note This RSD value is not the same value used in Data Review or the Compound Calibration Report. The application uses this RSD value when you select AverageRF as the curve type for the method. See Calibration Page.
Min RF	The minimum average response factor (RF) for an acceptable calibration (when in average RF mode).

Table 53. Calibration page parameters (Sheet 2 of 2)

Parameter	Description
Max Amt Diff (%)	The maximum deviation between the calculated and theoretical concentrations of the calibration curve data points (when in linear or quadratic mode).
CV Test (%)	Coefficient of Variance test. The coefficient of variance percentage is the standard deviation of the multiple samples of one level, multiplied by 100, and then divided by the average of the multiple samples of that level. This calculation is based on the areas of the peaks.

Chk Std

Use the Chk Std page to review the calibration on an ongoing basis. The application compares the quality check standard results for each compound in the sample to the initial calibration using values defined on this page.

In the Check Standard report, the application flags the calculated values for internal standard compounds that exceed these limits.

For linear and quadratic modes, the maximum difference for the calculated concentration in the Chk Std sample versus the theoretical value is set on the Compounds page.

Figure 86. Chk Std page

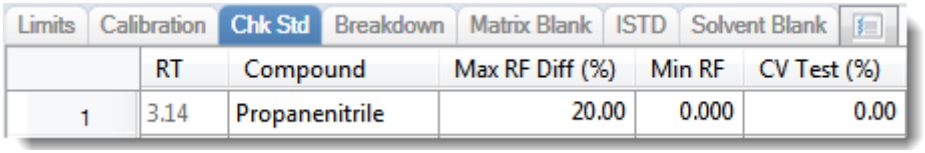


Table 54. Chk Std page parameters

Parameter	Description
RT	Retention time. The time after injection when the compound elutes. The total time that the compound is retained on the column.
Compound	The compound name.
Max RF Diff (%)	The maximum deviation between the response factor (RF) of the Chk Std sample and the average response factor from the calibration (when in average RF mode).
Min RF	The minimum response factor for the Chk Std sample (when in average RF mode).
CV Test (%)	Coefficient of Variance test. The coefficient of variance percentage is the standard deviation of the multiple samples of one level, multiplied by 100, and then divided by the average of the multiple samples of that level. This calculation is based on the areas of the peaks.

Breakdown

Use the Breakdown page to view and edit values used for the evaluation of breakdown and degradation reporting. The application makes the evaluation by calculating the ratio of breakdown compounds to native compounds.

❖ To display the list of compounds in a group

Click anywhere in the group row.

❖ To select a group for breakdown calculation

Select the **Active** check box in the group row.

You can select any group in the method for breakdown calculation, but the application calculates and reports only those that contain breakdown and native compounds.

Figure 87. Breakdown page

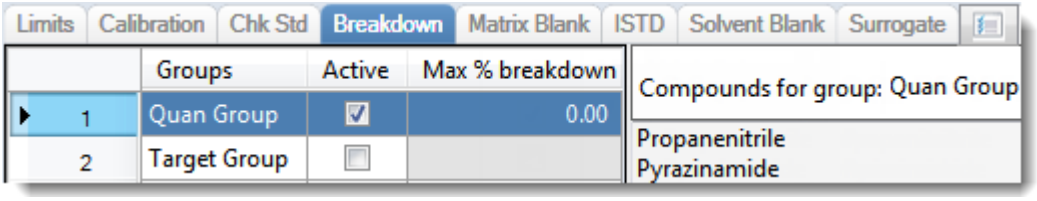


Table 55. Breakdown page parameters

Parameter	Description
Groups	Lists all groups created on the Groups page. See Editing the Groups Page.
Active	Specifies which groups are used for analysis.
Max % Breakdown	The maximum allowable percentage of breakdown to native compounds. This value is calculated by summing the responses of the breakdown compounds and dividing them by the sum of the native compounds. In the Breakdown Report, the application flags the calculated values for breakdown and native compounds that exceed these limits.
Compounds for Group	Lists all compounds in the selected group.

Matrix Blank

Use the Matrix Blank page parameters to define acceptable levels of target compounds in blank samples. The application compares the calculated concentration for each compound in the sample to the maximum concentration defined on this page. You can enter the maximum concentration as a percentage of a flag value or as a specified value.

In the Matrix Blank report, the application flags the calculated values for target compounds that exceed these limits.

❖ To specify the maximum concentration as a percentage

1. From the Method column list, select one of the following methods:
   - % of LOD
   - % of LOQ
   - % of LOR
2. In the Percentage column, type a percentage value.

❖ To specify the maximum concentration

1. From the Method column list, select **Concentration**.
2. In the Max Conc column, type an absolute value.

Figure 88. Matrix Blank page

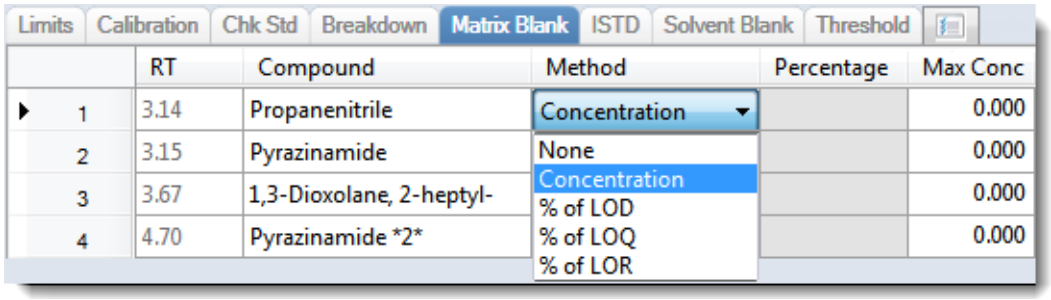


Table 56. Matrix Blank page parameters

Parameter	Description
RT	Retention time. The time after injection when the compound elutes. The total time that the compound is retained on the column.
Compound	The compound name.
Method	The evaluation process used for comparing the calculated concentration. You can specify no maximum, a specific concentration, or a percentage of the LOR, LOD, or LOQ.
Percentage	The percentage of the LOR, LOD, or LOQ if you are using the percentage approach.
Max Conc	The maximum concentration if you are using an absolute value.

ISTD

Use the ISTD page to review the response and retention time of internal standards (when available). The application compares the area and retention time results for each internal standard compound in the sample to a specified range.

If all of your target compounds are set to external calibration mode or if you have not identified any compounds as internal standards, this page does not show any values.

Figure 89. ISTD page

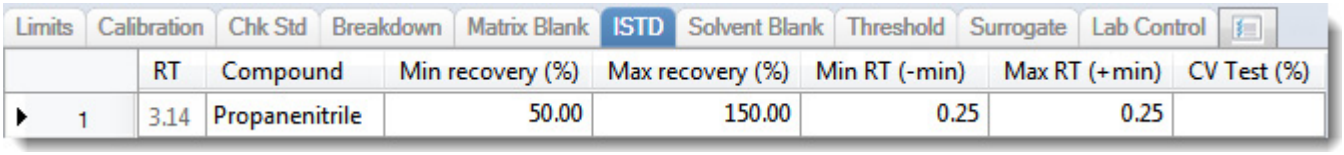


Table 57. ISTD page parameters

Parameter	Description
RT	Retention time. The time after injection when the compound elutes. The total time that the compound is retained on the column.
Compound	The compound name.
Min Recovery (%) Max Recovery (%)	The minimum and maximum percent recoveries for the internal standards to define an acceptable range. For check standards, the application compares the response of each internal standard in each sample to a range around the average of the responses of that compound in all of the calibration standards. For all other samples, the application calculates the comparison range around the check standard responses if a check standard is available in the batch. If no check standard is available, the application tests against the initial calibration.
Min RT (–min) Max RT (+min)	The minimum and maximum drift (in minutes) for the internal standards to define an acceptable range. For check standards, the application compares the retention time of each internal standard in each sample to a range around the average of the retention times of that compound in all of the calibration standards. For all other samples, the application calculates the comparison range around the check standard retention times if a check standard is available in the batch. If no check standard is available, the application tests against the initial calibration.
CV Test (%)	Coefficient of Variance test. The coefficient of variance percentage is the standard deviation of the multiple samples of one level, multiplied by 100, and then divided by the average of the multiple samples of that level. This calculation is based on the areas of the peaks.

Solvent Blank

Use the Solvent Blank page to view or edit QC values for solvent reporting. The application compares the calculated response for each compound in the sample to the maximum response defined on this page.

In the Solvent Blank report, the application flags the calculated values for target compounds that exceed these limits.

Figure 90. Solvent Blank page

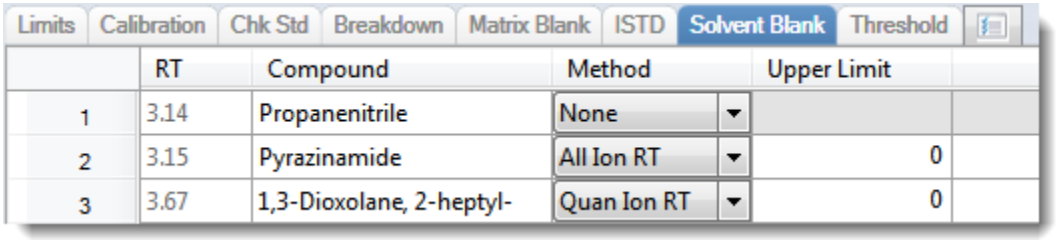


Table 58. Solvent Blank page parameters

Parameter	Description
RT	Retention time. The time after injection when the compound elutes. The total time that the compound is retained on the column.
Compound	The compound name.
Method	The evaluation process to use as a response for the quantitation ion only (Quan Ion RT) or as a summed response for the quantitation ion and any confirming ions (All Ion RT). To deactivate the solvent blank test for a specific compound, select **None**.
Upper Limit	Specifies an upper limit for each compound in the sample when you select an evaluation process. These values are not concentrations; they are raw response values.

Surrogate

Use the Surrogate page to view or edit values for surrogate recovery reporting. The application compares the calculated concentration for each compound in the sample to the theoretical concentration and range defined on this page.

You can use these parameters to evaluate the performance of your method. For this evaluation, prepare, analyze, and evaluate a number of samples (typically 4 to 10) to document method accuracy and precision as a comprehensive whole.

In the Surrogate Recovery report, the application flags the calculated values for method validation compounds that exceed these limits.

Figure 91. Surrogate page

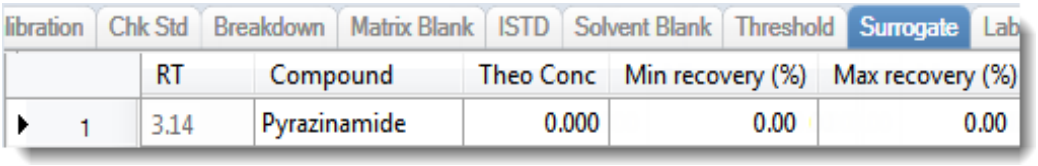


Table 59. Surrogate page parameters

Parameter	Description
RT	Retention time. The time after injection when the compound elutes. The total time that the compound is retained on the column.
Compound	The compound name.
Theo Conc	Values for the compounds that represent the expected theoretical concentration of that compound in the sample.
Min Recovery (%)	A range between the allowable minimum recovery percentage and the maximum recovery percentage that the application can determine by comparing the observed calculated concentration in the analysis to the expected concentration. Each method validation compound can have its own values for these fields, independent of other method validation compounds.
Max Recovery (%)	

Lab Control

Use the Lab Control page to view and edit QC values for lab control sample (LCS) and lab control sample duplicate (LCSD) analyses. The application compares the calculated concentration for each compound in the sample to the theoretical concentration and range defined on this page.

You can prepare samples (typically known as clean matrices) as LCS or LCSD. These represent samples where you have added known concentrations of target analytes. To define an LCS and its duplicate in a batch, select the appropriate sample type and a common sample ID. To group the samples for a Lab Control report, the samples must have the same Sample ID.

In the Lab Control report, the application flags the calculated values for spiked compounds that exceed these limits.

Figure 92. Lab Control page

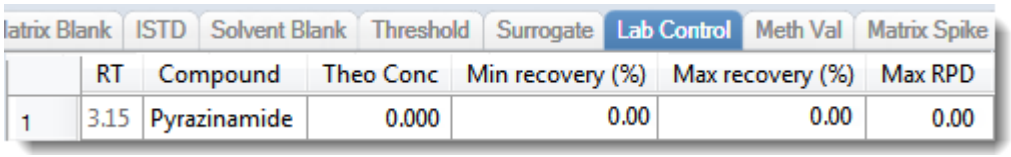


Table 60. Lab Control page parameters (Sheet 1 of 2)

Parameter	Description
RT	Retention time. The time after injection when the compound elutes. The total time that the compound is retained on the column.

Table 60. Lab Control page parameters (Sheet 2 of 2)

Parameter	Description
Compound	The compound name.
Theo Conc	Values for the lab control compounds that represent the expected theoretical concentration of that compound in the sample.
Min Recovery (%) Max Recovery (%)	A range of the allowable minimum recovery percentage and the maximum recovery percentage that the application can determine by comparing the observed calculated concentration in the analysis to the expected concentration. Each LCS or LCSD compound can have its own values for these fields, independent of other LCS or LCSD compounds.
Max RPD	Specifies a maximum value for the relative percent difference (RPD) between two spiked samples.

Meth Val

Use the Meth Val page to view or edit QC values for method validation reporting. The application compares the calculated concentration for each compound in the sample to the theoretical concentration and range defined on this page.

You can use these parameters to evaluate the performance of your method. For this evaluation, prepare, analyze, and evaluate a number of samples (typically 4 to 10) to document method accuracy and precision as a comprehensive whole. To define a method validation sample in the batch, select the appropriate sample type.

In the Method Validation report, the application flags the calculated values for method validation compounds that exceed these limits.

Figure 93. Meth Val page

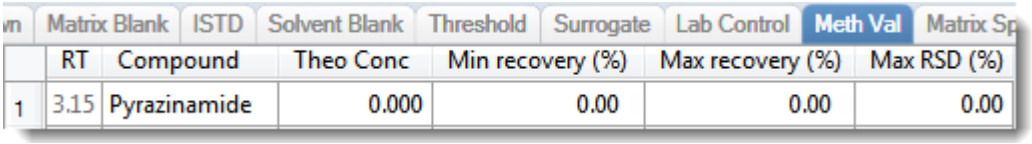


Table 61. Meth Val page parameters (Sheet 1 of 2)

Parameter	Description
RT	Retention time for the compound.
Compound	The compound name.
Theo Conc	Values for the compounds that represent the expected theoretical concentration of that compound in the sample.

Table 61. Meth Val page parameters (Sheet 2 of 2)

Parameter	Description
Min Recovery (%) Max Recovery (%)	A range between the allowable minimum recovery percentage and the maximum recovery percentage that the application can determine by comparing the observed calculated concentration in the analysis to the expected concentration. Each method validation compound can have its own values for these fields, independent of other method validation compounds.
Max RSD (%)	The maximum relative standard deviation of the set of observed concentrations for a component across the set of method validation samples.

Matrix Spike

Use the Matrix Spike page to view or edit QC values for matrix spike and matrix spike duplicate analyses. The application compares the calculated concentration for each compound in the sample (after subtracting the original sample value) to the theoretical concentration and range defined on this page.

To evaluate matrix spike or matrix spike duplicate compounds, prepare samples as MS or MSD. These represent samples where you have added known concentrations of target analytes. To define an Unknown sample, its MS, and its MSD in the batch, the samples must have the same Sample ID and be in Unknown/MS/MSD order; otherwise, data analysis and reporting cannot correctly calculate the MS and MSD sample values.

IMPORTANT To group samples for processing and to generate an MSMSD report, the samples must have the same Sample ID and must be in Unknown/MS/MSD order.

In the MSMSD report, the application flags the calculated values for spiked compounds that exceed these limits.

Figure 94. Matrix Spike page

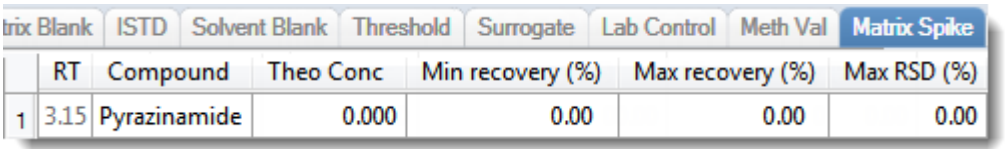


Table 62. Matrix Spike page parameters (Sheet 1 of 2)

Parameter	Description
RT	Retention time. The time after injection when the compound elutes. The total time that the compound is retained on the column.
Compound	The compound name.

Table 62. Matrix Spike page parameters (Sheet 2 of 2)

Parameter	Description
Theo Conc	Values for the matrix spike compounds that represent the expected theoretical concentration of that compound in the sample. You can apply or not apply the dilution factor for a sample to the calculated matrix spike concentrations.
Min Recovery (%)	A range between the allowable minimum recovery percentage and the maximum recovery percentage that can be determined by comparing the observed calculated concentration in the analysis to the expected concentration. Each matrix spike sample can have its own values for these fields, independent of other matrix spike samples.
Max Recovery (%)	
Max RSD (%)	The maximum relative standard deviation of the set of observed concentrations for a component across the set of method validation samples.

Tune

Use the Tune page to specify mass spectral comparison criteria according to Environmental Protection Agency (EPA) tune methods. Your method must include one (and only one) compound specified as an MSTune compound type. The QAQC evaluation compares the mass list of the compound to the values in the tune method.

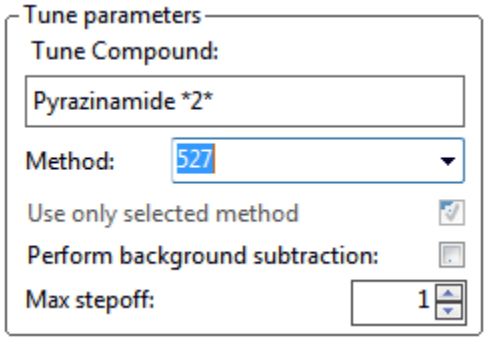
An MSTune sample contains Decafluorotriphenylphosphine (DFTPP) or bromofluorobenzene (BFB). The application handles the MSTune sample differently from other samples in the method, examining the mass spectrum of a specific compound on a qualitative, not a quantitative basis.

Follow these procedures:

- To select an EPA tune method
- To manually submit a mass spectrum for tune evaluation

❖ To select an EPA tune method

1. Select a method from the Method list.



- For some EPA tune methods, the Use Only Selected Method option is available.
- For some EPA tune methods, the Perform Background Subtraction option is available.

The application displays the mass spectral reference for the selected method.

Eval Mass	Base Peak?	Low Op	Lower Limit %	High Op	High Limit	Relative To
51		>=	10	<=	85	Base peak
68				<	2	69
70				<	2	69
127		>=	10	<=	80	Base peak
197				<	2	198
198	Yes	>	50	<=	100	Base peak
199		>=	5	<=	9	198
275		>=	10	<=	60	Base peak
365		>	0.5			198

2. (Optional) Select the **Use Only Selected Method** check box.

   The application uses only the specified EPA tune method for tune criteria testing.

   If this check box is not selected and the tune spectrum fails against the specified tune criteria, the application performs the comparison against other EPA tune method criteria. The Tune report displays the pass/fail status of the tune spectra against all methods used for comparison.

3. (Optional) Select the **Perform Background Subtraction** check box.

   - The 8000 and Contract Laboratory Program (CLP) series methods require background subtraction.
   - The 500 and 600 series methods do not require background subtraction but you can choose to use it.

   The Tune report displays the retention time and scan range of the spectrum.

4. (Optional) Click in the Max Stepoff box to select a number or type a number in the box.

   The Max Stepoff field defines the maximum number of scans as the range before and after the Tune compound peak that is used for background subtraction. For example, a maximum stepoff of 1 means that the background spectrum is taken one scan before the left edge of the tune peak or one scan after the right edge of the tune peak. If the left edge background spectrum fails the tune test, the system automatically tries the right edge of the spectrum. A maximum stepoff of 2 means trying up to four tests using four different background spectra: two scans before the left edge, one scan before the left edge, one scan after the right edge, and two scans after the right edge. EPA guidelines assign a maximum stepoff value of 20.

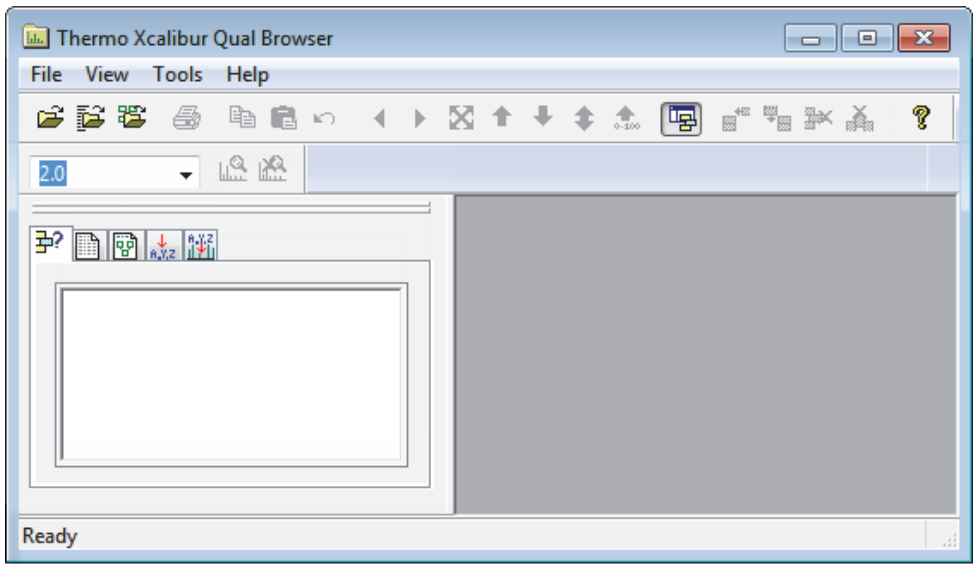
Note This Max Stepoff value is not related to the Stepoff Value parameter on the Acquisition page of the method. The Stepoff Value on the Acquisition page applies only to the reference spectrum set for the method and does not affect the Tune method.

❖ To manually submit a mass spectrum for tune evaluation

1. On the Tune page, click **Select File and Mass Spectrum**.
2. In the browser, select a raw data file and click **Open**.

   The Thermo Xcalibur Qual Browser application opens.

   Figure 95. Qual Browser main window



3. In the Qual Browser application, do the following:
   a. Select the tune compound peak that you want to use.
   b. Select the background subtraction parameters to use, and generate the mass spectrum.
   c. Right-click the spectrum view and choose **Export > Clipboard**.

   For detailed instructions about using the Qual Browser application, refer to the Help in the Qual Browser window.
4. On the TraceFinder Tune page, click **Create Tune Report as PDF**.

Figure 96. Tune page

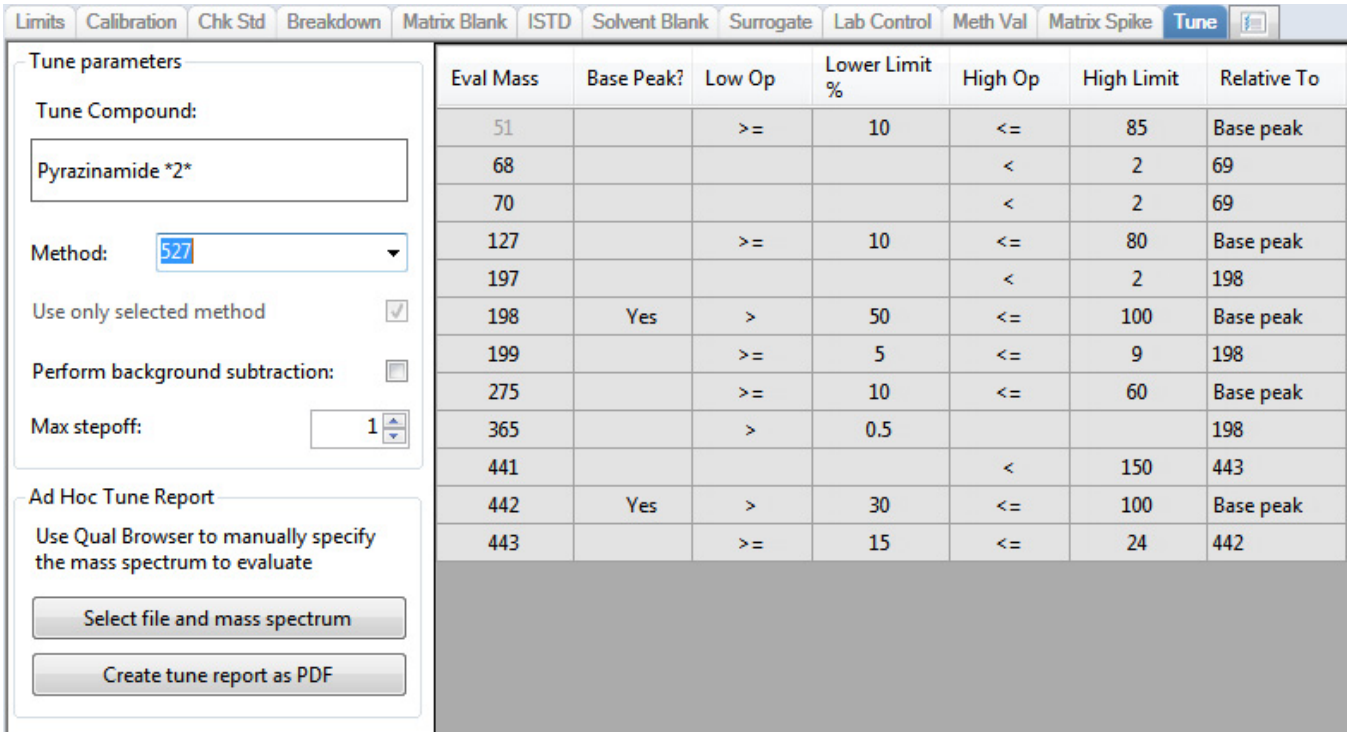


Table 63. Tune page parameters

Parameter	Description
Tune Parameters	
Tune Compound	Compound specified as a tune compound on the Identification page.
Method	Environmental Protection Agency (EPA) methods used for tuning.
Use Only Selected Method	Specifies that the application uses only the specified EPA tune method for tune criteria testing.
Perform Background Subtraction	Specifies that the application averages and subtracts the mass spectra as specified in the background subtraction settings on the Acquisition page for the method. See To set automated background subtraction options.
Max Stepoff	Specifies the maximum number of scans to be used for background subtraction as the range before and after the Tune compound peak.
Ad Hoc Tune Report	
Select File and Mass Spectrum	Opens a browser where you can select a raw data file to use for the tune report.
Create Tune Report as PDF	Creates an ad hoc tune report using the mass spectrum data saved to the Clipboard.

Threshold

Use the Threshold page to specify how to create a threshold guide to overlay on compounds in the Comparative View in the Data Review mode. For each compound, you can specify an absolute value or you can specify a percentage of the peak height. The application uses the selected threshold method and the specified amount to create a threshold guide in the Comparative View chromatograms.

When you create a batch, you can group samples and then specify a sample in the group as the threshold sample to use in the Comparative View. For instructions about specifying a threshold sample, refer to Chapter 4, "Using the Analysis Mode for Quantitation Batches" in the *TraceFinder User Guide*.

In the following figures, the threshold for the dibutyl phthalate compound is 50 percent of the peak height in the threshold sample; the samples Benzo26473, Benzo25557, and Benzo26154 are members of groupb; and the threshold sample for the group is Benzo26473. In the Comparative Data view, you can easily see that the peak height of dibutyl phthalate in the other samples in the group is less than 50 percent of the peak height in the threshold sample.

- Threshold page in the Method View
- Samples page in the Batch View
- Threshold Samples page in the Batch View
- Comparative View in the Data Review

Figure 97. Threshold page in the Method View

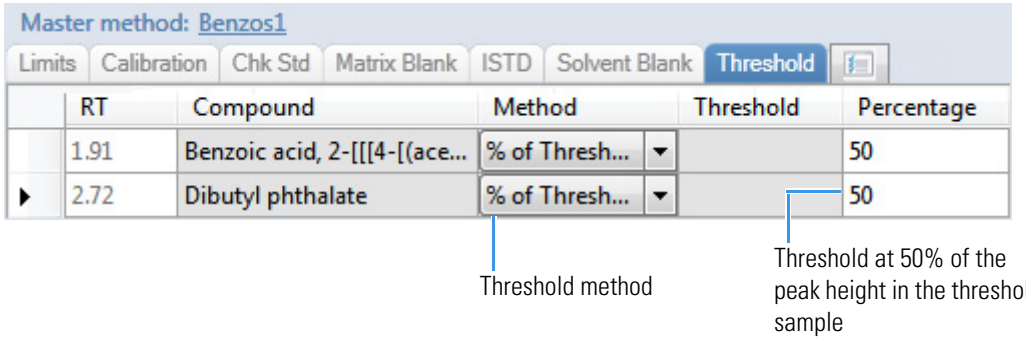


Figure 98. Samples page in the Batch View

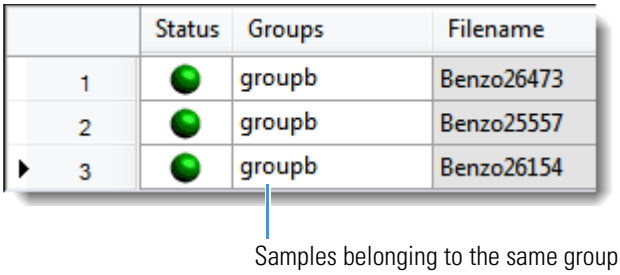


Figure 99. Threshold Samples page in the Batch View

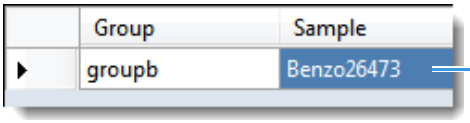


Figure 100. Comparative View in the Data Review

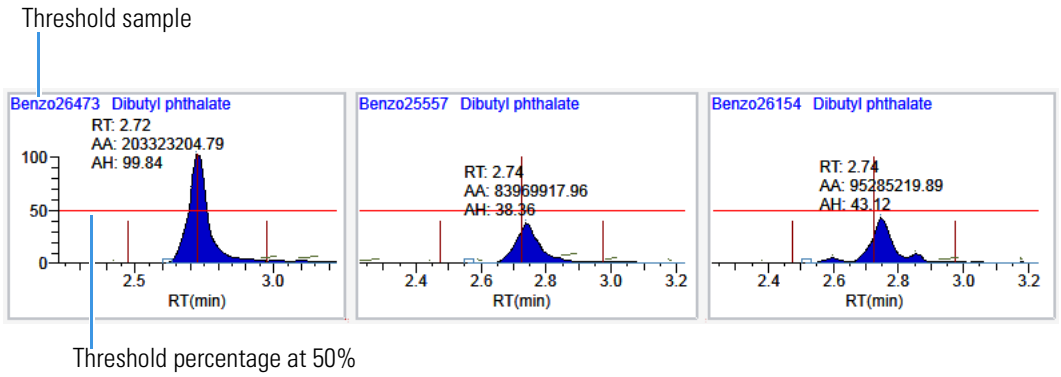


Figure 101. Threshold page

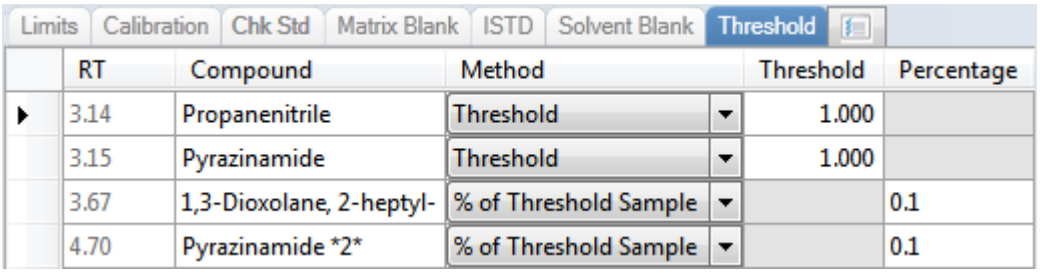


Table 64. Threshold page parameters (Sheet 1 of 2)

Parameter	Description
RT	Retention time. The time after injection when the compound elutes. The total time that the compound is retained on the column.
Compound	The compound name.
Method	Specifies the threshold method as a specific peak height value (Threshold) or as a percentage of the peak height in the threshold sample (% of Threshold Sample).

Table 64. Threshold page parameters (Sheet 2 of 2)

Parameter	Description
Threshold	Specifies the absolute peak height value to use when you select the Threshold method. This value represents the default threshold value to use when you do not specify a Threshold Sample for a group of samples. Default: 1.000
Percentage	Specifies the percentage of the peak height value to use when you select the % of Threshold Sample method. This value represents a percentage of the actual peak height in the Threshold Sample you select for a group of samples. For instructions about specifying a Threshold Sample, refer to Chapter 4, "Using the Analysis Mode for Quantitation Batches" in the *TraceFinder User Guide*. Valid range: 0.1 through 100.1 Default: 0.1

Editing the Groups Page

Use the Groups Page to organize compounds into functional or logical groups. You can use these groups for creating a subset of target compounds.

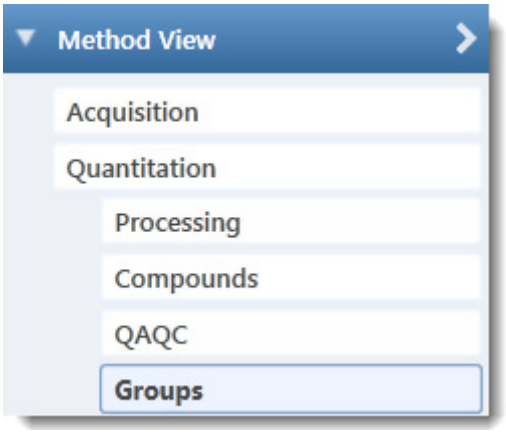
For quantitative processing, the application processes all compounds in the method and stores the complete result set, but only those in the selected group are visible in the Acquisition mode. Limiting the displayed compounds to those in the selected group can be useful when working with a method containing a large list of compounds, only some of which are required for analysis in certain samples. In that case, the application requires only a single method and can reduce the results.

You can create multiple groups and include the same compound in more than one group.

When the master method was created from a compound database, the Groups pane displays any compound groups defined in the compound database. See Compound Details Pane for Small Molecules.

❖ To open the Groups page

Click **Groups** in the Method View navigation pane.



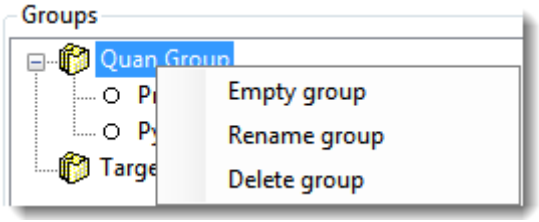
❖ To create a group

1. At the bottom of the Groups area, click **Add Group**.

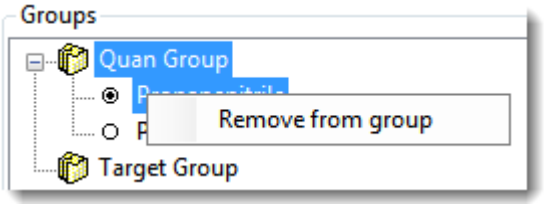
   The Add a New Group dialog box opens.
2. Type a name for the new group and click **OK**.

   The new group appears in the Groups area.
3. Drag a compound from the Compounds area onto a group name (as if you were moving files into a folder).

4. To remove all the compounds from a group, rename the group, or delete it, right-click the group name and choose the applicable command.



5. To remove a single compound, right-click the compound name in the group and choose **Remove from Group**.



Groups Page

Use the features on the Groups page to organize compounds into functional or logical groups.

Figure 102. Groups page

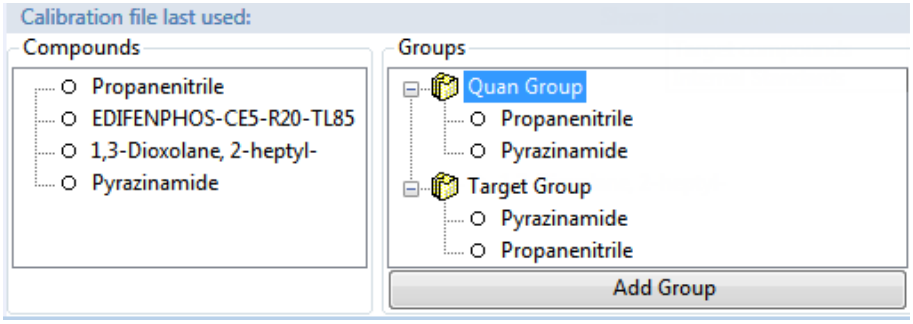


Table 65. Groups page parameters

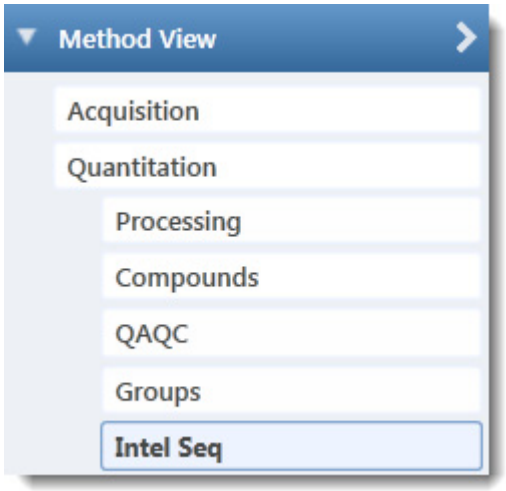
Parameter	Description
Compounds	Lists all available compounds.
Groups	Lists all available groups.
Add Group	Opens the Add a New Group dialog box where you can create a new group.
Shortcut menu	
Empty Group	Removes all compounds from the selected group.
Rename Group	Changes the name of the selected group.
Delete Group	Removes the selected group and all the compounds in it.
Remove From Group	Removes the selected compound from its group.

Editing the Intelligent Sequencing Page

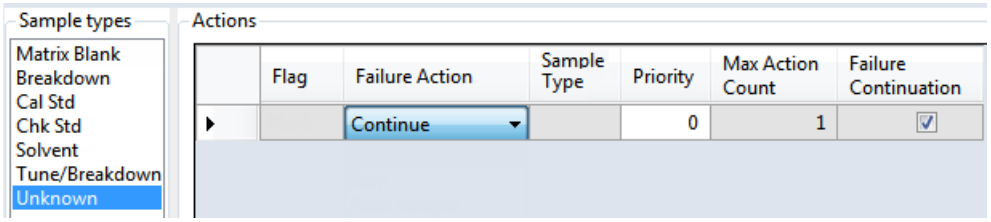
Use the Intelligent Sequencing page to specify the actions you want the application to take when there are acquisition failures with each sample type. The Intelligent Sequencing page is available only when you activate the Intelligent Sequencing option in the Configuration console.

❖ To open the Intelligent Sequencing page

Click **Intel Seq** in the Method View navigation pane.



The Intelligent Sequencing page opens.



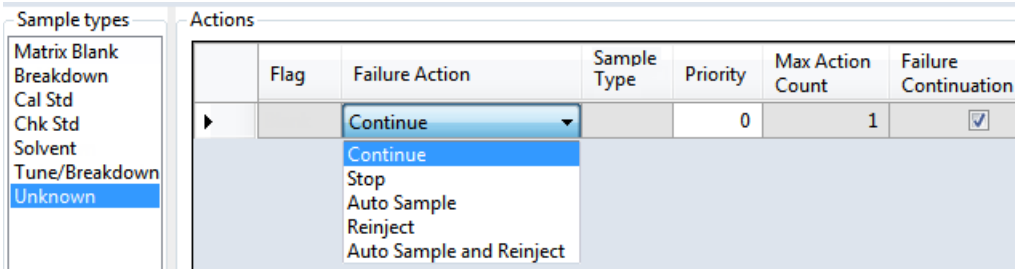
❖ To specify actions for sample acquisition failures

1. In the Sample Types list, select a sample type.

   Each sample type has a specific set of failure flags. See Sample-Specific Failure Flags.

2. For each failure flag, select a failure action.

   The failure action choices are the same for each failure flag except flags for Solvent or Matrix Blank sample types. The Solvent and Matrix Blank sample types do not have Auto Sample or Auto Sample and Reinject failure actions.



   Each Failure Action requires one or more of the following values:

   - Sample Type
   - Priority
   - Max Action Count
   - Failure Continuation

   For a detailed description of each of these parameters, see Actions.

3. Select a sample type to use for the failure action.

   This value is available only for Auto Sample and Auto Sample and Reinject failure actions. When you create your samples list on the Auto Samples page, you must include at least one sample with this sample type for the autosampler to use when it encounters this error condition. Refer to Chapter 4, "Using the Analysis Mode for Quantitation Batches" in the *TraceFinder User Guide*.

4. In the Priority column, type a priority value for this action.

   The priority value can be any positive or negative integer.

   - The application performs the failure action for the highest priority failure it encounters and ignores all others.
   - When you assign the same priority to two or more failures, the application performs the failure action for the first failure it encounters and ignores all others.

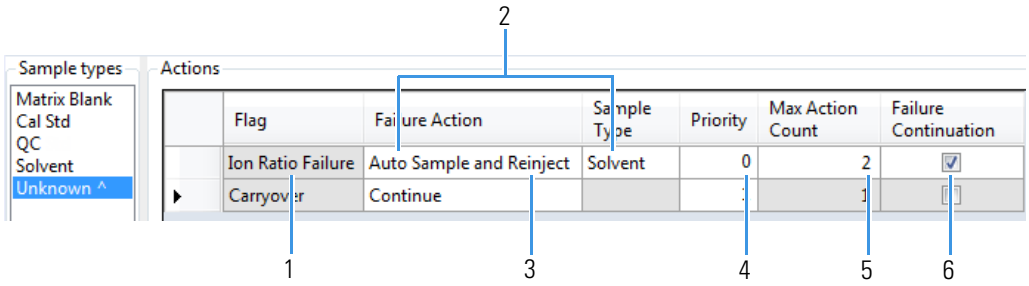
5. In the Max Action Count column, select a value for the maximum number of times the application should repeat a sample.

6. In the Failure Continuation column, do one of the following:

   - Select the check box to skip this sample and continue to the next sample when this sample exceeds the Max Action Count value.
   - Clear the check box to stop the batch when this sample exceeds the Max Action Count value.

Example

The following example shows the actions that the application uses when the acquisition for a sample fails.



1. The acquisition encounters an Ion Ratio Failure error on a sample.
2. The application runs a Solvent sample.
3. The application reinjects the original sample.
4. This action is priority 0 in the Acquisition queue.
5. The application repeats this autosample and reinject process two times.
6. After repeating the failure action two times, the application skips the sample and continues to the next sample.

Actions

Use the Actions pane to specify what action the application takes when it encounters a submission failure for the type of failure flag associated with each sample type.

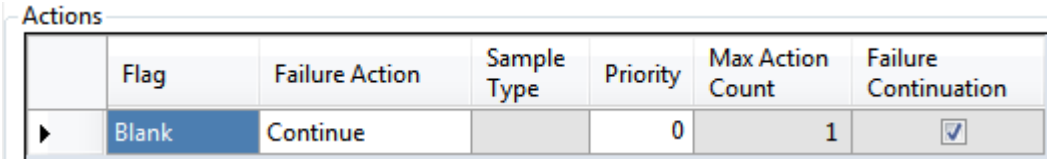


Table 66. Actions parameters (Sheet 1 of 2)

Parameter	Description
Flag	Flag (error) types specific to each sample type. See Sample-Specific Failure Flags. Each flag type has a set of user-specified actions that the application follows when it encounters this error.
Failure Action	In the event of a failed sample, the application does one of the following: • Continue: Continues to the next sample in the batch. • Stop: Stops the batch. • Auto Sample: Injects the sample type specified for the Auto Sample Type parameter and continues to the next sample. • Reinject: Reinjects the current sample by inserting a "reinject" sample in the batch. • Auto Sample and Reinject: Injects the sample type specified for the Auto Sample Type parameter and then reinjects the failed sample.

Table 66. Actions parameters (Sheet 2 of 2)

Parameter	Description
Sample Type	Specifies either a Solvent or Matrix Blank sample type to use for the auto sample injection. Default: Solvent
Priority	The priority value can be any positive or negative integer. When two or more failures have the same priority, the application performs the failure action for the first failure it encounters and ignores all others. The application performs the failure action for the highest priority failure and ignores all others.
Max Action Count	Specifies the maximum number of times the application should repeat a sample before it continues to the next sample or stops the sequence, as determined by the value in the Failure Continuation parameter. Default: 1
Failure Continuation	When this check box is selected, samples that exceed the value specified for the Max Action Count parameter cause the application to skip the sample and continue to the next sample. When this check box is cleared, samples that exceed the value specified for the Max Action Count parameter cause the application to stop the batch. Default: Selected

Sample-Specific Failure Flags

Each sample type has a specific set of failure flags.

Sample Type	Flag
Matrix Blank	• Blank
Breakdown	• Breakdown
Cal Std	• Amount Out of Range • Ion Ratio Failure • Carryover
Chk Std	• Ion Ratio Failure • QC Amount • Out of Range
Solvent	• Solvent Flag
Unknown	• Ion Ratio Failure • Carryover

Editing the Reports Page

Use the Reports Page to specify how you want to save or print your reports.

For the quantitation report types, you can modify quantitation limits flags, user interface options, and quantitation flag options on the Quan Report Settings page.

See the instructions for the following tasks:

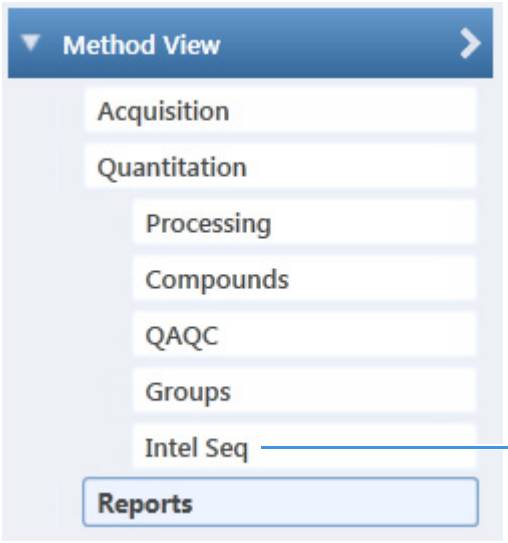
- Specifying Report Formats
- Specifying Quantitation Report Settings

Specifying Report Formats

For each report type, you can create a hard-copy printout, a PDF file, a CSV file, or an Excel file.

❖ To open the Reports page

Click **Reports** in the Method View navigation pane.



The Reports page opens with a list of all configured reports.

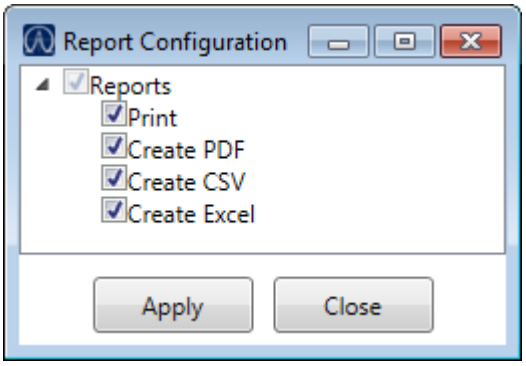
To configure which reports are available when you create a method or which reports create a batch-level report, see Specifying the Report Selections.

❖ To configure the visibility of report formats

1. Click the **Configuration** icon, , in the Report view header.

Report	Print	Create PDF	Create CSV	Create Excel
Batch Report	☐	☐	☐	☐
Blank Report	☐	☐	☐	☐
Calibration Report	☐	☐	☐	☐

The Report Configuration dialog box opens.



2. Select the check box for each report that you want to display.

Note This controls only the display of the format options in the view; it does not select these formats for processing.

3. Click **Apply**.

The Report view displays only the configured formats.

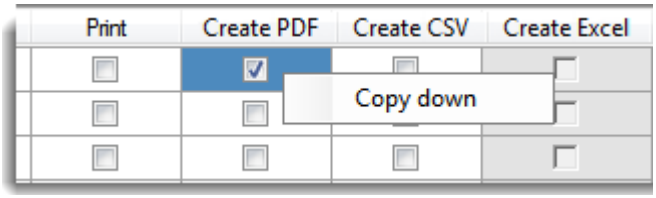
❖ To specify report types and output formats

1. To edit the Report Title, double-click the name and type your new custom title.

The application uses this title for all reports that use this method. You cannot edit the Report Title from other report views.

2. To specify the type of report output to create for each report type, select the check box in the appropriate column.

3. To duplicate an output type for all reports, click the cell to select it, and then right-click and choose **Copy Down**.



All check boxes in the column below the selected cell duplicate the selected or cleared state of the selected cell. This action applies only to reports where this output format is available. By default, all report types are cleared.

Reports Page

Use the features on the Reports page to specify how you want to save or print your reports.

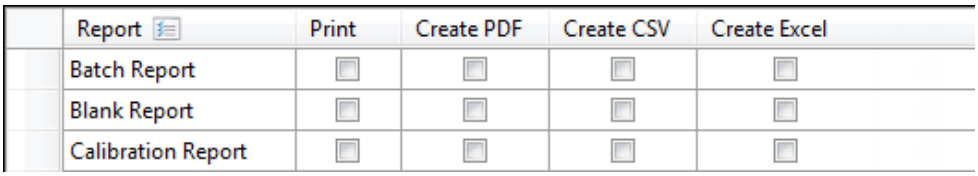


Table 67. Reports page parameters

Parameter	Description
Report list columns	
Report	The name of a report.
Configuration icon	Opens the Report Configuration dialog box where you can select which report formats are displayed in the view.
Print	Sends reports to the default printer.
Create PDF	Saves reports as PDF files.
Create CSV	Saves reports as CSV files.
Create Excel	Saves reports as Excel files.

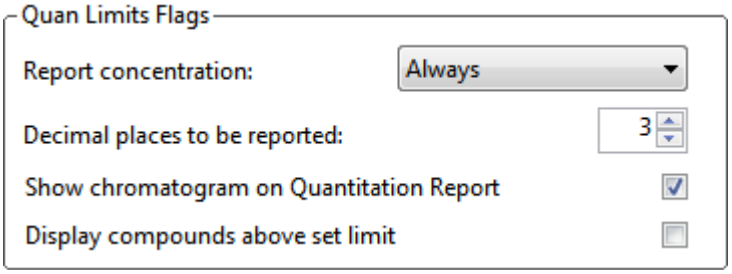
Specifying Quantitation Report Settings

Use the options on the Quantitation Page to choose parameters for flagging values and displaying information in reports.

Follow these procedures:

- To specify quantitation limits
- To specify user interface options
- To specify quantitation flag options
- To correct surrogates
- To track the tune file usage

❖ To specify quantitation limits



1. To report the calculated concentration at all times or only when the quantified value exceeds LOD, LOQ, or LOR, choose the appropriate value from the Report Concentration list.

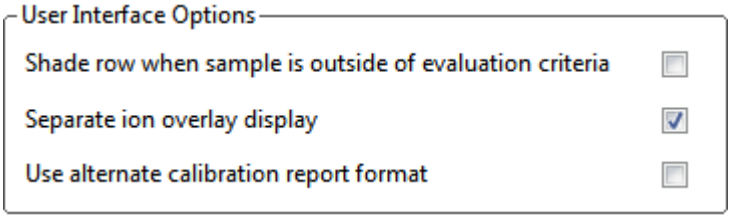
   For a description of concentration limits, see Editing the QAQC Page.

2. To select the number of decimal places to report for calculated concentrations, set the value in the Decimal Places to be Reported box.

3. To include a chromatogram of the sample in the Quantitation Report, select the **Show Chromatogram on Quantitation Report** check box.

4. To display only valid compounds, select the **Display Compounds Above Set Limit** check box.

❖ To specify user interface options

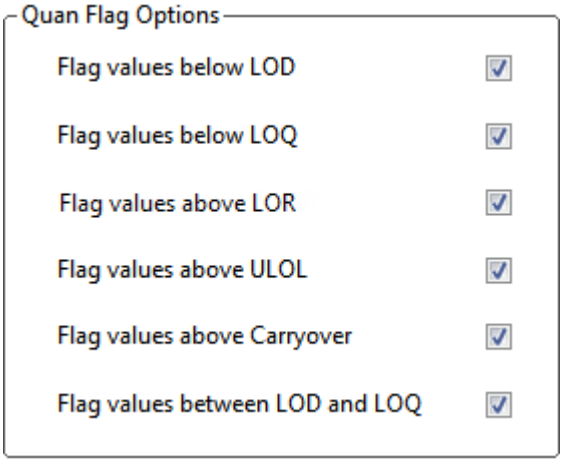


1. To shade a compound row in any of the reports if a value fails one of the criteria used for evaluation, select the **Shade Row when Sample is Outside of Evaluation Criteria** check box.

2. To separate the ion overlay pane from the confirming peak plots, select the **Separate Ion Overlay Display** check box.

3. To use an alternate format for the Calibration Report designed to print more concisely and limit the report to a maximum of seven calibration standards, select the **Use Alternate Calibration Report Format** check box.

❖ To specify quantitation flag options

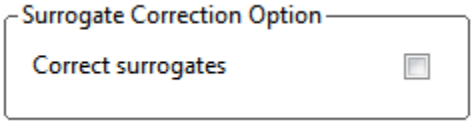


Select the values that you want to display in the report.

Values are above or below the limits defined on the Quan page.

These flags appear in a variety of reports and are defined in Quan Report Settings page parameters.

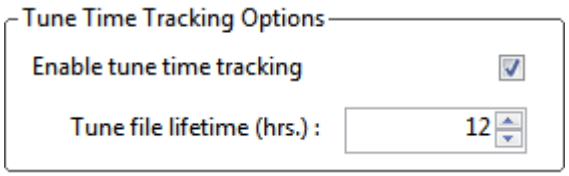
❖ To correct surrogates



Select the **Correct Surrogates** check box.

The application applies the conversion factor (specified in the sample row in the batch) to the sample's calculated concentrations for surrogates, as it applies the conversion factor to target compounds.

❖ To track the tune file usage



1. Select the **Enable Tune Time Tracking** check box.

   This option tracks the number of hours between the last instrument tune and each sample acquisition.

2. In the Tune File Lifetime box, enter the number of hours that you want to allow between the last instrument tune and a sample acquisition.

   Any sample acquired outside this maximum allowable time is flagged in the Batch report.

Quantitation Page

Use the features on the Quantitation page to specify parameters for flagging values and displaying information in reports.

Figure 103. Quantitation page

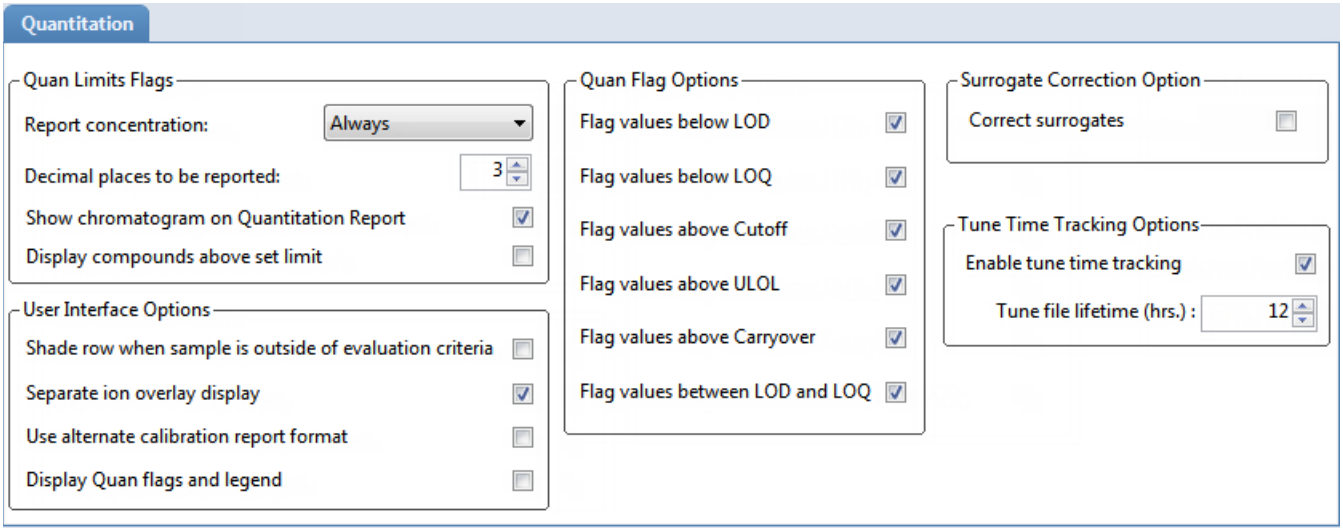


Table 68. Quan Report Settings page parameters (Sheet 1 of 2)

Parameter	Description
Quan Limits Flags	
Report Concentration	Reports the concentration at all times or only when the quantified value exceeds either the limit of detection (LOD), the limit of quantitation (LOQ), or the limit of reporting (LOR). Report concentration: Always, >LOD, >LOQ, or >LOR.
Decimal Places to be Reported	Number of decimal places to be included in the report. Maximum value is 6.
Show Chromatogram on Quantitation Report	Displays a chromatogram (TIC trace) of the sample in the quantitation report.
Display Compounds Above Set Limit	Prints reports for only the compounds that are found in a sample. If a compound is above the specified Quan Flag Options limits, the application reports the compound. This prevents generating “empty” reports for the compounds that are not found.
User Interface Options	
Shade Row When Sample is Outside of Evaluation Criteria	Shades a compound row in any of the reports if a value fails one of the criteria used for evaluation.
Separate Ion Overlay Display	Separates the ion overlay pane from the confirming peak plots in an analysis.

Table 68. Quan Report Settings page parameters (Sheet 2 of 2)

Parameter	Description
Use Alternate Calibration Report Format	Uses an alternate format for the Calibration Report that is designed to print more concisely (this report is limited to a maximum of seven calibration standards).
Display Quan Flags and Legend	Displays the flags and legend in reports.
Quan Flag Options	Values that are above or below limits defined on the Limits page. These flags appear in a variety of reports.
Flag Values Below LOD	Flags values below the limit of detection (LOD).
Flag Values Below LOQ	Flags values below the limit of quantitation (LOQ).
Flag Values Above Cutoff	Flags values above the cutoff or limit of reporting (LOR).
Flag Values Above ULOL	Flags values above the upper limit of linearity (ULOL).
Flag Values Above Carryover	Flags values above the carryover limit.
Flag Values Between LOD and LOQ	Flags values between the limit of detection and the limit of quantitation known as the J flag.
Surrogate Correction Option	
Correct Surrogates	Applies the conversion factor (specified in the sample row in the batch) to the sample's calculated concentrations for surrogates, as it applies the conversion factor to target compounds. For example, if you added surrogates to the sample as part of sample preparation and you require a dilution for analysis, the application dilutes the surrogates and target compounds and applies a dilution correction to correct for this dilution. However, if you added surrogates after a dilution has occurred, then you can leave the option cleared so that, while the target compounds are corrected for the dilution, the surrogates are reported "as is."
Tune Time Tracking Options	
Enable Tune Time Tracking	Tracks the number of hours between the last instrument tune and each sample acquisition.
Tune File Lifetime	Specifies the maximum number of hours between the last instrument tune and a sample acquisition. Any sample acquired outside this maximum allowable time is flagged in the Batch report.

Saving a Quantitation Method to a New Name

You can save any method to a new name, or you can use the current method data to overwrite an existing method. The new method contains all the data of the saved method.

❖ To save a method to a new name

1. From the main menu, choose **File > Save As.**

   The Save Master Method As dialog box opens, displaying all available quantitation methods.

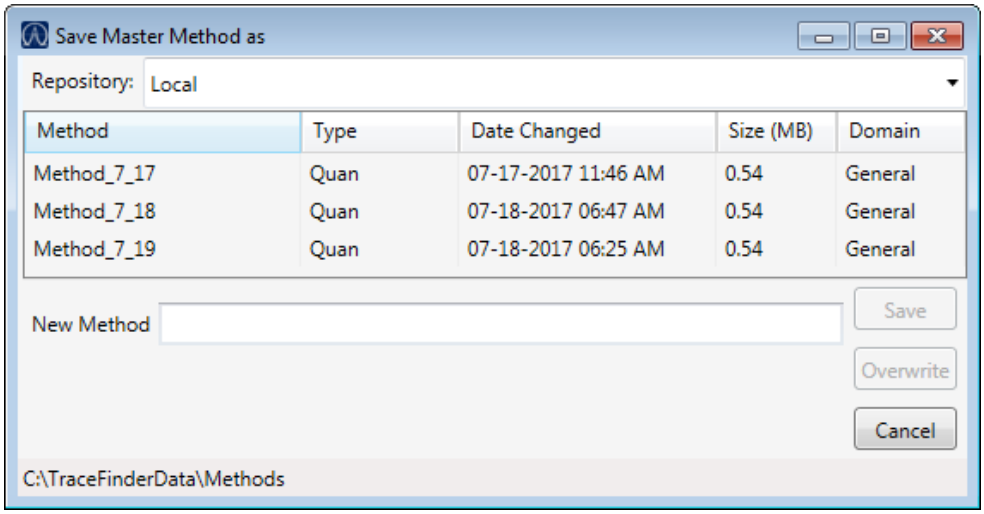


Table 69. Save Master Method As dialog box parameters

Parameter	Description
Repository	The data repository where you want to store the master method.
Method	Name of the quantitation methods in the selected repository.
Type	Type of method: Quan
Date Changed	Date the method was last updated.
Size	Size of the method in megabytes.
Domain	TraceFinder domain for which the method was created.
New Method	Name of the new method to create.
Path	Path to the selected method in the …\TraceFinderData\Methods folder.
Save	Saves the current method to a new name.
Overwrite	Overwrites an existing method.
Cancel	Closes the dialog box without saving the current method to a new name.

2. Do one of the following:
   - In the New Method box, type a name for the new method.
     The application enables the Save button.
   - In the New Method box, type the name of an existing method.
     The application enables the Overwrite button.
3. Click **Save** or **Overwrite**.
   The application saves all the method data using the specified name and opens the Acquisition page of the new method.

Creating a Method Template

In the TraceFinder application, you can create a processing method using a method template that contains common settings. You can use the Method Template Editor to create a method template that specifies peak detection criteria, screening libraries, confirming ion criteria, compound calibration, and qualitative peak processing.

The application uses the settings in the method template to identify the data to display in the Qualitative View. Refer to Chapter 4, "Using the Analysis Mode for Quantitation Batches" in the *TraceFinder User Guide*.

Only quantitation methods use method templates.

Follow these procedures:

- To specify a search type
- To open the Method Template Editor
- To specify peak criteria for a Trace search type
- To specify peak criteria for a Target List search type
- To identify the peaks for a Trace search type
- To identify the peaks for a Target List search type
- To specify the confirming ions to include
- To specify the target peaks to include
- To calibrate the compounds
- To save the method template

❖ To open the Method Template Editor

1. Click **Method Development** in the navigation pane.
   The Method Development navigation pane opens.

2. Click **Method View**.



3. From the main menu, choose **File > New > Method Template**.

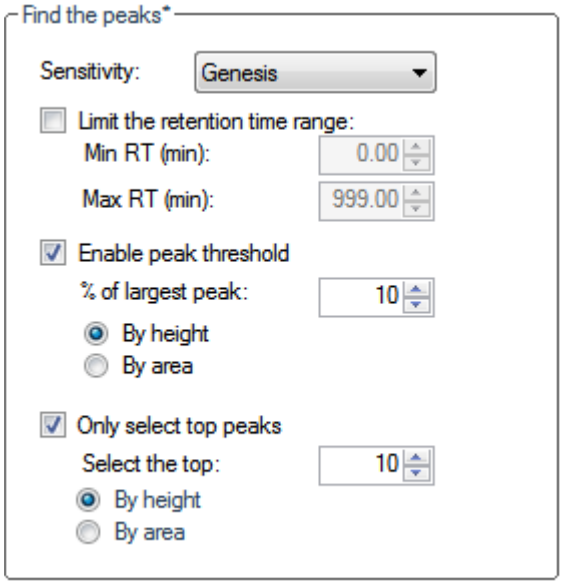
   The Method Template Editor opens.

❖ To specify a search type

From the Search Type list, select one of the following:

- **Trace**: Specifies that the method automatically selects peaks and ratios. Supports SRM and TSQ data.
- **Target List**: Specifies that the method uses a target list of peaks. Supports Exactive data.

❖ To specify peak criteria for a Trace search type



Note Parameters in the Find the Peaks area might also be used for qualitative peak processing.

1. In the Find the Peaks area, select a sensitivity level.

   In selecting the degree of sensitivity, you define how extensively the peak detector algorithm searches for low-level peaks.

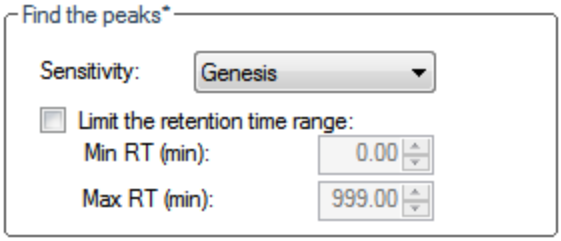
   - The Genesis peak detection algorithm provides backward compatibility with Xcalibur 1.0 studies.
   - The ICIS peak detection algorithm is designed for integrating UV/Vis and analog chromatograms.

- The Avalon peak detection algorithm is designed for MS data and has superior peak detection efficiency at low MS signal levels.
- The Auto peak detection algorithm is designed for integrating UV/Vis and analog chromatograms. The Auto algorithm uses parameters that are identical to ICIS parameters with the following exception: The Use Genesis Algorithm for Qual Processing parameter is required and cannot be cleared.

2. To look for peaks only in a certain range of the entire chromatogram, select the **Limit the Retention Time Range** check box and specify a retention time (RT) range.
3. To indicate whether to select peaks by relative height or area and the percentage of the highest peak that results in compound selection, select the **Enable Peak Threshold** check box.

   To consider a peak for a processing method, the application uses the Enable Peak Threshold filter to determine which peaks meet the specified percentage of the height or area of the largest peak.
4. To display a specific number of the largest peaks by height or area, select the **Only Select Top Peaks** check box and enter the number of peaks to display.

❖ To specify peak criteria for a Target List search type



Note Parameters in the Find the Peaks area might also be used for qualitative peak processing.

1. In the Find the Peaks area, select a sensitivity level.

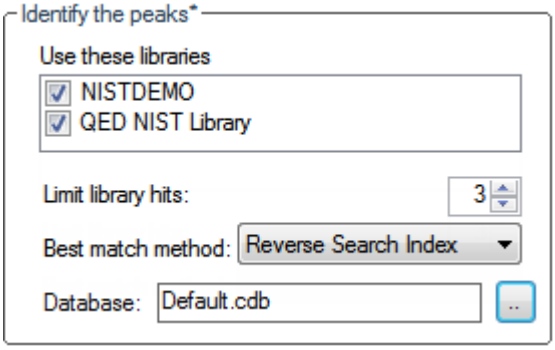
   In selecting the degree of sensitivity, you define how extensively the peak detector algorithm searches for low-level peaks.

   - The Genesis peak detection algorithm provides backward compatibility with Xcalibur 1.0 studies.
   - The ICIS peak detection algorithm is designed for integrating UV/Vis and analog chromatograms.
   - The Avalon peak detection algorithm is designed for MS data and has superior peak detection efficiency at low MS signal levels.

- The Auto peak detection algorithm is designed for integrating UV/Vis and analog chromatograms. The Auto algorithm uses parameters that are identical to ICIS parameters with the following exception: The Use Genesis Algorithm for Qual Processing parameter is required and cannot be cleared.

2. To look for peaks only in a certain range of the entire chromatogram, select the **Limit the Retention Time Range** check box and specify a retention time (RT) range.

❖ To identify the peaks for a Trace search type



Note Parameters in the Identify the Peaks area might also be used for qualitative peak processing.

1. In the Use these Libraries box, select the libraries that you want to search.

   All libraries installed on your instrument are displayed in the Use These Libraries box.

2. To limit the number of matches returned when the system searches a spectrum against the selected libraries, set a value in the Limit Library Hits box.

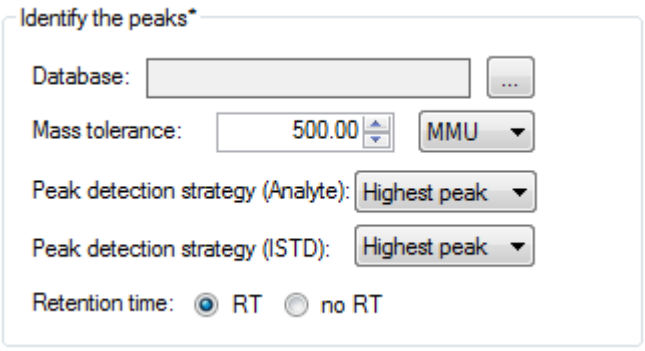
3. To specify how to sort the library searches, select a value from the Best Match Method list.

4. To specify a compound database, browse to and select a compound database to use for the method.

   When there are no libraries selected in the Use These Libraries box, the application searches this compound database to identify peaks.

   When there are libraries selected in the Use These Libraries box, the application first searches the libraries and, if it finds no matches, the application then searches the specified compound database.

❖ To identify the peaks for a Target List search type



Note The application can also use the parameters in the Identify the Peaks area for qualitative peak processing.

1. To specify the compound database to use for peak identification when no library is specified, browse to and select a compound database to use for the method.

   Note The application uses this compound database when you do not specify screening libraries in the method or when it cannot identify a compound in any of the specified libraries.

2. To specify the Mass Tolerance, do the following:

   a. Select the units of measure that you want to use.

   b. Specify the number of millimass units or parts per million to use as the *m/z* ± tolerance value.

   The application applies this mass tolerance to the extracted chromatograms.

3. To specify how the method identifies analyte peaks, select either peak detection strategy:

   - **Highest Peak**: Uses the highest peak in the analyte chromatogram for component identification.
   - **Nearest RT**: Uses the peak with the nearest retention time in the chromatogram for component identification.

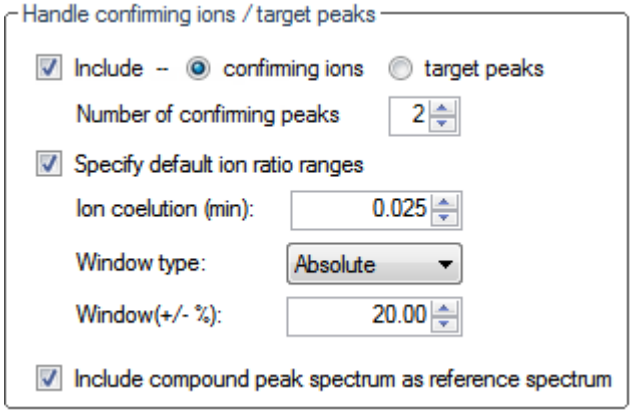
4. To specify how the method identifies internal standard peaks, select either peak detection strategy:

   - **Highest Peak**: Uses the highest peak in the internal standard chromatogram for component identification.
   - **Nearest RT**: Uses the internal standard peak with the nearest retention time in the chromatogram for component identification.

5. To specify that the method use retention time, select the **RT** option.

   To specify that the method not use retention time for peak detection, select the **No RT** option.

❖ To specify the confirming ions to include



1. To set the number of confirming ions, select the **Confirming Ions** option and enter a value in the Number of Confirming Peaks box.

   This value is the number of other ions in the spectrum whose ratio is compared to the quantitation ion. Using this ratio, you can then determine if it is the target compound or something else. You can set this value to integers from 1 through 10. This value defaults to 2 because you typically perform a 3-ion experiment with one quantitation mass and two confirming ions.

   Using the confirming ions in the target list, the application limits the search to the specified number of confirming ions, selects the most intense ion to use as the quantitation mass, and then uses this mass for the mathematical operations.

   When this parameter is not selected, the application searches all confirming ions in the target list.

2. To define the criteria for evaluating confirming or qualifying ions, select the **Specify Default Ion Ratio Ranges** check box and set the following values:

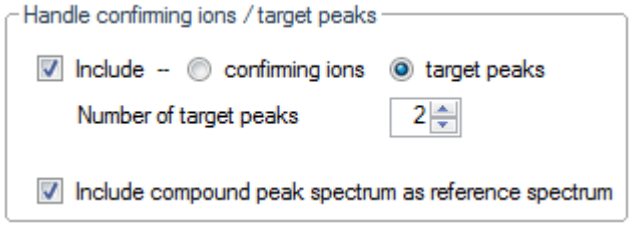
   a. To specify the maximum difference in retention time between a confirming peak and the quantification ion peak, set a value in the Ion Coelution (min) box.

   b. To specify an absolute or relative calculation approach for determining the acceptable ion ratio range, select **Absolute** or **Relative** from the Window Type list.

   c. To specify the acceptable ion ratio range, set a value in the Window (+/– %) box.

3. To include the peak spectrum in the processing method, select the **Include Compound Peak Spectrum as Reference Spectrum** check box.

❖ To specify the target peaks to include



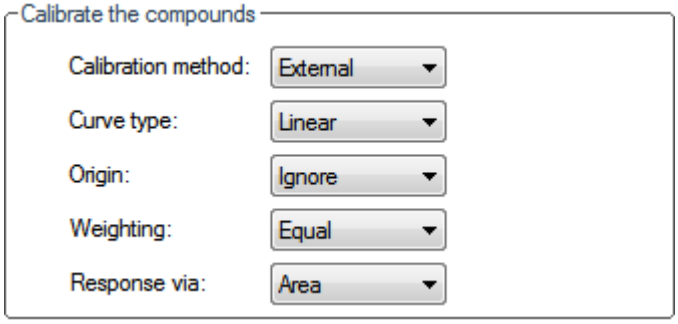
Note The Target Peaks option is not available when you set the Search Type to Target List. See To specify a search type.

1. To set the number of target peaks, select the **Target Peaks** option and enter a value in the Number of Target Peaks box.

   This value defaults to 2 to represent the total number of ions.

2. To include the peak spectrum in the processing method, select the **Include Compound Peak Spectrum as Reference Spectrum** check box.

❖ To calibrate the compounds

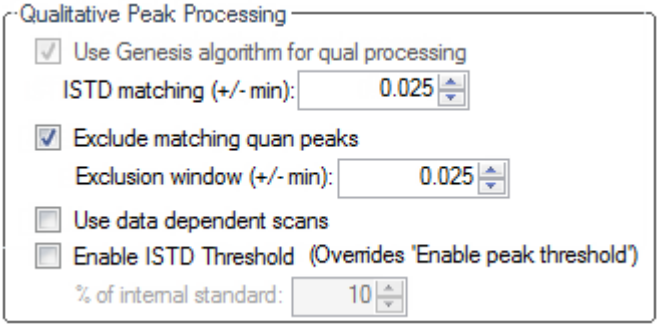


1. From the Calibration Method list, select **Internal** or **External**.
2. From the Curve Type list, select one of the following:
   - **Linear**: All other settings are available with this exception: When you select Include in the Origin list, the Weighting parameter is unavailable.
   - **Quadratic**: All other settings are available with this exception: When you select Include in the Origin list, the Weighting parameter is unavailable.
   - **Average RF**: The Weighting and Origin parameters are unavailable.
3. From the Origin list, select one of the following:
   - **Ignore**: Specifies that the origin is not included as a valid point in the calibration curve when the curve is generated. When you select Ignore, the calibration curve might or might not pass through the origin.
   - **Force**: Specifies that the calibration curve passes through the origin of the data point plot when the calibration curve is generated.

- **Include**: Specifies that the origin is included as a single data point in the calculation of the calibration curve. When you select Include, the calibration curve might or might not pass through the origin.

4. From the Weighting list, select one of the following:
   - **Equal**: Specifies that the origin is included as a single data point in the calculation of the calibration curve. When you select Equal, the calibration curve might or might not pass through the origin.
   - **1/X**: Specifies a weighting of 1/X for all calibration data points during the least-squares regression calculation of the calibration curve. Calibrants are weighted by the inverse of their quantity.
   - **1/X^2**: Specifies a weighting of 1/X^2 for all calibration data points during the least-squares regression calculation of the calibration curve. Calibrants are weighted by the inverse of the square of their quantity.
   - **1/Y**: Specifies a weighting of 1/Y for all calibration data points during the least-squares regression calculation of the calibration curve. Calibrants are weighted by the inverse of their response (or response ratio).
   - **1/Y^2**: Specifies a weighting of 1/Y^2 for all calibration data points during the least-squares regression calculation of the calibration curve. Calibrants are weighted by the inverse of the square of their response (or response ratio).
5. From the Response Via list, select Area or Height.
   - **Area**: Specifies that the application uses this area value in response calculations.
   - **Height**: Specifies that the application uses this height value in response calculations.

❖ To specify qualitative peak processing



1. Select the **Use Genesis Algorithm for Qual Processing** check box and specify a value for internal standard matching.

   The application uses the Genesis algorithm to match internal standards in a range plus/minus the value that you specify. For additional information about the Genesis algorithm, see Genesis Detection Method.

This parameter is available only when you set the Sensitivity parameter in the Find the Peaks area to ICIS or Avalon. When you select the Use Genesis Algorithm for Qual Processing check box, the application ignore the Sensitivity parameter in the Find the Peaks area.

2. Select or clear the **Exclude Matching Quan Peaks** check box and specify a value for the exclusion window.

   The application excludes target peaks in a range plus or minus the value that you specify.

3. To process samples that include data-dependent scans, select the **Use Data Dependent Scans** check box.

   When you process a sample using this feature, the application uses the TIC trace to find all data-dependent full scans, lists them, and performs a library search against the data-dependent MS/MS or MS^n scan.

   This option constrains the Data Review to only data-dependent scan spectra. Refer to Chapter 4, "Using the Analysis Mode for Quantitation Batches" in the *TraceFinder User Guide*.

   In addition to the peak information, the TIC Report and TIC Summary Report display information about the data-dependent filtered data.

4. To indicate whether to select peaks above a minimum percentage of the nearest internal standard peak that results in compound selection, select the **Enable ISTD Threshold** check box and specify a minimum percentage.

   To consider a peak for a processing method, the application uses the Enable ISTD Threshold filter to determine which peaks meet the specified percentage of the height of the nearest internal standard peak.

   When you select the Enable ISTD Threshold parameter, the method ignores values set for the Enable Peak Threshold and Only Select Top Peaks parameters. See To specify peak criteria for a Trace search type.

   Note When you create a method with the Method Forge, the application ignores the parameters in the Qualitative Peak Processing area.

❖ To save the method template

1. Choose **File > Save** from the Method Template Editor menu.

   The Save Method Template dialog box opens.

2. Do one of the following:

   - Type a name for the new method.

     The application enables the Save button.

   - Type the name of an existing method.

     The application enables the Overwrite button.

3. Click **Save** or **Overwrite**.

   The application saves the new method template in the following folder:

   …\TraceFinderData\Templates\Methods\EFS

   Saved method templates are available when you create a method using Method Forge. See Starting a New Method with Method Forge.

Method Template Editor

Use the features in the Method Template Editor to specify peak detection criteria, screening libraries, confirming ion criteria, compound calibration, and qualitative peak processing.

Figure 104. Method Template Editor dialog box

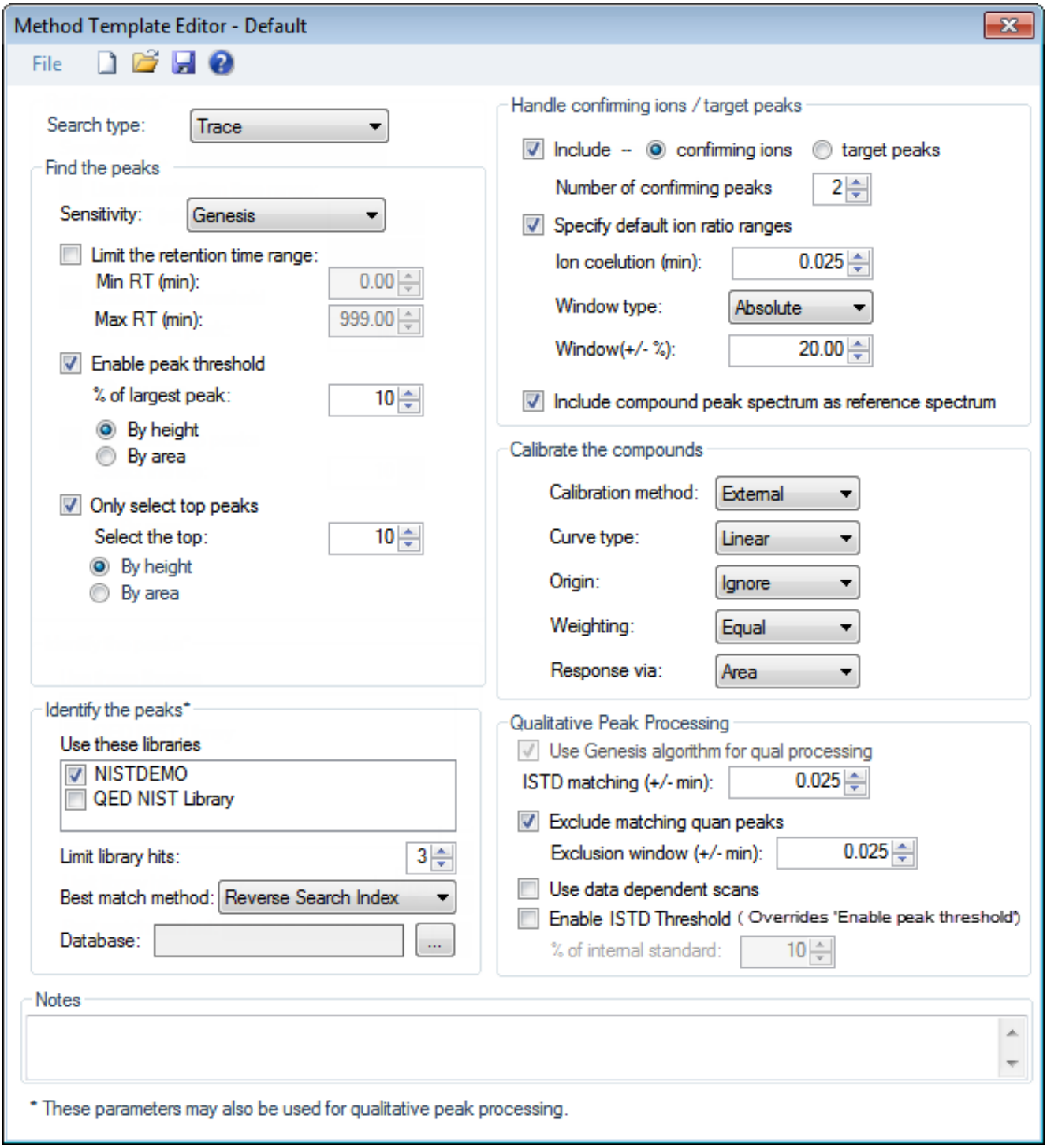


Table 70. Method Template Editor dialog box parameters (Sheet 1 of 4)

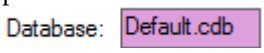
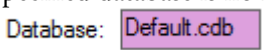
Parameter	Description
Search Type	Specifies the search type as Trace or Target List. Default: Trace
Find the Peaks	
Sensitivity	Defines how extensively the peak detector algorithm searches for low-level peaks.
Limit the Retention Time Range	Min RT specifies the beginning of the range. Max RT specifies the end of the range.
Enable Peak Threshold (Trace only)	Specifies whether to select peaks by relative height or area and the percentage of the highest peak that results in compound selection.
Only Select Top Peaks (Trace only)	Displays a specific number of the largest peaks by height or area.
Identify the Peaks (Trace)	
Use These Libraries	Lists the libraries that you can search.
Limit Library Hits	Specifies the number of matches returned when the system searches a spectrum against the selected libraries.
Best Match Method	Specifies how to sort the library searches. Valid values: Search Index, Reverse Search Index, Match Probability
Database	Specifies the compound database used to identify peaks. If you do not specify a compound database, the application uses the system database. If the specified database is no longer on the disk, the application highlights the database name. 
Identify the Peaks (Target List)	
Database	Specifies the compound database used to identify peaks. If you do not specify a compound database, the application uses the system database. If the specified database is no longer on the disk, the application highlights the database name. 

Table 70. Method Template Editor dialog box parameters (Sheet 2 of 4)

Parameter	Description
Mass Tolerance	Specifies the *m/z* ± tolerance value of MMU or PPM. Valid range: 0.01 through 50 000 • MMU (millimass units): MMU is a static calculation to the extracted mass. • PPM (parts per million): PPM is a variable calculation dependent on the actual mass. The smaller the mass, the narrower the tolerance range. The larger the mass, the wider the tolerance range. The default value and units are specified in the Configuration Console. See Specifying Peak Detection Defaults.
Peak Detection Strategy (Analyte)	Highest Peak: Uses the highest peak in the chromatogram for component identification. Nearest RT: Uses the peak with the nearest retention time in the chromatogram for component identification.
Peak Detection Strategy (ISTD)	Highest Peak: Uses the highest peak in the internal standard chromatogram for component identification. Nearest RT: Uses the peak with the nearest retention time in the chromatogram for component identification.
Retention Time	Specifies that the method use or not use retention time for peak identification.
Handle Confirming Ions / Target Peaks	
Include – Confirming Ions	Specifies the number of confirming ions, which are other ions in the spectrum whose ratio is compared to the quantitation ion to identify the compound.
Number of Confirming Peaks	This value defaults to 2 because you typically perform a 3-ion experiment with one quantitation mass and two confirming ions. Valid values: Integers from 1 through 10
Specify Default Ion Ratio Ranges	Enables the ion ratio range features. Ion Coelution specifies the maximum difference in retention time between a confirming peak and the target peak. Window Type specifies an Absolute or Relative calculation approach for determining the acceptable ion ratio range. Window (+/-%) specifies the acceptable ion ratio range.
Include – Target Peaks	
Number of Target Peaks	Valid values: Integers from 1 through 10 Default: 2
Include Compound Peak Spectrum as Reference Spectrum	Includes the peak spectrum in the processing method. Use this setting to perform a spectra comparison in Data Review.

Table 70. Method Template Editor dialog box parameters (Sheet 3 of 4)

Parameter	Description
Calibrate the Compounds	
Calibration Method	Specifies an internal or external calibration method.
Curve Type	Specifies a linear, quadratic, or average RF curve type.
Origin	Specifies that the origin is ignored, forced, or included in the generated calibration curve. • Ignore: Specifies that the origin is not included as a valid point in the calibration curve when the curve is generated. When you select Ignore, the calibration curve might or might not pass through the origin. • Force: Specifies that the calibration curve passes through the origin of the data point plot when the calibration curve is generated. • Include: Specifies that the origin is included as a single data point in the calculation of the calibration curve. When you select Include, the calibration curve might or might not pass through the origin.
Weighting	Specifies the weighting for the calibration data points. • Equal: Specifies that the origin is included as a single data point in the calculation of the calibration curve. When you select Equal, the calibration curve might or might not pass through the origin. • 1/X: Specifies a weighting of 1/X for all calibration data points during the least-squares regression calculation of the calibration curve. Calibrants are weighted by the inverse of their quantity. • 1/X^2: Specifies a weighting of 1/X^2 for all calibration data points during the least-squares regression calculation of the calibration curve. Calibrants are weighted by the inverse of the square of their quantity. • 1/Y: Specifies a weighting of 1/Y for all calibration data points during the least-squares regression calculation of the calibration curve. Calibrants are weighted by the inverse of their response (or response ratio). • 1/Y^2: Specifies a weighting of 1/Y^2 for all calibration data points during the least-squares regression calculation of the calibration curve. Calibrants are weighted by the inverse of the square of their response (or response ratio).
Response Via	Specifies if the application uses area or height in response calculations. • Area: Specifies that the application uses this peak area value in response calculations. • Height: Specifies that the application uses this peak height value in response calculations.
Qualitative Peak Processing	
Use Genesis Algorithm For Qual Processing	The application uses the Genesis algorithm to match internal standards.
ISTD Matching	Excludes all the target compounds found in the method and does not list these compounds in the TIC Report or in the Qual Mode view in the Data Review.

Table 70. Method Template Editor dialog box parameters (Sheet 4 of 4)

Parameter	Description
Exclude Matching Quan Peaks	Compares the retention time of the internal standard in the method to the found retention time of the internal standard in the library search and excludes peaks outside the Exclusion Window range.
Exclusion Window	Defines a range plus/minus the Exclusion Window value that you specify.
Use Data Dependent Scans	Constrains the Data Review to only data-dependent scan spectra. Refer to Chapter 4, "Using the Analysis Mode for Quantitation Batches" in the *TraceFinder User Guide*. In addition to the peak information, the TIC Report and TIC Summary Report display information about the data-dependent filtered data.
Enable ISTD Threshold	Specifies that, when identifying a peak, qualitative peak processing use the minimum threshold specified as a percentage of the nearest internal standard peak, rather than the threshold specified in the Enable Peak Threshold and Only Select Top Peaks parameters.
% of Internal Standard	Percentage of the nearest internal standard peak to use as the minimum threshold for identifying a peak.

Exporting Mass Data

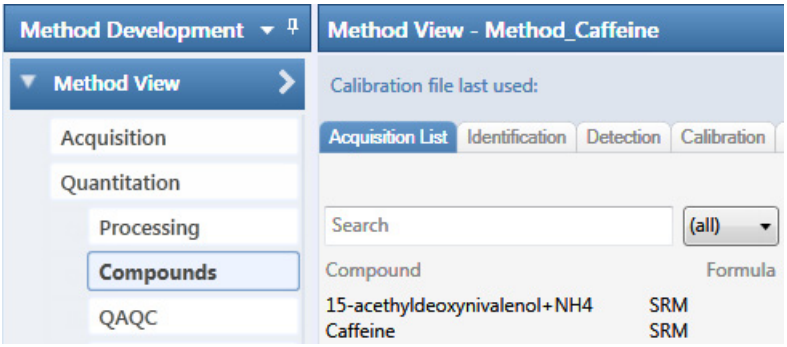
You can export the mass data list from the Compounds page to an XML file that can be read by the TSQ, ISQ, Q Exactive, TSQ Endura, or TSQ Quantiva applications. You can export mass data only from quantitation methods.

❖ To export mass data list to an XML file

1. Open the method whose mass data list you want to export.

   If you make changes to the method, you must save it before you can export the mass data list.

2. To view a list of your mass data, click the **Acquisition List** tab on the Compounds page.



   You do not have to display the Acquisition List to export the data. For information about displaying the Acquisition List, see Acquisition List.

3. Choose **Method View > Export Mass List** from the main menu.

   IMPORTANT If you have neither a TSQ, an ISQ, a Q Exactive, a TSQ Endura, nor TSQ Quantiva instrument configured, a message asks which format you want to export: Triple Quadrupole, Q Exactive, TSQ Quantiva/Endura SIM, or TSQ Quantiva/Endura SRM.

   The application writes the mass data in the Acquisition List to the following folder, using a format compatible with your configured instrument:

   …\TraceFinderData\Methods*Methodname*

5

Using the Method Development Mode for Target Screening Methods

This chapter includes method development tasks for creating and editing screening methods. (See Method Development navigation pane.)

The TraceFinder application uses a method to specify the types of acquisition, processing, and reporting that occur with a batch of samples. You can create a method designed specifically to test for compounds in your assay.

Contents

- Opening a Target Screening Method
- Starting a New Target Screening Method
- Editing a Target Screening Method
- Saving a Target Screening Method to a New Name

Information in the method determines how the application does the following:

- Processes a set of samples to provide meaningful results.
- Acquires and processes the raw data.
- Displays the results.
- Reports the output.

The application applies your method to a batch, which is a list of one or more samples. Together, the method and batch provide a workflow-oriented approach to the data processing and information reporting for batches of samples.

Figure 105. Method Development navigation pane



Table 71. Method Development navigation pane commands

Command	Description
Method View	
Acquisition	Displays the Acquisition page of the Method View. Use the features on the Acquisition page to define basic information about the method. See Editing the Acquisition Page.
Target Screening	
Processing	Displays the Processing page of the Method View. Use the features on the Processing page to specify peak filter settings, screening databases, and identification and confirmation settings for a target screening method. See Editing the Processing Page.
Peak Detection	Displays the Peak Detection page of the Method View. Use the features on the Peak Detection page to specify any of the following peak detection algorithms: Genesis, ICIS, or Avalon. See Editing the Peak Detection Page.
Reports	Displays the Reports page of the Method View. See Editing the Reports Page.
Compound Database	See Chapter 2, "Using Compound Databases in the Method Development Mode."
Instrument View	See Chapter 3, "Using Instrument Methods in the Method Development Mode."

Opening a Target Screening Method

A target screening method contains compound databases, identification and confirmation criteria used in detecting and processing, and settings for reporting compounds.

When you open a target screening method, the Method View navigation pane displays the available pages for target screening methods. For descriptions of all the features in the Method Development navigation pane, see Starting a New Target Screening Method.

❖ To open a target screening method in the Method Development mode

1. Click **Method Development** in the navigation pane.

   The Method Development navigation pane opens.

2. Choose **File > Open > Master Method** from the main menu.

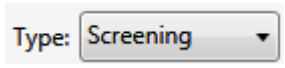
   The Open Master Method dialog box opens.

   Tip You can also open one of your most recently used method files. Choose **Files > Recent Files > *Method***.

3. (Optional) Select a different repository.

   The default repository is specified in the Administrator Console. Refer to the instructions in the *TraceFinder Administrator Console User Guide*.

4. Select **Screening** in the Type list.

   The method list displays only target screening methods in the selected repository.

5. Select a method and click **Open**.

   The Acquisition page for the selected method opens. To edit the method, see Editing a Target Screening Method.

   The navigation pane displays the available pages for target screening methods. For descriptions of all the features in the Method Development navigation pane, see Starting a New Target Screening Method.

Figure 106. Open Master Method dialog box

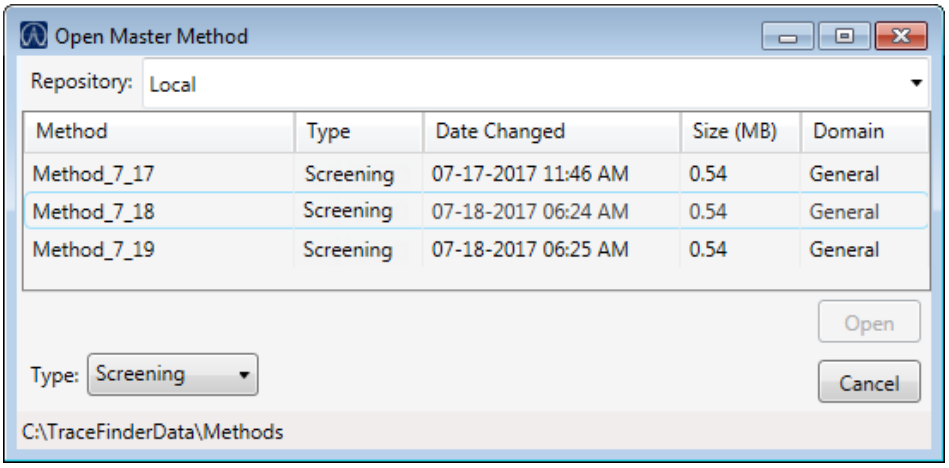


Table 72. Open Master Method dialog box parameters

Parameter	Description
Repository	The data repository where you want to store the master method.
Method	Name of the methods for the selected type.
Type	Type of method: Quan, Screening, or Unknown Only.
Date Changed	Date the method was last updated.
Size (MB)	Size of the method in megabytes.
Domain	TraceFinder domain for which the method was created.
Type	Type of method to display: Quan, Screening, Unknown Only, or Any.
Path	Path to the selected method in the …\TraceFinderData\Methods folder.

Starting a New Target Screening Method

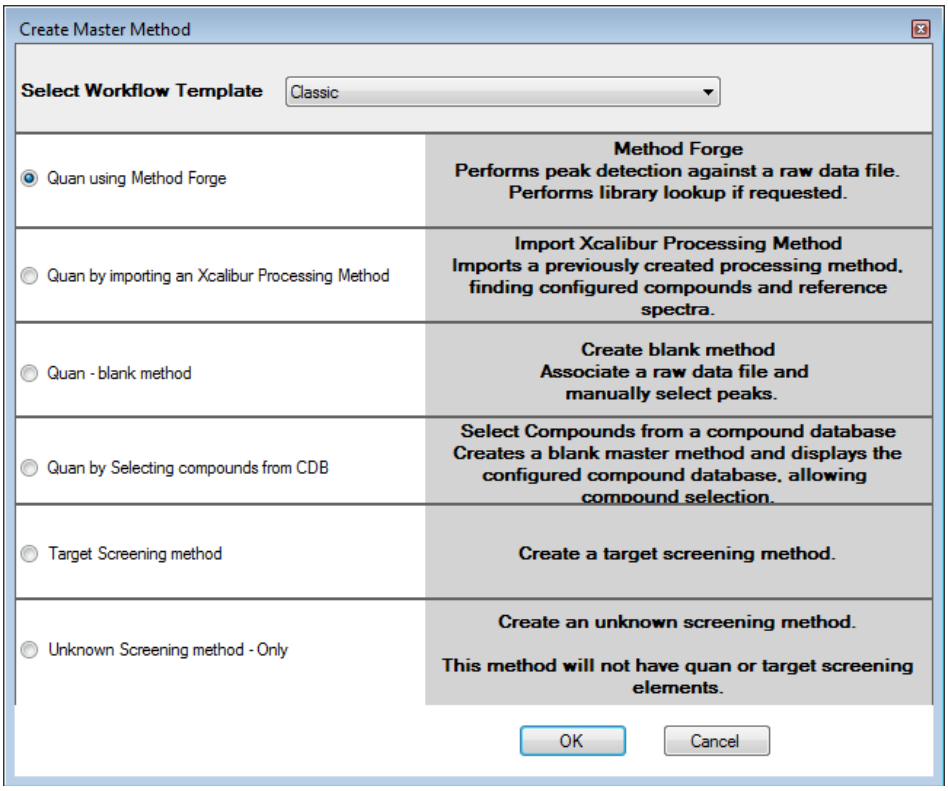
You can create a target screening method to use a target list of compounds in selected compound databases to identify the compounds in the samples. The following procedure includes the basic parameters you must define to create and save a target screening method. For a detailed description of how to modify all parameters in a method, see Editing a Target Screening Method.

❖ To create a new method

1. Choose **File > New > Master Method** from the main menu.

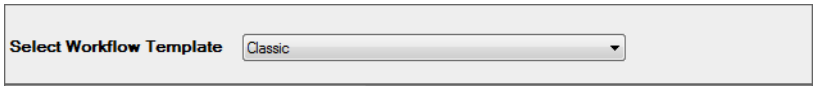
   The Create Master Method dialog box opens.

   Figure 107. Create Master Method dialog box



IMPORTANT The Unknown Screening Method - Only feature is available only when you select the Allow Unknown Screening option in the application configuration console. See Processing Options.

2. Select a workflow template from the list.



You can select the Classic or Screening template, or select any custom target screening workflow template previously saved to the following folder:

…\TraceFinderData\Templates\Workflows

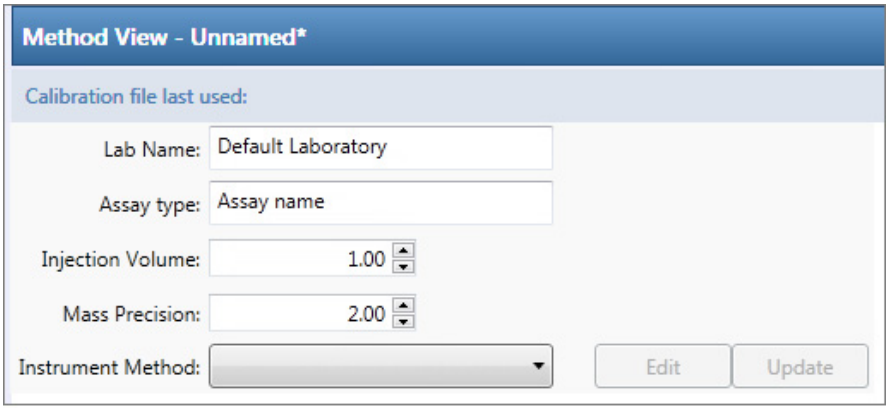
The Create Master Method dialog box displays only the method creation techniques that you can create with the selected workflow template.

3. Select the **Target Screening Method** option and click **OK**.



The Method View for a target screening method includes the Acquisition, Processing, Peak Detection, and Reports pages.

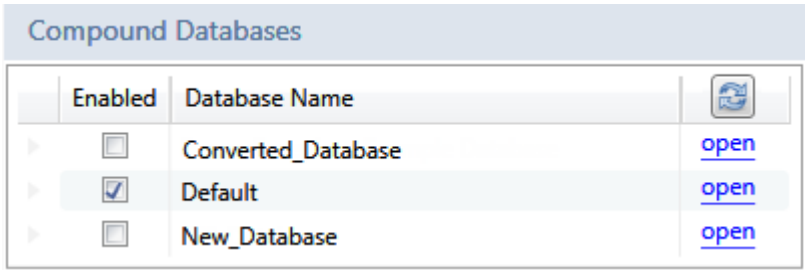
The Acquisition page for the method opens.



4. From the Instrument Method list, select an instrument method.
5. Click **Processing** in the Method View navigation pane.

The Processing page for the method opens. The application lists the compound databases (.cdb extension) that are stored in the following folder:

…\TraceFinderData\CompoundDatabases



6. Select the **Enabled** check box for at least one compound database.

You must select at least one compound database before you can save the method. The target screening method uses only the target list of compounds in the selected compound databases to identify the compounds in the samples.

7. To save the new method, choose **File > Save** from the main menu.
8. In the Save Master Method dialog box, type a name for the method and click **OK**.

   For a detailed description of how to modify a method, see Editing a Target Screening Method.



Tip (Animation) To view "Starting a Target Screening Method," choose **Help > Animations**.

Editing a Target Screening Method

You can open a method to specify method instructions, reporting options, peak filter settings, screening databases, identification and confirmation settings, and peak detection parameters.

See the instructions for the following tasks:

- Editing the Acquisition Page
- Editing the Processing Page
- Editing the Peak Detection Page
- Editing the Reports Page

Editing the Acquisition Page

Use the features on the Acquisition page to define basic information about the method.

❖ To edit the parameters on the Acquisition page

1. Click **Acquisition** in the Method View navigation pane.

   The Acquisition Page for the method opens.
2. In the Lab Name box, type the name to be displayed on the top of each printed, saved, or exported report.

   The default name is Default Laboratory.
3. In the Assay Type box, type the assay type to be targeted by the method.
4. From the Injection Volume box, select the injection volume to be used for sample injection, a value from **0.1** through **5000** µL.

   Use the up/down arrows to change the volume in increments/decrements of 1.0 µL, or use the keyboard to enter non-integer injection volumes.

IMPORTANT The application uses this injection volume in the method, not the injection volume in the instrument method.

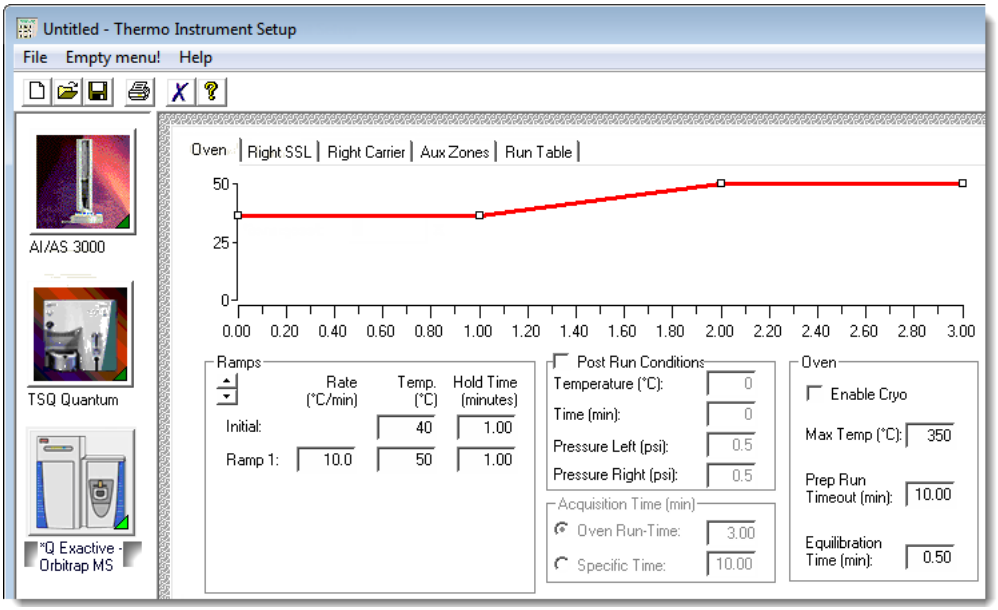
5. From the Mass Precision box, select the number of decimal places to be used in reports and in peak and spectrum displays, an integer from **2** through **6**.
6. From the Instrument Method list, select an instrument method.



7. To edit the instrument method, click **Edit**.

   The Thermo Instrument Setup window opens. This example of an instrument setup shows multiple configured instruments.

Figure 108. Thermo Instrument Setup window



8. Edit the values on the instrument page for your instrument.
9. From the main menu in the Thermo Instrument Setup window, choose **File > Save** and then choose **File > Exit**.

   The application returns you to the Acquisition page of the Method View.

Acquisition Page

Use the features on the Acquisition page to define basic information about the method.

Figure 109. Acquisition page for a target screening method

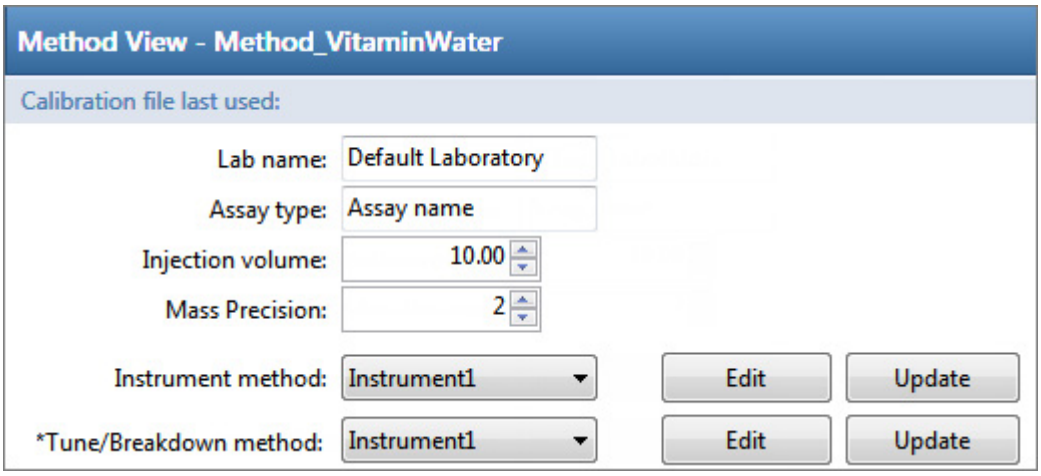


Table 73. Acquisition page parameters (Sheet 1 of 2)

Parameter	Description
Lab Name	The laboratory name to be displayed on the top of each printed, saved, or exported report. Default: Default Laboratory To specify this default laboratory name, see Specifying Method Defaults.
Assay Type	The name for the analysis type to be targeted by the method. The assay type associates the method with the analysis of a compound or specific class of compounds (for example, you might use an assay type of PAH for the analysis of Polynuclear Aromatic Hydrocarbons).
Injection Volume	The system uses the injection volume (in µL) for sample injection. For a more detailed explanation, refer to the documentation for the autosampler. The injection volume in the method overrides the injection volume in the instrument method. The injection volume in the batch overrides the injection volume in the method. Valid range: 0.1 through 5000 µL

Table 73. Acquisition page parameters (Sheet 2 of 2)

Parameter	Description
Mass Precision	Number of decimal places used in reports and in peak and spectrum displays. Valid values: Integers from 2 through 6
Instrument Method	Instrument method used for acquiring samples.
Tune/Breakdown Method	Tune/Breakdown method used for processing samples.
Edit	Opens the Thermo Instrument Setup window where you can edit the current instrument method.
Update	Choose one of the following: • Send to System Methods: Overwrites the instrument method in the …\TraceFinderData\InstrumentMethods folder with the current instrument method or tune/breakdown method. • Get from System Methods: Overwrites the current instrument method with the instrument method or tune/breakdown method in the …\TraceFinderData\Methods folder.

Editing the Processing Page

Use the features on the Processing page to specify peak filter settings, screening databases, and identification and confirmation settings for a method.

Follow these procedures:

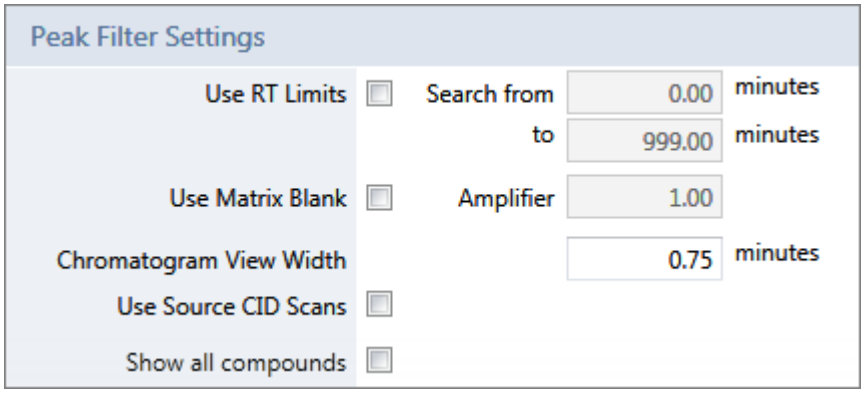
- To open the Processing page
- To specify peak filter settings for limiting the display of unwanted data
- To add unknown screening to the method
- To specify compound databases
- To specify identification and confirmation settings

❖ To open the Processing page

Click **Processing** in the Method View navigation pane.

The Processing Page for the method opens.

❖ To specify peak filter settings for limiting the display of unwanted data



1. To set a retention time range that excludes searching for peaks outside the range, do the following:
   a. Select the **Use RT Limits** check box.

      The application activates the Search From and To options.

   b. In the Search From box, enter the lower limit; in the To box, enter the upper limit.
2. To use one or more matrix blank samples for subtraction to filter the resulting peaks, do the following:
   a. Select the **Use Matrix Blank** check box.

      The application activates the Amplifier option.

      During automatic processing, the application subtracts the areas of the peaks in the matrix blank samples from the matching areas in the unknown samples.

      To determine the pair of peaks to subtract from each other, the application selects the two peaks with the mass and the retention time that are closest to each other (as defined in the compound database), within the mass tolerance range specified in the method.

      When the same compound peak (same mass and retention time within the predefined tolerance windows) is in multiple selected matrix blank samples, the application subtracts only the one with the highest area.

      When a compound peak in one matrix blank sample has the same primary ion as another peak in a different matrix blank sample but has different adducts, the application uses all the adducts from both these peaks for subtraction purposes. For example, if a compound has the M+H and M+Na ions in one matrix blank sample, and this same compound has the M+H and M+NH4 ions in another matrix blank sample, the application uses for subtraction the area that results from all of these ions.

      When no matrix blank sample exists in the sequence, or if one or more matrix blank samples exist but you do not select the Use Matrix Blank check box, then subtraction does not occur. If subtraction occurs and the subtracted area is less than 0, the application sets the subtracted area to 0.

b. In the Amplifier box, type an amplifier value.

Use the up/down arrows to change the value in increments/decrements of 1 unit, or use the keyboard to enter non-integer values.

The application multiplies a matrix blank area by this value before performing subtraction. The larger the amplifier value, the more peaks the application filters from the final results.

In the Batch View for sequences created with this method, you can select which matrix blank samples to use for subtraction. Refer to Chapter 5, "Using the Analysis Mode for Target Screening Batches" in the *TraceFinder User Guide*.

3. In the Chromatogram View Width box, type a value to define the chromatogram viewing range in the Data Review view.

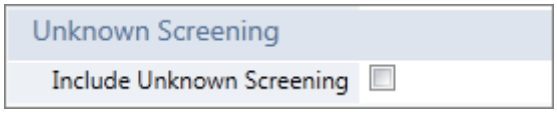
4. To use source CID scans for target screening confirmation (fragment ion or library search), select the **Use Source CID Scans** check box.

When you select this check box, the application uses the source CID scans when they are available in the data file. If they are not available, then the application uses AIF or MS/MS scans when available.

5. To display all compounds from the compound databases in the Data Review display (regardless of whether there is a match in the samples), select the **Show All Compounds** check box.

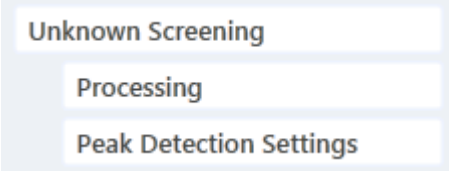
❖ To add unknown screening to the method

1. Select the **Include Unknown Screening** check box.



IMPORTANT The Include Unknown Screening check box is available only when you select the Allow Unknown Screening option in the application configuration console. See Processing Options.

The application adds the Unknown Screening features to the Method View navigation pane.



2. To specify unknown screening settings for peaks, libraries, databases, elements, and ChemSpider searches, click **Processing**.

For further instructions, see Editing the Processing Pages.

3. To specify unknown screening peak detection settings, click **Peak Detection Settings**.

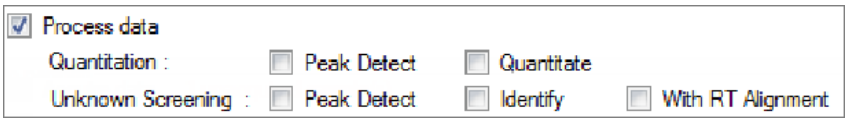
   For further instructions, see Editing the Peak Detection Settings Page.

   The application indicates that a target screening method includes unknown screening in the following locations:

   - In the Batch View in the Analysis mode, when you create a batch using a target screening method, the title bar indicates that the method includes unknown screening.

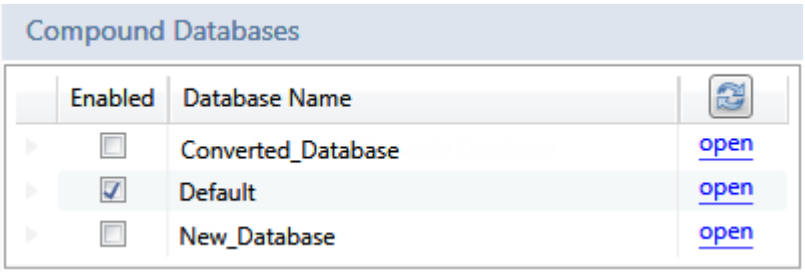
     

   - In the Analysis navigation pane, the application displays access to local methods for both target screening and unknown screening.
   - In the Submit Options dialog box, when you submit a batch, the Process Data area shows all processing method types for the batch.

     You can select the detection options for each processing method that you want to use when you submit the batch.

❖ To specify compound databases



1. Select the **Enabled** check box for at least one compound database.

   The Compound Databases pane displays all compound databases stored in the …\TraceFinderData\CompoundDatabases folder.

2. (Optional) To edit a database, click **Open** and do the following:

   a. Edit the database.

      See Chapter 2, "Using Compound Databases in the Method Development Mode."

   b. When you finish editing the database, click **Processing** in the Method View navigation pane to return to the Processing page.

❖ To specify identification and confirmation settings

Note The Identification and Confirmation Settings pane displays parameters for identifying or confirming compounds. For additional information about the confirmation and identification process, see Understanding the Identification and Confirmation Process.

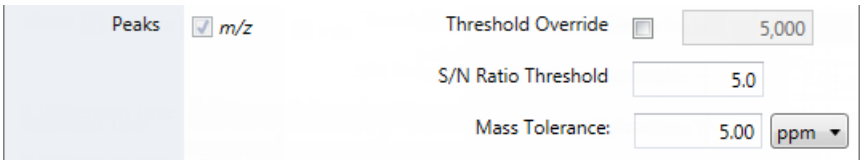
1. To set a target threshold override and include only peaks with areas above this designated threshold, specify the following Peaks parameters:
   a. Select the **Threshold Override** check box.
   b. In the associated box, type the threshold as an area value.

      This threshold overrides the Response Threshold value set in the compound database. The application ignores the peaks with areas below your specified threshold.
   c. To include only peaks with signal-to-noise ratios (S/Ns) that are above a specified value, in the S/N Ratio Threshold box, type the threshold as a ratio value.

      The application ignores the peaks with S/Ns that are below the specified threshold.
   d. In the Mass Tolerance box, type an *m/z* ± tolerance value and then select **ppm** or **mmu** for the mass tolerance units.

      The application applies this mass tolerance range to the extracted chromatograms.

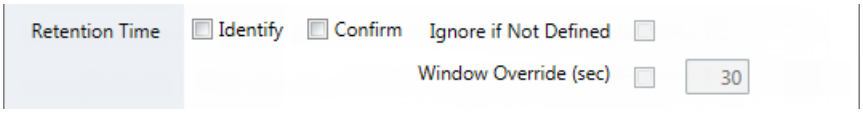
Figure 110. Peaks parameters



2. To specify the Retention Time parameters, do the following:
   a. Select either the **Identity** or **Confirm** check box.
   b. Select the **Window Override** check box and type the window value.

      This window overrides the RT Window value that was set in the compound database and includes only peaks within this designated window. The application identifies or confirms the presence of a compound only when its measured retention time matches the target compound's expected retention time within the specified Window Override retention time.

Figure 111. Retention Time parameters



For additional information about how the application identifies retention time, see Retention Time.

3. To specify the Fragment Ions parameters, do the following:
   a. Select either the **Identity** or **Confirm** check box.
   b. To ignore the Fragment Ions options when no fragment is defined in the compound database, select the **Ignore if Not Defined** check box.

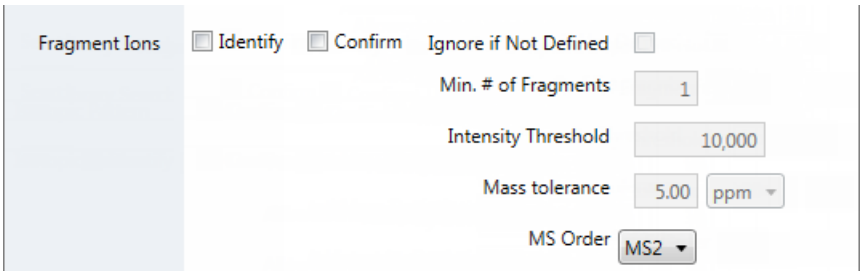
      When the compound database does not define fragments for a compound, the application does not include the results of identification or confirmation for fragment ions in the target screening results.
   c. In the Min. # of Fragments box, type the minimum number of fragments required to identify or confirm the presence of a compound.
   d. In the Intensity Threshold box, type the intensity threshold value.

      The intensity of a fragment must be above this threshold to be identified or confirmed.
   e. In the Mass Tolerance box, type an *m/z* ± tolerance value and then select **ppm** or **mmu** for the mass tolerance units.

      This mass tolerance value indicates the number of millimass units or parts per million to use as the *m/z* ± tolerance value for the fragment ions. It is separate from the mass tolerance value specified for the parent peak.

      Note When using ion trap data, the application uses 300 mmu regardless of the value you enter here.

Figure 112. Fragment Ions parameters



For additional information about how the application identifies fragment ions, see Fragment Ions.

4. To specify the Isotopic Pattern parameters, do the following:
   a. Select either the **Identity** or **Confirm** check box.
   b. In the Fit Threshold box, type the fit threshold percentage.

      To identify or confirm the presence of a compound, the resulting score percentage from isotopic pattern matching must be higher than the specified fit threshold percentage.

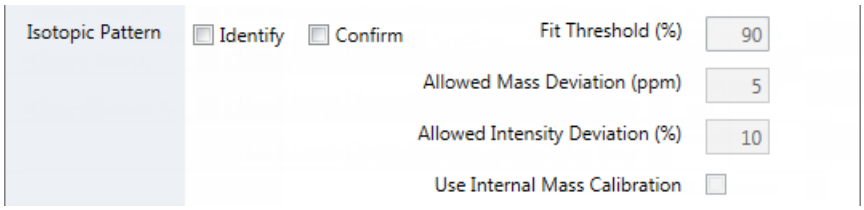
c. In the Allowed Intensity Deviation box, type a value to specify the allowed intensity deviation of the mass spectrometer relative to the monoisotopic ion, as a percentage of the base peak height.

The TraceFinder isotopic pattern algorithm considers an isotope peak as not found if its intensity, relative to the monoisotopic ion's intensity, is more than the deviation percentage from the theoretical relative intensity of the isotope ion. For best results, set this value to a number that causes up to 98% of all intensity deviations to be smaller than the allowed intensity deviation value.

d. To specify that isotopic pattern calculations use internal mass calibration instead of external mass calibration, select the **Use Internal Mass Calibration** check box.

When you select this check box, the application applies a requirement that an isotope's *m/z* must be closer to its theoretical value to avoid a score penalty.

Figure 113. Isotopic Pattern parameters

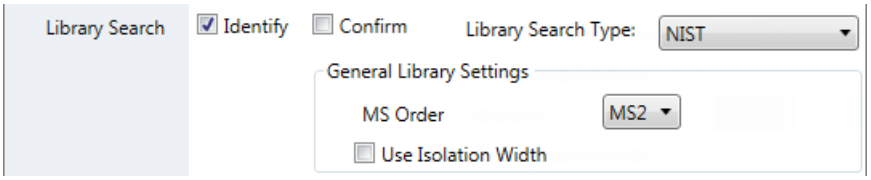


For additional information about how the application calculates isotopic pattern scores, see Isotopic Pattern.

5. To specify the General Library Search parameters, do the following:
   a. Select either the **Identity** or **Confirm** check box.
   b. Select a Library Search Type: **mzVault**, **NIST**, or **NIST High Resolution**.
   c. In the General Library Settings area, do the following:
      i. To specify the source for the peaks, set the MS Order to **MS**, **MS2**, or **MS–In Source CID.**
      ii. To specify that the application uses the isolation width to determine if there is an MS/MS scan within the same peak RT window that includes the precursor fragments, select the **Use Isolation Width** check box.

> **Note** This option is available only when you set the MS Order to MS2.

Figure 114. General Library Search parameters



d. To specify the NIST Settings parameters, do the following:

i. Specify the Search Type as **Normal**, **MS/MS**, or **In Source HiRes.**

ii. Specify the *m/z* ± tolerance value for the Precursor Tolerance and the Product Tolerance, and then specify the units of measure.

– For accurate mass libraries, choose **amu**.

– For exact mass libraries, choose **ppm** (default).

> **Note** These options are available only when you select the MS/MS or In-source HiRes search types.

iii. To ignore the mass spectral peaks within the specified precursor tolerance range, select the **Ignore Precursor** check box.

> **Note** This option is available only when you select the MS/MS or In-source HiRes search types.

iv. To use an alternate peak matching method for searching the mass spectra, select the **Use Alt Peak Matching** check box.

> **Note** This option is available only when you select the MS/MS or In-source HiRes search types.

v. To compare a library entry to an unknown compound, select the **Reverse Search** check box.

A forward search compares the mass spectrum of an unknown compound to a mass spectral library entry.

vi. To specify that the application uses presearch screening before calculating the spectrum-by-spectrum match factor, specify the Presearch as **Default**.

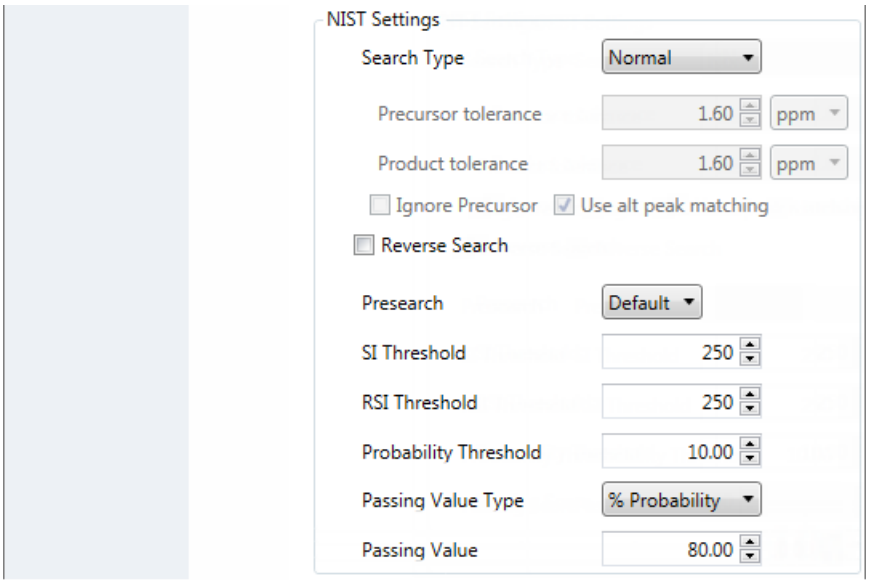
vii. Specify the lower SI Threshold value for the search index method to use in the NIST library search.

viii. Specify the lower RSI Threshold value for the reverse search index method to use in the NIST library.

ix. Specify the lower Probability Threshold value for the match probability.

x. Specify a value for Passing Score.

Figure 115. NIST Settings parameters



e. To specify the mzVault page parameters, do the following:

   i. From the Search Type list, select either **Classic** or **HighChem**.

   ii. Specify an *m/z* ± tolerance value for Precursor Tolerance, and then select the units of measure for precursor tolerance.

   iii. Specify an *m/z* ± tolerance value for Fragment Tolerance, and then select the units of measure for fragment tolerance.

   iv. Select a threshold value for Score Threshold.

   The application uses this lower threshold value for the Library Score in Data Review. The application does not return results below this value.

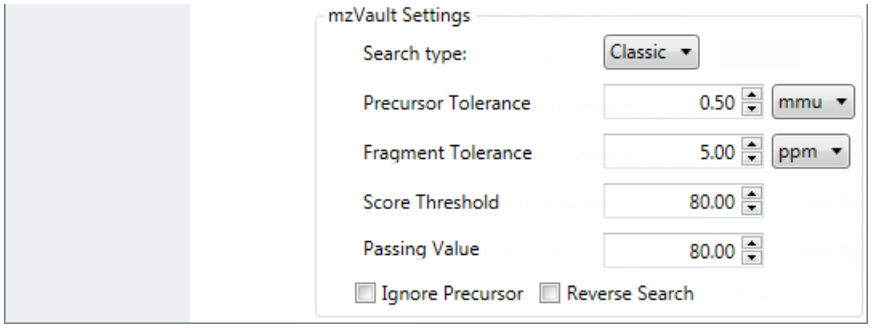
   v. Specify a value for Passing Score.

   vi. To ignore mass spectral peaks within the specified precursor tolerance range, select the **Ignore Precursor** check box.

   vii. To specify that the application use the reverse search method, select the **Reverse Search** check box.

   A reverse search compares a library entry to an unknown compound (whereas a forward search compares the mass spectrum of an unknown compound to a mass spectral library entry).

Figure 116. mzVault page parameters



For additional information about how the application performs library searches, see Library Search.

Processing Page

The Processing page includes the following panes:

- Settings

  Use the features in the Settings Pane to specify peak filter settings.

- Target Screening Settings

  Use the features in the Target Screening Settings Pane (part 1) to specify screening databases and identification and confirmation settings for Retention Time, Fragment Ions, and Isotopic Pattern, or use the features in the Target Screening Settings Pane (part 2) to specify identification and confirmation settings for Library Search.

Settings Pane

Use the features in the Settings pane to specify peak filter settings.

Figure 117. Settings pane on the Processing page

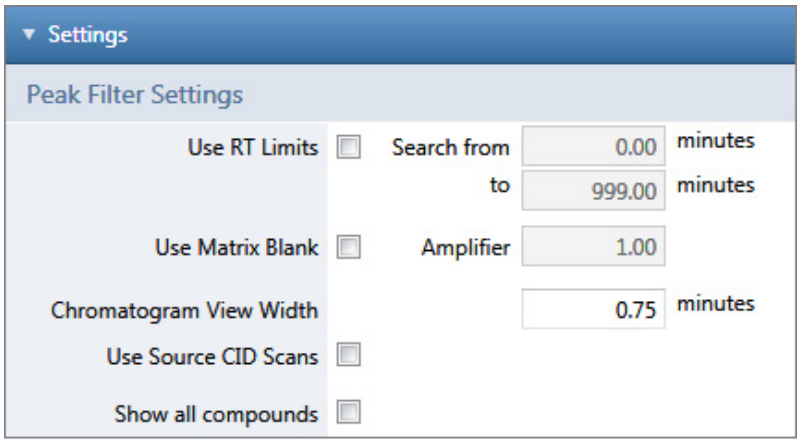


Table 74. Settings pane parameters

Parameter	Description
Peak Filter Settings	
Use RT Limits	Specifies a lower and upper limit for searches. Valid range: 0.00 through 999.99 minutes Default: 0.00 minutes for lower limit; 999.00 minutes for upper limit
Use Matrix Blank	Specifies that during automatic processing, the application subtracts the areas of the peaks in the selected matrix blank samples from the matching areas in the unknown samples.
Amplifier	The application multiplies a matrix blank area by this value before performing subtraction. The larger the amplifier value, the more peaks the application filters from the final results. Valid range: 0.01 through 1000.00 Default: 1.00
Chromatogram View Width	Specifies a window width to define the chromatogram viewing range in the Data Review view. Valid range: 0.10 through 999.00 minutes Default: 0.75 minutes
Use Source CID Scans	Specifies that the application uses the source CID scans when they are available in the data file. If they are not available, then the application uses available AIF or MS/MS scans.
Show All Compounds	Displays results for all compounds in the method, regardless of whether they are found in any samples.

Target Screening Settings Pane (part 1)

Use the features in the Target Screening Settings pane to specify screening databases and identification and confirmation settings for Retention Time, Fragment Ions, and Isotopic Pattern.

Figure 118. Target Screening Settings pane (part 1)

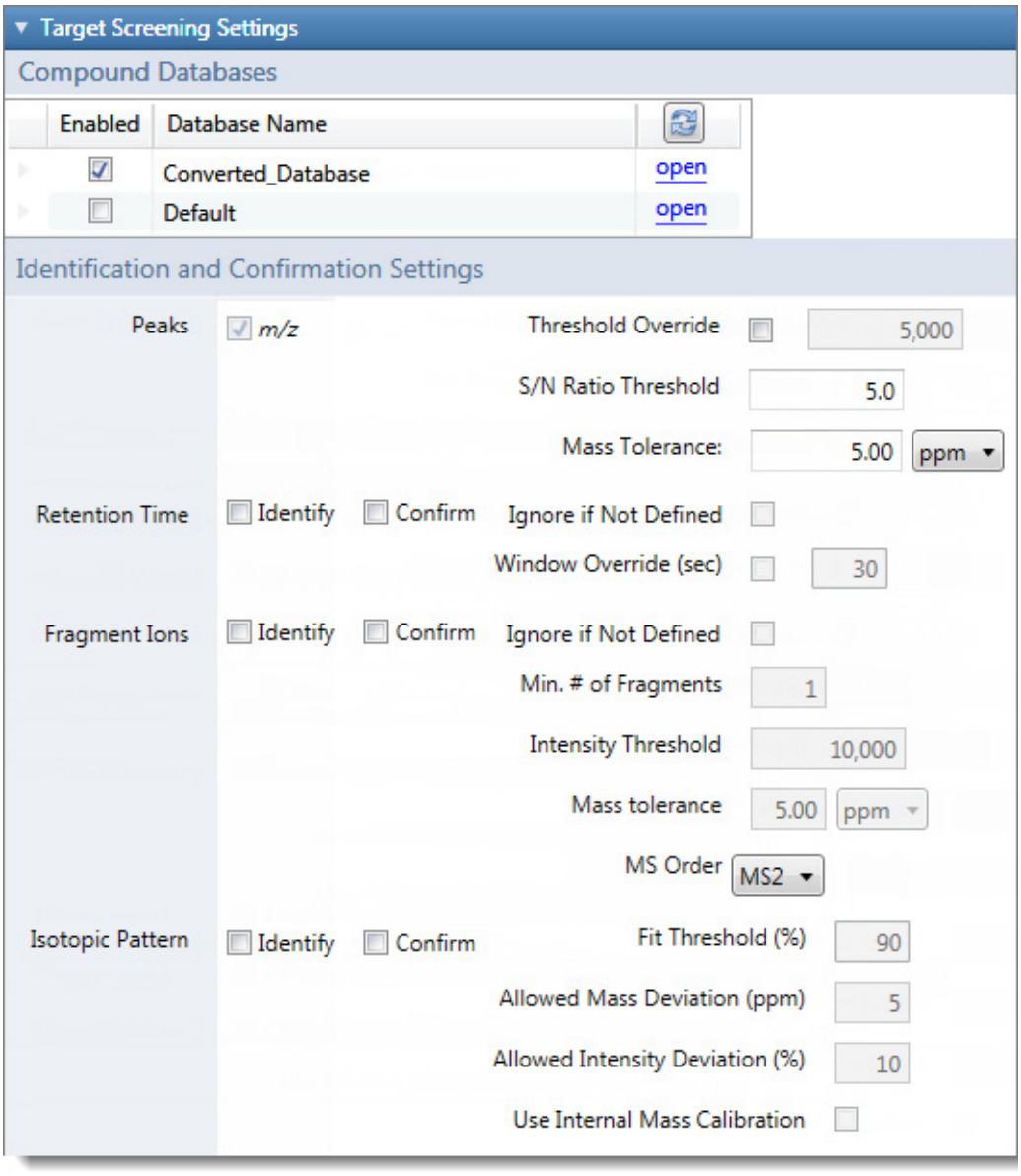


Table 75. Target Screening Settings pane parameters (part 1) (Sheet 1 of 4)

Parameter	Description
Compound Databases	
Enabled	Specifies databases to use for target screening processing.
Database Name	Lists available databases in the …\TraceFinderData\CompoundDatabases folder.

Table 75. Target Screening Settings pane parameters (part 1) (Sheet 2 of 4)

Parameter	Description
Identification and Confirmation Settings	
Peaks	Specifies that the application uses the mass-to-charge ratio (*m/z*) for filtering compound peaks.
Threshold Override	Overrides the Response Threshold value set in the compound database. The application ignores the peaks with areas below this specified threshold. Valid range: 1000 through 1 000 000 000 Default: 5000
S/N Ratio Threshold	Includes only peaks with signal-to-noise ratios (S/Ns) above the specified value. Valid range: 1.0 through 100 000 Default: 5.0
Mass Tolerance	Specifies the number of millimass units or parts per million to use as the *m/z* ± tolerance value for the peak identification. Valid range: 0 through 500 Default: 5 Unit: mmu or ppm
Retention Time	Specifies either the Identify or Confirm option for a retention time search. To identify a compound, the application searches the specified RT window for a match. To confirm a compound, the application searches the entire raw data file.
Ignore if Not Defined	Ignores the values you specify for Retention Time options when no retention time is defined in the compound database, and does not include the results of identification or confirmation for retention time in the Data Review target screening results.
Window Override	Specifies the number of seconds to override the RT Window value set in the compound database and include only peaks within this designated window. The application identifies or confirms the presence of a compound only when its measured retention time matches the target compound's expected retention time within the specified Window Override retention time. Valid range: 0 through 999 seconds Default: 30 seconds
Fragment Ions	Specifies either the Identify or Confirm option for a fragment ion match. To identify a fragment, the application searches the specified RT window for a match. To confirm a fragment, the application searches the entire raw data file.
Ignore if Not Defined	Ignores the values you specify for Fragment Ions options when no fragment is defined in the compound database, and does not include the results of identification or confirmation for fragment ions in the Data Review target screening results.

Table 75. Target Screening Settings pane parameters (part 1) (Sheet 3 of 4)

Parameter	Description
Min. # of Fragments	Specifies the minimum number of fragments required to identify or confirm the presence of a compound. Valid range: 1 through 5 Default: 1
Intensity Threshold	Specifies the minimum height of a fragment ion peak. The peak of a fragment ion must be above this intensity threshold to be identified or confirmed. Valid range: 1 through 1e9 Default: 10 000
Mass Tolerance	Specifies the number of millimass units or parts per million to use as the *m/z* ± tolerance value for the fragment ions and is separate from the mass tolerance specified for the parent (see Editing the Acquisition Page). Valid range: 0 through 500 Default: 5 Unit: mmu or ppm **Note** When using ion trap data, the application uses 300 mmu regardless of the value you enter here.
MS Order	Specifies that the confirming peaks come from the same scan (MS), are fragments from an adjacent scan (MS2), or come from the Source CID (MS–In Source CID).
Isotopic Pattern	Specifies either the Identify or Confirm option for an isotopic pattern match. To identify a compound, the application searches the specified RT window for a match. To confirm a compound, the application searches the entire raw data file.
Fit Threshold	To identify or confirm the presence of a compound, the resulting score percentage from isotopic pattern matching must be higher than the specified fit threshold percentage. Default: 90%
Allowed Mass Deviation	Specifies the allowed mass deviation in the spectrum data. The TraceFinder isotopic pattern algorithm considers an isotope peak as found if its measured *m/z* is less than this amount away from its expected *m/z*. For best results, set this value to a number that causes up to 98 percent of all mass deviations to be smaller than the allowed mass deviation value. Valid range: 3 through 100 ppm Default: 3 ppm

Table 75. Target Screening Settings pane parameters (part 1) (Sheet 4 of 4)

Parameter	Description
Allowed Intensity Deviation	Specifies the allowed intensity deviation of the mass spectrometer, relative to the monoisotopic ion, as a percentage of the base peak height. The TraceFinder isotopic pattern algorithm considers an isotope peak as not found if its intensity relative to the monoisotopic ion's intensity is more than this deviation percentage from the theoretical relative intensity of the isotope ion. For best results, set this value to a number that causes up to 98% of all intensity deviations to be smaller than the allowed intensity deviation value. Default: 10%
Use Internal Mass Calibration	Specifies that the application requires an isotope's *m/z* to be closer to its theoretical value to avoid a score penalty.

Target Screening Settings Pane (part 2)

Use the features in the Target Screening Settings pane to specify identification and confirmation settings for Library Search. See Target Screening Settings pane parameters (part 2).

Figure 119. Target Screening Settings pane (part 2)

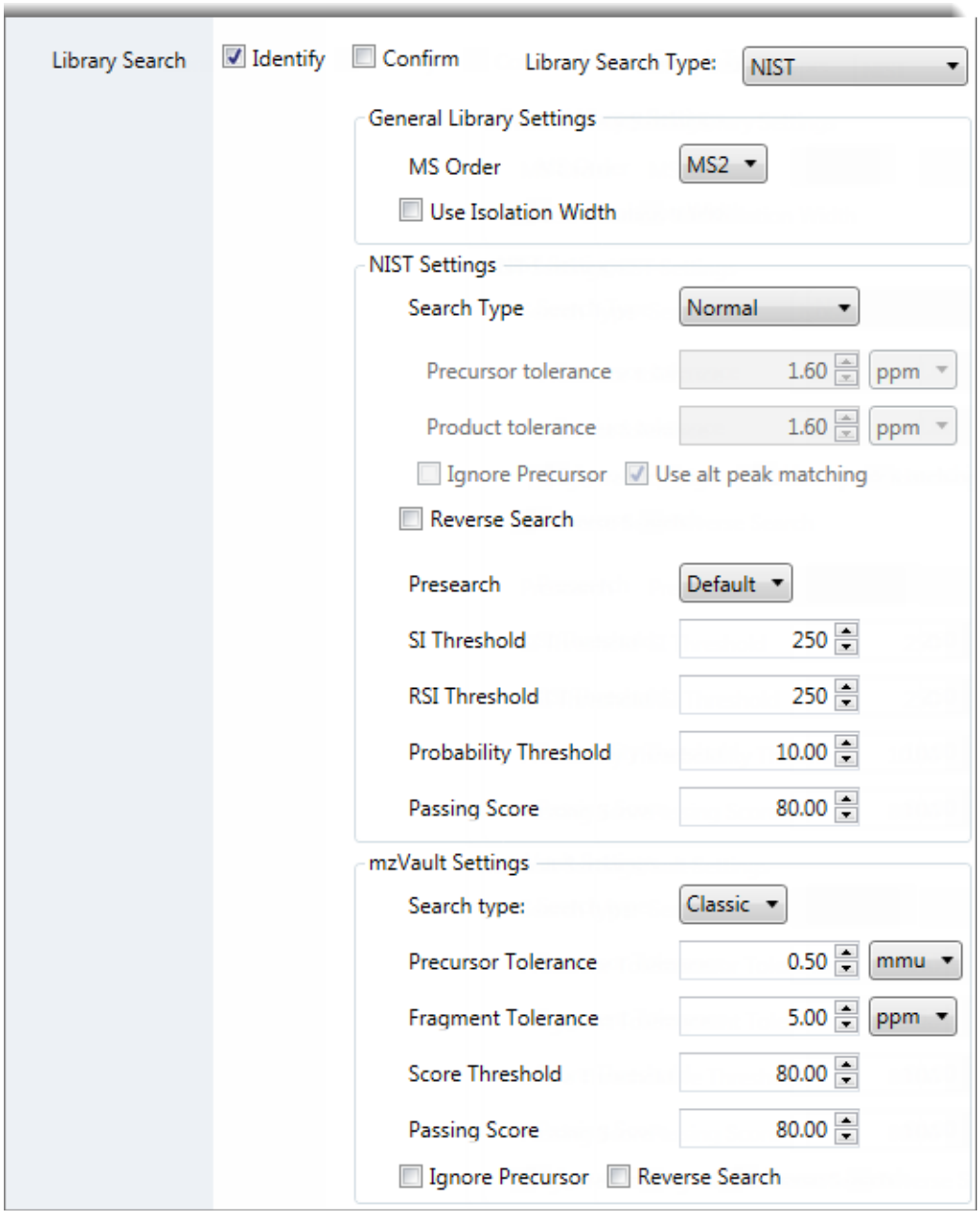


Table 76. Target Screening Settings pane parameters (part 2) (Sheet 1 of 3)

Parameter	Description
Identification and Confirmation Settings (continued)	
Library Search	Specifies either the Identify or Confirm option for a library search. To identify a compound, the application searches the specified RT window for a match. To confirm a compound, the application searches the entire raw data file.
Library Search Type	Specifies the type of library to search: mzVault, NIST, or NIST High Resolution.
General Library Settings	
MS Order	Specifies that the confirming peaks come from the same scan (MS), are fragments from an adjacent scan (MS2), or come from the Source CID (MS–In Source CID).
Use Isolation Width	Specifies that the application uses the isolation width to determine if there is an MS/MS scan within the same peak RT window that includes the precursor fragments. Available only when you set the MS Order to **MS2**.
NIST Settings	
Search Type	Specifies the type of library search algorithm to use. Normal: Uses a more extensive set of presearch screenings—a total of four different criteria. MS/MS: Searches for an MS/MS spectrum in a library of MS/MS spectra. In-source HiRes: Searches for an in-source/EI with an accurate ion *m/z* or an MS/MS spectrum in a library that contains in-source/EI accurate ion *m/z* or MS/MS spectra.
Precursor Tolerance	Specifies the precursor *m/z* ± tolerance value as either amu or ppm. Available only when you select MS/MS or In-source HiRes search types. **Note** Use amu for accurate mass libraries (such as libraries from Exactive, Q Exactive, or Orbitrap instruments). Valid range: 0.01 through 100 000.00 Default: 1.60 ppm
Product Tolerance	Specifies the product *m/z* ± tolerance value as either amu or ppm. Available only when you select MS/MS or In-source HiRes search types. **Note** Use amu for accurate mass libraries (such as libraries from Exactive, Q Exactive, or Orbitrap instruments). Valid range: 0.01 through 100 000.00 Default: 1.60 ppm
Ignore Precursor	Ignores mass spectral peaks within the specified precursor tolerance range. Available only when you select MS/MS or In-source HiRes search types.
Use Alt Peak Matching	Specifies that the application uses an alternate peak matching method for searching the mass spectra. Available only when you select MS/MS or In-source HiRes search types.

Table 76. Target Screening Settings pane parameters (part 2) (Sheet 2 of 3)

Parameter	Description
Reverse Search	Specifies that the NIST library search uses the reverse search method. A reverse search compares a library entry to an unknown compound (whereas a forward search compares the mass spectrum of an unknown compound to a mass spectral library entry).
Presearch	Specifies that the application uses presearch screening before calculating the spectrum-by-spectrum match factor.
SI Threshold	Specifies the lower threshold value for the search index method used to search the NIST library. The application does not return results below this value. Valid range: 0 through 999 Default: 250
RSI Threshold	Specifies the lower threshold value for the reverse search index method used to search the NIST library. The application does not return results below this value. Valid range: 0 through 999 Default: 250
Probability Threshold	Threshold value for the match probability.
Passing Score	Valid range: 0.00 through 100.00 Default: 80.00
mzVault Settings	
Search Type	Specifies the type of library search algorithm to use. • Classic: Uses the standard library search algorithm. • HighChem: Uses the HighChem library search algorithm.
Precursor Tolerance	Specifies the spectrum *m/z* ± tolerance value as either amu or ppm. Valid range: 0.1 through 1000.0 Default: 5.00 ppm
Fragment Tolerance	Specifies the spectrum *m/z* ± tolerance value as either amu or ppm. Valid range: 0.1 through 1000.0 Default: 5.00 ppm
Score Threshold	Specifies that the application does not return results below this value. The resulting score percentage from a library search match must be higher than your specified threshold value to identify or confirm the presence of a compound. Valid range: 0 through 100 Default: 80.00
Passing Score	Valid range: 0.00 through 100.00 Default: 80.00
Ignore Precursor	Specifies that the application ignores mass spectral peaks within the specified precursor tolerance range.

Table 76. Target Screening Settings pane parameters (part 2) (Sheet 3 of 3)

Parameter	Description
Reverse Search	Specifies using the reverse search method for an mzVault search. A reverse search compares a library entry to an unknown compound (whereas a forward search compares the mass spectrum of an unknown compound to a mass spectral library entry). **Note** When you are using all ions fragmentation (AIF), data-independent acquisition (DIA), variable data-independent acquisition (vDIA), or source collision-induced dissociation (CID) data, the application automatically uses reverse search even if you do not select this option.

Understanding the Identification and Confirmation Process

By default, the application uses the mass-to-charge ratio (*m/z*) for filtering compound peaks. In the Identification and Confirmation Settings area, you can select additional criteria to help increase confidence using either of the following:

- Compound identification for identifying a sample compound as a minimum requirement for a match to be displayed in the results.

  To identify a compound, the application searches within the specified RT window (or the RT window override if specified in the method) and compares the measured *m/z* of the sample peak against the expected *m/z* of the target compound. When the sample peak's *m/z* is within the default ±5 ppm tolerance of the target compound's *m/z*, the application considers that this target compound is identified.

- Compound confirmation for confirming a sample compound and to increase confidence in the match results.

  To confirm a compound, the application searches the entire raw data file and compares the measured *m/z* of the sample peak against the expected *m/z* of the target compound. When the sample peak's *m/z* is within the tolerance of the target compound's *m/z*, the application considers that this target compound is confirmed.

The application processes identification and confirmation settings in a specific order. When a compound passes identification at each point in the order of processing, the application continues to the next automatic or selected identification or confirmation test.

Note When you select the Ignore if Not Defined option and the related data is not defined in the compound databases, the application skips that criteria test.

When a compound fails identification at a point in the processing, the application skips the testing of all remaining criteria, even when they are selected, and the flags for those criteria are blank.

The application processes identification and confirmation settings in the following order:

1. *m/z* and Retention Time

   The application automatically identifies the mass to charge ratio for all compounds. When you select the Retention Time settings, the compound must pass this criteria first. When a compound fails the *m/z* or the Retention Time identification test, the application does not perform identification or confirmation testing for any other selected criteria lower in the processing order.

   For additional information about the *m/z* and Retention Time parameters, see Mass to Charge (m/z) and Retention Time.

2. Threshold

   The application automatically identifies the default area threshold (5000) for the compound after the compound passes the *m/z* and Retention Time criteria. When you specify a Threshold Override, the application uses the specified threshold value instead.

   When a compound fails the Threshold identification test, the application does not perform the selected Fragment Ions or Library Search testing for identification or confirmation.

   For additional information about the Threshold parameter, see Threshold.

3. Isotopic Pattern

   For additional information about the Isotopic Pattern parameter, see Isotopic Pattern.

4. Fragment Ions

   For additional information about the Fragment Ions parameter, see Fragment Ions.

5. Library Search

   For additional information about the Library Search parameter, see Library Search.

Mass to Charge (*m/z*)

The application automatically uses the *m/z* and mass tolerance ranges to identify each compound, using the mass tolerance value you specified on the Acquisition page. See Editing the Acquisition Page.

The application compares the measured *m/z* of the sample peak against the expected *m/z* of the target compound. When the measured *m/z* is within the mass tolerance range of the expected *m/z*, the application considers that this target compound is found and the *m/z* criteria passes.

On the Target Screening page in Data Review, the MZ column in the Compounds table indicates whether this criteria passes or fails. The Flag column indicates whether the target compound is identified, fully confirmed, or both. For details, refer to Chapter 5, “Using the Analysis Mode for Target Screening Batches” in the *TraceFinder User Guide*.

The Compounds table also displays the *m/z* Expected, *m/z* Measured, and *m/z* Delta values for each compound.

Threshold

To identify each sample compound, the application automatically uses the area and threshold values defined in the selected compound database.

In the Identification and Confirmation Settings pane, you can specify a threshold override to use instead of the area threshold defined in the compound database.

The application compares the area of the sample peak against the defined area thresholds. When the sample peak's area is greater than or equal to the corresponding area threshold from the compound database or the override area threshold, the target compound is identified.

On the Target Screening page in Data Review, the Flag column in the Compounds table indicates whether this criteria passes or fails. The Flag column indicates whether the target compound is identified, fully confirmed, or both. For details, refer to Chapter 5, "Using the Analysis Mode for Target Screening Batches" in the *TraceFinder User Guide*.

The Compounds table also displays Measured Area column displays the area value in green to indicate that the peak's area is greater than or equal to the area threshold; otherwise, this column displays the area value in red.

Retention Time

The application uses the expected retention time and retention time window values defined in the selected compound database to identify or confirm each sample compound.

In the Identification and Confirmation Settings pane, you can set an override value to use for the retention time window instead of using the retention time window that is specified in the compound database.

When the compound database defines an expected retention time for a compound, it uses the following process to identify or confirm the compound.

- Identify: The application searches within the specified retention time window (or the retention time window override) and compares the measured *m/z* of the sample peak against the expected *m/z* of the target compound. When the measured *m/z* is within the mass tolerance range of the expected *m/z*, this target compound is identified.
- Confirm: The application searches the entire raw data file and compares the measured *m/z* of the sample peak against the expected *m/z* of the target compound. When the measured *m/z* is within the specified mass tolerance range of the expected *m/z*, the target compound is confirmed.

When the compound database does not define an expected retention time for a compound, the application cannot identify or confirm the compound. If you select the Ignore if Not Defined option, the application does not perform testing for the retention time and the RT flag is blank on the Target Screening page in Data Review.

On the Target Screening page in Data Review, the RT column in the Compounds table indicates whether this criteria passes or fails. The RT column indicates whether the target compound is identified, fully confirmed, or both. For details, refer to Chapter 5, "Using the Analysis Mode for Target Screening Batches" in the *TraceFinder User Guide*. The Compounds table also displays the RT Expected, RT Measured, and RT Delta values for each compound.

Fragment Ions

To use fragment ions for identification or confirmation, the application requires the following conditions:

- The selected compound databases contain the charged mass for each defined fragment ion of interest for the compounds in the target list.
- The HCD (higher energy collision-induced dissociation), source CID (source collision-induced dissociation), or AIF (all ions fragmentation) ion spectra exist at a time point within the compound's elution time range.

When no fragment is defined in a compound database for a target compound, the following apply:

- When the Ignore if Not Defined option is selected in the method, the application does not perform filtering for the Fragment Ions. In the Data Review view, the FI column is blank.
- When the Ignore if Not Defined option is not selected in the method, the application considers that this target compound is not identified. The Fragment Ions filter fails.

The application uses the number of fragment masses defined in the compound database when it processes a sample for fragment ions. The value you specify for Min. # of Fragments cannot be greater than the number of fragments defined in the compound database.

For example, if a compound has three fragment ion masses defined in a compound database, any of the following scenarios can occur:

- You enter "2" in the Min. # of Fragments box of the method, and the application finds at least two out of the three defined fragment ions. The Fragment Ions filter passes.
- You enter "4" in the Min. # of Fragments box of the method, and the application finds only the three defined fragment ions. The Fragment Ions filter fails.
- You enter "2" in the Min. # of Fragments box of the method, but the application finds only one of the three defined fragment ions. The Fragment Ions filter fails.

The application repeats the following process for each of the fragment ions:

1. When the mass of the parent (plus or minus half of the isolation window value from the acquired raw data file) is not within the mass range of the extracted ion chromatogram shown in the Chromatogram pane of the Data Review view, the application considers the fragment ion as not found and the application fails the Fragment Ions filter; otherwise, the application continues.

2. The application inspects the processed fragment ion scan closest to the target compound's expected retention time and locates the MS/MS spectrum closest to the compound's apex.

   Within this spectrum, the application finds the intensity for the tallest fragment whose mass is within the mass tolerance range of an entered fragment ion mass. When this intensity is not found or when it is less than the intensity threshold defined in the method, the application determines that the entered fragment ion is not found; otherwise, it determines that the fragment ion is found.

Isotopic Pattern

You can choose to either identify or confirm the isotopic patterns for all compounds in a batch. The application calculates an isotopic pattern score (as a percentage value), using the target compound's formula.

For the isotopic pattern filter, enter the isotopic pattern parameters, including a fit threshold value, in the processing method. To identify or confirm the presence of a compound, the resulting score from isotopic pattern matching must be higher than the fit threshold.

To identify or confirm an isotopic pattern, the application must detect the compound for at least one of its defined adduct ions. The application identifies the elemental composition to match using the formula that is associated with the most intense adduct peak. The application then generates an isotopic pattern score (as a percentage value) for the match between the measured and expected isotopic patterns of the calculated elemental composition.

- For profile data, the application calculates the measured isotopic pattern using the average of all scans (within the allowed intensity deviation of the spectrum closest to the compound's apex retention time). In the Data Review view, the application displays both the expected and measured spectra.
- For centroid data, the application calculates the measured isotopic pattern using the apex scan. In the Data Review view, the application displays the expected spectra.

A high isotopic pattern score (approaching 100 percent) occurs when the measured isotope patterns, the expected isotope patterns, and the intensities are almost identical using the scoring parameters specified in the processing method. When the patterns are not similar, the score is closer to 0 percent. When the score is greater than or equal to the specified fit threshold and the number of isotopes matched is not 1 out of 1, this filter passes.

When you view results in the Data Review view, the IP column displays a green or red flag to indicate whether the compound passed or failed based on the criteria specified in the method.

For additional information about isotopic scorings, see Isotopic Pattern Details.

Library Search

For a target screening analysis, you can select the Library Search criterion for either identification or confirmation in the processing method. The application identifies or confirms the sample compound by searching the selected library and returning the library entry with the highest score (as a percentage value) for the fragment ion spectrum in that library that matches the compound's ion spectrum.

For this criterion, you enter a score threshold value in the processing method. The resulting score from a library search match must be higher than your entered threshold value, and the library entry must match a compound's name, a compound's formula, or both to identify or confirm the presence of the compound.

The application uses the following logic to determine when a match is successful:

- When a formula is available in the library entry and the formula matches the target compound formula:
  - A library score that is higher than or equal to the score threshold meets the criteria.
  - A library score that is lower than the score threshold fails to meet the criteria.
- When a formula is available in the library entry and the name in the library entry matches the target compound name but the formula does not match the target compound formula (or it is not available):
  - A library score that is higher than or equal to the score threshold meets the criteria.
  - A library score that is lower than the score threshold fails to meet the criteria.
- When neither the formula nor the library entry name match the target compound, the criteria fails and the Lib Match Name, Library Score, and Library Match Rank columns display N/A in black text.

  Note When the compound is identified, but no MS/MS scan has been performed, the Lib Match Name, Library Score, and Library Match Rank columns display N/A in red text.

- When an MS/MS scan has been performed and both the library entry and the formula match the target compound:
  - A library score that is higher than or equal to the score threshold meets the criteria. The Lib Match Name, Library Score, and Library Match Rank columns display their values in green text.
  - A library score that is lower than the score threshold fails to meet the criteria. The Lib Match Name, Library Score, and Library Match Rank columns display their values in red text.

To use a library search for identification or confirmation, the application requires that the data meet these conditions:

- The raw data file contains HCD (higher energy collision-induced dissociation), source CID (source collision-induced dissociation), or AIF (all ions fragmentation) ion spectra.
- The spectra exist at a time point within the compound's elution time range.

The application performs either a forward library search or a reverse library search. A forward search compares the mass spectrum of an unknown compound to a mass spectral library entry, whereas a reverse search compares a library entry to an unknown compound.

When a parent-selected MS/MS spectrum does not exist, the application performs a reverse library search because the compound scan contains a mixture of fragments from all co-eluting parents. A forward search with such a mixture biases the results toward the strongest parent because its fragments would be dominant, and a forward search also takes more time because the application must search every spectrum in the library.

When a parent-selected MS/MS spectrum exists, the application performs a forward library search because the scan usually contains fragments of only one or two parents, so matching this spectrum against the library is faster and more accurate. When you select the Use Reverse Library Searching Only check box in the processing method, the application performs only a reverse library search.

When you use profile data, the application uses the averaged spectrum of the unknown peak as the input spectrum for the library search. This averaged spectrum is based on the average of all scans within 10 percent of the spectrum closest to the peak's apex retention time. When you use centroid data, the application uses the apex scan as the input spectrum for the library search.

When the application locates a match, it generates a score percentage for the matching library entry. This library search can result in multiple matches with different scores.

After searching based on the *m/z* in the input spectrum, the application performs another search for only the matches from the first library search, but this time based on the name, and then the formula, of the target compound. If at least one match is found, the application displays the highest scoring match from the second search for data review. If no match is found from the second search, the application displays the highest scoring match from the first search.

Editing the Peak Detection Page

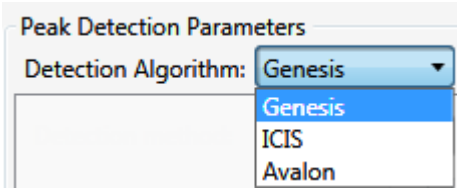
Use the features on the Peak Detection page to specify any of the following peak detection algorithms: Genesis, ICIS, or Avalon.

❖ To specify peak detection parameters

1. Click **Peak Detection** in the Method View navigation pane.

   The Peak Detection page for the method opens.

2. In the Peak Detection Parameters area, select one of the detection algorithms: **Genesis**, **ICIS**, or **Avalon**.



   - The Genesis peak detection algorithm provides backward compatibility with Xcalibur 1.0 studies. For detailed descriptions of the Genesis method parameters, see Genesis peak detection parameters.
   - The ICIS peak detection algorithm is designed for MS data and has superior peak detection efficiency at low MS signal levels. For detailed descriptions of the ICIS method parameters, see ICIS peak detection parameters.
   - The Avalon peak detection algorithm is designed for integrating UV/Vis and analog chromatograms. For detailed descriptions of the Avalon method parameters, see Avalon peak detection parameters.

3. Specify the parameters for the selected detection algorithm.

Genesis Detection Method for a Target Screening Method

The application provides the Genesis peak detection algorithm for backward compatibility with Xcalibur 1.0 studies.

Figure 120. Genesis peak detection

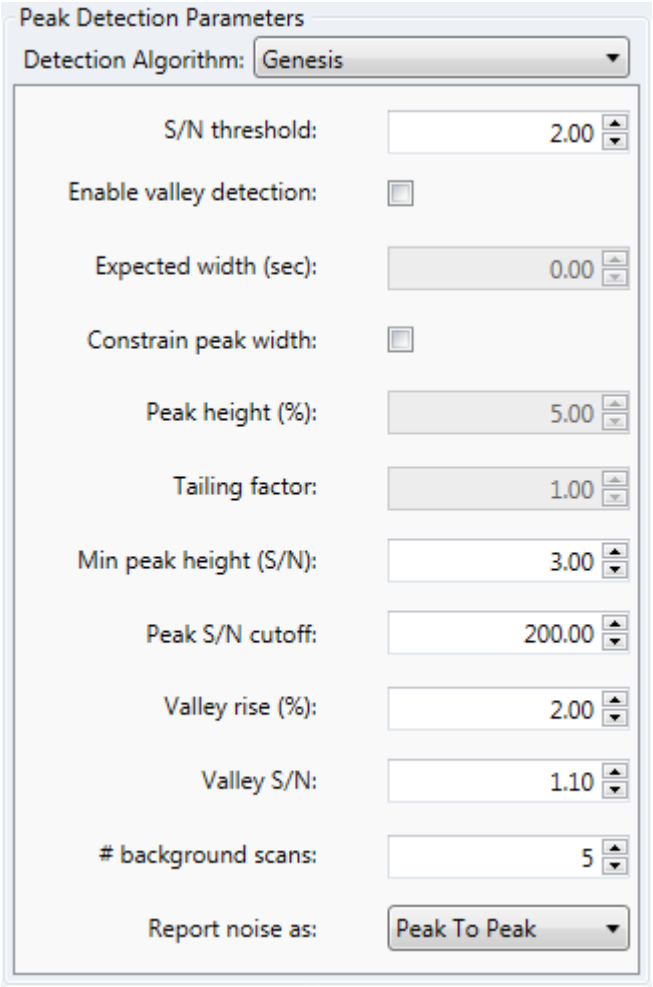


Table 77. Genesis peak detection parameters (Sheet 1 of 3)

Parameter	Description
Detection Algorithm	Specifies the Genesis peak detection algorithm.
S/N threshold	Current signal-to-noise threshold for peak integration. Peaks with signal-to-noise values less than this value are not integrated. Peaks with signal-to-noise values greater than this value are integrated. Valid range: 0.0 through 999.0

Table 77. Genesis peak detection parameters (Sheet 2 of 3)

Parameter	Description
Enable Valley Detection	Uses the valley detection approximation method to detect unresolved peaks. This method drops a vertical line from the apex of the valley between unresolved peaks to the baseline. The intersection of the vertical line and the baseline defines the end of the first peak and the beginning of the second peak.
Expected Width	The expected peak width parameter (in seconds). This parameter controls the minimum width that a peak is expected to have if you enable valley detection. With valley detection enabled, any valley points nearer than the *expected width*/2 to the top of the peak are ignored. If a valley point is found outside the expected peak width, the application terminates the peak at that point. The application always terminates a peak when the signal reaches the baseline, independent of the value set for the expected peak width. Valid range: 0.0 through 999.0
Constrain Peak Width	Constrains the peak width of a component during peak integration of a chromatogram. You can then set values that control when peak integration is turned on and off by specifying a threshold and a tailing factor. Selecting the Constrain Peak Width check box activates the Peak Height (%) and Tailing Factor options.
Peak Height (%)	A signal must be above the baseline percentage of the total peak height (100%) before integration is turned on or off. This text box is active only when you select the Constrain Peak Width check box. Valid range: 0.0 through 100.0%
Tailing Factor	A factor that controls how the application integrates the tail of a peak. This factor is the maximum ratio of the trailing edge to the leading side of a constrained peak. This text box is active only when you select the Constrain the Peak Width check box. Valid range: 0.5 through 9.0
Min Peak Height (S/N)	Specifies the minimum peak height measured as a signal-to-noise ratio. Valid range: 0.00 through 999.00 Default: 3.00
Peak S/N Cutoff	The peak edge is set to values below this signal-to-noise ratio. This test assumes it has found an edge of a peak when the baseline adjusted height of the edge is less than the ratio of the baseline adjusted apex height and the peak S/N cutoff ratio. When the S/N at the apex is 500 and the peak S/N cutoff value is 200, the application defines the right and left edges of the peak when the S/N reaches a value less than 200. Valid range: 50.0 through 10000.0

Table 77. Genesis peak detection parameters (Sheet 3 of 3)

Parameter	Description
Valley Rise (%)	The peak trace can rise above the baseline by this percentage after passing through a minimum (before or after the peak). This criteria is useful for integrating peaks with long tails. This method drops a vertical line from the apex of the valley between unresolved peaks to the baseline. The intersection of the vertical line and the baseline defines the end of the first peak and the beginning of the second peak. When the trace exceeds rise percentage, the application applies valley detection peak integration criteria. This test is applied to both the left and right edges of the peak. Valid range: 0.1 through 500.0
Valley S/N	Specifies a value to evaluate the valley bottom. Using this parameter ensures that the surrounding measurements are higher. Valid range: 1.0 through 100.0 Default: 2.0
# Background Scans	Number of background scans performed by the application.
Report Noise As	Determines if the noise used in calculating S/N values is calculated using an RMS calculation or peak-to-peak resolution threshold. Options are RMS or Peak To Peak.

ICIS Detection Method for a Target Screening Method

The ICIS peak detection algorithm is designed for MS data and has superior peak detection efficiency at low MS signal levels.

Figure 121. ICIS peak detection

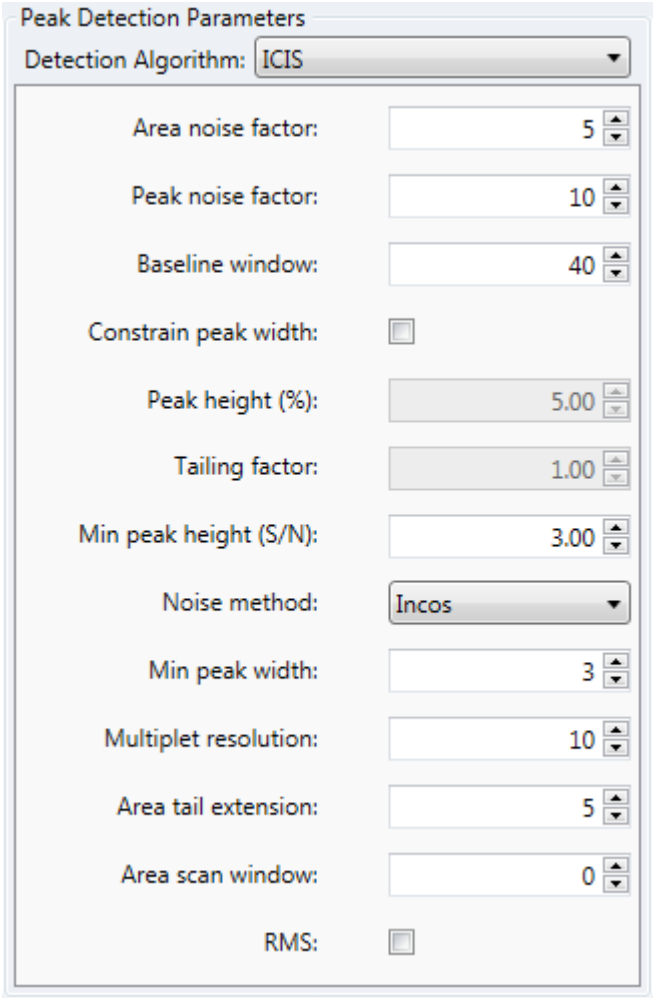


Table 78. ICIS peak detection parameters (Sheet 1 of 3)

Parameter	Description
Detection Algorithm	Specifies the ICIS peak detection algorithm.
Area Noise Factor	The noise level multiplier used to determine the peak edge after the location of the possible peak. The ICIS peak detection algorithm uses this value. Valid range: 1 through 500 Default: 5

Table 78. ICIS peak detection parameters (Sheet 2 of 3)

Parameter	Description
Peak Noise Factor	The noise level multiplier used to determine the potential peak signal threshold. The ICIS peak detection algorithm uses this value. Valid range: 1 through 1000 Default: 10
Baseline Window	The application looks for a local minima over this number of scans. The ICIS peak detection algorithm uses this value. Valid range: 1 through 500 Default: 40
Constrain Peak Width	Constrains the peak width of a component during peak integration of a chromatogram. You can then set values that control when peak integration is turned on and off by specifying a peak height threshold and a tailing factor. Selecting the Constrain Peak Width check box activates the Peak Height (%) and Tailing Factor options.
Peak Height (%)	A signal must be above the baseline percentage of the total peak height (100%) before integration is turned on or off. This text box is active only when you select the Constrain Peak Width check box. Valid range: 0.0 through 100.0%
Tailing Factor	A factor that controls how the application integrates the tail of a peak. This factor is the maximum ratio of the trailing edge to the leading side of a constrained peak. This text box is active only when you select the Constrain the Peak Width check box. Valid range: 0.5 through 9.0
Min Peak Height (S/N)	Specifies the minimum peak height measured as a signal-to-noise ratio. Valid range: 0.00 through 999.00 Default: 3.00
Noise Method	The options are INCOS or Repetitive. INCOS: Uses a single pass algorithm to determine the noise level. The ICIS peak detection algorithm uses this value. Repetitive: Uses a multiple pass algorithm to determine the noise level. The ICIS peak detection algorithm uses this value. In general, this algorithm is more accurate in analyzing the noise than the INCOS Noise algorithm, but the analysis takes longer.
Min Peak Width	The minimum number of scans required in a peak. The ICIS peak detection algorithm uses this value. Valid range: 0 through 100 scans Default: 3

Table 78. ICIS peak detection parameters (Sheet 3 of 3)

Parameter	Description
Multiplet Resolution	The minimum separation in scans between the apexes of two potential peaks. This is a criteria to determine if two peaks are resolved. The ICIS peak detection algorithm uses this value. Valid range: 1 through 500 scans Default: 10
Area Tail Extension	The number of scans past the peak endpoint to use in averaging the intensity. The ICIS peak detection algorithm uses this value. Valid range: 0 through 100 scans Default: 5
Area Scan Window	The number of allowable scans on each side of the peak apex. A zero value defines all scans (peak-start to peak-end) to be included in the area integration. Valid range: 0 through 100 scans Default: 0
RMS	Specifies that the application calculates noise as RMS. By default, the application uses Peak To Peak for the noise calculation. RMS is automatically selected if you manually determine the noise region.

Avalon Detection Method for a Target Screening Method

The Avalon peak detection algorithm is designed for UV data. The Avalon peak detection algorithm also supports negative peaks. You can edit the Event values from the Avalon Event List for a Target Screening Method.

Figure 122. Avalon peak detection

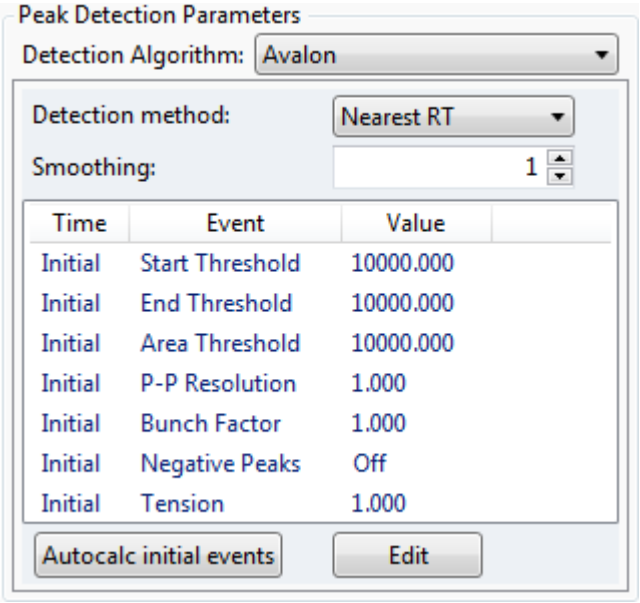


Table 79. Avalon peak detection parameters

Parameter	Description
Detection Algorithm	Specifies the Avalon peak detection algorithm.
Time/Event/Value	Displays the events specified in the Avalon Event List dialog box. Initially displays only the default events that cannot be edited or deleted.
Autocalc Initial Events	Automatically calculates the events in the Event list.
Edit	Opens the Avalon Event List for a Target Screening Method dialog box where you can edit the Time/Event/Value parameters.

Avalon Event List for a Target Screening Method

The event list includes both user-defined and noneditable default events. The application displays the default events when you choose Avalon sensitivity. You cannot delete these events or change their time or values. For a detailed list of events and value ranges, see Event types.

Figure 123. Avalon Event List dialog box

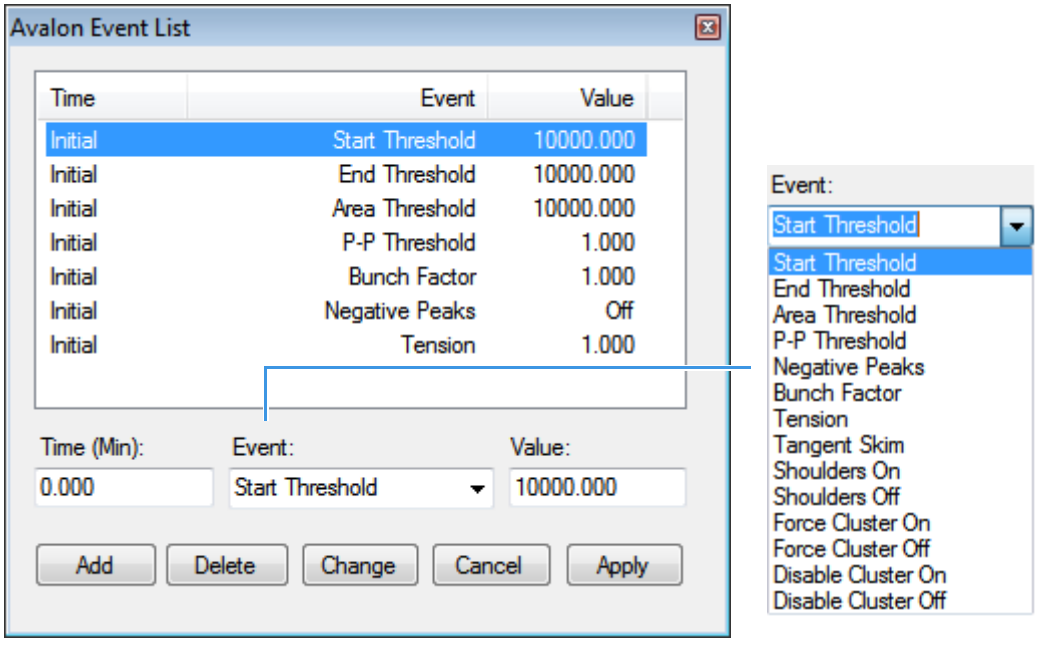


Table 80. Avalon Event List dialog box parameters (Sheet 1 of 2)

Parameter	Description
Time (Min)	Specifies the start time of the event.
Event	Specifies the type of event. For a detailed list of events and value ranges, see Event types.
Value	Specifies the value of the event.

Table 80. Avalon Event List dialog box parameters (Sheet 2 of 2)

Parameter	Description
Add	Adds a new event to the list with the current Time/Event/Value parameters.
Delete	Removes the selected Time/Event/Value parameter from the event list.
Change	Applies the current parameter values.
Cancel	Closes the dialog box without making any changes. Any additions, deletions, or changes revert to their original state.
Apply	Closes the dialog box.

Figure 124. Event types

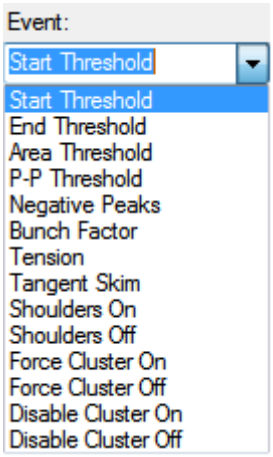


Table 81. Event type descriptions (Sheet 1 of 2)

Event type	Description
Start Threshold	Specifies the threshold at the start of a peak. The Start Threshold is directly related to the RMS noise in the chromatogram. Valid range: 0 through 999 999 999
End Threshold	Specifies the threshold at the end of a peak. The End Threshold is directly related to the RMS noise in the chromatogram. Valid range: 0 through 999 999 999
Area Threshold	Controls the area cutoff. Any peaks with a final area less than the area threshold will not be detected. This control is in units of area for the data. Valid range: 0 through 999 999 999

Table 81. Event type descriptions (Sheet 2 of 2)

Event type	Description
P-P Threshold	The peak-to-peak resolution threshold controls how much peak overlap must be present before two or more adjacent peaks create a peak cluster. Peak clusters have a baseline drop instead of valley-to-valley baselines. Specified as a percent of peak height overlap. Valid range: 0.1 through 99.99
Negative Peaks	Permits detection of a negative going peak. Automatically resets after finding a negative peak. Valid values: On or Off
Bunch Factor	Specifies the number of points grouped together during peak detection. This event controls the bunching of chromatographic points during integration and does not affect the final area calculation of the peak. A high bunch factor groups peaks into clusters. Valid range: 0 through 999
Tension	Controls how closely the baseline should follow the overall shape of the chromatogram. A lower tension traces the baseline to more closely follow changes in the chromatogram. A high baseline tension follows the baseline less closely, over longer time intervals. Valid range: 0 through 999.99 minutes
Tangent Skim	Using this event, you can tangent skim any peak clusters. By default, it chooses the tallest peak in a cluster as the parent. You can also identify which peak in the cluster is the parent. Tangent skim peaks are detected on either side (or both sides) of the parent peak. Tangent skim automatically resets at the end of the peak cluster. Valid range: 0 through 1
Shoulders On	Allows peak shoulders to be detected (peaks which are separated by an inflection rather than a valley) Sets a threshold for the derivative.
Shoulders Off	Disables peak shoulder detection. Valid range: 0 through 50
Force Cluster On	Force the following peaks to be treated as a cluster (single peak).
Force Cluster Off	End the forced clustering of peaks.
Disable Cluster On	Prevent any peaks from being clustered.
Disable Cluster Off	Permit clusters to occur again.

Editing the Reports Page

Use the Reports Page to specify the reports and report output formats that you want to create for the method.

Follow these procedures:

- To open the Reports page
- To configure the compounds in the reports
- To specify output formats

❖ To open the Reports page

Click **Reports** in the Method View navigation pane.

The Reports page for the method opens, displaying only the reports that are configured in the Configuration Console. To configure the reports that are available, see Specifying the Report Selections.

❖ To configure the compounds in the reports

In the Target Screening pane, select one of the following options:

- **Include All Compounds**: Generates reports that include all compounds from the screening library, whether or not they are found in the samples.
- **Include Only Found Compounds**: Generates reports that include only the compounds from the screening library that are found in the samples.

❖ To specify output formats

1. To edit the Report Title, double-click the name and type a new title.

   The application uses this title for all reports that use this method. You cannot edit the Report Title from other report views.

2. To specify the type of report output to create for each report (hard copy, PDF, CSV, or Excel), select the check box in the appropriate column.

3. To duplicate an output type for all reports, click the cell to select it, and then right-click and choose **Copy Down**.

   All check boxes in the column below the selected cell duplicate the selected or cleared state of the selected cell.

   By default, all report types are cleared.

Reports Page

Use the features on the Reports page to specify the reports and report output formats that you want to create with the method.

Figure 125. Reports page

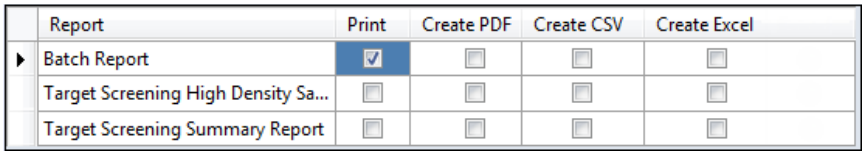


Table 82. Reports page parameters

Parameter	Description
Report list columns	
Report	The name of a report.
Print	Sends reports to the default printer.
Create PDF	Saves reports as PDF files.
Create CSV	Saves reports as CSV files.
Create Excel	Saves reports as Excel files.

Saving a Target Screening Method to a New Name

You can save any method to a new name, or you can use the current method data to overwrite an existing method. The new method contains all the data of the original method.

❖ **To save a method to a new name**

1. From the main menu, choose **File > Save As.**

   The Save Master Method As dialog box opens, displaying all available target screening methods.

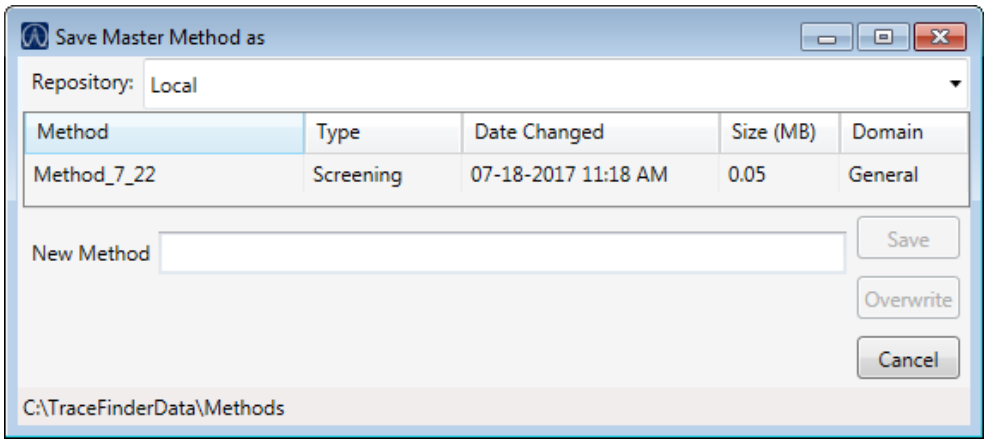


Table 83. Save Master Method As dialog box parameters

Parameter	Description
Repository	The data repository where you want to store the master method.
Method	Name of the quantitation methods in the selected repository.
Type	Type of method: Screening
Date Changed	Date the method was last updated.
Size	Size of the method in megabytes.
Domain	TraceFinder domain for which the method was created.
New Method	Name of the new method to create.
Path	Path to the selected method in the …\TraceFinderData\Methods folder.
Save	Saves the current method to a new name.
Overwrite	Overwrites an existing method.
Cancel	Closes the dialog box without saving the current method to a new name.

2. Do one of the following:
   - In the New Method box, type a name for the new method.
     The Save button is now available.
   - In the New Method box, type the name of an existing method.
     The Overwrite button is now available.
3. Click **Save** or **Overwrite**.
   The application saves all the method data using the specified name and opens the Acquisition page of the new method.

6

Using the Method Development Mode for Unknown Screening Methods

This chapter includes method development tasks for creating and editing an unknown screening method. (See Method Development navigation pane.)

Contents

- Opening an Unknown Screening Method
- Starting a New Unknown Screening Method
- Editing an Unknown Screening Method
- Saving an Unknown Method to a New Name

The TraceFinder application uses a method to specify the types of acquisition, processing, and reporting that occur with a batch of samples. You can create a method designed specifically to test for compounds in your assay.

The application identifies or confirms sample peaks that are in a database or library and performs preliminary analysis of unknown peaks by assigning probable elemental composition to them.

When you create a method, the TraceFinder application uses it to determine how to process a set of samples and provide a set of meaningful results. The application uses an instrument method to define how to acquire raw data. The rest of the method defines how to process the raw data, how the flags information displays the results, and how the reporting functionality defines the output for your data and results.

The application applies your method to a batch, which is a list of one or more samples. Together, the method and batch provide a workflow-oriented approach to the data processing and information reporting for batches of samples.

Figure 126. Method Development navigation pane

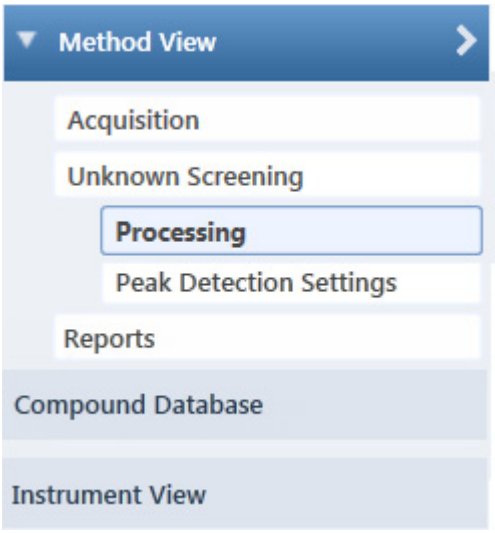


Table 84. Method Development navigation pane commands

Command	Description
Method View	Displays the Method View for the method.
Acquisition	Displays the Acquisition page of the Method View. See Editing the Acquisition Page.
Unknown Screening	
Processing	Displays the Processing page of the Method View. See Editing the Processing Pages.
Peak Detection Settings	Displays the Peak Detection Settings page of the Method View. See Editing the Peak Detection Settings Page.
Reports	Displays the Reports page of the Method View. See Editing the Reports Page.
Compound Database	See Chapter 2, "Using Compound Databases in the Method Development Mode."
Instrument View	See Chapter 3, "Using Instrument Methods in the Method Development Mode."

Opening an Unknown Screening Method

Use the TraceFinder application to open an unknown screening method that was created and saved in the TraceFinder application.

❖ To open an unknown screening method in the Method Development mode

1. Click **Method Development** in the navigation pane.

   The Method Development navigation pane opens.

   For descriptions of all the features in this navigation pane, see Starting a New Unknown Screening Method.

2. Choose **File > Open > Master Method** from the main menu.

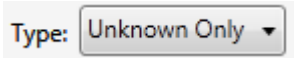
   Tip You can also open one of your most recently used method files. Choose **Files > Recent Files > *Method***.

   The Open Master Method dialog box opens.

3. (Optional) Select a different repository.

   The default repository is specified in the Administrator Console. Refer to the instructions in the *TraceFinder Administrator Console User Guide*.

4. Select **Unknown Only** in the Type list.

   The method list displays all unknown screening methods in the selected repository.

5. Select an unknown screening method and click **Open**.

   The Acquisition page for the selected method opens. For detailed descriptions of all the features on the Acquisition page, see Acquisition Page.

Figure 127. Open Master Method dialog box

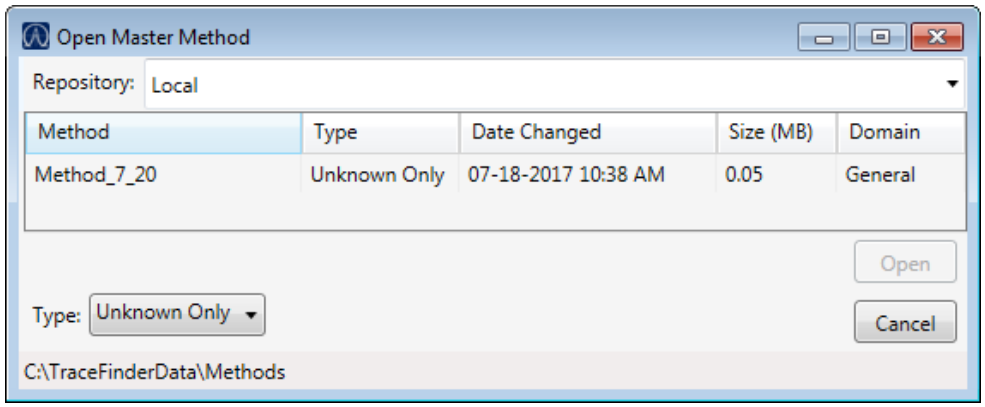


Table 85. Open Master Method dialog box parameters

Parameter	Description
Repository	The data repository where you want to store the master method.
Method	Name of the methods for the selected type.
Type	Type of method: Quan, Screening, or Unknown Only.
Date Changed	Date the method was last updated.
Size	Size of the method in megabytes.
Domain	TraceFinder domain for which the method was created.
Type	Type of method to display: Quan, Screening, Unknown Only, or Any.
Path	Path to the selected method in the …\TraceFinderData\Methods folder.

Starting a New Unknown Screening Method

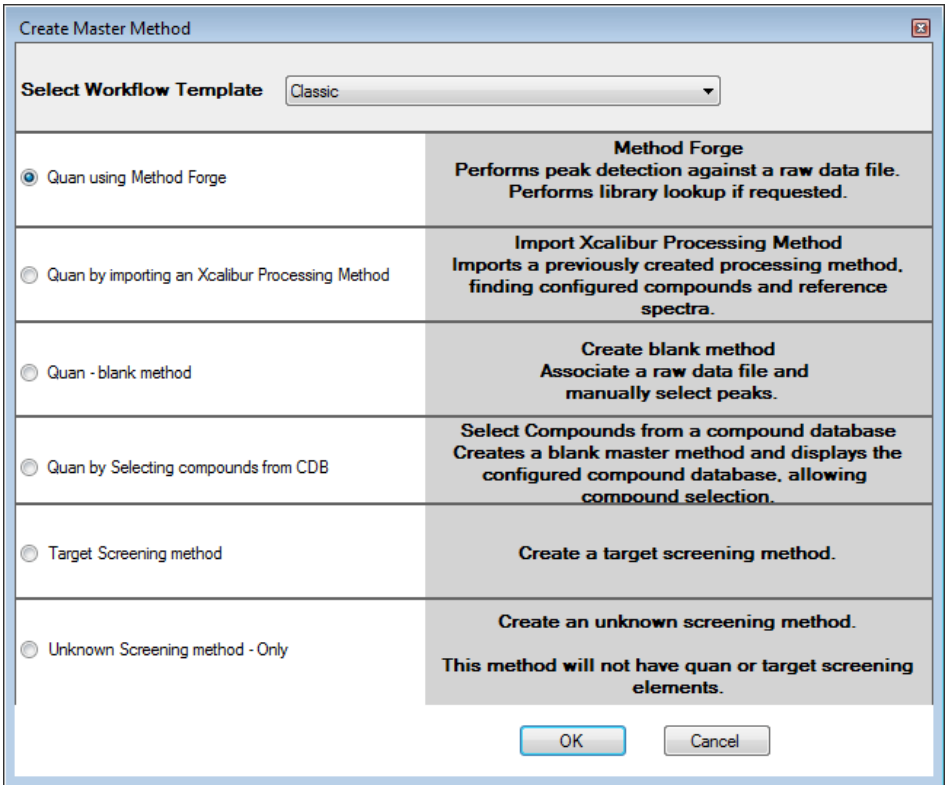
The following procedure includes the basic parameters you must define to create and save an unknown screening method. For a detailed description of how to modify all parameters in a method, see Editing an Unknown Screening Method.

❖ To create a new method

1. Choose **File > New > Master Method** from the main menu.

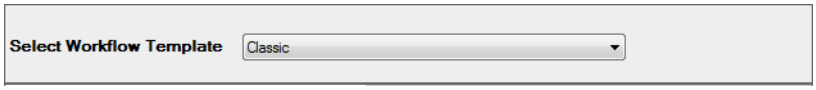
   The Create Master Method dialog box opens.

Figure 128. Create Master Method dialog box



IMPORTANT The Unknown Screening Method - Only feature is available only when you select the Allow Unknown Screening option in the application configuration console. See Processing Options.

2. Select a workflow template from the list.

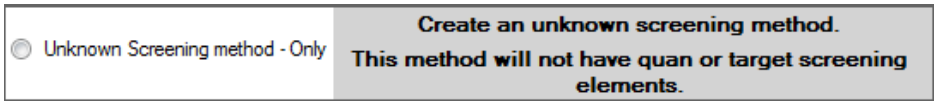


The Create Master Method dialog box displays only the method creation techniques that you can use with the selected workflow template. In addition to any custom workflows you created in your laboratory, the Classic workflow and Screening workflow support the Unknown Screening Method – Only option.

You can choose any of the workflow templates previously saved to the following folder:

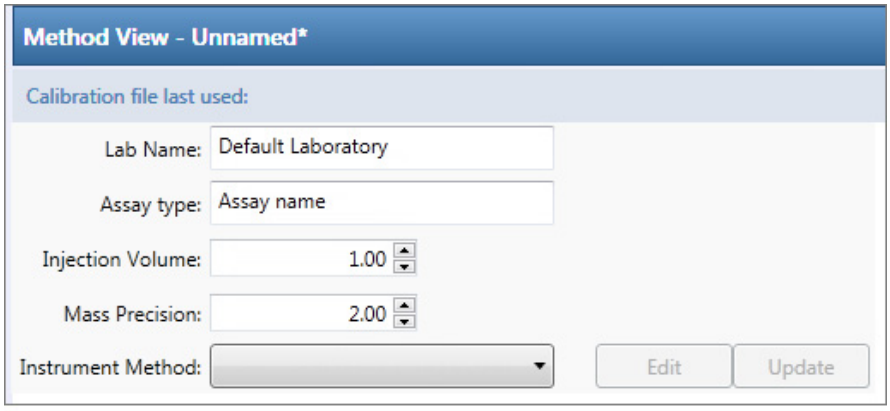
…\TraceFinderData\Templates\Workflows

3. Select the **Unknown Screening Method – Only** option and click **OK**.



The Method View for an unknown screening method includes the Acquisition, Processing, Peak Detection Settings, and Reports pages.

The Acquisition page for the method opens.

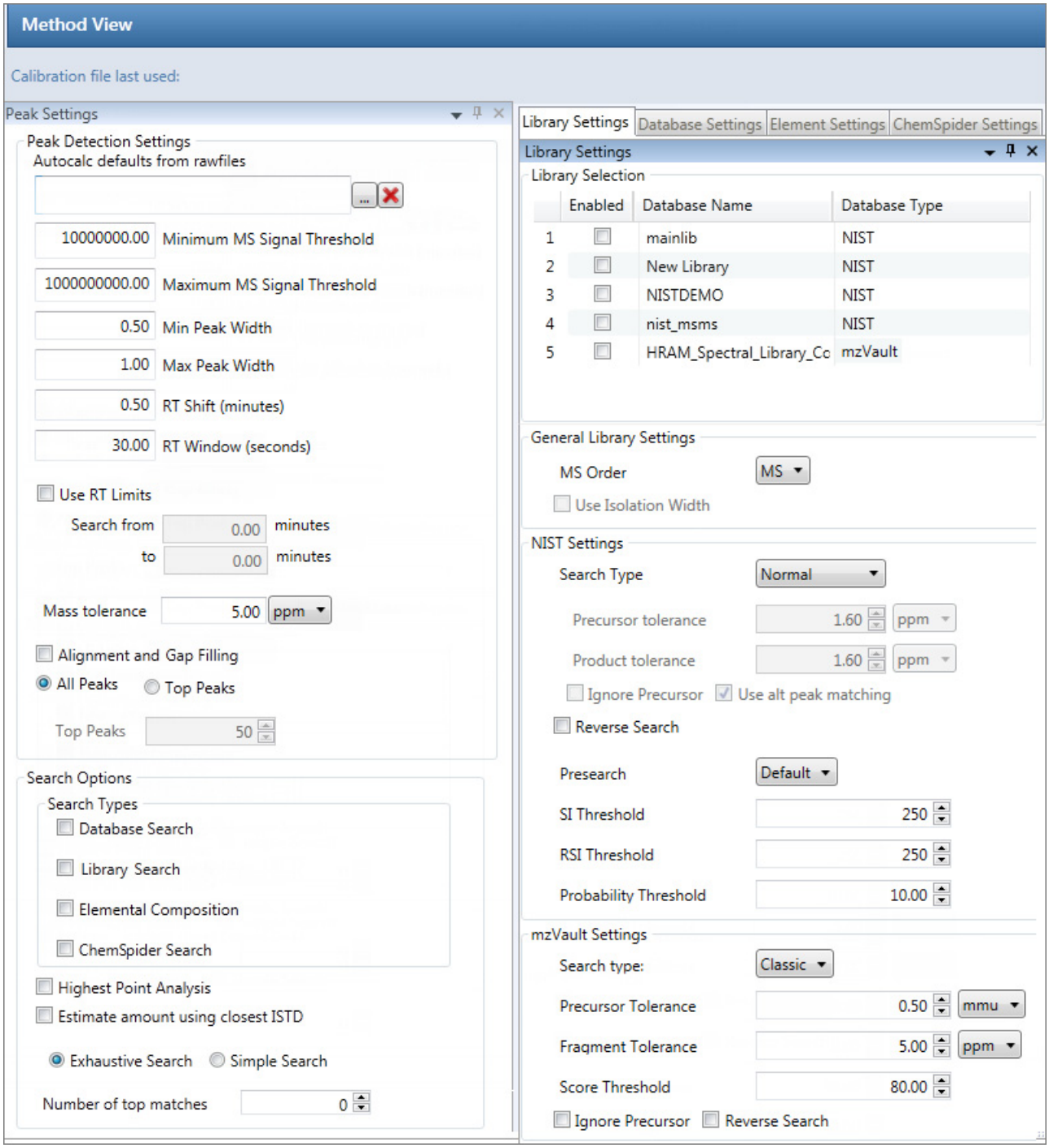


4. From the Instrument Method list, select an instrument method.
5. Click **Processing** in the Method View navigation pane.

   The Processing page for the method opens. See Processing page for an unknown screening method.

Tip (Animation) To view "Starting an Unknown Screening Method," choose **Help > Animations**.

Figure 129. Processing page for an unknown screening method



Editing an Unknown Screening Method

You can open an unknown screening method to specify acquisition instructions and the settings for processing, peak detection, and reports.

See the instructions for the following tasks:

- Editing the Acquisition Page
- Editing the Processing Pages
- Editing the Peak Detection Settings Page
- Editing the Reports Page

Editing the Acquisition Page

The Acquisition Page defines basic information used to acquire samples.

Follow these procedures:

- To open the Acquisition page
- To specify acquisition information for a method
- To edit an instrument method
- To edit a tune/breakdown method

❖ To open the Acquisition page

Click **Acquisition** in the Method View navigation pane.

The Acquisition Page opens.

❖ To specify acquisition information for a method

1. In the Lab Name box, type the name to be displayed at the top of each printed, saved, or exported report.

   The default name is Default Laboratory.

2. In the Assay Type box, type the assay type for the method.

3. From the Injection Volume box, select an injection volume (between **0.1** and **5000** µL) that the method uses for sample injection.

   Use the Up/Down arrows, , to change the volume in increments/decrements of 1 µL, or use the keyboard to enter non-integer injection volumes.

   IMPORTANT The application uses this injection volume in the method, not the injection volume from the instrument method.

4. From the Mass Precision box, select a precision value (**2** through **6**) for the number of decimal places to use in reports and in peak and spectrum displays.

❖ To edit an instrument method

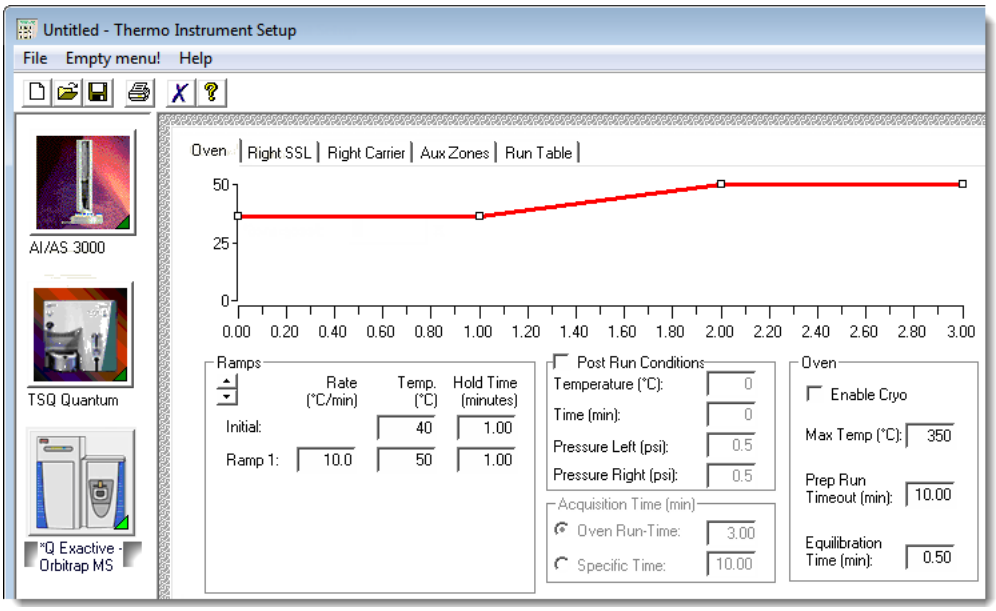
1. From the Instrument Method list on the Acquisition page, select an instrument method.



2. To edit the selected instrument method, click **Edit**.

   The Thermo Instrument Setup window opens. This example of an instrument setup shows multiple configured instruments.

   Figure 130. Thermo Instrument Setup window



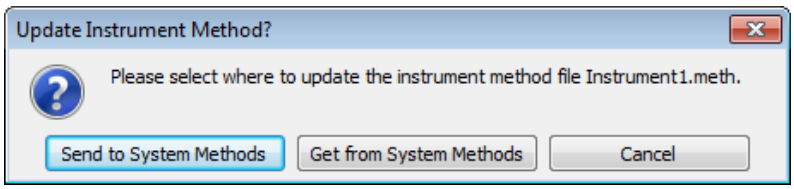
3. Edit the values on the instrument page for your instrument.

4. From the main menu in the Thermo Instrument Setup window, choose **File > Save** and then choose **File > Exit**.

   The application returns you to the Acquisition Page.

5. To update any changes that were made to the instrument method after you created this method, click **Update**.

   The Update Instrument Method dialog box opens.



6. Choose one of the following options:
   - **Send to System Methods**: Overwrites the instrument method in the …\TraceFinderData\InstrumentMethods folder with the current instrument method.
   - **Get from System Method**: Overwrites the current instrument method with the instrument method in the …\TraceFinderData\Methods folder.
   - **Cancel**: Makes no changes to the instrument method in the current method.

❖ **To edit a tune/breakdown method**

From the Tune/Breakdown Method list, select a tune/breakdown method.



The instructions for editing and updating a tune/breakdown method are the same as for an instrument method. Follow the instructions To edit an instrument method.

Acquisition Page

Use the Acquisition page parameters to define basic information about the method.

Figure 131. Acquisition page for an unknown screening method

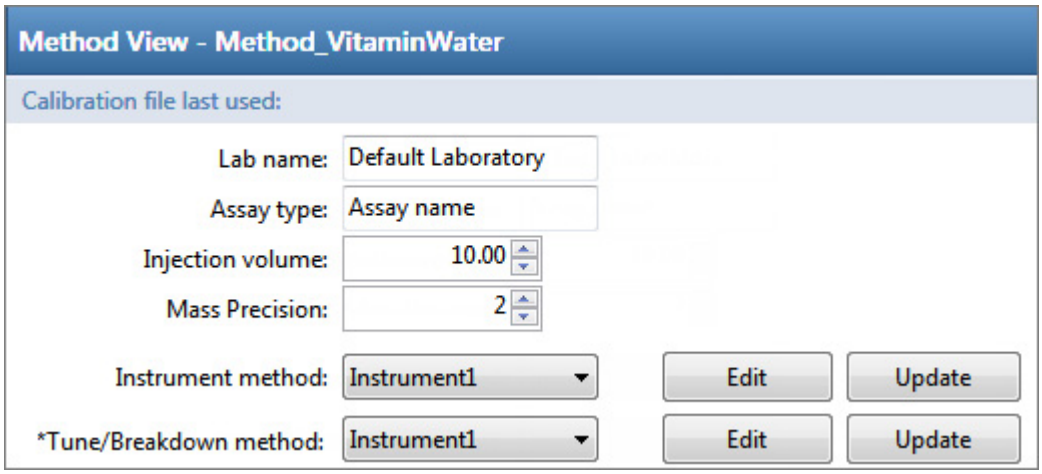


Table 86. Acquisition page parameters (Sheet 1 of 2)

Parameter	Description
Lab Name	Specifies the laboratory name to be displayed at the top of each printed, saved, or exported report. Default: Default Laboratory To specify this default laboratory name, see Specifying Method Defaults.

Table 86. Acquisition page parameters (Sheet 2 of 2)

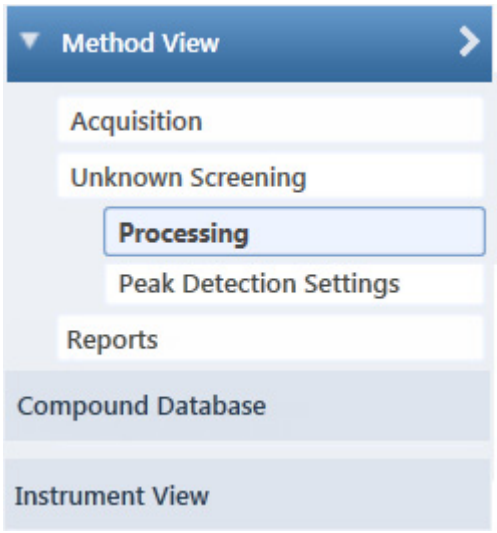
Parameter	Description
Assay Type	Specifies the name for the analysis type to be targeted by the method. The assay type associates the method with the analysis of a compound or specific class of compounds (for example, you might use an assay type of PAH for the analysis of Polynuclear Aromatic Hydrocarbons).
Injection Volume	Specifies the system uses the injection volume (in µL) for sample injection. For a more detailed explanation, refer to the documentation for the autosampler. The injection volume in the method overrides the injection volume in the instrument method. The injection volume in the batch overrides the injection volume in the method. Valid range: 0.1 through 5000 µL
Mass Precision	Specifies the number of decimal places used in reports and in peak and spectrum displays. Valid range: Integers from 2 through 6
Instrument Method	Specifies the instrument method used for acquiring samples.
Tune/Breakdown Method	Tune/Breakdown method used for processing samples.
Edit	Opens the Thermo Instrument Setup window where you can edit the instrument method or tune/breakdown method.
Update	Specifies one of the following: Send to System Methods: Overwrites the instrument method in the …\TraceFinderData\InstrumentMethods folder with the current instrument method or tune/breakdown method. Get from System Methods: Overwrites the current instrument method or tune/breakdown method with the instrument method in the …\TraceFinderData\Methods folder.

Editing the Processing Pages

The Unknown Screening – Processing pages define basic information about the method.

❖ **To open the Processing pages**

Click **Processing** in the Method View navigation pane.



The Processing pages include settings for peaks and settings for library, database, element, and ChemSpider searches.

See the following topics:

- Editing the Peak Settings
- Editing the Library Settings
- Editing the Database Settings
- Editing the Element Settings
- Editing the ChemSpider Settings

IMPORTANT After the first processing, you might see an error message stating that the total number of results is too large for a single batch (more than 500 000). Return to the Processing pages in the method and make one or more of these parameter adjustments:

- Limit the RT range.
- Shorten the signal range.
- Specify a lower value for the Number of Top Matches.
- Specify a Simple Search instead of an Exhaustive Search.
- Specify Top Peaks instead of All Peaks.
- Limit the number of search types.

Editing the Peak Settings

Use the Peak Settings page to define how the method detects and identifies peaks.

Follow these procedures:

- To open the Peak Settings page
- To calculate default peak detection values from a raw data file
- To specify the peak detection settings
- To specify the search options

❖ To open the Peak Settings page

Click **Processing** in the Method View navigation pane.

By default, the Peak Settings Page opens.

❖ To calculate default peak detection values from a raw data file

For the Autocalc Defaults from Rawfiles box, browse to and select a raw data file.

The application populates the parameters in the Peak Detection Settings area with the values from the raw data file. (To reset the default values in the method, click  .)

❖ To specify the peak detection settings

1. Do the following:
   a. In the Minimum MS Signal Threshold box, type the minimum number of counts for the application to detect an ion.
   b. In the Maximum MS Signal Threshold box, type the maximum number of counts for the application to detect an ion.

   The application calculates the MS signal threshold (noise level) from the raw data file and sets this value to reflect a signal-to-noise level of 20.

   Valid range: These values depends on the instrument. The minimum value is 0, and the maximum value is never larger than the maximum intensity component in the sample.
2. In the Min Peak Width box, type a value for the minimum acceptable peak width for the chromatographic peak.
3. In the Max Peak Width box, type a value for the maximum acceptable peak width for the chromatographic peak.
4. In the RT Shift box, type a value for the maximum allowable peak alignment correction for the chromatographic peak.

   The application applies this value only when you enable batch processing.
5. In the RT Window box, type a value for the maximum viewable window around the identified chromatographic peak.

6. To create one peak list for the batch regardless of threshold settings, select the **Alignment and Gap Filling** check box.

   The application processes the batch as a group instead of as individual samples. When the application finds a peak in one sample but not in another, this option adds the missing peak to all sample lists and displays N/A or the found peak information in the results. This option is similar to the Show All Compounds option in a quantitation or a target screening method. The application displays the results for the batch in a color-coded heat map grid. Refer to Chapter 6, "Using the Analysis Mode for Unknown Screening Batches" in the *TraceFinder User Guide*.

   When you clear this option, the application creates a unique peak list for each sample based on the method peak settings, similar to clearing the Show All Compounds option in a target screening method.

❖ To specify the search options

1. In the Search Types area, select the check box for the type of search that you want to perform:
   - Database Search
   - Library Search
   - Elemental Composition
   - ChemSpider Search

   You can select as many search types as you want.

2. To perform the search only on the sample that has the highest peak response in the batch, select the **Highest Point Analysis** check box.

   When you select this option, the application performs the search only on the sample in the batch that has the highest peak response.

   When you clear this option, the application uses all search criteria on a per-peak basis.

   For example, in a batch with samples 1, 2, and 3 with responses of 10, 1000, and 50, respectively, the application performs the search only on the peak from sample 2 with the greatest peak response. Because the mass and retention time for the peak are the same in each sample, the application identifies the same chemical. When you use this option with the Top Peaks option, the application uses the responses across the batch to filter the results and treats the entire batch of sample data as it would when returning the top *n* responses for a single sample.

3. Select one of the following options:
   - **Simple Search**: When the application identifies a peak by one of the identification points, it lists only that compound name in Data Review.
   - **Exhaustive Search**: The application lists all identified peaks from each search criterion and displays them as hierarchical entries under the top hit in Data Review.

4. To specify the number of matching peaks to display for each search type, enter a value for **Number of Top Matches**.

Peak Settings Page

Use the Peak Settings page to define how the method detects and identifies peaks.

Figure 132. Peak Settings page

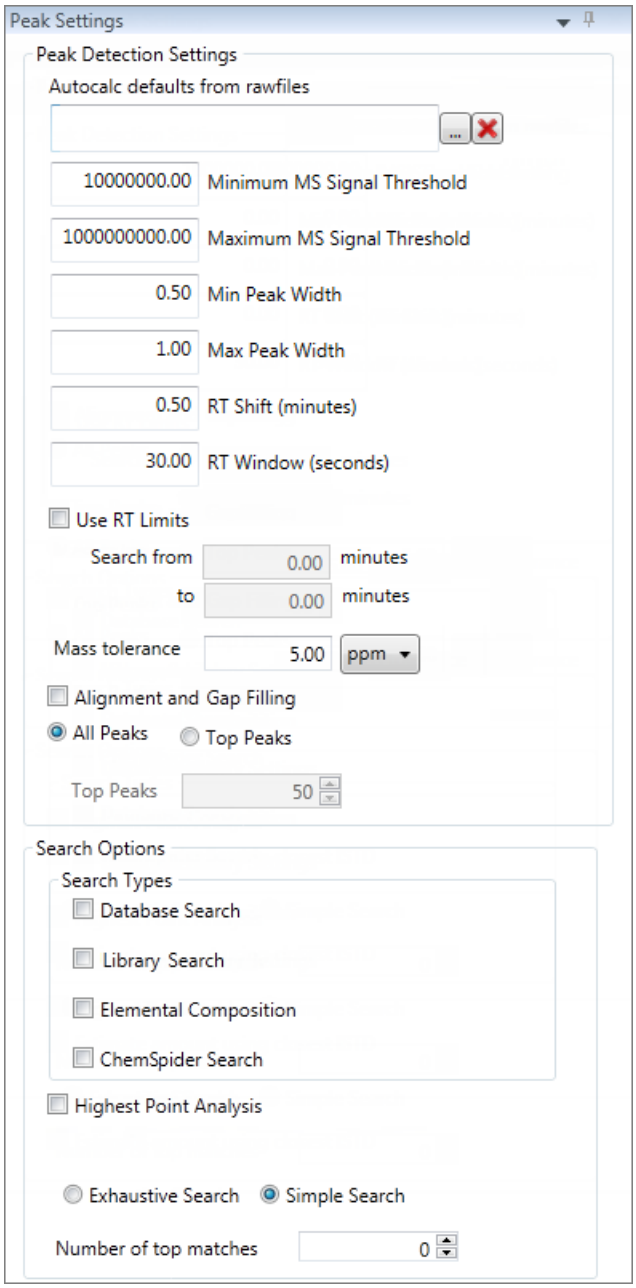


Table 87. Peak Settings page parameters (Sheet 1 of 3)

Parameter	Description
Peak Detection Settings	
Autocalc Defaults from Rawfiles	Populates the parameters in the Peak Detection Settings area with the values from the selected raw data file.

Table 87. Peak Settings page parameters (Sheet 2 of 3)

Parameter	Description
Minimum/Maximum MS Signal Threshold	Specifies the minimum and maximum number of counts that the application uses to detect an ion. The application calculates the MS signal threshold (noise level) from the raw data file and sets this value to reflect a signal-to-noise level of 20. Valid range: This value depends on the instrument. The minimum value is 0, and the maximum value is never larger than the maximum intensity component in the sample. Default minimum: 10 000 000.00 Default maximum: 1 000 000 000.00
Min Peak Width	Specifies the minimum allowable peak width for chromatographic peak detection. Default: 0.50
Max Peak Width	Specifies the maximum allowable peak width for chromatographic peak detection. Default: 1.00
RT Shift (minutes)	Specifies the maximum allowable peak alignment for chromatographic peak detection. Default: 1.0
RT Window (seconds)	Specifies the maximum viewable window around the identified chromatographic peak. Default: 30.00
Use RT Limits	Specifies a lower and upper limit for searches. Valid range: 0.00 through 999.99 minutes Default: 0.00 minutes for lower limit; 999.00 minutes for upper limit
Mass Tolerance	Specifies the number of millimass units or parts per million to use as the *m/z* ± tolerance value. Valid range: 0 through 500 Default: 5.00 Unit: mmu or ppm **Note** When using ion trap data, the application uses 300 mmu regardless of the value you enter here.
Alignment and Gap Filling	Specifies that the application creates a single peak list for the whole batch whether or not it finds a peak in a raw data file. When you clear this option, each data file has its own unique peak list based on the unknown peak settings.
All Peaks / Top Peaks	Specifies that the application displays results for the top *n* peaks or for all peaks.
(number of) Top Peaks	Specifies the maximum number of peaks to identify. Available only when you select the previous Top Peaks option. Default: 50

Table 87. Peak Settings page parameters (Sheet 3 of 3)

Parameter	Description
Search Options	
Search Types	Database Search: To specify the unknown database search parameters, see Editing the Database Settings. Library Search: To specify the unknown library search parameters, see Editing the Element Settings. Elemental Composition: To specify the unknown elemental search parameters, see Editing the Element Settings. ChemSpider Search: To specify the ChemSpider search parameters, see Editing the ChemSpider Settings.
Highest Point Analysis	Specifies that the application performs the search only on the sample that has the highest peak response of all samples in the batch, working in conjunction with the Alignment and Gap Filling option. For example, in a batch with samples 1, 2, and 3 with responses of 10, 1000, and 50, respectively, the application performs the search only on the peak from sample 2 with the greatest peak response. Because the mass and retention time for the peak are the same in each sample, the application identifies the same chemical. When you use this option with the Top Peaks option, the application uses the responses across the batch to filter the results and treats the entire batch of sample data as it would when returning the top *n* responses for a single sample. When you clear this option, the application uses single-sample logic.
Exhaustive Search / Simple Search	Simple Search (Default): When the application identifies a peak by one of the identification points, it lists only that compound name in Data Review. Exhaustive Search: The application lists all identified peaks from each search criterion and displays them as hierarchical entries under the top hit entry in Data Review.
Number of Top Matches	Specifies the maximum number of matches returned from a library search that you want to display in the Cross Sample Peak List in Data Review and in reports. Valid range: 1 through 99 Default: 3

Editing the Library Settings

Use the Library Settings page to specify the settings for library searches. The application uses these settings only when you select the Library Search option as a search type. See To specify the compound databases to use.

To find the identity of an unknown compound, the application searches the MS/MS spectrum against a mass spectral library using the library search parameters specified in the method. The application displays the results in Data Review. The application uses the MS/MS scan closest to the apex of the detected peak or from the associated data-dependent scan for the extracted mass chromatogram and submits it for a library search.

When the application finds a library match and the formula is present in the library record, the application reports the formula in Data Review.

When the application does not find a library match, or when the match does not have an associated formula, the application does the following:

- Performs a reverse search of the mass spectrum against the library.
- Returns the top *n* hits, where *n* is the value of the Number of Top Matches parameter.
- Displays each match as a separate row in Data Review.
- Shows the score for each match in Data Review (except matches with a score of zero).

Follow these procedures:

- To open the Library Settings page
- To specify the libraries to use
- To specify the general library settings
- To specify the NIST settings
- To specify the mzVault settings

❖ To open the Library Settings page

Click the **Library Settings** tab.

The Library Settings Page opens.

❖ To specify the libraries to use

In the Library Selection area, select the **Enabled** check box for each library that you want to use for unknown screening searches.

The Database Name list displays the libraries located in the following folders:

C:\Program Files (x86)\NISTMS\MSSEARCH

C:\TraceFinderData\New Libraries

❖ To specify the general library settings

1. To specify the source for the confirming peaks, select one of the following:
   - **MS**: Confirming peaks come from the same scan.
   - **MS2**: Confirming peaks are fragments from an adjacent scan.
   - **MS–In Source CID**: Confirming peaks come from the Source CID.
2. (Optional only when you select MS2) To use the isolation width when no precursor match is found, select the **Use Isolation Width** check box.

   The application uses the isolation width to determine if there is an MS/MS scan within the same peak RT window that includes the precursor fragments.

❖ To specify the NIST settings

IMPORTANT When you use the following combination of criteria,
- An accurate mass library (such as libraries from Exactive™, Q Exactive™, or Orbitrap instruments)
- Search Type set to MS/MS
- Presearch set to Default

and the application does not return results, you might be using a tighter tolerance than the selected library supports.

Make sure that the Precursor Tolerance parameter for NIST libraries specifies enough decimal precision to match the precursor in the library. Try setting the Precursor Tolerance to **.01 amu** (the minimum allowed), or try checking the decimal precision entered for the precursor in the specified library.

1. To specify the type of search to perform, select one of the following:
   - **Normal**: Uses a more extensive set of presearch screenings—a total of four different criteria.

     When you select this option, skip to step 6.
   - **MS/MS**: Searches for an MS/MS spectrum in a library of MS/MS spectra.
   - **In-source HiRes**: Searches for an in-source/EI with an accurate ion *m/z* or an MS/MS spectrum in a library that contains in-source/EI accurate ion *m/z* or MS/MS spectra.
2. Specify the *m/z* ± tolerance value for the Precursor Tolerance and the Product Tolerance, and then specify the units of measure.
   - For accurate mass libraries, select **amu**.
   - For exact mass libraries, select **ppm** (default).

Note These tolerance options are available only when you select the MS/MS or In-source HiRes search types.

3. To ignore mass spectral peaks within the specified precursor tolerance range, select the **Ignore Precursor** check box.

   Note This option is available only when you select the MS/MS or In-source HiRes search types.

4. To use alternate peak matching, select the **Use Alt Peak Matching** check box.

   Note This option is available only when you select the MS/MS or In-source HiRes search types.

5. To specify that the application use the reverse search method, select the **Reverse Search** check box.

   A reverse search compares a library entry to an unknown compound (whereas a forward search compares the mass spectrum of an unknown compound to a mass spectral library entry).

6. To choose a Presearch method, use these guidelines:

   - For libraries created before 2014, set Presearch to **Off**.
   - When you set the Search Type to **In-source HiRes**, set Presearch to **Off**.
   - For all other criteria, set Presearch to **Default**.

7. Select a threshold value for SI Threshold.

   The application uses this lower threshold value for the search index method used to search the NIST library. The application does not return results below this value.

8. Select a threshold value for RSI Threshold.

   The application uses this lower threshold value for the reverse search index method used to search the NIST library. The application does not return results below this value.

9. Select a threshold value for Probability Threshold.

   The application uses this lower threshold value for the match probability.

❖ To specify the mzVault settings

1. From the Search Type list, select one of the following search algorithms to use for matching: **Classic** or **HighChem**.
2. Specify an *m/z* ± tolerance value for Precursor Tolerance, and then select the units of measure for precursor tolerance.
3. Specify an *m/z* ± tolerance for Fragment Tolerance, and then select the units of measure for fragment tolerance.

4. Select a threshold value for Score Threshold.

   The application uses this lower threshold value for the Library Score in Data Review. The application does not return results below this value.

5. To ignore mass spectral peaks within the specified precursor tolerance range, select the **Ignore Precursor** check box.

6. To specify that the application use the reverse search method, select the **Reverse Search** check box.

   A reverse search compares a library entry to an unknown compound (whereas a forward search compares the mass spectrum of an unknown compound to a mass spectral library entry).

Library Settings Page

To perform library searches, specify the Library Settings page parameters. The application uses these settings only when you select the Library Search option as a search type.

Figure 133. Library Settings page

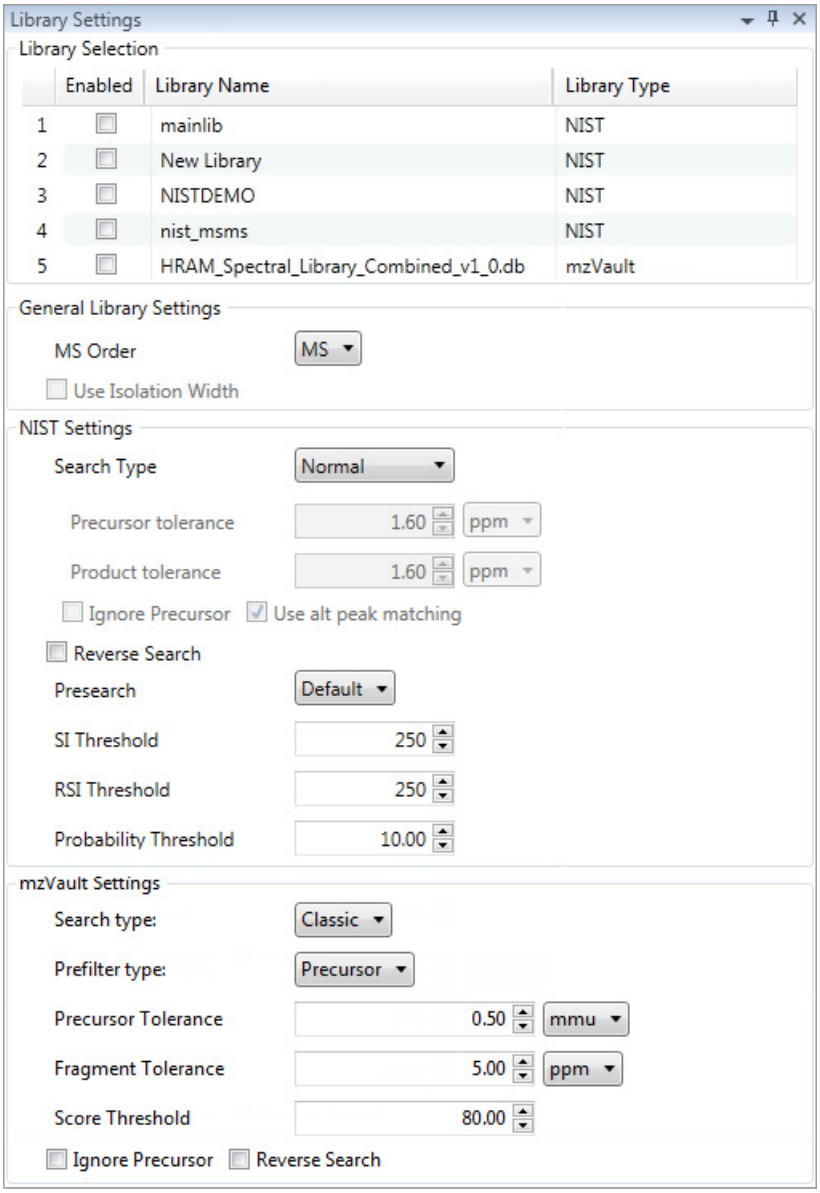


Table 88. Library Settings page parameters (Sheet 1 of 3)

Parameter	Description
Library Selection	
Enabled	The databases for unknown screening searches.
Library Name	Specifies all libraries that you configured in the Configuration Console. See Specifying mzVault and NIST Libraries.

Table 88. Library Settings page parameters (Sheet 2 of 3)

Parameter	Description
Library Type	Specifies the library type as either NIST or mzVault.
General Library Settings	
MS Order	Specifies that the confirming peaks come from the same scan (MS), are fragments from an adjacent scan (MS2), or come from the Source CID (MS–In Source CID).
Use Isolation Width	Specifies that the application uses the isolation width to determine if there is an MS/MS scan within the same peak RT window that includes the precursor fragments. Available only when you set the MS Order to MS2.
NIST Settings	
Search Type	Specifies the type of library search algorithm to use. Normal: Uses a more extensive set of presearch screenings—a total of four different criteria. MS/MS: Searches for an MS/MS spectrum in a library of MS/MS spectra. In-source HiRes: Searches for an in-source/EI with an accurate ion *m/z* or an MS/MS spectrum in a library that contains in-source/EI accurate ion *m/z* or MS/MS spectra.
Prefilter Type	Specifies the type of prefilter to use: Precursor, Compound, or None.
Precursor Tolerance	Specifies the precursor *m/z* ± tolerance as either amu or ppm. Available only when you select MS/MS or In-source HiRes search types. **Note** Use amu for accurate mass libraries (such as libraries from Exactive, Q Exactive, or Orbitrap instruments). Valid range: 0.01 through 100 000.00 Default: 1.60 ppm
Product Tolerance	Specifies the mass spectral peak *m/z* ± tolerance as either amu or ppm. Available only when you select MS/MS or In-source HiRes search types. **Note** Use amu for accurate mass libraries (such as libraries from Exactive, Q Exactive, or Orbitrap instruments). Valid range: 0.01 through 100 000.00 Default: 1.60 ppm
Ignore Precursor	Ignores mass spectral peaks within the specified precursor tolerance range. Available only when you select MS/MS or In-source HiRes search types.
Use Alt Peak Matching	Specifies that the application uses an alternate peak matching method for searching the mass spectra. Available only when you select MS/MS or In-source HiRes search types.
Reverse Search	Specifies that the NIST library search uses the reverse search method. A reverse search compares a library entry to an unknown compound (whereas a forward search compares the mass spectrum of an unknown compound to a mass spectral library entry).
Presearch	Specifies that the application uses presearch screening before calculating the spectrum-by-spectrum match factor.

Table 88. Library Settings page parameters (Sheet 3 of 3)

Parameter	Description
SI Threshold	Specifies the lower threshold value for the search index method used to search the NIST library. The application does not return results below this value. Valid range: 0 through 999 Default: 250
RSI Threshold	Specifies the lower threshold value for the reverse search index method used to search the NIST library. The application does not return results below this value. Valid range: 0 through 999 Default: 250
Probability Threshold	Threshold value for the match probability.
mzVault Settings	
Search Type	Specifies the type of library search algorithm to use. • Classic: Uses the standard library search algorithm. • HighChem: Uses the HighChem library search algorithm.
Precursor Tolerance	Specifies the precursor *m/z* ± tolerance as either amu or ppm. Valid range: 0.1 through 1000.0 Default: 0.50 mmu
Fragment Tolerance	Specifies the fragment *m/z* ± tolerance as either amu or ppm. Valid range: 0.1 through 1000.0 Default: 5.00 ppm
Score Threshold	Specifies that the application does not return results below this value. Valid range: 0 through 100 Default: 80.00
Ignore Precursor	Specifies that the application ignores mass spectral peaks within the specified precursor tolerance range.
Reverse Search	Specifies using the reverse search method for an mzVault search. A reverse search compares a library entry to an unknown compound (whereas a forward search compares the mass spectrum of an unknown compound to a mass spectral library entry). **Note** When you are using all ions fragmentation (AIF), data-independent (DIA), variable data-independent (vDIA), or source collision-induced dissociation (CID) data, the application automatically uses reverse search even if you do not select this option.

Editing the Database Settings

Use the Database Settings page to specify the compound databases and the settings for database searches. The application uses these settings only when you select the Database Search option as a search type. See To specify the compound databases to use. The application compares the specified compound databases with found peaks from the mass analyzer peak detector.

When a found peak is within the mass range of a compound in a specified database, the application scores it as a match and adds the labeled peak result to the Data Review results grid for unknown screening analysis.

If the application finds a conflict of information, then the application uses the next level of identification to compare the fragments of any associated data-dependent scan. The application chooses the peak with the most matching fragments as the name of the identified peak.

If there are no fragments available, then the application labels the compound database entry with the mass that is closest to the mass of the extracted mass analyzer peak.

Follow these procedures:

- To open the Database Settings page
- To specify the database matching settings
- To specify the fragments settings
- To specify the isotopes settings
- To specify the compound databases to use

❖ To open the Database Settings page

Click the **Database Settings** tab.

The Database Settings Page opens.

❖ To specify the database matching settings

1. In the CDB RT Window box, type a value (in seconds) for the database retention time window.
2. Specify the number of millimass units or parts per million for the Mass Tolerance.
3. Select the units of measure for the *m/z* ± tolerance value.

   The application applies this mass tolerance range to the extracted chromatograms.

❖ To specify the fragments settings

1. In the Intensity Threshold box, type a value for the minimum intensity value for fragments.

   Fragments with intensity values less than this value are ignored.

2. Specify the number of millimass units or parts per million for the Mass Tolerance.
3. Select the units of measure for the *m/z* ± tolerance value.

   The application applies this mass tolerance range to the fragment chromatograms.
4. To specify the source for the peaks, set the MS Order to **MS**, **MS2**, or **MS–In Source CID**.

❖ To specify the isotopes settings

1. In the Allowed Mass Deviation box, type a value (in parts per million) to specify the allowed mass deviation in the spectrum data.

   The isotopic pattern algorithm considers an isotope peak as found if its measured *m/z* is less than this deviation amount away from its expected *m/z*. For best results, set this value to a number that causes up to 98 percent of all mass deviations to be smaller than the allowed mass deviation value.
2. In the Allowed Intensity Deviation box, type a value to specify the allowed intensity deviation of the mass spectrometer relative to the monoisotopic ion, as a percentage of the base peak height.

   The isotopic pattern algorithm considers an isotope peak as not found if its intensity relative to the monoisotopic ion's intensity is more than this deviation percentage from the theoretical relative intensity of the isotope ion. For best results, set this value to a number that causes up to 98 percent of all intensity deviations to be smaller than the allowed intensity deviation value.
3. To specify that isotopic pattern calculations use internal mass calibration, select the **Use Internal Mass Calibration** check box. Otherwise, to use external mass calibration, clear the check box.

   When you select this check box, the application applies a requirement that an isotope's *m/z* must be closer to its theoretical value to avoid a score penalty. For more information, see Isotopic Pattern Details.

❖ To specify the compound databases to use

1. Select the **Selected** check box for each compound database that you want to use for the database search.
2. (Optional) To edit a database, click **Open** and do the following:
   a. Edit the database.

      See Chapter 2, "Using Compound Databases in the Method Development Mode."
   b. When you finish editing the database, click **Processing** in the Method View navigation pane to return to the Processing page.

Database Settings Page

Use the Database Settings page to specify the compound databases and settings for database searches. The application uses these settings only when you select the Database Search option as a search type.

Figure 134. Database Settings page

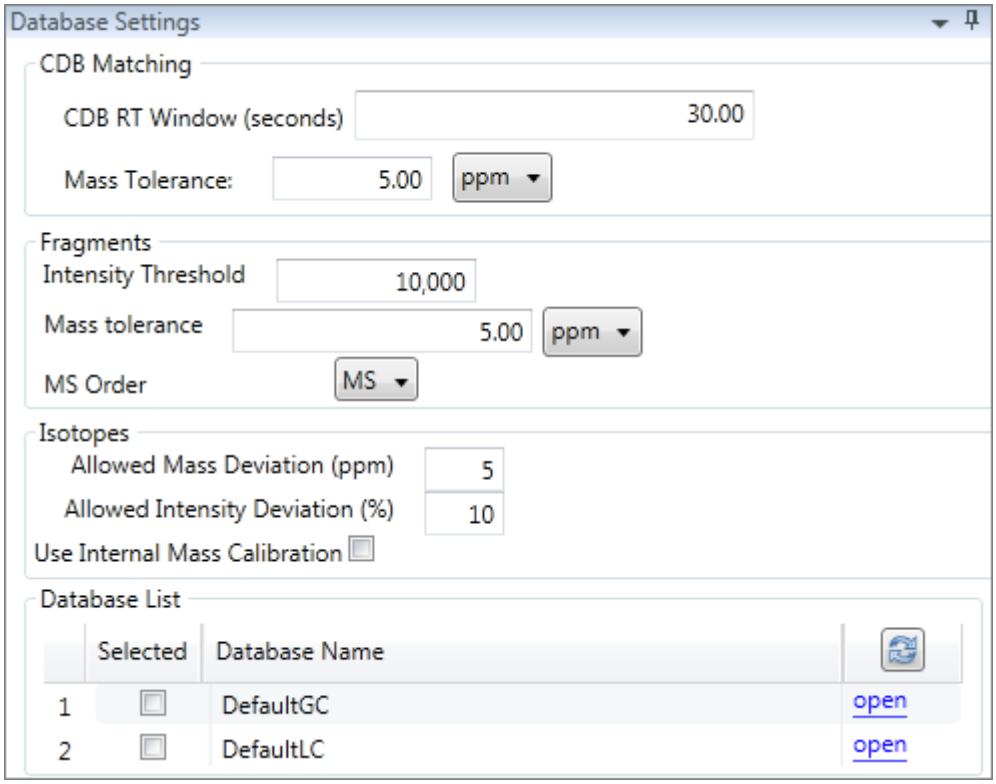


Table 89. Database Settings page parameters (Sheet 1 of 2)

Parameter	Description
CDB Matching	
CDB RT Window (seconds)	Specifies the database retention time window in seconds. Default: 30.00
Mass Tolerance	Specifies the *m/z* ± tolerance in units of mmu or ppm. • mmu (millimass units): A static calculation to the extracted mass. • (Default) ppm (parts per million): A variable calculation dependent on the actual mass. The smaller the mass, the narrower the tolerance range. The larger the mass, the wider the tolerance range. Default: 5.00 Valid range: 0.1 through 999

Table 89. Database Settings page parameters (Sheet 2 of 2)

Parameter	Description
Fragments	
Intensity Threshold	Fragments with intensity values less than this are ignored. Default: 10 000
Mass Tolerance	Specifies the *m/z* ± tolerance in units of mmu or ppm. • mmu (millimass units): A static calculation to the extracted mass. • ppm (parts per million): A variable calculation dependent on the actual mass. The smaller the mass, the narrower the tolerance range. The larger the mass, the wider the tolerance range. Default: 5.00 ppm Valid range: 0.1 through 999
MS Order	Specifies that the confirming peaks come from the same scan (MS), are fragments from an adjacent scan (MS2), or come from the Source CID (MS–In Source CID).
Isotopes	
Allowed Mass Deviation	Specifies the allowed mass deviation in the spectrum data. Valid range: 3 through 100 ppm Default: 5 ppm
Allowed Intensity Deviation	Specifies the allowed intensity deviation of the mass spectrometer relative to the monoisotopic ion, as a percentage of the base peak height. Valid range: 1 through 100% Default: 10%
Use Internal Mass Calibration	Specifies that isotopic pattern calculations use internal mass calibration.
Database List	
Selected	Specifies that the application uses the compound database for a database search.
Database Name	Specifies the name of the database.
Refresh 	Updates the database list.
Open	Opens the compound database in the Method Development mode.

Editing the Element Settings

Use the Element Settings page to specify settings to use for elemental composition searches. The application uses these settings only when you select the Elemental Composition option as a search type. See To specify the elemental composition settings.

Follow these procedures:

- To open the Element Settings page
- To specify the scoring settings
- To specify the elemental composition settings
- To select elements to use in the elemental formula

❖ To open the Element Settings page

Click the **Element Settings** tab.

The Element Settings Page opens.

❖ To specify the scoring settings

1. In the Allowed Mass Deviation box, type a value (in parts per million) to specify the allowed mass deviation in the spectrum data.

   The isotopic pattern algorithm considers an isotope peak as found if its measured *m/z* is less than this deviation amount away from its expected *m/z*. For best results, set this value to a number that causes up to 98 percent of all mass deviations to be smaller than the allowed mass deviation value.

2. In the Allowed Intensity Deviation box, type a value to specify the allowed intensity deviation of the mass spectrometer relative to the monoisotopic ion, as a percentage of the base peak height.

   The isotopic pattern algorithm considers an isotope peak as not found if its intensity relative to the monoisotopic ion's intensity is more than this deviation percentage from the theoretical relative intensity of the isotope ion. For best results, set this value to a number that causes up to 98 percent of all intensity deviations to be smaller than the allowed intensity deviation value.

3. To specify that isotopic pattern calculations use internal mass calibration, select the **Use Internal Mass Calibration** check box.

   Otherwise, to use external mass calibration, clear the check box.

   When using internal mass calibration, the application applies a requirement that an isotope's *m/z* must be closer to its theoretical value to avoid a score penalty.

For more information, see Appendix A, "Isotopic Pattern Details."

❖ To specify the elemental composition settings

1. From the Electron Rule list, select one of the following rules to use in the formula calculation: **Do Not Use**, **Even Electrons**, or **Odd Electrons**.
2. In the RDB Equiv Low and the RDB Equiv High boxes, type values for double bonds and ring equivalents—a measure of the number of unsaturated bonds in a compound—to limit the calculated formulas to only those that make sense.

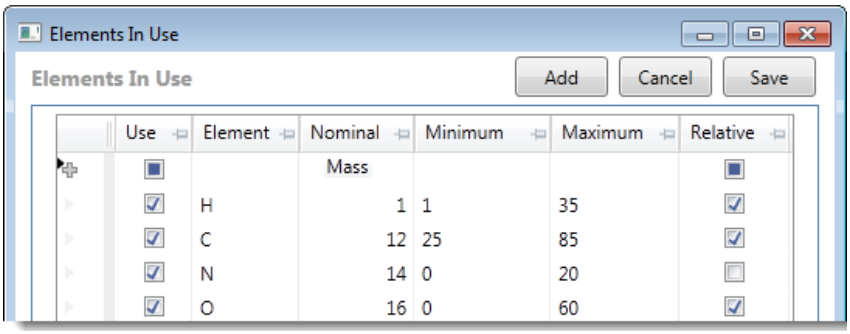
   The RDB Equiv Low value must be smaller than the RDB Equiv High value.

❖ To select elements to use in the elemental formula

1. Click **View Elements**.

   The Elements In Use dialog box opens. By default, all elements are selected for use.

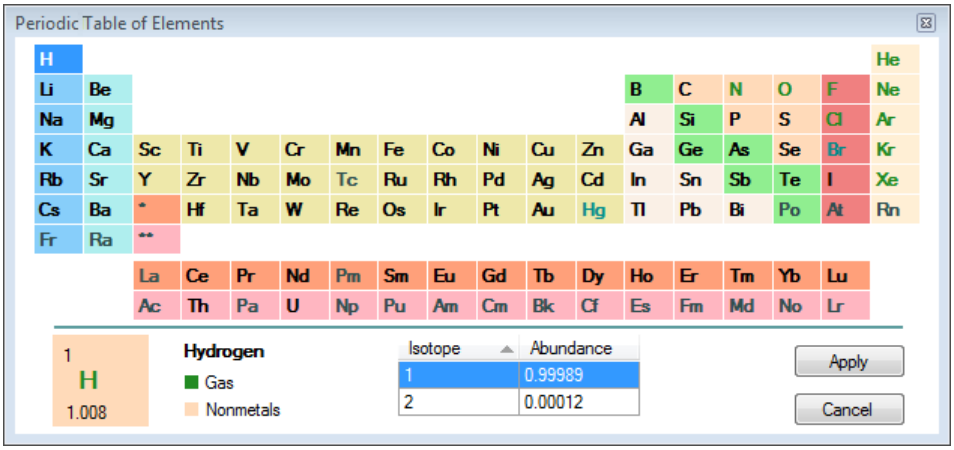
   Figure 135. Element in Use dialog box



2. To activate an element in the list, make sure its corresponding **Use** check box is selected.
3. To deactivate an element in the list, clear the corresponding **Use** check box.
4. To add an element to the list, do the following:
   a. Click **Add.**

      The Periodic Table of Elements opens.

      Figure 136. Periodic Table of Elements



b. Select an element and click **Apply**.

Tip If the element you select displays several isotopes, for best results, select the most abundant one before you click Apply.

Isotope	Abundance
1	0.99989
2	0.00012

The periodic table closes, and the application adds the selected element to the Elements In Use dialog box.

The values in the Element column and the Nominal Mass column are read-only. You can edit the values in the Minimum, Maximum, and Relative columns.

IMPORTANT Add candidates only for the neutral molecule, not for adducts.

5. In the Relative column, do one of the following:
   - To specify the maximum or minimum value as an absolute number of atoms, clear the check box.
   - To specify the maximum or minimum value as a relative percentage by weight, select the check box.

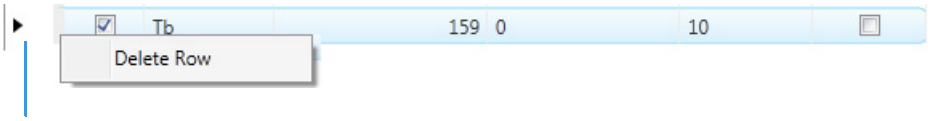
   Default: Selected for H, C, and O; cleared for all other elements

6. In the Minimum and Maximum columns, specify a minimum value and a maximum value to define the calculated element.

   You can type each of these values as either an absolute number of atoms (0 through 1000) or as a relative percentage by weight (0 through 100%), depending on the setting in the Relative column.

   The value you enter for the Minimum column must be smaller than the value for the Maximum column.

7. To remove elements from use in the elemental formula, do the following:
   a. Select the element row by clicking in the row selector area marked by the arrow icon.
   b. Right-click and choose **Delete Row** (or press the DELETE key).



Click here to select the row, right-click, and choose **Delete Row**.

   c. Click **Yes** in the confirmation dialog box to remove the selected rows from the table.

Tip Use the SHIFT or CTRL key to select contiguous or noncontiguous rows. Or, drag your pointer across the group of rows.

Tip You can also deactivate an element from use in the elemental formula without removing it from the table by clearing the element's Use check box.

Element Settings Page

Use the Element Settings page to specify settings to use for elemental composition searches.

Figure 137. Element Settings page

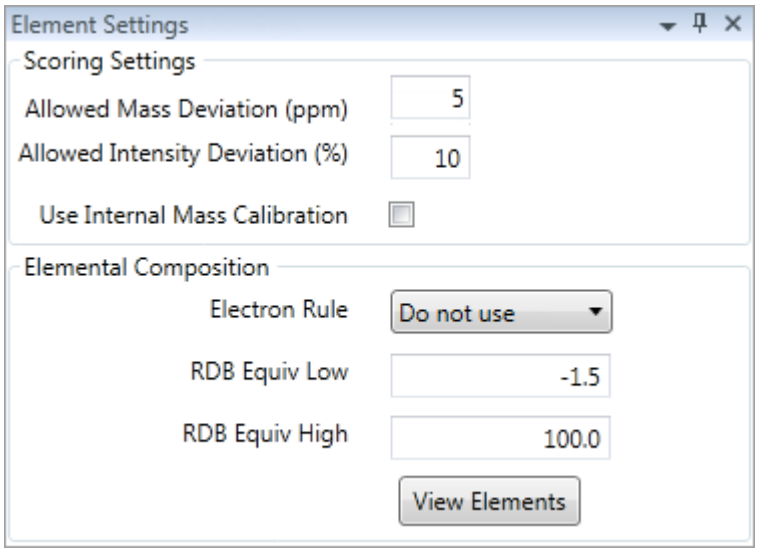


Figure 138. Element Settings page parameters (Sheet 1 of 2)

Parameter	Description
Scoring Settings	
Allowed Mass Deviation (ppm)	Specifies the allowed mass deviation in the spectrum data. Valid range: 3 through 100 ppm Default: 5 ppm
Allowed Intensity Deviation (%)	Specifies the allowed intensity deviation of the mass spectrometer relative to the monoisotopic ion, as a percentage of the base peak height. Valid range: 1 through 100% Default: 10%
Use Internal Mass Calibration	Specifies that isotopic pattern calculations use internal mass calibration.
Elemental Composition	
Electron Rule	Specifies whether to use a rule in the formula calculation. Valid values: Do Not Use, Even Electrons, Odd Electrons Default: Do Not Use
RDB Equiv Low	Specifies the lower limit value for double bonds and ring equivalents—a measure of the number of unsaturated bonds in a compound—to limit the calculated formulas to only those that make sense. The RDB Equiv Low value must be smaller than the RDB Equiv High value. Valid range: –1.5 through 99.9 Default: –1.5

Figure 138. Element Settings page parameters (Sheet 2 of 2)

Parameter	Description
RDB Equiv High	Specifies the upper limit value for double bonds and ring equivalents—a measure of the number of unsaturated bonds in a compound—to limit the calculated formulas to only those that make sense. Valid range: 0 through 100 Default: 100.00
View Elements	Opens the Elements in Use dialog box where you can specify the elements to use in the elemental formula.

Editing the ChemSpider Settings

Use the ChemSpider Settings page to specify settings to use for ChemSpider searches. The application uses these settings only when you select the ChemSpider Search option as a search type. See To open the ChemSpider Settings page.

ChemSpider is an online search engine that provides access to millions of chemical structures in databases. The ChemSpider search engine aggregates and indexes chemical structures and their associated information into a single, searchable repository.

IMPORTANT You must have an Internet connection to access the ChemSpider databases.

The application sends suggested elemental compositions to the ChemSpider search engine and retrieves compound names that match the searched elemental compositions. The application uses the active databases that you select and ranks the results according to the number of databases that return found references for each matched compound. The higher the number of databases with found references, the higher the ranking for that match. To speed processing, the application stores the results in a local cache. The cache maintains the age of each result from the time that the entry is saved. During a search, the application deletes any cached entry older than the time period specified in the Cached Entries Expire After list and replaces it with newer information.

Each time you update the list of active databases, the application clears the entire cache and starts over with new information from the next search. Unknown screening searches that use the ChemSpider search engine might take longer until the application populates the cache with results again. When you change the list of active databases, the ranking of the results might also change accordingly.

Follow these procedures:

- To open the ChemSpider Settings page
- To update the list of available databases
- To make databases active
- To specify an expiration time for cached entries

❖ To open the ChemSpider Settings page

Click the **ChemSpider Settings** tab.

The ChemSpider Settings Page opens.

❖ To update the list of available databases

Click **Refresh**.

The application updates the list of available databases with the most current list from the ChemSpider website.

IMPORTANT Each time you access the ChemSpider Settings page, the application automatically compares the list of available databases with the most current list from the ChemSpider website. The Refresh button becomes available only when there is a difference between the two lists.

❖ To make databases active

1. From the Available Databases list, select the ChemSpider databases that you want to search.

Note Use the CTRL or SHIFT keys to select multiple databases.

2. Click >> to move the selected databases to the Active Databases list.

The application searches all active ChemSpider databases for matching compound formulas.

IMPORTANT Whenever you change the list of active databases, the application deletes all previous results stored in the cache and starts over with new information from the next search. This might increase the time required to complete the search.

IMPORTANT If a database in the Active Databases box is no longer available on the website, the application automatically removes that database name.

❖ To specify an expiration time for cached entries

Select one of the following time periods from the Cached Entries Expire After list:

- ThreeMonths
- SixMonths
- OneYear
- Never

During a search, the application deletes any cached entry older than this value and replaces it with newer information.

ChemSpider Settings Page

Use the ChemSpider Settings page to specify settings to use for ChemSpider searches.

Figure 139. ChemSpider Settings page

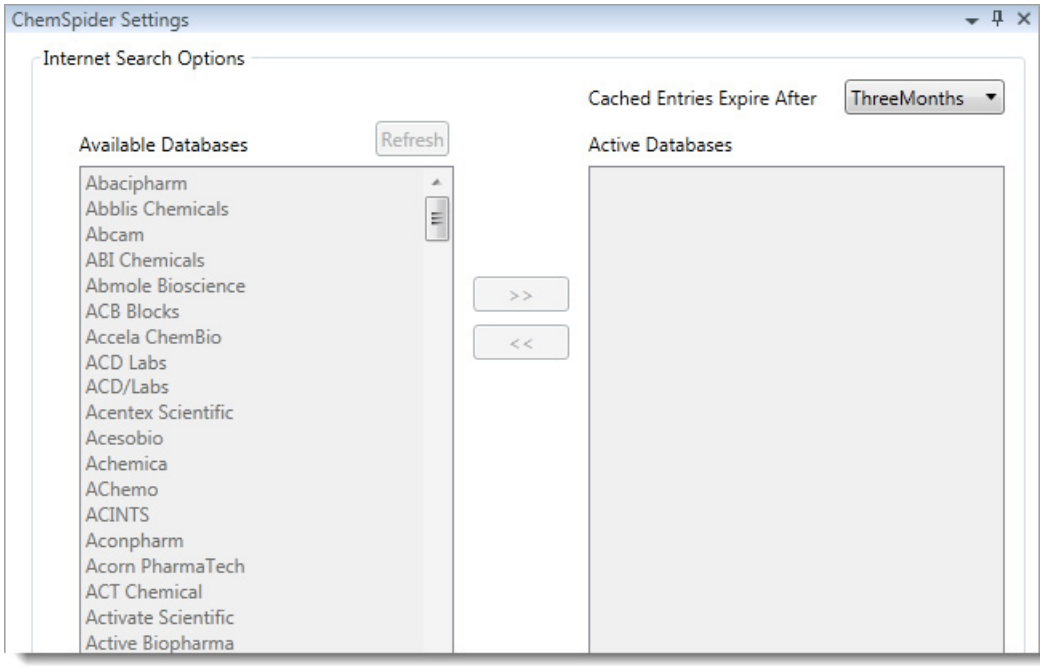


Table 90. ChemSpider Settings page parameters

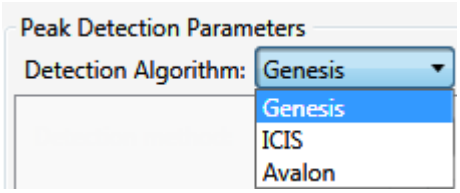
Parameter	Description
Available Databases	Lists the most current databases from the ChemSpider website.
Refresh	Updates the list of available databases with the most current list from the ChemSpider website.
Cached Entries Expire After	During a search, the application deletes any cached entry older than the specified value and replaces it with newer information.
Active Databases	Lists the ChemSpider databases that you can search.

Editing the Peak Detection Settings Page

Use the features on the Peak Detection page to specify any of the following peak detection algorithms: Genesis, ICIS, or Avalon.

❖ To specify peak detection parameters

1. Click **Peak Detection** in the Method View navigation pane.
2. In the Peak Detection Parameters area, select one of the detection algorithms: **Genesis**, **ICIS**, or **Avalon**.



   - The Genesis peak detection algorithm provides backward compatibility with Xcalibur 1.0 studies. For detailed descriptions of the Genesis method parameters, see Genesis peak detection parameters.
   - The ICIS peak detection algorithm is designed for MS data and has highly efficient peak detection at low MS signal levels. For detailed descriptions of the ICIS method parameters, see ICIS peak detection parameters.
   - The Avalon peak detection algorithm is designed for integrating UV/Vis and analog chromatograms. For detailed descriptions of the Avalon method parameters, see Avalon peak detection parameters.
3. Specify the parameters for the selected detection algorithm.

Genesis Detection Method

The application provides the Genesis peak detection algorithm for backward compatibility with Xcalibur 1.0 studies.

Figure 140. Genesis peak detection

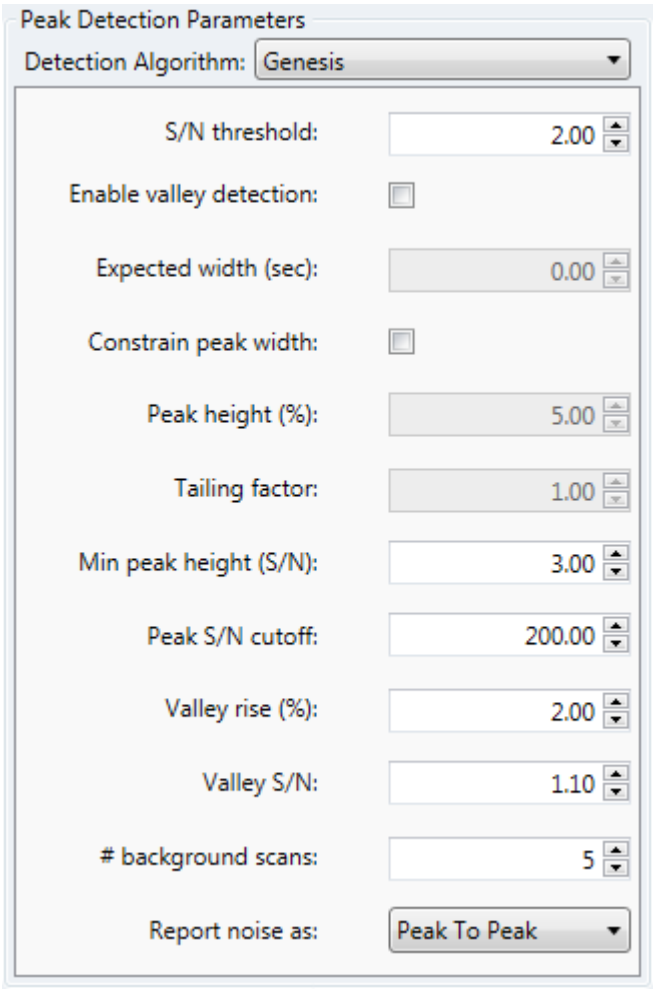


Table 91. Genesis peak detection parameters (Sheet 1 of 3)

Parameter	Description
Detection Algorithm	Specifies the Genesis peak detection algorithm.
S/N Threshold	Current signal-to-noise threshold for peak integration. Peaks with signal-to-noise values less than this value are not integrated. Peaks with signal-to-noise values greater than this value are integrated. Valid range: 0.0 through 999.0

Table 91. Genesis peak detection parameters (Sheet 2 of 3)

Parameter	Description
Enable Valley Detection	Uses the valley detection approximation method to detect unresolved peaks. This method drops a vertical line from the apex of the valley between unresolved peaks to the baseline. The intersection of the vertical line and the baseline defines the end of the first peak and the beginning of the second peak.
Expected Width (sec)	The expected peak width parameter (in seconds). This parameter controls the minimum width that a peak is expected to have if valley detection is enabled. With valley detection enabled, any valley points nearer than the *expected width*/2 to the top of the peak are ignored. If a valley point is found outside the expected peak width, the application terminates the peak at that point. The application always terminates a peak when the signal reaches the baseline, independent of the value set for the expected peak width. Valid range: 0.0 through 999.0
Constrain Peak Width	Constrains the peak width of a component during peak integration of a chromatogram. You can then set values that control when peak integration is turned on and off by specifying a threshold and a tailing factor. Selecting the Constrain Peak Width check box activates the Peak Height (%) and Tailing Factor options.
Peak Height (%)	A signal must be above the baseline percentage of the total peak height (100%) before integration is turned on or off. This text box is active only when you select the Constrain Peak Width check box. Valid range: 0.0 through 100.0%
Tailing Factor	A factor that controls how the application integrates the tail of a peak. This factor is the maximum ratio of the trailing edge to the leading side of a constrained peak. This text box is active only when you select the Constrain the Peak Width check box. Valid range: 0.5 through 9.0
Min Peak Height (S/N)	Specifies the minimum peak height measured as a signal-to-noise ratio. Valid range: 0.00 through 999.00 Default: 3.00

Table 91. Genesis peak detection parameters (Sheet 3 of 3)

Parameter	Description
Peak S/N Cutoff	The peak edge is set to values below this signal-to-noise ratio. This test assumes it has found an edge of a peak when the baseline adjusted height of the edge is less than the ratio of the baseline adjusted apex height and the peak S/N cutoff ratio. When the S/N at the apex is 500 and the peak S/N cutoff value is 200, the application defines the right and left edges of the peak when the S/N reaches a value less than 200. Valid range: 50.0 through 10000.0
Valley Rise (%)	The peak trace can rise above the baseline by this percentage after passing through a minimum (before or after the peak). This criteria is useful for integrating peaks with long tails. This method drops a vertical line from the apex of the valley between unresolved peaks to the baseline. The intersection of the vertical line and the baseline defines the end of the first peak and the beginning of the second peak. When the trace exceeds rise percentage, the application applies valley detection peak integration criteria. This test is applied to both the left and right edges of the peak. Valid range: 0.1 through 500.0
Valley S/N	Specifies a value to evaluate the valley bottom. Using this parameter ensures that the surrounding measurements are higher. Valid range: 1.0 through 100.0 Default: 2.0
# Background Scans	Number of background scans performed by the application.
Report Noise As	Determines if the noise used in calculating S/N values is calculated using an RMS calculation or peak-to-peak resolution threshold. Options are RMS or Peak To Peak.

ICIS Detection Method

The ICIS peak detection algorithm is designed for MS data and has highly efficient peak detection at low MS signal levels.

Figure 141. ICIS peak detection

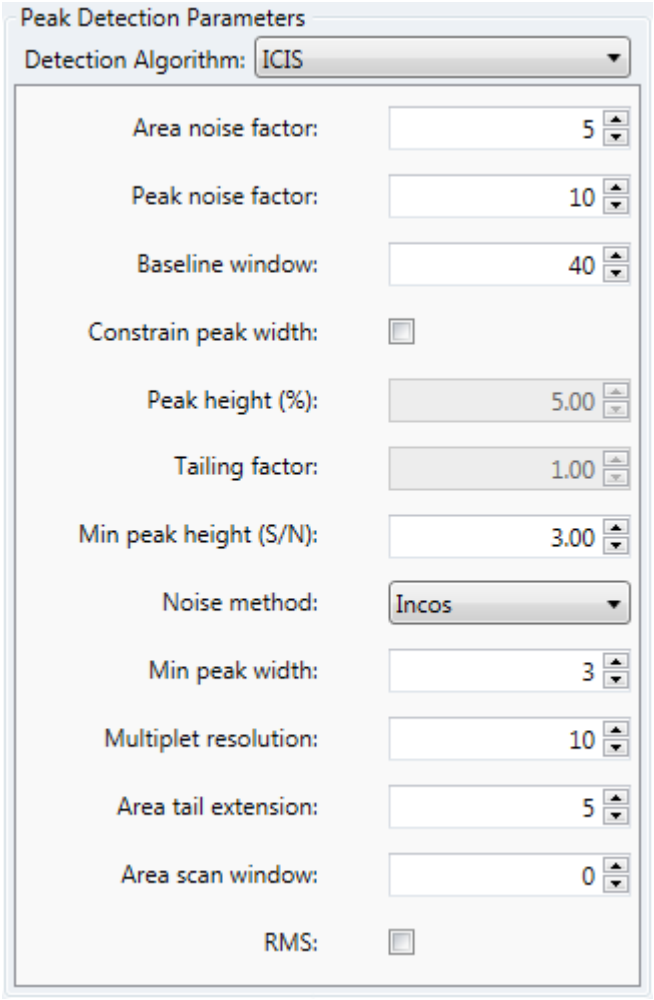


Table 92. ICIS peak detection parameters (Sheet 1 of 3)

Parameter	Description
Detection Algorithm	Specifies the ICIS peak detection algorithm.
Area Noise Factor	The noise level multiplier used to determine the peak edge after the location of the possible peak. The ICIS peak detection algorithm uses this value. Valid range: 1 through 500 Default: 5

Table 92. ICIS peak detection parameters (Sheet 2 of 3)

Parameter	Description
Peak Noise Factor	The noise level multiplier used to determine the potential peak signal threshold. The ICIS peak detection algorithm uses this value. Valid range: 1 through 1000 Default: 10
Baseline Window	The application looks for a local minima over this number of scans. The ICIS peak detection algorithm uses this value. Valid range: 1 through 500 Default: 40
Constrain Peak Width	Constrains the peak width of a component during peak integration of a chromatogram. You can then set values that control when peak integration is turned on and off by specifying a peak height threshold and a tailing factor. Selecting the Constrain Peak Width check box activates the Peak Height (%) and Tailing Factor options.
Peak Height (%)	A signal must be above the baseline percentage of the total peak height (100%) before integration is turned on or off. This text box is active only when you select the Constrain Peak Width check box. Valid range: 0.0 through 100.0%
Tailing Factor	A factor that controls how the application integrates the tail of a peak. This factor is the maximum ratio of the trailing edge to the leading side of a constrained peak. This text box is active only when you select the Constrain the Peak Width check box. Valid range: 0.5 through 9.0
Min Peak Height (S/N)	Specifies the minimum peak height measured as a signal-to-noise ratio. Valid range: 0.00 through 999.00 Default: 3.00
Noise Method	The options are INCOS or Repetitive. INCOS: Uses a single pass algorithm to determine the noise level. The ICIS peak detection algorithm uses this value. Repetitive: Uses a multiple pass algorithm to determine the noise level. The ICIS peak detection algorithm uses this value. In general, this algorithm is more accurate in analyzing the noise than the INCOS Noise algorithm, but the analysis takes longer.

Table 92. ICIS peak detection parameters (Sheet 3 of 3)

Parameter	Description
Min Peak Width	The minimum number of scans required in a peak. The ICIS peak detection algorithm uses this value. Valid range: 0 through 100 scans Default: 3
Multiplet Resolution	The minimum separation in scans between the apexes of two potential peaks. This is a criteria to determine if two peaks are resolved. The ICIS peak detection algorithm uses this value. Valid range: 1 through 500 scans Default: 10
Area Tail Extension	The number of scans past the peak endpoint to use in averaging the intensity. The ICIS peak detection algorithm uses this value. Valid range: 0 through 100 scans Default: 5
Area Scan Window	The number of allowable scans on each side of the peak apex. A zero value defines all scans (peak-start to peak-end) to be included in the area integration. Valid range: 0 through 100 scans Default: 0
RMS	Specifies that the application calculates noise as RMS. By default, the application uses Peak To Peak for the noise calculation. RMS is automatically selected if you manually determine the noise region.

Avalon Detection Method

The Avalon peak detection algorithm is designed for UV data. The Avalon peak detection algorithm also supports negative peaks. You can edit the Event values from the Avalon Event List.

Figure 142. Avalon peak detection

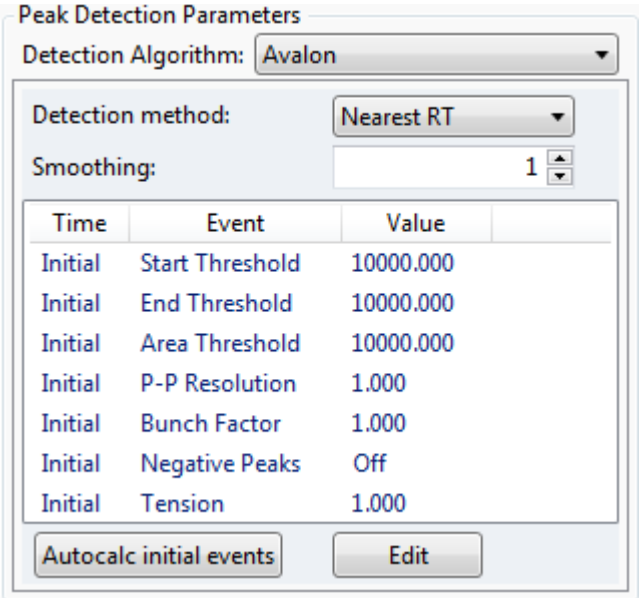


Table 93. Avalon peak detection parameters

Parameter	Description
Detection Algorithm	Specifies the Avalon peak detection algorithm.
Detection Method	Specifies the peak detection method used for analyte compounds. Highest Peak: Uses the highest peak in the chromatogram for component identification. Nearest RT: Uses the peak with the nearest retention time in the chromatogram for component identification.
Smoothing	Determines the degree of data smoothing to be performed on the active component peak prior to peak detection and integration. The ICIS peak detection algorithm uses this value. Valid values: Any odd integer from 1 through 15 points Default: 1
Time/Event/Value	Displays the events specified in the Avalon Event List dialog box. Initially displays only the default events that cannot be edited or deleted.
Autocalc Initial Events	Automatically calculates the events in the Event list.
Edit	Opens the Avalon Event List dialog box where you can edit the Time/Event/Value parameters.

Avalon Event List

The event list includes both user-defined and noneditable default events. The application displays the default events when you choose Avalon sensitivity. You cannot delete these events or change their time or values. For a detailed list of events and value ranges, see Event types.

Figure 143. Avalon Event List dialog box

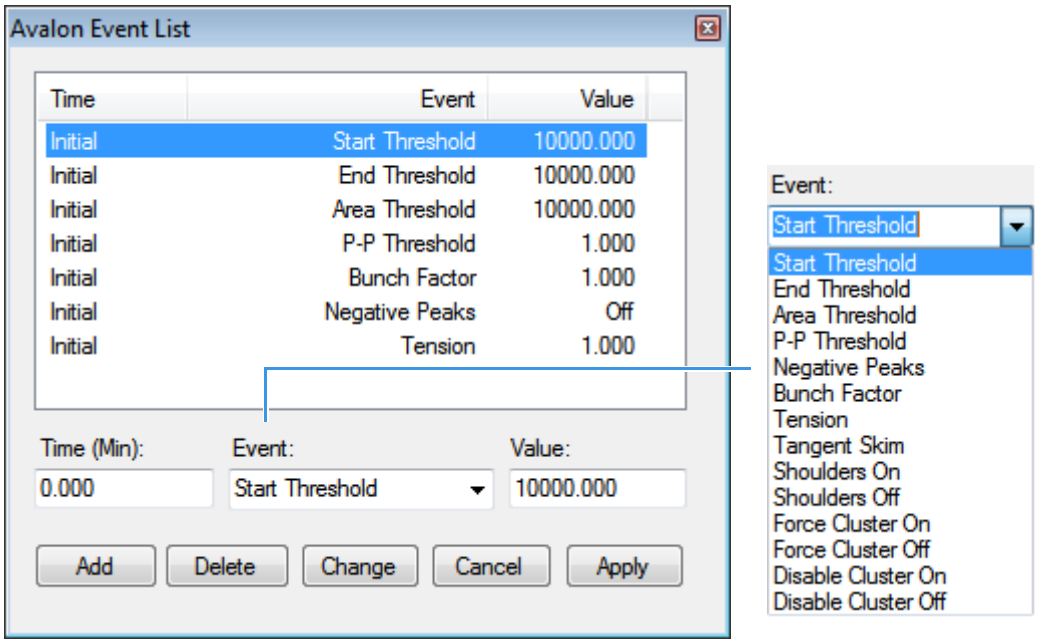


Table 94. Avalon Event List dialog box parameters

Parameter	Description
Time (Min)	Specifies the start time of the event.
Event	Specifies the type of event. For a detailed list of events and value ranges, see Event types.
Value	Specifies the value of the event.
Add	Adds a new event to the list with the current Time/Event/Value parameters.
Delete	Removes the selected Time/Event/Value parameter from the event list.
Change	Applies the current parameter values.
Cancel	Closes the dialog box without making any changes. Any additions, deletions, or changes revert to their original state.
Apply	Closes the dialog box.

Figure 144. Event types

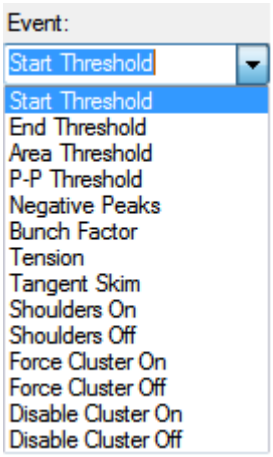


Table 95. Event type descriptions (Sheet 1 of 2)

Event type	Description
Start Threshold	Specifies the threshold at the start of a peak. The Start Threshold is directly related to the RMS noise in the chromatogram. Valid range: 0 through 999 999 999
End Threshold	Specifies the threshold at the end of a peak. The End Threshold is directly related to the RMS noise in the chromatogram. Valid range: 0 through 999 999 999
Area Threshold	Controls the area cutoff. Any peaks with a final area less than the area threshold will not be detected. This control is in units of area for the data. Valid range: 0 through 999 999 999
P-P Threshold	The peak-to-peak resolution threshold controls how much peak overlap must be present before two or more adjacent peaks create a peak cluster. Peak clusters have a baseline drop instead of valley-to-valley baselines. Specified as a percent of peak height overlap. Valid range: 0.1 through 99.99
Negative Peaks	Permits detection of a negative going peak. Automatically resets after finding a negative peak. Valid values: On or Off
Bunch Factor	Specifies the number of points grouped together during peak detection. This event controls the bunching of chromatographic points during integration and does not affect the final area calculation of the peak. A high bunch factor groups peaks into clusters. Valid range: 0 through 999

Table 95. Event type descriptions (Sheet 2 of 2)

Event type	Description
Tension	Controls how closely the baseline should follow the overall shape of the chromatogram. A lower tension traces the baseline to more closely follow changes in the chromatogram. A high baseline tension follows the baseline less closely, over longer time intervals. Valid range: 0 through 999.99 minutes
Tangent Skim	Using this event, you can tangent skim any peak clusters. By default, it chooses the tallest peak in a cluster as the parent. You can also identify which peak in the cluster is the parent. Tangent skim peaks are detected on either side (or both sides) of the parent peak. Tangent skim automatically resets at the end of the peak cluster. Valid range: 0 through 1
Shoulders On	Allows peak shoulders to be detected (peaks which are separated by an inflection rather than a valley) Sets a threshold for the derivative.
Shoulders Off	Disables peak shoulder detection. Valid range: 0 through 50
Force Cluster On	Force the following peaks to be treated as a cluster (single peak).
Force Cluster Off	End the forced clustering of peaks.
Disable Cluster On	Prevent any peaks from being clustered.
Disable Cluster Off	Permit clusters to occur again.

Editing the Reports Page

Use the Reports Page to specify how you want to save or print your reports.

See the instructions for the following tasks:

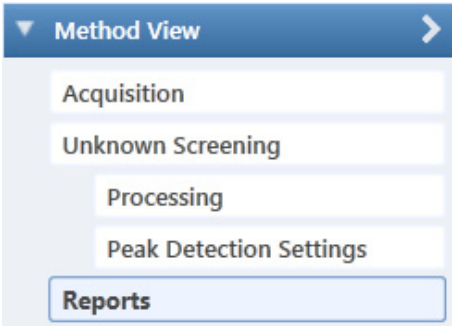
- Specifying Report Formats
- Specifying Report Options

Specifying Report Formats

For each report type, you can create a hard-copy printout, a PDF file, a CSV file, or an Excel file.

❖ To open the Reports page

Click **Reports** in the Method View navigation pane.

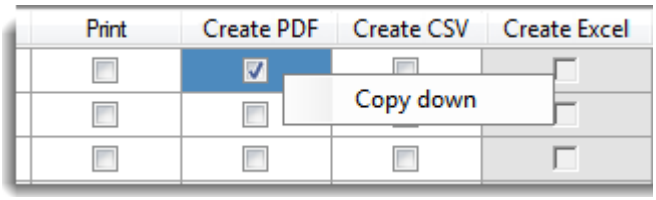


The Reports page opens with a list of all configured reports.

To configure which reports are available when you create a method or which reports create a batch-level report, see Specifying the Report Selections.

❖ To specify reports and output formats

1. To specify the type of report output to create for each report, select the check box in the appropriate column.
2. To duplicate an output type for all reports, click the cell to select it, and then right-click and choose **Copy Down**.



All check boxes in the column below the selected cell duplicate the selected or cleared state of the selected cell. This action applies only to reports where this output format is available. By default, all report type options are cleared.

Reports Page

Use the features on the Reports page to specify how you want to save or print your reports.

Figure 145. Reports page

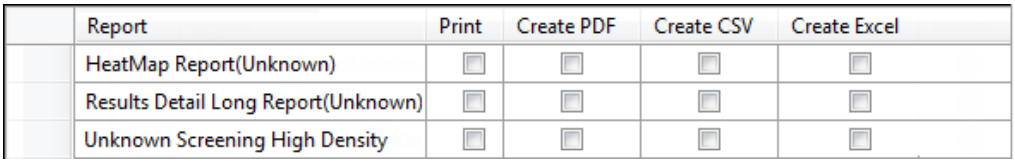


Table 96. Reports page parameters

Parameter	Description
Report list columns	
Report	The name of a report.
Print	Sends reports to the default printer.
Create PDF	Saves reports as PDF files.
Create CSV	Saves reports as CSV files.
Create Excel	Saves reports as Excel files.

Specifying Report Options

Use the options on the Report Options page to choose the peaks to display in reports.

Figure 146. Reports Options page

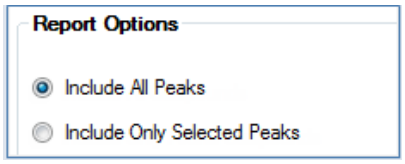


Table 97. Report Options page parameters

Parameter	Description
Include All Peaks	Generates reports that include all peak results.
Include Only Selected Peaks	Generates reports that include only selected peaks.

Saving an Unknown Method to a New Name

You can save any method to a new name, or you can use the current method data to overwrite an existing method. The new method contains all the data of the saved method.

❖ To save a method to a new name

1. From the main menu, choose **File > Save As.**

   The Save Master Method As dialog box opens, displaying all available unknown methods.

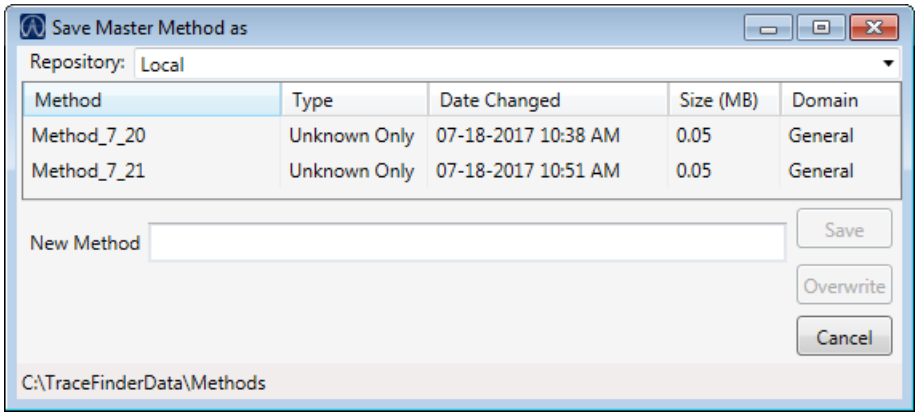


Table 98. Save Master Method As dialog box parameters

Parameter	Description
Repository	The data repository where you want to store the master method.
Method	Name of the quantitation methods in the selected repository.
Type	Type of method: Unknown Only
Date Changed	Date the method was last updated.
Size	Size of the method in megabytes.
Domain	TraceFinder domain for which the method was created.
New Method	Name of the new method to create.
Path	Path to the selected method in the …\TraceFinderData\Methods folder.
Save	Saves the current method to a new name.
Overwrite	Overwrites an existing method.
Cancel	Closes the dialog box without saving the current method to a new name.

2. Do one of the following:
   - In the New Method box, type a name for the new method.

     The Save button is now available.
   - In the New Method box, type the name of an existing method.

     The Overwrite button is now available.
3. Click **Save** or **Overwrite**.

   The application saves all the method data using the specified name and opens the Acquisition page of the new method.

A

Isotopic Pattern Details

The TraceFinder application calculates an isotopic pattern score based on the settings in the method. The application displays this score in the Data Review view. This appendix describes the isotopic distribution concepts and provides calculation details with examples for the isotopic pattern score.

Contents

- Isotopic Distribution in Exact Mass Spectra
- Isotopic Pattern Score Calculations

Isotopic Distribution in Exact Mass Spectra

To determine the elemental compositions, the product-name application uses an isotopic pattern matching algorithm that considers the isotope accurate mass and intensity ratios. Using a single exact mass, usually the monoisotopic mass of a measured isotope pattern, the application calculates all possible elemental compositions that lie within a mass tolerance window. You can filter this list of possible elemental compositions and narrow the results by using the natural isotopic distribution of elements.

Natural Isotopic Distribution

The following table lists the natural isotopic distribution of the most common elements.

Table 99. Natural isotopic distribution (Sheet 1 of 2)

Element	Isotope	Isotope order	Exact mass	Mass difference	Abundance (%)
Hydrogen	^{1}H	A0	1.0078		99.9985
	^{2}H	A1	2.014102	+1.006302	0.015
Carbon	^{12}C	A0	12.0		98.890
	^{13}C	A1	13.003355	+1.003355	1.110
Nitrogen	^{14}N	A0	14.003074		99.634
	^{15}N	A1	15.00109	+0.998016	0.366

Table 99. Natural isotopic distribution (Sheet 2 of 2)

Element	Isotope	Isotope order	Exact mass	Mass difference	Abundance (%)
Oxygen	^{16}O	A0	15.994915		99.762
	^{17}O	A1	16.999132	+1.004217	0.038
	^{18}O	A2	17.999161	+2.004246	0.200
Fluorine	^{19}F	A0	18.99840		100
Phosphorus	^{31}P	A0	30.971459		100
Sulfur	^{32}S	A0	31.972071		95.020
	^{33}S	A1	32.971459	+0.999388	0.750
	^{34}S	A2	33.967867	+1.995796	4.210
	^{36}S	A4	35.967081	+3.995010	0.020

where:

- A0 represents the monoisotopic peak, which is the most abundant and usually the isotope with the lowest mass.

  For example: ^{1}H, ^{12}C, ^{14}N, ^{16}O, ^{19}F, ^{31}P, and ^{32}S

- A1 represents the isotope where one atom in the molecule is statistically replaced by another atom approximately 1 amu heavier.

  For example: ^{2}H, ^{13}C, ^{15}N, ^{17}O, and ^{33}S

- A2 represents the isotope where one of the following is true:
  - Two atoms in the molecule are statistically replaced by two other atoms, each approximately 1 amu heavier.
  - One atom is replaced by another atom approximately 2 amu heavier.

  For example: ^{18}O and ^{34}S

- A3 represents the isotope where one of the following is true:
  - Three atoms in the molecule are statistically replaced by three other atoms, each approximately 1 amu heavier.
  - One atom is replaced by another atom approximately 1 amu heavier and one atom is replaced by another atom approximately 2 amu heavier.
  - One atom is replaced by another atom approximately 3 amu heavier.

- A4 represents the isotope where one of the following is true:
  - Four atoms in the molecule are statistically replaced by four other atoms, each approximately 1 amu heavier.

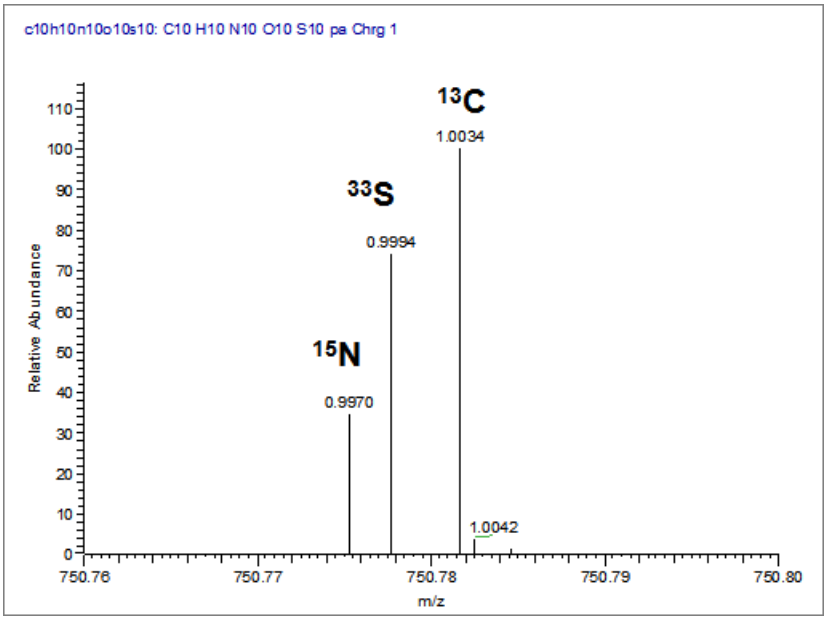
  - Two atoms are replaced by two other atoms, each approximately 1 amu heavier, and one atom is replaced by another atom approximately 2 amu heavier.
  - Each atom of the two atoms is replaced by another atom approximately 2 amu heavier.
  - One atom is replaced by another atom approximately 1 amu heavier, and one atom is replaced by another atom approximately 3 amu heavier.
  - One atom is replaced by another atom approximately 4 amu heavier.

  For example: ^{36}S

- Mass difference is the difference in mass between the A0 isotope and another isotope (A1, A2, A3, A4, and so on) of the same element.
- Abundance is the percentage of occurrence of each isotope normally in nature.

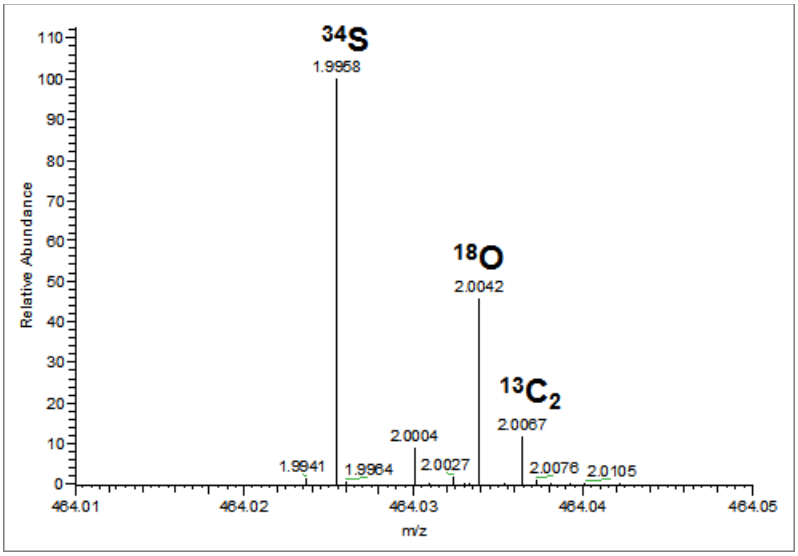
In the following figure, the *x* axis shows the mass difference of A1 relative to the monoisotopic peak (A0) of the ^{13}C, ^{15}N, and ^{33}S isotopes. The *y* axis shows relative abundance in intensity.

Figure 147. Mass difference and abundance of A1 relative to A0



In the following figure, the *x* axis shows the mass difference of A2 relative to the monoisotopic peak (A0) of the ^{18}O, ^{34}S, and $^{13}C2$ isotopes. The *y* axis shows relative abundance in intensity.

Figure 148. Mass difference and abundance of A2 relative to A0



Note For a particular isotopic spectrum, the mass difference is always the same between the A0 isotope and the other isotopes (A1, A2, and so on) of each specific element, but the intensity varies according to the composition of the molecule—that is, the number of each element in the molecule.

Isotopic Pattern Score Calculations

The product-name application follows the same isotopic distribution logic as described in Isotopic Distribution in Exact Mass Spectra, but in a different order and with numerical limits and scores to optimize automatic processing. After the application determines the possible elemental compositions for a particular compound of interest, it calculates an expected isotope pattern for each elemental composition candidate and an isotopic pattern score to represent the fit between the expected and measured isotope patterns.

The following data set example describes the isotopic pattern score data and provides the score calculation details for one specific data set.

Data Set Example

This example uses the **Metribuzin** target compound in an **Apple_PosHCD__40_5_01** sample.

For this compound, note that the Isotopic Pattern Score column shows 90% and the Num Isotopes Matched column shows "2 of 3" in the Compound Results pane in the Data Review. The compound's formula is C8H14N4OS and its adduct is H, so the modeled isotopic pattern is C8H15N4OS.

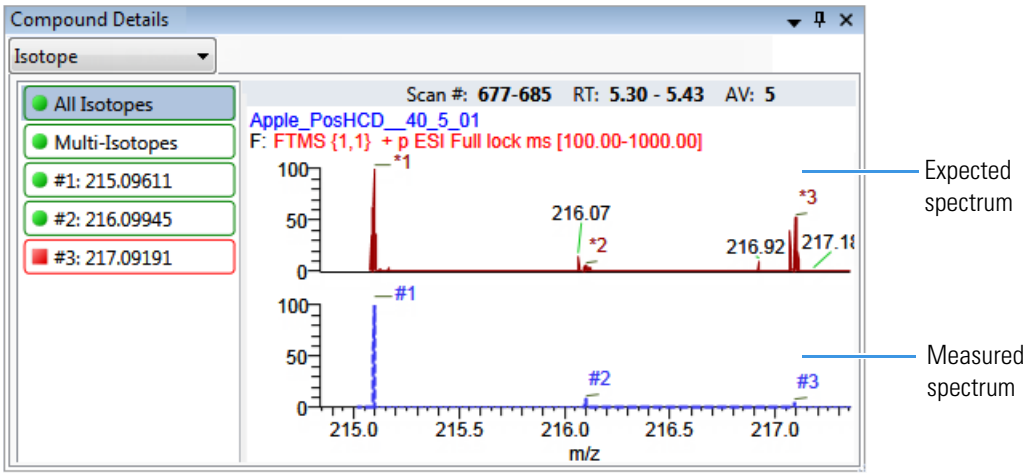
Figure 149. Isotopic data for Metribuzin

Compound Results

	Compound	Formula	Isotopic Pattern Score (%)	Num Isotopes Matched
640	Metribuzin	C8H14N4OS	90	2 of 3

In the Compound Details pane, select **Isotope** and zoom in to view the expected isotopic pattern spectrum compared to the acquired, measured spectrum. The resulting isotopic pattern score should correlate to a visual inspection of the difference between the expected isotope display and the measured display for the target compound.

Figure 150. Isotopic pattern spectra (stacked)



For this example, the processing method used to process the data contains the following isotopic pattern settings for target screening:

- Fit threshold = 90%
- Allowed Mass Deviation = 5 ppm
- Allowed Intensity Deviation = 10%
- Use Internal Mass Calibration = Cleared

The data for the **Metribuzin** compound follows.

Measured	Expected
A0 *m/z* = 215.09602	A0 *m/z* = 215.09611
A0 Noise = 1482	–
A0 intensity = 4.75E4	A0 intensity = 8.55E5

Because the measured and the expected spectra have different intensities, the spectral noise threshold must proportionally apply to the expected spectrum to decide which peaks are expected in the measured data.

Noise threshold (expected) = *Noise of A0 (measured)* × *Intensity of A0 (expected)* ÷ *Intensity of A0 (measured)*

Following the previous formula, the expected noise threshold is 1482 × 8.55E5 ÷ 4.75E4 = 2.6676E4.

The expected ions in the measured spectrum are those whose intensities are above the expected noise threshold. The following table lists the ions in the expected spectrum. A "✓" in the Above Threshold column indicates the expected ions.

Table 100. Ions in expected spectrum

Isotope order	*m/z* (expected)	Intensity (expected)	Above threshold	Present in measured spectrum
A0	215.09611	8.55E5	✓	Yes
A1	216.09945	7.50E4	✓	Yes
A2	217.09191	3.86E4	✓	Yes
A3	218.09521	3.39E3		No

Calculating Mass and Intensity Deviations

The expected number of ions is 3, as shown by those ions with a "✓" in the Above Threshold column of the Ions in expected spectrum table. These are the ions you focus on for the scoring calculations. This number "3" shows as the value of *y* in the Num Isotopes Matched column of the Compound Results pane in the Data Review. It indicates the number of expected isotopic pattern peaks based on the Fourier transform (FT) noise in the spectrum.

In this case, you expect to see the three most intense expected peaks in the measured spectrum. The masses of those peaks are (in order of intensity from high to low): 215.09611, 216.09945, and 217.09191. When the measured spectrum is more intense or the noise level is lower, you find more peaks passing the noise threshold and expected in the measured spectrum, eventually including other isotopic peaks.

The following table shows the mass deviation (delta *m/z*) data for each of the expected ions.

Table 101. Mass deviation data for the expected ions

Isotope order	*m/z* (expected)	*m/z* (measured)	Delta *m/z* (ppm)
A0	215.09611	215.09602	–0.42
A1	216.09945	216.09879	–3.05
A2	217.09191	217.09712	24

where:

Delta *m/z* (ppm) = 1 000 000 × ([*m/z (measured)* – *m/z (expected)*] ÷ *m/z (expected)*)

For example:

1 000 000 × ([216.09879 – 216.09945] ÷ 216.09945)] = –3.05 ppm

Tip You can see the expected, measured, and delta *m/z* values on the Isotope page of the Compound Details pane.

If you want more precision, you can see extra decimal digits for the A0 *m/z* values in an exported data file.

If the absolute value of the Delta *m/z* is less than 5 ppm (the Allowed Mass Deviation value set in the processing method), the product-name application determines that this ion is found—that is, the ion is present in the measured spectrum. For this data set example, the application finds only the A0 and A1 ions, so "2" shows as the value of *x* in the Num Isotopes Matched column of the Compound Results pane in the Data Review. The application does not find the A2 expected ion because the absolute value of its Delta *m/z* of 24 ppm is much higher than 5 ppm.

Note You can see from zooming in on the isotopic pattern spectra (see Isotopic pattern spectra (stacked)) that there are measured peaks in the measured spectrum closely corresponding to the first two expected ions, but there is not a measured peak closely corresponding to the 217.09191 expected ion.

The following table lists the intensity deviation (delta intensity) data for each of the expected ions, relative to the A0 ion's expected intensity of 8.55E5 and measured intensity of 4.75E4.

Table 102. Intensity deviation data for the expected ions

Isotope order	*m/z* (expected)	Intensity (expected)	Relative intensity (expected, %)	Intensity (measured)	Relative intensity (measured, %)	Delta intensity
A0	215.09611	8.55E5	100	4.75E4	100	0
A1	216.09945	7.50E4	8.77	8.75E3	18.42	9.65
A2	217.09191	3.86E4	4.51	3.47E4	73.05	68.54

where:

- Relative intensity (expected and measured) values are derived from the isotopic pattern spectra (see Isotopic pattern spectra (stacked)). Each value is a percentage of the isotope's intensity relative to the A0 ion's intensity.

  For example: 7.50E4 ÷ 8.55E5 = 8.77%

- Delta intensity = *Relative intensity (measured) – Relative intensity (expected)*

  For example: 18.42 – 8.77 = 9.65

In this example, the absolute values of the Delta *m/z* for the A0 and A1 ions (see Mass deviation data for the expected ions) are both less than the Allowed Mass Deviation of 5 ppm; therefore, the application considers that these two ions are present in the measured spectrum. The delta intensity of the A1 isotope ion is close to the Allowed Intensity Deviation of 10% and the delta intensity of the A2 isotope ion is much higher (see Intensity deviation data for the expected ions).

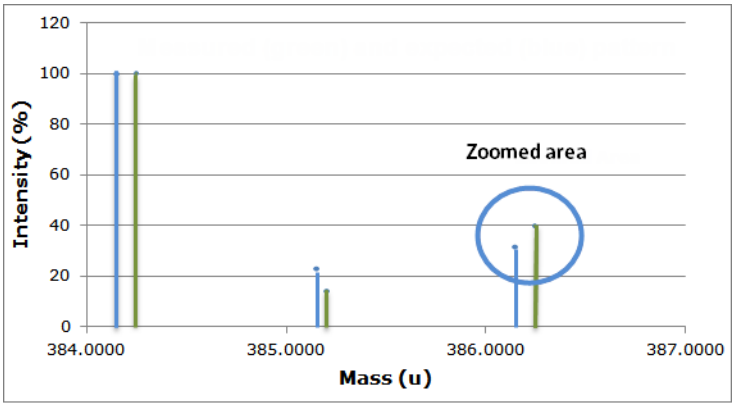
The application determines the isotopic pattern score value from a combination of the mass and intensity deviations between the expected and the measured spectra. In this case, the application reduces the isotopic pattern score value down to 90 from 100 to reflect the marginal quality of the intensities of the A1 and A2 isotopes and to penalize for not finding the A2 isotope.

Calculating Isotopic Pattern Score

To score the fit for an isotopic pattern, the product-name application calculates each expected ion's fit and then combines the individual fit scores, weighted by their expected intensities.

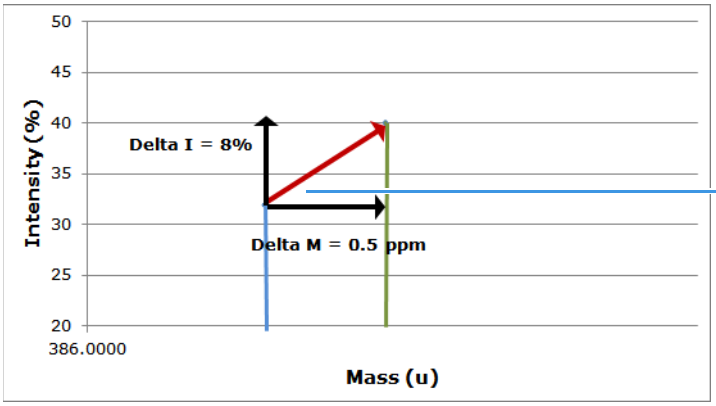
For each expected ion peak, the application measures the *m/z* and intensity differences between the expected and the measured patterns (see Measured and expected patterns). It then normalizes those differences (normalized deviation values) to the maximum allowed mass and intensity deviation values set in the processing method. The application then sums the normalized differences by vector addition (see Zoomed area showing the vector sum of intensity (I) and mass (M) deviations).

Figure 151. Measured and expected patterns



Expected spectrum in blue Measured spectrum in green

Figure 152. Zoomed area showing the vector sum of intensity (I) and mass (M) deviations



Expected spectrum in blue Measured spectrum in green

This example starts with the intensity deviations. The Allowed Intensity Deviation value set in the processing method is 10, so this is the normalization value. As shown in Intensity deviation data for the expected ions, the delta intensity value for the A1 isotope is close to 10%, resulting in a normalized intensity deviation close to 1.0.

Normalized Intensity Deviation

The following table lists the normalized intensity deviation data for each of the expected ions.

Table 103. Normalized intensity deviation data for the expected ions

Isotope order	*m/z* (expected)	Delta intensity	Allowed intensity deviation (%)	Normalized intensity deviation
A0	215.09611	0	10	0 ÷ 10 = 0.0
A1	216.09945	9.65	10	9.65 ÷ 10 = 0.965
A2	217.09191	68.54	10	68.54 ÷ 10 = 6.854

For mass deviations, you can control two settings in the processing method:

- The first setting is the Allowed Mass Deviation that functions as an outer limit in the same way that the Allowed Intensity Deviation functions as a limit for the intensity.
- The second setting is the Use Internal Mass Calibration check box. If you do not select this check box in the method, then the application considers any mass value within 2 ppm of the expected *m/z* as a perfect match (no deviation). If you select this check box, then the application considers only a mass value within 1 ppm of the expected *m/z* as a perfect match.

In this example, the Allowed Mass Deviation value set in the processing method is 5 ppm and the Use Internal Mass Calibration check box is cleared. The mass normalization is more complex than the intensity normalization because mass values < 2 ppm (Use Internal Mass Calibration setting) from the expected *m/z* are considered to have no deviation from theory; however, for values between 2 and 5 ppm (Allowed Mass Deviation value) from the expected *m/z*, the normalized deviation varies from 0 through 1.

The calculated normalized mass deviation value is as follows:

- 0 if absolute value (*Delta m/z)* < 2 ppm

  where 2 ppm is the value from the Use Internal Mass Calibration setting.
- [absolute value (*Delta m/z*) – 2 ppm] ÷ (5 ppm – 2 ppm) if absolute value (*Delta m/z)* ≥ 2 ppm

  where 2 ppm is the value from the Use Internal Mass Calibration setting and 5 ppm is the Allowed Mass Deviation value.

In this case, the absolute value of the mass deviation for the A0 ion is less than 2 ppm; therefore, its normalized mass deviation value is 0.

Normalized Mass Deviation

The following table lists the normalized mass deviation data for each of the expected ions.

Table 104. Normalized mass deviation for each of the expected ions

Isotope order	*m/z* (expected)	*m/z* (measured)	Delta *m/z* (ppm)	Internal calibration (ppm)	Allowed mass deviation (ppm)	Normalized mass deviation
A0	215.09611	306.10356	–0.42	2	5	0
A1	216.09945	307.10691	–3.05	2	5	0.35
A2	217.09191	308.11324	24	2	5	7.33

For example: [(24 – 2)] ÷ (5 – 2) = 7.33

To calculate the combined deviations, the application uses the Pythagorean theorem to calculate the vector sum of the normalized deviations. The calculation for the vector sum is as follows:

Vector sum = Square root [(*Normalized intensity deviation*)^2 + (*Normalized mass deviation*)^2].

However, if the vector sum > 1, then set it to 1.

Calculated Vector Sum

The following table lists the vector sum data for each of the expected ions.

Table 105. Calculated vector sum

Isotope order	*m/z* (expected)	Normalized intensity deviation	Normalized mass deviation	Vector sum
A0	215.09611	0	0	0
A1	216.09945	0.965	0.35	1
A2	217.09191	6.854	7.33	1

To calculate the final score, you must weigh the vector sum values and then express the result as a percentage value. Each ion's weighting contribution to the final isotopic pattern score is proportional to its intensity.

Weighting Factor Calculations

The following table lists the weighting factor of each of the three expected ions.

Table 106. Weighting factor calculations

Isotope order	*m/z* (expected)	Intensity (expected)	Weighting factor for final score
A0	215.09611	8.55E5	0.8827
A1	216.09945	7.50E4	0.0774
A2	217.09191	3.86E4	0.0399
		Sum = 9.686E5	Sum = 1.000

The weighting factor of each individual ion = *Intensity of each ion ÷ Sum of intensities of all expected ions*

For example: 8.55E5 ÷ 9.686E5 = 0.8827

When not all of the expected ions are present in the measured spectrum, the application applies a penalty value (1, 2, or 4) to the weighted deviation of each missing ion, lowering the final isotopic pattern score even further. The penalty value depends on how strong the ion signal is expected to be in the measured spectrum. For the A2 ion that is not found, the

application sets its penalty to a value of 1, causing its vector sum value of 1 (in Calculated vector sum) to be replaced with the penalty value of 1 (in Calculated isotopic pattern score). In this case, it is the same number, but for other cases, the penalty value might be different from the vector sum value.

Calculated Isotopic Pattern Score

The following table lists the calculated isotopic pattern score using the weighting factors.

Table 107. Calculated isotopic pattern score

Isotope order	*m/z* (expected)	Deviation (vector sum or penalty)	Weighting factor	Weighted deviation
A0	215.09611	0 (vector sum)	0.8827	0
A1	216.09945	1 (vector sum)	0.0774	0.0774
A2	217.09191	1 (penalty)	0.0399	0.0399
				Sum = 0.12

where:

- Weighted deviation of each individual ion = *Deviation* × *Weighting factor*

  For example: 1 × 0.0774 = 0.0774

- Isotopic pattern score = 100% × (1.0 – *Sum of all weighted deviation values*)

  For this example, the calculated isotopic pattern score of 100% × (1.0 – 0.12) is 88, which is close to the 90 score displayed in the application.

Note Use these calculations to approximate the score displayed in the application. The calculated score might not exactly match the score in the application because some internal calculation details are not listed here or because there is a discrepancy caused by decimal digit rounding.

In certain cases, closely matching isotopes exist because heavier isotopes contribute to the isotopic pattern observed in the mass spectra. For example, the isotopes 206.0941 and 206.1006 result from the contribution of one heavier isotope of carbon and one heavier isotope of nitrogen, respectively, which make up the split A1 isotopic peak. When the application performs isotopic pattern scoring, this situation appears as a main isotopic peak with a smaller peak to the side whose *m/z* is included in the calculations.

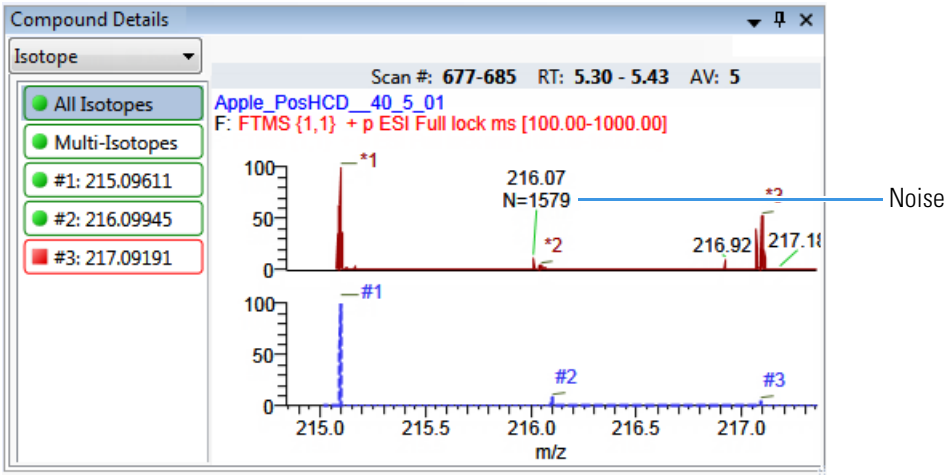
Finding the Noise Value

On the Isotope page of the Compound Details pane, you can view the averaged noise value (N) for a peak in the measured spectrum.

❖ To find the noise value associated with a mass spectral peak

1. Right-click the plot area and choose **Display Stack Spectra**.
2. Zoom in to the peak of interest.

   As an example, see Isotopic pattern spectra (stacked) for the compound **Metribuzin**.
3. Right-click the measured plot area and choose **Show Noise Label.**



Expected spectrum in blue Measured spectrum in red

In this example, the averaged noise value for the peak is 1579.