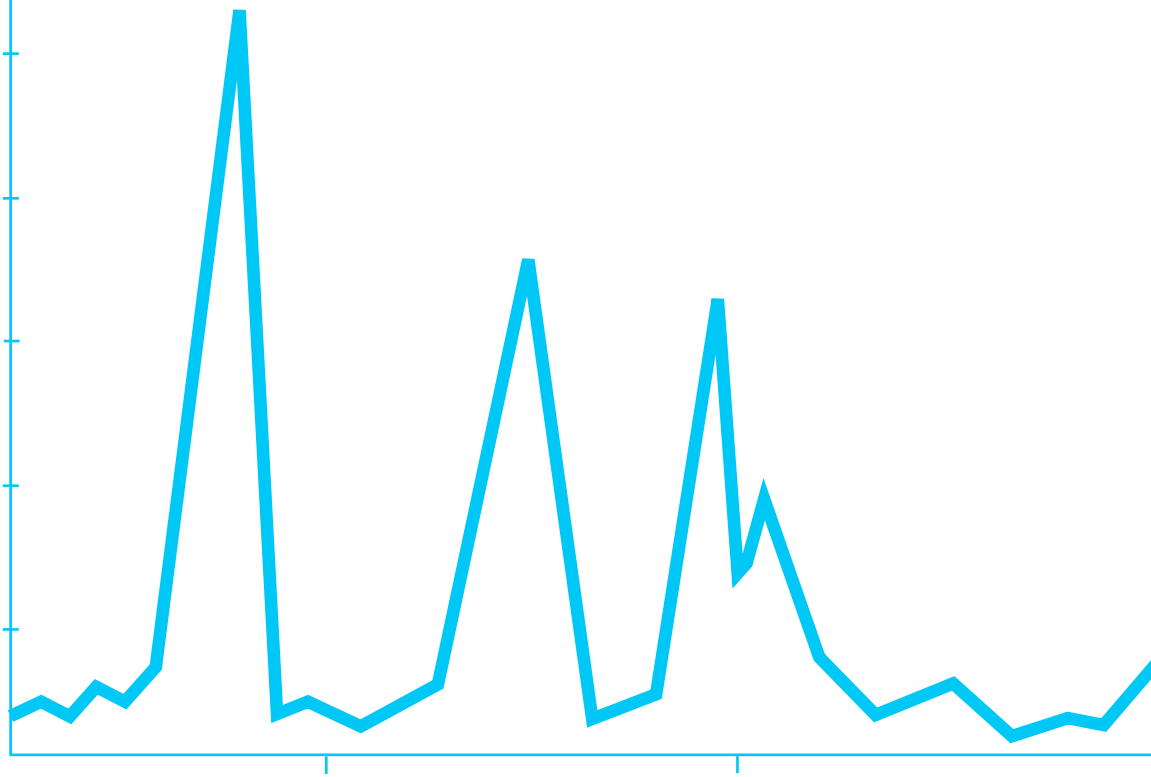
Analyst

Operator's Manual

P/N 1004314A
July 2002



Applied Biosystems | MDS SCIEX

http://www.appliedbiosystems.com

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Printed in Canada.

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About this Manual

Audience

This manual contains the instructions required to start up, calibrate, optimize, and operate the mass spectrometer. It provides instructions for the operation of system components and operational software. It is meant for the person with some knowledge of mass spectrometry as well as some experience with Analyst. Additional information is available in the Analyst online Help. The online Help is accessible from the Analyst application by choosing Help Topics from the Help menu.

Manual Organization

The *Analyst Operator's Manual* is organized as follows:

Chapter 2—Introduction to the Analyst Software

This section provides a general introduction to the Analyst software. This section also includes an overview of supported devices.

Chapter 3—Creating Projects

This section explains how to create projects and sub-projects to help you to organize your data.

Chapter 4—Creating a Hardware Configuration

This section explains how to create and edit hardware profiles and to configure your instrument.

Chapter 5—Tuning and Calibrating

This section describes how to tune and calibrate your instrument. It also includes procedures for building a tuning method.

Chapter 6—Optimizing for Your Analyte

This section provides an overview of how to optimize your instrument for a particular analyte of interest.

Chapter 7—Building an Acquisition Method

This section provides an overview of how to build an acquisition method using the Acquisition Method Editor.

Chapter 8—Creating Batches

This section describes how to create and submit an acquisition batch. It also includes procedures for creating sets and ExpressView templates.

Chapter 9—Running Your Samples

This section describes procedures for setting up queue options and controlling the acquisition. It also includes procedures for managing the batches and samples in the acquisition queue.

Chapter 10—Exploring your Data

This section describes procedures for viewing and organizing data. It also includes procedures for viewing contour plots, using libraries, and interpreting your fragments.

Chapter 11—Analyzing Quantitative Data

This section describes procedures for creating and using quantitation methods. It also includes procedures for reviewing peaks and the Results table.

Chapter 12—Creating Reports

This section describes procedures for creating reports and report templates and for modifying a report template. It also includes how to generate, print, and export a report.

Related Documentation

Additional information is available in the Analyst online Help system, the *Analyst Laboratory Director's Guide to Security and Regulatory Compliance*, and in the release notes that accompany the software. Information on configuring peripheral devices is also available in the online Help or in the *Peripheral Devices Setup Manual*.

Technical Support

Applied Biosystems/MDS Sciex and its representatives maintain a staff of fully-trained service and technical specialists located throughout the world. They can answer questions about the Analyst software for LC/MS/MS systems, API instruments, or any technical issues that may arise. For more information, visit the Applied Biosystems/MDS Sciex Web site:

http://www.appliedbiosystems.com

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Introducing the Analyst Software

This manual pertains to Analyst 1.3 and later versions of the Analyst software. Analyst 1.3 contains several new and enhanced features. Please note that not all features are available for all instruments. For more information, refer to the release notes that accompany the software.

This introductory section provides an overview of the Analyst software, the devices supported by Analyst, and the available scan types. An overview of tuning, calibration, and optimization is also provided.

The Analyst Software

Analyst enables you to control Applied Biosystems/MDS Sciex mass spectrometers and peripheral HPLC devices, such as autosamplers, pumps, column ovens, and valves. Analyst 1.3 supports single quadrupole, triple quadrupole, and linear ion trap (LIT) instruments. The easy-to-use interface allows you to perform all the activities necessary to analyze a sample. Using Analyst, you can configure the devices attached to your instrument station. You can also perform the following tasks:

- Tune and calibrate your instrument
- Optimize your system for specific analytes or masses
- Run your samples
- Explore your data

- Perform quantitative analysis
- Create and print reports

Not only can you perform a full array of activities with Analyst, you can also use its customization options to tailor your system to provide the level of performance you need.

Data System

The Analyst software requires an NT-based operating system, which allows you to operate it in a network environment. This means you can perform some activities from a remote location, thus saving you valuable time and effort.

When operating the instrument, the acquired data is relayed to the Analyst software where it can be displayed as either full mass spectra, intensity of single or multiple ions versus time, or total ion current versus time. For more information on hardware and operating system requirements, refer to the software release notes.

Supported Peripheral Devices

Analyst supports a number of different kinds of HPLC peripherals—devices that are used with the mass spectrometer. For a complete list of supported devices, please refer to the release notes that accompany the software.

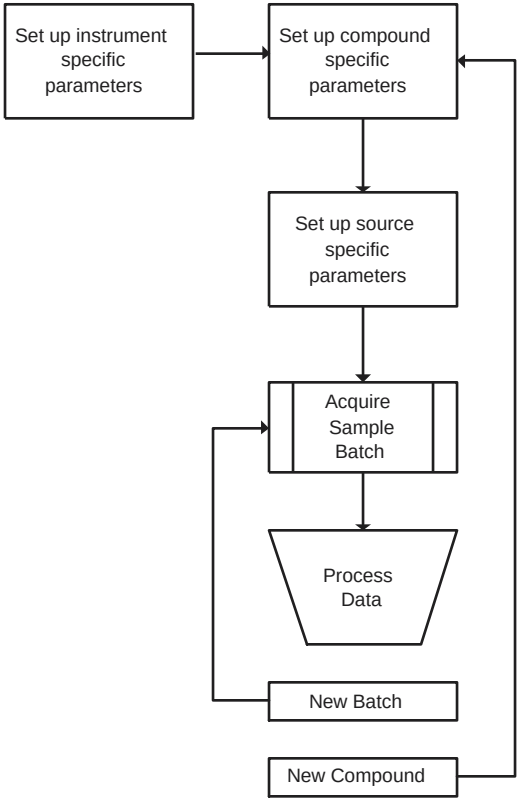
A number of different analog devices can be used with the Analyst software; however, their signals must first be read by an analog-to-digital converter card (ADC). The ADC card digitizes the analog device signal so it can be incorporated into the data file by the software.

Work Process Flow

Running samples can involve several modes and steps. The *Work flow process* diagram on page 7 illustrates a common process flow from beginning to end at a very basic level. The top half of the diagram depicts the instrument parameters, which can be divided into three types:

- Instrument-specific (set up at installation and on mass calibration)
- Compound-specific (set up for each analysis)
- Source-specific (set up for each analysis)

The bottom half of the diagram describes the acquisition and processing of sample data. To process a new compound, however, you may choose to redefine the compound- and source-specific parameters.



Work flow process

Setting Up Instrument-Specific Parameters

Setting up instrument parameters is only necessary on a periodic basis, either on initial installation, or if you know that you need to recalibrate the instrument. This process is not required for each analysis. Once you assign values to instrument parameters for a particular analysis, they become the working parameters describing the control parameters for the instrument.

Setting up Compound-Specific Parameters

Each time you analyze a new compound, you need to begin at this step and define the analysis conditions for the compound. You can set up compound-specific parameters in two different ways: automatically, using the Quantitation Optimization wizard, or manually, using Manual Tune.

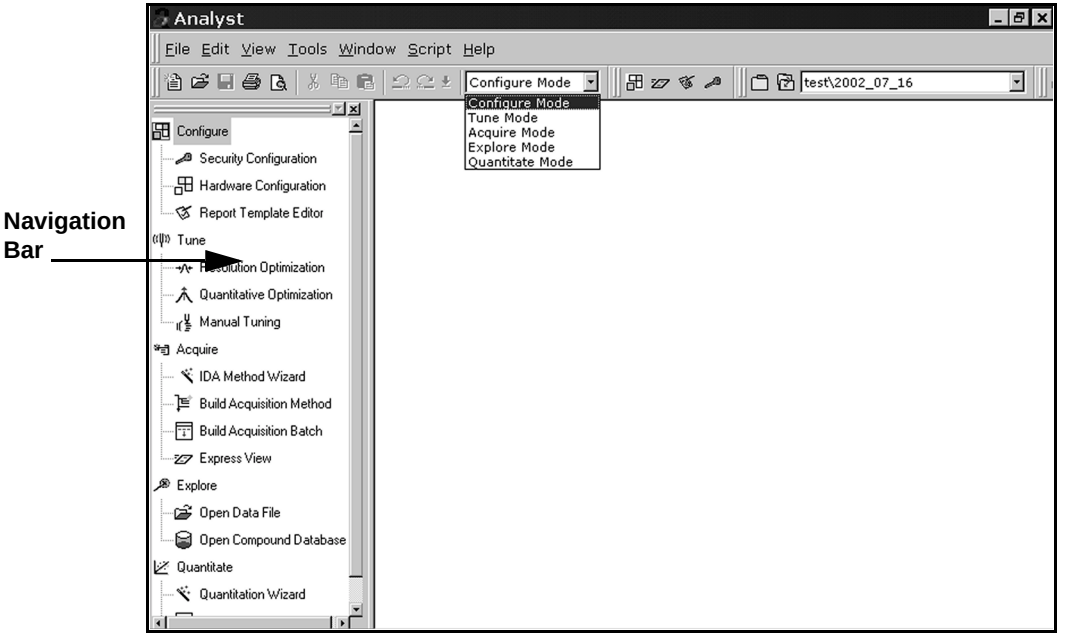
Setting up Source-Specific Parameters

Source parameters can be optimized for the LC conditions used during analysis. These parameters are accessed either by selecting the Source/Gas tab in the Manual Tune Method Editor window, or by clicking Edit Parameters in the Method Editor window in Acquire mode.

The bottom half of the diagram describes the processing of the sample data according to the instrument parameters; you introduce the samples and then analyze the acquired data.

Overview of Analyst Activities

This section provides an overview of the processes involved in operating your instrument. Analyst processes are divided into five main areas, known as modes. A mode is a separate functional area within Analyst, allowing you to perform a range of activities that all relate to a certain main task. The five modes in Analyst are: configure, tune, acquire, explore, and quantitate.



Analyst modes

You can switch from one mode to another, without having to close a mode or lose your work in the current mode. To switch between modes, click the desired mode in the Navigation bar, or choose a new mode from the Mode list box in the toolbar. The new mode appears on the screen. The previous mode retains all of its workspace information, but is no longer displayed.

Configure Mode

Configure mode is used to set various options and parameters for the Analyst application. In Configure mode, you can create, set or configure the following:

- Security options
- Hardware profiles
- Reports
- ExpressView options
- Tuning options

- Queue options

Tune Mode

Use Tune mode to set options for tuning your instrument to ensure optimal results. In Tune mode, you can

- Optimize resolution
- Perform mass calibration (manual or automatic)
- Optimize for quantitation
- Perform manual tuning
- Build a tuning method
- Build a quick single period, single experiment acquisition method.
- Set processing options
- Set appearance options

Acquire Mode

In Acquire mode you set options to determine how samples should be acquired. In Acquire mode, you can

- Build an acquisition method with the Acquisition Method Editor or IDA wizard
- Build a batch with the Batch Editor
- Enter batch information quickly with ExpressView
- View the queue with Queue Manager
- Monitor the acquisition status
- Display the data in real-time
- Set processing options
- Set appearance options

Explore Mode

Use Explore mode to perform qualitative analysis on samples. In Explore mode, you can

- View a graph
- View a chromatogram
- View a spectrum
- View a table
- Use calculators
- Process the data, including smoothing, background subtraction, and peak finding
- Display data in real time during batch acquisition

Quantitate Mode

Use Quantitate mode to perform activities related to quantitative analysis. In Quantitate mode, you can

- Build a quantitation method
- Quantitate a batch or batches of samples
- Review chromatographic peaks
- Compare results between batches
- Review the Results table
- Plot results
- Generate reports using the Report Template Editor

Audit Tracking

Audit tracking is a mechanism that ensures the integrity of the Analyst files by making sure that any change to any field is logged. That way, a third party (such as an auditor) can determine exactly what happened to the data and why. It is essentially an electronic paper trail that logs the following details about a change: who made the change, when they made it, what kind of data they changed, their reason for changing it (if enabled), and other details about the

change. For more information about audit tracking, refer to the *Analyst Laboratory Director's Guide to Security and Regulatory Compliance*.

Electronic Signatures

If the electronic signature (ESig) option is enabled, user verification is required when making a change of any nature to any file, for example a data file, method file, or quantitation results file. If you make a change, before it can be accepted an Audit Trail dialog box opens and you must provide a reason for the change. You must also enter an electronic signature (password) before the change is implemented. For more information on electronic signatures, refer to the online Help.

Scan Types

The scan type determines the operation mode of the mass spectrometer. Depending on the type of MS configured, different scan types are available.

Available scan types

Q1 scan: A full scan using the first quadrupole (Q1). The ion intensity is returned for every requested mass in the scan range.

Q1 Multiple Ion: A scan using the first quadrupole (Q1). The ion intensity is returned for specified point masses.

Q3 scan: A full scan using the third quadrupole (Q3). The ion intensity is returned for every requested mass in the scan range.

Q3 Multiple Ion: A scan using the third quadrupole (Q3). The ion intensity is returned for specified point masses.

MRM (Multiple Reaction Monitoring) scan: Mode of operating a triple quadrupole instrument so that an ion of given mass (Q1) must fragment or dissociate to give a product ion of specific mass (Q3) in order for a response to be detected. Used for very specific target compound analysis.

Product Ion: An MS/MS full scan where the first quadrupole (Q1) is fixed to transmit a specific precursor ion and the third quadrupole (Q3) sweeps a mass range. An experiment that will search for all of the products of a particular precursor ion.

Precursor Ion: An MS/MS scan where the third quadrupole (Q3) is fixed at a specified mass-to-charge ratio and the first quadrupole (Q1) sweeps a

mass range. A scan for the ion of a specific mass-to-charge ratio that is generating a specific product ion.

Neutral Loss: MS/MS scan where both the first quadrupole (Q1) and the third quadrupole (Q3) sweep a mass range, a fixed mass apart. A response will be observed if the ion chosen by the first analyzer fragments by losing the neutral loss specified.

The following enhanced scan types pertain to instruments with LIT capability only:

Enhanced MS (EMS): Ions are transferred directly from the ion source and orifice region to the third quadrupole (Q3) where they are collected. These ions are scanned out of Q3 to produce enhanced single-MS type spectra.

Enhanced Multi-Charge (EMC): This mode operates similarly to the Enhanced MS scan mode except that before scanning the ions out of the linear ion trap, there is a delay period in which low charge state ions (primarily singly charged ions) are allowed to preferentially escape from the linear ion trap. When the retained ions are scanned out, the multiply-charged ion population dominates the resulting spectrum.

Enhanced Product Ion (EPI): Product ions are generated in the Q2 collision cell by the precursor ions from Q1 colliding with the collision (CAD) gas in Q2. These characteristic product ions are transmitted and collected in Q3. These ions are scanned out of Q3 to produce enhanced product ion spectra. Use the EPI scan mode if you need good resolution and intensity.

Enhanced Resolution (ER): This mode is similar to the Enhanced Product Ion mode except that the Q1 precursor ions pass gently through the Q2 collision cell without fragmenting. A small range about the precursor mass is scanned out of Q3 at the slowest scan rate to produce a narrow window of the best-resolved spectra.

MS/MS/MS (MS3): In MS/MS/MS mode, product ions are generated in the Q2 collision cell by the precursor ions from Q1 colliding with the collision (CAD) gas in Q2. These characteristic product ions are transmitted and collected in Q3. Applying the normal mode resolving RF-DC voltages to the Q3 quadrupole isolates a specified mass (m/z) ion and removes all other ions from Q3. By applying a second auxiliary AC frequency to Q3, the specified ion can be resonantly excited. These excited ions collide with the residual nitrogen in Q3 and fragment producing a characteristic spectrum of ions.

Time Delayed Fragmentation (TDF): Product ions are generated and collected in the linear ion trap. During the first part of the collection period,

the lower mass ions are not collected in the linear ion trap. During the second part of the collection period, all masses over the mass range of interest are collected. The resultant enhanced product ion spectra are simplified compared to EPI scan type spectra. The nature of the spectra aids in the interpretation of the structure and fragmentation pathways of the molecule of interest.

Information Dependent Acquisition

Information Dependent Acquisition (IDA) allows you to build an acquisition method that will analyze data as it is being acquired and change experiment conditions on-the-fly according to the results of the analysis. You have control over the criteria that activate an IDA experiment and the parameters of the IDA experiment once activated.

IDA experiments can modify experiments and improve results based on the following criteria:

- Ion selection
- Ion charge states
- Isotope matching

Setting IDA parameters to optimize data acquisition settings on-the-fly drastically reduces sample acquisition time. Using Information Dependent Acquisition, an operator can conserve both sample and valuable working time on an instrument. For more information on Information Dependent Acquisition, see "Creating an IDA Experiment" on page 82.

Understanding Tuning, Calibrating, and Optimization

What is Tuning?

Tuning is the adjustment of working parameters to enable an operator to get the best signal from a specific ion or ions. Tuning can be automatic (as in Resolution Optimization) or manual (as in Manual Tuning). Tuning allows you to

- interactively control the acquisition of data.
- interactively control the working parameters and observe how this affects the intensity and shape of the signal.
- select the masses to be analyzed and calibrate the instrument. The calibration may use data from the current spectrum or data saved from a previous spectrum.
- set up one or more calibration standards. A calibration standard is a well-characterized compound.
- adjust the resolution offset settings while monitoring the ion signal.
- print mass calibration, manual turning, and resolution optimization reports.

It also allows an expert to manually adjust instrument parameters to obtain the best performance for a particular experiment or series of experiments. Before you can access the Tuning icons on the toolbar and Navigation bar, you must first reserve the acquisition queue for tuning. For more information, see "To reserve the acquisition queue for tuning" on page 32.

What is Calibrating?

Mass calibration ensures that the mass peaks are assigned to the correct mass-to-charge values. By performing a mass calibration using a calibrant such as polypropylene glycol (PPG) or taurocholic acid, for which the masses are known, you can compare the results with a previous calibration and either update the previous calibration, or replace it with the new calibration as required.

Analyst allows you to select multiple masses for calibration for each polarity. The results are stored in a calibration table. Analyst also allows you to update

the calibration table with data from the current calibration. Data for masses already in the calibration table are updated. All data for masses not calibrated in the current calibration are retained. If you choose to replace the mass calibration, all previous calibration masses are deleted.

You can perform a mass calibration using a newly acquired spectrum, or on a spectrum from a stored data file. For more information on mass calibration, see "Tuning and Calibrating Your Instrument" on page 31.

To perform a mass calibration, Analyst

- processes spectral data as specified by the tuning method.
- finds the largest peak in the search range for each selected mass.
- obtains the mass, intensity, and peak width values.
- compares the mass with the expected mass and determines the shift, if any.
- compares the peak width with the target peak width.
- compares the intensity with the previous calibration.
- displays the results in a real-time display.
- saves the calibration table in the instrument data file.

What is Optimizing?

Optimizing the resolution adjusts the peak resolution offset value for each selected mass so that it meets the criteria set up in the tuning options. You can set up the criteria for four resolution values: high, unit, open, and low.

Typical criteria are as follows:

- for high resolution, a peak width of 0.5 ± 0.1 amu at 50% of the maximum peak height.
- for unit resolution, a peak width of 0.7 ± 0.1 amu at 50% of the maximum peak height.
- for open resolution, unit with a predefined offset drop.
- for low resolution, unit with a predefined offset drop.

Analyst calculates the offsets for low and open resolutions for triple quadrupole instruments from the required drop of the offset from unit resolution. This varies from instrument to instrument.

To optimize the resolution, Analyst

- uses the calibration standard, the reference table, and the criteria from the tuning options to build an acquisition method.
- acquires data.
- adjusts the peak resolution offset values for each peak selected in the reference table.
- displays the result in a real-time display.
- displays a report.
- saves the new resolution table in the instrument data (if required).

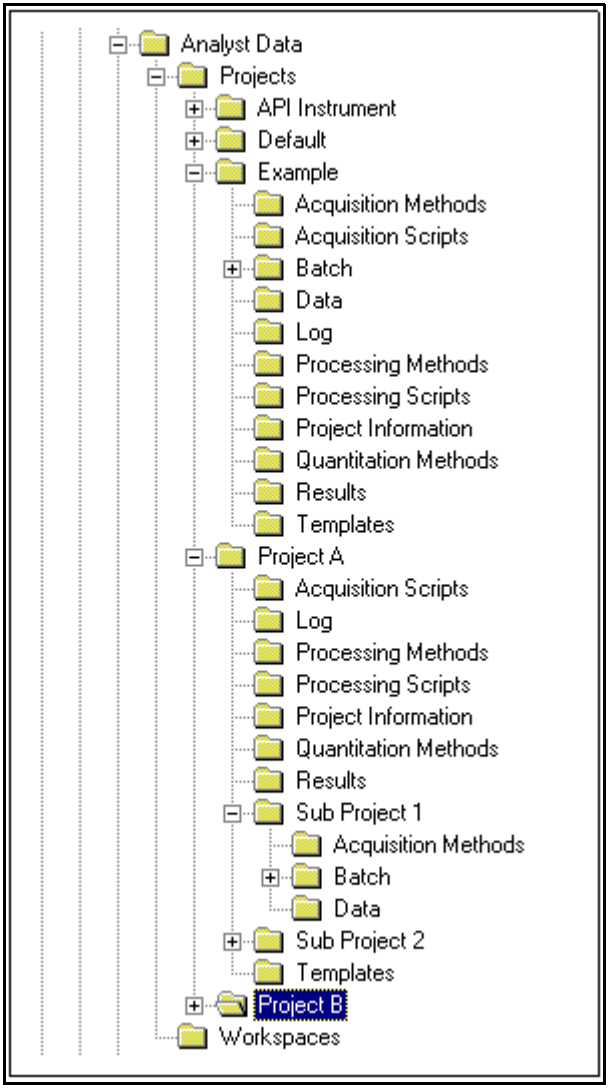
3

Creating Projects

This section explains how to create projects and sub-projects. Projects are used to help you organize your data. A file is a collection of data from the instrument that is stored together under a specified file name. A project is a collection of various types of files that are stored together under a specified project name.

When you create a project, Analyst sets up a project directory structure automatically and stores appropriate files within these directories. The default folders installed with Analyst are the API Instrument, Default, and Example folders.

There are numerous file types that are used with Analyst. Files can be created, opened, closed, saved, and some may be imported, and exported. Projects can be created, copied, and selected (opened). The figure below shows the typical contents of the different project directories. All directories are relative to the Project and Sub-Project levels.



Project Hierarchy

Directory	Contents
\Acquisition Methods	Contains all of the acquisition methods used by the project. Files in this folder have the extension .dam
\Acquisition Scripts	Contains all the acquisition batch scripts used in the project.
\Processing Methods	Contains all the qualitative data processing methods used in the project.
\Processing Scripts	Contains all the data processing scripts used in the project.
\Project Information	Contains all the information and settings about the project.
\Quantitation Methods	Contains all of the quantitation methods used by the project. Files in this folder have the extension .qmf
\Batch	Contains all of the acquisition batch files. Files in this folder have the extension .dab. The Templates sub-directory contains acquisition batch templates. Files in this folder have the extension .dat
\Data	Contains the acquisition data. Files in this folder have the extension .wiff

Directory	Contents
\Results	Contains the quantitation results table files.
\Templates	Contains all the report templates used in the project. Files in this folder have the extension .rpt
\Workspaces	Contains the workspace files.

Creating New Projects and Sub-Projects

By default, the Example folder stores your files. Therefore, if you want to manage your files better and be able to compare your results, before you start analyzing your compounds you should create a new project in which to store your data.

The Create New Project/Sub-Project dialog box enables you to create a new project or sub-project. You can use sub-projects for each compound you want to measure or for a specific period (such as a day).

To create a project or sub-project

NOTE: To create a sub-project, you must have an existing project.

1. On the **Tools** menu, point to **Project**, and then click **Create Project** to create a new project.

   —or—

   Click **Create Sub-project** to create a sub-project.

2. In the **Project Name** box, type a project name.

3. Complete the following fields as required.

   Sub-Project Name: After specifying any sub-project folders, enter the sub-project name. If creating a sub-project, enter the sub-project name.

   Project folders: Displays the folders you selected.

   Sub-Project Folders: Select the sub-project folders you want to move to the project folders list.

   Add All: Click Add All to move all project folders to the sub-project folders list.

   Remove All: Click Remove All to remove project folders.

   Set configuration as default for new projects: Select to set the project and sub-project folders as the default hierarchy for all new projects. Subsequent new projects will be created with this folder configuration as default.

4. Click **OK**.

To copy a project or sub-project

1. On the **Tools** menu, point to **Project**, and then click **Copy Project**.

   —or—

   Click **Copy Sub-project** to copy a sub-project.

2. In the **Project Source Directory** box, type the source drive and directory information, or click **Browse** and select the source location.
3. From the **Source Project Name** list, select the project to be copied.
4. In the **Project Destination Directory** box, type the drive and directory to which you wish to copy the files, or click **Browse** and select the destination location.
5. In the **Target Project Name** box, type the name for the copied project.
6. Click **Copy**.

   The project or sub-project is copied and the dialog box closes.

7. To cancel copying a project, click **Cancel**, or repeat the process to copy additional projects.

4

Creating a Hardware Profile

This section explains how to create hardware profiles for you instrument. A hardware profile is used to indicate which devices are available for use during acquisition and enables communication with these devices. Hardware profiles contain the mass spectrometer and can include peripheral HPLC devices.

Configuring a Hardware Profile

Configuring peripheral devices within Analyst consists of two processes: setting up the physical hardware connections and configuring the software to communicate with the devices. When Analyst is installed, most of the drivers required to configure the supported devices are also installed. To activate these devices, you must configure them using the Hardware Configuration Editor.

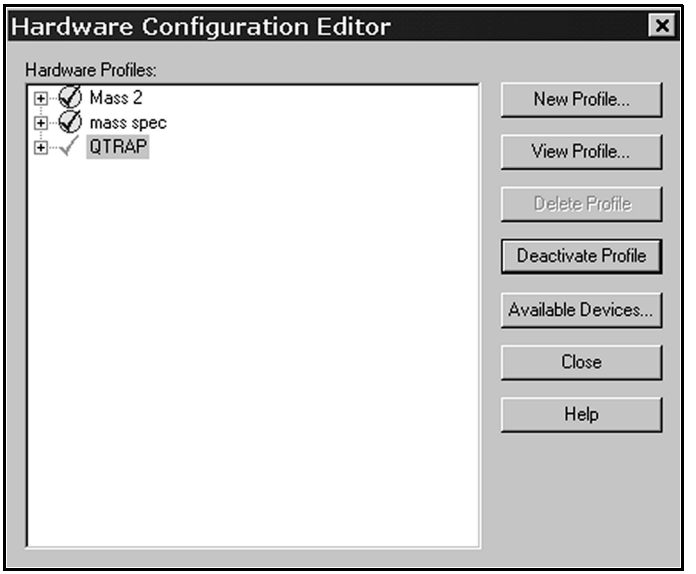
The Hardware Configuration Editor provides an interface to configure the devices and save them within hardware profiles. You may set up multiple hardware profiles but only one can be active at any one time. To activate a hardware profile, the device(s) for which the profile is configured must be connected to the computer through serial, general purpose interface bus (GPIB), or ethernet connections, depending on the devices.

To create and configure a new hardware profile

NOTE: We recommend that you do not change the default settings for the general purpose interface bus (GPIB) communications.

1. Double-click **Hardware Configuration** under **Configure** in the Navigation bar.

   The Hardware Configuration Editor dialog box opens. All existing profiles and their statuses (active or inactive) are shown.



2. In the **Hardware Configuration Editor** dialog box, click **New Profile**.

   The Create New Hardware Profile dialog box opens.

3. Type a description of the profile in the **Profile Name** field and click **Add Device**.

   The Available Devices dialog box opens.

4. Choose the appropriate device type from the **Device Type** list.

5. Choose the appropriate device from the **Devices** list and click **OK**.

6. Click **Setup Device** and type an alias, a name or other identifier, for the device. This feature is particularly useful if you are using two similar devices.

7. Complete the configuration dialog box as required.

   NOTE: For devices using serial communication, ensure that the serial port selected matches the serial port to which the device is physically connected. When using the serial expansion octopus cable, the number selected in the profile is the number on the cable plus two.

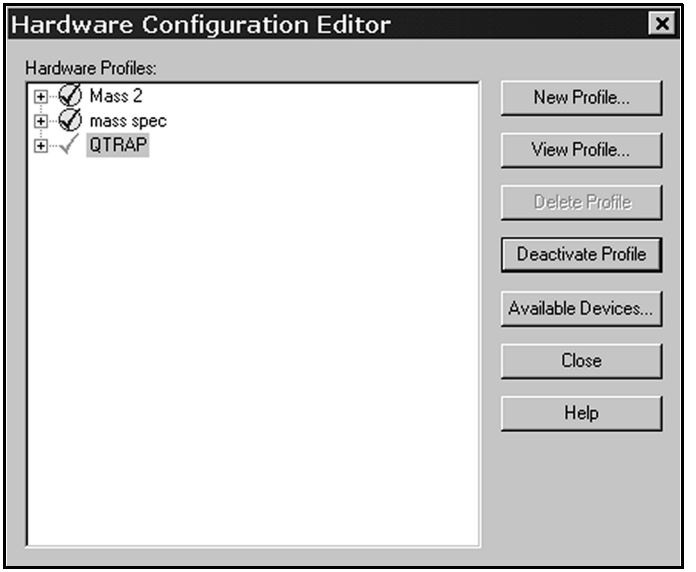
8. Click **OK** to save your settings.
9. Repeat steps 3 (from click Add Device) to 7 for each device you want to add to the hardware profile.
10. In the **Create New Hardware Profile** dialog box, click **OK.**
11. To activate a profile, click **Activate Profile** in the **Hardware Configuration Editor**.

    To restore the device defaults you can click Set Defaults on the Communication Tab.

To edit an existing profile

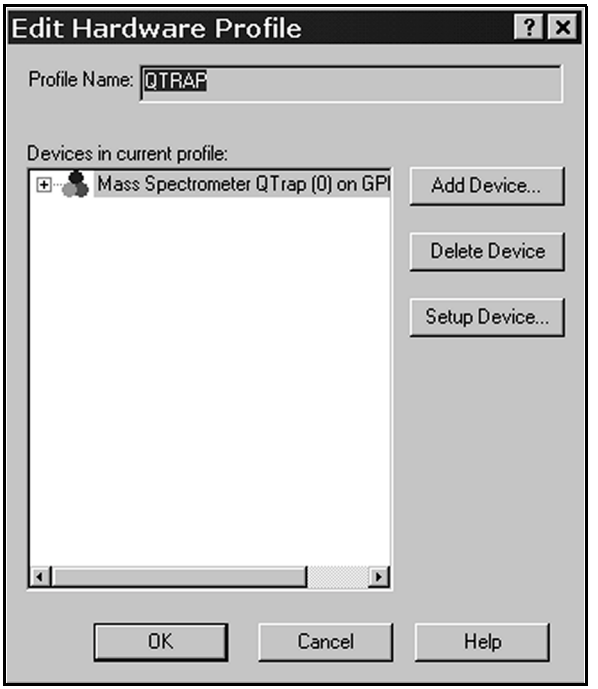
1. Double-click **Hardware Configuration** under **Configure** in the Navigation bar.

   The Hardware Configuration Editor dialog box opens.



2. If necessary, deactivate the hardware profile by clicking **Deactivate Profile**, and then click **Edit Profile**.

   The Edit Hardware Profile dialog box appears.



3. In the **Edit Hardware Profile** dialog box, click **Setup Device** and edit the configuration dialog box that appears.
4. Click **OK** to save your settings.
5. Click **OK** to save the changes to the profile.

5

Tuning and Calibrating Your Instrument

Before you use the mass spectrometer, you should ensure that you get the best results by tuning the instrument. This section describes how to use Analyst to tune and calibrate your instrument either automatically or manually.

Analyst gives you three ways to tune the mass spectrometer:

- automatic: Analyst completes the tasks for you using resolution optimization
- semi-automatic: you complete some tasks (resolution optimization, cycle-dependent parameters) with assistance from Analyst. For more information on using semi-automatic tuning, refer to the online Help
- manual: you complete all of the tasks yourself

Tuning with Resolution Optimization

Resolution optimization automatically tunes the resolution of your instrument and calibrates your instrument using the standards and settings you have selected in the Resolution Optimization Options dialog box. You can choose to perform a mass calibration automatically after optimizing the resolution successfully.

Before you begin a resolution optimization, you should set the calibration and resolution options for your instrument, and you should ensure that the acquisition queue has been reserved for tuning.

To reserve the acquisition queue for tuning

- From the **Tools** menu, click **Tuning Instrument**.

The Tuning icons become available on the toolbar and Navigation bar.

Setting the Calibration Options for the Resolution Optimization

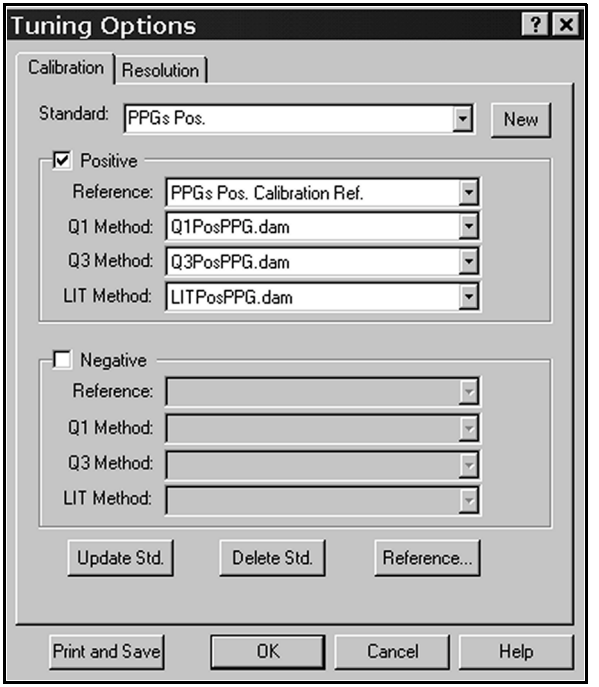
In the Calibration tab of the Tuning Options dialog box, you can select the standard, polarity, and reference to be used for the mass calibration. You can then edit the Reference table to select the mass or masses to be calibrated.

To set the calibration options for the resolution optimization

1. From the **Tools** menu, point to **Settings**, and click **Tuning Options**.

   The **Tuning Options** dialog box appears.

2. Click the **Calibration** tab.



3. Select the calibration **Standard**.

4. Select either **Positive** or **Negative** polarity (or both).
5. Select the **Reference** and the **Q1**, **Q3, or LIT Method** if applicable.
6. Click **OK**.

Setting the Resolution Options for the Resolution Optimization

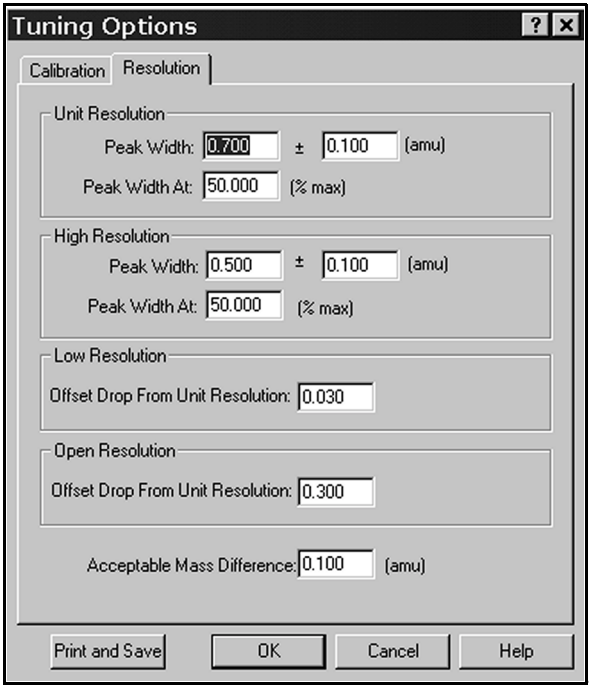
In the Resolution tab you can edit the resolution settings. You should set the value of unit, high, low, and open resolution before you optimize the resolution and perform a mass calibration.

To set the resolution options for the resolution optimization

1. From the **Tools** menu, point to **Settings**, and click **Tuning Options**.

   The **Tuning Options** dialog box appears.

2. Click the **Resolution** tab.



3. Type the **Peak Width** and tolerance for Unit and High Resolution.

4. Type the percentage of the peak height at which the **Peak Width At** is measured for both Unit and High Resolution.
5. Type the **Offset Drop From Unit Resolution** value for Low and Open Resolution.
6. Type the value for **Acceptable Mass Difference**.
7. Click **OK**.

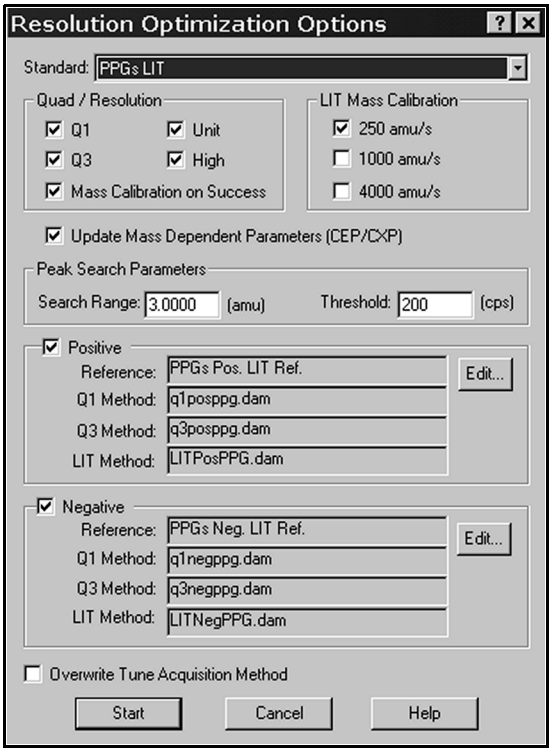
Automatically Optimizing the Resolution

Once you have set the calibration and resolution options, you are now ready to automatically tune your instrument.

To automatically optimize the resolution

1. Double-click **Resolution Optimization** under **Tune** in the Navigation bar.

   The **Resolution Optimization Options** dialog box appears.



2. Select the calibration standard on the **Standard** list.
3. To select the quadrupole or quadrupoles on which to perform the resolution optimization, select either **Quad 1** or **Quad 3** (or both).
4. To select the resolution or resolutions to be optimized, select either **Unit** or **High** (or both).
5. If you want to perform a mass calibration automatically if the resolution optimization operation is a success, select **Mass Calibration on Success**.

   For LIT instruments, select the scan rates for LIT mass calibration.
6. If you want to update the mass-dependent instrument parameters with new values resulting from the resolution optimization, select **Update Mass Dependent Parameters**.
7. Edit the **Peak Search Parameters**, if required.
8. If required, select the polarity to be optimized. Select either **Positive** or **Negative**, or both.

9. If you want to view the reference table to see or change the masses selected for use, click **Edit**.
10. Click **Start**.

    The automatic resolution optimization operation starts and the Automatic Resolution report appears. When the operation completes, a real-time display of the results of the operation appears.
11. When the **Save Resolution Table** message box appears, click **Yes** to save the Resolution table created by the operation.
12. In the **Auto Resolution** dialog box, click **OK** to complete the operation.

Resolution and Sensitivity

Resolution is one component that helps determine the sensitivity of your mass spectrometer. Analyst uses pre-defined high, unit, low, and open resolution values. Some methods may require a lower resolution of the mass spectrometer to obtain the required sensitivity levels. For typical MS/MS analysis, Q1 is at unit resolution and Q3 resolution is low. Decreasing the Q1 resolution will allow more ions to enter the collision cell, increasing sensitivity. Similar decreases in Q3 resolution can yield further gains in sensitivity.

Calibrating Single and Triple Quadrupole Instruments

Use this procedure to set the calibration standard, search range, peak width, and intensity threshold to be used in a mass calibration. Before you begin, ensure that the acquisition queue has been reserved for tuning.

Mass calibration consists of four stages:

- setting the tuning options for the calibration. This consists of identifying the appropriate calibration standard, the polarity, the reference, and the Q1 method
- identifying the search range, peak width, and threshold
- selecting the masses to be calibrated
- starting the calibration

Calibrating from a Spectrum

When performing a mass calibration, you identify the masses to calibrate by selecting them in the appropriate reference table for the scan and polarity. Different instruments require you to select different masses to calibrate. The procedures are similar in all cases, but the scan types and working parameters are different. For the tables containing the recommended calibration ions, refer to "Appendix H: Calibration Ions" on page 191.

Calibration Tables

Analyst contains a Calibration table for every combination of quadrupole, polarity (positive, negative), and resolution. Each Calibration table contains the results of one or more mass calibrations for the combination. When Analyst performs a mass calibration, it compares the results with the values in the calibration table. The Calibration tables are stored with the instrument data. For more information on updating calibration standards, refer to the online Help.

Reference Tables

There is a Reference table for each polarity of each calibration standard. A reference table contains information about the mass of each ion in the sample, the intensity of the ion, and the number of charges on the ion. Each reference table provides a check box for each ion to allow you to select specific ions for mass calibration and resolution optimization.

Performing Q1 and Q3 Mass Calibrations

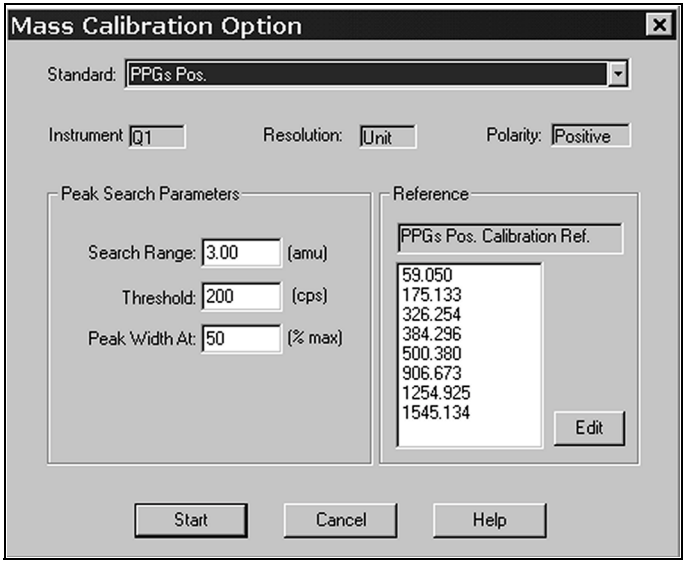
Perform a Q1 mass calibration when you want to calibrate the Q1 quadrupole in a single or triple quadrupole instrument. Perform a Q3 mass calibration when you want to calibrate the Q3 quadrupole in a triple quadrupole instrument. The Q3 mass calibration does not calibrate the Q1 quadrupole. Before you begin calibrating, ensure that the acquisition queue has been reserved for tuning.

To perform a Q1 or Q3 calibration

1. Acquire data. For the procedure on how to acquire data, refer to "To acquire data" on page 46.
2. Select the pane containing the spectrum.

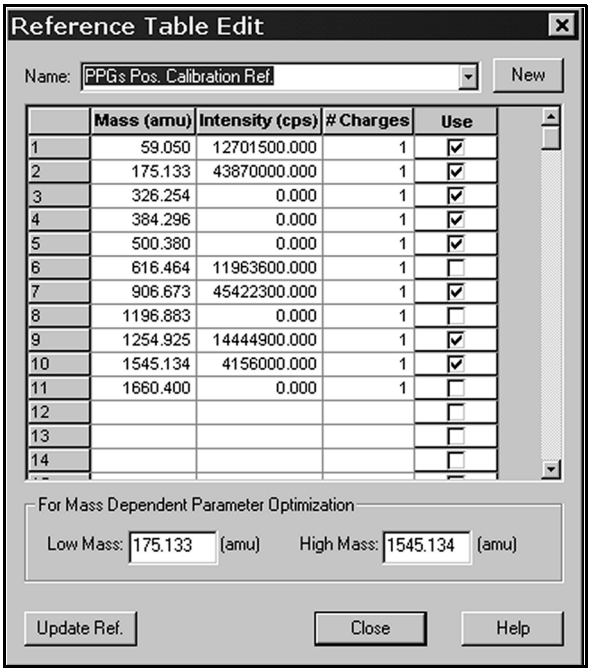
3. From the Tools menu, choose **Calibrate from Spectrum**.

   The **Mass Calibration Option** dialog box appears.



4. Select the calibration **Standard**.
5. Type the **Search Range**, **Threshold**, and **Peak Width At** you require.
6. Click **Edit**.

   The Reference Table Edit dialog box opens.



7. Select the appropriate **Use** check boxes to select the masses you want to monitor for calibration.
8. Click **Update Ref** and then click **Close**.
9. In the **Mass Calibration Options** dialog box, click **Start**.

   When the mass calibration ends, three graphs appear in the Calibration Report window.

   - The mass shift graph shows the difference between the measured masses from the current calibration and the true masses from the reference table.
   - The peak width graph shows the peak width for each mass. For unit resolution, this should be about 0.7 amu full width at half maximum (FWHM).
   - The intensity difference graph shows the intensity difference between the previous calibration and the current calibration.

10. You can update or replace the current calibration in the calibration table. If you do, Analyst verifies that the new calibration is valid (that is, 0.7 ± 0.1 amu FWHM for unit resolution, 0.5 ± 0.1 amu FWHM for high resolution).
    - If it is valid, to save the new calibration, click **Save**.
    - If it is not valid, Analyst displays a warning. To exclude a value, right-click on the value, and click **Exclude** from the popup list.

Updating the current calibration adds the values from the new mass calibration to the existing mass calibration. Replacing the current calibration deletes the existing mass calibration and replaces it with the new mass calibration.

Calibrating Linear Ion Trap Instruments

You can perform an LIT mass calibration for the following scan types: EMS, ER, EPI, and EMC. For more information, see "Scan Types" on page 12.

NOTE: Linear ion trap instruments must be calibrated at each scan speed.

Use this procedure to set the calibration standard, search range, peak width, and intensity threshold to be used in a mass calibration. Before you begin, ensure that the acquisition queue has been reserved for tuning.

To perform an LIT calibration

1. Acquire data.
2. Select the pane containing the spectrum.
3. From the **Tools** menu, click **Calibrate from Spectrum**.

   The **LIT Mass Calibration** dialog box appears.
4. Select the calibration **Standard**.
5. Type or edit the **Search Range**, **Threshold**, and **Peak Width At**.
6. Click **Edit**.

   The **Reference Table Edit** dialog box appears.
7. Click the appropriate **Use** check boxes to select the masses you want to monitor for calibration.
8. Click **Update Ref** and click **Close**.

9. Click **Start**.

   When the mass calibration ends, the Calibration Results window and the Calibration Report window appear. Three graphs appear in the Calibration Report window: mass shift, peak width, and intensity difference.

   - The mass shift graph shows the difference between the measured masses from the current calibration and the true masses from the reference table.
   - The peak width graph shows the peak width for each mass.
   - The intensity difference graph shows the intensity difference between the previous calibration and the current calibration.

10. To exclude a point from the mass calibration, right-click on a point in one of the graphs, then click Exclude from the popup menu that appears.
11. In the Calibration Report window, click **Update Mass Calibration** to add the values from the new mass calibration to the existing mass calibration, or click **Replace Mass Calibration** to delete the existing mass calibration and replace it with the new mass calibration.

Calibrating Using Resolution Optimization

You should automatically optimize the resolution and perform a mass calibration as part of a regular routine. The resolution optimization procedure assumes that you have already set up the values for unit and high resolution. For more information, refer to "Tuning with Resolution Optimization" on page 31.

For optimizing mass-dependent parameters, the reference table contains values for the low mass and high mass to allow Analyst to calculate the relationship between the mass and the value of the parameter. For more information on reference tables, refer to the online Help.

Optimizing the Detector

Detector parameters are instrument-dependent parameters. The detector wears out over time; therefore, you should optimize the bias voltage periodically using the standard positive PPG calibrant and monitoring the 907+ peak.

To optimize the detector

NOTE: The final setting must not exceed 3297 V.

1. Drop the current voltage by 200 V.
2. Increase the voltage in 100 V steps until a gain of 30% is no longer observed.

   The last value at which a 30% gain was obtained is your optimal voltage.

Tuning Your Instrument Manually

Manual tuning allows users with expert knowledge of the instrument to manually adjust the instrument parameters, working parameters, and other options to get the best performance from the instrument. Principal tools for performing manual tuning are

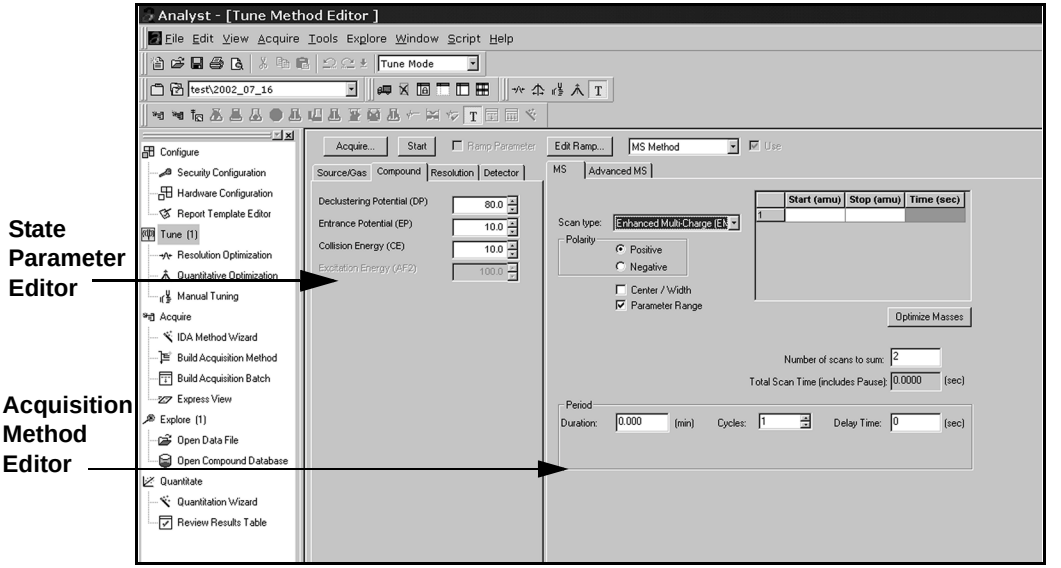
- Ramping a parameter. This feature allows you to automatically ramp a selected compound-dependent parameter while viewing the results.
- Optimizing working parameters manually. This allows you to change the value of one or more compound-dependent parameters as the experiment progresses. You can increase or decrease the value or values.

The Tune Method Editor allows you to perform manual tuning operations, such as creating a method, acquiring data, ramping a parameter, and manually optimizing a parameter.

To display the Tune Method Editor

- Double-click **Manual Tuning** on the Navigation bar.

  The Tune Method Editor window opens.



Tune Method Editor window

The Tune Method Editor consists of two panes and several buttons above the panes.

- The State Parameter Editor on the left has four tabs, including, the Source/Gas tab, the Compound tab, the Resolution tab, and the Detector tab.
- The Acquisition Method Editor pane is on the right. This pane displays various tabs depending on the method (MS, Syringe Pump, or LC), and the types of peripheral devices configured to work with the mass spectrometer.

Building a Tuning Method

To build a tuning method you must perform the following steps:

1. Reserve the acquisition queue for tuning.
2. Create a mass spectrometry (MS) method, and if required, a liquid chromatography (LC) method and a syringe pump method.

To reserve the acquisition queue for tuning

- From the Tools menu, click **Tuning Instrument**.

The Tuning icons become available on the toolbar and Navigation bar.

You can create an LC acquisition method, an MS acquisition method, or a syringe pump acquisition method in manual tuning mode. You can save and use these methods when building an acquisition batch. After creating an acquisition method in manual tuning, you are ready to acquire data. For more information, see "To create an MS acquisition method" on page 44, or "To add an LC acquisition method" on page 45, or "To add a syringe pump acquisition method" on page 45.

Creating an MS Acquisition Method

You can create a mass spectrometry (MS) acquisition method in manual tuning mode.

To create an MS acquisition method

1. On the **Tools** menu, click **Manual Tuning.**

   The Tune Method Editor appears.
2. From the method list, select **MS Method**.
3. Click the **MS** tab.
4. Choose the **Scan type** and configure values for all of the parameters as required.

   NOTE: Different parameters are displayed depending on the Scan type selected. For more information, see the online Help topic describing the MS tab for each scan type.
5. Type the **Duration**, number of **Cycles**, and the period **Delay Time**.
6. Click the **Advanced MS** tab.

7. Configure values for all of the parameters as required.

   NOTE: Different parameters are displayed depending on the scan type selected on the MS tab. For more information, see the online Help topic describing the Advanced MS tab for each scan type.

8. Click the **Source/Gas** tab and edit the values for the parameters.
9. Click the **Compound** tab and edit the values for the parameters.

   You are now either save your method or acquire data. See "Acquiring Data" on page 46.

Adding an LC Acquisition Method

You can add a liquid chromatography (LC) acquisition method in manual tuning mode. Before you begin, ensure that the active hardware profile contains an LC pump.

To add an LC acquisition method

1. On the **Tools** menu, choose **Manual Tuning**.

   The Tune Method Editor appears.

2. From the method list, select **LC Method**.
3. Click the **LC Pump Gradient** tab.
4. Type the values for the flow rate, gradient, and other parameters displayed in the table.
5. Click the **Limits** tab.
6. Type the values for maximum pressure, minimum pressure, shutdown time, and any other parameters displayed on the tab.

Adding a Syringe Pump Acquisition Method

You can add a syringe pump acquisition method in manual tuning mode. Before you begin, ensure that the active hardware profile contains a syringe pump.

To add a syringe pump acquisition method

1. On the **Tools** menu, choose **Manual Tuning**.

   The Tune Method Editor appears.

2. From the method list, select **Syringe Pump Method**.
3. On the **Syringe Pump Properties** tab, type the **Syringe Diameter**.
4. Type the **Flow Rate** value.
5. Select the **Unit** for the flow rate.
6. To start the syringe pump, click **Start Syringe Pump**.

   The syringe pump starts. The label on the Start Syringe Pump button changes to Stop Syringe Pump.
7. To change the flow rate while the pump is on, type the **Flow Rate** value and then click **Set Flow Rate**.

Acquiring Data

After you have created a method using the Tune Method Editor you can start data acquisition.

To acquire data

1. Create an MS acquisition method, and, if required, an LC method or a syringe pump method.
2. Click **Start** (if you want Analyst to name the data file) or **Acquire** (if you want to name the sample and data file).
   - If you clicked **Start**, the **Start** button becomes the **Stop** button; go to step 4.
   - If you clicked **Acquire**, go to step 3.
3. In the **Acquire to Disk** dialog box, type the name of the sample and the name of the data file where the results will be stored, and then click **OK**.
4. Data acquisition starts.

   The TIC and corresponding mass spectrum are appended to the Tune Method Editor as separate panes.
5. Right-click on the TIC pane to display the following three options:
   - If you select **Open File** the file currently displayed in the window opens with Explore and allows you to use the tools available in Explore.

- If you select **Fixed Intensity Display**, the **Fixed Intensity Display** dialog box opens, and you can specify the y-axis maximum intensity in cps.
- If you highlight an area of interest in the pane and then select **Normalize to Selection**, you can perform a y-axis zoom.

6. Right-click on the mass spectrum to display the following Explore options:

   - If you select **Open File**, the file currently displayed in the window opens with Explore and allows you to use all the tools available in Explore.
   - If you select **Acquire Product**, you can acquire the product of the highest intensity mass in the region that was selected.
   - If you select **Fixed Intensity Display**, the **Fixed Intensity Display** dialog box opens, and you can specify the y-axis maximum intensity in cps.
   - If you highlight an area of interest in the pane and then select **Normalize to Selection**, you can perform a y-axis zoom.
   - If there are multiple mass windows, you can select a particular mass window or the entire mass range in the MS pane by selecting **All Mass Ranges**.

   LIT scan types split the mass range and, by default, display the lowest mass range for the spectrum. The right-click list will include the Split Mass Ranges as well as the All Mass Ranges option.

6

Optimizing for Your Analyte

Once you have tuned and calibrated your instrument, you can optimize the instrument for best performance for the compounds of interest. Optimization can be done automatically or manually. This section provides an overview of how to optimize your instrument for a particular analyte of interest.

Optimizing the Source for an Analyte

This section describes how to optimize the source when the analyte of interest changes. The State Parameter Editor, which is part of the Tune Method Editor, provides the ability to adjust parameters when tuning the instrument. The parameters are stored in the Tune data file and consist of four groups: Source/Gas, Compound, Resolution, and Detector.

Source-dependent parameters vary depending on the type of source. Not all source-dependent parameters are available for all sources or all instruments. For more information on specific settings, refer to the relevant source manuals.

Optimizing the Temperature (TEM) (Heated Nebulizer)

The optimal heated nebulizer temperature is affected by the mobile phase and analyte. As the LC flow rates increase, the optimal temperature increases. As the organic content increases, the optimal temperature decreases. The starting point depends on the type of instrument and the flow rate. For more information on starting points for temperature optimization, refer to the online Help.

To optimize the temperature (TEM)

NOTE: Use a step size of 25 °C and leave a minute or two between steps to allow the source to stabilize. After optimizing, allow the source 10 minutes to allow the temperature of the entire source to reach equilibrium.

1. In the **Source/Gas** tab in the **Tune Method Editor**, type the desired temperature.
2. Increase the step size until you obtain the best sensitivity.

Optimizing the Turbo Gas (Gas 2) and Temperature (TEM)

TurboIonSpray sources

The flow rate of the turbo gas (Gas 2) and the temperature (TEM) parameters control the turbo gas, which provides heat for the TurboIonSpray source. These parameters interact and are affected by the LC flow rate and the organic content of the mobile phase. As the LC flow rates increase, the optimal temperature increases. As the organic content increases, the optimal temperature decreases.

The settings of these parameters depend on the type of instrument. In general, the best sensitivity is obtained when the combination of temperature and turbo gas flow rate causes the LC solvent to reach a point at which it is nearly all vaporized. Heating beyond this point produces negligible gains. Overheating can also cause the LC solvent to thermally degrade, the spray to become unstable, and the background noise to become excessive. If this happens, reduce the temperature or gas flow. Moving the IonSpray or TurboIonSpray probe away from the curtain plate may reduce the amount of heat reaching the sprayer tip.

Instrument	Starting value	Ending value	Step size
API 365	4 L/min	8 L/min	1 L/min
API 2000	40 psi	70 psi	10 psi
API 3000	4 L/min	8 L/min	1 L/min
API 4000	4 L/min	8 L/min	1 L/min
Q Trap	40 psi	70 psi	10 psi

To optimize the TurboIonSpray gas flow and temperature

NOTE: Use a step size of 25 °C or 1L/min and leave a minute or two between steps to allow the source to stabilize. The starting point depends on the type of instrument and the flow rate.

1. In the **Source/Gas** tab in the **Tune Method Editor**, set the parameter using the starting values from the following table.
2. Increase the flow using the step size until you obtain the best signal-to-noise ratio.

After optimizing, allow the source 10 minutes to allow the temperature of the entire source to reach equilibrium. If you exceed the maximum value, you can cause a significant increase in background noise, resulting in negligible gain in the signal-to-noise ratio.

For API 365, API 3000, and API 4000 instruments, turbo gas flows of about 5 L/min are optimal for conditions that require heat. For all liquid flow rates ionization efficiency improves with the input of heat; however, at lower flow rates (<20 µL/min) the gains are small.

NOTE: Some instruments have a heated spray plate. If the heater is on, the temperature is enough to vaporize up to 20–30 µL/min flow. The turbo gas does not need to be on.

The following table shows optimum liquid flow rates and temperatures. The upper end of the ranges is for aqueous solvents.

Flow rate	Temperature
1–20 µL/min	100–200 °C
20–100 µL/min	150–350 °C
100–300 µL/min	300–400 °C
300µL/min–1 mL/min	400–500 °C

Optimizing Gas Flows

Optimizing the Curtain Gas

The main function of the curtain gas is to prevent the contamination of the ion optics. The supply pressure at the instrument should be 60 psi. The curtain gas parameter should always be maintained as high as possible without losing sensitivity. The value depends on the type of instrument and source.

To optimize the Curtain gas

NOTE: Do not set the parameter below the starting value.

1. In the **Source/Gas** tab in the **Tune Method Editor**, set the parameter using the starting values from the following table.
2. Increase the value of the parameter until you observe a loss of signal.

The type of source you use will affect the curtain gas flow. For more information on optimizing the curtain gas, refer to the relevant source manuals.

Instrument and source	Recommended starting value
API 365	8
API 2000	20
API 3000	10
API 4000	10
Q Trap	20

Optimizing the Collision Activated Dissociation (CAD) Gas

Optimize the CAD gas for single and triple quadrupole instruments after optimizing all working parameters.

In Q1 scans, no CAD gas is needed. In Q3 scans, the CAD gas is fixed at 1 or 2. For MS/MS scans, set the CAD gas initially to 2 or 3, and then, after optimizing collision energy (CE or RO2), optimize the CAD gas value. Start with a setting of 1 and increase it until you reach maximum sensitivity. Use the lowest CAD gas value that produces the best sensitivity.

To optimize the CAD Gas

NOTE: Do not set the parameter below the starting value.

1. In the **Source/Gas** tab in the **Tune Method Editor**, set the parameter using the starting values in the above paragraph.
2. Increase the value of the parameter until you reach maximum sensitivity.

Once you have optimized your source and created your acquisition method, you can now create and submit your batches. For more information on creating batches see "Creating Batches" on page 85.

Optimizing CAD Gas for LIT Analysis

Optimize the CAD gas for LIT instruments after optimizing all working parameters.

- For Q1 scans, no CAD gas is needed. In Q3 scans, the CAD gas value is fixed according to the numerical value corresponding to Low.
- For MS/MS scans, the CAD values vary depending on whether the access type is Simplified or Operator. For more information on Operator access, refer to the online Help.

  The default access type for CAD is Simplified and uses a value initially set to medium. (Simplified uses values of Low, Medium, or High). After optimizing collision energy (RO2), optimize the CAD gas value. Start with a value of low and increase it until you reach maximum sensitivity. Use the lowest CAD gas value that produces the best sensitivity.
- For MS2, Prec, NL, and MRM scans, set the value to Medium.
- For LIT scans, the CAD values vary depending on whether the access type is Operator or Simplified. For more information on Operator access, refer to the online Help.

  The default access type for CAD is Simplified and uses a value initially set to high. (Simplified uses values of Low, Medium, or High). After optimizing collision energy (RO2), optimize the CAD gas value. The value you set depends on the scan type:
- For EMS and TDF scans, set the value to Low.
- For ER, EPI, EMC and MS3 scans, set the value to High.

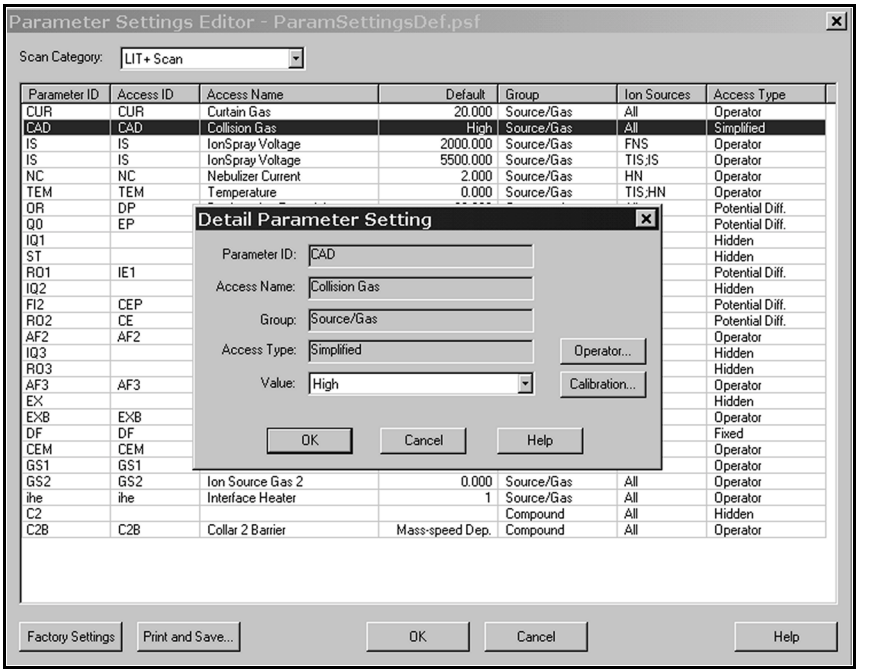
You can change the numerical values that apply to Low, Medium, or High by clicking the Calibration button in the Detail Parameter Setting dialog box for the CAD parameter (only for MS/MS and LIT scan categories).

To access the Detail Parameter Setting dialog box

1. From the **Tools** menu, point to **Settings** and click **Parameter Settings**.

   The Parameter Settings Editor dialog box appears.
2. Select the appropriate scan type from the **Scan Category** list.
3. Double-click on the **CAD** parameter row.

   The Detail Parameter Setting dialog box appears.



4. Click the **Calibration** button, edit the values as required and then click **OK**.
5. Click **OK** to close the **Detail Parameter Setting** dialog box.

The recommended vacuum gauge operating pressures for the Low, Medium, and High values are

- Low (2×10e-5 torr)
- Medium (3×10e-5 torr)
- High (4×10e-5 torr)

Optimizing the Discharge Needle Current

For heated nebulizer sources, the current applied to the corona discharge needle (NC) usually optimizes over a broad range (about 1 to 5 µA).

To optimize the discharge needle current

NOTE: If, on increasing the current, you observe no changes in signal, leave the current at the lowest setting that provides the best sensitivity (for example, 2 µA).

1. In the **Source/Gas** tab in the **Tune Method Editor**, set the parameter to 1.
2. Increase the value of the parameter until you achieve the best signal-to-noise ratio.

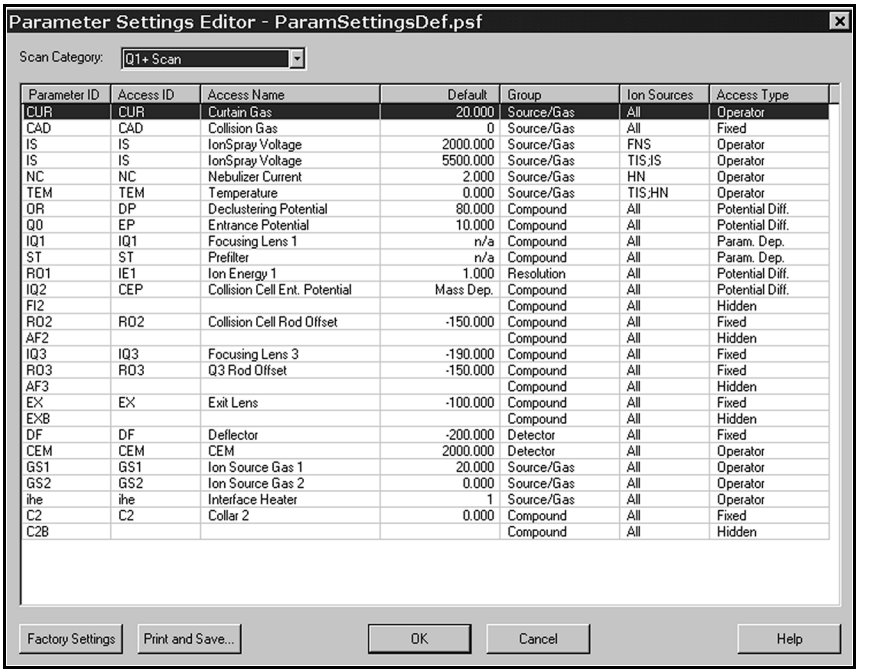
Optimizing Lens Voltages

This section describes how to optimize various voltages throughout the instrument. Voltages are set using the Detail Parameter Setting dialog box.

To access the Detail Parameter Setting dialog box

1. From the **Tools** menu, point to **Settings** and click **Parameter Settings**.

   The Parameter Settings Editor dialog box appears.



Parameter ID	Access ID	Access Name	Default	Group	Ion Sources	Access Type
CUR	CUR	Curtain Gas	20.000	Source/Gas	All	Operator
CAD	CAD	Collision Gas	0	Source/Gas	All	Fixed
IS	IS	IonSpray Voltage	2000.000	Source/Gas	FNS	Operator
IS	IS	IonSpray Voltage	5500.000	Source/Gas	TIS;IS	Operator
NC	NC	Nebulizer Current	2.000	Source/Gas	HN	Operator
TEM	TEM	Temperature	0.000	Source/Gas	TIS;HN	Operator
OR	DP	Declustering Potential	80.000	Compound	All	Potential Diff.
Q0	EP	Entrance Potential	10.000	Compound	All	Potential Diff.
IQ1	IQ1	Focusing Lens 1	n/a	Compound	All	Param. Dep.
ST	ST	Prefilter	n/a	Compound	All	Param. Dep.
RO1	IE1	Ion Energy 1	1.000	Resolution	All	Potential Diff.
IQ2	CEP	Collision Cell Ent. Potential	Mass Dep.	Compound	All	Potential Diff.
FI2				Compound	All	Hidden
RO2	RO2	Collision Cell Rod Offset	-150.000	Compound	All	Fixed
AF2				Compound	All	Hidden
IQ3	IQ3	Focusing Lens 3	-190.000	Compound	All	Fixed
RO3	RO3	Q3 Rod Offset	-150.000	Compound	All	Fixed
AF3				Compound	All	Hidden
EX	EX	Exit Lens	-100.000	Compound	All	Fixed
EXB				Compound	All	Hidden
DF	DF	Deflector	-200.000	Detector	All	Fixed
CEM	CEM	CEM	2000.000	Detector	All	Operator
GS1	GS1	Ion Source Gas 1	20.000	Source/Gas	All	Operator
GS2	GS2	Ion Source Gas 2	0.000	Source/Gas	All	Operator
ihe	ihe	Interface Heater	1	Source/Gas	All	Operator
C2	C2	Collar 2	0.000	Compound	All	Fixed
C2B				Compound	All	Hidden

2. Select the appropriate scan type from the **Scan Category** list.
3. Double-click on the desired parameter row.

   The Detail Parameter Setting dialog box appears.
4. Edit the values as required and click **OK**.
5. Click **OK** to close the **Detail Parameter Setting** dialog box.

Optimizing the Focusing Lens 3/Collision Cell Exit Potential (IQ3/ST3/CXP)

API 3000 and API 4000 instruments

For Q1 scans, set ST3 (prefilter) to between -100 V and -200 V (for positive ions) and to between 100 V and 200 V for negative ions.

For Q3 scans, CXP = RO2 - ST3, and is not mass-dependent. Set CXP to between 0 V and 55 V (for positive ions) and to between -55 V and 0 V (for negative ions).

API 365, API 2000, and Q Trap instruments

For Q1 scans, set IQ3 to between -200 V and -100 V (for positive ions) and to between 100 V and 200 V for negative ions.

For Q3 and MS/MS scans, CXP = RO2 - IQ3, and is mass-dependent on the Q3 mass. Set CXP to between 0 V and 200 V (for positive ions) and to between -200 V and 0 V (for negative ions).

Optimizing the Inter-Quadrupole Lens 2/Collision Cell Entrance Potential (IQ2/CEP)

API 3000 and API 4000 instruments

For all scans, IQ2 = Q0 + offset. Set the offset to 8 V to 10 V for positive ions and -10 V to -8 V for negative ions.

API 365, API 2000, and Q Trap instruments

In Q1 and MS/MS scans, CEP = Q0 - IQ2. CEP is mass-dependent on the mass of Q1; that is, its optimum value becomes increasingly positive with increasing mass values (in positive ion mode). Analyst calculates the value of CEP using the slope and intercept values from the Parameter Settings Table.

In Q3 scans, IQ2 = RO2 + an offset. Set the offset to 2 V for positive ions and -2 V for negative ions. In LIT scans CEP = Q0 + FI2.

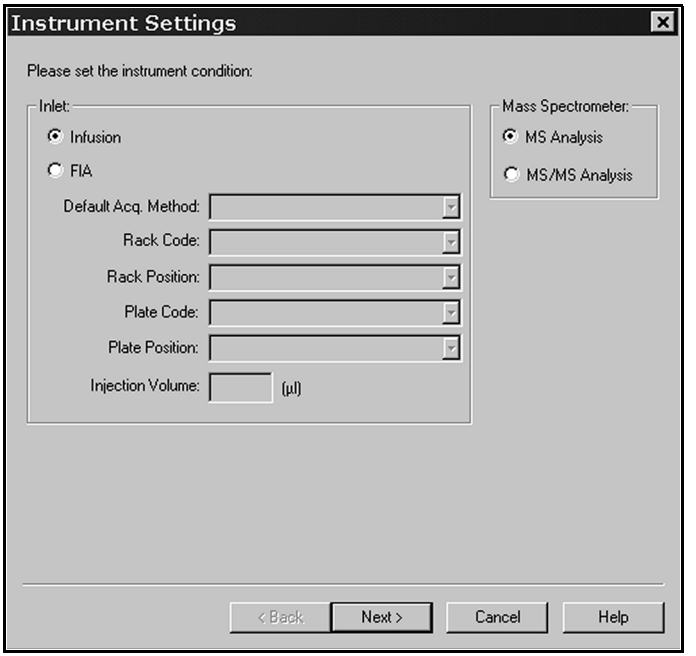
MS Analysis By Infusion

This procedure optimizes the quantitation process for an MS analysis using an instrument with an infusion device. Before you begin, ensure that the acquisition queue has been reserved for tuning.

To perform MS quantitative optimization using an infusion device

1. From the Tools menu, click **Quantitative Optimization**.

   The Instrument Settings dialog box appears.



2. Select **Infusion** and **MS Analysis**, and then click **Next**.

   The Ions to Use in MS Analysis page appears.

3. Select the target ion.

   - If you select **MW Ion**, Analyst can optimize multiple compounds.
   - If you select **Base Peak Ion**, Analyst optimizes only one compound. You can name the compound and edit the search range. Go to step 8.

4. Select the **Resolution** for the Target Ion from the list.
5. Select the polarity. If you select **Both**, Analyst optimizes using both **Positive** and **Negative** polarities and selects the best polarity for each target compound.
6. Click **Next**.

   The Target Components dialog box appears.
7. Type the list of compounds, molecular weights, and number of charges.
8. Click **Finish**.

   Quantitative optimization starts and when completed, the quantitative optimization report appears.

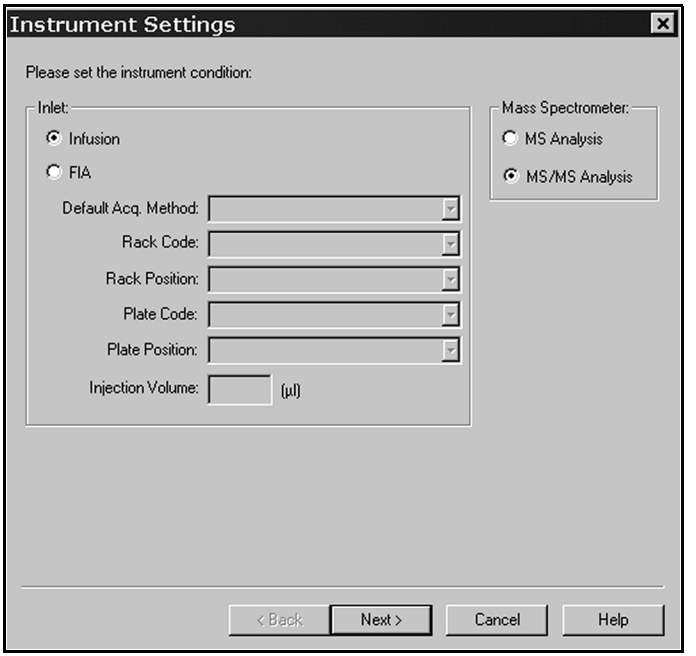
MS/MS Analysis By Infusion

This procedure optimizes the quantitative process for an MS/MS analysis using an instrument with an infusion device. Before you begin, ensure that the acquisition queue has been reserved for tuning.

To perform MS/MS quantitative optimization using an infusion device

1. From the **Tools** menu, click **Quantitative Optimization**.

   The Instrument Settings dialog box appears.



2. Select **Infusion** and **MS/MS Analysis**, and then click **Next**.

   The Ions to Use in MS/MS Analysis page appears.

3. Select a precursor ion.

   - If you select **MW Ion**, you can select **User Specified** to select the user-specified ion, or you can select **Auto Select** to automatically select the ion. If you select **Auto Select**, you can click **Criteria** to use the **Product Ion Auto Selection Criteria** dialog box to edit the criteria for selecting the product ion.
   - If you select **Base Peak Ion**, Analyst optimizes only one compound. You can name the compound and edit the search range. The product ion is auto-selected. If you click **Criteria**, you will be able to use the **Product Ion Auto Selection Criteria** dialog box to edit the criteria for selecting the product ion.

4. Select the **Resolution** for the product ion from the list.

   NOTE: User Specified and Auto Select are enabled or disabled depending on the type of Precursor Ion selected.

5. Select the polarity. If you select **Both**, Analyst will optimize using both the **Positive** and **Negative** polarities and will select the best polarity for each target compound.
6. If you selected **Base Peak Ion,** go to step 7.

   If you selected **MW Ion**, click **Next**.

   The Target Components page appears.

   - If you selected **Auto Select**, type or edit the list of compounds, molecular weights, and the number of charges for each compound.
   - If you selected **User Specified**, type or edit the list of compounds, and the Q1 and Q3 masses for each compound.
7. Click **Finish**.

   Quantitative Optimization starts and the quantitative optimization report appears.

Flow Injection Analysis

Before you start a flow injection analysis (FIA), you must build a method in the Method Editor that contains autosampler and LC methods and a simple Q1 MI or MRM experiment. FIA assumes that you have already identified the ions for your compounds using either Infusion or Manual Tune.

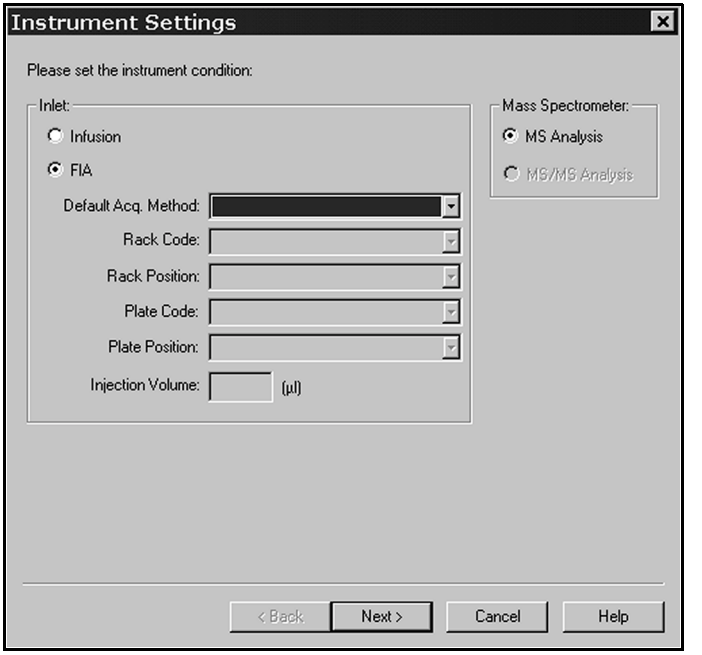
- If you use a method created by Quantitative Optimization in Infusion, add the necessary devices (an autosampler and a pump) and activate a hardware profile containing those devices.
- If you create or open a method in Manual Tune, you may add only an LC pump method to an acquisition method. If you open a method in the Acquisition Method Editor, you can add an LC pump or any other device to the acquisition method.

To run a flow injection analysis

NOTE: MS Analysis or MS/MS Analysis is set automatically based on the default method selected. Infusion optimizes the compound parameters only. FIA optimizes the source and gas parameters as well as the compound parameters.

1. Ensure that you are in Tune mode, and then from the **Tools** menu, click **Quantitative Optimization**.

   The Instrument Settings page appears.



2. Select **FIA**.
3. Choose a method from the **Default Acq. Method** list. The method must be an LC/MS method that includes an autosampler.

   The five boxes that allow you to select settings become available.
4. If you are using a rack, select the type of rack (**Rack Code**) and the position of the rack in the autosampler, if necessary (**Rack Position**).
5. If you are using a microlitre plate, select the type of plate (**Plate Code**) and the position of the plate in the autosampler, if necessary (**Plate Position**).

6. Type the injection volume in microliters, and then click **Next**.

   The FIA Target Compounds dialog box appears.

7. If required, edit the **Compound Name** field.

8. If you wish to use one of the compounds as an internal standard, select the check box in the **Int. Std.** column, and then click **Next**.

   The FIA Source Parameters dialog box appears.

9. In the **Values for Optimization** column for each parameter you have chosen to optimize, type at least two values, separated by a semicolon. The current value appears automatically. Click **Next**.

   The FIA Compound Parameters dialog box appears.

10. In the **Values for Optimization** column for each parameter you have chosen to optimize, type at least two values, separated by a semicolon. The current value appears automatically. Click **Finish**.

Quantitative optimization starts and the quantitative optimization report appears.

Flow Injection Analysis Using a Q1 MI Method

NOTE: Before you begin, ensure that you have selected a method and entered the relevant data in the Instrument Settings dialog box, (the first screen of the Quantitative Optimization wizard).

To perform a Q1 MI quantitative optimization using FIA

1. In the **Instrument Settings** page, click **Next**.

   The FIA Target Compounds page appears with the compounds and masses you entered in the method in the first two columns.

   NOTE: The Quantitative Optimization wizard reads the compounds, mass or masses, and polarity from the method.

2. If you wish to use one of the compounds as an internal standard, select the check box in the **Int. Std.** column.

3. In the **Vial Pos.** column, type the vial position for the compound of interest. For each compound, the default location is vial 1, position 1.

4. Click **Next**.

   The FIA Source Parameters page appears with the source parameters listed under Parameter Name.

5. In the **Values for Optimization** column for each parameter you have chosen to optimize, type at least two values, separated by a semicolon. The current value appears automatically.

6. Select the check box beside the parameters you wish to optimize.

7. Choose the number of times you wish to repeat the injection for each parameter. A separate number of injections will be made for each value of each parameter to be optimized.

8. Click **Next**.

   The FIA Compound Parameters page appears with the compound parameters listed.

9. Select the check box beside the parameters you wish to optimize.

10. In the **Values for Optimization** column for each parameter you have chosen to optimize, type at least two values, separated by a semicolon. The current value appears automatically.

11. Type the **Mass Spec. Duration** in minutes. The duration specified in the method is the default value.

12. Click **Finish**.

    Quantitative optimization starts and the quantitative optimization report appears. One looped experiment injection will be made for each parameter optimized.

Flow Injection Analysis Using an MRM Method

NOTE: Before you begin, ensure that you have selected a method and entered the relevant data in the Instrument Settings page, (the first screen of the Quantitative Optimization wizard).

To perform an MRM quantitative optimization using flow injection analysis

1. In the **Instrument Settings** page, click **Next**.

   The FIA Target Compounds page for an MRM method appears with the compounds and masses entered in the method in the first two columns.

2. If you wish to use one of the compounds as an internal standard, select the check box in the **Int. Std.** column.
3. In the **Vial Pos.** column, type the vial position for the compound of interest.

   The default location is 1 for each compound. You can use the same vial for each compound or you can specify different vials.
4. Select the **Resolution** and click **Next**.

   The FIA Source Parameters page appears with the source parameters listed under Parameter Name.
5. In the **Values for Optimization** column for each parameter you have chosen to optimize, type at least two values, separated by a semicolon.

   The current value appears automatically.
6. Select the check box beside the parameters you wish to optimize.
7. Choose the number of times you wish to repeat the injection for each parameter, and click **Next**.

   The FIA Compound Parameters page appears with the compound parameters listed.
8. In the **Values for Optimization** column for each parameter you have chosen to optimize, type at least two values, separated by a semicolon.

   The current value appears automatically.
9. Select the check box beside the parameters you wish to optimize.
10. Type the **Mass Spec. Duration** in minutes.
11. Click **Finish**.

Quantitative optimization starts and when completed, the quantitative optimization report appears.

7

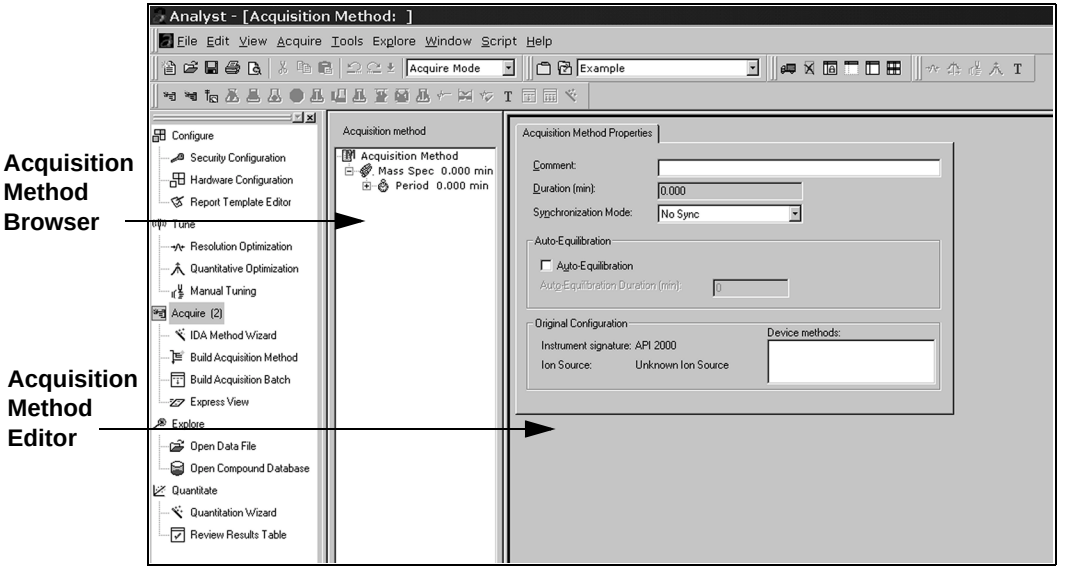
Building an Acquisition Method

This section provides an overview of how to build an acquisition method using the Acquisition Method Editor. Before you begin creating an acquisition method file, you should have a good understanding of the experiments you want to carry out and the devices that are required. You must also have access to the Acquisition Method Editor and be working on an acquisition workstation.

The Acquisition Method Editor offers two options for generating an acquisition method file:

- Create a method from scratch when you want to create a method that has never been done before or that is very different from any existing method.
- Modify an existing method file when you want to create a method that is only slightly different from an existing acquisition method file.

The Acquisition Method Editor is where you specify data acquisition parameters for the mass spectrometer and any configured external devices. It contains the programs to be executed by each device, including information about when they will start acquiring data.



Method Editor window

The Method Editor window contains two panes—the Acquisition Method Browser pane on the left side and the Acquisition Method Editor pane on the right side. The layout of the window can be changed and the panes can be resized.

You define instrument parameters in the parameter settings file; however, these parameters can be overwritten with the Method Editor. Resolution values are set in Tune mode and selected in Acquire mode using the Acquisition Method Editor.

What is an Acquisition Method?

In order to submit a batch and obtain data, you must create an acquisition method for the mass spectrometer and any external devices required. An acquisition method is a high-level description of a particular command sequence and its associated parameters for the data acquisition. In simple terms, an acquisition method is a recipe for data acquisition. It specifies which devices to use and how and when to put them to work to acquire data.

In the Acquisition Method Editor window, you create a sequence of acquisition periods and experiments for the mass spectrometer and

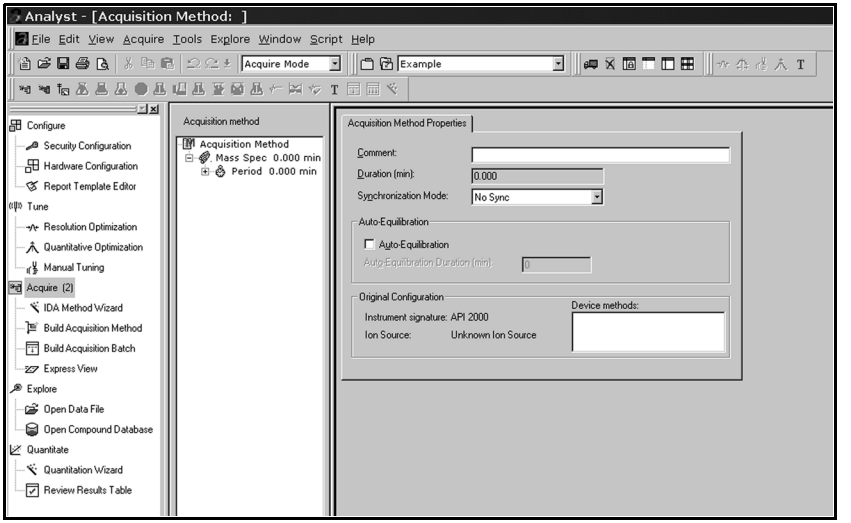
instructions for various devices to follow. An acquisition method is associated with each sample in the Batch Editor when submitted to the queue.

Specifying a Mass Spectrometer Method

The mass spectrometer acquisition method is specified in the Acquisition Method Editor pane. Depending on the type of mass spectrometer configured and the scan type selected, different fields and options are available. As you type the mass spectrometer parameters, the Acquisition Method Editor validates the data.

To specify a mass spectrometer acquisition method

1. Double-click **Build Acquisition Method** under **Acquire** in the Navigation bar.
2. With a method file open in the **Acquisition Method Editor** window, in the **Acquisition Method Browser** pane, click the Acquisition Method icon.



3. In the **Acquisition Method Properties** tab of the **Acquisition Method Editor** pane, select a **Synchronization Mode**.
4. Select **Auto-Equilibration**, if required.

5. In the **Acquisition Method Browser** pane, click the **Mass Spectrometer** icon.
6. In the **Acquisition Method Editor** pane, click the **MS** tab.
7. Choose the **Experiment** you want to edit.
8. Set the **MS** tab parameters to the desired values.
9. If required, click the **Advanced MS** tab and Set the **Advanced** tab parameters to the desired values.
10. Repeat steps 5 through 8 for each experiment in the method.
11. To save your method, on the **File** menu click **Save**.

    The Save Acquisition Method dialog box opens.
12. Type a file name and click **Save**.

Adding Peripheral Devices to Your Method

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. If you want, you can have more than one device in the same category (e.g. two pumps). With the Acquisition Method Editor, you can customize your acquisition method by adding or removing external device methods.

To add or remove an external device acquisition method

1. With a method file open in the Acquisition Method Editor, in the **Acquisition Method Browser** pane, right-click the Acquisition Method icon.
2. Choose **Add/Remove Device Method** from the menu that appears.

   The Add/Remove Device Method dialog box shows the devices configured in the active hardware profile.
3. Select or clear the appropriate check box(es) beside the device method to add or remove the method.

Configuring Devices

Configuring Analyst peripherals or devices consists of two processes: setting up the physical hardware connections and configuring the software to communicate with the devices. To learn more about setting up specific peripheral devices, refer to the *Peripheral Devices Setup Manual* or to "Creating a Hardware Profile" on page 25.

When Analyst is installed, all the drivers required to configure the supported devices are also installed. To activate these devices, you must configure them using the Hardware Configuration Editor. For more information, refer to the online Help.

Creating Methods for External Devices

Creating an acquisition method for an external device means specifying the operating parameters for that device. Methods can be created for any of the following devices if they are configured in the active hardware profile:

- Pumps
- Autosamplers
- Syringe pumps
- Column ovens
- Switching valves
- Diode array detector
- A/D converters
- Integrated system

For more information on configuring an integrated system refer to the online Help.

NOTE: Only devices configured in the active hardware profile and selected in the Add/Remove Device Method dialog box appear as icons in the Acquisition Method Browser pane. If the required device icon is not in the Acquisition Method Browser pane, you may add a device if it is part of the active hardware profile.

Specifying a Pump Method

The Acquisition Method Editor allows you to specify a pump method that includes information on the composition of the mobile phase, the gradient, and the pump's limits and support for timed events.

To display the pump parameters in the Acquisition Method Editor pane

1. With a method file open in the Acquisition Method Editor, in the **Acquisition Method Browser** pane, click the **pump** icon.

   The pump's properties tab appears in the Acquisition Method Editor pane.

2. Click one of the following possible tabs in the Acquisition Method Editor pane and edit the fields as required:

   - Gradient
   - Limits
   - Events
   - Column
   - Detector

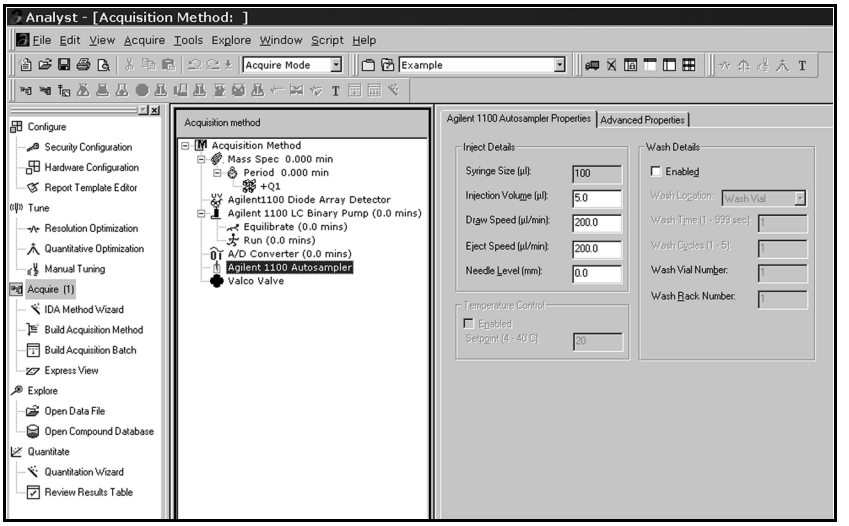
Specifying an Autosampler Method

The Acquisition Method Editor allows you to specify an autosampler method that includes details such as injection volume and the number of pre- and post-inject flushes. The procedure depends on the type of autosampler used.

To specify an autosampler acquisition method

1. With a method file open in the Acquisition Method Editor, in the **Acquisition Method Browser** pane, click the **autosampler** icon.

   The autosampler's properties tab appears in the Acquisition Method Editor pane.



2. In the **Acquisition Method Editor** pane, click the pump's **Properties** tab.
3. Specify inject and flush details.
4. Click the **Advanced Properties** tab, and specify needle and advanced injection information if required.

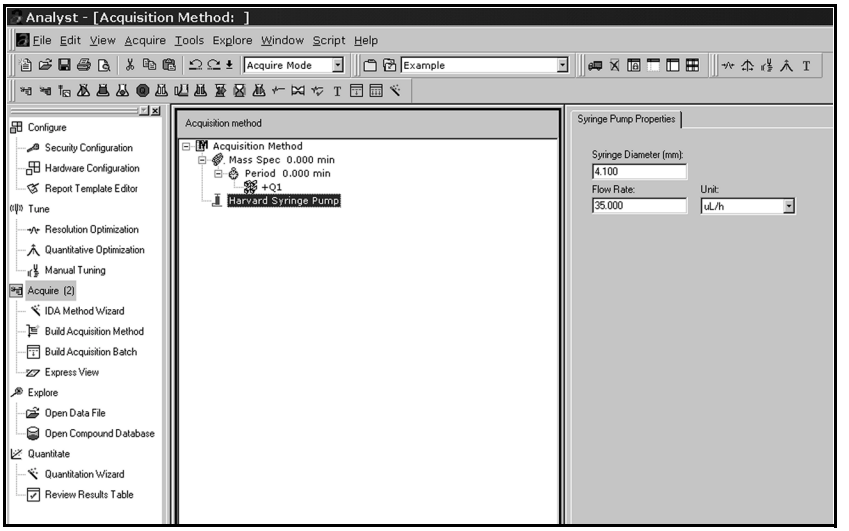
Specifying a Syringe Pump Method

NOTE: The API 2000 mass spectrometer includes an incorporated Harvard Syringe pump. In this case only, the pump must be configured with the mass spectrometer.

To configure a syringe pump

1. With a method file open in the Acquisition Method Editor, in the **Acquisition Method Browser** pane, click the **Syringe Pump** icon.

   The Syringe Pump Properties tab appears in the Acquisition Method Editor pane.



2. In the **Syringe Pump Properties** tab, type the syringe diameter in the **Syringe Diameter (mm)** field.
3. Type the **Flow Rate**.
4. Select the **Unit**.

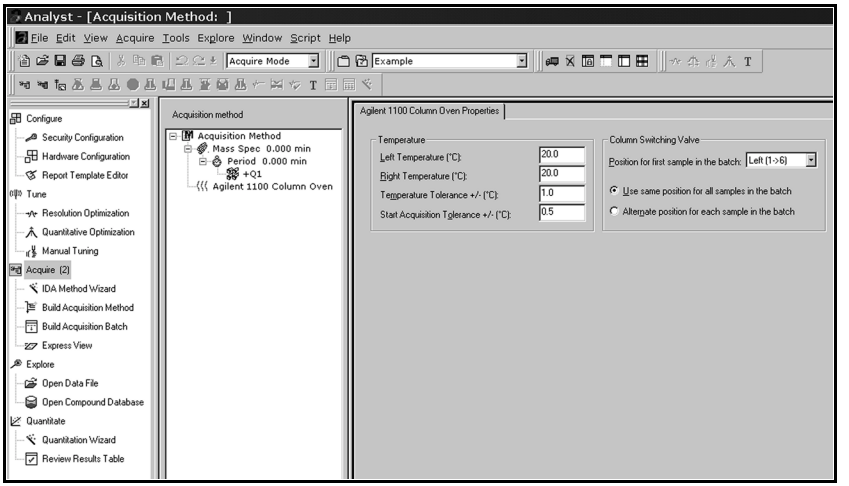
Specifying a Column Oven Method

The Acquisition Method Editor allows you to specify a column oven method that contains temperature and time information.

To specify a column oven acquisition method

1. With a method file open in the Acquisition Method Editor, in the **Acquisition Method Browser** pane, click the **Column Oven** icon.

   The Column Oven Properties tab appears in the Acquisition Method Editor pane.



2. In the **Column Oven Properties** tab, type the temperature of the column oven (PE200) or column oven compartments (Agilent 1100) in degrees Celsius.
3. Type the **Temperature Tolerance** in degrees Celsius.
4. Type the **Start Acquisition Tolerance** in degrees Celsius.

Specifying a Switching Valve Method

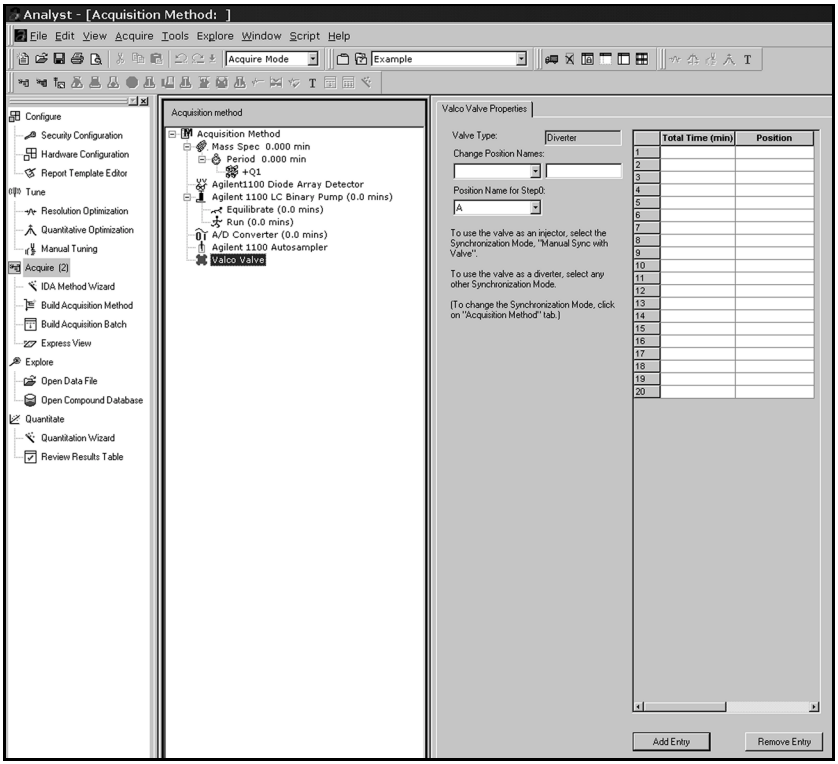
The Acquisition Method Editor allows you to specify a switching valve method, which contains information such as the valve type and position.

NOTE: Select the synchronization mode, Manual Sync with Valve, to operate the instrument and valve in synchronization. If you do not select Manual Sync with Valve, you must complete the Time Summary table manually. Synchronization modes are specified on the Acquisition Method Properties tab.

To specify a switching valve acquisition method

1. With a method file open in the Acquisition Method Editor, in the **Acquisition Method Browser** pane, click the **Valve** icon.

   The Valve Properties tab appears in the Acquisition Method Editor pane.



2. In the **Valve Properties** tab, choose a position from the **Change Position Names** list.
3. If you have not chosen **Manual Sync with Valve** as the **Synchronization mode** on the **Acquisition Method Properties** tab, click **Add Entry** to add an entry to the Time Summary table. Click **Remove Entry** to delete a step.
4. If required, change the position names. Click in a cell and choose a position name from the list. To create a different name for a position, choose the position in the **Change Position Names** list and type the new name in the adjacent field.

Specifying a Diode Array Detector Acquisition Method

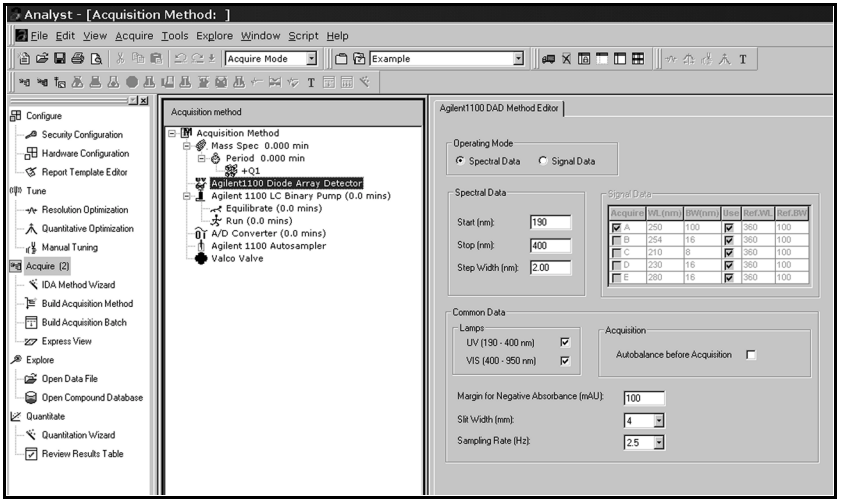
The diode array detector (DAD) uses UV wavelengths instead of the mass-to-charge ratio to characterize analysis. It provides complementary information to the total ion chromatogram (TIC). You must configure the device to operate in either Spectral Data or Signal Data mode.

Creating an acquisition method for an external device requires specifying the operating parameters for that device. Methods can be created for a diode array detector if the device is configured in the active hardware profile.

To specify a DAD acquisition method

1. With a method file open in the Acquisition Method Editor, in the **Acquisition Method Browser** pane, click the **DAD** icon.

   The DAD Method Editor tab appears in the Acquisition Method Editor pane.



2. In the **DAD Method Editor** tab, select the **Operating Mode,** Spectral Data or Signal Data**,** and specify the data requirements.

   - Configure the diode array detector to function in Signal Data mode if you want to scan one to five individual wavelengths.
   - Configure the diode array detector to function in Spectral Data mode if you want to scan over a wavelength range.

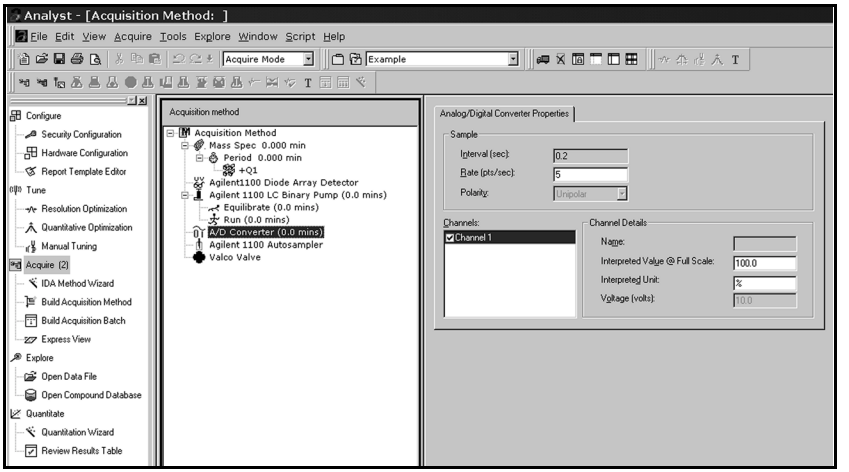
Specifying an Analog-to-Digital Converter Method

The Acquisition Method Editor allows you to specify a method for the analog-to-digital converter. This method contains information such as the sample rate and interval, and the voltage.

To specify an analog/digital converter acquisition method

1. With a method file open in the Acquisition Method Editor, in the **Acquisition Method Browser** pane, click the **ADC** icon.

   The Analog-to-digital Convertor Properties tab appears in the Acquisition Method Editor pane.



2. In **Analog/Digital Converter Properties** tab, type a value in the **Rate (pts/sec)** field.

   The interval and rate are proportional to each other. When you change the interval, the rate automatically re-calculates and vice versa.

3. In the **Channel** box, click the channel name you want to specify details for. If you select the check box beside a channel it is included in the method. If you clear the check box, the channel is not included.

4. In the **Interpreted Value @ Full Scale** field, type the appropriate value.

5. In the **Interpreted Unit** field, choose the appropriate unit from the list.

6. Repeat steps 3 to 5 for each channel you want to specify details for.

Saving Your Method

You can save a method you have created under any name you choose. Analyst automatically associates the extension .dam with any new method file saved.

If you have created a method file for one project that you also want to use for a different project, with or without some modifications, you can save the method under a new file name in the other project folder.

To save a new acquisition method file

1. From the **File** menu, choose **Save As** or **Save**.

   Analyst defaults to the Acquisition Method folder of the active project.

2. Choose a different drive and directory for the new file if required.
3. Type or edit the file name in the **File Name** box and click **Save.**

To save an acquisition method file to another project

1. Open an existing acquisition method file.
2. Modify the file as required.
3. Select a different project.
4. From the **File** menu, choose **Save As** or **Save**.
5. In the **File name** box, type a name for the method file and click **Save**.

Creating Additional Experiments and Periods

The Acquisition Method Editor also allows you to create multiple period, multiple experiment acquisition methods for the mass spectrometer in a single method file. This feature can be useful if, for example, you want to monitor different masses through different periods, use different scan types, or alternate between positive and negative scans. You can also create additional experiments for external devices.

An experiment is a sequence of measurements for the mass spectrometer, all of the same scan type. One or more experiments can be grouped to make a cycle. One or more cycles comprise a period.

A period is an analysis that is performed for a specific length of time or number of cycles. A period consists of one or more cycles, or sequential groups, of experiments performed during a period.

To add a new experiment

1. With a method file open in the Acquisition Method Editor, in the **Acquisition Method Browser** pane, click the device category where you want to add a new experiment.
2. Right-click the period where you want to add the experiment and choose **Add experiment** from the menu that appears.
3. In the **Acquisition Method Editor** pane, specify the appropriate device or instrument parameters.

You can make a copy of an existing experiment by using Analyst's drag and drop features. Experiments can be copied in the same acquisition method, or you can drag and drop between windows to copy them into a new acquisition method.

To copy an experiment

1. In the **Acquisition Method Browser** pane select the experiment you want to copy.
2. Right-click to select the experiment and to bring up the popup menu.
3. Select **Copy this experiment**.

To delete an experiment

1. With a method file open in the Acquisition Method Editor, in the **Acquisition Method Browser** pane, right-click the experiment you want to delete.
2. Choose **Delete this experiment** from the menu that appears.

Creating Periods

Adding a Period

The Acquisition Method Editor allows you to add a period (i.e. a step) to a device acquisition method, if desired. For example, you may want to add an equilibration step for the pump.

To add a period

1. With a method file open in the Acquisition Method Editor, in the **Acquisition Method Browser** pane, click the device category where you want to add a period.
2. Right-click on the device category and choose **Add period** from the menu that appears.

   A period is added below the last period created.

Copying Periods

You can make a copy of an existing period by using Analyst's drag and drop features. Periods can be copied in the same acquisition method, or you can drag and drop between windows to copy them into a new acquisition method.

To copy a period

1. In the **Acquisition Method Browser** pane select the period you want to copy.
2. Hold down CTRL and click the left mouse button.
3. Drag the period to the target Mass Spectrometer Method node.

Deleting a Period

You can delete a period from the mass spectrometer method.

To delete a period

1. With a method file open in the Acquisition Method Editor, in the **Acquisition Method Browser** pane, right-click the period you want to delete.
2. Choose **Delete this period** from the menu that appears.

   The period and the experiments it contains are deleted.

Creating an IDA Experiment

The Acquisition Method Editor allows you to create an Information Dependent Acquisition (IDA) Method with up to two survey scans in a single experiment. IDA is a process in which the mass spectrometer actions can be varied from scan to scan based on data acquired in a previous scan. IDA will analyze data as it is being acquired and determine the masses on which to perform dependent scans. You have control over the criteria that will activate an IDA experiment and the parameters of the IDA experiment once activated.

IDA experiments can modify experiments and improve results based on the following criteria:

- Ion Selection
- Ion Charge States
- Isotope Matching

Setting IDA parameters to optimize data acquisition settings on-the-fly, drastically reduces sample acquisition time. Using IDA, an operator can conserve both sample and valuable working time on an instrument.

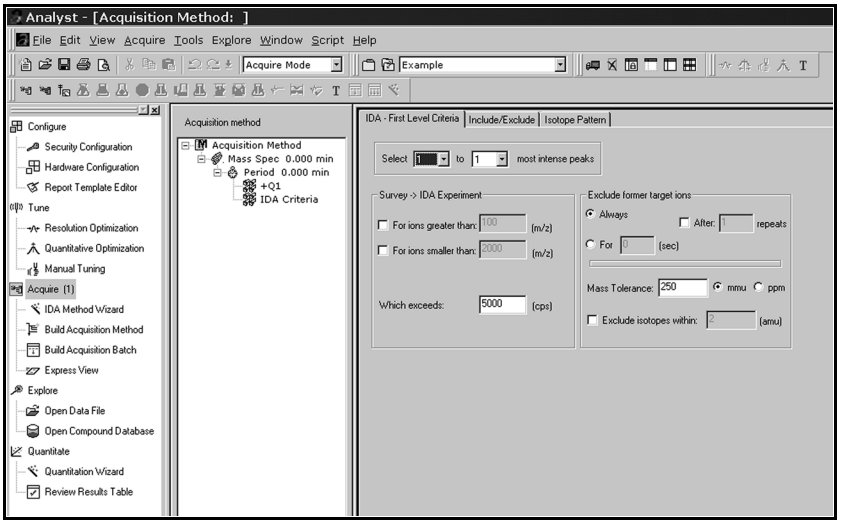
To create an IDA experiment in the Acquisition Method editor

1. Create a new Acquisition Method with at least one survey experiment. Any combination of two survey scans can be used.

   NOTE: If you have chosen EPI, MS2, or TDF as your survey experiment, you will only have access to the IDA–Second Level Criteria tab. If these survey experiments are used, you will not be able to set an additional IDA criteria level. To set the IDA selection criteria for these types of experiments go to step 8.

2. In the **Acquisition Method Browser** pane right-click on **Period** and select **Add IDA Criteria Level**.

   The IDA criteria tabs appear in the Acquisition Method Editor pane.



3. In the **Acquisition Method Editor** pane click the **IDA–First Level Criteria** tab. Set the tab parameters to the desired values.

4. If desired, on the **Include/Exclude** tab, select **Include List** or **Exclude List** checkboxes and specify the target ions or exclude undesired ions by modifying the appropriate list.

5. If desired, on the **Isotope Pattern** tab, select the **Match Isotopes** check box and specify the isotopic distribution pattern to be matched.

6. Create up to eight additional experiments defined as a dependent experiment that will be activated by the IDA criteria. Make sure that for

each level the number of dependent experiments is the same as the number of peaks selected to monitor from the IDA Criteria page.

7. If a second IDA criteria level is desired, continue with step 8. If no additional selection criteria are required go to step 11.
8. In the **Acquisition Method Browser** pane right-click **Period** and select **Add IDA Criteria Level**.
9. Set the **IDA–Second Level Criteria** tab parameters to the desired values.
10. Create an additional experiment(s) that will be activated by the second level IDA criteria.
11. Save the Acquisition Method.

Creating an IDA experiment using the IDA Wizard

A new IDA wizard has been introduced to allow for easy creation of LIT-IDA acquisition methods. For more information on using the wizard, refer to the online Help.

To display the Create IDA Experiments page of the IDA Method Wizard

- From the Navigation Bar, double-click the **IDA Method Wizard** icon.

  The Create IDA Experiments wizard launches.

- Complete the screens as required to automatically create an MS method. You can then add LC devices to the method.

8

Creating Batches

This section provides an overview of the Batch Editor and ExpressView. It also explains how to set up and submit samples and batches.

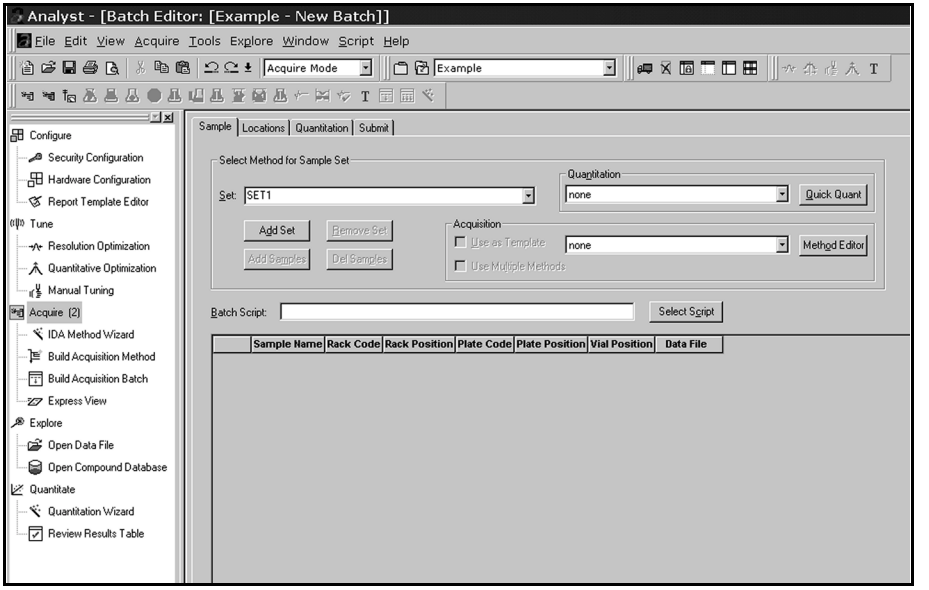
What is the Batch Editor?

The Batch Editor is used for creating, modifying, initiating, storing, and retrieving acquisition batches. The Batch Editor is also used for creating batch templates.

How Does the Batch Editor Work?

The Batch Editor window is composed of four tabs that are used to enter acquisition batch information. This information is then used by other Analyst modules to acquire and analyze samples.

For quick entry of batch acquisition data, you may use ExpressView instead of the Batch Editor. ExpressView allows you to select a Batch Editor template, enter minimal information, and submit a batch quickly. The ExpressView editor is an effective way to submit batches for both novice and expert users.



Batch Editor window

The Batch Editor allows full editing of sample information, including detailed sample, location, method, quantitation, and set information. To create a batch in the Batch Editor window, enter the sample data in each tab. You can then save it and/or submit it for acquisition.

For additional flexibility and efficiency, the Batch Editor allows you to import data in open database connectivity (ODBC) and text formats for transfer of data with the Laboratory Information Management System (LIMS), Microsoft Access, or Microsoft Excel.

Why Use the Batch Editor?

With the Batch Editor, you can construct batches to support all types of acquisition requirements, ranging from high throughput quantitation, where all samples are to be acquired and processed the same way, to method development where every sample may have different acquisition and processing requirements.

Why Use the Batch Editor Window Instead of ExpressView?

The Batch Editor window is the main editing component of the Batch Editor. It gives you access to all available fields in the acquisition batch. Use the Batch Editor for complicated batches with multiple types of samples and/or methods.

ExpressView uses Batch Editor templates to facilitate quick and easy submission of single or multiple samples with minimal information. It reduces the amount of information or options required to complete a batch. Use ExpressView when you want to create a batch quickly or when you simply need to add a sample name or change a small number of fields to an existing template. ExpressView is a good way to prevent inappropriate access to the system by unqualified or inexperienced users. An administrator password is required to exit ExpressView.

Creating Batches

A batch associates a collection of samples and acquisition methods. The Batch Editor creates a batch by linking together the following information:

- sample information such as name, ID, comment, etc.
- autosampler location (rack information)
- acquisition method
- processing method or script (optional)
- quantitation information (optional)
- custom sample data
- set information

A batch defines sample information for acquisition and is submitted to the Queue Manager for acquisition.

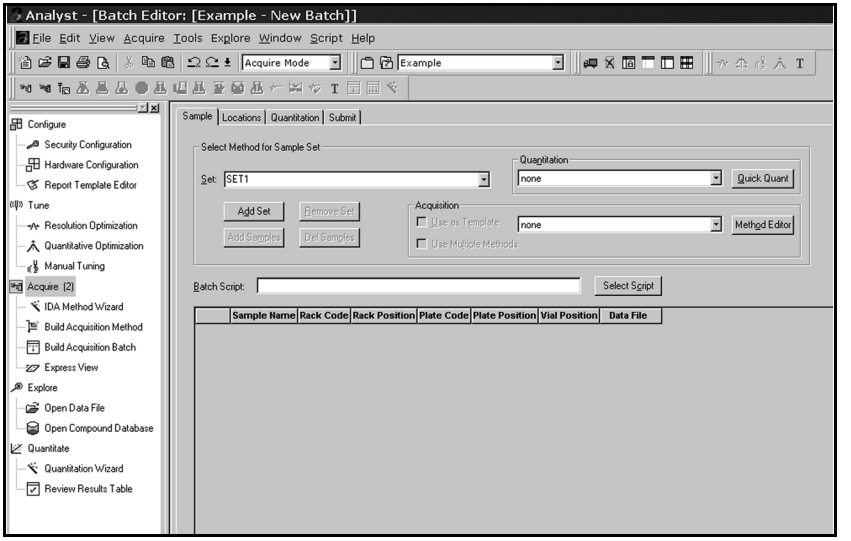
Creating a New Batch

Through the Batch Editor tabs, you can separate the batch into sets and specify every detail of the batch before submitting it for processing. You can only create and submit a batch from a station that is connected to a mass spectrometer.

To create a new batch

1. Double-click **Build Acquisition Batch** under **Acquire** in the Navigation bar.

   The Batch Editor window opens.



2. In the **Sample** tab, click **Add Set**.

   You may change the Set name first if desired.

3. Click **Add samples** to add the appropriate number of samples to the set.

   For more information on adding samples to sets, see "To add samples to a batch" on page 89.

4. Choose an **Acquisition** method from the list.

5. Specify remaining sample details in the **Sample** tab as desired. You may edit default settings such as the **Sample name** field.

6. Specify sample location on the autosampler in the **Locations** tab.

7. Specify quantitation information in the **Quantitation** tab, if required.

   NOTE: Some fields are mandatory and must be completed before you submit the batch. The Submit button in the Submit tab is disabled until you complete all mandatory fields.

8. Reorder samples in the set in the **Submit** tab, if required.
9. Save the batch file, if desired, and submit the file to queue.

To add samples to a batch

1. In the Batch Editor window, click the **Sample** tab.
2. Type a new set name or choose an existing set from the **Set** list and click **Add Set**.
3. Click **Add Samples** to add samples to the set you have selected.

   The **Add Sample** dialog box appears.
4. In the Sample name group, type a **Prefix** for the samples, if required.
5. Select the **Sample number** check box if you want a sample number to be added to the sample name.
6. If you have selected the **Sample number** check box, in the **Number of digits** field type the number of digits to include as the sample name. For example, if you type 3, the sample names will be samplename001, samplename002, etc.
7. In the Data file group, type a **Prefix** for the data file, if required.
8. Select the **Set name** check box if you want the set name to be part of the Data file name.
9. Select the **Auto Increment** check box to increment the data file names. This adds one (1) to the value of the row displayed above in the **Data File** column.
10. Type the number of new samples you want to add and click **OK**.

To remove samples from a batch

1. In the Batch Editor window, click the **Sample** tab.
2. Choose an existing set from the **Set** list and click **Remove Set** to delete a set from the batch.
3. Click **Yes** to confirm removal; click **No** to cancel.

Specifying Sample Details

In the Sample tab of the Batch Editor, you input information specific to individual samples. To type data in the tables, click in a cell and type the data. The Sample tab provides a way to verify the sample names against the rack and vial or well values and change them accordingly. Samples are created in the Add Sample dialog box. You can, however, edit the cells in the Sample Name column directly.

If you cannot see a column, right-click on the Sample table and choose Hide/Show Column from the menu that appears. Specify which columns you want to see. You can add or delete custom columns, and you can also save default column settings of subsequent batches by selecting Save Column Settings from the right-click menu.

You cannot use Acquisition Method templates if Multiple Methods is selected. If you are creating a set where some samples must have different acquisition methods, select Use Multiple Methods. You can select an acquisition method for each sample in the set from the list in the Acquisition Method column.

To specify sample details

NOTE: If you are creating a new batch, some of the fields are read-only and will remain empty until you specify sample location on the Locations tab.

1. With a batch file open in the Batch Editor window, click the **Sample** tab
2. Type a new set name or choose a set from the **Set** list and click **Add Set**.
3. Add samples to the set if required. (You cannot create a batch without adding samples.)
4. Select an **Acquisition** method from the list or click **Method Editor** to create or edit an acquisition method.
5. If desired, select the **Use Multiple Methods** to display the **Acquisition Method** column in the **Sample** table. You may select the method for each sample in the **Sample** table.
6. Select or clear the **Use as Template** check box. Select the check box if you want to enter masses individually for each sample. Clear the check box if you want to use the masses that are stored in the Acquisition method.

7. If required, select a **Quantitation** method from the list or click **Quick Quant** to create or edit a quantitation method.

   The system defaults to **None** or the method specified in the batch template (if applicable).

8. Edit the full file path for a script in the **Batch Script** field or click **Select Script** to locate a script file. You do not need to specify a script.

9. Specify the **Data File** name for each sample.

Specifying Sample Locations

In the Locations tab, you indicate the position of the rack on the autosampler, and the position of the samples on the rack. Locations can also be specified in the table in the Sample tab.

NOTE: Click  if you want to clear all the selected wells or vials in a rack.

To specify sample location

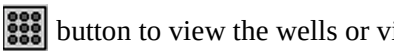
1. With a batch file open in the Batch Editor window, click the **Locations** tab.

2. Choose an autosampler from the **Autosampler** list.

   The appropriate number of racks appears in the graphic rack display.

3. Right-click on the rack where you want to specify the samples.

4. Choose a rack type from the list that appears.

5. Click the **Autosampler/Plate View**  button to view the wells or vials for the rack type.

6. Click the **Row/Column Selection**  button to specify whether samples are marked by row or column. If the button shows a red horizontal line, the Batch Editor marks the samples by row. If the button shows a red vertical line, the Batch Editor marks the samples by column. Click the button to change from row to column selection and vice versa.

7. Click the sample wells or vials you want to mark as occupied in the order that you want them to be analyzed. Click a selected well or vial to clear it.

   Click  to return to the Autosampler view.

NOTE: To select more than one well or vial at a time, hold down the SHIFT key and click the first and last well or vial. The Batch Editor marks every well or vial in between.

8. Repeat step 7 for each set in the batch.

Identifying Samples for Quantitation

Information in the **Quantitation** tab identifies the sample type and provides sample concentration information to the Analyst software. Quantitation information can be specified for a batch, a set, or even a single sample. You are not required to specify quantitation information before submitting your samples.

If you are using a batch template with a specified quantitation method, the quantitation information fields should already be filled in. Similarly, if you have imported batch information, the quantitation method file name will be present. The sample concentrations are not imported. In order to set the appropriate concentrations, you must click the Quantitation tab and edit the concentrations.

NOTE: If you change information in the Quantitation tab and then select a different quantitation method in the Sample tab, a message will warn you that you will lose the entered information.

The fields available in the Quantitation table depend on the internal standard and analyte columns in the selected quantitation method.

To specify quantitation information for a set

1. With a batch file open in the Batch Editor window, click the **Sample** tab.
2. Select the set you want to modify from the **Set** list.
3. Select a method from the **Quantitation** list or click **Quick Quant** to create and/or edit a quantitation method.

To specify quantitation information for a sample

1. With a batch file open in the Batch Editor window, click the **Quantitation** tab.
2. Select the **Set** containing the sample(s) you want to modify.
3. Select a **Quant Type** for the sample from the list in the cell.

4. Type the dilution factor for the sample.
5. Type the **Weight/Volume** for the sample.
6. If applicable, type the peak concentration in the **Analyte** column.
7. If applicable, type the **Internal Standard**.

NOTE: The appropriate Internal Standard and Standard columns appear in the Quantitation tab according to the current Quantitation method.

Submitting Samples

With the Batch Editor you can submit a single sample, a set of samples, or an entire batch to queue.

- A sample is a quantity of a substance that is introduced into the mass spectrometer for analysis. A sample may contain components and a solvent.
- A single sample or multiple samples make up a set. Samples in a set have the same quantitation method, but may have multiple acquisition methods. Group your samples into a set to reduce the amount of data you must manually enter.
- A batch consists of one or more sets. You can submit part of a batch in either a single sample or a set of samples.

To submit a sample or a set of samples

NOTE: Some fields are mandatory and must be completed before you submit the batch. The Submit button in the Submit tab is disabled until you complete all mandatory fields.

1. With a batch file open in the **Batch Editor** window, click the **Submit** tab.
2. Select the sample row(s) you want to submit.

   NOTE: To select multiple rows, press and hold the CTRL key or SHIFT key as you select the rows.
3. Click **Submit**.
4. In the **Acquisition** dialog box, click **Selected samples** and **OK** to confirm the selection.

   The Submit Status column and Submit Status field display the status of the set.

Submitting Samples with ExpressView

You can only create and submit an express batch from a station that is connected to a mass spectrometer. The amount of information required depends on the ExpressView configuration.

To create a batch with ExpressView

1. Double-click **Express View** under **Acquire** in the Navigation bar.
2. On the **Main** tab, select a template from the **Template** list.

   NOTE: If there are no templates in the Template list, contact your Analyst administrator.
3. Type or choose a **User Name**.
4. Type a **Sample Name**.
5. Type any batch notes or comments in the **Comment** field.
6. On the graphic representation of the rack, click the first sample well in the batch.

   Depending on the ExpressView configuration and the template selected, you will be required to specify one or all of the samples to be submitted. Express View marks the sample location based on the number of samples specified above and whether row or column selection is specified by the **Row/Column Selection**  button. If the button shows a red horizontal line, Express View marks the samples by row. If the button shows a red vertical line, ExpressView marks the samples by column. Click  to change from row to column selection or vice versa.
7. If custom parameters have been configured, click the **Custom Columns** tab and specify custom parameters as required.
8. On the **Main** tab, click **Submit**.
9. Place the samples in the rack and click **OK**.

Sorting a Batch or a Queue

The Queue Manager allows you to sort an individual batch or an entire queue in either ascending or descending order for viewing. The default order for processing is the time of submission in the Batch Editor.

To sort a Batch in a Queue

- Right-click on any column and choose **Sort** from the menu that appears.
- One click sorts the batch in descending order, while a second click sorts the batch in ascending order.

Saving Your Batch Files

You can use an existing batch file as you would use a template. You can modify a batch file, save it under a new file name, and submit it as a new batch. You can also save it as a template. Although you can save a batch to another project, it is recommended that you save the batch file in the project where it was created.

NOTE: To avoid overwriting an existing batch file, you should change the name and/or the destination of the Data File in the Sample tab of the Batch Editor window before you submit the batch.

To save a batch file

1. From the File menu, choose **Save As** or **Save**.
2. In the **File name** field, type the new file name.
3. Click **Normal Batch File** to create a standard batch file.

   —or—

   Click **Batch Template** to create a template file. (Minimal information including a set, samples, acquisition method, autosampler, and rack type are required to create a template.)
4. Click **OK**.

   By default, the file will save to the Batch folder of the current project.

9

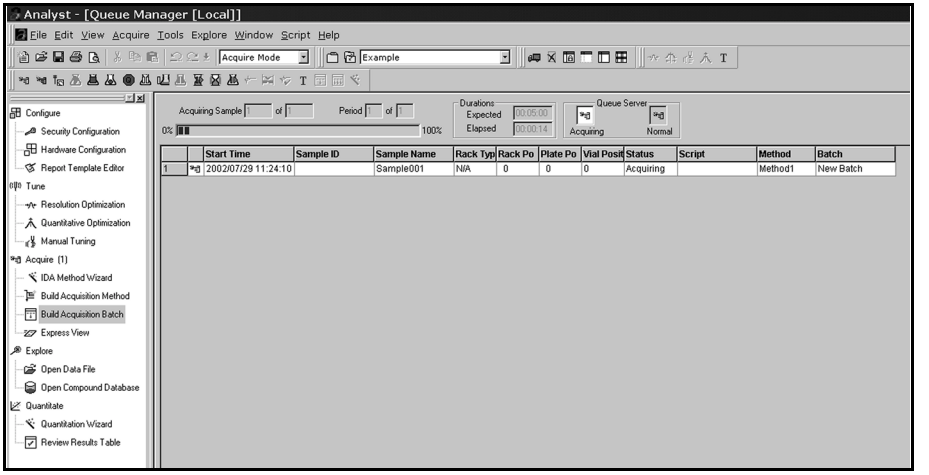
Running Your Samples

This section explains how data is acquired using the Queue Manager. It also provides an overview of how the instrument and devices are monitored.

Controlling and Monitoring the Acquisition Queue

The main function of the Queue Manager is to send instructions to instruments. You can access the Queue Manager in either Acquire or Tune mode.

The Queue Manager shows queue, batch, and sample status, and allows you to manage samples and batches in the queue. It also allows you to view the sample queues on any instrument stations to which you are directly connected and to view the sample queue on instruments to which you are connected through a network. You can also request detailed information about a particular sample in a queue.



Queue Manager window

Setting Queue Options

You must set the queue conditions in Configure mode before you begin creating a batch or sample.

To set queue options:

1. Click **Configure** in the Navigation bar.
2. From the **Tools** menu, choose **Settings**, then **Queue Options**.

   The Queue Options dialog box appears.
3. To change the default values for any of the queue options, click in the relevant area and type the new value.
4. Click **OK**.

Managing the Queue

The Queue Manager provides various functions to help you to manage and maintain the queue.

- You can select the operation mode of the instrument, start, or stop the queue.

- You can start, stop, or pause an acquisition, or you can continue a paused acquisition.
- You can also extend the acquisition time or skip a period within a sample scan.

You can also delete samples or batches. Which functions are available depends on your security level. See your System Administrator for details.

Selecting Operation Mode

The Queue Manager allows you to operate instruments from an instrument station. There are three instrument states: standby, equilibration, and ready. Equilibration functions as a type of warm-up and is done after an overnight quit or between samples in an acquisition queue. During equilibration, the instrument status displays Standby. When the instrument is ready for acquisition, the status changes to Ready.

In the Method Editor, there is also an autoequilibration feature that can be engaged when specifying a method. Autoequilibration is the automatic insertion of an equilibration between samples. If you choose Auto-Equilibration on the Method Properties tab in the Method Editor, the equilibration period will be longer than the duration specified if the methods are different and shorter if the methods are the same.

To modify an instrument state

1. Click **Acquire** in the Navigation bar.
2. From the **Acquire** menu, choose **Equilibrate**, **Standby**, or **Ready**, or click the appropriate icon on the toolbar.

   The equilibrate step can be skipped by choosing Ready directly from the menu.

Stopping the Queue

If you need to make adjustments to the queue, you can stop the queue before it has completed processing all the samples. All batches in the queue are stopped.

To stop the queue

1. Click the sample at the point where you want acquisition to stop.
2. From the **Acquire** menu, choose **Stop Queue**.

   The queue stops acquiring samples after the current sample has been acquired. The status of the sample where you stopped the queue on the Queue Manager (Local) window changes to Partial and all other samples following in the same batch are Suspended.
3. To continue the queue, choose **Start Sample** from the **Acquire** menu.

   The Queue Manager continues to process the next sample in the queue.

Starting a Sample Acquisition

When you submit a batch of samples to be acquired, the Batch Editor passes it to the Queue Manager. Processing starts in the Queue Manager.

To start a sample acquisition

1. Submit a batch in the Batch Editor.

   The Queue Manager (Local) dialog box appears.
2. From the **Acquire** menu, choose **Ready**.

   The Queue Server status icon on the Queue Manager (Local) window changes to Ready.
3. From the **Acquire** menu, choose **Start Sample**.

Stopping a Sample Acquisition

If a problem occurs, you can stop sample acquisition before a batch is completed. Stopping a sample acquisition allows the current sample to finish before stopping further processing of samples in the queue.

To stop a sample acquisition

1. Click the sample in the queue where you wish to stop acquisition.
2. From the **Acquire** menu, choose **Stop Sample**.

   The queue stops after the current scan in the sample you selected. The sample status on the Queue Manager (Local) window changes to Terminated, and all others following in the queue are Waiting.

3. When you are ready to continue processing the batch, choose **Start Sample** from the **Acquire** menu.

Aborting a Sample Acquisition

If a problem occurs, you can abort a sample acquisition in the middle of the processing of that sample. For example, if a sample is configured to run for five minutes and it is aborted two minutes into the run, it will stop at two minutes.

To abort a sample acquisition

1. Click the sample you wish to abort.
2. From the **Acquire** menu, choose **Abort Sample**.

   The Queue Manager stops acquiring the current sample immediately, tags it with the Partial status, and stops the queue. All samples following in the same batch are Waiting.
3. To continue sample acquisition, choose **Start Sample** from the **Acquire** menu.

   The Queue Manager processes the next sample in the queue.

Pausing a Sample Acquisition

There are two types of pauses in Queue Manager. You can insert a delay between samples in a queue, or you can put the currently acquiring sample in Pause mode. You would do this if you need to make adjustments, such as, to agitate the sample tray if it has been standing too long. Pausing the acquisition activates the **Continue**  icon.

To pause an acquiring sample

1. Click the sample currently being acquired.
2. From the **Acquire** menu, choose **Pause Sample Now**.

   The sample status changes to Paused in the Queue Manager (Local) window.

Continuing a Sample Acquisition After a Pause

You can restart processing of the sample after you have paused a sample acquisition.

To continue a sample acquisition after a pause

1. In the **Queue Manager (Local)** window, click anywhere in the paused sample.
2. From the **Acquire** menu, choose **Continue Sample**.

   The sample status shown in the Queue Manager (Local) window changes to Acquiring and the acquiring continues.

Skipping a Sample

You can skip a sample in the queue and move on to the next one if you wish, provided that the machine has begun acquiring it. This applies only to the current sample.

To skip a sample

- From the **Acquire** menu, choose **Next Sample**.

  When processing reaches this sample, it skips it and goes to the next sample in the queue.

Reacquiring a Sample

After a sample has been acquired, you may reacquire it at the end of the batch. You may need to do this when a problem is detected with a sample while it is being acquired. Both data files are kept, so it is similar to adding another sample to the batch.

To reacquire a sample

- Right-click the sample, and choose **Reacquire** from the menu that appears.

  The sample is added to the end of the batch. Its status changes to Acquiring.

Deleting a Sample or a Batch

The Queue Manager allows you to delete samples from a batch, or batches from the queue.

To select and delete a sample or batch

1. Right-click the sample you wish to delete. Hold down the SHIFT key while right-clicking to choose multiple samples for deletion.

   The Delete dialog box appears. The items in the first group are read only and come from the spot that you clicked in the Queue Manager window.

2. Click **Delete sample(s)**.
3. Click **OK**.

Viewing Detailed Information about a Sample

You can view detailed information about any sample at any time. Additional details about samples in the queue are available from your instrument station. You can also check the status of a queue on another instrument before connecting to it.

To view details of samples in the local queue:

1. Right-click the desired sample and choose **Sample Details** from the menu that appears.

   The Sample Details dialog box appears. Information in this dialog box is supplied by the Queue Server and can only be viewed not changed.

2. Click **OK** to close the dialog box.

To view details of samples in other queues:

1. From the **View** menu, choose **Sample Queue for Instrument**

   The Select Remote Instrument window appears.

2. Select an instrument from the list in the dialog box and click **Connect**.

   Detailed information about the chosen queue appears in the Queue Manager (Local) dialog box.

3. Click **OK** to close the dialog box.

More Options for Controlling the Acquisition

More options for managing a batch, queue, or sample acquisition are available. These include the following:

- Inserting a pause between samples or batches.
- Skipping a period within sample acquisition time.
- Extending a period within a scan.

For further details on each of these options, refer to the online Help.

Monitoring the Instrument and Device Status

Analyst allows you to view the detailed status of each instrument and peripheral device to which you are connected. You can view the detailed status of an instrument or device by double-clicking its icon in the task bar at the bottom right of the computer screen. Detailed status dialog boxes are device-dependent, so there is a different dialog box for each device from each manufacturer and model supported.

Instrument Status Overview

Status allows you to request information on and monitor the status and configuration of any instrument station(s) to which you are connected.

Monitoring Instruments

Analyst communicates with the mass spectrometer and with remote instruments.

To view the status of a remote instrument

1. From the **View** menu, click **Status for Remote Instrument**.

   The Connect to Remote Instrument dialog box displays a list of instruments.

2. Highlight the instrument of your choice and click **Connect**.

   —or—

   Click the **Computer** button and type the instrument name and then click **Connect**.

The Remote Instrument Status window displays the instrument to which you have just connected and icons representing each active peripheral device. Double-clicking any of these icons displays the detailed status dialog.

Icons representing the mass spectrometer and configured devices.

The possible status of devices and the corresponding background colors are as follows:

Status	Background color
equilibrate up	yellow
equilibrate down	yellow
fault	red
initializing	yellow
offline	gray
ready	green
shutdown	yellow
stand by	yellow

Viewing the Detailed Status of Instruments

You can view the current status of instrument stations on the network. You can also view the current status of any mass spectrometer and peripheral devices to which you are directly connected. For each device there is a Detailed Status dialog box. An icon representing each device in your configuration, including the mass spectrometer, is displayed on the Analyst task bar, as well as the status of the devices. Double-clicking an icon displays the detailed status for that device.

To view the detailed status of an instrument to which you are connected

- Double-click the icon representing the device you want to display.

  The Instrument Status dialog box appears. The device's status is indicated by both the background color of the icon, with one word, for example, ready, or idle, and in the **Method** field of the dialog box, for example "Ready", or "Idle".

To view the detailed status of an instrument on the network to which you are not connected

1. From the View menu, click **Status for Remote Instrument**.

   The Connect to Remote Instrument dialog box displays a list of instruments.

2. Highlight the instrument of your choice and click **Connect**.

   —or—

   Click the **Computer** button and type the instrument name and then click **Connect**.

3. Double-click a device icon to see the **Detailed Status** dialog box for that device.

10

Exploring Your Data

Analyst provides many tools that you can use to view and process your acquired data. This section explains how to use some of these tools.

Viewing Your Data

The Explore mode of Analyst is used for viewing and manipulating acquired data, whether it is obtained during or after acquisition or from an imported file. This data may be in one of two forms, data previously acquired to disk (imported) or real time data, which is the data stream directly from instrument firmware or peripheral devices, such as UV detectors (analog-to-digital converter data). Explore, along with the Report Generator component, is responsible for processing, printing, and exporting data.

Explore, together with Quantitation, is the heart of Analyst's data processing. You can also use Explore to view data as it is acquired, to verify the instrument's performance, and to provide initial data processing capability. It also provides the function of saving data to be included in reports and to provide information to an analyst.

You can view the data in tabular or graphical forms. Graphical data is presented as either a chromatogram or a spectrum. You can view from either of these table, and perform various sorting operations can be performed.

The commands and operations available in Explore are organized to maximize the ease with which you can view and manipulate the data. The user interface is designed so that the most frequent operations can be performed using the Explore Toolbar or by right-clicking in a pane and selecting a command from the shortcut menu.

Real Time Viewing

The Real-time Automatic "Show Last Scan" offset determines the time period during which a spectrum, generated from a real-time chromatogram during acquisition, will update as scans are acquired. If the difference between the last acquired scan and the point clicked in an updating chromatogram is less than the time entered here, the spectrum will update.

Labeling Peaks

To label peaks of interest, you can use captions. Captions are text that you place in a pane. When you place a caption beside a peak, the caption stays with the peak when you zoom in or out. Captions also stay with the sample as you move between samples.

You can also insert text into a pane to add additional information that may not be readily available or to simply label a graph. This text remains in the same location as the view is zoomed in and out, unlike the captions, which move as you zoom. Text also does not stay with samples as you move between samples.

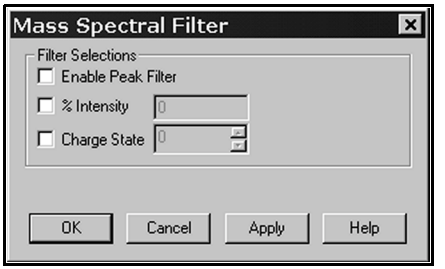
Mass Spectral Filter Dialog Box

In this dialog box, you can specify parameters to limit peak labeling. You may want to use this feature to reduce the number of labels on your screen.

To specify labeling parameters in the Mass Spectral Filter dialog box

1. Right-click in a spectrum and choose **Select Peaks for Label** from the popup menu.

   The Mass Spectral Filter dialog box appears.



2. Select the following fields as required:

   Enable Peak Filter: Turns the filter on or off. Select this check box to apply the filter to peak labels. Clear this check box to label peaks without filtering.

   % Intensity: Specifies the minimum height required for a peak to be labeled when the filter is enabled. Select the check box and type the percent height required for labeling, relative to the most intense peak in the spectrum. For example, if you want all peaks greater than half the height of the most intense peak to be labeled, enter a value of 50.

   Charge State: Specifies the charge state required for a peak to be labeled if the filter is enabled. Select the check box and enter the charge state required for labeling.

3. Once you have specified the labeling criteria, click **Apply** to filter the peak labels.
4. Click **OK** to close the dialog box.

Changing Labeling Precision in Graphs

Analyst allows you to change the peak label precision from zero to four decimal places in spectra and chromatograms.

To change peak labeling precision

1. On the **Tools** menu, point to **Settings**, and click **Appearance Options**.
2. Click the **Other Graph Options** tab.
3. Click the down-arrow beside the **Label Precision** list under the graph type you wish to change.

4. Choose the number of decimal places you wish to display (0 to 4), and click **OK**.

   Labels are displayed with the appropriate number of decimal places.

Adding text to a graph

In this dialog box, you can overlay text on a spectrum or chromatogram.

To add text

1. Right-click the text you want to edit, and then choose **Add User Text** from the menu that appears.

   The Add User Text dialog box appears with the text highlighted.

2. In the **User Text** field, type the text required.
3. Select the **Center Text** check box center the text; clear it to uncenter.
4. Click **Font** to display the Windows NT **Font** dialog box. Make appropriate selections to format your text as desired.
5. Click **OK** to close the dialog box.

Editing text in a pane or graph

The Edit User Text dialog box is used to change or format user text.

To edit text in a pane or graph

1. Right-click on the text you wish to change, and then click **Edit User Text** from the popup menu.

   The Edit User Text dialog box appears with the text highlighted.

2. In the **User Text** field, type the text required.
3. Select the **Center Text** check box center the text; clear it to uncenter.
4. Click **Font** to display the Windows NT **Font** dialog box. Make appropriate selections to format your text as desired.
5. Click **OK** to close the dialog box.

Changing Fonts in a Graph

The Appearance Options dialog box sets preferred fonts in chromatograms and in spectra. Analyst uses the standard Windows Font dialog box to change fonts for Title font, Axis font, Automatic Label font, and Default Caption font.

To change fonts in a graph

1. From the **Tools** menu, point to **Settings**, and then click **Appearance Options**.
2. Click the **Graph Colors and Fonts** tab.
3. Click the **Set** button beside the text you want to change. For example, to change the heading click **Title Font**.

   The Font dialog box is displayed.
4. Select a font from the **Font** list box, then select other font characteristics (i.e. font size and style) and click **OK**, then click **OK** again. (For more information on the **Font** dialog box, consult Microsoft Windows documentation.)

   The new font is displayed in all graphs.

Smoothing Data

Smoothing a data set removes local variations that are most likely due to noise. The smoothing operation involves replacing each data point with the average of the data point and a number of data points on either side. Data can be smoothed several times, but you can only undo the last operation.

NOTE: This operation is not available for MI/MRM spectra, but is available for TIC spectrum.

Smoothing Options Dialog Box

The Smoothing Options dialog box allows you to set the point weighting values for three data points before the smoothing operation is performed. This allows you to set the values for the current, or center, data point and the preceding and following data points. The algorithm used by Analyst multiplies the data points by the assigned weighting factors, sums these values and divides the total by the sum of the point weight values.

To perform data smoothing

1. Select a pane containing a chromatogram or spectrum.
2. On the **Explore** menu click **Smooth**.

   The Smoothing Options dialog box appears.
3. Assign values for **Previous**, **Current** and **Next** point weights.

   Once a data set is smoothed, it replaces the original data set on screen.

To undo smoothing

NOTE: Analyst currently supports one level of undo.

- From the **Edit** menu choose **Undo,** or click  on the toolbar.

Gaussian Smoothing

Smoothing a data set removes local variations that are most likely due to noise. Gaussian Smoothing involves replacing each data point with a weighted average of a number of data points on either side of it. The weighting for each new data point is determined on the basis of a Gaussian curve.

Processing Options Dialog Box: Smooth Tab

The Smooth tab of the Processing Options dialog box contains a group of Smoothing parameters and a group of Gaussian Smoothing parameters. The values you enter on this tab are used as defaults for Smoothing and for Gaussian Smoothing processes.

To access the Smooth tab

1. From the **Tools** menu, point to **Settings**, and then click **Processing Options**.
2. Click the **Smooth** tab.

Centroiding Data

Centroiding a spectrum converts peak distribution values into a single value that represents the peak, providing more accurate peak assignment and reducing the amount of data. This operation simplifies the data, making it easier to view or process further.

The algorithm used by Analyst converts peaks to single values by using an intensity-weighted average to calculate the center of gravity of the peak.

The output of the algorithm is a list of peaks that have three parameters:

- Centroid Value (units of mass or time), which is the value of the centroided data
- Intensity (cps), which is the intensity of each peak
- Width (amu), which represents the width of the centroided peak

NOTE: Creating a centroid changes the way data is displayed. To be able to compare the result with the original data, make a copy of the graph before centroiding.

Summing Overlays

You can sum several overlays of similar data types in order to make subsequent processing operations easier and faster. For example, you can overlay several Extracted Ion Chromatograms (XICs) and sum them, and then smooth the summed overlay to remove noise.

Summing overlays is similar to generating a Total Ion Chromatogram (TIC) with the benefit of being able to choose which graphs to overlay. Right clicking in the title bar displays a color-coded list of the overlaid graphs.

To overlay graphs

You can visually compare two sets of data by overlaying two similar data types.

1. Select the first pane that you want to overlay.
2. From the **Explore** menu, click **Overlay**.
3. Click in the pane that you wish to overlay.

   The display shows the two traces in contrasting colors.

To sum overlays

NOTE: By default, choosing three or less panes will result in each XIC being displayed in a separate pane. To overlay less than four XICs in the same pane, turn on the Overlay Multiple Panes option in the Multiple Graph Options tab of the Appearance Options dialog box.

1. Overlay the graphs you want to sum.
2. From the **Explore** menu, click **Sum Overlays**.

   —or—

   In a spectrum or chromatogram, select several peaks to overlay.
3. On the **Explore** menu, point to **Extract Ions**, and then click **Using Maximum**.

Viewing IDA Data

To make it easier to view IDA data in Analyst, you can set the IDA Explorer as your default viewer. The IDA Explorer tab in the Appearance Options dialog box allows you select the IDA Explorer to be used when displaying IDA data. You can also select the columns to be displayed in the Mass-List List view pane. By default, the Use IDA Explorer to display IDA samples is selected.

To set and configure IDA Explorer as your viewer

1. On the **Tools** menu, point to **Settings**, and click **Appearance Options**.
2. Click the **IDA Explore**r tab in the **Appearance Options** dialog box.
3. Select the **Use IDA Explorer to Display IDA Samples** check box.

4. Select the following check boxes options as required:

   Column Options

   Intensity: Select to display the intensity for a particular m/z.

   Molecular Weight (MW): Select to display the calculated molecular weight for a particular m/z.

   Scan: Select to display the scan type used for a particular m/z.

   Collision Energy (CE): Select to display the collision energy for a particular m/z.

   Charge (Z): Select to display the charge for a particular ion.

   LIT Column Options

   These options are only applicable to LIT Instruments.

   Excitation Energy (AF2): Select to display the excitation energy for the 2nd precursor ion.

   MS3 2nd Precursor (2nd Pre): Select to display the MS/MS/MS 2nd precursor ion.

To view IDA Data

1. On the **File** menu click **Open**.
2. Select the desired .wiff data file, and click **Open**. IDA files will automatically open in the IDA Viewer.

To view only the active graph

- Click the  button at the top of the viewer.

To return to multi-graph view

- Click the  button at the top of the viewer.

Saving Processed Data

You can save processed data, such as specific layouts and captions that can be re-opened in Explore mode only. These Explore status files also contain relevant history information and are similar to workspace files (.wiff extension) except they will only contain the data from the active pane in Explore. These files have the .pdt extension.

To save a processed data file

1. Select the pane of data you wish to save.
2. From the **File** menu click **Save Processed Data File**.
3. In the **Save Processed Data File** dialog box, type the file name.
4. Click **Save**.

To open a processed data file

1. On the **File** menu click **Open Processed Data File**.
2. In the **Load Processed Data File** dialog box, select the file to open.
3. Click **Open**.

Viewing Contour Plots

A Contour Plot graph is a color-coded plot of a complete data set where colour is used to represent a third dimension in the plot. In a Contour Plot of a Total Ion Chromatogram (TIC), the x-axis represents retention time or scan number, the y-axis represents mass, and the color represents the intensity of the data at that point. In a Contour Plot of a Total Wavelength Chromatogram (TWC) for a DAD, the y-axis represents wavelength, and the color represents absorbance. For both types of displays, and for immediate comparison, the x-axis spacing initially is the same as the graph pane. The user can define colors to provide a contrast that is most useful to them. Contour Plot is a post-acquisition tool that does not function in a real-time scan acquisition.

Contour Plot supports the following scan types:

- Q1 Scan
- Q3 Scan
- Product Ion

- Precursor Ion
- Neutral Loss
- TOF MS
- TOF Product
- Agilent DAD
- Enhanced MS
- Enhanced Multi-Charge
- Enhanced Product Ion
- Time Delayed Fragmentation
- MS/MS/MS (MS3)
- Enhanced Resolution

Displaying the Contour Plot Pane

You can display a Contour Plot from TIC, XIC, TWC, or XWC chromatogram graphs. TICs and XICs are available for all .wiff data files. TWCs and XWCs are available only for data acquired by a DAD.

To display a Contour Plot pane

1. In Explore mode, open a data file.
2. Drag the pointer over the area of the graph you want to examine. If no selection is made, the Contour Plot graph displays the entire range.
3. From the **Explore** menu, point to **Show**, and click **Show Contour Plot**.

   A Contour Plot graph of the selected area opens in a separate pane.

To close a Contour Plot pane

- Right-click in the Contour Plot pane, and then from the pop-up menu that appears, click **Delete Pane**.

Selecting Areas in a Contour Plot

There are three methods you can use to select an area within a Contour Plot.

To select a standard area within a box

- Click and drag the pointer to create a box around an area in the Contour Plot.

To make a vertical selection

- CTRL + click, and then drag the pointer in a vertical direction.

To make a horizontal selection

- Press the space bar + click, and then drag the pointer in a horizontal direction.

Displaying other Graphs from a Contour Plot

Contour Plot can display selected data as either a spectrum graph or an XIC. Additionally, for data acquired by a DAD, Contour Plot can display selected data as either a DAD spectrum or an XWC.

To show an XIC from Contour Plot

1. Using the pointer, select the data you want to display, then right-click.

   A pop-up menu appears.

2. Click **Show XIC**.

   An XIC graph of the selected data appears.

To show a spectrum from Contour Plot

1. Using the pointer, select the data you want to display, then right-click.

   A pop-up menu appears.

2. Click **Show Spectrum**.

   A spectrum graph of the selected data appears.

To show a DAD spectrum from Contour Plot

1. Using the pointer, select the data you want to display, then right-click.

   A pop-up menu appears.

2. Click **Show DAD Spectrum**.

   A DAD spectrum graph of the selected data appears.

To show an XWC from Contour Plot

1. Using the pointer, select the data you want to display, and then right-click.

   A pop-up menu opens.

2. Click **Extract Wavelengths (Use Range)** or **Extract Wavelengths (Use Maximum)**.

   A wavelength graph of the selected data appears.

Interpreting Your Fragments

The Fragment Interpretation tool generates a list of theoretical fragment masses from single, non-cyclic bond cleavage of a molecular structure. The user creates the molecular structure in a third-party drawing program and saves it as a .mol file. Fragment Interpretation displays the theoretical fragments in the fragment list and compares the fragment masses to peaks in the mass spectrum. Peaks above the threshold intensity and within the user defined mass tolerance (maximum 2 amu) of fragment masses are considered matched and display in **bold** text in the fragment list.

NOTE: Fragment Interpretation will not connect to a spectrum if the scan type is one of the following:

- Precursor Ion
- Neutral Loss
- Q1 Multiple Ion
- Q3 Multiple Ion
- Multiple Reaction Monitoring (MRM).

Displaying the Fragment Interpretation Pane

To automatically connect Fragment Interpretation to a spectrum, have a spectrum graph open before you open Fragment Interpretation. If you have multiple spectrum panes displayed, the Fragment Interpretation tool connects to the active spectrum. If the .wiff file contains more than one sample, the Fragment Interpretation tool connects to the active spectrum.

To display the Fragment Interpretation pane

- From the Explore menu, point to **Show**, and click **Show Fragment Interpretation Tool**.

Connecting Fragment Interpretation to a Spectrum

To get the most information from the Fragment Interpretation tool, connect Fragment Interpretation to a spectrum graph. If you have a spectrum open when you open Fragment Interpretation, it automatically connects to the spectrum. To connect an open fragment interpretation pane to a spectrum, use the following procedure.

To connect Fragment Interpretation to a spectrum

1. From the lower right corner of the Fragment Interpretation pane, click  .

   The pointer changes to the connecting tool.

2. Point to the spectrum graph you want to connect to the Fragment Interpretation tool and click the graph.

The Connected graph indicator in the lower left corner displays the name of the graph connected to the Fragment Interpretation pane. The connection is broken when either the graph or Fragment Interpretation is closed. If the connected .wiff file has more than one sample, the Fragment Interpretation pane automatically updates as you scroll through the samples.

Matching Fragments with Peaks

Fragment Interpretation automatically calculates the single, non-cyclic bond cleavage fragments from a .mol file. When the Fragment Interpretation tool is connected to a spectrum, theoretical fragments displayed in **bold** text indicate a matching peak in the spectrum within the specified mass tolerance and intensity threshold.

To display a fragment mass in a spectrum

- With an open .mol file in Fragment Interpretation, click a cell in the Fragment List that appears in **bold**.

  In the spectrum, the matching spectral peak highlights in the color selected under the Options tab. In the molecular structure, the bond highlights.

- If you click on a row that has more than one matching fragment, the spectral peak that is closest to its monoisotopic mass is highlighted in the mass spectrum in the color specified under the Options tab.

Selecting Bonds in a Molecular Structure

Selecting a single, non-cyclic bond in the molecular structure causes Fragment Interpretation to highlight the two fragments created when the bond is cleaved, and to display (in the color defined by the user) the matching peaks in the connected spectrum.

To select a bond in a molecular structure

1. From the Fragment Interpretation pane, open a .mol file.
2. Click a single, non-cyclic bond in the molecular structure.

   The two resulting fragments highlight in the fragment list. The masses of the two fragments appear above and below the bond (in the colors specified by the user in the Appearance Options dialog box).

   If a spectrum is connected, Fragment Interpretation displays any matching peaks in the graph.

   If you click a fragment in the list and the fragment is matched to a peak, the Fragment Interpretation window zooms in on that peak.

Sorting Data in Fragment List Columns

You can sort fragment list data by double-clicking on column headings. Data is sorted in ascending or descending alphabetical or numerical order.

To sort fragment list columns

- On the column heading, double-click.

  The column is sorted in either ascending or descending order (depending on the order before double-clicking the column heading). The data in the other columns organize in relation to the data in the sorted column.

Showing Isotopes

Fragment Interpretation can display the theoretical isotopic distribution for a peak matching a fragment in the fragment list.

To show isotopes

1. From the Fragment Interpretation pane, click the **Options** tab.
2. From the **Options** tab, select the **Show Isotopes** check box.
3. Click **Apply**.
4. From the fragment list, click a fragment that matches a peak.

   The isotopic distribution for matched peaks displays in the spectrum. The Show Isotopes feature can also be set from the Fragment Interpretation Tool Options tab in the Appearance Options dialog box.

Using Libraries

Library Search is a component of Analyst that enables you to manage library databases as well as individual spectra contained in the library.

Library Search finds and matches unknown spectra to known MS spectra. A library search compares unknown spectra to the stored spectra in the library resulting in a Hit List in the Search Results dialog box.

With Library Search you can:

- List and search library contents against an unknown spectrum
- Add records to the library
- Edit existing records

You can store library data in any of the following network locations:

- MS Access on a local database
- MS SQL Server

These network locations are established in the Optimization Options dialog box.

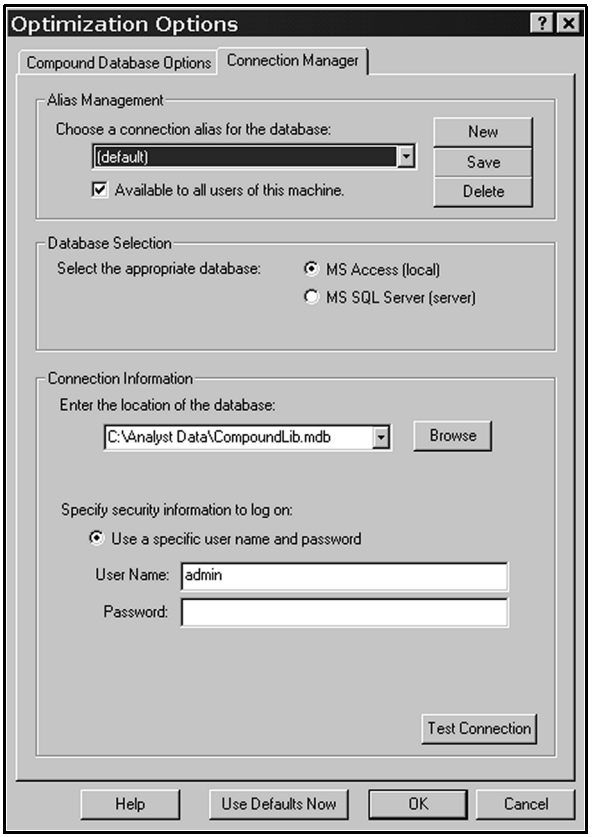
Connecting a Library Database Location

Before performing a Library search you must establish a network location where the library data is to be stored. A database server must be defined prior to making a connection.

To set optimization options

1. On the **Tools** menu, point to **Settings**, and then click **Optimization Options**.

   The Optimization Options dialog box appears.



2. Click the **Connection Manager** tab.
3. Select the database default alias or create a new database connection alias if more than one person has access to the computer.
4. To create a new database connection alias, click **New**.

   The New Connection dialog box appears.
5. Type a name for the connection alias. Click **OK**.
6. Select the **Available to all users of this machine** checkbox to allow other users to access the database.
7. Select the type of database to be used. Depending on the database selected complete the following steps:

For MS Access (local)

a) Click **Browse** and select the location of the database.

b) If desired, type your user name.

c) If desired, type your password.

For MS SQL Server (server)

a) Click **Refresh** to update the possible database servers connected to the computer.

b) Select the database server from the list of available database server

c) Select **Use Windows NT Integrated Security** to use Windows NT Integrated Security. The **User Name** and **Password** fields are unavailable.

—or—

Select **Use a Specific User Name and Password**

a) If desired, type your user name.

b) If desired, type your password.

c) Type the name of the database in the **Enter the name of the database on the server**.

8. Click **Test Connection** to verify that the connection to the database is successful. A connection success or failure message will appear.

9. Click **Cancel** to close the dialog box without saving your settings.

   —or—

   Click **OK** to save your settings.

Listing Library Contents

All records in the library can be listed in the Librarian table. The records can be listed with or without constraints (criteria). When you list the library contents without constraints, all records will be listed. When you list the library with constraints, only those records that match the constraints you specified will appear in the Librarian table. The more constraints you specify the more precise the list becomes and fewer, more relevant matches are displayed.

To list library contents without constraints

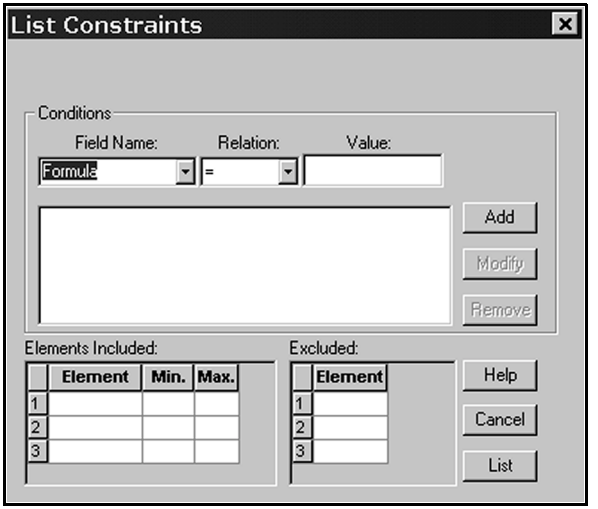
- On the **Explore** menu, point to **Library Search**, and then click **List**.

  The Librarian dialog box appears displaying all records in the library.

To list library contents with constraints

1. On the **Explore** menu, point to **Library Search**, and then click **List With Constraints**.

   The List Constraints dialog box appears.



2. From the **Field Name** list, choose a field as one of the constraints.
3. From the **Relation** list, choose the relation (operator) that applies to the Field Name.
4. In the **Value** field, type the value of the field name based on the relation.
5. Click **Add** to add the selected constraint to the Conditions list.
6. Continue adding constraints to the Conditions list as desired.
7. If desired, from the **Elements Included** table, select or type in an element to be included in the molecular formula.

   Elements are case sensitive, for example Hydrogen is H not h and Sodium is Na not NA or na.

8. Type a minimum and maximum number of the selected element to be included in the compound.
9. If desired, from the **Excluded** table, select or type in an element you want excluded from the molecular formula.
10. Click **Cancel** to close the dialog box without saving your settings.

    —or—

    Click **List**. The records matching all the constraints are displayed in the Records table. Listing constraints are saved.

Adding a Record

You may add records and compound related data including spectra to the library. You must have an active spectrum to perform this task.

To add a record to your library

1. On the **Explore** menu, point to **Library Search**, and then click **Add**

   —or—

   Right-click on an active spectrum, and then click **Add a Record**.

   The spectrum will be centroided automatically if you have not already done so. The Add a Record dialog box appears.
2. On the **Mass Spectral Information** tab, fill in the **Compound Name** field. The compound name is mandatory and must be unique.
3. Type or edit any other desired fields. Many of the fields are automatically filled in from the data associated with spectrum.
4. Click the **General Information** tab.
5. Type or edit any desired fields.
6. Click **Cancel** to close the dialog box without saving your settings.

   —or—

   Click **OK**. The settings are saved and the dialog box closes.

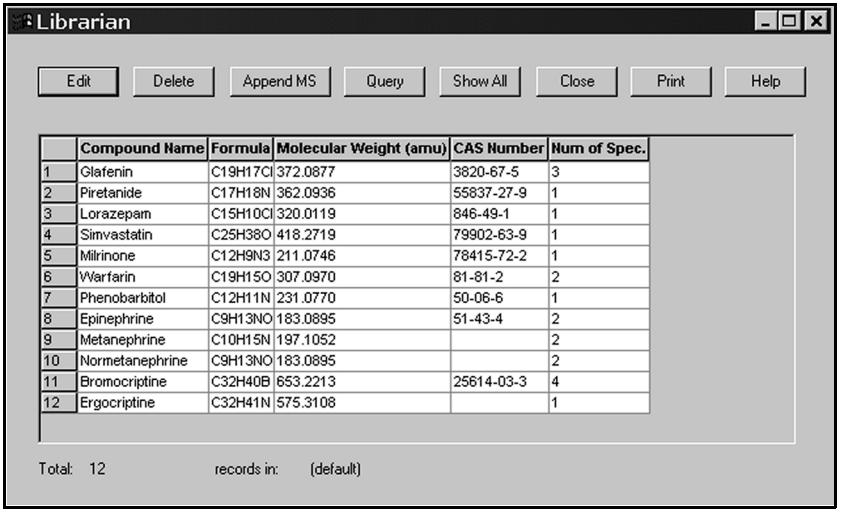
Deleting a Record

You can delete selected records from the library.

To delete a record

1. On the **Explore** menu, point to **Library Search**, and then click **List**.

   The Librarian dialog box appears.



	Compound Name	Formula	Molecular Weight (amu)	CAS Number	Num of Spec.
1	Glafenin	C19H17Cl	372.0877	3820-67-5	3
2	Piretanide	C17H18N	362.0936	55837-27-9	1
3	Lorazepam	C15H10Cl	320.0119	846-49-1	1
4	Simvastatin	C25H38O	418.2719	79902-63-9	1
5	Milrinone	C12H9N3	211.0746	78415-72-2	1
6	Warfarin	C19H15O	307.0970	81-81-2	2
7	Phenobarbitol	C12H11N	231.0770	50-06-6	1
8	Epinephrine	C9H13NO	183.0895	51-43-4	2
9	Metanephrine	C10H15N	197.1052		2
10	Normetanephrine	C9H13NO	183.0895		2
11	Bromocriptine	C32H40B	653.2213	25614-03-3	4
12	Ergocriptine	C32H41N	575.3108		1

2. In the **Librarian** table, select the record you wish to delete.
3. Click **Delete**. The following message displays "This record will be removed from the database, Are you sure?".
4. Click **No**. The record is not deleted.

   —or—

   Click **Yes** to delete the record.

Searching Library Contents

You can search the library for a spectrum (and its related compound information) that matches an active spectrum. Searches can be performed with or without constraints. If you search with constraints, only records matching all the criteria will be displayed. All peaks above the threshold set in Analyst will be used in the search the constraints you specified will appear. The more constraints you specify the more precise the list becomes and fewer, more relevant matches are displayed. Once constraints have been defined all subsequent searches will have the same constraints applied unless constraints are edited.

To search without constraints or with previously saved constraints

You must have an active spectrum to search the library.

- On the **Explore** menu, point to **Library Search**, and then click **Search**

  —or—

  Right-click on an active spectrum, and then click **Search Library**.

  The spectrum will be centroided automatically if you have not already done so.

  The Search Results dialog box appears.

To define constraints and search

When performing a search, you can add or remove peaks from the Peak List.

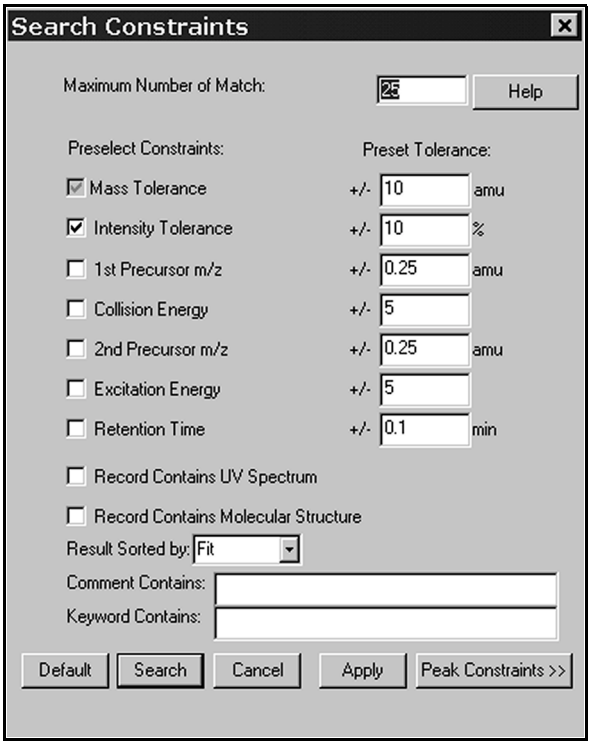
1. On the **Explore** menu, point to **Library Search**, and then click **Search With Constraints**

   —or—

   Right-click on an active spectrum, and then click **Search With Constraints**.

   The spectrum will be centroided automatically if you have not already done so.

2. The Search Constraints dialog box appears.



3. Select the desired constraints.
4. For each constraint selected, type the desired tolerance.
5. If desired, select a method of sorting records from the **Result sorted by** list.
6. If desired, type in text in the **Comment Contains** box.
7. If desired, type in text in the **Keyword Contains** box.
8. To apply peak constraints, click **Peak Constraints**.

   The **Peaks Included** table appears.
9. To add peak(s) to the list you want to search against, click **Add** and then type the m/z and the corresponding intensity in the empty cell.
10. To remove peak(s) from the **Peaks Included** table, select the peak(s) that you do not want to search against and click **Remove** to remove the peaks from the table.

11. Click **Apply** to save the constraints without searching.
12. Click **Cancel** to close the dialog box without saving the constraints or searching the library.

    —or—

    Click **Search** to save the constraints and begin the search.
13. Click **Default** to reset with the default constraints settings.

Viewing Library Records from Search Results

You can view compound information on individual records in the library.

To display a record

1. On the **Explore** menu, point to **Library Search**, and then click **List**.

   The Librarian dialog box appears displaying all the records from the library.
2. In the Librarian table, double-click the row number of the desired record. The View Record dialog box appears.

   You may view but not edit the record details.
3. Click **Close**.

   The dialog box closes.

11

Analyzing Quantitative Data

This section provides an overview of the various quantitative method creation tools offered by Analyst. It also explains how to create sample sets prior to quantitation, how to choose default queries and create table-specific queries, and how to manipulate the acquired data.

What is Quantitation in Analyst?

Quantitation is used to determine the concentration—the amount of analyte per volume—of a particular substance in a sample.

Researchers often know what is in the sample they are testing, but not *how much* of it. For example, if a pharmaceutical company needs to test a drug's absorption in a patient's bloodstream, they might analyze a blood sample taken 10 minutes before the patient took the drug, then 10 minutes afterward, then 30 minutes afterward and so on. They would know which substance they were looking for and its structural makeup. They would not know how much of it was present in a given sample.

That is where quantitation comes in. By analyzing a sample and comparing it to another sample or samples containing the same substance in a known concentration (standard), Analyst can help determine the concentration of the substance in the unknown sample. It does this by building a calibration curve using the standards, and then using it to interpolate the concentration for the samples of unknown concentration. The software then reports the concentrations of each sample in a Results table.

NOTE: In Analyst, the term quantitation is used to describe the post-acquisition phase also known as *quantitative data analysis*.

Choosing a Quantitation Method Creation Tool

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

Use the Standard option of the wizard to create a method when all of the following statements are true:

- You have already acquired all samples to be quantitated.
- You want to choose a representative sample, specify analyte and internal standard peaks, adjust peak-finding and integration parameters, review peaks during method creation, and specify calibration.

Use the Automatic option of the wizard to create a method when all of the following statements are true:

- You have already acquired all samples to be quantitated.
- You want to specify calibration.
- You do not want to adjust peak-finding and integration parameters.
- You do not want to specify analyte peak names.
- You do not have any internal standards.
- You do not need to review peaks during method creation or you have different compounds in every sample (you are calculating areas rather than quantitating).

Use the Full Method Editor to create a method when all of the following statements are true:

- You have already acquired a representative sample.
- You want to adjust peak-finding and integration parameters, specify analyte and internal standard peaks, and specify calibration.
- You need to complete any of the following tasks:
    - sum multiple ions for a single analyte or internal standard peak
    - use an internal standard from a different period or experiment from the analyte
    - edit an existing method.

Use the Semi-Automatic Method Editor (in the Batch Editor) to create a method when all of the following statements are true:

- You have not yet acquired any samples.
- You want to specify names and masses for analyte and internal standard peaks.
- You want to specify concentrations and sample types on the Quantitation tab in the Batch Editor (you require a quantitation method to complete these tasks), but don't have any other quantitation method.
- You may want to edit the method, if necessary, at a later time.

Choosing the Sample Set or Sets

A sample set is a group of samples whose data is to be processed together. A quantitation sample set can consist of one or more batches, which must be acquired prior to quantitative data processing.

Creating a Sample Set

Before you can begin quantitation, you must first create a sample set. After the Results table has been created, you can add samples to the set or remove samples from the set.

To create a sample set

NOTE: If you have many sample files to select, you can speed up the process by clicking Add All, which adds all of the samples to the Selected Samples list. Then remove any that you do not need by clicking their names and then clicking the left arrow.

1. Double-click the **Quantitation** folder on the Navigation bar.
2. Double-click **Quantitation Wizard**.

   The Create Quantitation Set: Select Samples page appears.
3. From the **Available Data Files** list, choose the data file containing the samples you want to use.

   The samples in the selected data file appear in the Available Samples list.

NOTE: If you do not see the data file or files you need, it does not exist within the current project. You must change the project to see data files residing in another project.

4. From the **Available Samples** list, select the sample you want to use.
5. Click the right arrow to add the sample you want to use to the **Selected Samples** list.
6. Repeat steps 4 and 5 for each sample you want to add to your sample set.

Adding a Sample to a Set

Once you have created a Results table with a sample set, you can add samples to the set. Any samples you add must be compatible with the quantitation method used to create the Results table.

To add a sample to the sample set

1. From the **Tools** menu, choose **Results table**, and then choose **Add/Remove Samples**.

   The Add/Remove Samples Dialog Box appears.

2. From the **Available Data Files** list, choose the data file containing the sample you want to add.

   NOTE: If you do not see the data file or files you need, it does not exist within the current project. You must change the project to see data files residing in another project.

3. From the **Available Samples** list, select the sample you want to add.
4. Click the right arrow to add the sample you want to use to the **Selected Samples** list.
5. Repeat steps 2 to 4 for each sample you want to add.
6. Click **OK**.

   Analyst automatically integrates the samples into the Results table and adjusts peaks and calibration curves accordingly.

Removing a Sample

Once you have created a Results table, you might want to remove a sample from the sample set. This feature is useful if a sample was either added by accident or does not work with the current sample set.

To remove a sample from the sample set

1. From the **Tools** menu, choose **Results table**, and then choose **Add/Remove Samples**.

   The Add/Remove Samples dialog box appears.

2. From the **Selected Samples** list, choose the sample you want to remove.
3. Click the left arrow to remove the sample from the **Selected Sample** list.
4. Repeat steps 2 and 3 for each sample you want to remove.
5. Click **OK**.

   Analyst removes the samples from the Results table and adjusts peaks and calibration curves accordingly.

Choosing the Setting and Default Query

You 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 check that runs against Results table columns to determine which records meet certain criteria. You can define a default or table-specific query.

What is a Query and Why is it Used

A query is a method of selecting only those records that meet certain criteria. You create a query using textual or mathematical selection criteria. Any matches to the criteria appear in the Results table.

If you create a query as a Standard Query using the Query wizard, the Pass or Fail status of the query will be displayed in the Standard Status Query column of the Results table. If the query is applied from the Results table or is executed as a "Non-Standard Query" (in the Query wizard), the Results table shows only the rows of data that meet the specified criteria.

When the query is applied from the Results table, you can further refine your selection by running a second query on the rows displayed by the first query.

If the query is so selective that no record meets the criteria, the table displays no data.

Creating a Default Query

A default query is usually used to identify samples that do not meet certain criteria, and it is only available before a Results table is created. You can choose a default query from the Create Quantitation Set: Select Settings & Query page. If you want to run a query after a table has been created, you need to create a table-specific query.

To create a default query

1. In the **Default Query** area on the second page of the Quantitation Wizard choose **Create New Standard Query** and type a name to save the query.
2. Click **Next**.

   The Create Default Query page appears.
3. In the left (quality control) **QCs** box under **Max. Variation**, type the maximum allowable percent of variation for each QC (for example, 5 is ±5%) in the same row as the corresponding concentration. (If the concentrations were not specified during acquisition, they do not appear here. In that case, you will have to type them in the **Concentration** column.)
4. In the right (**Standards**) box under **Max. Variation** type the maximum allowable percent of variation for each standard (for example, 10 is ±10%) in the same row as the corresponding concentration. (If the concentrations were not specified during acquisition, they do not appear here. In that case, you will have to type them in the **Concentration** column.)
5. Click **Next**.
6. Select or create a method in the **Select Method** dialog box.
7. Click **Finish**.

The query is applied as a standard query. Therefore the query results are displayed as a Pass or Fail entry in the Standard Query Status column of the Results table.

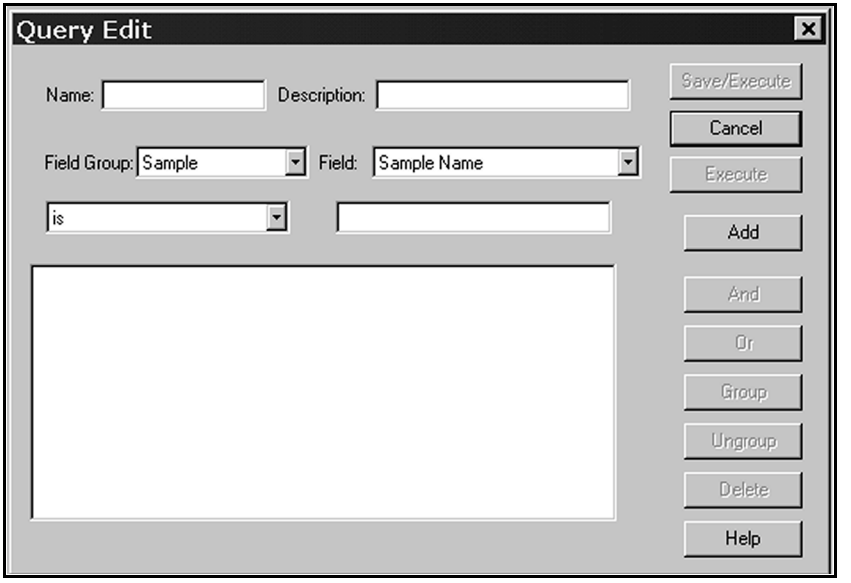
Creating Table-Specific Queries

Table-specific queries, as part of the current table settings, are available only to the current Results table and are generally used to identify records that meet certain criteria. If you want to create a query that runs before a table is created, you need to create a default query.

To create a table-specific query

1. Right-click anywhere in the Results table.
2. Point to **Query** and select **New** from the menu that appears.

   The Query Edit dialog box appears.



3. Type a name for the query in the **Name** box so that you can save it.
4. If desired, type a description in the **Description** box.
5. Choose a Field Group such as **Analyte** from the **Field Group** box.
6. Choose a Field such as **Analyte Peak Area** from the **Field** box.
7. Choose a criterion function in the next box, such as **is greater than** or **equal to**.
8. Type the criterion operand in the next (small) box, such as **2.00e2**.

9. Click **Add**.

   The query is displayed in the large box.

10. If desired, to add a second condition to the query, repeat steps 5 through 9.

    Analyst adds the second condition to the query, appending the Boolean AND operator between the two conditions. To change the operator from AND to OR, click **AND**, then click **OR**.

11. Click **Save**/**Execute** to save the settings and run the query.

    The results of the query appear and the system saves the current settings.

12. To display the entire table again, right-click anywhere in the Results table and choose **Show All** from the **Query** menu. Analyst allows you to apply the query again or edit the query, if you wish.

Creating a Quantitation Method

There are four different ways to create a new quantitation method. Each one involves the same four basic steps. Creating a semi-automatic method is the only exception; it only has one step–step 2 below.

To create a new method

NOTE: You can change the default values that Analyst uses during method creation on the Quantitation Method Options Dialog Box.

1. Choose a representative sample.
2. Describe the compound or compounds of interest, along with applicable internal standards.
3. Verify that the peaks found by the software represent the analyte of interest and correct where necessary. This step is not necessary when you use the Automatic Method Wizard.
4. Specify the calibration to be used for the regression. The calibration type and fit are stored in the method file.

Using the Standard Wizard

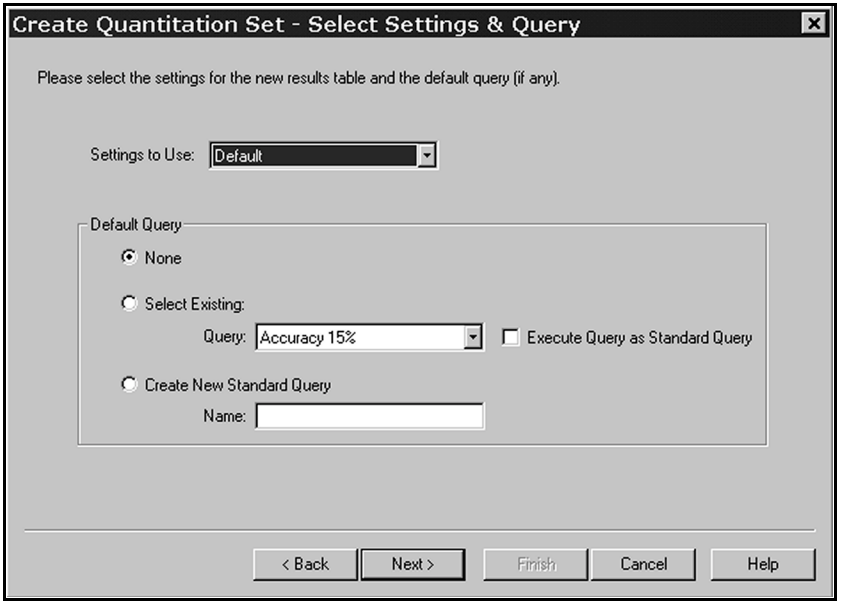
The Standard wizard helps you to select a batch, create a method, do a peak confirmation, and integrate the sample set. You can also analyze ADC and DAD data. Use this wizard for all quantitation tasks that involve finding concentrations.

To use the Standard wizard

1. Double-click the **Quantitate** folder to open it. Alternately, you can choose **Open** from the **File** menu and then choose **Quantitation Results Files**.
2. Double-click **Quantitation Wizard**.

   The Create Quantitation Set: Select Samples page appears.
3. Create a sample set and click **Next**.

   The Create Quantitation Set: Select Settings & Query page appears.



4. From the **Settings to Use** list, choose the settings file you wish to use. Analyst uses the last used setting if you do not specify one.
5. In the **Default Query** group box, specify the type of query you want to use by selecting the appropriate option.

- If you do not want to apply a query, select **None**.

—or—

- If you want to use an existing query, select **Select Existing** and choose a query from the box.

—or—

- If you want to create a new query, select **Create New Standard Query** and type a name in the box. If you select this option, the query creation process begins. You must complete the query creation process before going on to step 6.

6. Click **Next**.

   The Create Quantitation Set: Select Method page appears.

7. Specify the method you want to use.

   - If you want to use an existing method, select **Choose Existing Method** and select a method from the **Method** list.

   Analyst applies the method and creates the Results table. The wizard ends here.

   —or—

   - If you want to create a new automatic method, select **Create Automatic Method** and proceed to "Using the Automatic Wizard" on page 144.

   —or—

   - If you want to create a new method, select **Create New Method** and type a name for it in the **Method Name** box.

   The Create Quantitation Method: Select Representative Sample page appears.

8. Click the name of the sample you want to use as a representative sample and click **Next**.

   The Create Quantitation Method: Define Peaks page appears. If you selected an ADC or DAD file, the Create Quantitation Set: Select Integration Data page appears.

9. Type the name of the analyte or analytes you want to quantify in the **Name** column of the **Analytes** box, one analyte per row. If analyte names

were specified during acquisition, you can select the appropriate name from the list.

The names of the analytes may be stored within the sample file, so you must use the exact same spelling as was used during acquisition.

10. Choose the analyte's mass from the **Q1/Q3** column (or the **Q1** or **Start-Stop** column, depending upon how the data was acquired). Specify one analyte per row.
11. Type the name of the internal standard or standards being used in the first row of the **Name** column of the **Internal Standards** box. Specify one standard per row. If internal standard names were specified during acquisition, you can select the appropriate name from the list.

    The names of the internal standards may be stored within the sample file, so you must use the exact same spelling as was used during acquisition.
12. Choose the standard's mass from the **Q1/Q3** column (or the **Q1** or **Start-Stop** column, depending upon how the data was acquired). Specify one standard per row.
13. In the **Analyte** box, choose the internal standard to be associated with each analyte from the **Internal Standard** column from the list.
14. If you want to specify the number of smooths to be used for all peaks, click the **Default Number of Smooths** box and choose the number of smooths. (You can also specify the number of smooths for individual peaks during peak review.)
15. Click **Next**.

    If an ADC or DAD file was selected, additional Define Peak pages appear. (If you prefer to accept all of the default integration parameters, click **Finish**. Analyst produces the Results table; you do not need to complete the remainder of the steps.)

    The Create Quantitation Method: Define Integration page appears.
16. Confirm the peak of the internal standard or standards (if one exists) and of the analyte or analytes. Click **Next** to move to the next analyte or internal standard. When all peaks have been confirmed, click **Next** to move to the next step.

    The Create Quantitation Method: Specify Calibration page appears.
17. Choose the calibration options.

18. Click **Finish**.

    Analyst completes quantitation and produces the Results table. If you want to delete the Results table window, right-click and choose **Delete Pane** from the menu that appears.

Using the Automatic Wizard

The Automatic wizard helps you to select a batch, create a method (without peak confirmation) and integrate the sample data. Generally, use this wizard when you are only integrating, not finding concentrations. It 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 specify an internal standard—all ions are treated as analytes.

To use the Automatic wizard

1. Complete steps 1 through 6 of the Standard wizard.

   The Create Quantitation Set: Select Method page appears.

2. Click **Create Automatic Method**.

   The Create Quantitation Set: Automatic Method page appears.

3. Adjust the void volume retention time, bunching factor, and number of smooths as necessary, then click **Next**.

   If you prefer to accept all of the default integration parameters, click **Finish**. Analyst produces the Results table; you do not need to complete the remainder of the steps.

   The Create Quantitation Method: Specify Calibration page appears.

4. Choose the calibration options.

5. Click **Finish**.

   Analyst completes quantitation and produces the Results table. If you want to delete the Results table window, right-click and choose **Delete Pane** from the context sensitive menu.

Using the Full Method Editor

The Full Method Editor, like the wizards, helps you create a new quantitation method. Unlike the wizards, it allows you to do three additional things:

- sum ions for integration
- use an internal standard from a different period or experiment, if it was acquired in a different period or experiment than the analyte.
- edit an existing method.

To create a new method using the Full Method Editor

1. From the **File** menu, choose **New**.
2. Choose **Quantitation Method Files (.qmf)**.

   Analyst starts the Full Method Editor.
3. In the **Select Sample** dialog box, click the name of the file containing the representative sample in the **Data Files** box.
4. In the **Select Sample** dialog box, click the name of the representative sample to be used in the **Samples** box. Choose one that is a standard.
5. Describe the method components (compounds and internal standards of interest).
6. Define the method integration.
7. Set the method calibration.
8. Choose **Save** from the **File** menu.

   The Save dialog box appears.
9. Type the name of the new method in the **File Name** box. Analyst does not permit you to save quantitation methods outside the **Quantitation Methods** folder.

   Analyst saves the new method.

Using the Semi-Automatic Method Editor

Semi-automatic methods allow you to specify concentrations and sample types on the Quantitation tab in the Batch Editor before any samples are acquired. Standard methods require that you have acquired at least one typical sample. Because you have not yet acquired a sample, you cannot choose a representative sample or review any peaks—you are simply defining the method components.

To create a semi-automatic method

1. On the **Sample** tab of the **Batch Editor** window, click **Quick Quant**.

   The Create Semi-Automatic Method dialog box appears.

2. Type the name of the analyte or analytes you want to quantify in the **Name** column of the **Analytes** box. Specify one analyte per row.
3. Choose the analyte mass from the **Q1/Q3** column (or the **Q1** or **Start-Stop** column, depending upon how the data will be acquired). Specify one analyte per row.
4. Type the name of the internal standard or standards being used in the first row of the **Name** column of the **Internal Standards** box. Specify one standard per row.
5. Choose the internal standard mass from the **Q1/Q3** column (or the **Q1** or **Start-Stop** column, depending upon how the data will be acquired). Specify one standard per row.
6. In the **Analyte** box, choose the internal standard to be associated with each analyte from the list in the **Internal Standard** column.
7. If you want to specify a particular number of smooths, choose a number from the **Num. Smooths** box.
8. Click **OK**.

   The Save Quantitation Method dialog box appears.

9. Type a name for the new method and click **OK**.

Analyst creates the method using the default calibration (linear). It then returns you to the Sample tab. You can now define concentrations and sample types by clicking the Quantitation tab.

Customizing a Results Table

The Results table contains the report of each analyte sample in spreadsheet format. It appears when you have completed the Automatic or Standard wizard. The system lets you customize the table and display predefined layouts.

Analyst allows you to export the data from a Results table to a plain text file for use in other applications, such as Microsoft Excel. You can choose to export all possible data in the table or only data that is visible to the user. Along with the data from the table, Analyst also exports the parameters that describe the regression.

The Results table fields are grouped as follows:

- sample fields
- analyte results fields
- internal standard results fields
- record fields
- formula field
- custom fields

You can customize the current results table by adjusting the column width, moving columns, or locking columns.

To adjust the width of a column

- Move your mouse over the column's right division line (the line dividing it from the next column) until the cursor changes to a double-headed arrow. Click and drag the line to the width you want.

To move a column

1. Double-click anywhere in the desired column. A square handle appears at the bottom of the cursor.
2. Click and then drag the column to the new position. Locked columns cannot be moved; unlocked columns cannot be moved to the position before a locked column.

To lock a column

1. Select the column you want to lock by clicking the cell heading of the desired column.
2. Click  on the Results Table toolbar. (If this button is grayed out, you cannot lock the selected column.)

The column is locked and will not move when the table is scrolled. The lock button changes to a closed padlock (). You can unlock the column by clicking the lock button again.

You can also customize the table by modifying table-specific settings as follows:

- adjusting the columns displayed
- sorting and saving the results or just sorting the results
- querying the results
- plotting the results
- adjusting the audit trail criteria
- customizing the peak review window

If you want to change the settings that affect future results tables, you need to modify the global settings. You can also export the settings for the current table as global settings. When you set global preferences, you modify either the default settings or a group of settings that you create when you export table-specific settings. Default settings are unique because the system uses them if the user does not specify a group of settings and because they can never be removed from Analyst. For more information about global settings, refer to the online Help.

Defining the Table Layout

Analyst provides four different pre-defined views or layouts of the Results table:

- Full Layout
- Summary Layout
- Analyte Layout
- Analyte Group Layout

You would normally view the default Full Layout or the Summary Layout. If you have multiple analytes per sample you can see each analyte in the Analyte Layout.

To view a specific layout

1. Right-click anywhere in the Results table.
2. Do one of the following:
   - Choose **Full** from the drop-down list to view the Full Layout.

   —or—

   - Choose **Summary**, then choose a field name from the menu to view the Summary Layout.

   —or—

   - Choose **Analyte**, then choose a single analyte from the menu to view the Analyte Layout.

   —or—

   - Choose **Analyte Group**, then choose an analyte group from the menu to view the Analyte Group Layout.

Sorting the Results

You sort the data in the Results table in three different ways:

- Use the Sort button to sort the current table without saving the sort criteria.
- Create a table-specific sort to save the sort criteria with the current table.
- Create a global sort to save the sort criteria for use with future Results tables.

Sorting with the Sort Button

You can use the sort button to sort the current results table. This type of sort does not allow you to save the sort criteria.

To sort the Results table you are viewing without saving the sort criteria

1. Choose up to three columns in the Results table in the order you want Analyst to sort them. To select multiple columns, hold the Ctrl key while you click each column.
2. You can use the Sort button to sort in ascending or descending order.
   - To sort in ascending order click  on the Results table toolbar.
   - To sort in descending order click  on the Results table toolbar.

Creating Table-Specific Sorts

Table-specific sorts allow you to sort the current table and save the criteria for use again with the current table.

To sort the Results table you are viewing and save the criteria

1. Right-click anywhere in the Results table.
2. Choose **New** from the **Sort** menu.

   The Sort dialog box appears.
3. Type a name in the **Name** box for the sort.
4. Choose up to three sort criteria. For example,

   Sort By:

   Group: **Analyte**

   Column: **Analyte Peak Area**

   Then By:

   Group: **Analyte**

   Column: **Analyte Retention Time**

5. Click **Ascending** or **Descending** for each sort criterion.
6. Click **Save/Execute** to perform the sort and save the criteria.

The table is sorted based on the order in which you chose the columns. The set of criteria are available under the name you assigned to them for future sorts of the table or criteria list in the Sort menu. Analyst also allows you to edit an existing sort.

In the above example, the data is sorted in order of the analyte retention time within the order of the analyte peak area. If two analytes have the same peak area, the one with the highest retention time is shown first.

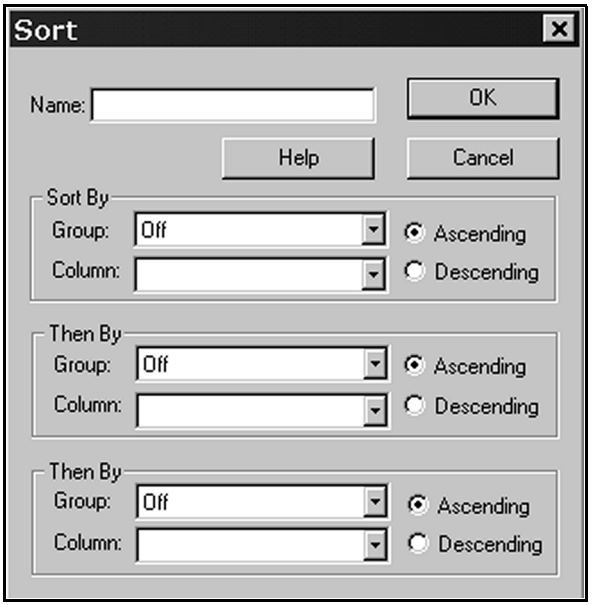
Creating Global Sorts

You can create a default sort to apply to future results tables when you choose that group of settings from the Quantitation wizard's Create Quantitation Set: Select Settings & Query page.

To create default sort criteria for future Results tables

1. From the **Tools** menu, point to **Settings**, and then choose **New Quantitation Results table Settings**.
2. Open the folder you want to edit (the Default folder or one containing settings that you have exported) in the Table Settings folder.
3. Choose the **Sorts** folder, and then click **New**.

   The Sort dialog box appears.



4. In the **Name** field, type the name for the new setting.
5. Choose up to three sort criteria. For example, two are shown here:

   Sort By:

   Group: **Analyte**

   Column: **Analyte Peak Area**

   Then By:

   Group: **Analyte**

   Column: **Analyte Retention Time**
6. Click **Ascending** or **Descending** for each column.
7. Click **OK** to save the criteria.
8. Click **Done** to close the Table Settings dialog box.

In this example, the data is sorted in order of the analyte retention time within the order of the analyte peak area. If two analytes have the same peak area, the one with the highest retention time is shown first.

Saving the Results Table

If you save a Results table, you can recall it later.

To save the Results table you are viewing

1. Click **Save** or **Save As** in the **File** menu.
2. Type a name in the **File Name** box and click **OK**. Analyst does not permit you to save a results table outside of the **Results table** folder.

The system adds .RDB to the file name and saves it in the Results folder.

Reviewing the Calibration Curve

The calibration curve is used to determine the calculated concentration of samples including QC samples. It is a curve that results from plotting the standard's concentration 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's 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.

You can choose to plot the calibration by either area or height. If there is an internal standard present in the samples, the curve plots the concentration ratio against the area or height ratio. You can choose to calibrate using analyte height or area (or ratio, if an internal standard is used).

Analyst 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 the weighting type that you choose. This curve is used, along with the area (or height) for the unknowns to interpolate the concentration.

Metric plots allow you to plot a given column of information, such as Analyte Peak Area, Accuracy, and Calculated Concentration, from the Results table. You can also plot two Results table fields against each other. You can then click a point to investigate points that appear outside the normal range.

Analyst allows you to create metric plots in three different ways:

- Use the plot button to plot a column or columns of the current Results table, but not save the plotting criteria.
- Create a table-specific plot to save the plot criteria with the current table.

- Create a global plot to save the plotting criteria for use with future Results tables.

Plotting the Calibration Curve

Plot a calibration curve to see the curve used for regression. When two or more curves are open, you can overlay them.

NOTE: This option is available only when a Results table appears in the workspace.

To plot a calibration curve

1. From the **Tools** menu, choose **Calibration** and then:

   Choose **Window** from the **Calibration** menu if you want the Calibration Control dialog box displayed in a separate window.

   —or—

   Choose **Pane** from the **Calibration** menu if you want the Calibration Control dialog box to appear in the same window as the Results table. If you want to delete the calibration pane later, you can right-click it and choose **Delete Pane** from the context sensitive menu.

   The Quantitation Calibration Control pane appears.

2. Choose an Analyte from the **Analyte** drop-down list.
3. Choose **Area** or **Height** from the next drop-down list.
4. Click **Regression**.
5. On the **Regression Options** dialog box choose the **Fit** that works best and the **Weighting** that works best for your project.
6. Click **OK**.

   The regression curve appears. The Calculated Concentration field reflects any changes resulting from the fit of the curve to the standard's points. You can review individual peaks on the curve. You can also exclude points from the curve to produce a better curve.

7. If necessary, click **Regression** and change the **Fit** or **Weighting** or both to create a more appropriate curve. You can also change from **Area** to **Height** or vice-versa.

8. Click **Accept** to save your changes, or **Revert** to cancel the changes. If audit trail logging is enabled, specify a reason for the change only after you accept the changes.

Overlaying Calibration Curves

When you have two or more Results tables open, Analyst allows you to overlay their calibration curves (plot them on the same graph). To overlay curves, the method used to create the tables being used must be the same.

To overlay calibration curves

NOTE: If you want to examine the curve for one table more closely, right-click anywhere in the curve, choose Active Plot, and choose the curve to be plotted on top.

1. Create or open two or more Results tables.
2. Plot a calibration curve for one of the tables.
3. Right-click and choose **Overlay** from the menu.

   Analyst produces a list of open Results tables.
4. Select the tables whose data are to be overlaid with that on the current curve.
5. Click **OK**.

   Analyst plots the curves for all selected tables on the same graph.

Reviewing Sample Statistics

Use the Statistics window to view the statistics for standards and quality control samples (QCs). The data from each available batch in the Results table is displayed in tabular form in the grid. There is a row of data for each standard or QC at a given concentration.

Reviewing the Accuracy of Quantitative Analysis

To ensure accuracy, Analyst allows you to build in the following controls:

- an internal standard, which behaves similarly to the analyte in question

- quality control samples (QCs), which you include as part of the set to measure the accuracy of the unknown samples. QCs are not part of the regression
- the option of triple quadrupole analysis (provided you have the appropriate hardware), which provides more specific results than single quadrupole analysis
- a batch-to-batch comparison, which allows you to check accuracy

The accuracy of quantitative analysis is determined by the accuracy of the standards and the QCs at each concentration level. View the grid in the Statistics pane to check for variations in the standards and QCs.

To view the statistics for standards and QCs

1. Display a Results table.
2. From the **Tools** menu, choose **Statistics**.

   The Statistics window appears.
3. Choose **Concentration** from the **Statistics Metric** box.
4. Choose **Standard** from the upper right hand box.
5. Choose an analyte from the **Analyte** box.

   The results display. You can examine an individual peak from here.
6. Examine the **Low**, **High**, and **Mean** for each row in the grid. Each row represents standards that have the same concentration levels.
7. Choose another analyte.
8. Choose **QC** in the upper right-hand box to check for Quality Control variations at the same concentration levels.

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.

Reviewing Integrated Peaks

Once you have obtained a Results table you can use the Peak Review window or the Peak Review pane to review any of the peaks for each analyte and internal standard in a batch. Both the Peak Review window and the Peak Review pane display the same information. However, the Peak Review window displays the peaks in a separate window, while the Peak Review pane displays the peaks in the same window as the Results table.

Why Should I Review Peaks?

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

Peak review allows you to survey the peaks that Analyst has selected, and redefine the peak or the start and end points where necessary.

Reviewing Individual Peaks Manually

Once you have obtained a Results table, you can review individual peaks on a one-by-one basis. Alternatively, you can choose to review all peaks for that batch.

To review an individual peak

1. Click **Display the Data Set**, and then double-click a data point in the **Data Point** column of the Statistics Table.

   —or—

   Right-click a point in a calibration or metric plot, and then click **Show Peak** from the submenu.

   Analyst displays only the Peak Review window with the peak you chose. Regardless of changes made to the Results table, this Peak Review window will continue to show the same peak. If you make a change to the Results table and want to see the change in the peak, you must close the Peak Review window and then open a new one using one of the methods described in step 1.

2. Examine the peak.
3. Redefine the peak as necessary. If you then find that the peak was correct as originally selected, revert to the default method.

Reviewing all Peaks Manually

Once you have obtained a Results table, you can manually review all of the peaks for each analyte and internal standard in that batch. Alternatively, you can choose to review only certain peaks in the batch.

To review all peaks manually

1. From the **Tools** menu, choose **Peak Review**
2. Choose **Window** from the **Peak Review** menu if you want the peaks displayed in a separate window.

   —or—

   Choose **Pane** from the **Peak Review** menu if you want the peaks to appear in the same window as the Results table.

   Analyst displays the peaks.

   The Peak Review window contains exactly the same peaks as are visible in the Results table; if some samples are hidden in the table, if you applied a query, for example, then they are also hidden in peak review. In order to review all peaks, you should ensure that all samples are listed on the Results table.
3. Examine the peaks that Analyst found.
4. Redefine peaks as necessary. If you find that a peak was correct as originally selected, revert to the default method.
5. Click the right arrow or left arrow to move through the peaks. To move to the first peak in the batch, click  on the Quantitation menu, or right-click anywhere in the peak-review window and choose **Show First Page** from the menu. To move to the last peak in the batch, click  on the Quantitation menu, or right-click anywhere in the peak review window and choose **Show Last Page**.

Defining a Peak Manually

To force the software to find a peak, you can define the exact start and end points manually. These changes apply only to that individual peak unless you then update the global method.

NOTE: This tool is only available during peak review, not during the peak confirmation portion of method creation.

To define a peak

1. In the **Peak Review** window, click the Manual Integration Mode  icon.
2. Position the crosshair at the start point of the peak.
3. Click and hold the left mouse button.
4. Drag the crosshair to draw a line to the end point of the peak.
5. Release the mouse button.

Analyst shades the area bounded by the base of the peak and the sides of the peak. Peak parameters are grayed because they are no longer applicable, since the peak was drawn manually instead of defined by the parameters.

6. To make this change permanent, click **Accept**.

   The audit trail dialog box appears if audit trail logging is enabled.

Examining an Individual Peak

During peak review, you may want to view a peak in its entirety—or you may want to examine the baseline to find out how well Analyst found the start and end points of the peak. Use the automatic zooming feature to help you do either.

To examine an individual peak

1. Right-click in the **Peak Review** window and choose **Options**.

   The Peak Review Default Options dialog box appears.

2. In the Peak Review Default Options dialog box, select the **Automatic Zooming** options to tailor the display of the peaks for both the Intensity and the Time axes.
3. Click **OK**.

Analyzing Data in a Results Table

The Results table contains the report of each analyte sample in spreadsheet format. You can add columns to the standard Analyst Results table to display Diode Array Detector (DAD) data for the Analyte, Internal Standard, and Record fields. The following fields can be added to the Results table for data acquired by a DAD.

- **Analyte Results Fields**

  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).

- **Internal Standard Results Fields**

  IS Peak Area for DAD: The area of the internal standard peak (mAU-min).

  IS Peak Height for DAD: The height of the internal standard peak (mAU).

  IS Wavelength Ranges: The range of wavelengths (nm).

- **Record Results Field**

  Calculated Concentration for DAD: The calculated concentration of the analyte (ng/mL).

12

Creating Reports

This section describes procedures for creating and printing reports as well as creating new report templates and modifying an existing report template.

Analyst allows you to either print or export reports. When you print a report, you send it to a printer to be printed immediately. When you export a report, you send the data to a file.

You can export reports in the following formats:

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

Although the process of printing varies slightly from the process of publishing, the four main steps are the same.

1. Create a template to determine the look of the report.

   —or—

   Modify an existing template.
2. Choose the report template to use for printing or exporting.
3. Select the window, pane, or workspace you want to print.
4. Print the report.

Previewing a Report

You can preview, scale or copy your graphs using the Print Preview feature.

To preview your report

1. On the **File** menu, choose **Print Preview**, and click **Pane**.
2. Edit the **Print Preview** dialog box.
3. Click **Print** to print the report, or click **Close** to close the **Print Preview** dialog box.

Printing a Report

You can print with or without the use of a report template.

To print without a template

- On the **File** menu, point to **Print**, and then choose the item you want to print.

To print with a template

1. Create a template or open one that already exists.
2. From the **File** menu, choose **Print & Report Setup**.

   The **Print & Report Setup Dialog Box** appears.
3. Select the appropriate file from the list available in the **Report Template** group and click **OK**.
4. On the **File** menu, click **Print**, and then choose the item you want to print.

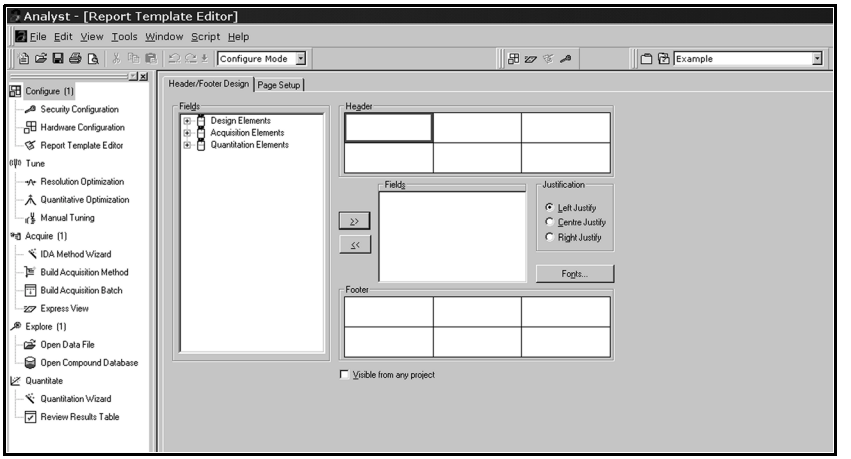
Creating a Report Template

If you are not using an existing template to print or export a report, you must create a new one.

To create a new template

1. On the **File** menu, choose **New**, and then double-click **Report Template**.

   The Report Template Editor Dialog Box: Header/Footer Design tab appears.



NOTE: Individual options are available to users depending upon your security level.

2. Select a header or footer box.

   The box appears outlined in red on the screen. This outline will not print on the document.

3. Click the desired element from the **Fields** Box on the left.

4. Double-click the desired design element. If the field chosen is **Date**, **Time**, or **Custom Field**, fill in the dialog box that appears and click **OK**.

   The design element will be added to the selected header or footer box.

5. Repeat steps 3 to 4 until you have completed your template. You do not need to fill in all of the boxes.

6. Click the **Page Setup** tab.

7. Select **Landscape** or **Portrait** from the **Page Orientation**.

8. Set margins to provide the appropriate space for the header and footer you have just created.

   The value must be set to some value other than zero to enable a proper print out. Default margins are provided for a total of 4 lines of header or footer using the default font.

9. On the **File** menu, click **Save**. Type a file name in the **Save Report Template** dialog box, click **Save**. On the **File** menu, click **Close**.

Modifying an Existing Report Template

You may wish to edit a template you have created, or you may wish to modify an existing template to create a new one that suits your own needs. Start with a template that is similar to what you want to create.

To modify an existing template

1. Click the **Report Template Editor** from the navigation bar.
2. Click **Open** from the **File** Menu. Make sure "Report Template (*.rpt)" is selected for the Files of type field.
3. Double click on one of the files listed in the **Files** window.
4. Modify the template as necessary. For more information, see "Adding a Design Element to Your Template".
   - To change the header and footer fields **Date**, **Time**, and **Custom Field**, double-click the field to get the relevant dialog box for modifying the values.
   - To change the margins, follow instructions for setting margins.
   - To insert a field, highlight the desired design element, and click  or double-click the desired element.
   - To remove a field, highlight the desired design element, and click  or press Delete.
5. Save the file with either the same or a new name, depending on whether you want to write over the original template or not.

Adding a Design Element to Your Template

You can customize you reports by adding different types of information to the headers and footers. There are three types of information that can be added to your reports.

- Basic design elements
- Quantitation elements
- Acquisition elements

To add an element to the header or footer

1. On the **File** menu click **Open**.
2. Select **Report Template (*.rpt)** from the **Files of Type**: combo box.
3. Select the template you wish to edit.
4. On the **Header/Footer Design** tab of the **Report Template Editor Dialog Box** select the area in which you wish your information to appear.
5. Select one of the information elements from the left window.
6. Double-click an element and the element appears in the location you selected.
7. On the **File** menu, click **Save**.

Adding Text to Reports

You can add customized text to an area in the header or footer of a report.

To add customized text

1. In the **Report Template Editor** window, click the Header/Footer Design tab.
2. Click **Design Elements**.
3. Double-click **Custom Field**.

   The Custom Field Settings Dialog Box appears.
4. Click the **Text** option.
5. Type the desired text in the box and click **OK**.

   The boxes in the header and footer are of fixed size. Any text that is too long for the box will be truncated.

Exporting a Report

You can export four types of reports: acquisition methods, batches, quantitation results tables, and graph results tables.

To export a report

1. From the **File** menu, click **Export**.

   The **Save As** dialog box appears.

2. Type the name of the file.
3. Choose the file type (.csv, .doc, .txt, .pdf) depending on the type of report you are exporting.
4. If you are exporting a report from Quantitate mode, choose either **All Columns**, or **Visible Columns** from the Export group and click **Save**.

Appendix A: Generic API 100 Series Parameters

The first figure in each cell is a typical value. Below this is the range possible for each parameter.

Parameter ID	Access ID	Positive	Negative
NEB	**NEB**	8 1 to 15	8 1 to 15
CUR	**CUR**	8 6 to 15	8 6 to 15
IS(1)(2)	**IS(1)(2)**	5000 3500 to 5500	-4000 -(3000 to 5500)
NC(3)	**NC(3)**	2 1 to 5	-2 -(1 to 5)
TEM(2)(3)	**TEM(2)(3)**	350 200 to 500	350 (200 to 500)
OR	**DP**	30 0 to 200	-30 (0 to -200)
RNG	**FP**	300 100 to 400	-300 -(100 to 400)
Q0(4)	**EP**	-10 or -5 -(2 to 12)	10 or 5 2 to 12
IQ1 **IQ1 = Q0 + offset**	**IQ1**	Q0-1 -(5 to 1)	Q0+1 1 to 5
ST **ST= Q0 + offset**	**ST**	Q0 - 5 -(6 to 2)	Q0+5 2 to 6
RO1 **IE1 = Q0 - RO1**	**IE1(5)**	1 0.5 to 2	-1 -(2 to 0.5)
(1) IonSpray (2)TurboIonSpray (3) Heated Nebulizer **(4) Use ± 5 V for fragile analytes (5) Resolution Parameter**			

Parameter ID	Access ID	Positive	Negative
DF	**DF**	-300 (0 to -400)	300 0 to 400
CEM	**CEM**	1800 1800 to 3300	1800 1800 to 3300
(1) IonSpray (2)TurboIonSpray (3) Heated Nebulizer **(4) Use ± 5 V for fragile analytes (5) Resolution Parameter**			

Appendix B: Generic API 300 Series Parameters

The first figure in each cell is a typical value. Below this is the range of settings possible.

Parameter ID	Access ID	Positive Ion Mode			Negative Ion Mode		
		Q1	Q3	MS/MS	Q1	Q3	MS/MS
NEB	**NEB**	8 1 to 15	8 1 to 15	8 1 to 15	8 1 to 15	8 1 to 15	8 1 to 15
CUR	**CUR**	8 6 to 15	8 6 to 15	8 6 to 15	8 6 to 15	8 6 to 15	8 6 to 15
CAD	**CAD**	0	3 0 to 12	3 0 to 12	0	3 0 to 12	3 0 to 12
IS(1)(2)	**IS(1)(2)**	5000 3500 to 6000	5000 3500 to 6000	5000 3500 to 6000	-4000 -(3000 to 5000)	-4000 -(3000 to 5000)	-4000 -(3000 to 5000)
NC(3)	**NC(3)**	2 1 to 5	2 1 to 5	2 1 to 5	-2 -(1 to 5)	-2 -(1 to 5)	-2 -(1 to 5)
TEM(2)(3)	**TEM(2)(3)**	350 200 to 500	350 200 to 500	350 200 to 500	350 200 to 500	350 200 to 500	350 200 to 500
OR	**DP**	30 0 to 200	30 0 to 200	30 0 to 200	-30 0 to -200	-30 0 to -200	-30 0 to -200
RNG	**FP**	300 100 to 400	300 100 to 400	300 100 to 400	-300 -(100 to 400)	-300 -(100 to 400)	-300 -(100 to 400)
Q0(4)	**EP(4)**	-10 or -5 -(2 to 12)	-10 or -5 -(2 to 12)	-10 or -5 -(2 to 12)	10 or 5 2 to 12	10 or 5 2 to 12	10 or 5 2 to 12
(1) IonSpray (2) TurboIonSpray (3) Heated Nebulizer (4) Use ± 5 V for fragile analytes (5) Mass Dependent (6) Resolution Parameter							

Parameter ID	Access ID	Positive Ion Mode			Negative Ion Mode		
		Q1	Q3	MS/MS	Q1	Q3	MS/MS
IQ1	**IQ1**	Q0-1	Q0-1	Q0-1	Q0+1	Q0+1	Q0+1
IQ1 = Q0 + offset		-(5 to 1)	-(5 to 1)	-(5 to 1)	1 to 5	1 to 5	1 to 5
ST	**ST**	Q0-5	Q0-5	Q0-5	Q0+5	Q0+5	Q0+5
ST = Q0 + offset		-(6 to 2)	-(6 to2)	-(6 to 2)	2 to 6	2 to 6	2 to 6
RO1	**IE1(6)**	1		1	-1		-1
IE1 = Q0 - RO1		0.5 to 2		0.5 to 2	-(2 to 0.5)		-(2 to 0.5)
RO1	**RO1**		Q0-1			Q0+1	
RO1 = Q0 + offset			-(2 to 0.5)			0.5 to 2	
IQ2	**CEP(5)**	Q0 - IQ2		Q0 - IQ2	Q0 - IQ2		Q0 - IQ2
CEP = Q0 - IQ2		0 to 188		0 to 188	-(188 to 0)		-(188 to 0)
IQ2	**IQ2**		RO2 +2			RO2-2	
IQ2 = RO2 + offset			2 to 5			(-5 to 2)	
RO2	**CE**			30			-30
CE = (Q0 - RO2)				5 to 130			-(130 to 5)
RO2	**RO2**	-60	-20		60	20	
			-(180 to 20)			20 to 180	
IQ3	**CXP(5)**		Mass Dependent			Mass Dependent	
CXP = (RO2 - RO3)			0 to 58	0 to 58		-(58 to 0)	-(58 to 0)
IQ3	**IQ3**	-150			150		
		-(200 to 100)			100 to 200		
RO3	**IE3(6)**		4	4		-4	-4
IE3 = RO2 - RO3			1 to 8	1 to 8		-(8 to 1)	-(8 to 1)
RO3(5)	**RO3(5)**	-150			150		
		-(200 to 100)			100 to 200		

(1) IonSpray (2) TurboIonSpray (3) Heated Nebulizer (4) Use ± 5 V for fragile analytes
(5) Mass Dependent (6) Resolution Parameter

Parameter ID	Access ID	Positive Ion Mode			Negative Ion Mode		
		Q1	Q3	MS/MS	Q1	Q3	MS/MS
DF	DF	0	0	0	0	0	0
		0 to -400	0 to -400	0 to -400	0 to 400	0 to 400	0 to 400
CEM	CEM	1800	1800	1800	1800	1800	1800
		1800 to 3300	1800 to 3300	1800 to 3300	1800 to 3300	1800 to 3300	1800 to 3300
(1) IonSpray (2) TurboIonSpray (3) Heated Nebulizer (4) Use ± 5 V for fragile analytes (5) Mass Dependent (6) Resolution Parameter							

Appendix C: Generic API 2000 Parameters

The first figure in each cell is a typical value. Below this is the range of settings possible.

Parameter ID	Access ID	TurboIonSpray		Heated Nebulizer
		Solvent flow 2 to 20 µL/min	Solvent flow 200 µL/min	Solvent flow 1 mL/min
GAS 1[(1)]	**GAS 1** [(1)]	25 15 to 40	45 30 to 75	60 40 to 90
GAS 2 [(2)(3)]	**GAS 2** [(2)(3)]	0 0 to 90	80 60 to 90	15 5 to 25
CUR	**CUR**	25 15 to 55	40 30 to 55	45 35 to 55
TEM[(2)(3)]	**TEM**[(2)(3)]	0	<400	450
(1) Ion Spray Mode (2) TurboIon Spray Mode (3) Heated Nebulizer (4) Resolution Parameter				

Parameter ID	Access ID	Positive Ion Mode			Negative Ion Mode		
		Q1	Q3	MS/MS	Q1	Q3	MS/MS
CAD	**CAD**	0 1 to 2	1 2 to 12	3 2 to 12	0 1 to 2	1 2 to 12	3 2 to 12
IS[(1)(2)]	**IS[(1)(2)]**	5000 1000 to 5500	5000 1000 to 5500	5000 1000 to 5500	-4200 -(1000 to 4500)	-4200 -(1000 to 4500)	-4200 -(1000 to 4500)
NC[(3)]	**NC[(3)]**	2 1 to 5	2 1 to 5	2 1 to 5	-2 -(5 to 1)	-2 -(5 to 1)	-2 -(5 to 1)
OR	**DP**	30 0 to 120	30 0 to 120	30 0 to 120	-30 (0 to -120)	-30 (0 to -120)	-30 (0 to -120)
RNG	**FP**	350 50 to 400	350 50 to 400	350 50 to 400	-350 -(50 to 400)	-350 -(50 to 400)	-350 -(50 to 400)
Q0	**EP**	-10 -(2 to 12)	-10 -(2 to 12)	-10 -(2 to 12)	10 2 to 12	10 2 to 12	10 2 to 12
IQ1 **IQ1 = Q0 - offset**	**IQ1**	Q0 -1 -(2 to 1)	Q0 -1 -(2 to 1)	Q0 -1 -(2 to 1)	Q0 +1 1 to 2	Q0 +1 1 to 2	Q0 +1 1 to 2
ST **ST = Q0 - offset**	**ST**	Q0 -5 -(6 to 2)	Q0 -5 -(6 to 2)	Q0 -5 -(6 to 2)	Q0 +5 2 to 6	Q0 +5 2 to 6	Q0 +5 2 to 6
RO1 **IE1 = Q0 - RO1**	**IE1[(4)]**	1 0.5 to 2		1 0.5 to 2	-1 -(2 to 0.5)		-1 -(2 to 0.5)
RO1 **RO1 = Q0 + offset**	**RO1**		Q0 -1 -(2 to 0.5)			Q0 +1 0.5 to 2	
IQ2 **CEP = Q0 - IQ2**	**CEP**	Mass Dependent 0 to 188		0 to 188	Mass Dependent -(188 to 0)		-(188 to 0)
(1) Ion Spray Mode (2) TurboIon Spray Mode (3) Heated Nebulizer (4) Resolution Parameter							

Parameter ID	Access ID	Positive Ion Mode			Negative Ion Mode		
		Q1	Q3	MS/MS	Q1	Q3	MS/MS
IQ2	**IQ2**		RO2 + 2			RO2 - 2	
IQ2 = RO2 + offset			0 to 2			-(2 to 0)	
RO2	**CE**			30			-30
CE = Q0 - RO2				5 to 130			-(130 to 5)
RO2	**RO2**	-60	-18		60	20	
		-(20 to 150)	-(5 to 130)		20 to 150	5 to 130	
IQ3	**CXP**		Mass Dependent			Mass Dependent	
CXP = RO2 - IQ3			0 to 58	0 to 58		-(58 to 0)	-(58 to 0)
IQ3	**IQ3**	-150			150		
		-(200 to 100)			100 to 200		
RO3	**IE3**		4	4		-4	-4
IE3 = RO2 - RO3			1 to 5	1 to 5		-(5 to 1)	-(5 to 1)
RO3	**RO3**	-150			150		
		-(200 to 150)			150 to 200		
DF	**DF**	0	0	0	0	0	0
		-(400 to 0)	-(400 to 0)	-(400 to 0)	0 to 400	0 to 400	0 to 400
CEM	**CEM**	1800	1800	1800	1800	1800	1800
		1600 to 3300	1600 to 3300	1600 to 3300	1600 to 3300	1600 to 3300	1600 to 3300
(1) Ion Spray Mode (2) TurboIon Spray Mode (3) Heated Nebulizer (4) Resolution Parameter							

Appendix D: Generic API 3000 Parameters

The first figure in each cell is a typical value. Below this is the range of possible values.

Parameter ID	Access ID	Positive Ion Mode			Negative Ion Mode		
		Q1	Q3	MS/MS	Q1	Q3	MS/MS
NEB	NEB	5 2 to 10	5 2 to 10	5 2 to 10	5 2 to 10	5 2 to 10	5 2 to 10
CUR	CUR	8 6 to 15	8 6 to 15	8 6 to 15	8 6 to 15	8 6 to 15	8 6 to 15
CAD	CAD	0	1 1 to 2	4 2 to 12	0	1 1 to 2	4 2 to 12
IS(1)(2)	IS(1)(2)	4800 1000 to 5500	4800 1000 to 5500	4800 1000 to 5500	-3800 -(1000 to 4500)	-3800 -(1000 to 4500)	-3800 - (1000 to 4500)
NC(3)	NC(3)	2 1 to 5	2 1 to 5	2 1 to 5	-2 -(5 to 1)	-2 -(5 to 1)	-2 -(5 to 1)
TEM(3)	TEM(3)	450 300 to 500	450 300 to 500	450 300 to 500	450 300 to 500	450 300 to 500	450 300 to 500
OR	DP	30 0 to 120	30 0 to 120	30 0 to 120	-30 0 to -120	-30 0 to -120	-30 0 to -120
RNG	FP	250 50 to 400	250 50 to 400	250 50 to 400	-250 -(50 to 400)	-250 -(50 to400)	-250 -(50 to 400)
Q0	EP	-10 -(3 to -15)	-10 -(3 to -15)	-10 -(3 to -15)	10 3 to 15	10 3 to 15	10 3 to 15
(1) IonSpray Source (2) TurboIonSpray Source (3) Heated Nebulizer (4) Resolution Parameter							

Parameter ID	Access ID	Positive Ion Mode			Negative Ion Mode		
		Q1	Q3	MS/MS	Q1	Q3	MS/MS
IQ1	**IQ1**	Q0 - 1	Q0 - 1	Q0 - 1	Q0 + 1	Q0 + 1	Q0 + 1
IQ1 = Q0 + offset		-(2 to 1)	-(2 to 1)	-(2 to 1)	1 to 2	1 to 2	1 to 2
ST	**ST**	Q0 - 5	Q0 - 5	Q0 - 5	Q0 + 5	Q0 + 5	Q0 + 5
ST = Q0 + offset		-(7 to 2)	-(7 to 2)	-(7 to 2)	2 to 7	2 to 7	2 to 7
RO1	**IE1(4)**	1		1	-1		-1
IE1 = Q0 - RO1		0.5 to 2		0.5 to 2	-(2 to 0.5)		-(2 to 0.5)
RO1	**RO1**		Q0 - 1			Q0 + 1	
RO1 = Q0 + offset			-(2 to 0.5)			0.5 to 2	
IQ2	**IQ2**	Q0 - 8	Q0 - 8	Q0 - 8	Q0 + 8	Q0 + 8	Q0 + 8
IQ2 = Q0 + offset		-(10 to 8)	-(10 to 8)	-(10 to 8)	8 to 10	8 to 10	8 to 10
RO2	**CE**			30			-30
CE = Q0 - RO2				5 to 130			-(130 to 5)
RO2	**RO2**	-60	-20		60	20	
		-(100 to 60)	-(180 to 20)		60 to 100	20 to 180	
ST3	**CXP**		15	15		-15	-15
CXP = RO2 - ST3			0 to 55	0 to 55		-(55 to 0)	-(55 to 0)
ST3	**ST3**	-80			80		
		-(100 to 60)			60 to180		
RO3	**IE3(4)**		2	2		-2	-2
IE3 = RO2 - RO3			0.5 to 5	0.5 to 5		-(5 to 0)	-(5 to 0)
RO3	**RO3**	-60			60		
		-(100 to 60)			60 to 100		
DF	**DF**	0	0	0	0	0	0
		-(400 to 0)	-(400 to 0)	-(400 to 0)	0 to 400	0 to 400	0 to 400
(1) IonSpray Source (2) TurboIonSpray Source (3) Heated Nebulizer (4) Resolution Parameter							

Parameter ID	Access ID	Positive Ion Mode			Negative Ion Mode		
		Q1	Q3	MS/MS	Q1	Q3	MS/MS
CEM	CEM	1800 1600 to 3300	1800 1600 to 3300	1800 1600 to 3300	1800 1600 to 3300	1800 1600 to 3300	1800 1600 to 3300
(1) IonSpray Source (2) TurboIonSpray Source (3) Heated Nebulizer (4) Resolution Parameter							

Appendix E: Generic API 4000 Parameters

The first figure in each cell is a typical value. Below this is the range of possible values.

Parameter ID	Access ID	Positive Ion Mode Q1	Q3	MS/MS	Negative Ion Mode Q1	Q3	MS/MS
CUR	**CUR**	20 10 to 50	20 10 to 50	20 10 to 50	20 10 to 50	20 10 to 50	20 10 to 50
CAD	**CAD**	0	1	4 0 to 12	0	1	4 2 to 12
IS	**IS**	5000 0 to 5500	5000 0 to 5500	5000 0 to 5500	-4200 -4500 to 0	-4200 -4500 to 0	-4200 -4500 to 0
NC	**NC**	3 0 to 5	3 0 to 5	3 0 to 5	-3 -5 to 0	-3 -5 to 0	-3 -5 to 0
TEM	**TEM**	350 0 to 800	350 0 to 800	350 0 to 800	350 0 to 800	350 0 to 800	350 0 to 800
OR	**DP** **DP = OR**	20 0 to 400	20 0 to 400	20 0 to 400	-20 -400 to 0	-20 -400 to 0	-20 -400 to 0
Q0	**EP** **EP = Q0**	-10 -15 to -2	-10 -15 to -2	-10 -15 to -2	10 2 to 15	10 2 to 15	10 2 to 15
IQ1	**IQ1** **IQ1 = Q0 + offset**	Q0 + (-1)	Q0 + (-1)	Q0 + (-1)	Q0 + 1	Q0 + 1	Q0 + 1
ST	**ST** **ST = Q0 + offset**	Q0 + (-5)	Q0 + (-5)	Q0 + (-5)	Q0 + 5	Q0 + 5	Q0 + 5
RO1	**IE1** **IE1 = Q0 - RO1**	1 0.5 to 2		1 0.5 to 2	-1 -2 to -0.5		-1 -2 to -0.5
RO1	**IE1**		Q0 + (-1)			Q0 + 1	

Parameter ID	Access ID	Positive Ion Mode Q1	Q3	MS/MS	Negative Ion Mode Q1	Q3	MS/MS
IE1 = Q0 + offset							
IQ2	**IQ2**	Q0+ (-8)	Q0+ (-8)	Q0+ (-8)	Q0 + 8	Q0 + 8	Q0 + 8
IQ2 = Q0 + offset							
RO2	**RO2**	-60	-20 -145 to -2		60 60 to 100	20 2 to 145	
RO2	**CE**			30	60	20	-30
CE = Q0 - RO2				5 to 130	60 to 100	2 to 145	-130 to -5
ST3	**ST3**	-80			80		
ST3	**CXP**		15	15		-15	-15
CXP = RO2 - ST3			0 to 55	0 to 55		-55 to 0	-55 to 0
RO3	**RO3**	-62			62		
RO3	**IE3**		2	2		-2	-2
IE3 = RO2 - RO3			-0.5 to 5	-0.5 to 5		-5 to 0	-5 to 0
DF	**DF**	0 -400 to 0	0 -400 to 0	0 -400 to 0	0 0 to 400	0 0 to 400	0 0 to 400
CEM	**CEM**	1800 500 to 3000	1800 500 to 3000	1800 500 to 3000	1800 500 to 3000	1800 500 to 3000	1800 500 to 3000
GS1	**GS1**	20 0 to 90	20 0 to 90	20 0 to 90	20 0 to 90	20 0 to 90	20 0 to 90
GS2	**GS2**	0 0 to 90	0 0 to 90	0 0 to 90	0 0 to 90	0 0 to 90	0 0 to 90
ihe	**ihe**	0 0 or 1	0 0 or 1	0 0 or 1	0 0 or 1	0 0 or 1	0 0 or 1

Appendix F: Generic Q Trap Parameters

The first figure in each cell is a typical value. Below this is the range of settings possible.

Parameter ID	Access ID	TurboIonSpray		Heated Nebulizer
		Solvent flow 2 to 20 µL/min	Solvent flow 200 µL/min	Solvent flow 1 mL/min
GAS 1[(1)]	**GAS 1** [(1)]	25 15 to 40	45 30 to 75	60 40 to 90
GAS 2 [(2)(3)]	**GAS 2** [(2)(3)]	0 0 to 90	80 60 to 90	15 5 to 25
CUR	**CUR**	25 15 to 55	40 30 to 55	45 35 to 55
TEM[(2)(3)]	**TEM**[(2)(3)]	0	<400	450
(1) Ion Spray Mode (2) TurboIon Spray Mode (3) Heated Nebulizer (4) Resolution Parameter *** For LIT scans DF = -400 positive mode; +400 negative mode**				

Parameter ID	Access ID	Positive Ion Mode			Negative Ion Mode		
		Q1	Q3	MS/MS	Q1	Q3	MS/MS
CAD	**CAD**	0	Low	Medium	0	Low	Medium
IS[(1)(2)]	**IS[(1)(2)]**	5000 1000 to 5500	5000 1000 to 5500	5000 1000 to 5500	-4200 -(1000 to 4500)	-4200 -(1000 to 4500)	-4200 -(1000 to 4500)
NC[(3)]	**NC[(3)]**	2 1 to 5	2 1 to 5	2 1 to 5	-2 -(5 to 1)	-2 -(5 to 1)	-2 -(5 to 1)
OR	**DP**	30 0 to 120	30 0 to 120	30 0 to 120	-30 (0 to -120)	-30 (0 to -120)	-30 (0 to -120)
Q0	**EP**	-10 -(2 to 12)	-10 -(2 to 12)	-10 -(2 to 12)	10 2 to 12	10 2 to 12	10 2 to 12
IQ1 **IQ1 = Q0 - offset**	**IQ1**	Q0 -1 -(2 to 1)	Q0 -1 -(2 to 1)	Q0 -1 -(2 to 1)	Q0 +1 1 to 2	Q0 +1 1 to 2	Q0 +1 1 to 2
ST **ST = Q0 - offset**	**ST**	Q0 -5 -(6 to 2)	Q0 -5 -(6 to 2)	Q0 -5 -(6 to 2)	Q0 +5 2 to 6	Q0 +5 2 to 6	Q0 +5 2 to 6
RO1 **IE1 = Q0 - RO1**	**IE1[(4)]**	1 0.5 to 2		1 0.5 to 2	-1 -(2 to 0.5)		-1 -(2 to 0.5)
RO1 **RO1 = Q0 + offset**	**RO1**		Q0 -1 -(2 to 0.5)			Q0 +1 0.5 to 2	
IQ2 **CEP = Q0 - IQ2**	**CEP**	Mass Dependent 0 to 188		0 to 188	Mass Dependent -(188 to 0)		-(188 to 0)
IQ2 **IQ2 = RO2 + offset**	**IQ2**		RO2 + 2 0 to 2			RO2 - 2 -(2 to 0)	
RO2 **CE = Q0 - RO2**	**CE**			30 5 to 130			-30 -(130 to 5)

(1) Ion Spray Mode (2) TurboIon Spray Mode (3) Heated Nebulizer (4) Resolution Parameter
*** For LIT scans DF = -400 positive mode; +400 negative mode**

Parameter ID	Access ID	Positive Ion Mode			Negative Ion Mode		
		Q1	Q3	MS/MS	Q1	Q3	MS/MS
RO2	**RO2**	-60 -(20 to 150)	-18 -(5 to 130)		60 20 to 150	20 5 to 130	
IQ3	**CXP**		Mass Dependent			Mass Dependent	
CXP = RO2 - IQ3			0 to 58	0 to 58		-(58 to 0)	-(58 to 0)
IQ3	**IQ3**	-150 -(200 to 100)			150 100 to 200		
RO3	**IE3**		4	4		-4	-4
IE3 = RO2 - RO3			1 to 5	1 to 5		-(5 to 1)	-(5 to 1)
RO3	**RO3**	-150 -(200 to 150)			150 150 to 200		
DF*	**DF***	-200 -(400 to 0)	-200 -(400 to 0)	-200 -(400 to 0)	200 0 to 400	200 0 to 400	200 0 to 400
CEM	**CEM**	1800 1600 to 3300	1800 1600 to 3300	1800 1600 to 3300	1800 1600 to 3300	1800 1600 to 3300	1800 1600 to 3300
EX*	**EX***	-100	-100	-100	100	100	100

(1) Ion Spray Mode (2) TurboIon Spray Mode (3) Heated Nebulizer (4) Resolution Parameter
*** For LIT scans DF = -400 positive mode; +400 negative mode**

For LIT scan types only

Parameter ID	Access ID	Positive Ion Mode	Negative Ion Mode
CAD	**CAD**	High Low–High	High Low–High
FI2	**CEP**	Mass Dependent 0 to 188	Mass Dependent -188 to 0
AF2**	**AF2****	100 0 to 200	100 0 to 200
AF3	**AF3**	Mass-Speed Dependent 0 to 5	Mass-Speed Dependent 0 to 5
EXB	**EXB**	Mass-Speed Dependent -200 to 0	Mass-Speed Dependent 0 to 200
C2B	**C2B**	Mass-Speed Dependent -500 to 500	Mass-Speed Dependent -500 to 500
**** MS/MS/MS only**			

Appendix G: PPG Exact Mass Table

Then following table contains 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, ensure that the correct isotope peak is used.

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

n	Exact Mass (M)	$(M + NH_4)^+$	MS/MS Fragments	$(M + 2NH_4)^{2+}$	$(M + COOH)^-$
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
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

n	Exact Mass (M)	$(M + NH_4)^+$	MS/MS Fragments	$(M + 2NH_4)^{2+}$	$(M + COOH)^-$
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
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	2000464	4009.855

n	Exact Mass (M)	$(M + NH_4)^+$	MS/MS Fragments	$(M + 2NH_4)^{2+}$	$(M + COOH)^-$
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

Appendix H: Calibration Ions

For Q1 PPG positive ion scans:

Instrument	Masses							
100, 100B	59.0	175.1	616.5	906.7	1254.9	1545.1	2010.5	2242.6
100LC	59.0	175.1	384.3	616.5	906.7	1196.9		
150*EX*	59.0	175.1	616.5	906.7	1254.9	1545.1	2010.5	2242.6
150MCA	59.0	175.1	384.3	616.5	906.7	1196.9		
165,165B	59.0	175.1	616.5	906.7	1254.9	1545.1	2010.5	2242.6
300	59.0	175.1	616.5	906.7	1254.9	1545.1	2010.5	2242.6
365	59.0	175.1	616.5	906.7	1254.9	1545.1	2010.5	2242.6
2000	59.0	175.1	616.5	906.7	1254.9	1545.1	1778.3	
3000	59.0	175.1	616.5	906.7	1254.9	1545.1	2010.5	2242.6
4000	59.0	175.1	616.5	906.7	1254.9	1545.1	2010.5	2242.6
Q Trap	59.0	175.1	616.5	906.7	1254.9	1545.1		

For Q1 negative ion scans:

Instrument	Masses							
100, 100B	45.0	527.3	933.6	1165.8	1572.1	1863.3	2037.4	2211.6
100LC	45.0	527.3	933.6	1165.8				
150Ex	45.0	527.3	933.6	1165.8	1572.1	1863.3	2037.4	2211.6
150MCA	45.0	527.3	933.6	1165.8				
165,165B	45.0	527.3	933.6	1165.8	1572.1	1863.3	2037.4	2211.6
300	45.0	527.3	933.6	1165.8	1572.1	1863.3	2037.4	2211.6
365	45.0	527.3	933.6	1165.8	1572.1	1863.3	2037.4	2211.6
2000	45.0	527.3	933.6	1223.8	1572.1	1747.2		
3000	45.0	527.3	933.6	1165.8	1572.1	1863.3	2037.4	2211.6
4000	45.0	527.3	933.6	1165.8	1572.1	1863.3	2037.4	2211.6
Q Trap	45.0	527.3	933.6	1223.8	1572.1			

For Q3 positive ion scans:

Instrument	Masses							
300	59.0	175.1	616.5	906.7	1254.9	1545.1	2010.5	2242.6
365	59.0	175.1	616.5	906.7	1254.9	1545.1	2010.5	2242.6
2000	59.0	175.1	616.5	906.7	1254.9	1545.1	1778.3	
3000	59.0	175.1	616.5	906.7	1254.9	1545.1	2010.5	2242.6
4000	59.0	175.1	616.5	906.7	1254.9	1545.1	2010.5	2242.6
Q Trap	59.0	175.1	616.5	906.7	1254.9	1545.1		

For Q3 negative ion scans:

Instrument	Masses							
300	45.0	527.3	933.6	1165.8	1572.1	1863.3	2037.4	2211.6
365	45.0	527.3	933.6	1165.8	1572.1	1863.3	2037.4	2211.6
2000	45.0	527.3	933.6	1223.8	1572.1	1747.2		
3000	45.0	527.3	933.6	1165.8	1572.1	1863.3	2037.4	2211.6
4000	45.0	527.3	933.6	1165.8	1572.1	1863.3	2037.4	2211.6
Q Trap	45.0	527.3	933.6	1223.8	1572.1	1747.2		

For Q3 negative ion scans:

Instrument	Masses							
300	45.0	527.3	933.6	1165.8	1572.1	1863.3	2037.4	2211.6
365	45.0	527.3	933.6	1165.8	1572.1	1863.3	2037.4	2211.6
2000	45.0	527.3	933.6	1223.8	1572.1	1747.2		
3000	45.0	527.3	933.6	1165.8	1572.1	1863.3	2037.4	2211.6
4000	45.0	527.3	933.6	1165.8	1572.1	1863.3	2037.4	2211.6
Q Trap	45.0	527.3	933.6	1223.8	1572.1			

For LIT scans:

Instrument/Polarity	Masses			
Q Trap positive	115.0	500.38	1080.799	1661.218
Q Trap negative	121.0	585.385	933.636	1630.138

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