

Analyst[®] 1.6 Software

Software Reference Guide



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This software reference guide provides information about the Analyst[®] software features.

Related Documentation

The guides and tutorials for the instrument and the Analyst software are installed automatically with the software and are available from the Start menu: All Programs > AB SCIEX > Analyst. A complete list of the available documentation can be found in the online Help. To view the Analyst software Help, press F1.

Technical Support

AB SCIEX and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the instrument or any technical issues that may arise. For more information, visit the Web site at www.absciex.com.



Analyst Software Modes

The software is divided into modes, which are discrete functional areas you can perform a range of activities related to a main task. You can access modes through the Navigation Bar or the Mode list in the toolbar and can switch from one mode to another without losing any work. For more information, see the Analyst[®] software *Show Me* tutorial.

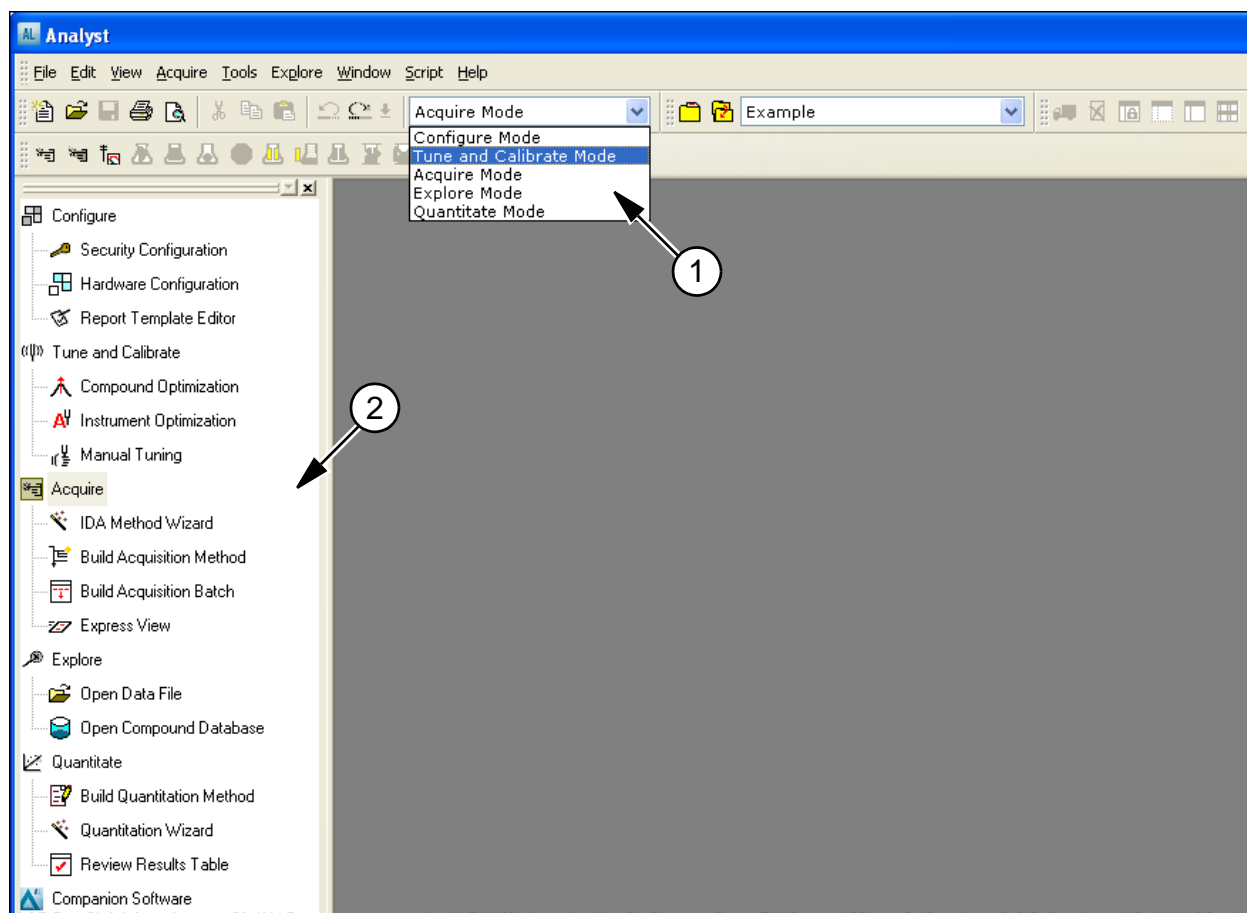


Figure 1-1 Analyst Software Window

| Item | Description |
|------|----------------|
| 1 | Mode list |
| 2 | Navigation Bar |

Analyst Software Features

As well as the main features of acquisition and processing, the software has some features that help you run your experiments more efficiently and more quickly.

IDA

IDA (Information Dependent Acquisition) is an acquisition method that analyzes data during acquisition. You can use IDA to change the experimental conditions depending on the analysis results. These real-time changes are controlled by criteria set in the acquisition method, including:

- Ion intensity and charge state.
- Inclusion and exclusion lists.
- Isotope pattern.

Being able to optimize data acquisition settings while the data is being acquired enables you to conserve both the sample and working time on an instrument.

Queue Manager

The Queue Manager shows queue, batch, and sample status and lets you manage samples in the queue.

Library Search

Library Search lets you create and manage a mass spectra database by helping you to search for and match unknown spectra against the mass spectra stored in the database.

Fragment Interpretation Tool

The Fragment Interpretation tool helps you interpret MS/MS data. Given the chemical structure of a molecule, this tool can generate a list of theoretical fragment masses from single non-cyclic bond cleavage of that molecular structure. The tool can then match the theoretical list with peaks in the current mass spectrum.

Express View

Use Express View when you want to create a batch quickly or when you need to add a sample name or change a small number of fields to an existing template. It provides a simplified interface for sample submission, while restricting further access to other functions. To use Express View, you select a batch template, type user information, and then submit a batch.

Workspaces

A workspace is a particular arrangement of windows and panes, including any associated file or files. For example, while working on a particular data set, you have opened and sized various windows to help you with your analysis. You can save this arrangement, or workspace, so that the next time you look at the data, the window arrangement is identical.

In Quantitate and Explore modes, you can have multiple workspaces per session. This means that you can design different workspaces to suit different tasks within these modes, and then save them for future use. When in one of these two modes, you can open a particular workspace without exiting that mode.

Analyst Service

The Analyst Service is the communication path between the mass spectrometer and attached peripheral devices.

The service is started each time you launch the Analyst® software. In general, however, the Analyst Service starts automatically when you log on to Windows. If for any reason the service is not running when you try to start the Analyst software, perhaps there was a problem starting the service at the time of log on, or if it was manually stopped, the Analyst Service will start up at that time. For this reason, starting or restarting the Analyst Service is almost always done automatically.

API Instrument Project Folders

The following are some of the folders found in the API Instrument project.

- **API BioAnalyst:** Contains the BTB Data Dictionary.csv file, the file containing the default Data Dictionary information required for the BioAnalyst™ software. The appropriate folders appear only when the BioAnalyst software is installed.
- **Bundler:** Contains a program that takes all aspects of a .wiff file and automatically combines them when the sample is completed.
- **Configuration:** Contains all the hardware profiles (.hwpf files).
- **Example Scripts:** Contains some of the scripts that are used throughout the software.
- **Instrument Data:** Contains a file called InstrumentData.ins, which stores all the critical calibration information and more.
- **Method Tables:** Contains all instrument settings that define the enhanced scan functions. Do not change the files in this folder. Changing the contents of this folder will affect the performance of the enhanced scan modes.
- **Parameter Settings:** Contains all the instrument settings and linkages. Instrument settings are saved as ParamSettingsdef.psf files.
- **Preferences:** Contains the Tunedata.tun file. All settings (parameter, tuning, instrument, processing, appearances and queue) are saved as Tunedata.tun in this folder.
- **Processing Scripts:** Contains the scripts for data processing in Explore mode. They are found in the Script menu in the Analyst software.
- **Queue Data:** Contains information from the queue.
- **Tuning Cache:** Contains all the data created in Manual Tuning by clicking Start instead of Acquire. Files are saved with a generic time and date stamp for their names. The tuning cache holds a limited number of files and will overwrite files as needed. Rename and move the files immediately if they need to be saved.

Area Threshold Parameters

See [Noise and Area Threshold Parameters on page 25](#).

Autotune Instrument Optimization™ Algorithm

The Autotune Instrument Optimization™ algorithm tunes both quadrupole and LIT modes and performs mass calibration. For quadrupole mode, it adjusts the resolution offsets. For LIT mode, it optimizes AF3 and EXB. For MS3, it adjusts the excitation and isolation coefficients.

Background Subtract

Background subtract reduces the amount of noise in a spectrum by subtracting either one or two ranges that contain noise from a range that contains a peak. You can move the ranges independently or lock them and move them as a single entity within the graph. Locked Background Subtract is the preset setting.

You can use background subtraction to isolate a peak of interest. You can highlight and subtract up to two selected ranges from your peak. You can also lock your ranges and move them within the graph to optimize peak isolation or to isolate another peak.

Background Subtract to File

You can use Background Subtract to File to save a new file that has a defined noise region subtracted from each scan. When a range is selected in the TIC, all the scans from that selection are internally averaged, and the resulting spectrum is subtracted from all the scans. Use the Background Subtract to File dialog to select where the file is to be saved. You also have the option of naming the new file. In some cases, the resulting file might look close to the original scan

Baseline Subtract

Baseline subtract removes a constant or slowly varying offset from a set of data. This is useful in locating small peaks that are obscured by noise. The software uses the following algorithm in performing a baseline subtraction.

1. Every data point in the data set is considered as the center of a window (in mass or time) with a user-definable width measured in amu or minutes.
2. The minimum values on either side of the current data point (minima) within the window are located.
3. A straight line is fitted between the two minima and the height (intensity) of the current data point above the line is calculated. The end points of the data are regarded as minima.
4. The data point is replaced with the new calculated value.

Batches

A batch is a collection of information about the samples that you want to analyze. Samples are usually grouped into sets to make it easier for you to submit them. Grouping your samples into a set also reduces the amount of data you must manually enter. A set can consist of a single

sample or multiple samples. All of the sets in a batch use the same hardware profile; however, samples in a set can have different acquisition methods. A batch can be submitted only from an acquisition station.

Batches link together:

- Sample information, such as name, ID, and comment.
- Autosampler location (rack information).
- Acquisition methods.
- Processing method or script (optional).
- Quantitation information (optional).
- Custom sample data (optional).
- Set information.

Batch Editor

The Batch Editor is used for creating and modifying batches and for creating batch templates. To run samples, each using different acquisition methods, use the Batch Editor to select multiple acquisition methods in the same set. In the Batch Editor, you can also use an acquisition method as a template. In this case, the same method is used for each sample, but you can select different masses or mass ranges for each sample.

You can also modify every detail of the batch before submitting it for processing. When you submit a batch for analysis, you can submit the entire batch, specific sets within the batch, or specific samples within a set.

For example, you may want to analyze ten samples, five using one acquisition method and five using a different acquisition method. You could create a batch of two sets, one for each method that you want to use.

You can also use the Batch Editor to import sample lists created in external programs, such as Microsoft Excel. The Batch Editor has the following tabs:

Table 1-1 Batch Editor Tabs

| Tab | Description |
|--------------|---|
| Sample | Used to create the sample list and to select sample details such as the sample name and the acquisition method to be used to acquire the sample. |
| Locations | Used to select the positions of samples in the autosampler. Sample locations can be specified numerically in the Sample tab; however, the Locations tab provides a graphical interface for selecting sample locations. |
| Quantitation | Used to select the sample types and concentrations for quantitation batches. Because quantitation information can be specified post-acquisition in the quantitation Results Table, you do not have to use the Quantitation tab in the Batch Editor. Instead, you can use the Quantitation Wizard. |
| Submit | Used to verify sample information and to submit samples to the acquisition queue. |

About Importing Batch Files

To make data entry easier, you can import batch file information from other applications. The Batch Editor can import text and other files that are formatted correctly. For examples of correctly formatted files, refer to the Batch folder in the Example project.

The information in a batch file can also be exported for use with other applications, such as Microsoft Excel, Microsoft Access, and certain LIMS (Laboratory Information Management System) software.

Calculators

With the Calculator window, you can perform calculations on the basis of collected data. Although the calculator is a separate window, it is connected to the active graph within the software.

The following calculators are available.

- [Elemental Composition Calculator](#)
- [Hypermass Calculator](#)
- [Elemental Targeting Calculator](#)
- [Mass Property Calculator](#)
- [Isotopic Distribution Calculator](#)

You can cut and paste from one text box to another between the different windows in the calculators. You can also print data from any of the calculators by clicking the Print icon in the top left corner of the window. For more information about using calculators, see the Help.

Data from the Elemental Composition, Mass Property, and Isotopic Distribution calculators can be exported to a separate file. Use the Elemental Targeting calculator to modify the data within the active graph. Data from the HyperMass and Isotopic Distribution calculators can be overlaid on the active spectrum.



Tip! You can set the precision of calculator data in the Calculators tab of the Appearance Options dialog. To open the dialog, click **Tools > Settings > Appearance Options**.

Elemental Composition Calculator

The Elemental Composition calculator determines potential molecular or amino acid compositions based on a target mass-to-charge ratio. You can type this ratio manually or select it from an active spectrum. This calculator creates a table with the possible element or amino acid combinations making up the mass of interest and the characteristics of each.

You can type or select values for such parameters as tolerance, electron state, and number of charges. You can also type a list of possible elements and put a limit on the number of each.

Hypermass Calculator

The Hypermass calculator determines the distribution of a multiply charged envelope based on an uncharged mass. You can select the uncharged mass, including the adduct and its polarity.

The calculator displays a graphical representation of the Hypermass series, which can be overlaid onto the active spectrum. A list of the Hypermass data is also available.

Elemental Targeting Calculator

The Elemental Targeting calculator reduces the data spectrum based on a specific pattern, primarily one corresponding to isotopic distributions. It can also search an MS data spectrum for a specific pattern of peaks, which can be entered either as a formula or as an isotopic distribution.

If the calculator finds a match, it creates a reduced plot containing only data pertaining to the specified pattern. For a spectrum, the calculator removes all unmatched data. For a chromatogram, the calculator calculates the elemental target for each of the underlying spectra and regenerates each point in the chromatogram on the basis of these new spectra.

Mass Property Calculator

The Mass Property calculator determines various properties such as exact mass, the average mass, the mass accuracy, and the mass defect of a mass of interest. The results generated by this calculator depend upon the number of input fields completed.

Isotopic Distribution Calculator

The Isotopic Distribution calculator determines the isotopic distribution based on an entered formula. This allows you to distinguish between compounds with the same mass based on relative intensities of isotopes.

The calculated isotopic distribution can be displayed in graphical or text format on the Isotopic Distribution pane, overlaid on the active spectrum, or exported to a separate file.

Calibration Options

The calibration options define the parameters for a calibration curve, which are used to determine the calculated concentration of the samples. The curve is a plot of the concentration of the standard against the area or height of the standard if no internal standard is used. If an internal standard is used, the curve is a plot of the concentration ratio against the area or height ratio. This curve is used, along with the area (or height) for the unknowns, to interpolate the calculated concentration.

You must choose the best regression type or fit to fit the curve to the points and the best weighting factor for your project.

About Calibration Curves

The calibration curve is used to determine the calculated concentration of samples, including QC samples. It is a curve that results from plotting the concentration of the standard versus its area or height, or ratios, if you are using an internal standard. The area or height of a sample is then applied to this curve to determine the sample concentration, as displayed in the Results Table. The regression equation generated by this calibration curve is used to calculate the concentration of the unknown samples.

The software places the known concentrations (or ratios) on the x-axis and the calculated area or height (or ratios) on the y-axis. It then plots the points for all the standards in the batch. The system produces a best-fit curve to those points through regression and weighting type that you choose. This curve is used, along with the area (or height), for the unknowns to interpolate the concentration.

Selecting the Best Regression Type

After you select a regression type (fit), you cannot see the calibration curve from the wizard. Instead, you may use the preset values, rely on your experience, or use your corporate policy to choose a regression type.

After you change the fit, check the Accuracy column in the Results Table for changes. You will find that the better the fit is, the better the accuracy of the quantitative analysis will be.

The calibration curve plots the standards concentration against its peak area or height (or ratios, if an internal standard is used). When the points for the standards are plotted, determine the best fit for the curve to these points and indicate your choice in the Specify Calibration dialog of the wizard. The preset fit is linear, which assumes that all your standards will fall on a straight line. Select from the types of fit in [Table 1-2](#).

Table 1-2 Types of Fit

| Fit | Description |
|----------------------|---|
| Linear | Linear regression assumes that the standard points fall on a straight line. |
| Linear Through Zero | Linear Through Zero regression assumes that the standard points fall on a straight line and that the points line up with the zero point on the x- and y- axes. Use this setting to force the line to go through the zero point. |
| Quadratic | If the standard points do not fall on a straight line, use quadratic regression to produce a quadratic fit to the data points. |
| Mean Response Factor | If the standard points fall on a straight line and you want to average the points, use mean response factor regression to produce an average of the slope for every point on the curve. |
| Power | If there is some linear and some curvature in the line of points, use power regression instead of linear or quadratic regression to produce a line somewhere between these fits. |

Choosing the Best Weighting Factor

The calibration curve plots the standards concentration against its peak area or height. When the points for the standards are plotted, you must determine the best weighting factor for these points and indicate your choice in the Specify Calibration dialog. The default fit is None, which assumes that all points along the curve have the same importance. Select from the types of weighting in [Table 1-3](#).

Table 1-3 Types of Weighting

| Weighting | Description |
|-----------|---|
| 1/x | To place some additional emphasis on lower-value points, use a weighting factor of 1/x. |

Table 1-3 Types of Weighting (Continued)

| Weighting | Description |
|-----------|---|
| 1/(an) | Use a weighting of $1/x^2$ to place much higher emphasis on lower-value points. |
| 1/y | Use a weighting factor of $1/y$ when you are calibrating by the area (y-axis) rather than by the concentration (x-axis), and you want to place some emphasis on lower-value points. A weighting of $1/y$ is a variant of $1/x$ where y and x should be proportional to each other. |
| 1/(y*y) | Use a weighting factor of $1/y^2$ when calibrating by the area (y-axis) rather than by the Concentration (x-axis), and you want to place much higher emphasis on lower-value points. A weighting of $1/y$ squared is a variant of $1/x$ squared where y and x should be proportional to each other. |
| In x | Use the logarithm of x to place more emphasis on higher-value points. |
| In y | Use the logarithm of y to place more weight on higher-value points. Use when calibrating by the area (y-axis) rather than by the concentration (x-axis). |

Chromatograms

A chromatogram displays the variation of some quantity with respect to time in a repetitive experiment; for example, when the instrument is programmed to repeat a given set of mass spectral scans several times. Chromatographic data is contiguous, even if the intensity of the data is zero. Chromatograms are not generated directly by the instrument, but are generated from mass spectra.

In the chromatogram display, the intensity, in counts per second (cps), is shown on the y-axis versus time on the x-axis. Peaks are automatically labeled.

In the case of LC/MS, the chromatogram is often displayed as a function of time, the time at which a particular scan was obtained, which can be derived from the scan number.

Compound Database

The compound database stores information about compounds, including optimization specifications.

Use the compound database when you have large numbers of samples and need to optimize a large number of compounds quickly. The Automaton software automatically fills the database. The Compound Database window stores optimized conditions for compounds that can be retrieved to run samples.

Compound Optimization

The Compound Optimization software wizard automatically optimizes an analyte. You can introduce the sample using infusion or FIA (flow injection analysis.) The software first checks for the presence of the compounds. The voltages of the various ion path parameters are gradually increased or decreased to determine the maximum signal intensity (Q1 scan) for each ion. A text

file is generated and displayed during the optimization process. This file records the various experiments performed and the optimal values for each ion optic parameter. A file folder containing all the experiments performed is also generated and can be found by opening the data file folder in Explore mode. For each experiment performed, an acquisition method is also generated and saved in the acquisition method folder.

See also [Flow Injection Analysis \(FIA\) on page 17](#) and [Infusion on page 19](#).

Dynamic Background Subtraction™ Algorithm

Dynamic Background Subtraction™ algorithm improves detection of precursor ions in an IDA (Information Dependant Acquisition) experiment. When the algorithm is activated, IDA uses a spectrum that has been background subtracted to select the ion of interest for MS/MS analysis, as opposed to selecting the precursor from the survey spectrum directly. Because this process takes place during LC analysis, the algorithm enables detection of species as their signal increases in intensity, therefore focusing on detection and analysis of the precursor ions on the rising portion of the LC peak, up to the top of the LC peaks (maximum intensity).

Dynamic Fill Time

Dynamic Fill Time (DFT) is a feature specifically designed to optimize the data obtained in every spectrum for the linear ion trap scan functions. DFT will automatically adjust the fill time used to fill the ion trap based on the ion flux coming from the source. For more intense ions, the fill time will be automatically reduced to ensure the trap is not overfilled with ions.

For less intense ions, the fill time will be automatically increased, ensuring that good ion statistics are obtained in the spectrum. DFT is applicable for the following scan types:

- Enhanced MS (EMS)
- Enhanced Resolution (ER)
- Enhanced Product Ion Scan (EPI)
- MS/MS/MS (MS3)

You can adjust the DFT settings by selecting **Tools > Settings > Method Options** in the Analyst software.

Experiments and Periods

The mass spectrometer acquisition method consists of experiments and periods. In the Acquisition Method Browser pane, you can create a sequence of acquisition periods and experiments for the mass spectrometer. You can also open a method previously created in the Tune Method Editor.

Experiments

An experiment includes the mass spectrometer settings and scan type during an MS scan. A set of MS scans performed for a specific amount of time is called a period. An acquisition method in which the MS parameters and actions are the same through the entire duration is called a single-period, single-experiment method.

In looped experiments, MS settings are changed on a scan-by-scan basis. For example, if the sample contains two compounds, A and B, you may want to loop an MS/MS experiment of compound A with an MS/MS experiment of compound B to obtain information on both compounds in the same run. The mass spectrometer method will alternate between the two scans. Other examples of looped experiments include alternating between positive and negative mode in a run and IDA methods.

Periods

A period can contain one or more looped experiments. In a multi-period acquisition method, experiments are performed for a specified amount of time and then switch to another set of experiments. Periods are useful when you know the elution time of the compounds in an LC run. You can have the mass spectrometer perform different experiments according to when the compounds elute to get as much information as possible in the same run.

In [Figure 1-2](#), a three-period method is shown. The first period has a duration of 3.015 minutes; the second period is 4.986 minutes; and the third period is 7.000 minutes.

The screenshot displays the software interface for configuring a multi-period experiment. On the left, the 'Acquisition method' tree shows a sequence of events: 'Mass Spec' (24.003 min), followed by three 'Period' blocks (13.970 min, 5.025 min, and 5.008 min), each containing '4MS2' experiments. Below these are 'Agilent 1100 LC Binary Pump (0.0 mins)', 'Equilibrate (0.0 mins)', 'Run (0.0 mins)', 'Agilent 1100 Column Oven', and 'Agilent 1100 Autosampler'. The right pane, titled 'MS Advanced MS', shows settings for the first period: 'Experiment: 1', 'Scan type: Product Ion (MS2)', 'Polarity: Positive', 'MCA: unchecked', 'Product Of: 592 (Da)', and 'Total Scan Time (includes pauses): 1:00:50 (sec)'. A table below these settings lists mass ranges and times for three periods:

| | Start (Da) | Stop (Da) | Time (sec) |
|---|------------|-----------|------------|
| 1 | 100.000 | 700.000 | 1.0000 |
| 2 | | | |
| 3 | | | |

At the bottom right, a 'Period Summary' section shows: 'Duration: 13.970 (min)', 'Delay Time: 0 (sec)', 'Cycles: 417', and 'Cycle: 2.0100 (sec)'.

Figure 1-2 Example of a multi-period experiment

Flow Injection Analysis (FIA)

FIA is the injection of a small quantity of a sample by an autosampler into the LC stream. During the FIA optimization process, multiple sample injections are performed for various source- or compound-dependent, or both, parameter types that are changed between injections. FIA optimizes for declustering potential, collision energy, and collision cell exit potential by performing looped experiments in succession, that is, one compound-dependent parameter followed by then next compound-dependent parameter. It optimizes for source-dependant parameters by making an injection for each parameter.

Use FIA optimization to optimize both compound- and source-dependent parameters using LC at higher flow rates. See also [Infusion on page 19](#).

IDA Explorer

The IDA (Information Dependent Acquisition) Explorer is used to display data acquired through an IDA method.

The IDA Explorer can be turned off and on by going to the IDA Explorer tab in the Appearance Options dialog. Columns present in the List View can be defined under this tab as well.

The left side of the viewer shown in [Figure 1-3](#) displays the masses on which a product ion scan was performed. In this area, you can examine the mass, intensity, time, and collision energy of ions on which product ion scans were performed. You can view these in either a list view or a tree view. In list view, the list can be sorted by double-clicking on any column header. In the Appearance Options dialog, you can customize the columns you want to view in the list view.

On the right side, the viewer is split into four panes. The top left pane displays the survey TIC data. The bottom left pane shows the XIC of the mass. The top and bottom right panes show the survey and product scans, respectively.

The IDA viewer lists all the masses on which Enhanced Product Ion scans or Enhanced Resolution scans were performed. Click a mass in the list or tree view to display plots relevant to that mass. You can view the survey spectrum from which the mass was identified and the product spectrum of that mass. It also displays the TIC of the survey scan and the XIC for each mass.



Note: Brackets around a mass indicate that the mass is merged. A merged mass is contiguous across a number of cycles. When a merged mass is displayed, it indicates an averaged spectrum, containing the average of all contiguous spectra.

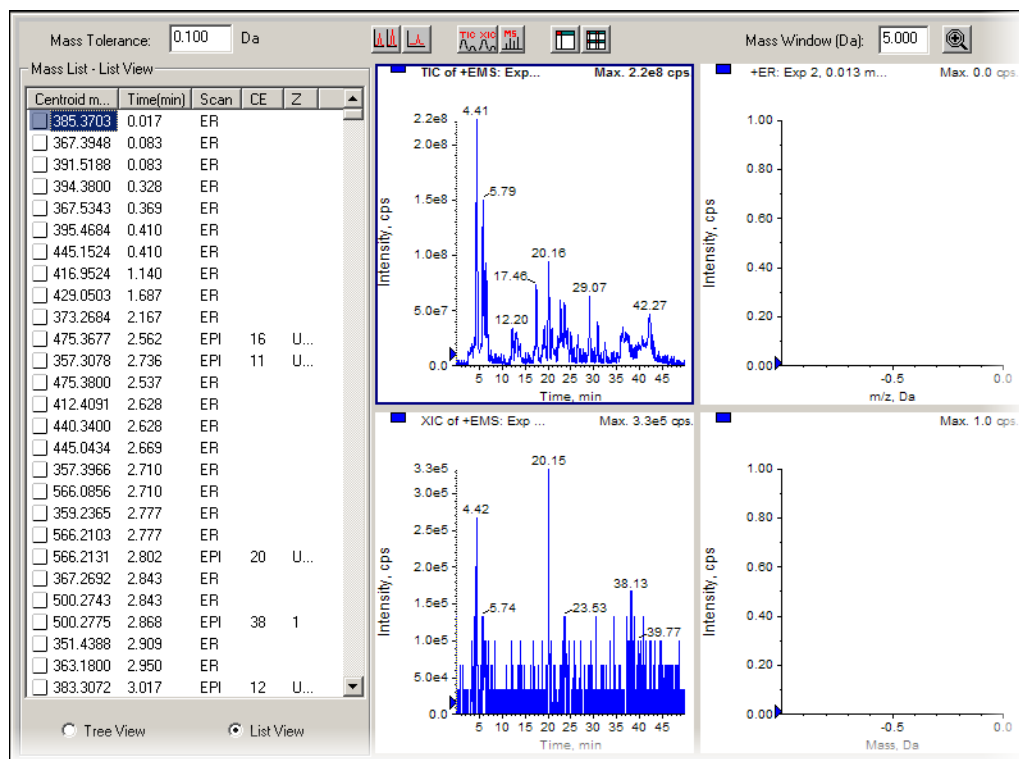


Figure 1-3 IDA Viewer

IDA Methods

An IDA (Information Dependent Acquisition) method automatically runs experiments based on results obtained from previous experiments. Use IDA criteria to optimize data acquisition settings while acquiring data to reduce the sample acquisition time in a single injection. With IDA, you can conserve both the sample amount required and working time.

Create an IDA method with up to two survey scans and eight dependent scans in a single experiment. A survey scan is used in IDA to trigger additional experiments. You can use the following scans as a survey scan:

- Enhanced Multiply-Charged (EMC).
- Enhanced Product Ion (EPI) (second survey scan).
- Enhanced MS (EMS).
- MRM.
- Neutral Loss (NL).
- Precursor Ion (Prec).
- Q3 MS.

You can use the following dependent scans:

- EPI.
- MS/MS.

In an IDA experiment, the mass spectrometer actions are varied from scan to scan based on the data acquired in a previous scan. The software analyzes data as it is being acquired and then determines the masses on which to perform dependent scans. You can set the criteria that will activate an IDA experiment and the method parameters to be used.

IDA experiments modify experiments and improve results based on the following user-defined criteria:

- Ion intensity and charge state.
- Inclusion and exclusion lists.
- Isotope pattern.
- Dynamic exclusion.
- Rate of change in ion intensity (See [Dynamic Background Subtraction™ Algorithm on page 16](#)).

Infusion

Infusion is the continuous flow of the sample at low flow rates into the ion source using a syringe pump. During the infusion optimization process, the software can select precursor and product ions and optimize for declustering potential, collision energy, and collision cell exit potential for both. The voltages of these ion path parameters are gradually increased or decreased to determine the maximum signal intensity for the precursor and product ions.

Use infusion optimization to optimize compound-dependent parameters only at much lower flow rates than those used during LC/MS analysis. See also [Flow Injection Analysis \(FIA\) on page 17](#).

Integration Algorithms

The Analyst[®] software has two integration algorithms: the original Analyst Classic integration algorithm and the IntelliQuan integration algorithms. The IntelliQuan algorithm provides more consistent peak-finding and integrated functionality, with fewer parameters that require adjustment.

Analyst Classic and IntelliQuan Integration Algorithms

The IntelliQuan algorithm uses one of two peak-finding parameters: Automatic IQA II, which is a parameterless setting, or Specify Parameters MQ III. After integrating peaks using the IntelliQuan algorithm, you can choose which peak-finding parameter best fits the data set. This is done in the displayed peak integration parameters in the Peak Review pane or window.

Table 1-4 shows the parameters available with the Analyst Classic algorithm.

Table 1-4 Analyst Classic Algorithm

| Parameter | Definition |
|--|---|
| Default Bunching Factor | The number of points to be averaged together and considered as a single point for the purpose of peak-finding. |
| Default Number of Smooths | The number of times to smooth the chromatogram. |
| Default Void Volume Retention Time | Any peaks that appear before this time are ignored. |
| Default Concentration Units | The concentration units used to describe the sample concentration, for example, pg/ μ L. |
| Default Calculated Concentration Units | The concentration units used to describe the calculated sample concentration, for example, pg/ μ L. |
| Default RT Window | The time window centered at the expected retention time for peak-finding. For example, a 30 second retention time window gives an additional 15 seconds before and after the expected retention time. |

Table 1-5 shows the parameters available with the MQ III algorithm, but not the IQA II algorithm.

Table 1-5 MQ III Algorithm

| Parameter | Definition |
|-------------------------------------|---|
| Default Noise Percentage | The threshold used in peak-finding. Only peaks higher than this specified percentage will be detected. |
| Default Baseline Subtraction Window | A time window around each data point that is used to determine the height of the baseline correction to be applied to that point. This time window helps remove excessive noise from the chromatogram. The baseline is defined as the line connecting the point of minimum intensity on the left side of a given data point to the point of minimum intensity on the right side, within the specified window. |

Table 1-5 MQ III Algorithm (Continued)

| Parameter | Definition |
|------------------------------------|---|
| Default Peak-Splitting Factor | Controls whether a given peak cluster consists of multiple adjacent peaks or one (possibly noisy) peak. If the intensity dip is less than the value specified, then a single peak is reported; otherwise the point with minimum intensity in the dip splits the cluster into two separate peaks. Setting a large factor will prevent clusters from being split into more than one peak. |
| Default Void Volume Retention Time | Any peaks that appear before this time are ignored. |
| Report Largest Peak | Selecting this parameter returns the largest peak in the retention time window. If this parameter is not selected, the closest peak to the expected retention time is found. The expected retention time is automatically calculated in the Quantitation Wizard. |

Table 1-6 shows the parameters available for use with both IntelliQuan algorithms.

Table 1-6 IntelliQuan Algorithm

| Parameter | Definition |
|--|---|
| Default Minimum Peak Height | The minimum height of a peak required for peak integration. |
| Default Minimum Peak Width | The minimum width of a peak required for peak integration. |
| Default RT Window | Specifies the time window centered at the expected retention time for peak-finding. For example, a 30 second retention time window gives an additional 15 seconds before and after the expected retention time. |
| Default Smoothing Width | The number of points used in data smoothing. |
| Default Concentration Units | The concentration units used to describe the sample concentration, for example, pg/ μ L. |
| Default Calculated Concentration Units | The concentration units used to describe the calculated sample concentration, for example, pg/ μ L. |

Interface Heater Temperature (IHT)

The IHT parameter controls the temperature of the NanoSpray[®] ion source interface heater. The optimal heater temperature depends on the type of sample you are analyzing and the solvent used. If the heater temperature is too high, the signal degrades. The maximum heater temperature you can set is 250 °C, but this is too high for most applications. Typically, heater temperatures are in the 130 to 180 °C range.

LC Methods

If you are creating a new acquisition method file from an existing file, you may decide to use some or all of the device methods in the acquisition method. With the Acquisition Method Editor,

you can customize the acquisition method by adding or removing HPLC peripheral device methods.

You can create an acquisition method for an HPLC peripheral device means by selecting the operating parameters for that peripheral device. Methods can be created for any of the following peripheral devices if they are configured in the active hardware profile:

- Pumps.
- Autosamplers.
- Syringe pumps.
- Column ovens.
- Switching valves.
- Diode array detector.
- Analog-to-digital converters.
- Integrated systems.

For information about setting properties for peripheral devices, see the *Peripheral Devices Setup Guide*.

Library Databases

The Library Search feature compares unknown spectra to known MS spectra contained in a library database and generates a list of possible matches.

With Library Search you can:

- Compare library contents against an unknown spectrum.
- Add records to the library.
- Edit existing records.

You can store library data in the following locations:

- MS Access on a local database.
- MS SQL Server.

Before you can use the Library Search feature, determine where the library database is stored and connect your computer to that location. Library databases can be stored locally and over a network.

To connect to a database, use an alias. In this case, the alias specifies a connection to a specific database and may include the user name and password required to access the database. For example, you may have a small library database of your own identified compounds on your computer and your organization may have a central database that you use occasionally. Creating aliases for each database allows you to switch between them quickly. For information about creating aliases and connecting to databases, see the Help.

Method-Creation Tools

The software offers four quantitation method-creation tools, each of which creates a fully functional method. The best choice of tool depends on the tasks you need to accomplish.

Wizards

There are two available method-creation wizards: the Standard Quantitation wizard and the Automatic Quantitation wizard. Both allow you to select the batch or batches to be quantified, create or select a quantitation method, and then integrate the sample data.

The difference between the two is the type of method you create. The standard wizard creates a standard method, while the automatic wizard creating methods. You do not verify the peaks as part of the method creation (you can still review the peaks after integration has taken place).

There is only one common occasion for which you would not need to verify the peaks: when you are doing quantitation simply to integrate, not to find concentrations. You might want to do this, for example, if you have a batch that contains different compounds in every sample, or when the mass is not the same from sample to sample. If this is the case, use the automatic wizard. Otherwise, to perform quantitation, use the standard wizard.

Use the Standard Quantitation wizard after you have acquired your sample and you want to:

- Choose a representative sample.
- Select analyte and internal standard peaks.
- Adjust peak-finding and integration parameters.
- Review peaks during method creation.
- Select calibration.

Use the Automatic Quantitation wizard to select a batch, create a method (without peak confirmation), and then integrate the sample data. This wizard is quicker than the Standard wizard and does not require that the masses scanned be the same for all samples. It does not, however, allow you to select an internal standard—all ions are treated as analytes.

Use the Automatic Quantitation wizard after you have acquired your sample and you:

- Want to select calibration.
- Do not want to adjust peak-finding and integration parameters.
- Do not want to select analyte peak names.
- Do not want any internal standards.
- Do not want to review peaks during method creation, or you have different compounds in every sample.

When you are only integrating peaks, you do not need to verify them as no concentration calculation is required. In this case, use the Automatic quantitation method creation, which allows you to review the peaks after integration has taken place.

Finding Peaks Using an Automatic Method

The software uses the standard peak-detection process, with the following exceptions:

- It uses the bunching factor and the number of smooths (from the wizard) as is.
- It calculates the expected retention time and noise and area thresholds separately for every peak.

Quantitation Method Editor

Use this option after you have acquired your sample and you want to:

- Adjust peak-finding and integration parameters.
- Select analyte and internal standard peaks.
- Select calibration.

You can use the Quantitation Method Editor to do three additional tasks:

- Sum ions for integration.
- Use an internal standard from a different period or experiment (if the internal standard was acquired in a different period or experiment than the analyte).
- Edit an existing method.

The Semi-Automatic Method Editor

The Semi-Automatic Quantitation Method Editor is part of the Batch Editor. You can use the Semi-Automatic Quantitation Method Editor to select quantitation information, such as sample type and sample concentration, prior to data acquisition. This preparation makes performing subsequent quantitative analysis easier. Alternatively, you can select a full method in the Batch Editor, which is then automatically applied at the end of the batch run to generate the quantitation Results Table.

Use this option when you:

- Have not yet acquired any samples.
- Want to select names and masses for analyte and internal standard peaks.
- Want to select concentrations and sample types on the Quantitation tab in the Batch Editor, but do not have any other quantitation method.
- Want to edit the quantitation method, if necessary, at a later time.

Finding Peaks Using a Semi-Automatic Method

The software uses the standard peak-detection process, with the following exceptions:

- It uses the bunching factor (from the Quantitation Method Options dialog) and the number of smooths (from the Create Semi-Automatic Quantitation Method dialog) as is.
- It uses the most concentrated standard as the representative sample. To establish a retention time, it uses the largest peak within that chromatogram.
- To set noise and area thresholds, it uses the resulting baseline noise. (This process is identical to how the default values are set for peaks in normal methods.) These integration parameters are applied to all other samples.
- If the batch being examined does not contain quantitation information (sample type and concentrations), the retention time and thresholds are calculated separately for every peak (as for fully automatic methods).

Noise and Area Threshold Parameters

To identify peaks, the software requires a set of noise and area threshold parameters. The software sets these parameters initially, but you can change them later. It sets the parameters as follows:

1. The software calculates the largest intensity difference between any two sequential data points. This number represents the difference between two intensities, not the actual intensity itself.
2. For each sequential pair with an intensity difference of less than 5% of the value calculated in Step 1, it calculates the standard deviation (using a mean of zero) of the intensity differences. (The software does not use those pairs of points with an intensity difference larger than 5% of the maximum.)

It sets the noise and area thresholds as follows:

3. The noise threshold is equal to the standard deviation calculated in [step 4](#).
4. The area threshold is equal to five times the noise threshold.



Note: The minimum value for both the noise and area thresholds is 0.000001. If the calculations produce a value that is lower than this minimum, then the software resets the value of that threshold at 0.000001.

Recalculating the Noise and Area Threshold

If you define a new background area, the software recalculates the noise and area thresholds as follows. For each sequential pair of data points, the software calculates the standard deviation, using a mean of zero, of the intensity difference. The Analyst software uses all points within the selected range because you are explicitly telling it that the selected area is background noise.

It sets the noise and area thresholds as follows:

1. The noise threshold is equal to the standard deviation calculated for the selected range.
2. The area threshold is equal to five times the noise threshold.



Note: The minimum value for both the noise and area thresholds is 0.000001. If the calculations produce a value that is lower than this minimum, then the software resets the value of that threshold at 0.000001.

Peak Integration

The following are integration types by which the baseline was found and integrated when the peak was found.

- **Manual:** The peak was manually integrated by the user.
- **Automatic:** The peak was automatically integrated as follows:
 - **Baseline-to-baseline:** The peak area is defined by vertical droplines at the beginning and end of the peak which extend to the baseline. This integration type is possible only for peaks that do not have another peak immediately preceding or following.

- **Valley:** Same as baseline-to-baseline, except that it applies only to peaks that do have another peak immediately preceding or following.
- **Exponential Skim:** The peak area is the main or parent peak in an exponential skim.
- **Exponential Child:** The peak area is the child peak resulting in an exponential skim.

Peak Review

During peak review you can survey the peaks that the software selected and then redefine the peak or the start and end points where necessary.

Reviewing Peaks

In general, the software is adept at accurately identifying analyte and internal standard peaks. For a variety of reasons, including sample acquisition and quantitation method definition, sometimes the software misses the correct peak, chooses the wrong one, or is unable to locate a peak at all. Other times, although the software may correctly identify the peak, you might not agree with the start or end points selected.

Detecting Peaks

The software detects peaks in four stages.

1. First, it finds the potential peak start by examining the distance between each bunched point and the preceding one. When the distance exceeds the current noise threshold, a potential peak start has been found.
2. Then it confirms the peak start by making sure that enough points exist in a row to exceed the area threshold.
3. Next, it finds the peak top by searching for a point that is lower than the previous point.
4. Finally, it finds the end of the peak by identifying the place where the distance between one bunched point and the next falls below the noise threshold. If necessary, it then separates peaks.

Finding the Potential Peak Start

To find the potential start of a peak, the software measures the intensity difference between sequential pairs of bunched points, starting at the first point. When it finds a difference that exceeds the current noise threshold, the software declares the first point a potential peak start.

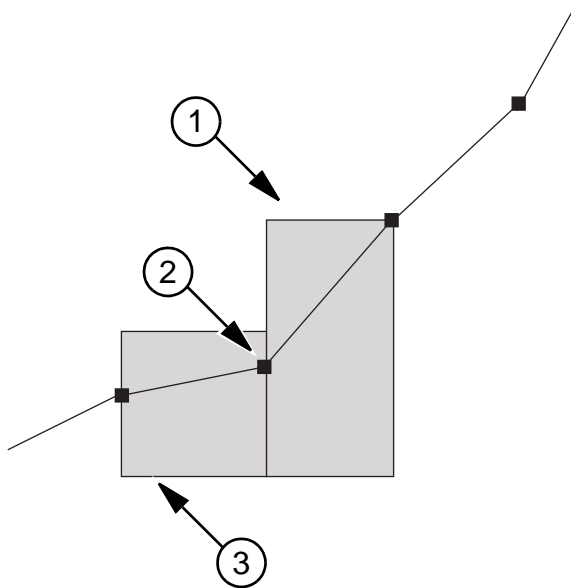


Figure 1-4 Finding the potential peak start

| Item | Description |
|------|---------------------------------|
| 1 | Exceeds noise threshold |
| 2 | Potential peak start |
| 3 | Does not exceed noise threshold |

Confirming the Peak Start

To make sure that it has found a real peak, the software moves along the curve, adding the intensity difference between each bunched data point from the intensity at the potential peak start to a total sum. This process stops when the intensity difference between successive points is less than the noise threshold. This sum is an approximation of the area of the leading edge of the peak. If this sum exceeds the area threshold, then the software confirms the peak start.

Next, the software determines the actual start of the peak by moving backward from the potential peak start until it finds the lowest point in the peak. It moves back through five bunches of raw data. This point is the actual peak start.

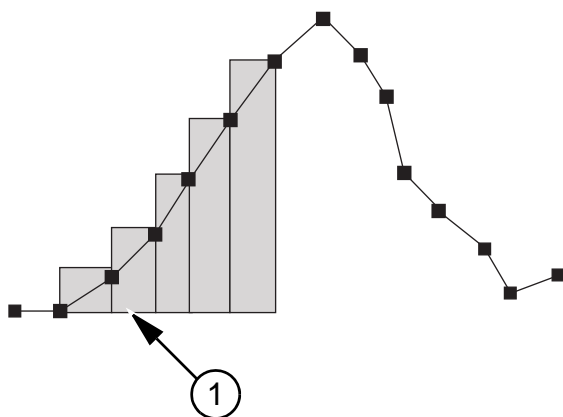


Figure 1-5 Confirming the peak start

| Item | Description |
|------|---|
| 1 | Sum of area slices greater than threshold |

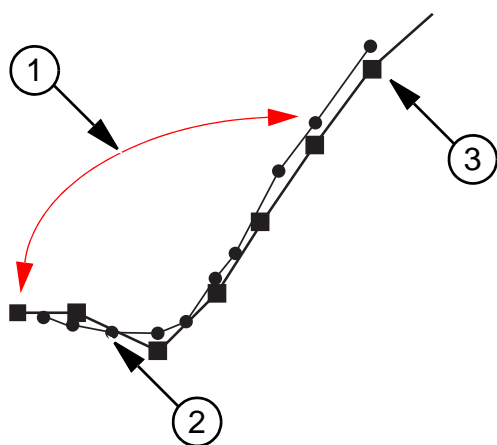


Figure 1-6 Confirming the actual peak start

| Item | Description |
|------|---|
| 1 | Look through data points in this region |
| 2 | Minimum data point |
| 3 | Potential peak start |

Finding the Peak Top

To find the peak top, the software first looks for a point that is lower than the preceding point. Then, to confirm that it has found the top correctly, it sums the intensity differences between the potential top and subsequent bunched points until it reaches the end of the peak. If the total distance between points exceeds two-thirds of the area threshold, then the peak top is confirmed. That is, the software makes sure it has a peak first, and then works backward to find the top of it.

If, however, the software finds a higher bunched point before the area test has been passed, then it identifies a new top and restarts the area test.



Note: The actual retention time for a peak is not simply the point identified as described previously. Instead, it is determined from a quadratic fit based on the three highest data points.

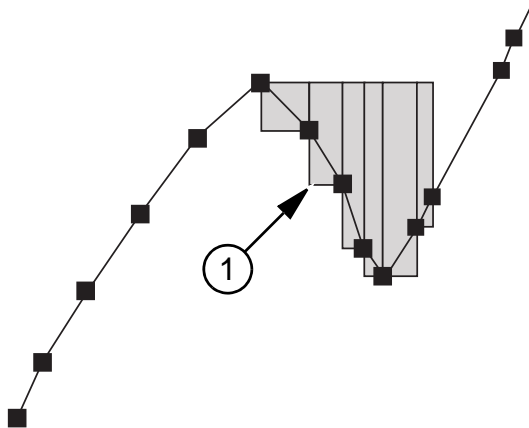


Figure 1-7 Finding the peak top

| Item | Description |
|------|---|
| 1 | Sum of area slices is greater than two thirds of the area threshold |

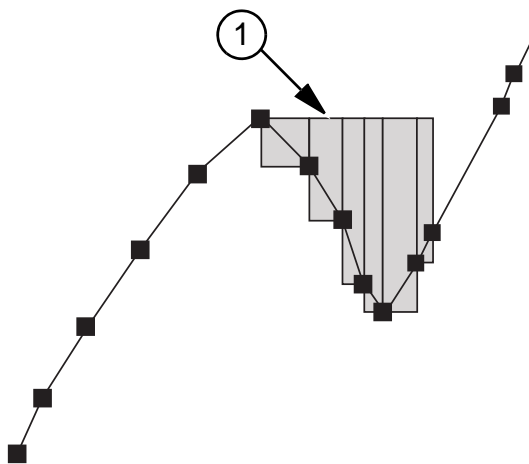


Figure 1-8 Identifying a new peak top

| Item | Description |
|------|--|
| 1 | The shoulder has a maximum, but the cumulative crest area is not greater than two thirds of the area threshold |

Finding the Peak End

The software declares a peak end point when one of the following occurs:

- The difference between two consecutive points fails the noise threshold test.
- The software detects the start of a new peak.

In either case, the lowest bunched point from the last five bunches is considered to be the actual end point of the peak.

In general, the software finds several peaks for each chromatogram. The peak it selects is the one whose retention time is the closest to the expected retention time, specified in the method. If no peak has a retention time within the specifications, the software marks the peak as not found.

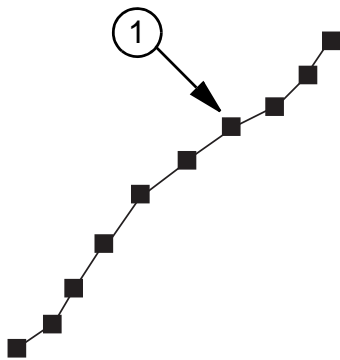


Figure 1-9 Finding peaks

| Item | Description |
|------|--|
| 1 | The shoulder has no separate maximum point |

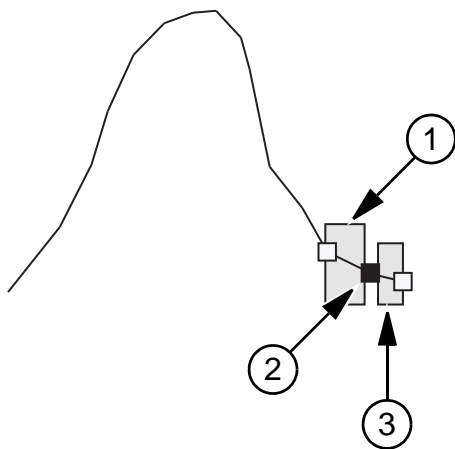
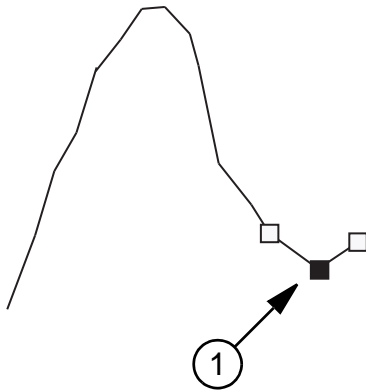


Figure 1-10 Finding the peak end: case 1

| Item | Description |
|------|-------------------------|
| 1 | Exceeds noise threshold |

Figure 1-10 Finding the peak end: case 1 (Continued)

| Item | Description |
|------|---------------------------------|
| 2 | Peak end |
| 3 | Does not exceed noise threshold |

**Figure 1-11 Finding the peak end: case 2**

| Item | Description |
|------|-------------|
| 1 | Peak end |

Separating Peaks

If a new peak begins before the current peak hits the baseline, the software decides, based on the following criteria, whether to resolve the baseline by using exponential skims. The skim passes under one or more peaks that follow the precursor. These peaks are called product peaks.

When the software performs an exponential skim, it subtracts the area underneath the skim from the product peaks and gives it to the precursor peak. It then subtracts the small area above the skim from the precursor peak and adds it to the first product peak.

The software uses the following criteria to determine whether it will use exponential skimming:

- Exponential Peak Ratio.
- Exponential Adjusted Ratio.
- Exponential Valley Ratio.

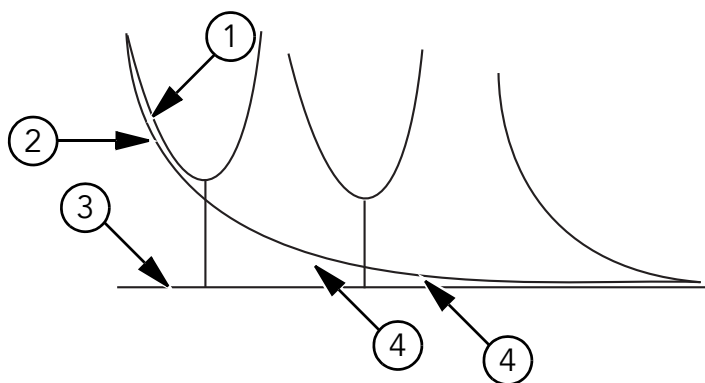


Figure 1-12 Separating peaks: an exponential skim

| Item | Description |
|------|---|
| 1 | This area is subtracted from the precursor peak and added to the first product peak |
| 2 | Exponential skim |
| 3 | Cluster baseline |
| 4 | These areas are subtracted from the product peaks and added to the precursor peaks |

Program Files

The following folders are found within the Program Files\Analyst folder.

- **bin:** This folder contains the Analyst® software program files. Contents of this folder should not be changed, as this will affect the software functionality.
- **BioAnalyst Tutorial:** This folder contains a tutorial that guides new users through all the features of the BioAnalyst™ software. There is also a multimedia overview of the BioAnalyst software.
- **Firmware:** This folder contains the instrument system controller software (scu21.exe) and the instrument firmware files. These files can be used to download new firmware to the instrument when required. For more information, see the software installation guide included with the software.
- **Help:** This folder contains the help files, as accessed from the Help menu in the program, and the release notes.
- **Scripts:** This folder contains all the available scripts that can be installed.
- **Simulation:** This folder contains the instrument data files needed to run the software in simulation mode.

Qualitative Data

You can view the information contained in a data file in table or graph form. Graphical data is presented either as a chromatogram or as a spectrum. You can view data from either of these displays as a table of data points, and you can perform various sorting operations on the data.

When you open a data file in the software, different panes appear depending on the type of experiment you performed.

If the MCA check box is selected in the Tune Method Editor, the data file will open to the MS (mass spectrum). If the MCA check box is not selected, the data file opens with the TIC. You then have to select a range and double-click in the TIC pane at a particular time to show the MS for this range.

The software stores data in files with a .wiff extension. Wiff files can contain data for more than one sample. In addition to .wiff files, the software can open .txt files; .txt files contain data for only one sample.

Quantitative Analysis

Quantitative analysis is the process of determining the concentration of analyte in a sample; it occurs after the data acquisition phase.

Quantitative analysis is used to determine the concentration—the amount of analyte per volume—of a particular substance in a sample. By analyzing an unknown sample and comparing it to other samples containing the same substance in a known concentration (standard), the software can calculate the concentration of the unknown sample. The process involves building a calibration curve using a standard and then using it to interpolate the concentration for the unknown sample. The calculated concentrations of each sample are then available in a Results Table.

Quantitative Methods

A quantitative method can be created either before or after data acquisition; it is a set of instructions used to generate a quantitation Results Table. The quantitation method can include parameters used to locate and integrate peaks, generate standard curves, and calculate unknown concentrations. It can also include a query to sort the data.

Queries

A query is a method of selecting only those records that meet certain criteria. You can use queries to view particular parts of the data in the Results Table that interest you, based on textual or mathematical selections.

When you use a query, the table displays only the rows of data that meet the criteria you select. All columns are displayed. You can further refine your selection by running a second query on the rows displayed by the first query.

Use pre-defined choices and typed entries to create a query that can be executed, saved, or modified. Each line of the query works like a Boolean search that runs against Results Table

columns to determine which records to display. Each line of the query selects only the records for display that meet its criteria. You can define a default or table-specific query.

Queries on Sample Type

If you design a query that selects only the standard sample type, the Results Table displays only those rows of data that contain Standard in the Sample Type column.

Default Queries and Table-Specific Queries

A default query is generally used to identify samples that do not meet certain criteria. A table-specific query is generally used to identify records that meet certain criteria.

The default query is generally used to find problems with quality control or standards. If you select the concentration and maximum variation of the QCs and standards in the Quantitation Method Wizard, then the Results Table displays only those samples that lie outside this range. If the Results Table displays nothing, then the samples are all right.

Table-specific queries are run against a displayed Results Table to select records that meet certain criteria. Design these queries through the menu that is available when you right-click in the table. You can save and export a query to make it available for future Results Tables.

Table-Specific or Global Settings

When you are working with table setting options, you will notice procedures that are table-specific or global.

- **Table-specific settings:** When you modify the table settings on the table itself, you are making changes to the settings that are available only to that table; however, you can export them as global settings.
- **Global settings:** When you modify global settings, you are making changes to a group of settings that can be applied to future Results Tables. You can customize a Results Table that you are in the process of creating by choosing a group of settings on the Create Quantitation Set: Select Settings & Query page. If you do not choose a group of settings, the software automatically uses the default settings.

How Accuracy Variations Affect the Results

For default queries, accuracy is expressed as a percent and implemented as plus or minus that number. For example, if you type 10 for Maximum Variation for standards in the Create Default Query dialog, then all records containing standards whose accuracy falls outside 90% and 110% are displayed in the Results Table. If you typed 5, only standards whose accuracy is less than 95% and greater than 105% would appear in the Results Table. For more information, see [Results Tables on page 40](#).

Regression

This section gives the equations for each of the regression types. In the following equations, x , y , and w are as described in [Weighting Factors on page 52](#).

In the following equations, x represents the analyte concentration for standard sample types, and y represents the corresponding peak area or height. The exact variables used for the regression

depend on whether or not an internal standard is being used and whether the peak area or the peak height is used as shown in the following table.

Table 1-7 Regression Formulae

| Internal Standard Used? | Area Used? | x | y |
|-------------------------|------------|-----------------|--------------|
| Yes | Yes | $C_a/C_{is}/DF$ | A_a/A_{is} |
| Yes | No | $C_a/C_{is}/DF$ | H_a/H_{is} |
| No | Yes | C_a/DF | A_a |
| No | No | C_a/DF | H_a |

Where:

- C_a = actual analyte concentration
- C_{is} = internal standard concentration
- DF = dilution factor
- A_a = analyte peak area
- A_{is} = internal standard peak area
- H_a = analyte peak height
- H_{is} = internal standard peak height

Linear Regression

Linear regression assumes that the points of the standard fall on a straight line.

The linear calibration equation is calculated as:

$$y = m x + b$$

The slope and intercept are calculated as:

$$m = (\sum w \sum wxy - \sum wx \sum wy) / D_x$$

$$b = (\sum wx^2 \sum wy - \sum wx \sum wxy) / D_x$$

The correlation co-efficient is calculated as:

$$r = (\sum w \sum wxy - \sum wx \sum wy) / \sqrt{(D_x D_y)}$$

where:

$$D_x = \sum w \sum wx^2 - (\sum wx)^2$$

$$D_y = \sum w \sum wy^2 - (\sum wy)^2$$

Linear Through Zero regression

Linear through zero regression assumes that the points of the standard fall on a straight line and that the points do line up with the zero point on the x and y axes. Use this setting to force the line to go through the zero point.

The linear through zero calibration equation is calculated as:

$$y = m x$$

The slope is calculated as:

$$m = \Sigma wxy / \Sigma wx^2$$

The correlation co-efficient is calculated as:

$$r = \Sigma wxy / \sqrt{(\Sigma wx^2 \Sigma wy^2)}$$

If the standard points fall on a straight line and you want to average the points, then use mean response factor regression to produce an average of the slope for every point on the curve.

Mean Response Factor

The mean response factor calibration is calculated as:

$$y = m x$$

This is the same equation as for the linear through zero case. However the slope is calculated differently as:

$$m = \Sigma w(y/x) / \Sigma w$$

The standard deviation of the response factor as:

$$\sigma = \sqrt{\langle nD / \langle n - 1 \rangle \rangle} / \Sigma w$$

Where:

$$D = \Sigma w * \Sigma wy^2 / x^2 - \langle \Sigma wy / x \rangle^2$$



Note: Points whose x value is zero are excluded from the sums.

If there is some linear and some curvature in the line of points, then use power regression instead of linear or quadratic regression to produce a line somewhere between these fits.

Power

The power function calibration equation is calculated as follows:

$$y = ax^p$$

The equations for the linear calibration are used as described previously to calculate the slope (m) and intercept (b), except that x in those equations is replaced by ln x and y replaced by ln y. When this is done a, p and the correlation co-efficient (r) are calculated as:

$$a = \exp (b)$$

$$p = m$$

$$r = r$$

If the points of the standard do not fall on a straight line, use quadratic regression to produce a quadratic fit to the data points. This setting is frequently used.

Quadratic Calibration Equation

The quadratic calibration equation is calculated as:

$$y = a_2x^2 + a_1x + a_0$$

The polynomial co-efficients are calculated as:

$$a_2 = \langle b_2/b_0 - b_5/b_3 \rangle / \langle b_1/b_0 - b_4/b_3 \rangle$$

$$a_1 = b_5/b_3 - a_2 b_4/b_3$$

$$a_0 = \langle \Sigma w y - a_1 \Sigma w x - a_2 \Sigma w x^2 \rangle / \Sigma w$$

The correlation co-efficient as:

$$r = \sqrt{\langle 1 - \Sigma w^* \Sigma w \langle y - a_2 x^2 - a_1 x - a_0 \rangle^2 / D_y \rangle}$$

Where:

$$D_y = \Sigma w \Sigma w y^2 - \langle \Sigma w y \rangle^2$$

$$b_0 = \Sigma w x / \Sigma w - \Sigma w x^2 / \Sigma w x$$

$$b_1 = \Sigma w x^2 / \Sigma w - \Sigma w x^3 / \Sigma w x$$

$$b_2 = \Sigma w y / \Sigma w - \Sigma w x y / \Sigma w x$$

$$b_3 = \Sigma w x^2 / \Sigma w x - \Sigma w x^3 / \Sigma w x^2$$

$$b_4 = \Sigma w x^3 / \Sigma w x - \Sigma w x^4 / \Sigma w x^2$$

$$b_5 = \Sigma w x y / \Sigma w x - \Sigma w x^2 y / \Sigma w x^2$$

Report Templates

You can add the following information to report headers and footers.



Tip! Make a backup of the existing report templates before editing them.

Table 1-8 Basic Design Elements

| Element | Definition |
|----------------------|---|
| Printing Date | The date the document was printed. |
| Printing Time | The time the document was printed. |
| Operator | The operator who printed the document. |
| Workstation | The workstation from which the document was printed from. |
| Page n of N | Indicates the page number of total number of pages. |
| Custom Field | Create custom text here. |
| Analyst Version | Indicates the version of the Analyst [®] software. |
| User Type | Indicates the user type (Security). |
| Electronic Signature | Indicates whether the electronic signature feature (security) is enabled or disabled. |

Table 1-9 Acquisition Elements

| Element | Definition |
|------------------|--|
| Acquisition File | The name of the data file with the sample acquisition information. |

Table 1-9 Acquisition Elements (Continued)

| Element | Definition |
|------------------------|---|
| Acquisition Date | The date the sample acquisition was run. |
| Acquisition Time | The time of the sample acquisition was run. |
| Operator | The name of the operator who ran the sample batch. |
| Batch Name | The name of the batch. |
| Sample Number | The number related to the sample. |
| Sample Name | The name of the sample. |
| Sample Comment | Shows any comments about the sample entered through the Acquisition Method Editor. |
| Sample ID | The identification number of the sample. |
| Scan Mode | Indicates the method in which the system calculates the mass points for a scan for a full mass range scan. |
| Scan Type and Polarity | Indicates the acquisition scan type (Q1, Q3, MRM, Product ion, Precursor ion, neutral loss/gain) and acquisition method polarity (positive or negative). |
| Scan Mass(es) | Ions or ion fragments to be scanned. |
| Dwell Time | The time the system takes to scan a particular mass. |
| Pause Time | Indicates the pause between the scanning of mass ranges or between experiments. |
| Ion Energy | Ion energy comes from the acquisition method and is related to the IonSpray™ ion source voltage or the collision energy. |
| Collision Energy | Collision energy comes from the acquisition method and is related to the IonSpray ion source voltage. |
| Period and Experiment | A period contains a collection of experiments; an experiment contains a number of properties, such as Scan Type, Scan Mode, Resolution, Ion Source Parameters, and a collection of mass ranges or masses. |
| State Table Parameters | The mass spectrometer parameters used in the experiment. |
| Pump | The name of the pump used for the experiment. |
| Autosampler | The name of the autosampler used for the experiment. |
| Custom Annotation | The custom text added via the Batch Editor. |
| Collected By | The name of the person who collected the data. |

Table 1-10 Quantitation Elements

| Element | Definition |
|--------------------|--------------------------------------|
| Results Table Name | The name of the Results Table. |
| Results Table Path | The location of the Results Table. |
| Method Name | The name of the quantitation method. |
| Method Path | The location of the method file. |
| Project Name | The name of the project. |

Table 1-10 Quantitation Elements (Continued)

| Element | Definition |
|--------------------|--------------------------------|
| Results Table Name | The name of the Results Table. |

Customizing Reports

The Report Template Editor provides a way to customize reports by setting up headers, footers, and page layouts. You can use report templates with both printed output and data exported to another application.

Printed output includes several types of elements.

- **Window:** Windows in the Analyst software appear in the working area of the software window, below the toolbar and to the right of the Navigation bar. When you print a window, you are printing everything that appears in that space.
- **Pane:** Panes are parts of a window arranged in such a way that they do not overlap and are always fully visible. For example, the Method Editor window contains two panes: the Browser pane and the Method Editor pane. You can print information from each pane in the window.
- **Report:** Reports are structured sets of information created in the software. Some reports can be directly printed, such as calibration reports; other information must be exported, such as batches and quantitation Results Tables.
- **Workspace:** A workspace is a particular arrangement of windows and panes along with an associated file or files. When you print a workspace, you print each open window and pane in the current mode.

Previewing, Printing, and Exporting Reports

You can export acquisition methods, batches, quantitation Results Tables, and graph Results Tables as reports. Other forms of information, such as calculator data, can be exported but cannot be customized with a report template.

You can print most areas that you see on the screen. You can preview, scale, or copy graphs using the Print Preview feature.

When you export a report, you save the data in a file format that is appropriate for programs such as Notepad, Microsoft Word or Excel, or certain LIMS (Laboratory Information Management System) software.

You can export reports in the following formats:

- .csv
- .doc
- .pdf
- .txt

The formats available depend on the information being exported. For example, if you are exporting a graph, you can export it as a .pdf; if you are exporting a table of data, you can export it as a .txt file.

If you want to include additional information in the header and footer of the report, print the report using an appropriate template.

Table 1-11 Previewing, Printing, and Exporting Reports

| To do this... | ...do this |
|--------------------------------------|--|
| To preview a graph | <ol style="list-style-type: none"> 1. Click File > Print Preview > Pane. 2. Edit the Print Preview dialog. 3. Click Print. |
| To print a report without a template | <ul style="list-style-type: none"> • Click File > Print, and then click the report that you want to print. |
| To print a report with a template | <ol style="list-style-type: none"> 1. Click File > Print & Report Setup. 2. In the Report Template section, select the template that you want to use and then click OK. |
| To export a report | <ol style="list-style-type: none"> 1. Click File > Export. 2. In the File field, type the name of the file. 3. In the Save as type list, select the appropriate file type for the type of report you are exporting. 4. If you are exporting a report in Quantitate mode, select either All Columns or Visible Columns from the Export section and then click Save. |

Results Tables

Results Tables summarize the calculated concentration of analyte in each unknown sample based on the calibration curve. They also include the calibration curves themselves and statistics for the results.

With the software you can export the data from a Results Table to a .txt file for use in other applications, such as Microsoft Excel. You can choose to export all possible data in the table or just the data in the visible columns.

About Using Queries with Results Tables

A query is a request for records in a Results Table that the certain conditions set using textual or mathematical selection criteria. You can apply a query either during the process of generating a Results Table or after one has been generated. These two types of queries are called default and table-specific queries. For more information, see [Default Queries and Table-Specific Queries on page 34](#).

Comparing Results Between Batches

When more than one Results Table is displayed, you can obtain statistical information on the standards and QCs for additional batches in the Statistics window. You normally compare results between batches to look for trends in the standards or QCs or to verify that the method is valid.

If you have two or more Results Tables open, you can compare results in the Statistics window. Both sets of statistics appear in the Statistics window.



Note: The number of analytes and the number of analyte names must be the same for the data to be combined in the Statistics window.

How Concentration Levels Affect Results

The concentration is defined for all QCs and standards. If there is a change in the accuracy of the concentration level by more than the amount defined in the Max. Variation field in the Create Default Query dialog, then this information appears in the Results Table.

Defining the Layout of Results Tables

The software has four predefined views of the Results Table.

- [Full Layout View](#)
- [Summary Layout View](#)
- [Analyte Layout View](#)
- [Analyte Group Layout View](#)

If you have multiple analytes per sample, you can see each analyte in the Analyte Layout view. The default view is Full layout.

Full Layout View

The default Full Layout view shows the data for all analytes in the quantitation batch. The columns that are displayed depend on the columns selected in the Results Table Columns dialog, and the settings selected on the second page of the Quantitation Method Wizard.

| | Sample Name | Sample Type | File Name | Analyte Peak Name | Analyte Peak Area | Analyte Peak Height | Analyte Concentration |
|----|---------------------|-------------|--------------|-------------------|-------------------|---------------------|-----------------------|
| 1 | B series 0 blank | Blank | QuanData.Wif | Peak 1 | 2.45e+002 | 6.02e+001 | 0.00 |
| 2 | B series 0 blank | Blank | QuanData.Wif | Peak 2 | 1.25e+004 | 4.53e+003 | 0.00 |
| 3 | B series 0.1 ng/mL | Standard | QuanData.Wif | Peak 1 | 7.60e+002 | 2.53e+002 | 0.00 |
| 4 | B series 0.1 ng/mL | Standard | QuanData.Wif | Peak 2 | 1.39e+004 | 4.93e+003 | 0.00 |
| 5 | B series 0.2 ng/mL | Standard | QuanData.Wif | Peak 1 | 1.55e+003 | 5.08e+002 | 0.00 |
| 6 | B series 0.2 ng/mL | Standard | QuanData.Wif | Peak 2 | 1.28e+004 | 4.27e+003 | 0.00 |
| 7 | B series 0.5 ng/mL | Standard | QuanData.Wif | Peak 1 | 3.32e+003 | 1.04e+003 | 0.00 |
| 8 | B series 0.5 ng/mL | Standard | QuanData.Wif | Peak 2 | 1.14e+004 | 4.20e+003 | 0.00 |
| 9 | B series 1.0 ng/mL | Standard | QuanData.Wif | Peak 1 | 7.12e+003 | 2.33e+003 | 0.00 |
| 10 | B series 1.0 ng/mL | Standard | QuanData.Wif | Peak 2 | 1.23e+004 | 4.35e+003 | 0.00 |
| 11 | B series 2.0 ng/mL | Standard | QuanData.Wif | Peak 1 | 1.50e+004 | 4.77e+003 | 0.00 |
| 12 | B series 2.0 ng/mL | Standard | QuanData.Wif | Peak 2 | 1.34e+004 | 4.63e+003 | 0.00 |
| 13 | B series 5.0 ng/mL | Standard | QuanData.Wif | Peak 1 | 3.70e+004 | 1.20e+004 | 0.00 |
| 14 | B series 5.0 ng/mL | Standard | QuanData.Wif | Peak 2 | 1.51e+004 | 5.29e+003 | 0.00 |
| 15 | B series 10.0 ng/mL | Standard | QuanData.Wif | Peak 1 | 7.73e+004 | 2.49e+004 | 0.00 |
| 16 | B series 10.0 ng/mL | Standard | QuanData.Wif | Peak 2 | 1.50e+004 | 5.41e+003 | 0.00 |
| 17 | B series 20.0 ng/mL | Standard | QuanData.Wif | Peak 1 | 7.61e+004 | 2.44e+004 | 0.00 |
| 18 | B series 20.0 ng/mL | Standard | QuanData.Wif | Peak 2 | 8.04e+003 | 3.13e+003 | 0.00 |

Figure 1-13 Sample Full Layout view

Summary Layout View

The Summary Layout view contains the locked columns and the chosen field for each analyte in the remaining columns. For example, if you have two analytes and choose Analyte Peak Area from the menu, then you see the Sample Name and Analyte Peak Area columns for those analyte names. The Summary Layout view also includes the Formula and Custom columns, if these exist.

| | Sample Name | Peak 1 | Peak 2 |
|----|---------------------|-----------|-----------|
| 1 | B series 0 blank | 2.45e+002 | 1.25e+004 |
| 3 | B series 0.1 ng/mL | 7.80e+002 | 1.39e+004 |
| 5 | B series 0.2 ng/mL | 1.55e+003 | 1.28e+004 |
| 7 | B series 0.5 ng/mL | 3.32e+003 | 1.14e+004 |
| 9 | B series 1.0 ng/mL | 7.12e+003 | 1.23e+004 |
| 11 | B series 2.0 ng/mL | 1.50e+004 | 1.34e+004 |
| 13 | B series 5.0 ng/mL | 3.70e+004 | 1.51e+004 |
| 15 | B series 10.0 ng/mL | 7.73e+004 | 1.50e+004 |
| 17 | B series 20.0 ng/mL | 7.61e+004 | 8.04e+003 |
| 19 | Unknown concentra | 1.23e+004 | 8.39e+003 |
| 21 | Unknown concentra | 8.71e+003 | 5.71e+003 |
| 23 | Unknown concentra | 1.12e+004 | 7.18e+003 |
| 25 | Unknown concentra | 1.32e+004 | 7.36e+003 |
| 27 | Unknown concentra | 1.25e+004 | 7.14e+003 |
| 29 | Unknown concentra | 1.10e+004 | 6.50e+003 |
| 31 | Unknown concentra | 1.36e+004 | 7.94e+003 |

Figure 1-14 Sample Summary Layout View

Analyte Layout View

The Analyte Layout view contains the data for a particular analyte; all other analytes are hidden. For example, if you choose analyte A you see all the data for analyte A. The columns that are displayed depend on the columns selected in the Results Table Columns dialog, and the settings selected on the second page of the Quantitation Method Wizard.

An Analyte Layout view, with Peak 1 selected, might look like [Figure 1-15](#). If you compare this layout with the Full Layout view, you will notice that every other row has been excluded.

| | Sample Name | File Name | Analyte Peak Area | Analyte Peak Height | Analyte Concentration | Use Record | Record Modified |
|----|---------------------|--------------|-------------------|---------------------|-----------------------|-------------------------------------|--------------------------|
| 1 | B series 0 blank | QuanData.Wif | 2.45e+002 | 5.02e+001 | 0.00 | | <input type="checkbox"/> |
| 3 | B series 0.1 ng/mL | QuanData.Wif | 7.80e+002 | 2.53e+002 | 0.00 | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| 5 | B series 0.2 ng/mL | QuanData.Wif | 1.55e+003 | 5.08e+002 | 0.00 | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| 7 | B series 0.5 ng/mL | QuanData.Wif | 3.32e+003 | 1.04e+003 | 0.00 | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| 9 | B series 1.0 ng/mL | QuanData.Wif | 7.12e+003 | 2.33e+003 | 0.00 | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| 11 | B series 2.0 ng/mL | QuanData.Wif | 1.50e+004 | 4.77e+003 | 0.00 | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| 13 | B series 5.0 ng/mL | QuanData.Wif | 3.70e+004 | 1.20e+004 | 0.00 | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| 15 | B series 10.0 ng/mL | QuanData.Wif | 7.73e+004 | 2.49e+004 | 0.00 | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| 17 | B series 20.0 ng/mL | QuanData.Wif | 7.61e+004 | 2.44e+004 | 0.00 | <input checked="" type="checkbox"/> | <input type="checkbox"/> |
| 19 | Unknown concentra | QuanData.Wif | 1.23e+004 | 4.30e+003 | N/A | | <input type="checkbox"/> |
| 21 | Unknown concentra | QuanData.Wif | 8.71e+003 | 2.53e+003 | N/A | | <input type="checkbox"/> |
| 23 | Unknown concentra | QuanData.Wif | 1.12e+004 | 3.40e+003 | N/A | | <input type="checkbox"/> |
| 25 | Unknown concentra | QuanData.Wif | 1.32e+004 | 4.24e+003 | N/A | | <input type="checkbox"/> |
| 27 | Unknown concentra | QuanData.Wif | 1.25e+004 | 4.04e+003 | N/A | | <input type="checkbox"/> |
| 29 | Unknown concentra | QuanData.Wif | 1.10e+004 | 3.96e+003 | N/A | | <input type="checkbox"/> |
| 31 | Unknown concentra | QuanData.Wif | 1.36e+004 | 5.16e+003 | N/A | | <input type="checkbox"/> |

Figure 1-15 Sample Analyte Layout view

Analyte Group Layout View

The Analyte Group Layout view contains the data for the analytes that belong to a particular group. Columns that you select as shown in the Results Table Columns dialog appear in the Results Table as shown in [Figure 1-16](#). Display the Analyte Peak Name column in the Results Table to show the names of the analytes that belong in the group.

| | Sample Name | Sample ID | Sample Type | File Name | Analyte Peak Name |
|---|-------------|-----------|-------------|------------------|-------------------|
| 1 | STD 1 | | Standard | Mix_batch_1.wiff | minoxidol |
| 2 | STD 1 | | Standard | Mix_batch_1.wiff | minoxidol |
| 3 | STD 2 | | Standard | Mix_batch_1.wiff | minoxidol |
| 4 | STD 2 | | Standard | Mix_batch_1.wiff | minoxidol |
| 5 | STD 3 | | Standard | Mix_batch_1.wiff | minoxidol |
| 6 | STD 3 | | Standard | Mix_batch_1.wiff | minoxidol |

Figure 1-16 Sample Analyte Group Layout view

Results Table Fields

You can add columns to the standard Results Table to display DAD (diode array detector) data for the Analyte, Internal Standard, and Record fields.

Formula Fields

The Formula fields display the result of a spreadsheet-style formula that you have defined.

The Formula field located at the top of the Results Table appears only if at least one Formula column is in the Results Table. The Formula field becomes active when Formula column cells are selected.

The Delete Formula Column button below the Formula field also becomes available when the Formula column is selected.

Custom Fields

Custom fields contain information defined during the acquisition process. When you acquire samples, you can create custom columns and define the type of data that goes in them. Once the custom column is part of the Results Table, you can treat it like any other column (for example, move it, hide it, base a formula on it).

Internal Standards Column Fields

The Internal Standard Results fields display information about the internal standard after analysis. [Table 1-12](#) shows the available fields.

Table 1-12 Internal Standards Column Fields

| Field | Definition |
|------------------|---|
| IS Peak Name | The name of the internal standard peak. |
| IS Units | The units in which the internal standard is given. |
| IS Peak Area | The area of the internal standard peak. |
| IS Peak Height | The height of the internal standard peak. |
| IS Concentration | The known concentration of the internal standard, this applies to standard and quality control sample types. Zeroes are displayed for solvent, blank, and double blank sample types; N/A is displayed for unknowns. |

Table 1-12 Internal Standards Column Fields (Continued)

| Field | Definition |
|------------------------------------|---|
| IS Retention Time | The chromatographic retention time as determined by the software. |
| IS Expected Retention Time | The retention time of the representative sample. Taken from the quantitation method. |
| IS Retention Time Window | The retention time window as specified in the quantitation method. |
| IS Centroid Location | The intensity-weighted average retention time for the analyte. The peak areas up to and after this time are identified. |
| IS Start Scan | The cycle number associated with the period/experiment combination where the peak begins. |
| IS Start Time | The time associated with the period/experiment combination where the peak begins. |
| IS Stop Scan | The cycle number associated with the period/experiment combination where the peak ends. |
| IS Stop Time | The time associated with the period/experiment combination where the peak ends. |
| IS Integration Type | The method by which the baseline was found and integrated when the peak was found. The types are: manual, automatic (Baseline-to-Baseline, Valley, Exponential Skim, and Exponential Child). |
| IS Signal to Noise | The signal-to-noise ratio of the peak. |
| IS Peak Width | The ratio of the peak height to its width. |
| IS UV Range | The UV range of the internal standard. |
| IS UV Channel | The UV channel of the internal standard. |
| IS Peak Width at 50 Percent (min.) | A read-only column displaying the peak width at 50% of peak height. |
| IS Baseline Slope (%/min.) | A read-only column displaying the slope of the baseline. |
| IS Peak Asymmetry | A read-only column displaying the peak asymmetry which is calculated by the following formula: $\frac{[(\text{Peak End Time}) - (\text{Retention Time})]}{[(\text{Retention Time}) - (\text{Peak Start Time})]}$ Values near 1.0 indicate symmetric peaks, values greater than 1.0 indicate tailing peaks, and values less than 1.0 indicate fronting peaks. |
| IS Processing Alg | A read-only column displaying the processing algorithm used. |
| IS Integration Quality | The Integration Quality™ Index indicates how well the peak is integrated. Values closer to 1 indicate well-integrated peaks and values closer to 0 indicate poorly integrated peaks. |

Record Fields

The Record fields display additional information about each sample record (information that is applicable only to the analyte, not the internal standard). [Table 1-13](#) shows the available fields.

Table 1-13 Record Fields

| Field | Definition |
|--------------------------|--|
| Use Record | Indicates whether this record should be included for calibration. Applies to standards and QCs. If the check box is cleared, then the unused standards and QCs are struck out in the Statistics Table. |
| Record Modified | Indicates whether the quantitation method used for the record was modified in any way from the original. |
| Calculated Concentration | The calculated concentration of the analyte as calculated using the calibration curve. |
| Relative Retention Time | The ratio of the retention times of the internal standard and the analyte. |
| Accuracy | The calculated concentration divided by the known concentration (as a percentage). |
| Response Factor | The peak area or height (depending on the regression option) divided by the analyte concentration. |

Sample Column Fields

The Analyte Results fields display information about each analyte and internal standard (if one was used) after analysis. [Table 1-14](#) shows the available fields.

Table 1-14 Results Tables: Sample Column Fields

| Field | Definition |
|---------------------------------|---|
| Analyte Peak Name | The name of the analyte. |
| Analyte Units | The units in which the analyte concentrations are given. |
| Analyte Peak Area | The area of the analyte. |
| Analyte Peak Height | The height of the analyte peak. |
| Analyte Concentration | The actual, known concentration of the analyte, this applies to standard and quality control sample types. Zeroes are displayed for solvent, blank, and double blank sample types; N/A is displayed for unknowns. |
| Analyte Retention Time | The chromatographic retention time as determined by the software. |
| Analyte Expected Retention Time | The retention time of the representative sample as taken from the quantitation method. |
| Analyte Retention Time Window | The retention time window as specified in the quantitation method. |
| Analyte Centroid Location | The intensity-weighted average retention time for the analyte. The peak areas up to and after this time are identified. |
| Analyte Start Scan | The cycle number associated with the period/experiment combination where the peak begins. |

Table 1-14 Results Tables: Sample Column Fields (Continued)

| Field | Definition |
|---|---|
| Analyte Start Time | The time associated with the period/experiment combination where the peak begins. |
| Analyte Stop Scan | The cycle number associated with the period/experiment combination where the peak ends. |
| Analyte Stop Time | The time associated with the period/experiment combination where the peak ends. |
| Analyte Integration Type | The method by which the baseline was found and integrated when the peak was found. The types are: manual, automatic (Baseline-to-Baseline, Valley, Exponential Skim, and Exponential Child). |
| Analyte Signal to Noise | The signal-to-noise ratio of the peak compared to the baseline. |
| Analyte Peak Width | The ratio of the peak height to its width. |
| Analyte UV Range | The UV range of the analyte. |
| Analyte UV Channel | The UV channel of the analyte. |
| Analyte Peak Width at 50 Percent (min.) | A read-only column displaying the peak width at 50% of peak height. |
| Analyte Baseline Slope (%/min.) | A read-only column displaying the slope of the baseline. |
| Analyte Peak Asymmetry | <p>A read-only column displaying the peak asymmetry which is calculated by the following formula:</p> $\frac{[(\text{Peak End Time}) - (\text{Retention Time})]}{[(\text{Retention Time}) - (\text{Peak Start Time})]}$ <p>Values near 1.0 indicate symmetric peaks, values greater than 1.0 indicate tailing peaks, and values less than 1.0 indicate fronting peaks.</p> |
| Analyte Processing Alg | A read-only column displaying the processing algorithm used |
| Analyte Integration Quality | Integration Quality™ Index indicates how well the peak is integrated. Values closer to 1 indicate well-integrated peaks and values closer to 0 indicate poorly integrated peaks. This facilitates peak review as you can see the peaks with low Analyte Integration Quality values for manual review. In addition, you can query the data for the Analyte Integration Quality values that are less than a value you consider acceptable in order to display and manually review a subset of the data. |

Sample Column Fields

The Sample Column fields display information about the sample that is common to all analytes. Blank and double blank can be defined differently from lab to lab. [Table 1-15](#) shows the available fields:

Table 1-15 Sample Column Fields

| Field | Definition |
|------------------------|---|
| Sample Name | The name that the user assigned to the sample when it was acquired. |
| Sample ID | A user-defined identifier associated with the sample. |
| Sample Type | <p>All analytes within a sample must have the same sample type. One of the following sample types is displayed:</p> <p>Unknown: Contains analytes whose concentrations are to be determined.</p> <p>Standard: A sample with known analyte concentration; used for calibration purposes.</p> <p>Quality Control: A sample with known analyte concentration; used to check the accuracy of the standard curve.</p> <p>Solvent: Confirms that the instrument is clean. Solvents are not run through the sample preparation process.</p> <p>Blank: A zero concentration sample that is not used in regression.</p> <p>Double Blank: A sample prepared without an internal standard or sample analyte; confirms that the extraction process added nothing.</p> |
| Sample Comment | A comment describing the sample. |
| Set Number | A number that identifies a subset of an entire batch. |
| Acquisition Method | The name of the method used to acquire the samples. |
| Acquisition Date | The date and time when the acquisition was run. |
| Rack Type | An identifier associated with the kind of autosampler rack used (if any). |
| Rack Number | The rack position in which the sample was placed when it was acquired. (In single-rack autosamplers, this is always 1.) |
| Vial Position | The position in the autosampler plate where the vial was located. |
| Plate Type | An identifier for the kind of plate used (multiplate racks only.) |
| Plate Number | The plate position on the rack (multiplate racks only.) |
| File Name | The name of the raw data file. This name is not unique because the data from many samples may be contained in a single data file. |
| Dilution Factor | The amount by which the sample was diluted; used to determine the calculated concentration. |
| Sample Annotation | Additional comments describing the sample. |
| Weight-to-Volume Ratio | Gives the weight to volume ratio for the sample. |

Table 1-16 DAD Fields

| Field | Definition |
|-----------------------------|---|
| Analyte Peak Area for DAD | The area of the analyte peak (mAU/min). |
| Analyte Peak Height for DAD | The height of the analyte peak (mAu). |
| Analyte Wavelength Ranges | The range of wavelengths (nm). |
| IS Peak Area for DAD | The area of the internal standard peak (mAU/min). |
| IS Wavelength Ranges | The range of wavelengths (nm). |
| IS Peak Height for DAD | The height of the internal standard peak (mAU) |
| | Calculated Concentration for DAD. |

[Table 1-17](#) shows the fields that can be added to the Results Table for data acquired by an ADC (analog-to-digital converter).

Table 1-17 ADC Fields

| Field | Definition |
|---------------------------|--|
| Analyte Channel | The ADC channel from which the analyte was acquired. |
| Analyte Wavelength Ranges | The range of wavelengths (nm). |
| IS Channel | The ADC channel from which the internal standard was acquired. |
| IS Wavelength Ranges | The range of wavelengths (nm). |

Scripts

Research-grade scripts are available to extend the functionality of the Analyst[®] software. Some scripts are installed automatically with the Analyst software. The remaining scripts can be installed individually. For more information, see the *Scripts User Guide* for the Analyst software.

Signal-to-Noise Ratio

The signal-to-noise ratio is the peak height divided by the noise.

To calculate the noise, the software uses the standard deviation (using a mean of zero) of all data points in the chromatogram from the Background Start to Background End time (both shown in the advanced parameters for the Quantitation Method Editor and Peak Review window.) These times are set when you define a new background range.

If you build a method without defining a new background range (which is possible if you accept the default integration with no changes), then the value for both Background Start and Background End is N/A. As a result, the signal-to-noise ratio is not calculated and the corresponding field in the Results Table reads N/A.

Smoothing Algorithms

You can select either the smoothing algorithm or the Gaussian smoothing algorithm as your smoothing method. The smoothing operation involves replacing each data point with the average of the data point before and after it. The smoothed data set replaces the old set.

Data can be smoothed more than once, but you can undo only the last smooth. Smoothing is not available for MI or MRM spectra.

Software Security

The software has a number of functions for configuring and managing security. The Analyst[®] software administrator can:

- Choose a security mode to best suit the needs of the operating environment.
- Add and delete users and roles.
- Set access rights to users and roles as required.
- Control access to remote instrument stations.
- Control access to project files.

For more information about Analyst software security, see the *Laboratory Director's Guide*.

Location of Security Information

When the software is running on a single workstation, all security information is stored in the Bin folder on that workstation.

When the software is running in a networked environment, the Windows security information is stored on the server and workstation. The security information is stored separately on each workstation in the bin folder.

If you are using the Administrator Console, a copy of the instrument security database is saved in both a local folder and a network folder. The local copy is the working copy. The network copy is used to replace the local copy when there are changes to the database.



Note: Projects and other files may be stored on any workstation that is part of a network.

Solvent Compressibility Values

Table 1-18 HPLC Solvent Compressibility Values

| Solvent | Compressibility (10^{-6} /bar) |
|----------------------|-----------------------------------|
| Acetone | 126 |
| Acetonitrile | 115 |
| Benzene | 95 |
| Carbon Tetrachloride | 110 |

Table 1-18 HPLC Solvent Compressibility Values (Continued)

| Solvent | Compressibility (10^{-6} /bar) |
|---------------|-----------------------------------|
| Chloroform | 100 |
| Cyclohexane | 118 |
| Ethanol | 114 |
| Ethyl acetate | 104 |
| Heptane | 120 |
| Hexane | 150 |
| Isobutanol | 100 |
| Isopropanol | 100 |
| Methanol | 120 |
| 1-Propanol | 100 |
| Toluene | 87 |
| Water | 46 |

Spectral Arithmetic Wizard

You can use the Spectral Arithmetic Wizard to perform arithmetic operations using two complete chromatograms to create a new chromatogram, which is saved as a new data file. For more information, see the Help.

- **Subtraction:** Use the subtraction option to subtract a control or background from a sample.
- **Addition:** Use the addition option to add separate chromatograms together to create a single, more complete profile for a compound. For example, you could create several chromatograms using different collision energies and then add the chromatograms together to create a single profile.
- **Average:** Use the average option to statistically improve the profile for a compound and to generate more reliable spectra.

Spectrum

A spectrum is the data that is obtained directly from the mass spectrometer and normally represents the number of ions detected with particular mass-to-charge (m/z) values. It is displayed as a graph with the m/z values on the x-axis and intensity (cps) represented on the y-axis.

Syringe Size Versus Flow Rate

The flow rate of a syringe pump depends on the syringe installed in the pump. The following tables show the relationship between flow rate and syringe size.

Table 1-19 Syringe Size and Flow Rate at L/hour

| Syringe size | L/hour | |
|--------------|---------|---------|
| (μ L) | Minimum | Maximum |
| 0.5 | .002 | 23.8 |
| 1.0 | .003 | 47.8 |
| 2.0 | .006 | 95.2 |
| 5.0 | .015 | 238.0 |
| 10.0 | .029 | 474.0 |
| 25.0 | .073 | 1193.0 |

Table 1-20 Syringe Size and Flow Rate at μ L/minute

| Syringe size | μ L/minute | |
|--------------|----------------|---------|
| (μ L) | Minimum | Maximum |
| 50 | .002 | 39.7 |
| 100 | .005 | 79.7 |
| 250 | .012 | 197.8 |
| 500 | .024 | 397.0 |
| 1000 | .048 | 795.0 |
| 1.0 | .049 | 805.0 |

Table 1-21 Syringe Size and Flow Rate at mL/hour

| Syringe size | mL/hour | |
|--------------|---------|---------|
| (mL) | Minimum | Maximum |
| 2.0 | .011 | 186.8 |
| 2.5 | .010 | 168.2 |
| 3.0 | .011 | 181.4 |
| 5.0 | .019 | 317.0 |
| 10.0 | .028 | 461.0 |
| 20.0 | .050 | 821.0 |
| 30.0 | .074 | 1208.0 |

Table 1-22 Syringe Size and Flow Rate at mL/minute

| Syringe size | mL/minute | |
|--------------|-----------|---------|
| (mL) | Minimum | Maximum |
| 50.0 | .002 | 28.40 |
| 100.0 | .003 | 47.60 |
| 140.0 | .004 | 55.10 |

Weighting Factors

The following table shows how the weighting factor (w in the equations below) is calculated for each of the seven weighting types. See also [Regression on page 34](#).

Table 1-23 Weighting Factors

| Weighting Type | Weight (w) |
|----------------|---|
| None | Always 1.0 |
| $1 / x$ | If $ x < 10^{-5}$ then $w = 10^5$, otherwise $w = 1 / x $. To place some additional emphasis on lower-value points, use a weighting factor of $1/x$. |
| $1 / x^2$ | If $ x < 10^{-5}$ then $w = 10^{10}$, otherwise $w = 1 / x^2$. Use a weighting of $1/x$ squared to provide a much higher emphasis on lower-value points. |
| $1 / y$ | If $ y < 10^{-8}$ then $w = 10^8$, otherwise $w = 1 / y $. Use a weighting factor of $1/y$ when calibrating by the area (y-axis) rather than by the concentration (x-axis), and you want to place some emphasis on lower-value points. A weighting of $1/y$ is a variant of $1/x$ where y and x should be proportional to each other. |
| $1 / y^2$ | If $ y < 10^{-8}$ then $w = 10^{16}$, otherwise $w = 1 / y^2$. Use a weighting factor of $1/y$ squared when calibrating by the area (y-axis) rather than by the concentration (x-axis), and you want to place extra emphasis on lower-value points. A weighting of $1/y^2$ is a variant of $1/x^2$ where y and x should be proportional to each other. |
| $\ln x$ | If $x < 0$, then an error is generated, otherwise if $x < 10^{-5}$ then $w = \ln 10^5$, otherwise $w = \ln x $. Use the logarithm of x to place more weight on higher value points. |
| $\ln y$ | If $y < 0$, then an error is generated, otherwise if $y < 10^{-8}$ then $w = \ln 10^8$, otherwise $w = \ln y $. Use the logarithm of y to place more weight on higher value points. Use when calibrating by the area (y-axis) rather than by the concentration (x-axis). |

Table 2-1 lists the exact monoisotopic masses and charged species (positive and negative) observed with the PPG (polypropylene glycol) calibration solutions. The masses and ions were calculated using the formula $M = H[OC_3H_6]_nOH$, while the positive ion MS/MS fragments used the formula, $[OC_3H_6]_n(H^+)$. In all calculations, $H = 1.007825$, $O = 15.99491$, $C = 12.00000$, and $N = 14.00307$.



Note: When performing calibration with the PPG solutions, make sure that the correct isotope peak is used.

Table 2-1 PPG Exact Masses

| n | Exact mass (M) | $(M + NH_4)^+$ | MS/MS fragments | $(M + 2NH_4)^{2+}$ | $(M + COOH)^-$ |
|----|----------------|----------------|-----------------|--------------------|----------------|
| 1 | 76.052 | 94.087 | 59.0 | 56.061 | 121.050 |
| 2 | 134.094 | 152.129 | 117.1 | 85.082 | 179.092 |
| 3 | 192.136 | 210.171 | 175.1 | 114.102 | 237.134 |
| 4 | 250.178 | 268.212 | 233.2 | 143.123 | 295.176 |
| 5 | 308.220 | 326.254 | 291.2 | 172.144 | 353.218 |
| 6 | 366.262 | 384.296 | 349.2 | 201.165 | 411.259 |
| 7 | 424.304 | 442.338 | 407.3 | 230.186 | 469.301 |
| 8 | 482.346 | 500.380 | 465.3 | 259.207 | 527.343 |
| 9 | 540.388 | 558.422 | 523.4 | 288.228 | 585.385 |
| 10 | 598.430 | 616.464 | 581.4 | 317.249 | 643.427 |
| 11 | 656.471 | 674.506 | 639.4 | 346.270 | 701.469 |
| 12 | 714.513 | 732.548 | 697.5 | 375.291 | 759.511 |
| 13 | 772.555 | 790.590 | 755.5 | 404.312 | 817.552 |
| 14 | 830.597 | 848.631 | 813.6 | 433.333 | 875.594 |
| 15 | 888.639 | 906.673 | 871.6 | 462.354 | 933.636 |
| 16 | 946.681 | 964.715 | 929.7 | 491.373 | 991.678 |
| 17 | 1004.723 | 1022.757 | 987.7 | 520.396 | 1049.720 |
| 18 | 1062.765 | 1080.799 | 1045.7 | 549.417 | 1107.762 |
| 19 | 1120.807 | 1138.841 | 1103.8 | 578.438 | 1165.804 |
| 20 | 1178.849 | 1196.883 | 1161.8 | 607.459 | 1223.845 |
| 21 | 1236.890 | 1254.925 | 1219.9 | 636.480 | 1281.887 |
| 22 | 1294.932 | 1312.967 | 1277.9 | 665.501 | 1339.929 |
| 23 | 1352.974 | 1371.009 | 1335.9 | 694.521 | 1397.971 |
| 24 | 1411.016 | 1429.050 | 1394.0 | 723.542 | 1456.013 |
| 25 | 1469.058 | 1487.092 | 1452.0 | 752.563 | 1514.055 |

Table 2-1 PPG Exact Masses (Continued)

| n | Exact mass (M) | (M + NH ₄) ⁺ | MS/MS fragments | (M + 2NH ₄) ²⁺ | (M + COOH) ⁻ |
|----|----------------|-------------------------------------|-----------------|---------------------------------------|-------------------------|
| 26 | 1527.100 | 1545.134 | 1510.1 | 781.584 | 1572.097 |
| 27 | 1585.142 | 1603.176 | 1568.1 | 810.605 | 1630.138 |
| 28 | 1643.184 | 1661.218 | 1626.2 | 839.626 | 1688.180 |
| 29 | 1701.226 | 1719.260 | 1684.2 | 868.647 | 1746.222 |
| 30 | 1759.268 | 1777.302 | 1742.2 | 897.668 | 1804.264 |
| 31 | 1817.309 | 1835.344 | 1800.3 | 926.689 | 1862.306 |
| 32 | 1875.351 | 1893.386 | 1858.3 | 955.710 | 1920.348 |
| 33 | 1933.393 | 1951.428 | 1916.4 | 984.731 | 1978.390 |
| 34 | 1991.435 | 2009.469 | 1974.4 | 1013.752 | 2036.431 |
| 35 | 2049.477 | 2067.511 | 2032.5 | 1042.773 | 2094.473 |
| 36 | 2107.519 | 2125.553 | 2090.5 | 1071.794 | 2152.515 |
| 37 | 2165.561 | 2183.595 | 2148.5 | 1100.815 | 2210.557 |
| 38 | 2223.603 | 2241.637 | 2206.6 | 1129.836 | 2268.599 |
| 39 | 2281.645 | 2299.679 | 2264.6 | 1158.857 | 2326.641 |
| 40 | 2339.687 | 2357.721 | 2322.7 | 1187.878 | 2384.683 |
| 41 | 2397.728 | 2415.783 | 2380.7 | 1216.899 | 2442.724 |
| 42 | 2455.770 | 2473.805 | 2438.7 | 1245.920 | 2500.766 |
| 43 | 2513.812 | 2531.847 | 2496.8 | 1274.940 | 2558.808 |
| 44 | 2571.854 | 2589.888 | 2554.8 | 1303.961 | 2616.850 |
| 45 | 2629.896 | 2647.930 | 2612.9 | 1332.982 | 2674.892 |
| 46 | 2687.938 | 2705.972 | 2670.9 | 1362.003 | 2732.934 |
| 47 | 2745.980 | 2764.014 | 2729.0 | 1391.024 | 2790.976 |
| 48 | 2804.022 | 2822.056 | 2787.0 | 1420.045 | 2849.017 |
| 49 | 2862.064 | 2880.098 | 2845.0 | 1449.066 | 2907.059 |
| 50 | 2920.106 | 2938.140 | 2903.1 | 1478.087 | 2965.101 |
| 51 | 2978.147 | 2996.182 | 2961.1 | 1507.108 | 3023.143 |
| 52 | 3036.189 | 3054.224 | 3019.2 | 1536.129 | 3081.185 |
| 53 | 3094.231 | 3112.266 | 3077.2 | 1565.150 | 3139.227 |
| 54 | 3152.273 | 3170.307 | 3135.2 | 1594.171 | 3197.269 |
| 55 | 3210.315 | 3228.349 | 3193.3 | 1623.192 | 3255.311 |
| 56 | 3268.357 | 3286.391 | 3251.3 | 1652.213 | 3313.352 |
| 57 | 3326.399 | 3344.433 | 3309.4 | 1681.234 | 3371.394 |
| 58 | 3384.441 | 3402.475 | 3367.4 | 1710.255 | 3429.436 |
| 59 | 3442.483 | 3460.517 | 3425.5 | 1739.276 | 3487.478 |
| 60 | 3500.525 | 3518.559 | 3483.5 | 1768.297 | 3545.5202 |

Table 2-1 PPG Exact Masses (Continued)

| n | Exact mass (M) | (M + NH ₄) ⁺ | MS/MS fragments | (M + 2NH ₄) ²⁺ | (M + COOH) ⁻ |
|----|----------------|-------------------------------------|-----------------|---------------------------------------|-------------------------|
| 61 | 3558.566 | 3576.601 | 3541.5 | 1797.318 | 3603.562 |
| 62 | 3616.608 | 3634.643 | 3599.6 | 1826.339 | 3661.604 |
| 63 | 3674.650 | 3692.685 | 3657.6 | 1855.359 | 3719.645 |
| 64 | 3732.692 | 3750.726 | 3715.7 | 1884.380 | 3777.687 |
| 65 | 3790.734 | 3808.768 | 3773.7 | 1913.401 | 3835.729 |
| 66 | 3848.776 | 3866.810 | 3831.7 | 1942.422 | 3893.771 |
| 67 | 3906.818 | 3924.852 | 3889.8 | 1971.443 | 3951.813 |
| 68 | 3964.860 | 3982.894 | 3947.8 | 2000.464 | 4009.855 |
| 69 | 4022.902 | 4040.936 | 4005.9 | 2029.485 | 4067.897 |
| 70 | 4080.944 | 4098.978 | 4063.9 | 2058.506 | 4125.938 |
| 71 | 4138.985 | 4157.020 | 4122.0 | 2087.527 | 4183.980 |
| 72 | 4197.027 | 4215.062 | 4180.0 | 2116.548 | 4242.022 |
| 73 | 4255.069 | 4273.104 | 4238.0 | 2145.569 | 4300.064 |
| 74 | 4313.111 | 4331.145 | 4296.1 | 2174.590 | 4358.106 |
| 75 | 4371.153 | 4389.187 | 4354.1 | 2203.611 | 4416.148 |
| 76 | 4429.195 | 4447.229 | 4412.2 | 2232.632 | 4474.190 |
| 77 | 4487.237 | 4505.271 | 4470.2 | 2261.653 | 4532.231 |
| 78 | 4545.279 | 4563.313 | 4528.3 | 2290.674 | 4590.273 |
| 79 | 4603.321 | 4621.355 | 4586.3 | 2319.695 | 4648.315 |
| 80 | 4661.363 | 4679.397 | 4644.3 | 2348.716 | 4706.357 |
| 81 | 4719.404 | 4737.439 | 4702.4 | 2377.737 | 4764.399 |
| 82 | 4777.446 | 4795.481 | 4760.4 | 2406.758 | 4822.441 |

