QuanLynx User's Guide

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Waters Part No – 715000407

Micromass Part No – 6666671

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QuanLynx User's Guide

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Overview

QuanLynx performs automated quantitation - providing a simple way to perform high-throughput analysis of large numbers of samples.

QuanLynx allows samples to be acquired, processed and reviewed, and Reports to be generated. In addition, Quan-Optimize can be used to automatically generate the Methods that are used to acquire and process samples. For details on how to use Quan-Optimize, refer to Chapter 4.

The QuanLynx Browser, which is accessed from the MassLynx top-level Screen, is used to review and reprocess quantitation results, produce reports and export information for use by third-party applications. For details on how to use the QuanLynx Browser, refer to Chapter 3.

All the data, methods and results associated with an instance of processing a batch of samples are stored together in a single file known as a QuanLynx Dataset.

Installing the QuanLynx Software

To install QuanLynx during the MassLynx Installation, check the QuanLynx option when it appears. You will need to insert the QuanLynx key disk when prompted. QuanLynx can also be installed to an existing MassLynx installation, by selecting Modify from the Setup Wizard and checking the QuanLynx option when prompted. For further details on installation see Chapter 2 of the main MassLynx NT User's Guide. If in any doubt contact your Micromass customer service representative for assistance.

Chapter 2 Quantitation Overview

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Introduction

QuanLynx enables Quantify calibration curves to be generated, using **Standard** samples containing compounds of known concentrations. The calibration curves are then used to calculate the concentrations of compounds in **Analyte** samples.

The user provides a list of the samples and a Quantify Method, which describes how to process each of the compounds within these samples.

Raw data, methods, calibrations and results associated with an instance of processing a set of samples are stored in a QuanLynx Dataset file.

QuanLynx Automated Quantitation - an Overview

There are seven basic stages involved in automated quantitation:

1. Creation of a list of samples using the Sample List Editor.
2. Acquisition of each sample in the analysis.
3. Creation of a QuanLynx Dataset from the sample data.
4. Integration of the data file chromatograms.
5. Generation of Quantify calibration curves.
6. Calculation of the compound concentrations.
7. Displaying the Quantify results and production of Reports.

How Does QuanLynx Quantify and Report a List of Samples?

After data for all of the samples has been acquired by MassLynx, QuanLynx must perform several tasks to create a printed Report of their concentrations. Whilst the user does have considerable flexibility in the control of these processes, quantitation is still a straightforward operation, consisting of the following basic steps:

Creation of a QuanLynx Dataset

A new Dataset is created containing information from the Sample List, sample data and Quantify Method. Sample headers, experimental records and the chromatograms identified in the Quantify Method are extracted from the raw data files and stored in the Dataset, along with a copy of the Quantify Method.

Integration of the Data File Chromatograms

Chromatogram integration is made up of two processes; smoothing and peak detection; exactly how these are to be applied is specified in the Quantify Method.

Each compound in the Quantify Method specifies a chromatogram trace that is to be used to Quantify that compound. The chromatogram for each of the Quantify Method compounds is integrated and the resulting peaks are saved to the Dataset.

Generation of Quantify Calibration Curves

A calibration curve is generated for each of the compounds in the Quantify Method. Samples, which are to be used when generating a calibration curve, are marked as being of type **Standard** in the Sample List. The Sample List also specifies the concentration of each of the calibration standards.

The peak, which represents each compound, must be located within a sample's detected peaks. A response value for each of the located peaks can then be calculated. For located peaks, information, such as compound name and peak response, is saved in the Dataset.

For each compound, one calibration point is obtained from each Standard sample. Calibration points are plotted as response against concentration. A polynomial is fitted to these points to form the compound’s calibration curve. The calibration curves are saved to the Dataset.

The Quantify Method specifies how to locate peaks, calculate responses and fit curves.

Calculation of the Compound Concentrations

QuanLynx calculates the concentration of each of the Quantify Method compounds for the samples in the Dataset.

A concentration is calculated for each of a compound’s located peaks by applying the compound’s calibration curve. Concentration information is saved in the Dataset.

Displaying the Quantify Results and Producing Reports

The QuanLynx Browser is used to display and report the results contained in the Dataset; refer to Chapter 3, The QuanLynx Browser, for more information.

A Step by Step Guide to Quantitation

1. Create a Sample List

A list of samples to be used to perform the analysis must be first created using MassLynx; refer to the MassLynx User's Guide for details. These samples can be acquired manually, but more often they are acquired automatically using an autosampler. The Sample List editor display is part of the MassLynx top-level screen; it has user-selectable columns, e.g. Filename, Bottle Number and Sample Type, into which the appropriate information for each sample is entered. Each sample is displayed as a single row in the Sample List.

To enable MassLynx to perform a complete analysis, the following must be described:

- The Sample Type for each bottle in the autosampler, i.e. whether it is a standard, an analyte, a blank, or a Quality Control (QC) sample.
- How the sample is to be acquired.
- If the sample is a standard or QC, its concentration(s).

In addition, MassLynx must be given a file name in which to store the data. Management information, such as Sample ID, the submitter’s name, or a sample description may also be specified.

Projects

MassLynx allows the work to be organized in a Project, which is a simple way of organizing all the data files, methods and results for a particular assay into one directory structure. When a MassLynx Project is opened, a new directory is created to hold all the files associated with the Project.

The types of file that can be saved in a MassLynx Project are:

- Raw data files.
- QuanLynx Datasets.
- Sample Lists.
- Quantify Methods.
- Quantify calibration curves.
- Tuning files.
- Scan methods.
- Instrument calibration files.
- Inlet methods.

Projects are created and selected from the MassLynx menu bar **File** command. See the MassLynx User’s Guide for details of how to create or open a Project.

2. Create a Quantify Method

General

A Quantify Method must be created, using MassLynx, before a QuanLynx Dataset can be produced.

The Quantify Method describes how a data file is processed to produce calibration curves and quantitative information. Details must be entered into the Method for each of the compounds being used in the analysis.

The Quantify Method specifies information for performing the following tasks:

- Integration of a chromatogram trace to obtain peak information.
- Location of the chromatogram peak relating to a specific compound from the list of detected peaks.
- Calculation of a response factor for the located peak.
- Generation of a Quantify calibration curve.

The Quantify Method Editor

The Quantify **Method Editor** is used to create new Methods and modify existing ones; it is invoked by selecting **QuanLynx, Edit Method** of the MassLynx shortcut bar

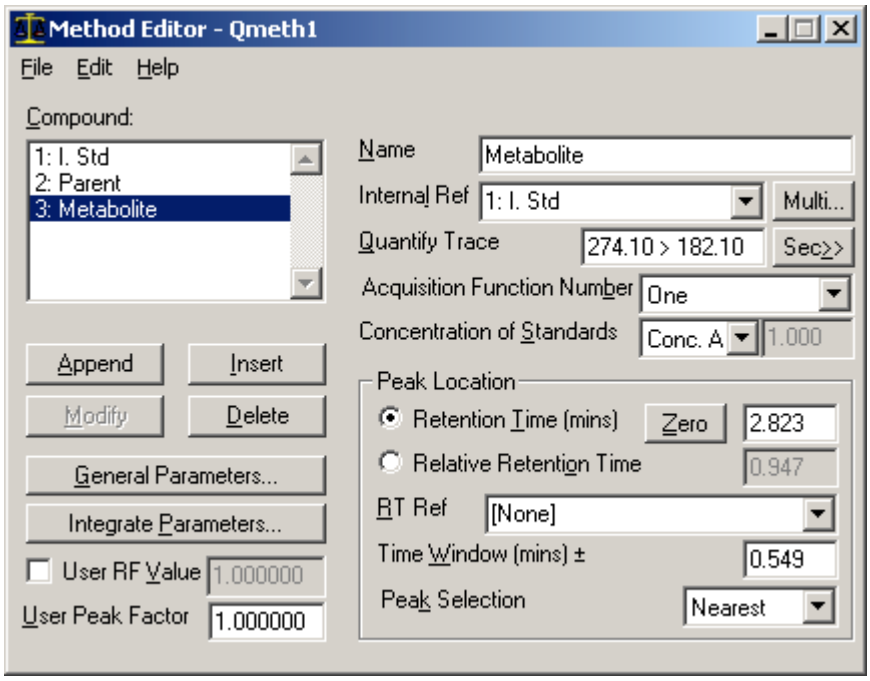


Figure 2.1 The Quantify Method Editor

When invoked, the Quantify **Method Editor** contains the current MassLynx Method; if this is not available, the Editor will contain default values and the name of the current Method in the Editor title bar is set to [Untitled].

The current Quantify Method Editor Method is the current system Method file; it is used when performing quantitation.

Changes made to the Method are not made permanent until they have been saved to disk. Consequently, the Method must be saved before it can be used to perform quantitation; select the Menu Bar **File**, **Save** command to update the current Method file, or **File**, **Save As** to save to a new Method file.

Setting Method Parameters

1. Enter the name of the compound in the Quantify **Method Editor**, **Name** box. (This can be up to 40 characters in length.)

2. Press the appropriate button to add the compound to the list in the **Compound:** box:

Append: Adds the compound to the end of the list.

Insert: Inserts the compound before the currently selected compound in the list.

Modify: Changes the currently selected compound to that entered in the Name box.

Delete: Deletes the currently selected compound from the list.

3. Select the internal reference compound in the **Internal Ref** box. (Set this to **[None]** if the compound is not using an internal reference. Only compounds that appear in the compound list can be selected.). If you wish to set up multiple internal standards select the **Multi…** button. This invokes the Internal Standards dialog box (Figure 2.2), up to six compounds can be selected. When **Multi…** is selected, [Multiple] is displayed in the adjoining combo box.

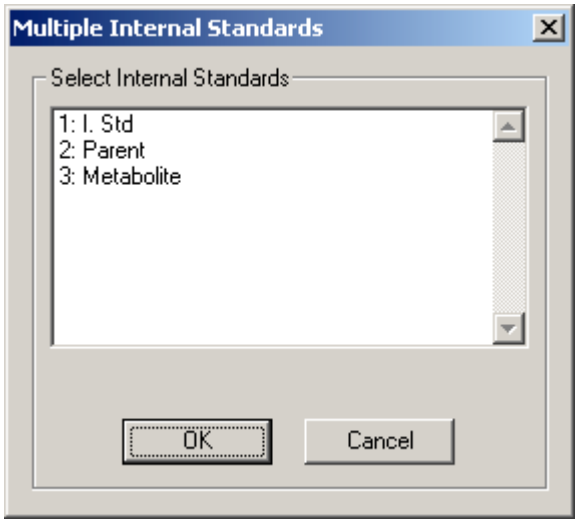


Figure 2.2 The Multiple Internal Standards dialog

4. Set the **Quantify Trace** edit control to the trace descriptor of the chromatogram being used to quantify the compound. (The **Quantify Trace** specifies a chromatogram to be integrated when performing automatic peak detection and is used during the locate phase when matching peak list entries against Method compounds). The trace descriptor should be:

   - A single decimal number for mass chromatograms.

   - Two decimal numbers separated by a “>” for a Multi Reaction Monitoring (MRM) function, e.g. 274.10 > 182.10. The first number represents the parent mass; the second number represents the daughter mass. A reaction can be specified for MRM data. The chromatogram used can be constructed from multiple chromatograms using the Add (+), Subtract (-) and Range (:) operators.

- **TIC** for Total Ion Current chromatograms.
- **BPI** for Base Peak Intensity chromatograms.
- **An1**, **An2**, **An3** or **An4** for analog data, depending on the channel required.
- The wavelength for Diode Array Detector (DAD) data.
- Ch1, Ch2, etc. for Selective Ion Recording (SIR) data to use one Quantify Method with multiple SIR functions, where Ch1 is the first mass in the list, Ch2 is the second, etc.

Note: The trace descriptor value will be entered automatically in **Quantify Trace** edit control if the **Peak Location** parameters are entered using the mouse, see Step 8.

5. In specific cases, it may be necessary to specify a secondary ion; if so, press the **Sec>>** button. The **Secondary** dialog is invoked.

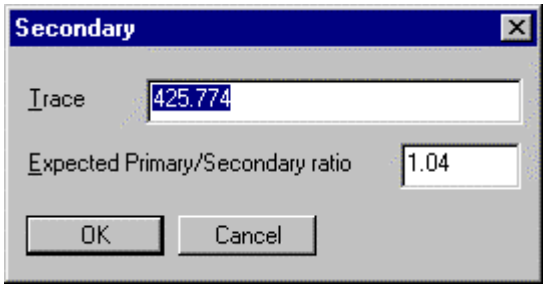


Figure 2.3 The Secondary dialog

- Enter the mass of the secondary ion in the **Trace** field. (If this field is left blank, the secondary ion will not be used during peak location.)
- Enter the expected ratio between the size of the Primary and Secondary peaks in the **Expected Primary/Secondary ratio** field. (If this field is set to zero the peak ratio will not be used for compound location.)
- Press **OK** to accept the new settings. (The display returns to the Quantify Method Editor.)

6. For multifunction data, select which function number is to be used to quantify the current compound in the **Acquisition Function Number** control. (Any number between **One** and **Thirty-two**, or **Any**, may be selected.)

7. Set the **Concentration of Standards** box to the Sample List column that contains the compound's concentration level within each Standard or QC sample; e.g. Conc A if the concentration is defined in the **CONC_A** column in the Sample List. (The software allows up to 20 concentration levels within a single sample. If the compound is an Internal Standard and is at the same concentration in all samples the **Fixed** option can be selected. The parameter box adjacent to the **Concentration of Standards** box becomes active when the **Fixed** option is selected; enter the concentration level of the International Standard in the box.)

8. Select the Peak Location Method by selecting either the **Retention Time (mins)** or **Relative Retention Time** radio button. Alternatively, a method compound to use as the retention time reference can be selected from the **RT Ref** drop-down list. (The Peak Location Method determines how a peak within a Peak List is identified as matching a method compound. If a reference is entered, the expected retention time of the compound will be shifted by the same amount as the found reference peak from its predicted time.). Clicking the **Zero** button will zero all the retention times.

Note: The Peak Location **Time Window (mins) ±** (see below) and **Retention Time (mins)** or **Relative Retention Time** parameter values can be entered by using the mouse, or with the keyboard.

To use the mouse, proceed as follows:

- Arrange the MassLynx display so that the Quantify Method Editor and the Chromatogram window showing the chromatogram to be used can both be seen. Select the Compound for which parameters are to be set in the Method Editor.

- On the Chromatogram window, press the right-hand mouse button at one end of the chromatogram region of interest and, without releasing the button, drag horizontally to the other end. As the mouse is dragged, a "rubber band" will be displayed to indicate the range selected. The Quantify Method Editor window will be updated to show the new Peak Location **Time Window (mins) ±**, and the **Retention Time (mins)** or **Relative Retention Time** will be set to the middle point of the **Time Window (mins) ±**.

  The **Quantify Trace** parameter will be set to the same type as the selected chromatogram [i.e. Total Ion Count (TIC), Base Peak Intensity (BPI), mass chromatogram or MRM].

- The **Retention Time (mins)** or **Relative Retention Time** parameters can also be set with a single click of the right-hand mouse button on the chromatogram trace.

To use the keyboard, proceed as follows:

- If **Retention Time (mins)** has been selected, set it to the time, in decimal minutes, at which the compound is expected to elute. Set the Peak Location **Time Window (mins) ±** parameter to specify by how much the compound elution time may vary. (The **Time Window (mins) ±** is applied either side of the predicted retention time to give a valid window. The **Time Window (mins) ±** parameter multiplied by the factor entered in the The Integrate chromatogram dialog (see page 2-12) defines the chromatogram range that will be integrated.)

- If **Relative Retention Time** has been selected, set it to the time at which the compound is expected to elute relative to the compound specified in the **Internal Ref** control. (The value specified here is a multiplication factor that is applied to the time at which the internal reference compound elutes. This can be used to deal with situations where some drift may occur in the time at which compounds elute but their relative retention times remain constant.)

9. Set the **Peak Selection** parameter to specify which peak should be located when more than one peak is detected within the Peak Location **Time Window**. (By default, the peak **Nearest** to the specified retention time will be selected. Other options that can be selected are **Largest** peak, **First** peak or **Last** peak in the specified time window, and **Totals. Totals** allows the sum of the valid peaks within the window to be calculated; see the Totals Compounds section, on page 2-27, for further details.)

10. If required, set the **User Peak Factor**. (This value is a multiplication factor that will be applied to all calculated concentrations for the current compound. If the **User Peak Factor** is left at **0**, or set to **1**, the concentration values will not be changed.)

11. If required, select the **User RF Value** option and enter a Response Factor (RF) value in the control. (The **User RF Value** is used in cases where there are no calibration standards to plot a calibration curve. It represents the gradient of a curve and is used as a multiplication factor, which will be applied to peak responses for the current compound to determine concentrations.)

12. Press the **General Parameters** button; the **General Method** dialog is invoked. (To use these General Parameters for all compounds in the Method, choose the Quantify Method Editor **Edit**, **Propagate**, **General Parameters** command. A tick mark will appear next to this option and the General Parameters will be copied to all compounds in the Method.)

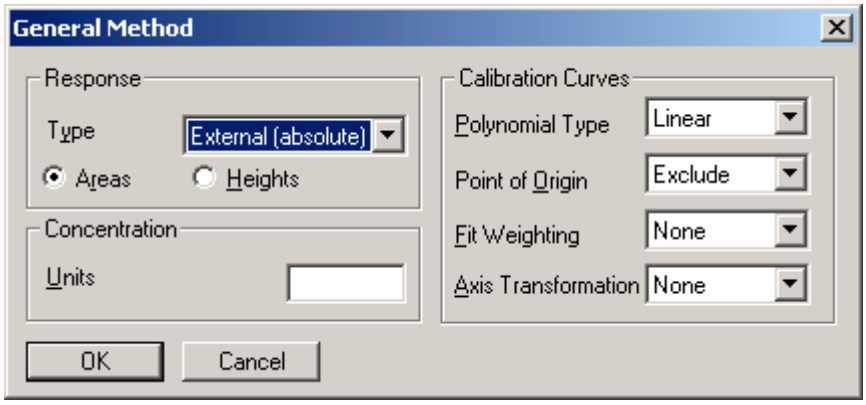


Figure 2.4 The General Method dialog

The **Response** parameters determine how the response value of a located peak is to be calculated. The response values are used to form calibration curves for compounds from standard samples and to calculate the concentration of compounds within analyte samples.

13. Set the **Response**, **Type** option to **Internal (relative)** or **External (absolute)**:

Internal (relative) Should be selected if a compound's response is to be calculated using an Internal Standard, in which case the Quantify Method Editor **Internal Ref** control must have the Internal Standard compound selected.

External (absolute) Should be selected if compound does not have an Internal Standard; the response is then taken as the absolute peak height/area.

14. Select **Response**, **Areas** or **Heights** to specify whether the compound responses will be based upon peak areas or heights respectively.

15. Next, set the **Calibration Curves** Parameters, as shown in Steps 16 to 19. (The calibration curve parameters determine how a compound's calibration curve is to be formed.)

16. Select the type of calibration curve in the **Polynomial Type** control; the following options are available:

Average RF Produces a calibration which is a straight line through the origin and through the mean response factor of the calibration points. A response factor is the response of a calibration point divided by its concentration. This option should be selected for compounds where the **Method Editor**, **Concentration of Standards** box is set to the **Fixed** option.

Linear Performs a linear regression on the compound's calibration points.

Quadratic Performs a second order regression on the compound's calibration points.

Cubic: Performs a third order regression on the compound's calibration points.

Quartic Performs a fourth order regression on the compound's calibration points.

17. Set the **Point of Origin** option to **Exclude**, **Include** or **Force**. (At the point of origin, it is assumed that zero concentration has a response of zero. If **Polynomial Type** is set to **RF** this parameter is not used.)

Force	The calibration curve will always pass through the origin
Include	The point of origin will be included in the calibration curve regression; the curve will not usually pass through the origin.
Exclude	The origin will be ignored when forming the calibration curve.

18. Set the **Fit Weighting** option to **None**, **1/X**, **1/X^2**, **1/Y** or **1/Y^2**. (This parameter is used to give higher priority to calibration points with a low concentration or response when using regression to fit a calibration curve. This generally results in the calibration curve being fitted closer to points at low concentrations, hence reducing the relative error at these points.)

19. Set the **Axis Transformation** parameter to the required option. (The available options are **None**, **LN** (Natural Log), **Log** (Base 10 Log) and **Square Root**. The transformation is applied to the concentration and response values before the calibration curve is fitted.)

    Note: Axis transformations cannot be used with RF-type curves, curves which use point weighting, or curves which include or force the origin.

20. If required, set the **Concentration Units** parameter. (The value set here will be used on the concentration axis of calibration curves and in the concentration column header in the Summary Report.)

21. Press **OK** to accept the new settings. (The display returns to the Quantify Method Editor.)

Setting Quantify Method Peak Integration Parameters

The Peak Integration parameters are used when automated chromatogram peak detection is being performed. The integration parameters can either be set on a per compound basis or for all compounds within the Method.

The facility to set different integration parameters for different compounds can be useful where peak characteristics such as peak width or shape vary between different compounds. For more detailed information on integration see the Chromatogram Processing section of the MassLynx User's Guide.

To use the same integration parameters for all compounds in the Method select the Quantify Method Editor **Edit**, **Propagate**, **Integration Parameters** command. A tick mark will appear next to this command and the integration parameters will be copied to all compounds in the Method.

By default, integration will take place over the chromatogram range defined by the Quantify Method Editor **Time Window** parameter. If you wish to integrate over a larger window, select the Quantify Method Editor **Edit**, **Integrate Window** command; the **Integration Window** dialog is invoked. Enter a multiplication factor in the **Factor applied to location window to calculate integration window** text box. This factor will be applied to the Peak Location **Time Window** to calculate the integration window; it is the same for all compounds in the Method.

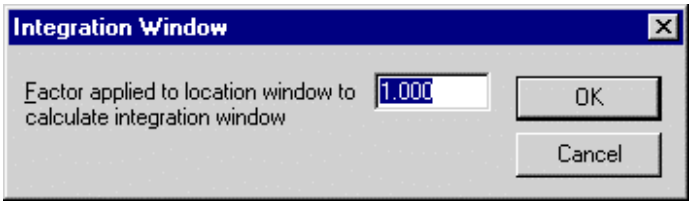


Figure 2.5 The Integration Window dialog

To define the integration parameters, press the Quantify Method Editor **Integrate Parameters** button; the **Integrate chromatogram** dialog is invoked.

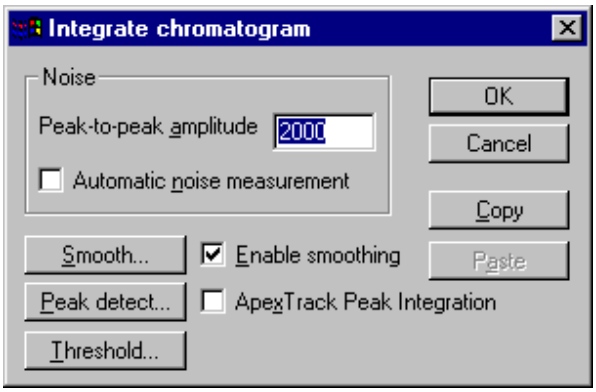


Figure 2.6 The Integrate chromatogram dialog

The peak-to-peak noise amplitude value is entered in the **Peak-to-peak amplitude** text box; this is used by the integration software to pre-filter the chromatogram. A suitable value can be measured directly from a chromatogram by pressing the right-hand mouse button, and dragging the mouse across a section of noise in the chromatogram. The sensitivity of the integration algorithm can be fine-tuned by manually adjusting this value.

The **Copy** and **Paste** buttons allow integration parameters to be written to, and read from, the Windows Clipboard. This enables integration parameters to be transferred easily between the chromatogram and the Quantify Method. This can be useful when experimenting to find the correct integration parameters using a chromatogram.

Select the **ApexTrack Peak Integration** option to use an alternative peak detection algorithm.

Smoothing

The chromatogram may be smoothed, before integrating, by selecting the **Integrate chromatogram** dialog **Enable smoothing** option. The parameters for the smooth may be examined and altered by pressing the **Smooth** button; this invokes the **Smooth chromatogram** dialog.

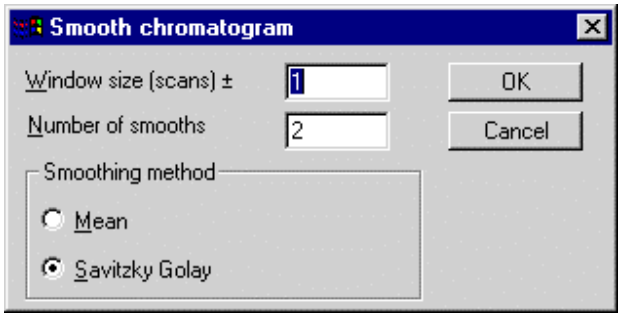


Figure 2.7 The Smooth chromatogram dialog

The **Window size (scans) ±** parameter should be set to the half-width of the smoothing window in scans. This parameter can be set automatically by clicking the right-hand mouse button, and dragging across a chromatogram peak.

Set the number of times the smooth is repeated, by changing the **Number of smooths** parameter from its default value of two. Increasing this parameter gives a heavier smooth.

Two types of smoothing are available for chromatograms; **Moving Mean** and **Savitzky Golay**. Both methods slide a window along the chromatogram, averaging the data points in the window to

produce a point in the smoothed spectrum. **Moving Mean** takes the arithmetical mean of the intensities of the data points in the window. **Savitzky Golay** takes an average of the intensities weighted by a quadratic curve. This tends to enhance peak and valley shapes, as well as preserving the height of the peaks better than the **Moving Mean**. However, **Savitzky Golay** does tend to produce small artefacts on either side of the real peaks.

Once the parameters have been selected, press the **OK** button to return to the **Integrate chromatogram** dialog.

Peak Thresholding

Small peaks may be optionally removed by setting one of the four available threshold parameters. To examine or modify these parameters, press the **Integrate chromatogram** dialog **Threshold** button; the **Response Threshold** dialog is invoked.

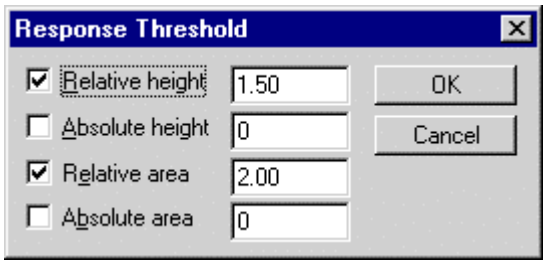


Figure 2.8 The Response Threshold dialog

Relative height Select this option to remove the peaks whose height is less than the specified percentage of the highest peak.

Absolute height Select this option to remove the peaks whose height is less than the specified value.

Relative area: Select this option to remove the peaks whose area is less than the specified percentage of the largest peak area.

Absolute area Select this option to remove the peaks whose area is less than the specified value

Once the parameters have been selected, press the **OK** button to return to the **Integrate chromatogram** dialog.

Peak Detection

The parameters controlling the positioning of peak baselines may be examined and modified by pressing the **Integrate chromatogram** dialog **Peak detect** button. The dialog invoked, and corresponding parameters, will depend on the Peak Detection method previously selected by the **Integrate chromatogram** dialog **ApexTrack Peak Integration** option.

A brief description of each of the Peak Detection parameters is given below; for a more detailed discussion see the MassLynx User's Guide.

Standard Peak Detection Parameters

If the **Integrate chromatogram** dialog **ApexTrack Peak Integration** option is not selected, the **Peak Detect** dialog is invoked when the **Integrate chromatogram** dialog **Peak detect** button is pressed.

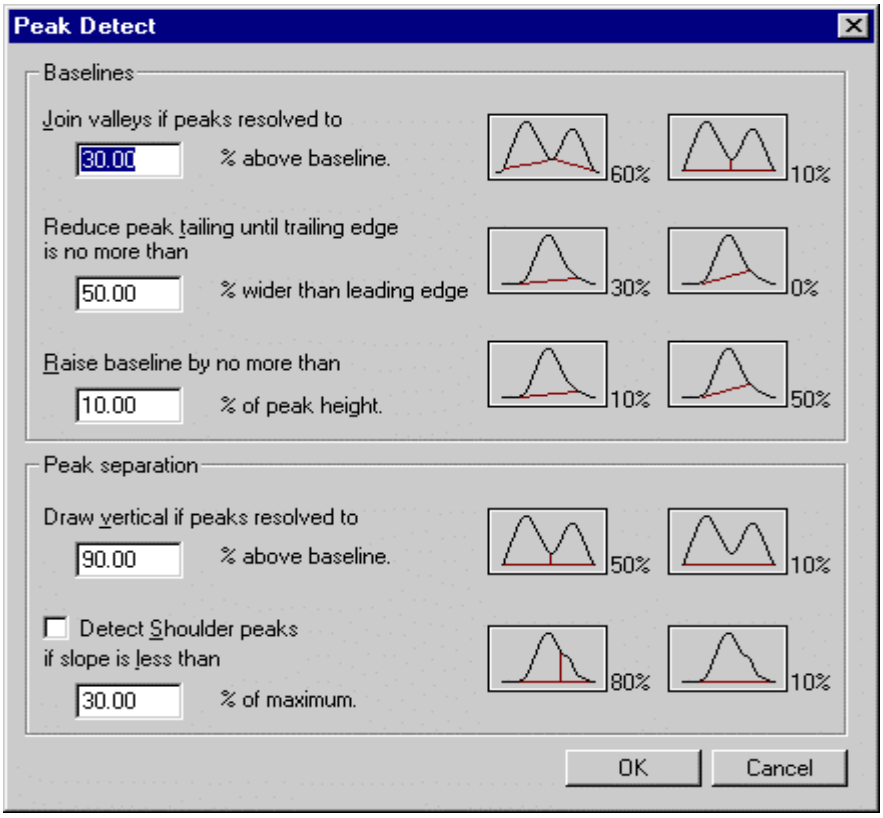


Figure 2.9 The Peak Detect dialog

Join valleys: Affects how baselines for partially resolved peaks are drawn. The larger the value of this parameter, the more peak baselines will be drawn up to the valleys between unresolved peaks. The default value for this parameter is 30%, and the normal operating range is 5% to 75%.

Reduce peak tailing and Raise baseline These parameters control the positioning of baseline end points. The default value for the **Reduce peak tailing** parameter is 50%, and the normal operating range is between 25% and 300%.

Raise baseline This parameter prevents the baseline end point being moved too high up the peak. To prevent the baseline endpoints moving up the peaks, reduce the value of this parameter. The default value is 10%, and normal operating range is 5% to 20%. This parameter is only relevant when the **Reduce peak tailing** parameter has a small value (less than 50%).

Draw vertical This parameter determines how well resolved peaks must be before they are separated by a dropline (or baselines are drawn up into the valleys, depending on the value of the **Join valleys** parameter). Increase the value of this parameter to separate poorly resolved peaks. The default value is 90%, and normal operating range is 50% to 100%.

Detect Shoulder peaks

Select this option to optionally attempt to detect completely unresolved peaks, or shoulders. The algorithm will detect a shoulder if the slope of the shoulder top is less than the specified percentage of the steepest slope on the peak. Therefore, to make shoulder detection more sensitive, increase the value of this parameter. The default value is 30%, and normal operating range is 20% to 90%.

Once the parameters have been selected, press the **OK** button to return to the **Integrate chromatogram** dialog.

ApexTrack Peak Detection Parameters

If the **Integrate chromatogram** dialog **ApexTrack Peak Integration** option is selected, the **ApexTrack Peak Detection Parameters** dialog is invoked when the **Integrate chromatogram** dialog **Peak detect** button is pressed.

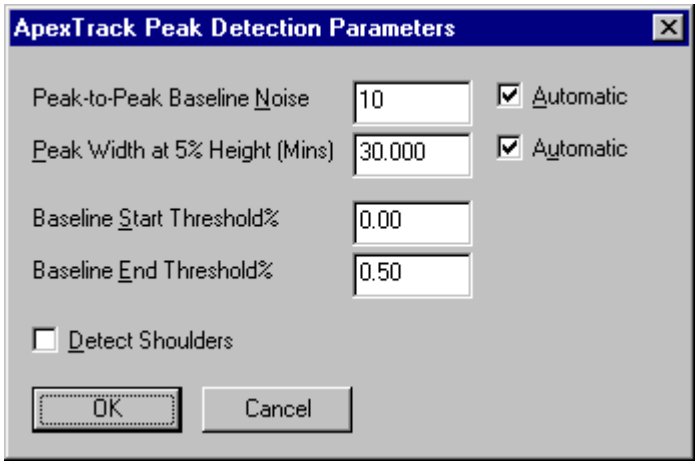


Figure 2.10 The ApexTrack Peak Detection Parameters dialog

Peak-to-Peak Baseline Noise

This is the Apex Detection Threshold. The value of this threshold is the maximum (peak-to-peak) excursion of the baseline noise.

ApexTrack converts Peak-to-Peak Baseline Noise to a second derivative threshold. Peaks that have an inverted second derivative apex higher than this internal value are considered to be valid peaks. If **Automatic** is selected, the second derivative threshold is automatically determined from the chromatogram. This value is converted to the Peak-to-Peak Baseline Noise, which is then displayed in the text box; the user-specified value is ignored. The conversion between Peak-to-Peak Baseline Noise and the second derivative noise threshold is a factor that depends on the peak width.

The Peak-to-Peak Baseline Noise is proportional to the second derivative threshold. Making the Peak-to-Peak Baseline Noise parameter larger will filter out some of the smaller peaks

Peak Width at 5% Height

Sets the width, in minutes, of a filter that is used to smooth the second derivative. If **Automatic** is selected, the Peak Width is proportional to the distance between the inflection points (see below) of the highest peak. The automatic value used is then displayed in the text box and the user-specified value is ignored. Making this parameter larger will filter out some of the narrower peaks.

Baseline Start Threshold%: Defines how high the baseline is raised at the start of each peak. It is given as a percentage of the height of the first inflection point of the peak, i.e. the point on the leading edge of the peak where the second derivative is zero.

Baseline End Threshold%: Defines how high the baseline is raised at the end of each peak. It is given as a percentage of the height of the last inflection point of the peak, i.e. the point on the trailing edge of the peak where the second derivative is zero.

If **Baseline Start Threshold%** and **Baseline End Threshold%** are both set to 100%, the baseline will be placed at the peak's inflection points (see below).

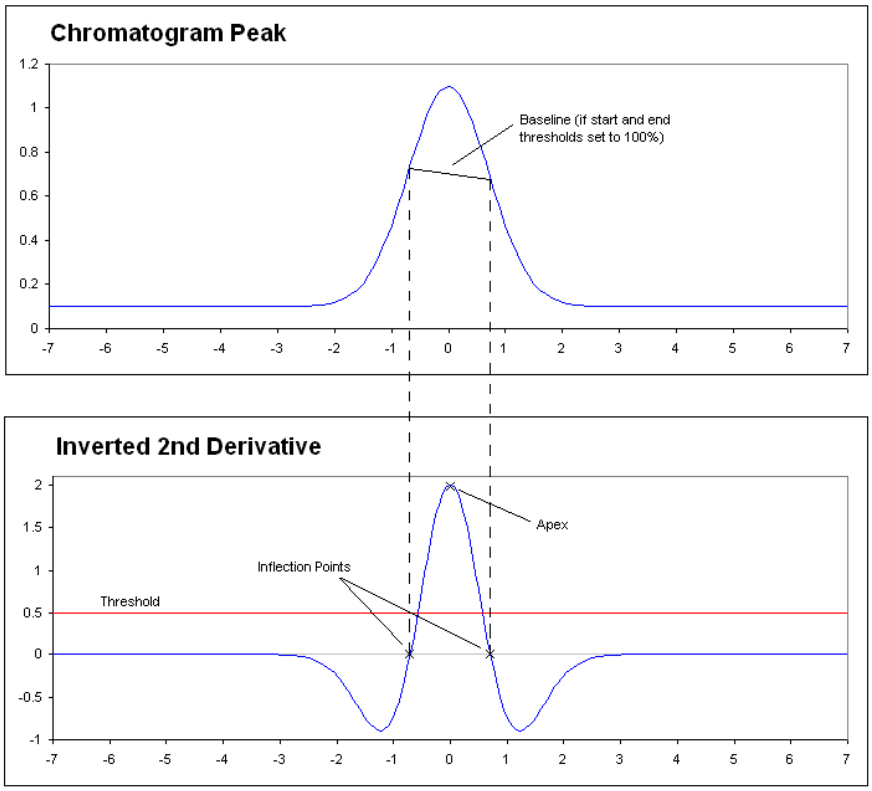


Figure 2.11 Chromatogram Peak and Inverted Second Derivative

Detect shoulders: Determines whether any detected shoulders will be treated as separate peaks or part of the parent peak.

Once the parameters have been selected, press the **OK** button to return to the **Integrate chromatogram** dialog.

Creating a New Quantify Method

1. Select the Quantify Method Editor dialog **File**, **New** command. (The Quantify Method Editor controls are set to default values and the **Compound:** list box is empty. The name of the current Method in the Quantify Method Editor title bar is set to [Untitled].)

2. Add the desired compounds as described below.

3. Select the **File**, **Save As** command; the **Save As** dialog is invoked.

4. Enter the name of the new Method into the **Save As** dialog.

5. Press the **OK** button.

Selecting an Existing Quantify Method

1. Select the Quantify Method Editor **File**, **Open** command; the **Open** dialog is invoked.

2. Choose the required Method file from the **Open** dialog.

3. Press the **Open** button. (The compounds held within the Method are loaded into the Quantify Method Editor **Compound:** list box. The first compound within the Method is selected.)

To Propagate General Parameters to All Compounds in the Quantify Method

To use the same general parameters for all compounds in the method, select the Quantify Method Editor **Edit**, **Propagate**, **General Parameters** command. (A tick mark will appear next to this command and the general parameters will be copied to all compounds in the method.)

To Propagate Integration Parameters to All Compounds in the Quantify Method

To use the same integration parameters for all compounds in the method select the Quantify Method Editor **Edit**, **Propagate**, **Integrate Parameters** command. A tick mark will appear next to this command and the integration parameters will be copied to all compounds in the method.

To Add a New Compound to the Quantify Method

1. Enter the required information for the new compound in the Quantify Method Editor.

2. Press the **Append** button. (The new compound will be added to the end of the **Compound:** list.)

To Insert a New Compound in the Quantify Method

1. Select the entry in the Quantify Method Editor **Compound:** list before which the new compound is to be inserted.

2. Enter the required information for the new compound.

3. Press the **Insert** button. (The new compound will be inserted in the **Compound:** list.)

To Modify Information for an Existing Compound in the Quantify Method

1. Select the entry in the Quantify Method Editor **Compound:** list that is to be modified.

2. Enter the updated information.

3. Press the **Modify** button.

To Delete a Compound from the Quantify Method

1. Select the entry in the Quantify Method Editor **Compound:** list that is to be deleted.

2. Press the **Delete** button, or the Delete key.

To Delete all Compounds in the Method

1. Choose the Quantify Method Editor **Edit**, **Delete All Compounds** command, the **Method Editor**, **Delete all entries** dialog is invoked.

2. Press the **OK** button to delete all compounds in the method.

3. Start the Analysis

Before starting an analysis, save any changes made to the MassLynx Sample List by selecting the MassLynx **File**, **Save** command.

To begin acquiring data select the MassLynx **Run**, **Start** command, or press the  toolbar button; this invokes the **Start Sample List Run** dialog.

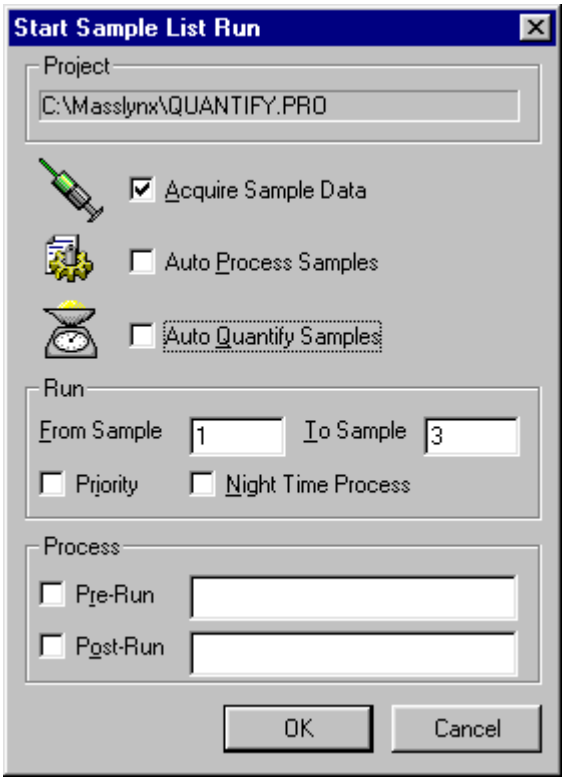


Figure 2.12 The Start Sample List Run dialog

Project — The name of the current project. To acquire to a different project, exit this dialog, open another project and start acquisition again.

Acquire Sample Data — Selecting this option will acquire data for all the samples in the Sample List. See the Acquiring Data section of the MassLynx Data Acquisition Guide for more information on acquisitions.

Auto Process Samples — Selecting this option will process the acquired data as specified in the **Process** column of the Sample List.

Auto Quantify Samples — Selecting this option will quantify the acquired data using the method specified in the **Quantify Samples** dialog (see below). If a method is not defined in the **Quantify Samples** dialog, the current method will be used. If selected, this option will generate a QuanLynx Dataset with the same name as the sample list; if a Dataset of this name already exists, a numeric postfix will be appended to the name. The Dataset can be viewed in the QuanLynx Browser, which is invoked by the **Quantify**, **View Results** command.

The above three actions can be run together or independently, i.e. data can be acquired, processed and quantified in one go, or acquired in one run and processed or quantified it at a later date.

Run

From Sample
To Sample — Sets the range of samples in the Sample List which will be acquired/and or analyzed.

Priority — Select this option to mark this entry as a Priority process.

> The MassLynx **Queue Properties** dialog **Pre-emptive Scheduling** option must be selected. See the Getting Started Chapter of the MassLynx User's Guide for details.

Night Time Process — Select this option to mark this entry as a night time process.

> The MassLynx **Queue Properties** dialog **Night Time Scheduling** option must be selected. See the Getting Started Chapter of the MassLynx User's Guide for details.

Process

Pre-Run, **Post-Run** — When submitting a batch to the MassLynx Queue to be acquired, processed, etc., these fields allow the user to specify an executable to be run before the batch starts and when the batch has finished. Any .EXE file can be run, hence this allows Users to write their own applications to perform some task before or after a batch is executed

4. Quantify the Data

Once data has been acquired, it can be Quantified by creating a Dataset containing the samples specified in the Sample List. Select the **QuanLynx**, **Process Samples** from the MassLynx shortcut bar, to invoke the **Create QuanLynx Dataset** dialog. Select the options as required (details below) and press the **OK** button.

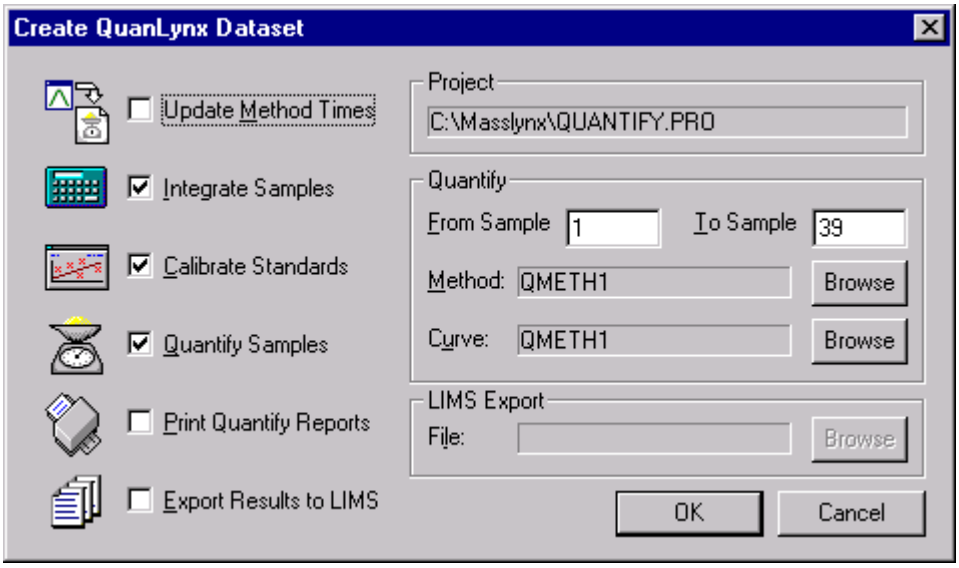


Figure 2.13 The Create QuanLynx Dataset dialog

Update Method Times	Select this option to update the Peak Location Retention Time of compounds in the Quantify Method. This operation will modify the Dataset Method. This is useful if compound elution times have changed due to different Liquid Chromatography (LC) conditions. A reference sample is required for this process to operate; a high-level calibration standard could be used for this purpose. The reference sample should be indicated by placing an 'x' in the MassLynx Sample List **Quan Reference** column before the Dataset is created.
Integrate Samples	Integrates all the sample data files named in the Sample List.
Calibrate Standards	Uses Integration results to form Quantify calibration curves. Do not select this option if an existing calibration is to be used; in this case use the **Curve:**, **Browse** button to select the desired calibration file
Quantify Samples	Uses Integration results and Quantify calibration curves to calculate compound concentrations.
Print Quantify Reports	Produces hard copies of the results of integration and quantitation.
Export Results to LIMS	Produces a text file containing the quantitation results details for use with LIMS systems. If this option is selected, the **LIMS Export File: Browse** button is enabled; press the **Browse** button and select a file, or enter the name of a new one, and press **Save**. Refer to the Export section of Chapter 3, The QuanLynx Browser, for further details.
Project:	The name of the current project. To quantify using a different project, exit this dialog, change the current project and select the MassLynx **Quantify**, **Process Samples** command again.

Quantify

From Sample **To Sample**	Sets the range of samples in the Sample List which will be quantified.
Method: or **Curve**	To change the**:** files press the appropriate **Browse** button and select a new file.

Press the **OK** button to start the analysis.

5. Examine the Results

The quantitation results are viewed using the QuanLynx Browser, which is invoked by selecting the MassLynx **QuanLynx**, **View Results** form the MassLynx shortcut bar. When creating a new Dataset, it will automatically be loaded into the Browser.

To save the quantitation results to a file, select the QuanLynx Browser **File**, **Save As** command.

> If the **Start Sample List Run** dialog **Auto Quantify Samples** option has been used, the quantitation results will be automatically saved to a Dataset, see Chapter 3, The QuanLynx Browser for further details.

If the required Dataset is not loaded, select the QuanLynx Browser **File**, **Open** command and use the **Open** dialog to select the required Dataset.

For information on using the QuanLynx Browser to examine results, refer to Chapter 3, The QuanLynx Browser.

Files used during Quantify

The Quantify program uses four types of files; these are Sample List, Quantify Method, Calibration Curves and QuanLynx Datasets. It is recommended that Projects are used when performing quantitation, as this allows the data to be easily stored and accessed. For more information on creating Projects, refer to the Getting Started Chapter of the MassLynx User's Guide.

The Sample List (.SPL) File

The Sample List files are normally stored in the \SAMPLEDB directory. Three items in the Sample List are required for quantitation:

File Name Specifies the sample data file name, which will be the same name as the corresponding Peak List file.

Sample Type Specifies the type of sample. This should be set to **Standard** if the sample is to be used to form a calibration curve, **Analyte** if the concentration of the compounds within the samples is to be calculated, **QC** if it is a Quality Control sample, or **Blank** if the sample doesn't contain any analyte compounds.

Concentration Only required if the sample is a **Standard** and is optional for **QC** samples. Specifies the known concentrations of the compounds within the standard. This does not apply to compounds whose concentration has been specified as being constant (**Fixed**), within all samples.

The Quantify Method (.MDB) File

The Quantify Method contains an entry for each of the compounds being analyzed, determining how the data is to be processed. The same method is applied to all the samples in the analysis. For more information, see The Quantify Method Editor section, on page 2-6. The Quantify Method files are normally stored in the \METHDB directory.

Calibration Curves (.CDB) File

Stores the Quantify Calibration Curves which are produced for each of the compounds within the Method. Calibration curves can be exported from QuanLynx Datasets. The Calibration files are normally stored in the \CURVEDB directory.

QuanLynx Datasets (.QLD) File

A Dataset contains the raw data, methods, calibrations and results associated with an instance of processing a set of samples. The Datasets are normally stored in the current Project directory.

The Edit Menu

Further options for setting method parameters are available on the Edit menu (only options not previously described as part of the Edit Method dialog are described).

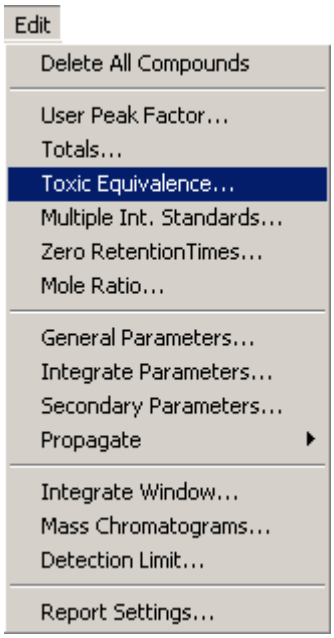


Figure 2.14 The Edit Menu

Delete all compounds

Deletes all compounds from the current method. When selected a confirmation dialog is displayed.

User Peak Factor

Invokes the User Peak Factor dialog box below. This is the same as the User Peak Factor on the main Edit Method dialog. Changes entered through this option do not show in the Edit Method dialog until the method is closed and reopened.

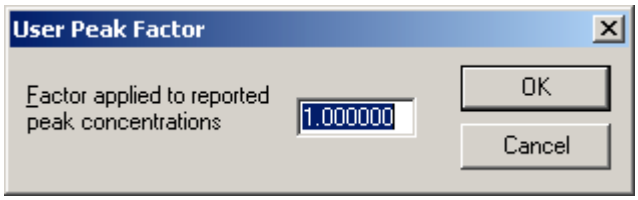


Figure 2.15 The User Peak Factor dialog

Totals

Invokes the dialog shown in Figure 2.24. Further information on this option can be found on page 2-27.

Toxic Equivalence

Invokes the following dialog:

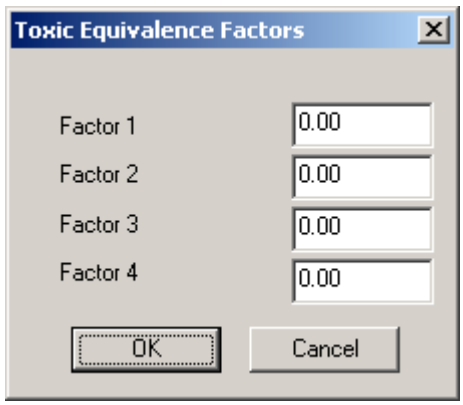


Figure 2.16 The Toxic Equivalence dialog

Enter toxic equivalence factors into the relevant edit box. On clicking OK, the current method is updated and entered values are used to calculate the toxic equivalence of a compound using its calculated concentration.

Mole Ratio

Invokes the dialog box shown below and converts experimentally measured isotope ratios into mole ratios.

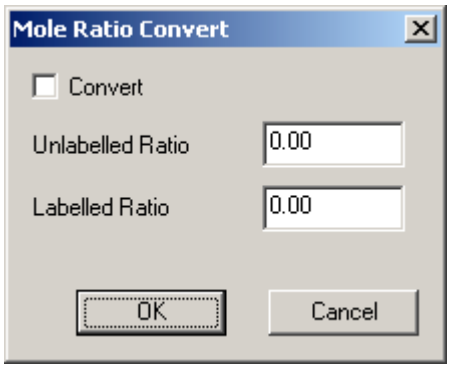


Figure 2.17 The Mole Ratio Convert dialog

For example, to quantify chloroform levels in human blood using a 13C-chloroform internal standard, the 13C-chloroform is contaminated with approximately 5% 12C-chloroform. So regardless of resolution, the addition of internal standard contributes to the native compound signal. Also the naturally occurring level of 13C leads to the native analyte (12C-chloroform) contributing to the internal standard signal. To adjust for this cross contamination enter unlabelled and labeled ratios for the internal standard only and for the native analyte.

Secondary Parameters

Select this option to display the dialog box shown below:

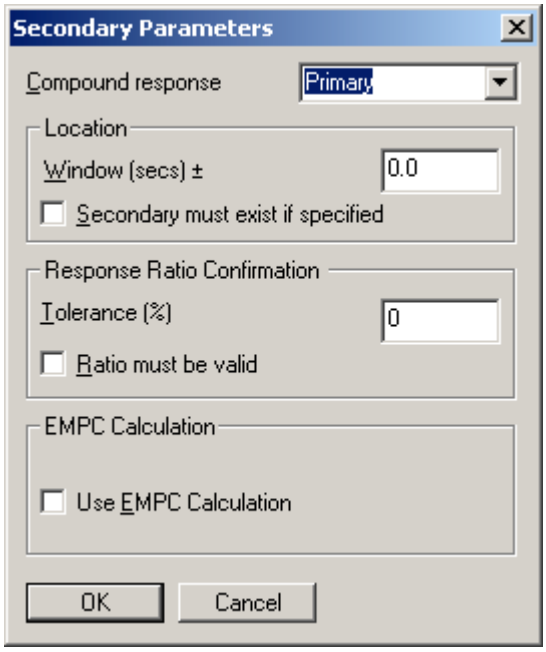


Figure 2.18 The Secondary Parameters dialog

Compound Response

Select from the drop down list if Primary or Secondary peak height / area is to be used to calculate peak responses. The options available are **Primary: Secondary** or **Both** (The sum of primary and secondary peaks is used).

Location

Window (secs) ± The size of the time window used to locate the Secondary peaks in relation to the found Primary Peaks. The window is centered about the RT of the Primary peak and the Secondary peak RT must be within this window to be located.

Secondary must exist if specified If selected the compound will not be located unless a Secondary peak is found within the secondary location window. If no Secondary Trace is specified for a compound this parameter has no effect.

Response Ratio Confirmation

Tolerance (%) Specifies the percentage Tolerance allowed when comparing the actual ratio between the Primary and Secondary peaks

Ratio must be valid If selected the compound will not be located unless the ratio of the Primary and Secondary peaks is within the specified tolerance. If the Primary and Secondary peaks fail the ratio test, it will be flagged as such. If no Secondary trace is specified for a compound or a zero value was set for the predicted ratio this parameter has no effect.

EMPC Calculation

If the Use EMPC Calculation in selected the calculated and predicted ion ratios are compared. If the calculated ion ratio is found to be out of tolerance, then the predicted ion ratio is used to calculate the Estimated Maximum Possible Concentration that will then be used in further calculations.

Propagate

Figure 2.19 The Propagate Menu Options

Selecting Propagate results in three choices, when enabled, making a change in each of the parameter dialog boxes will update the corresponding parameters for all entries in the method. Selecting each parameter results in confirmatory dialog box.

Integrate Window

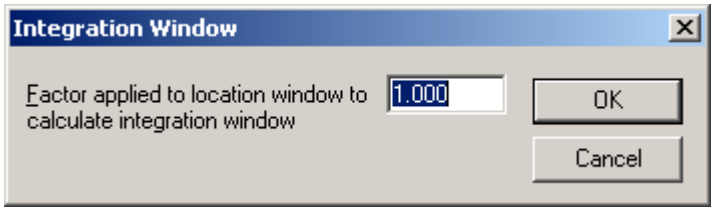


Figure 2.20 The Integrate Window dialog

Entering a number in the available Edit Box sets the integration Window for the current method. The value is used as a multiplication factor for the Time Window when a compounds chromatogram integration range is calculated.

Note. The factor must be equal to or less than 1.

Mass Chromatograms

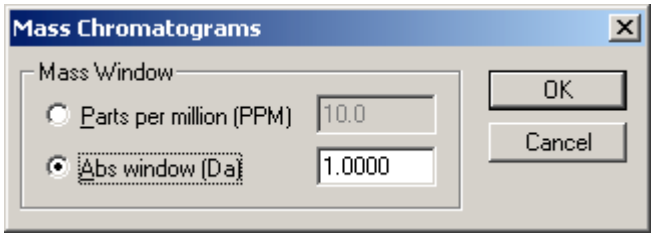


Figure 2.21 The Mass Chromatograms dialog

Parts per million (PPM) Check the radio button to use the specified PPM setting to determine the mass window, around the specified mass, used when generating mass chromatograms.

Abs. Window (Da) Check the radio button to use the specified Da setting as the mass window, around the specified mass, used when generating mass chromatograms.

Detection Limit

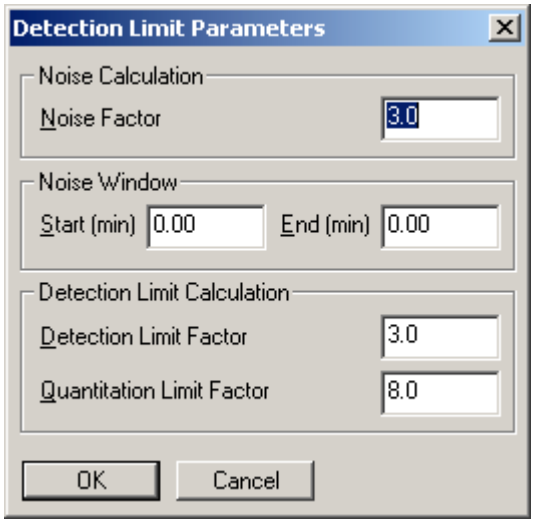


Figure 2.22 The Detection Limit dialog

Noise Calculation

Noise Factor The resulting noise value is the standard deviation of the data, in the specified region, multiplied by the user specified factor

A multiplication factor of 3 is commonly used as, for gaussian deviates, this corresponds to a range within which 95% of the data will lie.

Noise Window

Start (min)
End (min) If supplied the range entered is used, if not auto detect noise is used.

Detection Limit Calculation

Detection Limit Factor The signal to noise ratio at which a particular peak can be reliably detected, if below this value LOD (Limit of Detection) the results are flagged.

Quantitation Limit Factor The signal to noise ratio at which a particular peak can be reliable quantified, if below this value LOQ (Limit of Quantification) the results are flagged.

Report Settings

Invokes the following dialog:

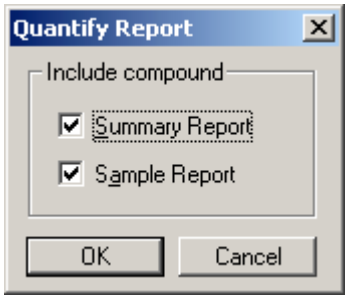


Figure 2.23 The Report Settings dialog

Summary Report Includes the currently selected method compound in printed Summary reports.

Sample Report Includes the currently selected method compound in printed Sample reports.

Totals Compounds

Totals Compounds are unique entries in the Method that are used to calculate and report a concentration, based on a group of peaks within a retention time window.

A Totals entry is used to form the sum of a number of compounds from the same family, where each compound appears as a separate peak on the same chromatogram trace. The Method also looks for individual **Named** compounds from the Totals Group, i.e. each has its own entry in the Method, which corresponds to a single chromatogram peak at a specified time. Peaks that are detected on the chromatogram trace that are not identified by a **Named** compound are referred to as **Unnamed** compounds; they are chemicals of the same type, but are not mentioned specifically in the Method.

A Totals entry can, therefore, sum just the peaks that are **Named** specifically in the Method, just the detected **Unnamed** peaks that are not in the Method, or sum **All** the peaks that have been detected on the chromatogram trace. (The Totals entry will take notice of the Peak Location **Time Window** and only include peaks in that **Retention Time** range).

All compounds within the Totals Group must use the same **Primary** and **Secondary** Traces, see the The Secondary dialog on page 2-8. The integration parameters specified in the Totals Compound will be used for all members of the Group.

To Identify a Totals Compound

1. Select the compound that is to be identified as a Totals Compound in the Quantify Method Editor **Compound:** list.

2. Select **Totals** from the **Peak Selection** drop-down list box.

To Identify Totals Groups

A Totals Group is used to identify all Compounds that are to be included within a Totals calculation. Groups are identified by a Group Name. All compounds that are members of the Totals Group, including the Totals Compound itself, must be given the same Group Name. A

Group Name can be any text string, between one and forty characters in length; it is entered in the **Totals** dialog **Group name** field, see below.

The Group Name must be assigned to each compound in the Totals Group individually.

To Set Totals Parameters

1. Select the compound to which the Totals parameters are to be applied in the QuanLynx Method Editor **Compound:** list.

2. Select the **Edit**, **Totals** command. (The **Totals** dialog is invoked.)

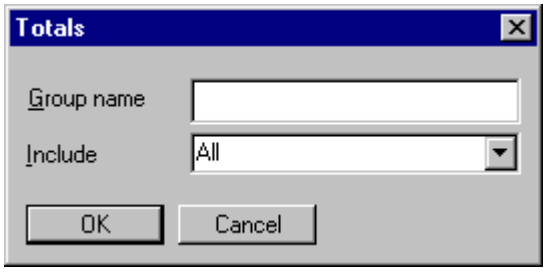


Figure 2.24 The Totals dialog

3. Enter the group identifier n the **Group Name** field. (If the field is left blank, the selected compound will not be included in a Totals Group.)

4. Select the compounds that to be included in this Totals Group from the **Include** drop-down list box. (**All** of the compounds, or just the **Named** or **Unnamed** compounds can be included.)

5. Press the **OK** button to accept the settings.

Peak Integration Settings

The group's Totals Compound is used to specify the peak integration parameters for the whole group. The integration settings for the other members of the group will be ignored.

The Retention Time, over which peak integration takes place, is based on the Totals Compound Peak Location **Time Window**. Peaks that are outside the integration range will not be identified by any of the compounds in the group.

Secondary Ion Criteria

To use Secondary Ion Criteria, both primary and secondary ions must have been specified for the compounds in the Totals Group. Only those peaks that meet the criteria will be included in the Totals calculations.

For **Named** compounds, the compound that has identified the peak will specify the criteria.

For **Unnamed** compounds, the secondary ion criteria will be specified by the Totals Compound method entry.

Setting Location Retention Time Range

For a Totals Group, the retention time at which peaks are included can be restricted, or peaks over the full time of the acquired function can be included. When specifying a retention time range, the time of the center of the window plus the size of the window must be identified.

To Include All Peaks Regardless of Retention Time

1. Select the Totals Compound from the Quantify Method Editor **Compound:** list.

2. Set the Peak Location **Time Window** to zero.

> If the **Time Window** is zero, the **Retention Time**, **Relative Retention Time** and **RT Ref**. field settings will be ignored.

To Specify a Retention Time for Peak Inclusion

1. Select the Totals Compound from the Quantify Method Editor **Compound:** list.

2. Specify the central retention time of the window using the Peak Location **Retention Time**, **Relative Retention Time** and **RT Ref**. fields.

3. Set the Peak Location **Time Window** to the desired time tolerance.

Peak Response Settings

Unnamed peaks are peaks that are part of a Totals Group but have not been specifically identified by another Compound in the group.

The response calculation for these peaks is based on the settings in the group Totals Compound. If **Response** type is set to **Internal**, an Internal Standard peak will be used when calculating peak responses.

To Use a Group Average Internal Standard

1. Select the Totals Compound from the Quantify Method Editor **Compound:** list.

2. Select **None** from the **Internal Ref** drop-down list box.

The average of the group's Named compound Internal Standard responses will be used when calculating **Unnamed** peak responses.

To Use a Specific Internal Standard

1. Select the Totals Compound from the Quantify Method Editor **Compound:** list.

2. Select the name of the compound that is to be used as the Internal Standard from the **Internal Ref** drop down list box.

Totals Compound Calibration Curves

Averaging the calibration curves of the Named compounds in the Totals Group forms Totals Compound calibration curves. The resulting calibration is used to calculate a concentration for the Unnamed peaks in the Totals Group.

The **Concentration of Standards** and **Calibration Curve** settings are ignored for Totals Compounds.

Chapter 3 The QuanLynx Browser

Contents

Figures

Overview

The QuanLynx Browser is used to create and view QuanLynx Datasets, print results or export results to other applications (such as LIMS or Excel) via the clipboard or text files. A Dataset contains the raw data, methods, calibrations and results associated with an instance of processing a set of samples.

Once a Dataset has been created, any reprocessing required can be performed from within the QuanLynx Browser.

If MassLynx Security Signatures are enabled, all operations that modify the Dataset will require the change to be electronically signed; this uses a common signature dialog. See the Other Menu Commands, File Menu section, on page 3-43, for further details.

Creating QuanLynx Datasets

A new QuanLynx Dataset (*.QLD) is created by processing a MassLynx Sample List.

To create a new QuanLynx Dataset, select the **QuanLynx**, **Process Samples** from the shortcut bar; the **Create QuanLynx Dataset** dialog is invoked.

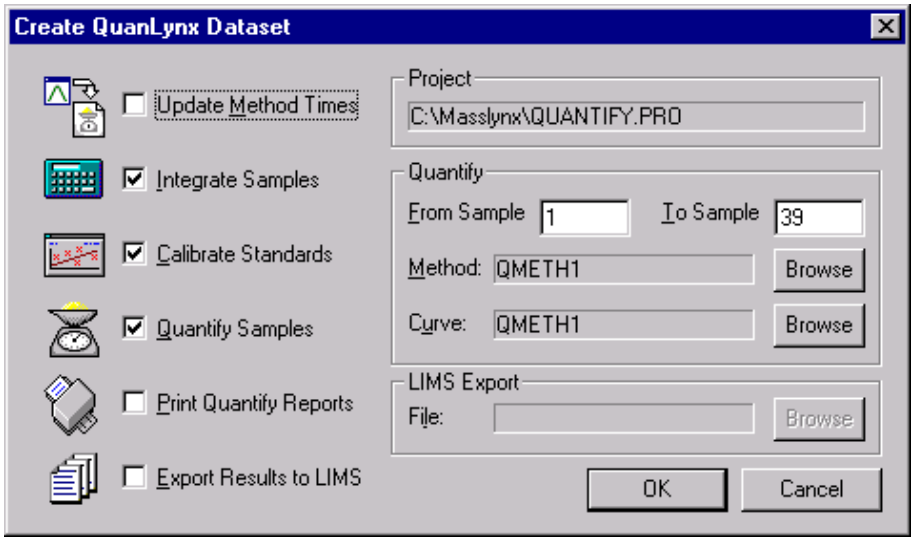


Figure 3.1 The Create QuanLynx Dataset dialog

Select the required Quantify Method, Calibration and processing options, refer to Chapter 2, Quantitation Overview, for details.

A portion of the Sample List can be used to create the Dataset by highlighting the desired samples before the **Quantify**, **Process Samples** command is selected.

The QuanLynx Browser is invoked when the **QuanLynx Dataset** dialog **OK** button is pressed; it will contain the results of the Quantify process. Data review, modification or subsequent processing can then be performed from within the Browser. The Dataset should be saved to a named file using the **File**, **Save** command.

Quantify processing can also be performed in a batch mode; select the MassLynx **Run**, **Start** command to invoke the **Start Sample List Run** dialog.

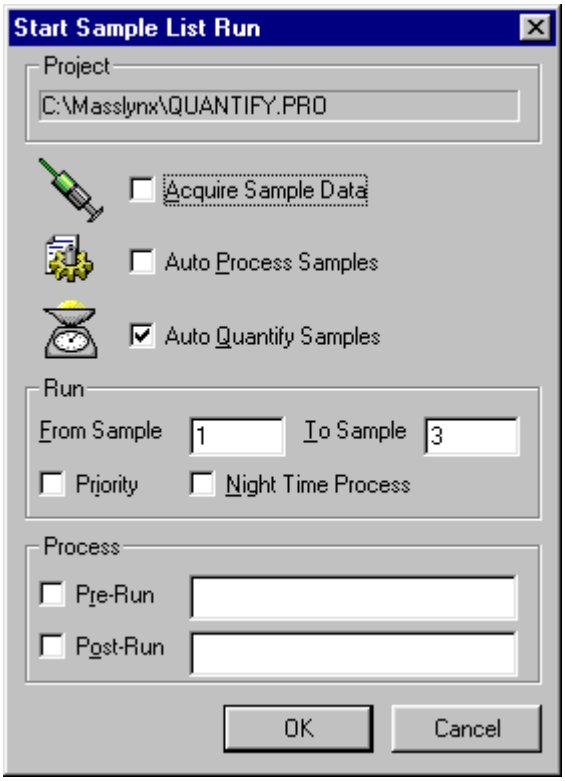


Figure 3.2 The Start Sample List Run dialog

Select the **Auto Quantify Samples** option and select **OK;** the **Create QuanLynx Dataset** dialog is invoked, see Figure 3.1. Select the desired Quantify processing options. In batch mode the created Dataset will be automatically saved to disk.

Accessing the QuanLynx Browser

The QuanLynx Browser file is automatically displayed when a Sample List is quantified from MassLynx.

Alternatively, to access the QuanLynx Browser directly, select **QuanLynx**, **View Results** form the MassLynx shortcut bar..

The QuanLynx Browser Screen

The default screen is split into three main windows, which can be displayed, or hidden, as required by selecting the appropriate option from the **View** menu or **Toolbar**:

- The Summary Window, showing a list of the samples or compounds processed and the quantification results. The summary list can be ordered by sample or by compound.
- The Calibration Window , showing a graphical display of the current compound's calibration curve.
- The Chromatogram Window , showing the chromatograms for the current selection in the Summary window.
- The Totals Bar showing information for peaks that have been included in the currently selected Totals compound.

- The Audit Log Bar displays audit information for the current dataset.
- The **Information** bar displays displays information about the current browser selection. The bar position is fixed at the top of the Browser window.
- The **Status** bar contains information about the current state of the browser and is fixed at the bottom of the Browser window.

Two modeless windows, The Experimental Record Window and The Statistics Window, can be displayed by selecting the appropriate command from the **View** menu or **Toolbar**.

- The Experimental Record Window displays the experimental record for the currently selected sample.
- The Statistics Window displays statistics for QC samples within the current group.

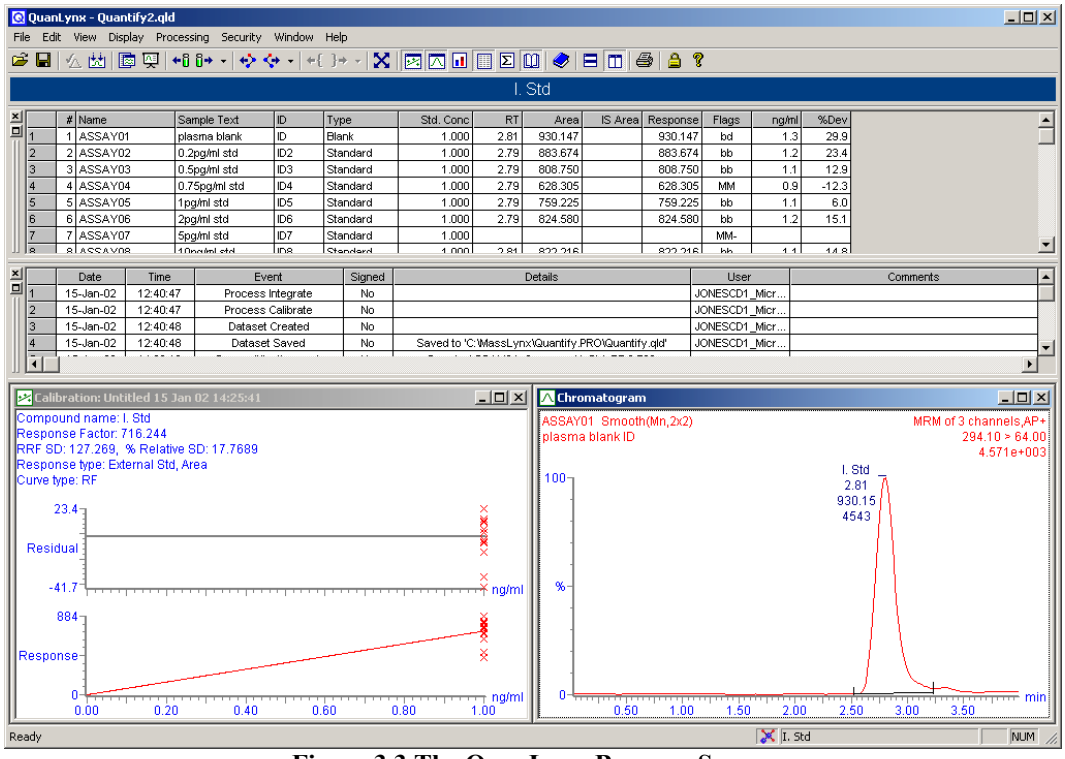


Figure 3.3 The QuanLynx Browser Screen

The QuanLynx Browser Toolbar

The Toolbar can be displayed, or hidden, by selecting the **View**, **Toolbar** command.

Toolbar button	Menu equivalent	Purpose
	File, Open	Opens an existing QuanLynx Dataset.
	File, Save or File, Save As	Saves the current QuanLynx Dataset.

	Save Modified Peaks	Updates any user-modified peak baselines. (Disabled if no modifications have taken place).
	Processing, Execute	Invokes the QuanLynx processing dialog.
	Display, Show Chromatograms	Displays the chromatograms and peak integrations associated with the current Summary selection.
	Display, Slideshow	Starts and stops the slideshow. (Two-state button.)
	Display, Previous Sample	Displays details for the previous sample. (Disabled if there is only one sample.)
	Display, Next Sample	Display details for the next sample. (Disabled if there is only one sample.) The associated drop-down displays a pop-up menu containing the names of all the samples in the Dataset; selecting a sample makes it current.
	Display, Previous Compound	Displays details for the previous compound. (Disabled if there is only one compound.).
	Display, Next Compound	Displays details for the next compound. (Disabled if there is only one compound.). The associated drop-down displays a pop-up menu containing the names of all the samples in the Dataset; selecting a sample makes it current.
	Display, Previous Sample Group	Displays details for the previous sample group. (Disabled if there is only one group.).
	Display, Next Sample Group	Displays details for the next sample group. (Disabled if there is only one group.). The associated drop-down displays a pop-up menu containing the names of all the sample groups in the Dataset; selecting a sample group makes it current.
	Display, Default	Displays the default range for the selected Calibration or Chromatogram window.
	View, Calibration	Shows/hides the Calibration window. (Two-state button..)
	View, Chromatogram	Shows/hides the Chromatogram window. (Two-state button.).

	View, Statistics	Shows/hides the Statistics window. (Two-state button.).
	View, Summary Bar	Toggles the Summary bar. (Two-state button.).
	View, Totals Bar	Toggles the Totals bar. (Two-state button.).
	View, Experimental Record	Toggles the Experimental Record window. (Two-state button.).
	View, Audit Log	Toggles the Audit Log bar. (Two-state button.).
	Window, Tile Horizontally	Tiles the windows horizontally. (Two-state button; if depressed, the windows are re-tiled when the main window is resized.).
	Window, Tile Vertically	Tiles the windows vertically. (Two-state button; if depressed, the windows are re-tiled when the main window is resized.).
	File, Print	Prints a QuanLynx Report.
	Help, About Quantify	Displays program information, version number and copyright.

Getting Started

To Open an Existing QuanLynx Dataset

1. Press the  toolbar button, or select the **File**, **Open** command. (The **Open** dialog is invoked.)

> The four most recently used (MRU) Datasets are displayed at the bottom of the **File** menu and can be selected directly.

2. Select the required QuanLynx Dataset file (*.QLD).

3. Press the **Open** button. (The Dataset file is loaded into the QuanLynx Browser.)

> The contents of the QuanLynx Browser Screen will depend on the options currently selected in the **View** menu.

To Import an Existing Project

1. Select the QuanLynx **File**, **Import** command. (The **Import** dialog is invoked.)

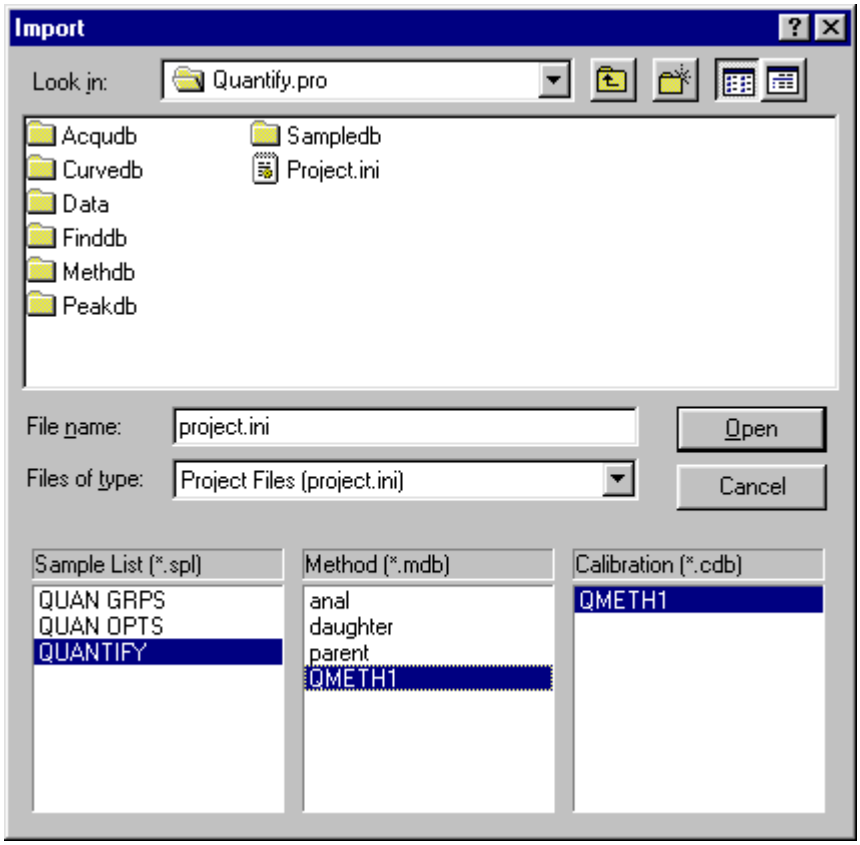


Figure 3.4 The Import dialog

2. Select the required Project in the Browser view.

3. Select the required **Sample list**, Quantify **Method** and **Calibration** from the list boxes. (These default to the Project's current settings.)

   Ensure that selected Quantify **Method** is appropriate for the samples that have been acquired.

4. Press the **Open** button. (The Dataset file is loaded into the QuanLynx Browser).

> The contents of the QuanLynx Browser Screen will depend on the options currently selected in the **View** menu.

5. Save the imported Dataset to a file by selecting the **File**, **Save** command.

The Summary Window

	#	Name	Sample Text	ID	Type	Std. Conc	RT	Area	IS Area	Response	Flags	ng/ml	%Dev
1	1	ASSAY01	plasma blank	ID	Blank	1.000	2.81	930.147		930.147	bd	1.3	26.3
2	2	ASSAY02	0.2pg/ml std	ID2	Standard	1.000	2.79	883.674		883.674	bb	1.2	20.0
3	3	ASSAY03	0.5pg/ml std	ID3	Standard	1.000	2.79	808.750		808.750	bb	1.1	9.8
4	4	ASSAY04	0.75pg/ml std	ID4	Standard	1.000	2.79	753.757		753.757	bb	1.0	2.3
5	5	ASSAY05	1pg/ml std	ID5	Standard	1.000	2.79	759.225		759.225	bb	1.0	3.1
6	6	ASSAY06	2pg/ml std	ID6	Standard	1.000	2.79	824.580		824.580	bb	1.1	11.9
7	7	ASSAY07	5pg/ml std	ID7	Standard	1.000	2.79	916.398		916.398	bb	1.2	24.4
8	8	ASSAY08	10pg/ml std	ID8	Standard	1.000	2.81	822.216		822.216	bb	1.1	11.6
9	9	ASSAY09	15pg/ml std	ID9	Standard	1.000	2.79	801.626		801.626	bb	1.1	8.8

Figure 3.5 The Summary window, List by Compound view

	#	Name	Trace	RT	Area	IS Area	Response	Flags	ng/ml	%Dev
1	1	I. Std	294.10 > 64.00	2.79	884.385		884.385	bb	1.1	7.8
2	2	Parent	288.10 > 58.00	2.79	101.248	884.385	0.114	bb	0.2	5.7
3	3	Metabolite	274.10 > 182.10	2.63	55.113	884.385	0.062	bb	0.2	7.3

Figure 3.6 The Summary window, list by Sample view

The **Summary** window displays a summary of the results of quantification. The results can be listed either by compound or by sample. If a peak has not been located for a compound entry the peak information fields will contain their default values.

Many columns of quantification information can be displayed in the **Summary** window; for details on amending the column format, see the Modifying Column Formats section on page 3-20.

Note: A multiple row selection can be made by clicking and on the row index. The chromatograms associated with the selection can then be viewed by pressing the **Show Chromatograms** toolbar button,  , by selecting the **Display**, **Show Chromatograms** command, or by clicking with the right-hand mouse button on the **Summary** Window and selecting the **Show Chromatograms** option from the pop-up menu. Once a multiple selection has been viewed, it will be maintained when selecting Next and Previous sample or compound, and also for Slide Show operations.

List by Sample

To list by sample, select the **Display**, **Options**, **Summary** page **List By: Sample** command, or click with the right-hand mouse button on the **Summary** window and select **List by Sample** from the pop-up menu. When in the List by Sample view, the  symbol, followed by the name of the sample selected, is shown on the status bar.

The compounds for the sample are shown in the **Summary** window and the chromatograms for the compound highlighted in the **Summary** window are shown in the **Chromatogram** window.

To view details of the selected compound (e.g. standard, metabolite or parent) for another sample, press the  or  toolbar buttons, or select **Previous Sample** or **Next Sample** from the **Display** menu.

To view details for another compound for the current sample, press the  or  toolbar buttons, or select **Previous Compound** and **Next Compound** from the **Display** menu. A different compound can also be selected by double clicking anywhere on the row of a compound in the **Summary** window.

Press Ctrl+Up Arrow or Ctrl+Down Arrow to select the previous or next compound. Press Ctrl+Left Arrow or Ctrl+Right Arrow to select the previous or next sample.

List by Compound

To list by compound, select the **Display**, **Options**, **Summary** page **List By: Compound** command, or click with the right-hand mouse button on the **Summary** window and select **List by Compound** from the pop-up menu. When in the List by Compound view, the  symbol, followed by the name of the compound selected, is shown on the status bar.

The list of samples is shown in the **Summary** window and the chromatograms for the highlighted samples are shown in the **Chromatogram** window.

To view details of the selected compound (e.g. standard, metabolite or parent) for another sample, press the  or  toolbar buttons, or select **Previous Sample** or **Next Sample** from the **Display** menu. Alternatively, press Ctrl+Up Arrow or Ctrl+Down Arrow to select the previous or next sample respectively. A different sample can also be selected by double-clicking, with the left-hand mouse button, anywhere on the row of a sample in the **Summary** window or on a point in the **Calibration** window.

To view details for another compound for the current sample, press the  or  toolbar buttons, or select **Previous Compound** or **Next Compound** from the **Display** menu. A different compound can also be selected by clicking, with the right-hand mouse button, on the **Calibration** window and selecting **Previous Compound** and **Next Compound** from the pop-up menu.

The Calibration Window

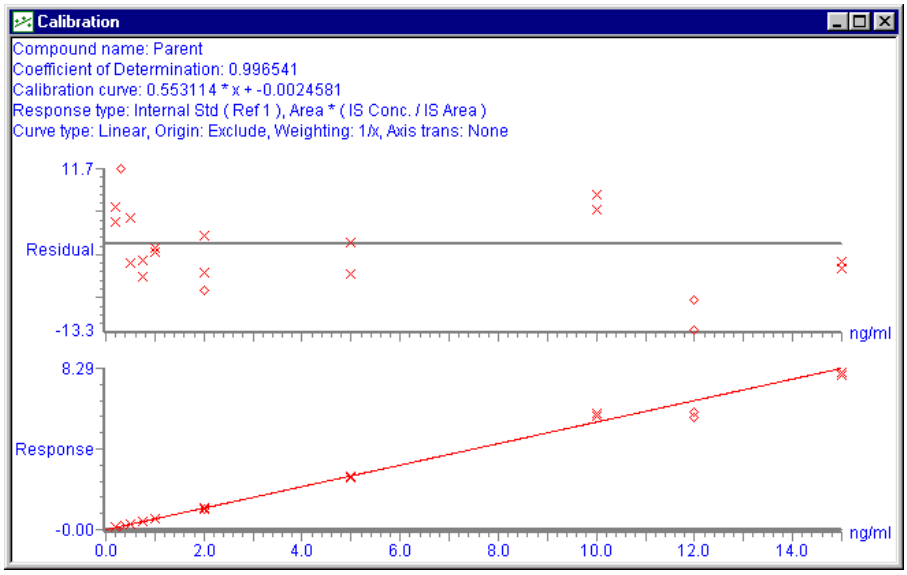


Figure 3.7 The Calibration window

The **Calibration** window contains a graphical display of the current calibration curve and/or its residual plot. Statistical information on the calibration curve is displayed above the graphs.

The calibration curve graph displays peak response value against concentration. The vertical axis is labeled Response. The horizontal axis is labeled with the concentration units specified in the method. The displayed calibration curve shows the response value measured for particular concentrations. Crosses mark the calibration points used to form the curve.

The Residual plot displays concentration against relative delta concentration at the calibration points. This shows the percentage difference between the concentration predicted by the calibration curve and the actual concentration at the calibration points.

The calculated response of the compound within any Quality Control samples can be optionally plotted on the calibration curve; these points are indicated by diamonds, for more information see the The Options Dialog, Calibration Page section, on page 3-26.

Changing the Display Range of the Calibration Window

Both the horizontal and vertical display ranges of the **Calibration** window can be expanded. Press the left-hand mouse button at one end of the region of interest, and without releasing the button, drag the mouse horizontally or vertically, or in both directions, to the other end. As the mouse is dragged, a “rubber band” is stretched-out to indicate the range selected. When the mouse button is released, the selected range will be re-displayed to fill the current window.

This operation can be repeated as often as required.

Pressing the  toolbar button once restores the previous display range; pressing it a second time restores the default display range.

Displaying More Information about a Particular Calibration Point

Place the cursor over the calibration point in the **Calibration** window; an Information ToolTip is displayed.

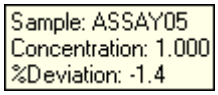


Figure 3.8 Typical Information ToolTip

Displaying a Calibration Point Chromatogram Peak

To display the chromatogram peak associated with a calibration point, double-click on the desired calibration point, or click with the right-hand mouse button and select **Show Chromatograms** on the pop-up menu.

To Exclude, or Include, Calibration Points

If, once the calibration curves have been created, a calibration point is seen to be erroneous it can be removed from the calibration. Click with the right-hand mouse button on the calibration point and select **Exclude** from the pop-up menu. A circle is drawn around the calibration to point to show that it has been excluded.

The calibration curve will be re-plotted using only the included calibration points; concentrations are recalculated using the updated calibration. Excluded points are denoted in the Summary Reports by the addition of an "X" to the **Detection Flags** column.

To include a calibration point that has been excluded, click with the right-hand mouse button on the calibration point and select **Exclude** (which will have an adjacent tick mark) from the pop-up menu.

> If MassLynx Security Signatures are enabled, an electronic signature will be required, see the Other Menu Commands, File Menu section, on page 3-43, for further details.

To Change the Calibration Properties

Select the **Edit**, **Properties** command; the **Calibration Properties** dialog is invoked.

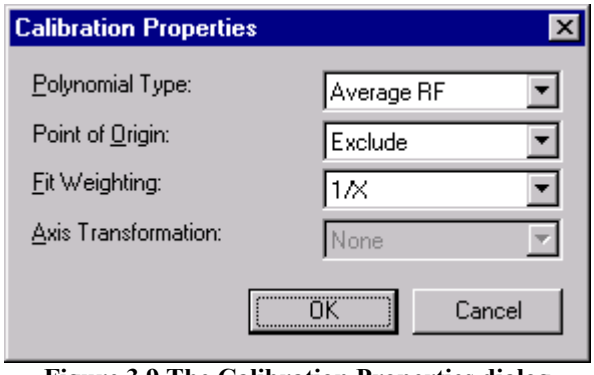


Figure 3.9 The Calibration Properties dialog

These parameters determine how a compound's calibration curve is to be formed.

Polynomial Type:

Select the type of calibration curve from the drop-down list box. The options are:

Average RF Produces a calibration that is a straight line through the origin and the mean response factor of the calibration points. A response factor is the response of a calibration point divided by its concentration. This option should be selected for compounds with a **Fixed** concentration.

Linear Performs a linear regression on the compound's calibration points.

Quadratic Performs a second order regression on the compound's calibration points.

Cubic Performs a third order regression on the compound's calibration points.

Quartic Performs a fourth order regression on the compound's calibration points.

Point of Origin

Select the type of calibration curve from the drop-down list box.

> At the point of origin it is assumed that zero concentration has a response of zero. If **Polynomial Type** is set to **Average RF** this parameter is not used.

The options are:

Force The calibration curve will always pass through the origin.

Include The point of origin will be included in the calibration curve regression, the curve will not usually pass through the origin.

Exclude The origin will be ignored when forming the calibration curve.

Fit Weighting

Select from , **None**, **1/X**, **1/X^2**, **1/Y** or **1/Y^2** in the drop-down list box. This parameter is used to give higher priority to calibration points with a low concentration or response when using regression to fit a calibration curve. This generally results in the calibration curve being fitted closer to points at low concentrations, hence reducing the relative error at these points.

Axis Transformation

Select the required option from the drop-down list box. The available options are **None**, **Ln** (Natural Log), **Log** (Base 10 Log) and **Square Root**. The transformation is applied to the concentration and response values before the calibration curve is fitted.

> Axis transformations cannot be used with RF-type curves, curves that use point weighting, or curves that include, or force, the origin.

The Chromatogram Window

The **Chromatogram** window displays the processed chromatograms associated with the currently selected samples or compounds.

The text on the left-hand side of the window displays the name of the raw data file from which the chromatogram was extracted and a description of any processing that has been performed.

The text on the right-hand side of the window displays a description of the chromatogram, including the maximum intensity.

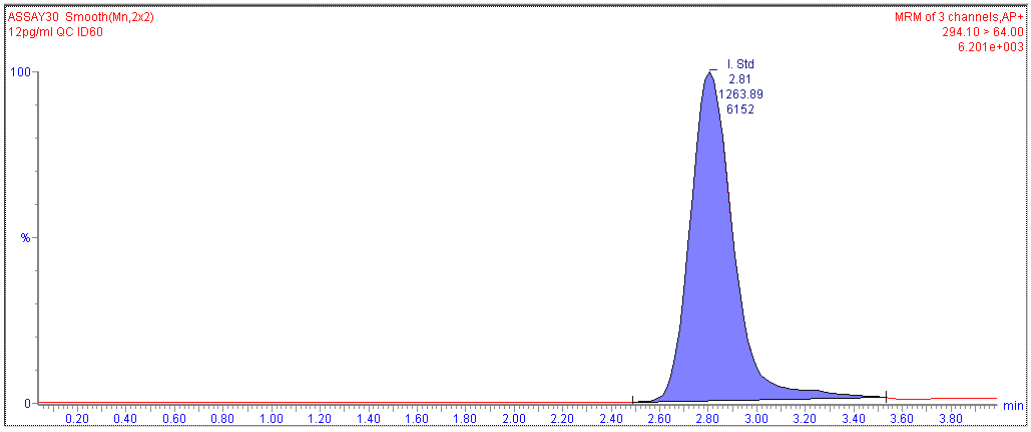


Figure 3.10 The Chromatogram window

Altering the Range of the Horizontal Axis (Zoom) with the Mouse

Move the cursor to one end of the region of interest, press the left-hand mouse button and, without releasing the button, drag the cursor horizontally to the other end. As the cursor is dragged, a "rubber band" is stretched-out to indicate the range selected. When the mouse button is released the selected range will be re-displayed to fill the current window.

This operation can be repeated as often as required.

Pressing the  toolbar button once restores the previous display range; pressing it a second time restores the default display range.

Viewing Other Chromatograms

Each entry in the results table can have one or more chromatograms associated with it. To view another chromatogram page down using either the scroll bar or the arrow keys.

Editing Detected Peaks

The results of integration can be changed by, moving the position of an individual baseline, adding a single peak, or deleting a peak. Manual changes made to peaks are not saved automatically. If multiple chromatograms are displayed, changes can be made to the peaks on each chromatogram, which can then be saved in a single action.

A "*" is displayed in the peak information when the peak has been modified, but the modifications have not been saved; the "*" is removed when modifications have been saved. "**MM**" denotes that the peak has been manually modified.

If the currently displayed chromatograms are refreshed without peak modifications being saved, the modifications will be lost.

To Edit a Peak Baseline

Click with the left-hand mouse button on a peak. The end points of the peak baseline are displayed as two small squares.

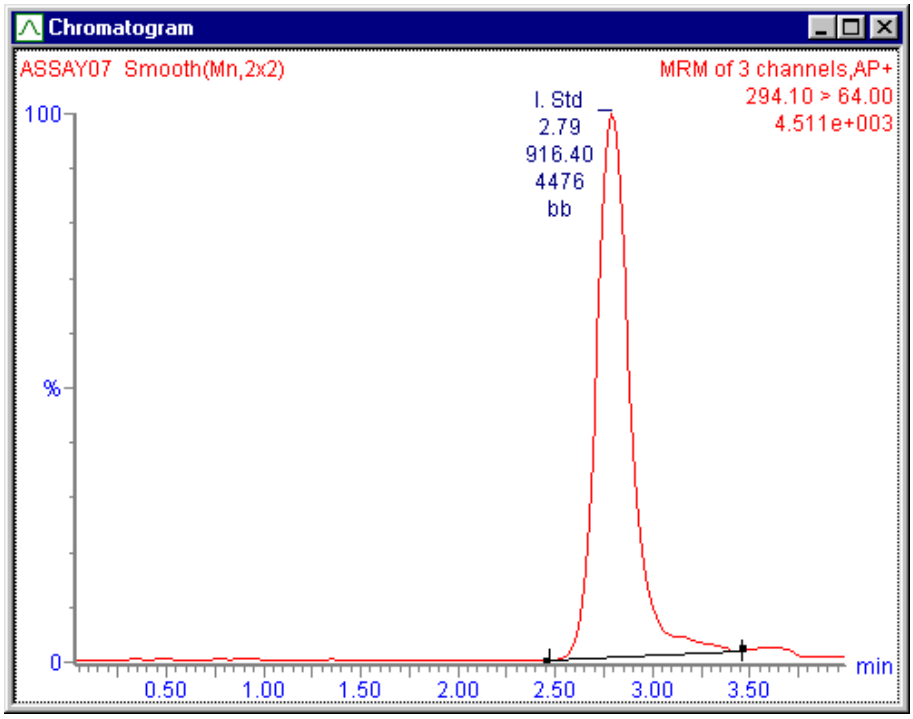


Figure 3.11 The Chromatogram window showing a selected peak

Move the cursor over one of the end points until it changes to a double-ended arrow, click with the left-hand mouse button and drag the end point to the required position. Repeat until the baseline is in the required position.

To Add a New Peak

Right Click on the peak in the **Chromatogram** window and select **Add Peak** from the pop-up menu.

A peak can only be added if one doesn't currently exist for the chromatogram.

To Delete a Peak

Right Click on the peak in the **Chromatogram** window and select **Delete Peak** from the pop-up menu.

The figures in the **Peak Information** group will update to reflect the new peak.

To Save Peak Modifications

To save the changes, press the  toolbar button or Right Click, on the **Chromatogram** window and select **Save Peak Modifications** from the pop up menu displayed. The Quantify results are automatically updated for the changes.

If MassLynx Security Signatures are enabled, an electronic signature will be required, see the Other Menu Commands, File Menu section, on page 3-43, for further details and Chapter 6.

Adding a Flag to a Peak

The user can use a Flag to highlight a peak as an *aide-mémoire*. To add a flag to a peak, right-click on the peak and select the **Flag Peak** option from the pop-up menu. (Selecting this option again will remove the flag from the peak; the option is ticked when the peak is flagged.) The option is

disabled if no peak has been selected. A "**!**" is displayed in the peak information when the peak is flagged.

Flagged peaks must be saved using the **Save Peak Modifications** command described above. The "**!**" will appear in the **Flags** field (if displayed) in the Summary window.

Slideshow

The Slideshow browses through each item in the list displayed in the **Summary** window in turn. The calibration curve and the chromatograms associated with the sample or compound are displayed in the relevant windows.

To start the slideshow, press the  toolbar button, or select the **Display**, **Slideshow** command. Each item in the list is displayed for the time defined in the **Display**, **Options**, **Summary** page **Slideshow Interval (seconds):** field.

To stop the slideshow, press any key, click the mouse button anywhere in the Browser window, or press the  toolbar button again.

The Statistics Window

The **Statistics** window is displayed by pressing the  button, or by selecting the **View**, **Statistics** command; it displays statistics relating to the Quality Control (QC) standards in the current QuanLynx summary.

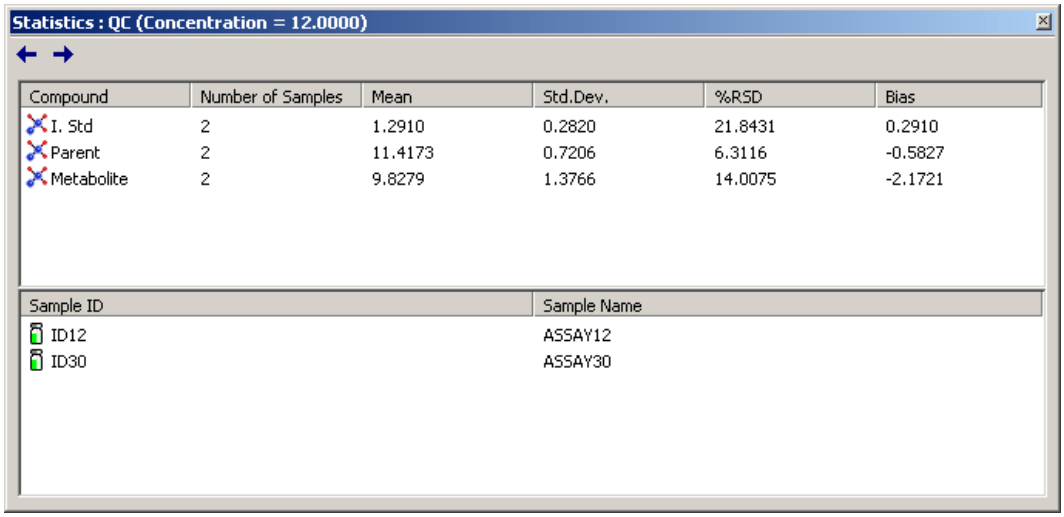


Figure 3.12 The Statistics window

Where there are multiple QC standards at any concentration, the Mean, Standard Deviation and %RSD of the peak areas for each compound in the method are displayed for all of the QC standards at each concentration.

Selecting the QC Standards

Pressing the  and the  buttons at the top of the **Statistics** window selects the previous or next concentration of QC standards.

The Experimental Record Window

The **Experimental Record** window is displayed by pressing the  button, or by selecting the **View**, **Experimental Record** command; it displays the experimental record for the currently selected sample.

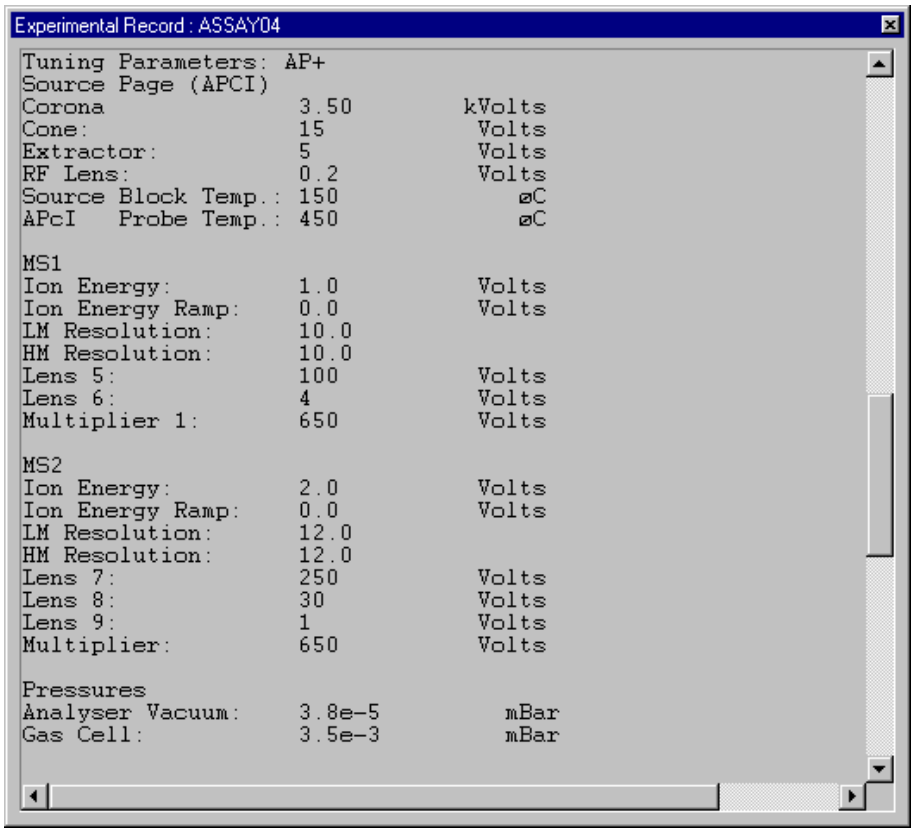
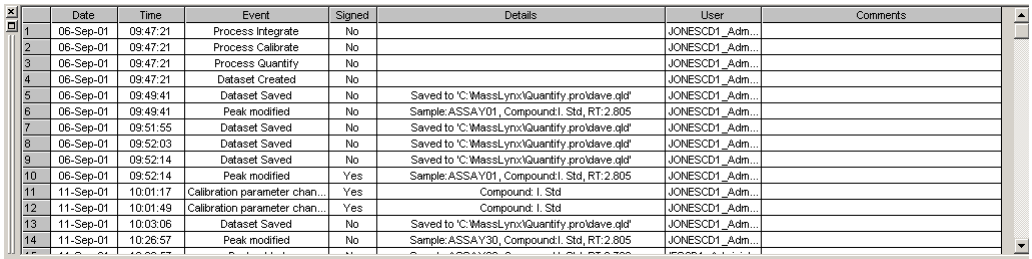


Figure 3.13 The Experimental Record window

The Audit Log Bar

The **Audit Log** window is displayed by pressing the  button, or by selecting the **View**, **Audit Log** command; it displays the audit information for the current Dataset.



	Date	Time	Event	Signed	Details	User	Comments
1	06-Sep-01	09:47:21	Process Integrate	No		JONESCD1_Adm...	
2	06-Sep-01	09:47:21	Process Calibrate	No		JONESCD1_Adm...	
3	06-Sep-01	09:47:21	Process Quantify	No		JONESCD1_Adm...	
4	06-Sep-01	09:47:21	Dataset Created	No		JONESCD1_Adm...	
5	06-Sep-01	09:49:41	Dataset Saved	No	Saved to 'C:\MassLynx\Quantify.pro\dave.qld'	JONESCD1_Adm...	
6	06-Sep-01	09:49:41	Peak modified	No	Sample:ASSAY01, Compound:I. Std, RT:2.805	JONESCD1_Adm...	
7	06-Sep-01	09:51:55	Dataset Saved	No	Saved to 'C:\MassLynx\Quantify.pro\dave.qld'	JONESCD1_Adm...	
8	06-Sep-01	09:52:03	Dataset Saved	No	Saved to 'C:\MassLynx\Quantify.pro\dave.qld'	JONESCD1_Adm...	
9	06-Sep-01	09:52:14	Dataset Saved	No	Saved to 'C:\MassLynx\Quantify.pro\dave.qld'	JONESCD1_Adm...	
10	06-Sep-01	09:52:14	Peak modified	Yes	Sample:ASSAY01, Compound:I. Std, RT:2.805	JONESCD1_Adm...	
11	11-Sep-01	10:01:17	Calibration parameter chan...	Yes	Compound: I. Std	JONESCD1_Adm...	
12	11-Sep-01	10:01:49	Calibration parameter chan...	Yes	Compound: I. Std	JONESCD1_Adm...	
13	11-Sep-01	10:03:06	Dataset Saved	No	Saved to 'C:\MassLynx\Quantify.pro\dave.qld'	JONESCD1_Adm...	
14	11-Sep-01	10:26:57	Peak modified	No	Sample:ASSAY30, Compound:I. Std, RT:2.805	JONESCD1_Adm...	

Figure 3.14 The Audit Log bar

The audit information is displayed in columns with each line being a single auditable event.

The columns can be resized by using the mouse to click and drag the relevant column boundary in the column name bar.

The columns displayed are:

Date	The date on which the event was logged. A signature bitmap is displayed in the column if the event was electronically signed.
Time	The time at which the event was logged.
Event	A brief description of the event.
Signed	Displays **Yes** if the event was electronically signed, **No** if it was not electronically signed.
Details	Displays further event specific information.
User	The full name of the user who created the event
Comment	Information entered by the user.
Start RT	The Retention time at the beginning of the new peak.
Top RT	The Retention time at the top of the new peak.
End RT	The Retention time at the end of the new peak.
Start Intensity	The intensity at the start of the new peak.
End Intensity	The intensity at the start of the new peak.
Peak Area	The area of the new peak.

The Totals Bar

The Totals Bar is displayed by pressing the  button, or by selecting the **View**, **Totals Bar** command; it displays the list of peaks that make up a Totals entry.

	#	Name	Trace	RT	Area	IS Area	Response	Flags	pg	%Dev
1	5	123678-HxCDF	373.821	34.08	266026.781	1567939.250	10.385	bb	9.5	-4.7
2	4	123478-HxCDF	373.821	33.86	256445.016	1476927.125	10.542	bb	9.4	-5.6
3	40	Totals Hexa-Furans	373.821	31.71	194.644	1476927.125	0.008	bb	0.0	-99.3
4	7	123789-HxCDF	373.821	36.50	234451.969	1353719.625	10.580	bd	9.5	-4.8
5	40	Totals Hexa-Furans	373.821	36.04	227.662	1476927.125	0.010	db	0.0	-99.1
6	6	234678-HxCDF	373.821	35.13	258223.313	1474938.625	10.739	bd	9.5	-4.8
7	40	Totals Hexa-Furans	373.821	34.73	2093.870	1476927.125	0.089	bb	0.1	-92.0

Figure 3.15 The Totals Bar

The Totals Bar displays the breakdown of peaks that have contributed to a Totals Compound for the sample and compound currently selected in the **Summary** Window. When the current sample or compound selection is changed, the Totals Bar will update automatically. If the currently selected compound is not a Totals Compound, the Totals Bar will be empty.

The columns can be resized by using the mouse to click and drag the relevant column boundary in the column name bar.

The column formats can be modified; see the Modifying Column Formats section on page 3-20.

Modifying Column Formats

The Columns used in the Summary window, Totals Bar, Audit Bar and Reports can be selected and formatted as follows.

Note: When the QuanLynx Browser is closed, the current column format is retained for when the Browser is next opened.

To Add a Column

Right Click, on the table and select **Show/Hide Columns** from the pop up menu; the **Show/Hide Columns** dialog is displayed.

The **Show/Hide Columns** option is only available in Report format. If the Summary window, or Totals Bar is being amended, only the **Hide Column** menu option is available; columns must be added using the **Change Column Order** option, see below.

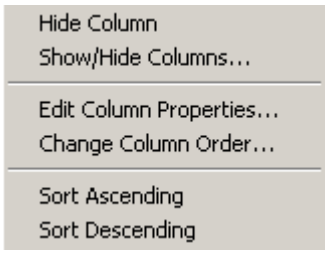


Figure 3.16 Column Format Pop up menu.

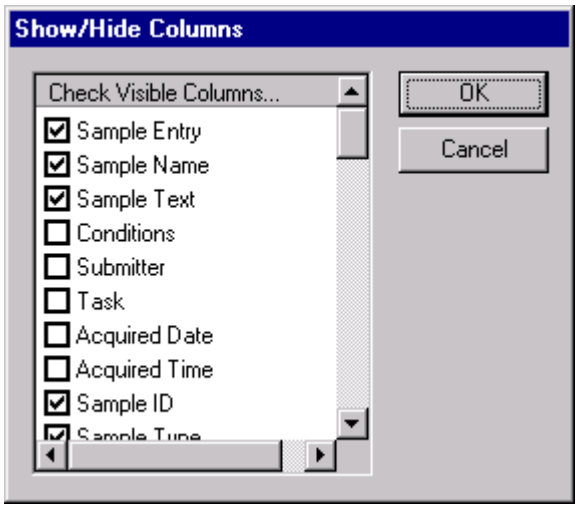


Figure 3.17 The Show/Hide Columns dialog

Select the boxes for the columns to be displayed; deselect those for columns to be hidden.

To Remove a Column

To remove a column, either:

Click with the right-hand mouse button on the column in the table and select **Hide Column** from the pop-up menu.

Or:

Click with the right-hand mouse button on the table and select **Show/Hide Column** from the pop-up menu. Deselect the boxes for the columns to be removed on the **Show/Hide Columns** dialog displayed.

To Hide/Show Columns and Change the Column Order

Right click, on the table and select **Change Column Order** from the pop-up menu. The **Column Order** dialog is invoked.

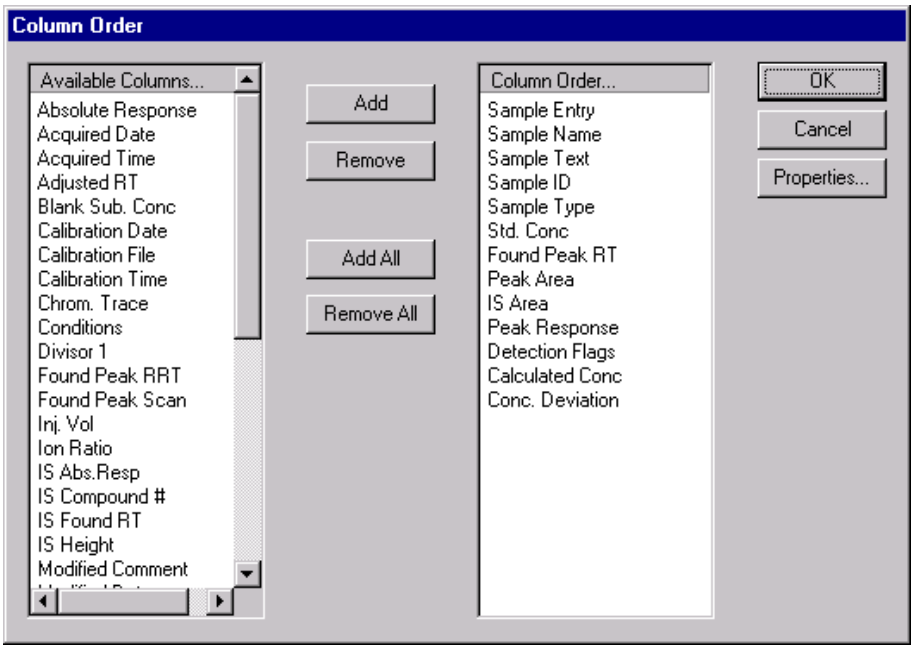


Figure 3.18 The Column Order dialog

Changing the Column Order

Click, with the left-hand mouse button, on the column name in the **Column Order** list, hold the mouse button down and drag the column name to the required position in the list.

To place a column before another one, release the mouse button when the column name is over the name of the column before which it is to be positioned. For example, in the above figure, dragging **Detection Flags** and positioning it on top of **Peak Response**, then releasing the mouse button will place the **Detection Flags** column before the **Peak Response** column.

Adding a Column

To add a column, either:

Click on the column name in the **Available Columns** list, hold the mouse button down and drag the column name to the required position in the **Column Order** list.

Or:

Click on the column name in the **Available Columns** list and press the **Add** button; the column is appended to the **Column Order** list. Use the **Add All** button to add all of the available columns to the **Column Order** list.

Removing a Column

To remove a column, either:

Click on the column name in the **Column Order** list, hold the mouse button down and drag the column name to the **Available Columns** list.

Or:

Click on the column name in the **Column Order** list and press the **Remove** button. The removed column will then be displayed in the **Available Columns** list. Use the **Remove All** button to remove all of the columns from the **Column Order** list.

To Edit Column Properties

Click, with the right-hand mouse button, on a column in the table and select **Edit Column Properties** from the pop-up menu. The **Column Properties** dialog will be invoked.

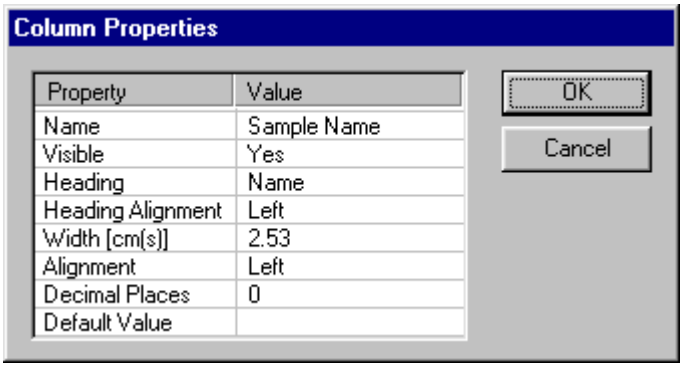


Figure 3.19 The Column Properties dialog

Name — This cannot be changed.

Visible — Click on the value and select the required option from the drop-down list box. If **Yes** is selected the column is displayed, if **No** is selected it is not.

Heading — The text that appears as the column heading. To change it, highlight the text and enter new text.

Heading Alignment — Defines the alignment of the column header. Click on the value and select the required option from the drop-down list box

Width — Defines the width of the column. Click on the value and either enter a new value or use the arrows to change the value by ±0.01 inches or centimeters.

Alignment — Defines the alignment of the column values. Click on the value and select the required option from the drop-down list box.

Format Type — Defines the format of the column, either Decimal Places, Significant Figures or Scientific, chosen from a list box.

Decimal Places / Significant Figures — Toggles between the two, depending on the **Format Type** selected. Defines the number of decimal places to use for this column. Click on the value and either enter a new value or use the arrows to change the value by one decimal place.

Default Value Defines what will be displayed if there is no value for the field. For example, in the Std. Conc column, only samples defined as a Standard will have a value, **---** or **0** could be displayed rather than leaving the field blank. Click on the value and enter the text or value to print.

To Change the Column Width

To change the column width either:

Position the cursor between two column headings; the cursor will change to a double-ended arrow (↔), press the left-hand mouse button and drag until the column is the required width.

Or:

Enter the required **Width** value in the **Column Properties** dialog described above.

To Change the Column Sorting

The order of rows in the table can be sorted based upon the contents of a selected column. To sort using a column, click with the right-hand mouse button on the column and select either **Sort Ascending** or **Sort Descending** from the pop-up menu.

Columns containing numbers will be sorted in numeric order, columns containing text will be sorted in alphabetical order.

Display Options

A number of options are available to change the appearance of the Browser screen; select the **Display**, **Options** command to invoke the **Options** dialog, which has four pages.

The Options Dialog, Summary Page

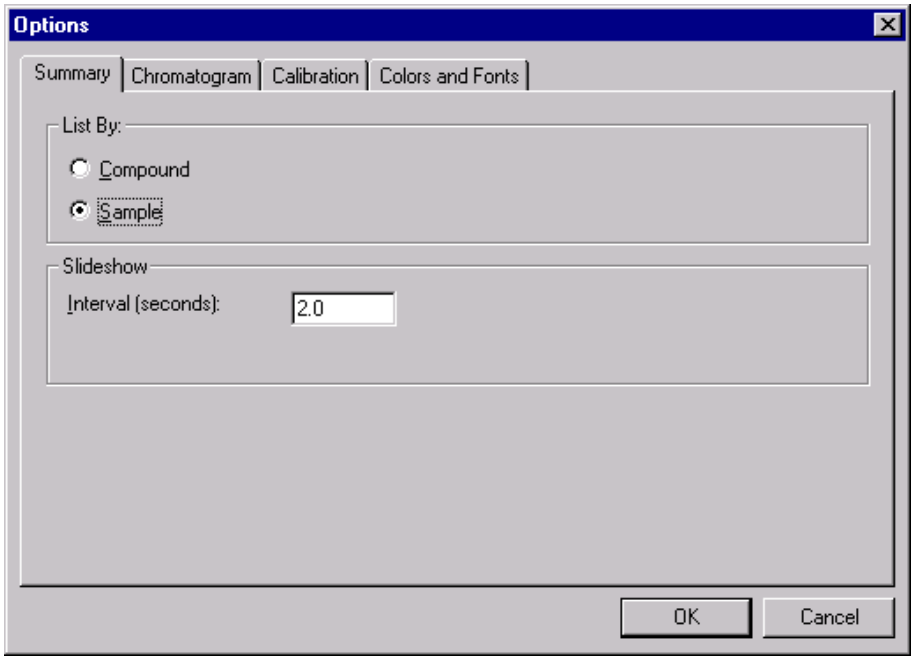


Figure 3.20 The Options Dialog, Summary page

List By

Compound Select this option to display all the samples in the Summary window.

> If **Show all Chromatograms** is checked on the **Options** dialog **Chromatogram** page **Style** list, then the chromatogram for all the compounds for the selected sample will be displayed in the Chromatogram window.

Sample Select this option to display all the compounds for the current sample in the Summary window.

Slideshow

Interval (seconds): Enter the time, in seconds, to display each sample in the Results List window when the slideshow option is selected. See the Slideshow section on page 3-17.

The Options Dialog, Chromatogram Page

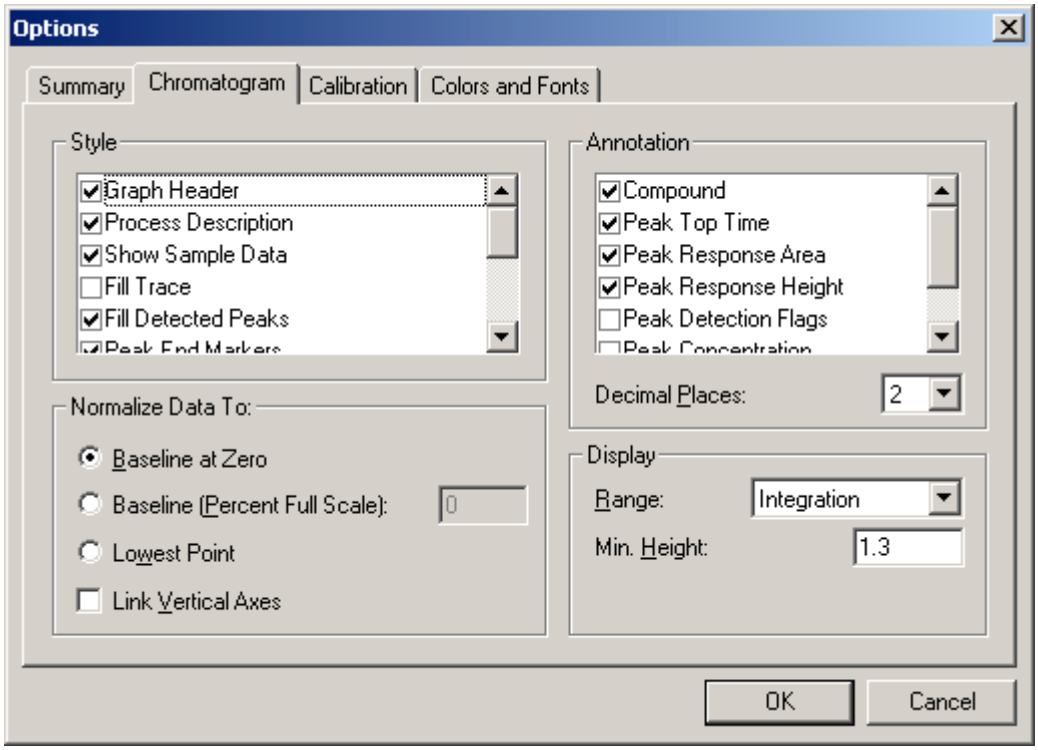


Figure 3.21 The Options Dialog, Chromatogram page

Style

Select the box(es) for the options required.

Graph Header Select this box to display the graph header information at the top of the chromatogram.

Process Description Each process performed on a chromatogram adds a summary of its parameters to the chromatogram header. Select this box to display this process information.

Show Sample Data Select this box to display sample data information at the top of the chromatogram.

Fill Trace	Select this box to fill the area under the chromatogram trace with the Trace color defined on the **Options** dialog **Colors and Fonts** page.
Fill Detected Peaks	Select this box to fill peaks detected by integration in the Filled Peaks color, **Options** dialog **Colors and Fonts** page.
Peak End Markers	Select this box to display the end markers for peaks detected by integration.
Show Zero Level	Select this box to display a horizontal line to indicate the zero intensity level.
Show Original Peak Baselines	Select this box to display the position of the original automatically integrated baseline. This is only applicable if the baseline has been manually modified.
Show Internal Standard	Select this box to display the chromatogram for the compound's Internal Standard.
Show All Chromatograms	Select this box to display all chromatograms related to the selected sample.
Show All Peaks	Select this box to display all peaks that Integration detected on a chromatogram. Otherwise, just the specific chromatogram peak that has been identified with a compound is displayed.

Annotation

Select the box(es) related to the annotations to be displayed on the chromatogram.

Compound	Select this box to annotate the peak with the compound name.
Peak Top Time	Select this box to annotate the peak with the retention time of the peak top in decimal minutes
Peak Response Area	Select this box to annotate the peak with the detected area.
Peak Response Height	Select this box to annotate the peak with the detected height.
Peak Detection Flags	Select this box to annotate the peak with detection flags.
Decimal Places	Select the number of decimal places to display for peak annotation, from the drop down list box

Normalize Data To

These controls specify the scale on the intensity axis

Baseline at Zero	Select this option to scale the vertical axis from 0%.
Baseline (Percent Full Scale)	Select this option and enter an intensity offset in the adjacent control, the vertical axis is scaled from the specified intensity. This option can be useful for displaying chromatograms that have a raised baseline, or entering a negative value allows the zero baseline to be lifted to make viewing of peak integration easier.

Lowest Point Select this option to automatically scale the display so that the lowest point on the trace is at the bottom of the display. This can be useful for displaying Diode Array data if the trace has dropped below zero and the data is negative, or to display chromatograms if there is a high baseline.

Link vertical axes When more than one chromatogram is displayed, select this option to plot both chromatograms on the same intensity scale.

Display Range

Select the required default display range from the drop-down list box.

Acquisition: Display the chromatogram using the range that the data was acquired over.

Integration: Display the chromatogram using the range that the data was integrated over.

Keep Current Display the chromatogram using the currently selected horizontal range.

Min Height Enter the minimum height for a displayed chromatogram. If there is only one chromatogram, it will fill the chromatogram window; if there are two, each will occupy half the window; if there are three, each will occupy a third of the window, etc. A scroll bar is added to the window if the number of chromatograms to be displayed means that the height of each would otherwise be below this value.

The Options Dialog, Calibration Page

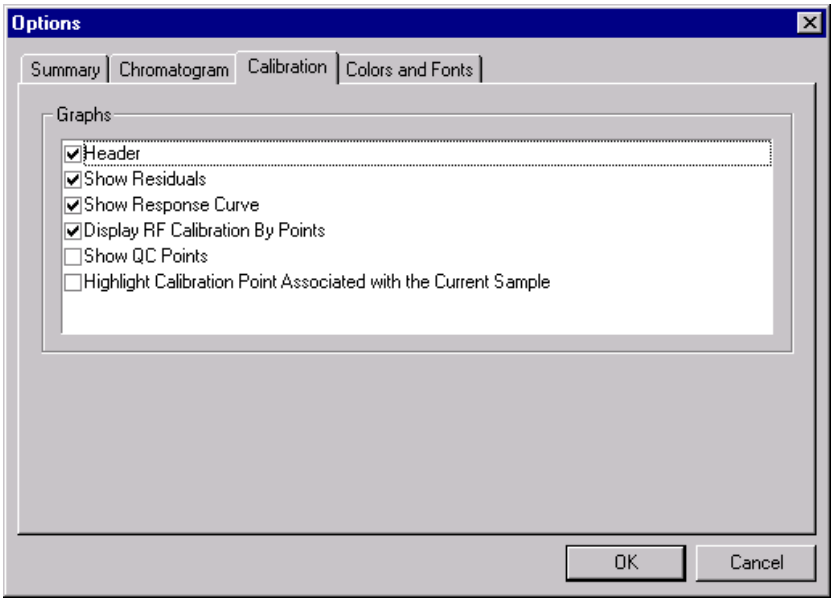


Figure 3.22 The Options Dialog, Calibration page

Graphs

Header Select this box to display the header information at the top of the calibration curve.

Show Residuals Select this box to display the calibration residuals graph.

Show Response Curve Select this box to display the calibration curve graph.

Display RF Calibration by Point	Select this box to display the calibration graphs with the horizontal value as a point number instead of a concentration. This is only applicable for RF-type calibrations.
Show QC Points	Select this box to plot the positions of any QC samples on the calibration graphs; these points are indicated by diamonds.
Highlight Calibration Point Associated with the Current Sample	Select this box to highlight calibration points that correspond to the currently selected Summary sample. The highlight color is defined on the **Options** Dialog **Colors and Fonts** page

The Options Dialog, Colors and Fonts Page

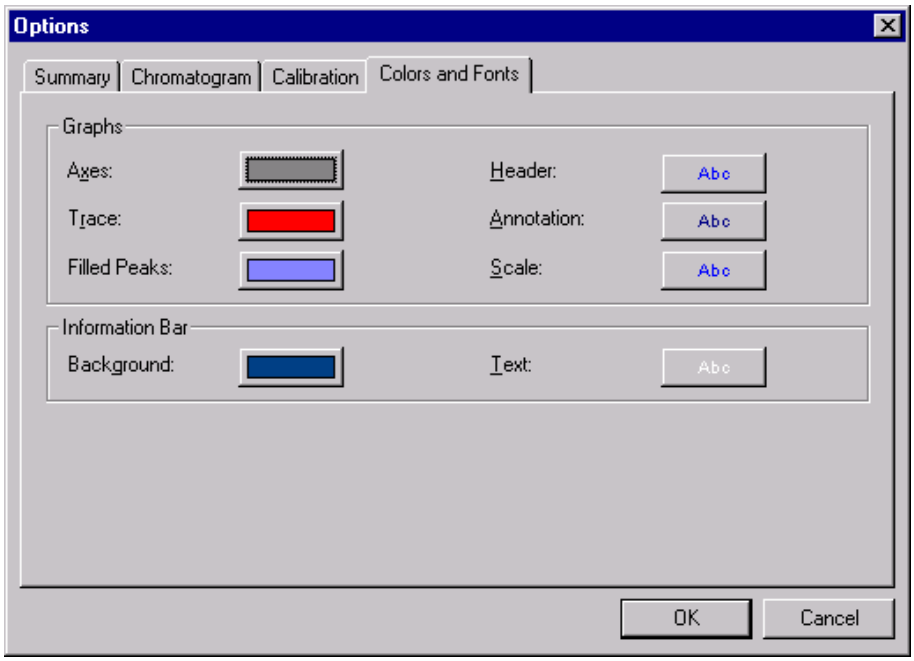


Figure 3.23 The Options Dialog, Colors and Fonts page

The colors and fonts used to display information in the QuanLynx Browser can be selected from this page.

To change the color for the Graph's **Axes:**, **Trace:**, **Filled Peaks:** and Information Bar **Background:** press the appropriate button to display the **Color** dialog.

For the **Basic colors:**, select the required color and press **OK**. **Custom colors:** can also be defined, see the Defining Custom Colors section in the MassLynx NT User's Guide, Getting Started chapter.

To change the font for the Graph's **Header:**, **Annotation:**, **Scale:** and Information Bar **Text:** press the appropriate button to display the **Font** dialog.

Select the required font, style and size, along with any effects or colour, and press **OK**.

Other Display Options

To Change the Size of a Window

To change the size of the windows on display, position the mouse pointer on the line between the two windows until a double-ended arrow appears. Hold down the left-hand mouse button and drag until the window is the required size.

Change the Size of the Display

On a standard screen, set to 800 by 600 pixels, there may not be enough room to display all the information in all of the windows. Changing the desktop settings will display more information:

1. Press the Windows Taskbar **Start** button. Select **Settings** and then **Control Panel**.
2. From the Control Panel select **Display**.
3. On the **Settings** page change the **Desktop Area** to 1024 by 768 pixels, or above.
4. Press the **Test** button and if the test page displays correctly press the **OK** button.

Layouts

Quantify Layouts contain Display Options and Report Format settings; multiple Layouts can be saved for later retrieval. When the QuanLynx Browser is invoked it loads the *default* layout; this is automatically saved when the Browser is exited.

Layouts can be saved to named files so that they can subsequently be reapplied.

To Save the Current Layout

Select the **File**, **Save Layout As** command and enter a name for the layout in the **Save Layout As** browser. Layout files are only modified via this command; they are not automatically updated when changes are made to the Display options or Report format.

To Apply a Saved Layout

Select the **File**, **Apply Layout** command and select the required layout file (*.QLT) from the **Apply Layout** browser. Any changes to the current layout settings will be lost if they haven't already been saved.

Modifying the Method

The Dataset Method parameters can be modified by selecting the **Edit**, **Method** command; this invokes the Quantify **Method Editor** dialog. Changing method parameters may require the information in the Dataset to be reprocessed, see the Processing section, on page 3-29.

> Changing the Method updates the method parameters held in the Dataset; to save these parameters to a Quantify Method file for use with other Datasets, use the **File**, **Export**, **Method** command; this invokes the **Export method** dialog.

For more information see the Method section in Chapter 2, Quantify Overview.

Processing

If processing is not selected when the Dataset is created, or new Data is acquired, or if the Method is edited, reprocessing may have to be performed from within the QuanLynx Browser.

The operations below can be applied individually by selecting them from the **Processing** menu. Multiple processing operations can be performed in a single action by selecting the **Processing**, **Execute** command (which invokes the **Processing** dialog, allowing the required processing operations to be selected), or by pressing the Toolbar Process,  , button.

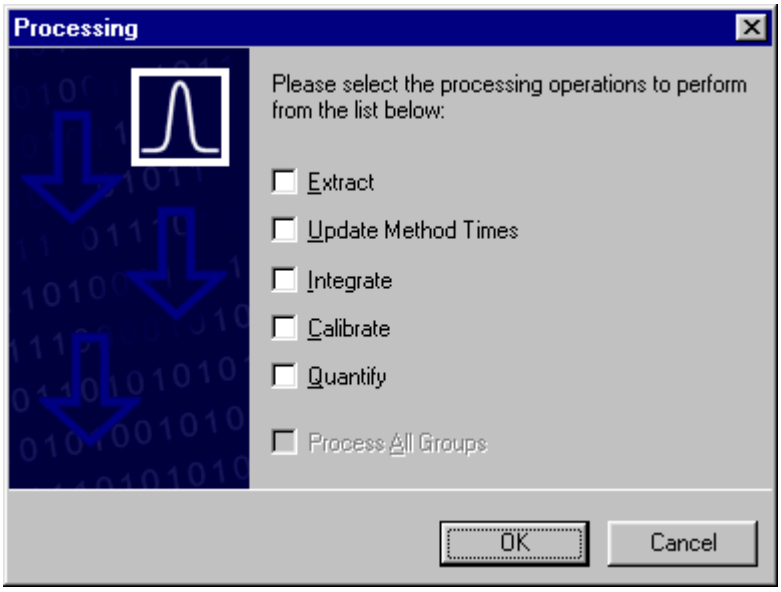


Figure 3.24 The Processing dialog

Extract

This process extracts all the information that is required to perform quantitation from the Raw data files and stores it within the Dataset. This information includes Sample Information, experimental record and chromatogram data. Once this data has been extracted, further processing, data review and reporting are performed using the information encapsulated within the Dataset.

The Extract process is required to access raw data acquired after a Dataset was created and to generate any new chromatograms that are defined in the Method.

Performing the Extract operation will remove any peak information held in the Dataset.

Update Method Times

This process updates the Locate retention time of compounds in the Quantify Method. This operation will modify the Dataset Method. This is useful if compound elution times have changed due to different LC conditions.

A reference sample is required for this process to operate; a high-level calibration standard could be used for this purpose. The reference sample should be indicated by placing an ‘x’ in the MassLynx Sample List **Quan_Ref** column before the Dataset is created.

If this operation is performed by selecting the **Processing**, **Update Method Times** command, the currently selected sample will be used as the reference.

Integrate

This process integrates the chromatograms defined in the Method and assigns detected peaks to the Method compounds.

The Integrate process is required if the automatic peak integration parameters are modified in the Method.

Performing the Integrate operation will remove any manual peak modifications that have been made.

Calibrate

This process generates a calibration curve for each Method compound based on samples that have been identified as Calibration Standards.

The Calibrate process should be performed if any of the Calibration parameters have been modified in the Method. Do not perform calibration processing if an existing calibration is being used.

Performing the Calibrate operation will remove any manual modifications that have been made to the calibration curve.

Quantify

This process applies the current calibration to the detected peak to produce a calculated concentration.

Sample Groups

The QuanLynx Browser can support multiple groups of samples within a single Dataset. Each group consists of a number of Samples, a Quantify Method and a Calibration. The Browser displays the samples and calibration for the currently selected group.

Setting the SAMPLE_GROUP column of the MassLynx Sample List indicates sample groups. Multiple group Datasets can also be automatically generated by Quan-Optimize processing.

To view details for a different sample group, press the  and  toolbar buttons or select **Previous Sample Group** and **Next Sample Group** from the **Display** menu. These buttons are only available if the Dataset contains multiple group information.

Printing Reports

Press the  toolbar button, or select the **File**, **Print** command; the **Print** dialog is invoked.

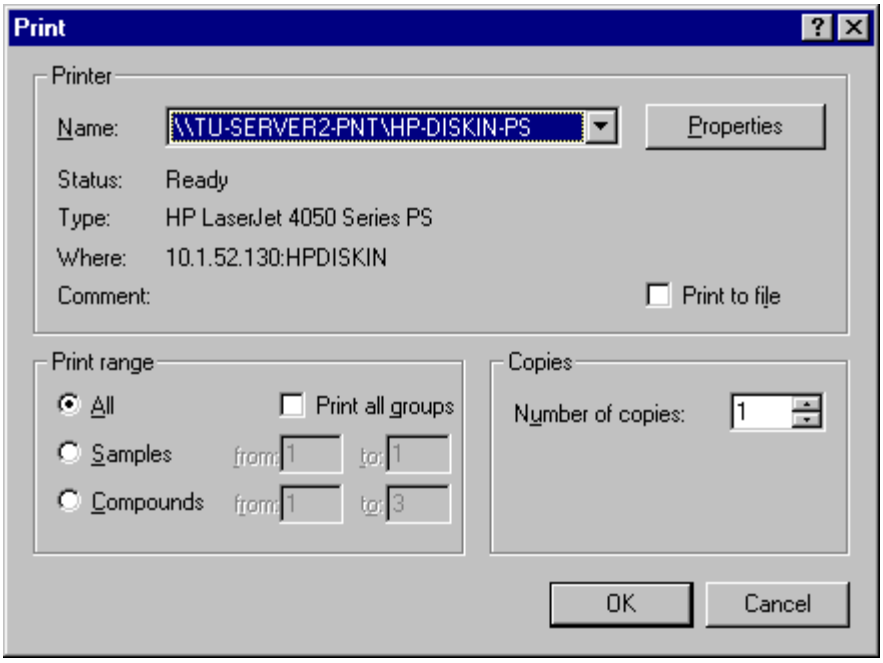


Figure 3.25 The Print dialog

All: Select this option to include all of the results in the printed Report.

Samples Select this option and enter the range of samples to print in the **from:** and **to:** boxes. By default, all of the samples in the list are printed.

Compounds Select this option and enter the range of compounds to print in the **from:** and **to:** boxes

Print all groups Check if the dataset includes groups and all should be printed

Report Formats

The information on printed Reports can be defined using the **Report Format Options** dialog, which has six pages. To access the dialog, select the QuanLynx Browser **File**, **Report Format** command.

The Report Format Options dialog, General Page

Page Numbers Select this option to print page numbers on the Report.

Header: Select this option and enter the text to be printed on each page, below the main Report title.

Footer Select this option and enter the text to be printed at the bottom of each page.

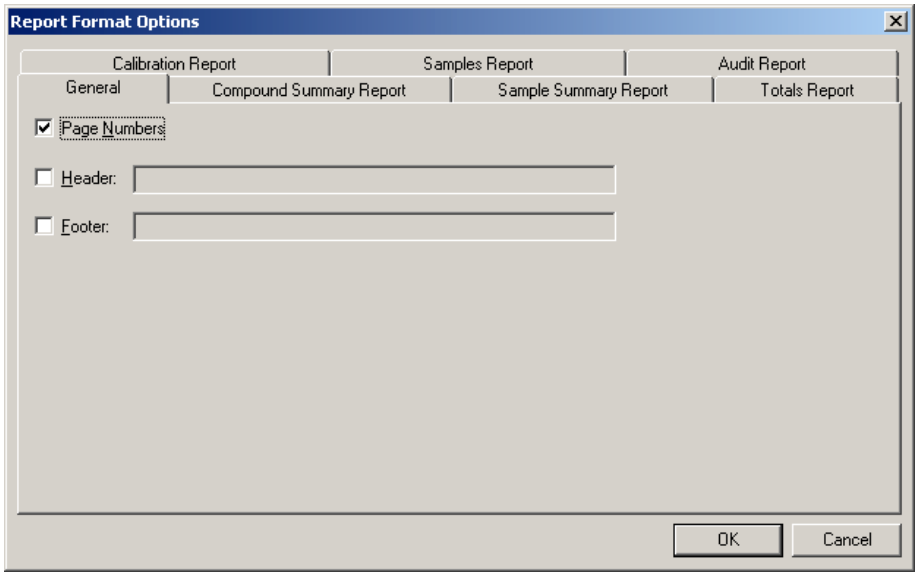


Figure 3.26 The Report Format Options dialog, General page

Calibration Report Page

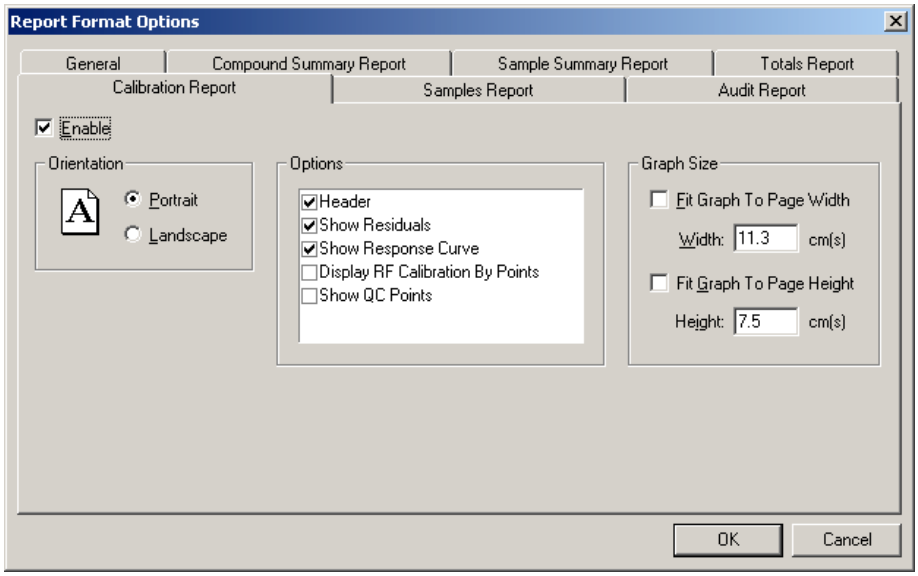


Figure 3.27 The Report Format Options dialog, Calibration page

Enable — Select this option to print calibration information on the Report. If not selected, calibration information will not be printed.

Orientation — Select the page orientation required for the Report: **Portrait** or **Landscape**.

Options — Select the boxes for the calibration information required on the Report. For more information see the Calibrate section on page 3-30.

Fit Graph to Page Width — Select this option to print the calibration graphs the width of the page. To print the graphs a different width, deselect this option and enter the required size in the **Width:** box.

Fit Graph to Page Height — Select this option to print the calibration graphs the height of the page. To print the graphs a different height, deselect this option and enter the required size in the **Height:** box.

Report Column Format Pages

The basic format of each of the Report Column Format pages is the same; each has an **Enable** option, a Page **Orientation** section, an **Options** section and a **Column Format** section. The Report Column Selection pages are:

- Compound Summary Report page.
- Sample Summary Report page.
- Totals Report page.
- Samples Report page.

Compound Summary Report Page

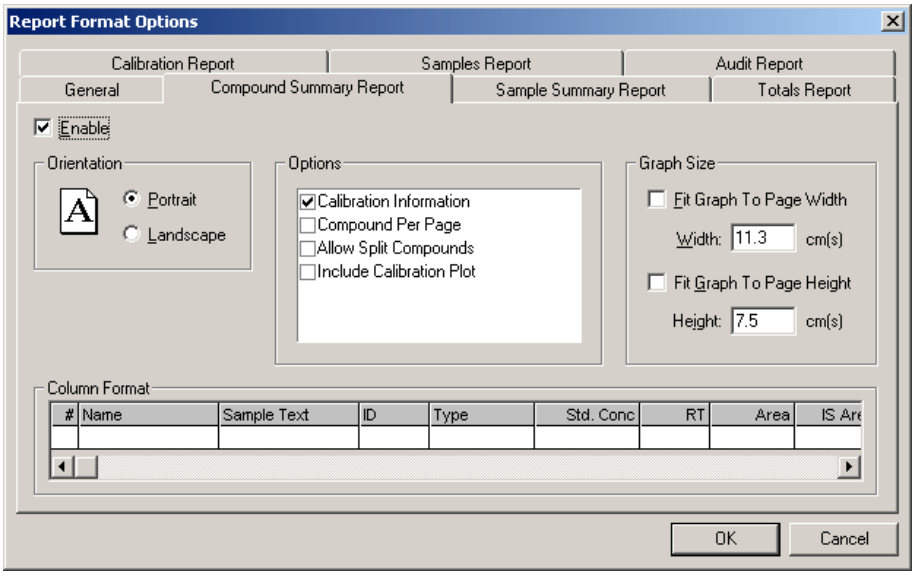


Figure 3.28 The Report Format Options dialog, Compound Summary Report page

Enable	Select this option to print the selected information on the page. If this option is not selected, the information for the page will not be printed
Orientation	Select the page orientation required for the Report: **Portrait** or **Landscape**.
Options	Select the boxes for the options required
Calibration Information	Select this box to display the compound calibration information before the compound summary.
Compound Per Page	Select this box to print information for each compound on a new page.
Allow Split Compounds	Select this box to print the next compound immediately after the previous one.
Column Format	This allows the columns on the Report to be selected and formatted; for details, see the Modifying Column Formats section on page 3-20.
Include Calibration Plot	Allows for plotting of a residual calibration curve underneath the summary.

Sample Summary Report Page

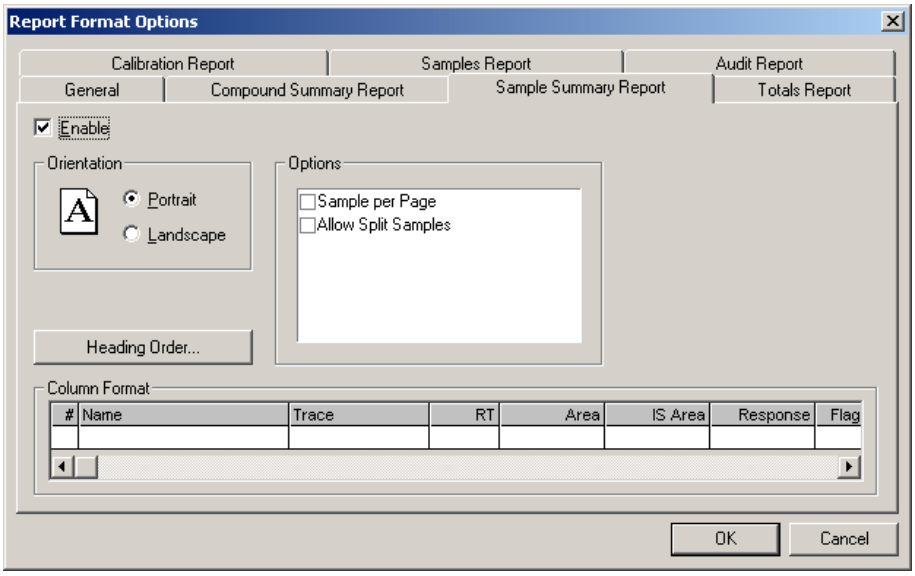


Figure 3.29 The Report Format Options dialog, Sample Summary Report page

Enable	Select this option to print the selected information on the page. If this option is not selected, the information for the page will not be printed.
Orientation	Select the page orientation required for the Report: **Portrait** or **Landscape**.
Options	Select the boxes for the options required.
Sample Per Page	Select this box to print information for each sample on a new page.
Allow Split Samples	Select this box to print the next sample immediately after the previous one.
Column Format	This allows the columns on the Report to be selected and formatted; for details, see the Modifying Column Formats section on page 3-20.

Heading Order

Pressing this button invokes the **Heading Order** dialog, which allows selected sample header information to be included in the Report. The sample header is printed before each sample table

Changing the Heading Order

Click on the heading name in the **Heading Order** list, hold the mouse button down and drag the column name to the required position in the list.

> To place a heading before another one, release the mouse button when the heading name is over the name of the heading it is to be positioned before.

Adding a Heading

To add a heading, either:

Click,on the heading name in the **Available Headings** list, hold the mouse button down and drag the heading name to the required position in the **Heading Order** list.

Or:

Click on the heading name in the **Available Headings** list and press the **Add** button; the heading is appended to the **Heading Order** list. Use the **Add All** button to add all of the available headings to the **Heading Order** list.

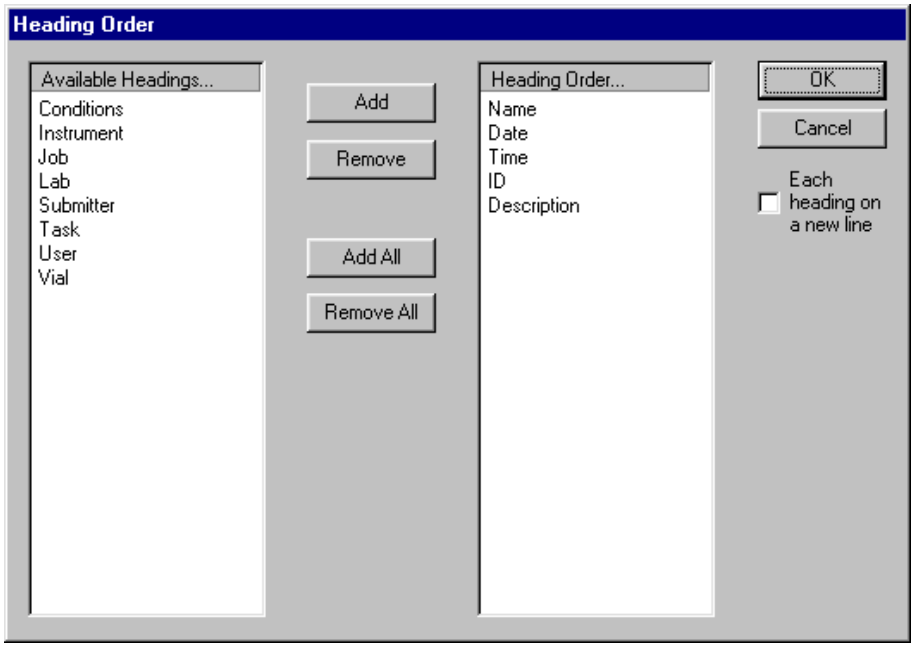


Figure 3.30 The Heading Order dialog

Removing a Heading

To remove a heading, either:

Click on the column name in the **Heading Order** list, hold the mouse button down and drag the column name to the **Available Headings** list.

Or:

Click on the column name in the **Heading Order** list and press the **Remove** button. The removed column will then be displayed in the **Available Headings** list. Use the **Remove All** button to remove all of the headings from the **Heading Order** list.

Totals Report Page

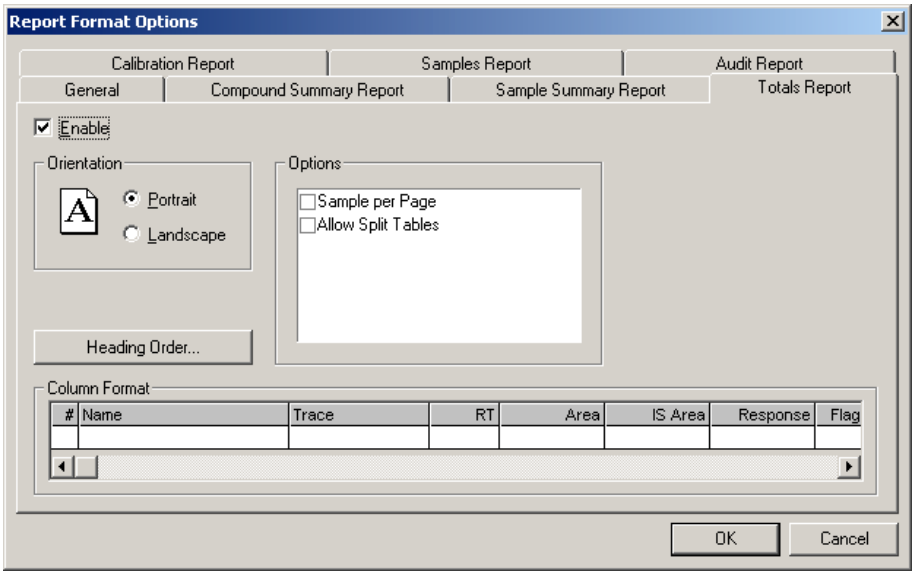


Figure 3.31 The Report Format Options dialog, Totals Report page

This page is virtually identical to the Sample Summary Report Page, see above for details.

Samples Report Page

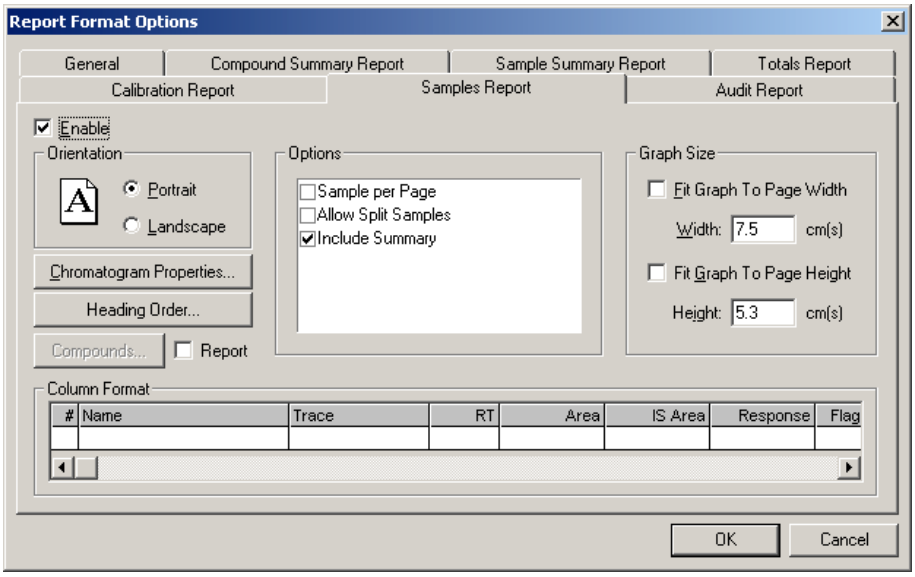


Figure 3.32 The Report Format Options dialog, Samples Report page

Enable: Select this option to print the selected information on the page. If this option is not selected, the information for the page will not be printed.

Orientation Select the page orientation required for the Report: **Portrait** or **Landscape.**

Chromatogram Properties Invokes the Chromatogram Properties dialog; for details refer to The Options Dialog, Chromatogram Page section on page 3-24.

Heading Order Invokes the The Heading Order dialog see page 3-35.

Compounds This button is available when the **Report** option is selected; if it is not selected, all compounds will be printed in the order they appear in the method. When the **Compounds** button is pressed, the **Compound Order** dialog is invoked; this allows the compounds and their order in the Report, to be specified. To display a compound, move it from the **Available Compounds** list to the **Compound Order** list. This dialog operates in a similar manner to the **Column Order** dialog, see the Modifying Column Formats section, on page 3-20.

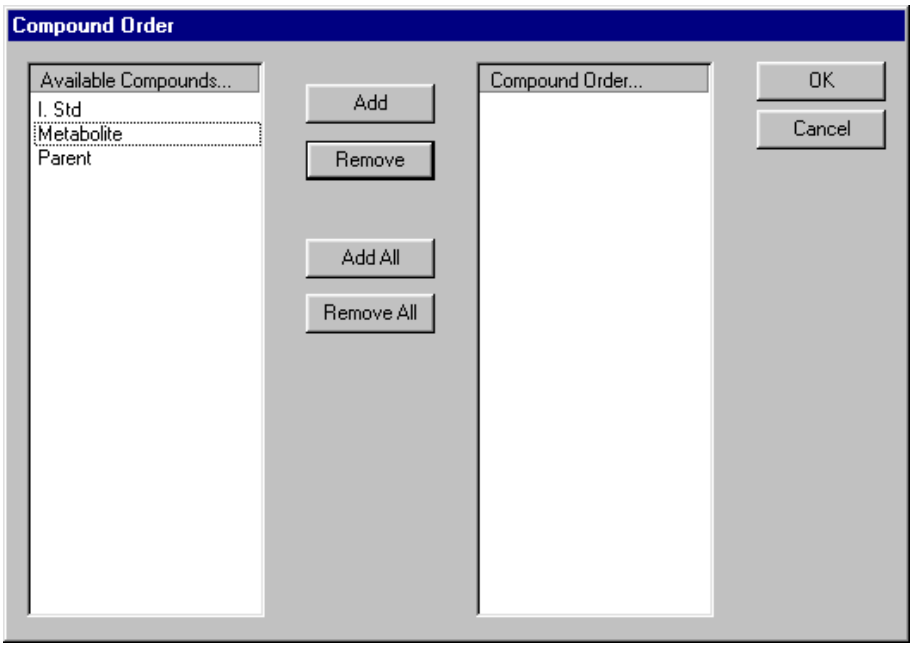


Figure 3.33 The Compound Order dialog

Options.

Select the boxes for the options required:

Sample Per Page Select this box to print information for each sample on a new page.

Allow Split Samples Select this box to print the next sample immediately after the previous one.

Include Summary .Select this box to include a summary for each sample.

Graph Size

Fit Graph to Page Width Select this option to print the chromatograms associated with a sample to the width of the page. To print the chromatograms a different width, deselect this option and enter the required size in the **Width:** box.

Fit Graph to Page Height Select this option to print the chromatograms associated with a sample to the height of the page. To print the chromatograms a different height, deselect this option and enter the required size in the **Height:** box.

Column Format This allows the columns on the Report to be selected and formatted; for details, see the Modifying Column Formats section on page 3-20.

Audit Report Page

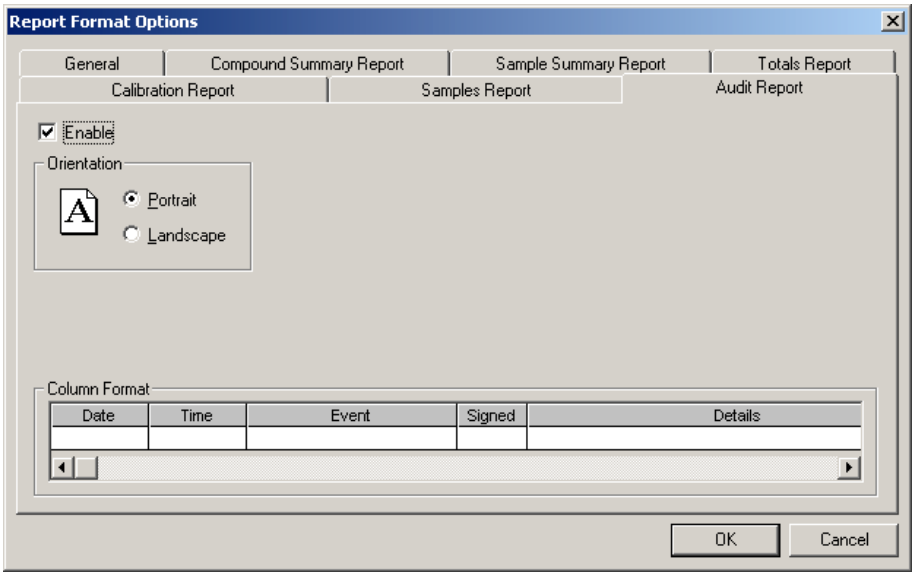


Figure 3.34 The Audit Report page

Enable Check to enable all the other items on the page.

Column Format This allows the columns on the Audit Report to be selected and formatted; for details, see the Modifying Column Formats section on page 3-20.

Orientation Select either Portrait or Landscape format to print the report.

Importing and Exporting Information

The **Import** and **Export** commands on the QuanLynx Browser **File** menu are used to import data from existing MassLynx projects, export methods and calibrations for reuse by MassLynx, and export results for use by third-party programs. The **Edit**, **Copy** command is used to write information to the Windows Clipboard.

Importing Information

Select the **File**, **Import** command to import MassLynx Quantify data into a new QuanLynx Dataset. Select the required project from the **Import** dialog displayed. Select the Sample List, Method, and Calibration files to be imported from the project. If Peak List files exist for the samples in the Sample List, the information from these will also be imported into the new Dataset.

Data can only be imported from an existing MassLynx project.

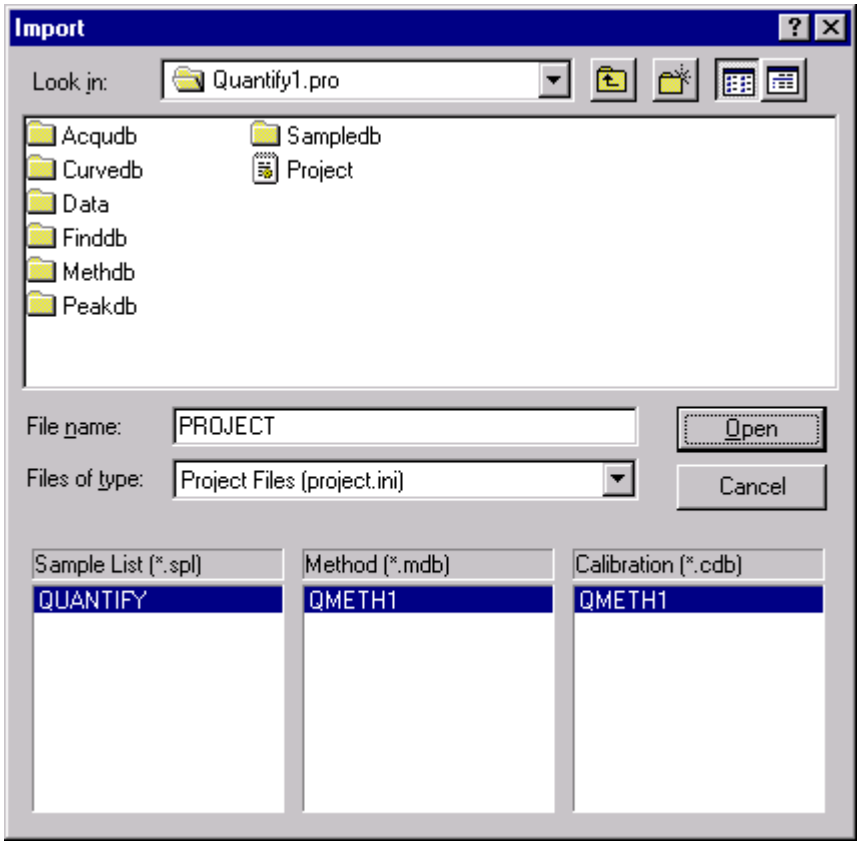


Figure 3.35 The Import dialog

Exporting Information

Information from the QuanLynx Browser can be exported for use with other Datasets or by third party programs.

Exporting to File

Method

To export the method, select the **File**, **Export**, **Method** command, this invokes the **Export Method** dialog. Select the METHDB folder of the project to export the data to. The file is saved as a standard MassLynx Quantify Method (.MDB) file.

Calibration

To export the calibration, select the **File**, **Export**, **Calibration** command; this invokes the **Export Calibration** dialog. Select the CURVEDB folder of the project to export the data to. The file is saved as a standard MassLynx Quantify Calibration (.CDB) file.

LIMS

To export the data into a text file for use with a LIMS, select the **File**, **Export**, **LIMS** command; this invokes the **Export** dialog. The file is written as comma-separated text.

The file generated will consist of three areas: the Header Section, the Samples Section and the Calibration Section.

The Header Section

The Header Section contains the following four sections. Each shows the full path name of the file generated by, or used to create, the Report, and the date and time that the file was last modified.

- LIMS EXPORT FILE The LIMS file generated.
- DATASET The QuanLynx Dataset.
- SAMPLELIST The Sample List file.
- QUANMETHOD The quantitation method file.
- QUANCALIBRATION The quantify curve file.

The Samples Section

The Samples Section will include an entry for each sample in the current sample list. For each sample there will be one entry for each compound named in the **Compound:** list box in the Quantify **Method Editor**. Each entry will have the following fields, separated by a comma:

- The compound number shown in the **Compound:** list box in the Quantify **Method Editor**.
- The text name of this compound.
- The scan at which the matching peak was found in the current sample data file.
- The Retention Time of the matching peak.
- The Relative Retention Time to the referenced peak at which the matching peak was found.
- The area of the matching peak.
- The height of the matching peak.
- The response of the sample for this compound.
- The flags associated with the peak.
- The concentration of compound recorded for this sample.
- The blank subtracted concentration of the compound for this sample.
- The chromatogram trace used to locate peaks for this compound.
- The error between the expected concentration and the calculated concentration for this sample for a fixed concentration compound.
- The ordinal number of the compound in the quantitation method that is used as the reference peak for this compound.
- The area of the reference peak.
- The height of the reference peak
- The Retention Time of the reference peak.

- The modification date of the peak used to quantify this compound for this sample. This refers to manually modifying the peak, for example, by double clicking on the entry in the peak display in the quantitation window.
- The modification time of the peak.
- The modification text (modification comment) of the peak.
- The MassLynx user who altered the peak.
- The mass of the peak.
- The Retention Time the peak was expected at for this compound.
- The Relative Retention Time the peak was expected at for this compound.
- The user factor associated with this compound.
- The User RF Value associated with this compound.
- Start Retention Time of the detected peak.
- End Retention Time of the detected peak.

The Calibration Section

The Calibration Section will have a subsection for each calibration curve calculated for the current quantitation calibration.

Each subsection will contain information as displayed on the calibration graphs window. Where a line entry is inappropriate it will not be entered in the report file.

- Correlation coefficient: or Coefficient of Determination:
- Response Factor: or Calibration Curve:
- Response Type:
- Curve Type:, Origin:, and Weighting:

XML

To export the data into a text file for use with a LIMS, select the **File**, **Export**, **XML** command; this invokes the **Export** dialog. The file is written using XML format.

Complete Summary

To export the Summary information into a text file, select the **File**, **Export**, **Complete Summary** command; this invokes the **Export Compound Summary** dialog. The file is written as Tab delimited text using the currently selected Summary format.

Current Summary

To export the Summary information for the currently selected sample or compound, into a text file, select the **File**, **Export**, **Current Summary** command; this invokes the **Export Compound Summary** dialog. The file is written as Tab delimited text using the currently selected Summary format.

Exporting to Windows Clipboard

Summary information, or a picture of the chromatogram or calibration curve, can be copied to the clipboard and pasted into other Windows applications.

To copy a picture of the chromatogram, highlight the chromatogram window and select the **Edit**, **Copy**, **Image** command.

To copy a picture of the current calibration curve, highlight the calibration curve window and select the **Edit**, **Copy**, **Image** command.

To copy summary information for all samples or compounds, select the **Edit**, **Copy**, **Complete Summary** command. The information displayed in the Summary window will be copied to the clipboard, information for columns not displayed will not be copied.

To copy summary information for the sample or compound currently highlighted in the Summary window, select the **Edit**, **Copy**, **Current Summary** command. The information displayed in the Summary window for the highlighted sample will be copied to the clipboard, information for columns not displayed will not be copied.

When copying summary information to the clipboard, the format, by sample or by compound, is determined by the current Summary View format.

Locking Datasets

The **File, Accept Dataset** command is used to lock the Dataset so that data in the Dataset cannot be accidentally modified; i.e. all the User Interface commands that can be used to modify data are disabled.

Signing and Reviewing Datasets

Datasets can be electronically signed and reviewed by using the following File Menu commands, the level of access that each user is entitled to is dependant upon the security settings of MassLynx, the details of which can be found in the MassLynx Security Users Guide. An overview of security relating to QuanLynx is described in Chapter 6.

Accept Dataset Selecting this command initially invokes a **Sign Dataset** warning dialog. Selecting **OK** locks the Dataset to prevent unauthorised modification of the data, i.e. all the User Interface commands that can be used to modify data are disabled.

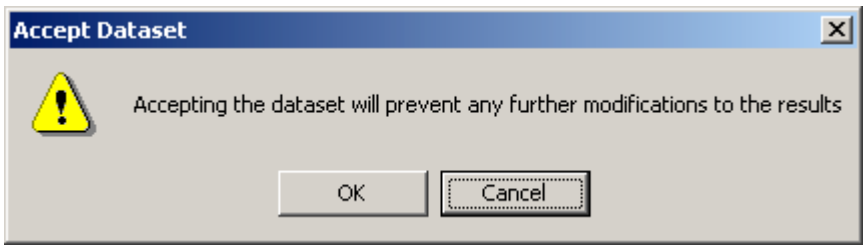


Figure 3.36 The Sign Dataset warning dialog

Review Dataset This allows a second user to review the Dataset and store a second electronic signature in the Dataset, indicating that the Dataset has been reviewed and approved.

Unaccept Dataset This unlocks the dataset and allows further modification.

Other Menu Commands

File Menu

Refresh Selecting this command rereads the current Dataset and updates the display information. This command should be used if the content of the Dataset has changed as a result of processing further samples.

Properties Select this command to display the **File Properties** dialog; this displays information about the current QuanLynx Dataset, including creation and last modification timestamps of the Dataset itself and the Methods and Calibrations it contains.

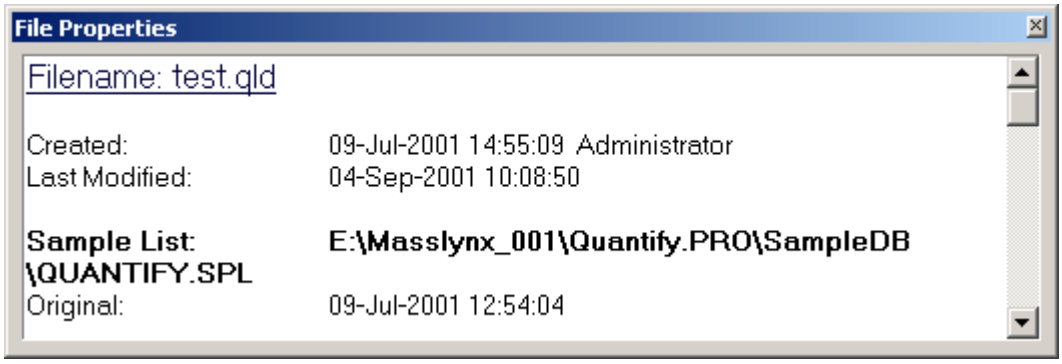


Figure 3.37 The File Properties dialog

View Menu

The **View** menu options control which windows or information windows are displayed, e.g. the Chromatogram window or the toolbar. To display a window, select the option from the menu. A tick mark appears next to the option when selected; selecting the option again will turn it off.

Window Menu

Cascade Select this command to arrange document windows so that the title bar of each window is visible.

Tile Horizontally Select this command to arrange open windows one above an other, dividing the available space equally between the open windows so that they are all visible.

Tile Vertically Select this command to arrange open windows side by side on the screen, dividing the available space equally between the open windows so that they are all visible.

When **Tile Horizontally** or **Tile Vertically** is selected, the menu item will become checked and the open windows will be retiled automatically when the QuanLynx Browser main window is resized. To cancel this operation, select the menu item again or manually reposition one of the open windows.

Chapter 4 Quan-Optimize

Contents

Figures

Overview

Advances in automation have increased the number of compounds being produced in drug discovery in the pharmaceutical industry. The initial bottleneck in lead generation has been alleviated by the acceleration of High Throughput Screening (HTS) and library verification, resulting in larger numbers of potential drug candidates. The pharmaceutical industry does not have the resources to take all of these compounds through traditional drug development avenues, which are both time-consuming and costly, so an alternative approach has to be used. This approach involves the high throughput screening of a number of compound properties, which determine how effectively that compound will perform as a drug. Experiments are performed *in vitro* and *in vivo* and are used to determine properties such as pharmacokinetics, stability and absorption. In many of the experiments LC-MS(/MS) is used to determine these properties using rapid quantitation.

Creating a Quan-Optimize Method

Before Quan-Optimize can be used, an acquisition using a general sample must be performed; ideally this should have all the compounds in it. Ensure that the instrument is calibrated to at least 1000 amu per second. Use the data acquired to set up the following files. This should only need to be done the first time Quan-Optimize is used.

Create Tune Files

Create an MS Tune File with details of the tune parameters for MS acquisition.

Create an MSMS Tune File with details of the tune parameters for MSMS acquisition.

Create an Acquisition Tune File with details of the tune parameters for acquisition.

The files created above will normally have the same Source parameters. For the MS file, set the LM and HM Res parameters for unit mass resolution. For the MSMS and Acquisition files, the LM and HM Res parameters may be lowered to gain more sensitivity.

These files should only need to be created the first time Quan-Optimize is used.

Create LC Method Files

Create an optimization LC method. The method can control any valve switching required to ensure that the LC column is bypassed whilst optimization is taking place; alternatively, this can be defined in the column method (see below). This file should only need to be created the first time Quan-Optimize is used, unless the loop configuration changes, when a new method will have to be created.

Create an acquisition LC method. The method can control any valve switching required to ensure that flow passes through the LC column during acquisition; alternatively, this can be defined in the loop switching method (see below). This file should only need to be created the first time Quan-Optimize is used, unless the loop configuration changes, when a new method will have to be created.

Create a column valve switching method (this is optional). The column valve switching method is run between analysis and optimization in order to allow the flow through the system to stabilize before optimization commences.

Create a loop valve switching method (this is optional). The loop valve switching method is run between optimization and analysis in order to allow the flow through the LC column to stabilize before analysis commences.

Several acquisition loop and column methods may need to be created, depending on the characteristics of the compounds in the compound Sample List.

Create Quantify Method Templates

Create an MS Quantify Method template containing the details of the peak detection parameters (smoothing and integration) to be used when optimizing MS analysis. This file should only need to be created the first time Quan-Optimize is used, unless the loop configuration changes, when a new method template will have to be created.

Create an MSMS Quantify Method template containing the details of the peak detection parameters to be used when optimizing MSMS analysis. This file should only need to be created the first time Quan-Optimize is used, unless the loop configuration changes, when a new method template will have to be created.

Create an Acquisition Quantify Method template containing the details of the peak detection parameters to be used quantifying acquired data. This method may need to be created on a per project basis, depending on the characteristics of the compounds in the compound Sample List.

For the MS and MSMS files, ensure that the peak to peak amplitude is suitable for full scan acquisition, and use a gentle smooth (use a more gentle smooth for MSMS). For the Acquisition file, use the data acquired in the test run for peak-to-peak amplitude and increase the smoothing. For all templates, ensure that **Peak Selection** is set to **Nearest** if using a Quan reference or **Largest** if not using a Quan reference.

Create a Project Template

Create a Project Template and put the files created above in the correct directories. **Important:** This step is essential.

ACQUDB Tune files, LC files, column and valve switching files.

METHDB Quantify templates.

Create Sample Lists

Create a Sample List containing details of the pure compounds (the Compound Sample List).

Create a Sample List containing details of the compound mix for analysis (the Analysis Sample List).

These Sample Lists can be created via the MassLynx screen, Access, Excel or a LIMS system; see the MassLynx User's Guide and Interfacing Guide for details.

Contents of Compound Sample List

The Compound Sample List is used to create the optimization method files and should contain the following information for the compounds. Other fields may be present in the Compound Sample List, but failure to specify the fields marked as mandatory could lead to an unexpected failure of the optimization stage of the Quan-Optimize procedure.

	Compound	Vial	Formula/Mass	Sample Group	Internal Stand...
1	sulfaguanidine	A,1	C7H10N4O2S	A	IS
2	sulfadiazine	B,1	C10H10N4O2S	A	
3	sulfapyridine	C,1	C11H11N3O2S	A	
4	sulfachloropyridazine	D,1	C10H9ClN4O2S	A	
5	sulfamethazine	E,1	C12H14N4O2S	A	
6	sulfamethoxine	F,1	C12H14N4O4S	A	
7	sulfamethizole	G,1	C9H10N4O2S2	A	
8	acetamidophenol	A,2	C8H9NO2	B	IS
9	doxepin	B,2	C19H21NO	B	
10	chlormipramine	C,2	C19H23ClN2	B	
11	amitriptyline	D,2	C20H23N	B	
12	nordoxepin	E,2	C18H19NO	B	
13	notriptyline	F,2	C19H21N	B	
14	chlorodiazepam	G,2	C16H12Cl2N2O	B	

Figure 4.1 Compound Sample List

In the field descriptions below the first name is the Field ID, it is the name in brackets on the MassLynx **Customize Field Display** dialog and should be used when generating Sample Lists externally (Access, Excel, LIMS systems, etc.). The following name is a more descriptive name (used for the column headings) and is defined using the MassLynx **Field Properties** dialog.

- FILE_TEXT Compound (Mandatory)

The compound name/identifier. If optimization is successful, the method file created will be in the format MS_filetext.exp (or MSMS_filetext.exp for MSMS acquisitions). Do not use spaces or punctuation characters in this field.

- SAMPLE_LOCATION Vial (Mandatory)

The autosampler vial position that contains this compound.

- MASS_A Formula/Mass (Mandatory)

The mass of the compound. This field can be specified as either a decimal mass value or a formula, which will automatically be converted into a decimal mass.

- SPARE_2 Internal Standard (Optional)

This field is used to explicitly specify which compounds are used as internal standards. Each group can only have one internal standard.

- SAMPLE_GROUP Sample Group (Optional)

This field is used to define the compounds present in a given sample group.

- AUTO_FILE Auto File (Optional)

This field is only necessary when the hardware setup requires an external autosampler method.

Contents of the Analysis Sample List

The Analysis Sample List is used for acquiring sample data and should contain the following information. Other fields may be present in the Analysis Sample List, but failure to specify the fields marked as mandatory could lead to an unexpected failure of the acquisition and quantification stage of the Quan-Optimize procedure.

	File Name	Vial	Sample Type	Conc A	Sample Group	Quan Reference
1	QLYNX15	H,1	Analyte		A	X
2	QLYNX16	A,3	Blank		A	
3	QLYNX17	B,3	Blank		A	
4	QLYNX18	B3	Blank		A	
5	QLYNX19	C,3	Standard	10	A	
6	QLYNX20	C,3	Standard	10	A	
7	QLYNX21	C,3	Standard	10	A	
8	QLYNX22	D,3	Standard	50	A	
9	QLYNX23	D,3	Standard	50	A	
10	QLYNX24	D,3	Standard	50	A	
11	QLYNX25	E,3	Standard	100	A	
12	QLYNX26	E,3	Standard	100	A	
13	QLYNX27	E,3	Standard	100	A	
14	QLYNX28	F,3	Standard	500	A	

Figure 4.2 Analysis Sample List

In the field descriptions below the first name is the Field ID, it is the name in brackets on the MassLynx **Customize Field Display** dialog and is the name that should be used when generating Sample Lists externally (Access, Excel, LIMS systems, etc.). The following name is a more descriptive name (used for the column headings) and is defined using the MassLynx **Field Properties** dialog.

- FILE_NAME File Name (Mandatory)

The name of the file that acquisition data will be held in. These filenames must be unique. If **Run Unattended** is selected on the Quan-Optimize Wizard (see Chapter 5, Quan-Optimize Setup), and a file exists with this name, files will be overwritten and no warning will be given. If **Run Unattended** is not selected and a file exists with this name, Quan-Optimize will display a warning and pause until the overwriting of the file is confirmed.

- SAMPLE_LOCATION Vial (Mandatory)

The autosampler vial position that contains this sample.

- TYPE Sample Type (Mandatory)

The type of the sample, e.g. QC, STANDARD, ANALYTE, or BLANK. This is required for quantitation.

- CONC_A Conc A (Mandatory)

All standards must have a value in the CONC_A field, as this will be used to determine the concentration for all compounds in that standard. For reasons of simplicity, it is assumed that the concentration of each compound in a standard will be the same (e.g. if concentration is 0.1 mg/ml then the standard will contain 0.1 mg/ml of A and 0.1 mg/ml of B etc.).

- SAMPLE_GROUP Sample Group (Optional)

The Sample Group Column is used to inform Quan-Optimize which optimization files for an Analysis sample should be combined to form the analytical method for that sample.

Each sample in the Compound Sample List only contains one compound. Each sample in the Analysis Sample List may contain a single compound or a mixture of compounds. Each sample group name can be any alphanumeric string; e.g. ‘Internal1’, ‘A2’ or ‘BB’ are all valid group

names. However, it is recommended that one or two letter/number group names be used for simplicity, e.g. ‘A’, ‘AB’, ‘C1’, etc.

The sample group names used in the Sample Analysis List and Compound Analysis List must be the same.

Multiple sample group names in the Sample Analysis List can be separated by any of the following characters: <space> . , : ; | used singly, or in multiple; e.g. ‘A:B:C’, ‘AA BB CC’ and ‘A….ZZ….QP’ are all valid three item groups.

If the sample group field is empty, then all the compounds will be optimized and one method file will be created and used for all the analysis samples.

If a sample group in the Analysis Sample List does not have a corresponding entry in the Compound Sample List, then that group will not be run.

Using the example below, Quan-Optimize reads the Analysis Sample List and sees that the first entry is for Sample Group A. It then reads the compound Sample List and performs optimization for each compound in Group A. The results for each compound are stored in separate acquisition files. The acquisition files are combined into one method file when all Sample Group A compounds have been optimized. IN a similar fashion Quantitation methods are created using the Quan template provided by the user.

This method file is then used to acquire the samples from the Analysis Sample List until an entry with a different Sample Group is encountered. Quan-Optimize then returns to the Compound Sample List and optimizes each entry for the new sample group; therefore, it is important that samples with the same sample group are next to each other in the Analysis Sample List.

In the example below, Analysis sample 1 contains sample groups A and B. A and B from the Compound Sample List are optimized and combined to produce a method file, which is used to acquired and quantify Analysis samples 1 and 2. Compound C will then be optimized, a method created with A and C, and samples 3 and 4 acquired and quantified. Compound D will then be optimized, a method created for B and D, and samples 5 and 6 acquired and quantified.

Analysis		**Compound**	
1	A B	Std 1	A
2	A B	Drug 1	B
3	A C	Drug 2	C
4	A C	Drug 3	D
5	B D		
6	B D		

Defining the Sample Lists in the following way will also obtain the same results.

Analysis		Compound	
1	A	Std 1	A, B
2	A	Drug 1	A, C
3	B	Drug 2	B
4	B	Drug 3	C
5	C		
6	C		

- QUAN_REF Quan Reference (Optional)

  This field specifies which sample (if any) is to be used to adjust the retention time window of the quantification method created for the analysis. The first sample in the current group that has this field set to anything other than blank will be used. The Quan Reference should be a high concentration standard (so that it has the largest peak for every compound present in the group).

  The retention time of the peak top in this standard will be used to set the predicted retention time in the quantify method. The window for peak detection will be set as the greater of the leading and trailing edges of the reference peak plus 25%.

- AUTO_FILE Auto File (Optional)

This field is only necessary when the hardware setup requires an external autosampler method.

Create a Quan-Optimize Method

Using the files created above, create a Quan-Optimize Method as described in Chapter 5, Quan-Optimize Setup.

Running Quan-Optimize

Note: If the optimization fails for any of the compounds in the Compound Sample List, Quan-Optimize will continue with acquisition and quantitation of the Analysis Sample List without this compound.

Stages in Optimization

MS

- Check for pre-existing MS methods.

Quan-Optimize checks the ACQUDB directory of the current project for an MS_filename.exp file. If this file already exists, it will be used for the analysis stage (unless the **Optimise all Compounds** option is selected on the **Import File Selection** page of the Quan-Optimize Wizard, when the optimization file will be recreated).

- Production of MS methods.

If not already present, MS methods will be created for each compound in the Compound Sample List. The MS methods will consist of full scan centroid acquisitions over the range MASS_A (±1 for ionization mode), with a safety margin of 50 amu below and above, in order to allow adducts

to be optimized. A number of functions will be generated, starting at the minimum cone voltage, specified on the Quan-Optimize Method Editor, **Optimisation** page (see Chapter 5, Quan-Optimize Setup), increasing in steps from that value to the maximum value. The requested step size is validated to make sure that it does not result in greater than the maximum number of functions (currently eight). Each of the functions will have the same length as the selected Optimization LC method. If both ionization modes have been selected, then the compound list will be acquired twice, once with a positive set of functions and then again with a negative set.

Alteration of Compound Sample List.

The Compound Sample List will be altered so that the MS method field corresponds to the method just created for each compound.

- Acquisitions carried out.

The data in the MS methods will be acquired.

- Post processing.

For each sample:

The data in the acquired files will be analysed to determine at what point the mass chromatogram of the [M+/-H] ion is maximized for each function.

The intensity and mass of the most intense ion in the spectra, belonging to the maximum point of the mass chromatogram representing any requested adduct mass, will be calculated for each function.

For each function the mass, Ionization Mode and Cone Voltage of the most intense spectral responses will be stored and used for creation of the MS method for that compound.

The result of the optimization procedure will be to create files of the name MS_<compoundname>.exp.

MSMS

- Creation of MS methods.

The optimization files are created as above but not saved; they are temporary files used as a first stage in the creation of MSMS methods.

- Daughter ion optimization.

MSMS methods are created using the ionization mode and cone voltage from the temporary files described above. The functions will be daughter scans of the ion selected above. The range of the daughter scans will be from mass 50 (or the minimum **Fragment Size** specified in the Quan-Optimize Method Editor **Optimisation** page) to 50 greater than the mass of the parent ion. The selected minimum, maximum and step sizes for collision energy will be used to provide duplicate scans of different collision energies (2 to 8 again).

- Alteration of Compound Sample List.

- Acquisitions carried out.

- Post processing.

For each sample, the most intense response for any ion in any of the functions will be selected as the daughter mass for the MRM acquisition method created. Daughter ions with masses in the window Parent ion ±2 amu will be ignored, as these will be unfragmented parent ions. Daughter

ions whose mass loss has been excluded in the **Losses** page of the Quan-Optimize Method Editor will also be excluded.

The result of the optimization procedure will be to create files of the name MSMS_<compoundname>.exp.

The Acquisition and Quantitation Procedure

The acquisition and quantitation procedure has the following steps:

- Create acquisition method.

For all the compounds in the required Sample Group, a channel will be added to the method. All these channels will be MRM if MSMS mode was selected, or SIR if MS mode was selected. If there are more than 32 compounds in a sample group in the Compound Sample List, additional functions will be used in the acquisition so that all components can be acquired.

If the optimized MSMS parameters include a mixture of positive and negative ion modes, these will be sorted into separate functions so that ion mode switching is minimized.

The dwell times of each channel will be adjusted so that the **Total Cycle Time** of all functions is as specified in the Quan-Optimize Method Editor **Acquisition** page. Note that, for large numbers of compounds, the definition in the response will suffer and, hence, the margin of error on the quantitation will be greater.

If the calculated dwell time is greater than the maximum specified dwell time, it will be replaced by that maximum dwell time. The acquisition time of the methods will be for the whole of the LC method.

- Acquire samples.

This stage will perform the acquisition from the Analysis Sample List as specified. Any process macros specified will also be carried out.

- Calculate retention times.

If a quantify reference standard is specified for the sample group, the chromatograms for each channel in the ‘reference standard’ acquisition will be generated. The greatest peak in each chromatogram will be selected as the retention time of the required compound. There should only be one peak in each channel for MRM or SIR acquisitions. The retention time window will be set as specified in the quantify reference standard field description.

- Create Quantify Method.

If **Auto Quantify Samples** is selected in the Quan-Optimize Wizard, the quantify method will be created using the defaults contained in the existing method and the calculated retention times for each compound. The quantify method is saved as according to the conventions described in "Quan-Optimize File Naming Conventions" page 4-11

- Quantify samples.

The Quantify Method created in the stage above will be used to quantify all acquired samples. The results of quantitation are written to a QuanLynx Dataset.

- Output results.

If the **Export to LIMS file** option was selected on the Quan-Optimize Wizard, **Quantify Options** page, the results will be exported to the file selected there.

- Repeat all stages for the next Sample Group.
- Concatenate Group Export Files.

If the **Concatenate Group Export Files** option was selected on the Quan-Optimize Wizard, **Quantify Options** page, the results for all samples groups will be concatenated into one file. The name of the concatenated file will be that specified in the LIMS export file selection box.

Quan-Optimize File Naming Conventions

Quantitation and acquisition methods generated for the analysis stage, are based on the QuanLynx results filename. The QuanLynx results filename is based on the sample list name, and will be incremented if a file with that name exists already.

The method names have the group name appended to the end of the filename, since each group has its own specific set of methods (1 quan and 1 acquisition method). If the sample has multiple groups, these will be concatenated into a single identification string.

The Quan method has QM_ appended to the front of the filename.

The acquisition method has MSMS_ appended to the front of MRM type methods and MS_ for SIR type methods.

If the methods have the same name as an already existing file, then the original file is overwritten. This is to ensure that the sample list, method names and QuanLynx results files all truly correspond, i.e. The same sample list maybe used to generate a number of QuanLynx results files, each with a unique name, with a corresponding set of methods with the same unique base name.

E.g. 1

Sample List:	AnalysisList.spl
QuanLynx results file:	AnalysisList.qld
Quan method:	QM_AnalysisList_A.mdb (for group A)
Quan method:	QM_AnalysisList_AB.mdb (for a sample with group A and B specified)
Acquisition method:	MSMS_AnalysisList_A.exp (for group A)
Acquisition method:	MSMS_AnalysisList_AB.exp (for a sample with group A and B specified)

E.g.2 If the QuanLynx results file exisits already.

Sample List:	AnalysisList.spl
QuanLynx results file:	AnalysisList-001.qld
Quan method:	QM_AnalysisList-001_A.mdb
Acquisition method:	MSMS_AnalysisList-001_A.exp

Reviewing and Reporting Results

The quantitation results are written to a QuanLynx Dataset; this can be viewed, edited and re-processed in the QuanLynx Browser. For details, refer to Chapter 3, QuanLynx Browser.

Chapter 5 Quan-Optimize Setup

Contents

Figures

The Quan-Optimize Method Editor

To display the Quan-Optimize Method Editor, select **Quan-Optimize Method Editor** from the **Quantify** menu. The Quan-Optimize Method Editor has six pages and if a MUX system is installed there is an extra page.

Optimisation Page

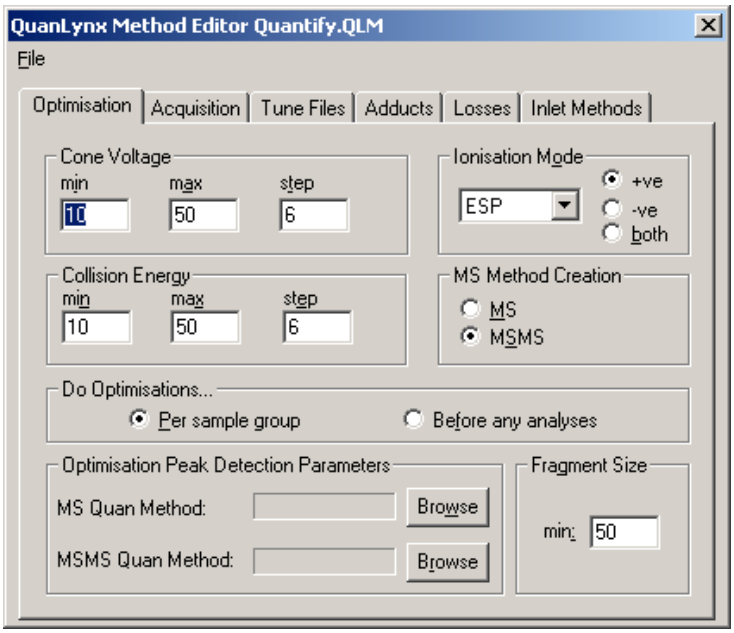


Figure 5.1 The Quan-Optimize Method Editor, Optimisation Page

Cone Voltage Enter the cone voltage to use for the first function (**min**), the last function (**max**) and the number of volts to jump for each **step**. For example, in the above example, data will be collected for 5, 10, 15 and 20 V. A maximum of eight steps is currently allowed. Attempting to set a step size that gives more than eight steps between maximum and minimum (inclusive of both endpoints) will show an error

Collision Energy These values are only used if the **MS Method Creation** is defined as **MSMS**. Enter the collision energy to use for the first function (**min**), the last function (**max**) and the number of eV to jump for each **step**, e.g. in the above example, data will be collected for 5, 10, 15 and 20 eV. A maximum of eight steps is currently allowed. Attempting to set a step size that gives more than eight steps between maximum and minimum (inclusive of both endpoints) will show an error.

Ionization Mode Select the required ionization mode from the drop-down list box and one of the **+ve** (positive), **-ve** (negative), or **both** ion modes. If **both** is selected, the best response from either positive or negative ion modes will be used to calculate the optimized method for each standard compound. An extra injection of each standard is automatically performed in order to optimize both positive and negative ion modes

MS Method Creation

This defines the experiment type for the analytical sample run. If **MS** mode is selected, the optimization process will create optimized SIR methods. If the **MSMS** mode is selected, the optimization process will create optimized MRM methods.

Note:- If the **MSMS** mode is selected, the optimized first stage MS files are not saved.

Both optimized SIR and MRM compound methods can exist in a project at the same time. These methods do not conflict with each other, but optimized SIR methods created during an acquisition using an MS Quan-Optimize method are not used as a first stage in an acquisition using an MSMS Quan-Optimize method.

DoOptimisations

Select **Per sample group** to run optimization and analysis one group at a time.

Select **Before all analysis** to optimize compounds for all groups bfore any analysis stage acquisitions**.**

Optimisation Peak Detection Parameters

Press the **Browse** buttons (which invoke the **Select Quantify Method File** dialog) and select the required MS and MSMS Quan Methods (*.MDB files). These method files will be read to determine the smoothing and integration parameters used when looking for the loop injection peak during an optimization acquisition. A gentler smooth is normally necessary in the MSMS Quantify Method, as the MSMS loop injection acquisitions have fewer data points than those for MS. A warning will be generated if these files are not specified, however, the current default values (as specified in the chromatogram window) for integration and smoothing will be used.

Fragment Size

Enter the minimum size of an acceptable daughter ion. The default value is 50, as fragments smaller than this will not usually give any useful information. Before acquisition, the minimum fragment size is checked and, if it is greater than the molecular mass of any compound, Quan-Optimize will terminate, as no MRM transition would ever be found.

Acquisition Page

Total Cycle Time

Enter the number of seconds that a scan will last. When all the SIR or MRM traces to be monitored are concatenated, the time taken to complete a cycle around all these traces should not exceed the total cycle time.

Max Dwell Time

Since it may be possible that there are not many traces present in the concatenated method used to monitor analytes, the **Max Dwell Time** is the longest time at which a particular trace can be monitored during the cycle time. This may result in the cycle time being reduced to below the requested total cycle time.

Inter Mode Delay

If **both** was selected for the **Ionization Mode** on the **Optimisation** page, enter the delay time, in seconds, to switch between ion modes.

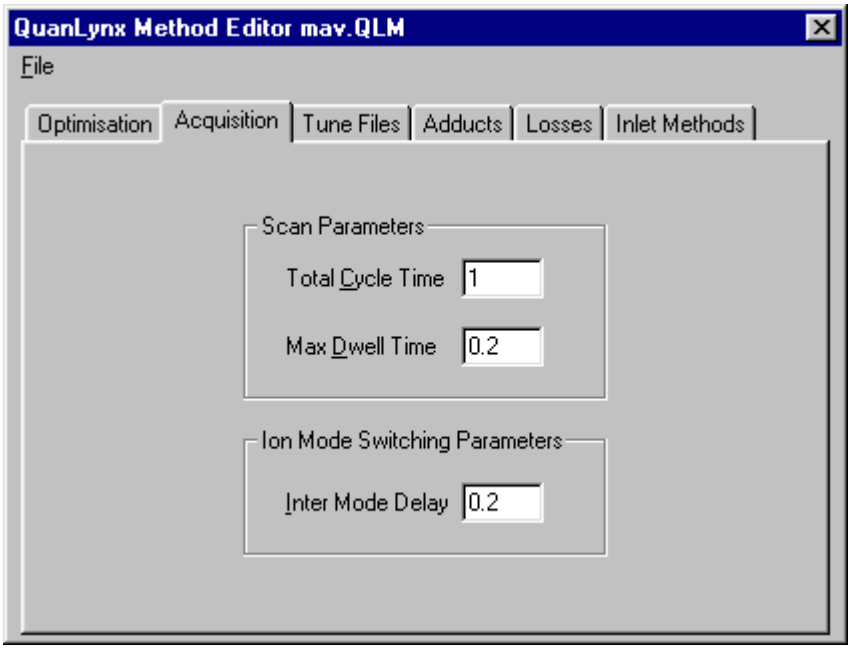


Figure 5.2 The Quan-Optimize Method Editor, Acquisition Page

Tune Files Page

MS Optimisation — Press the **Browse** button (which invokes the **Select Tune File** dialog) and select the required MS optimization tune file. This should contain tune parameters that give good MS response and peak shape for the desired ion mode. For a Quattro LC this will probably involve setting the entrance, exit and ion energy for the second quadruple appropriately.

MSMS Optimisation — Press the **Browse** button (which invokes the **Select Tune File** dialog)and select the required MSMS optimization tune file. This should contain tune parameters appropriate for MSMS scanning. It is not necessary to specify this file for MS only optimizations.

Acquisition — Press the **Browse** button (which invokes the **Select Tune File** dialog) and select the required Acquisition optimization tune file. This should contain tune parameters that give a good response for the SIR or MRM analysis being carried out during the main acquisition. For example, it may be decided that the high and low mass resolutions of both quadruples should be reduced during the main acquisition in order to improve sensitivity at low compound concentrations

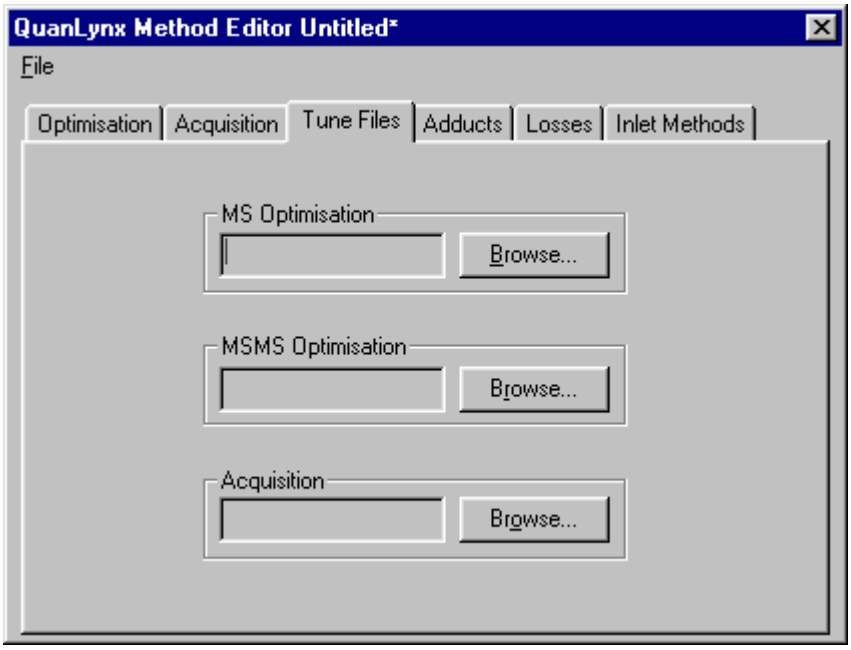


Figure 5.3 The Quan-Optimize Method Editor, Tune Files Page

Adducts Page

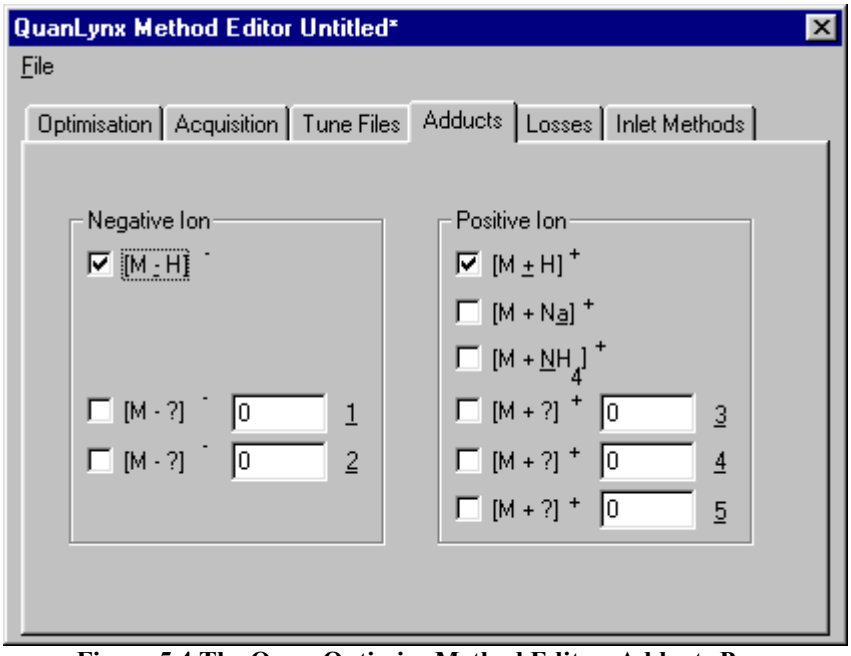


Figure 5.4 The Quan-Optimize Method Editor, Adducts Page

This page allows parent ion adducts to be selected. During MS scanning, the masses monitored are those of the given monoisotopic molecular mass (specified in the Compound Sample List) adjusted by the adducts that have been selected for the current ionization mode. Only masses falling within a ±0.5 amu boundary of the allowed masses are available for selection as the MS mass for SIR or further fragmentation. The most intense allowed mass signal over the range of cone voltage steps will be selected as the 'best' MS mass for each compound.

Select the box next to the required adduct and, for the **[M - ?]** and **[M + ?]** adducts, enter the adduct mass.

The sodium adduct is not recommended for **MSMS** mode as it tends to give poor fragmentation.

Losses Page

This page allows some fragmentations of the selected MS ion to be excluded.

These settings are only appropriate for **MSMS** mode optimizations.

Check the boxes for the losses that are not to monitored. Some common losses that may not be desired, i.e. water and carbon dioxide, are already displayed for selection. The three boxes at the bottom of the page are available for user-specified mass transitions that should be avoided.

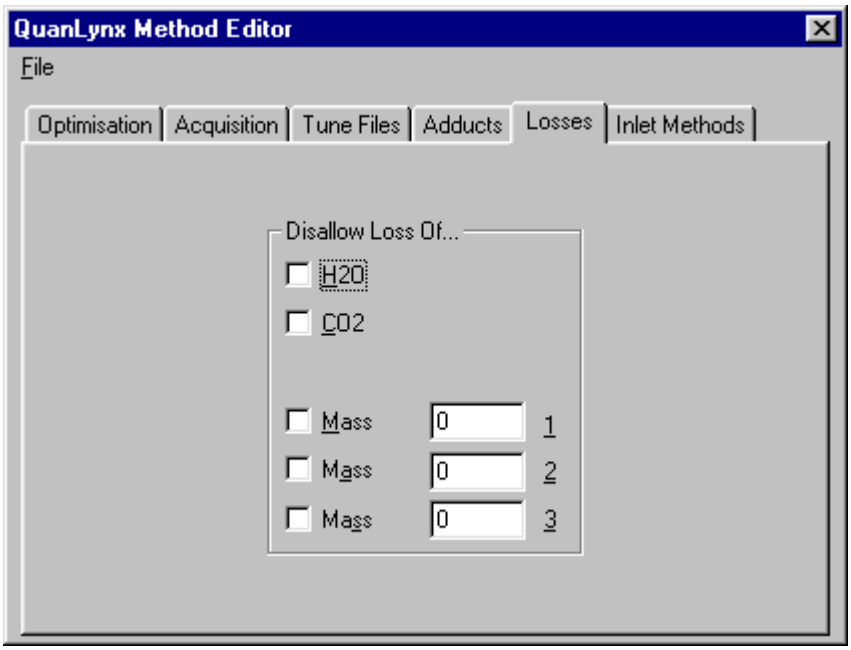


Figure 5.5 The Quan-Optimize Method Editor, Losses Page

Inlet Methods Page

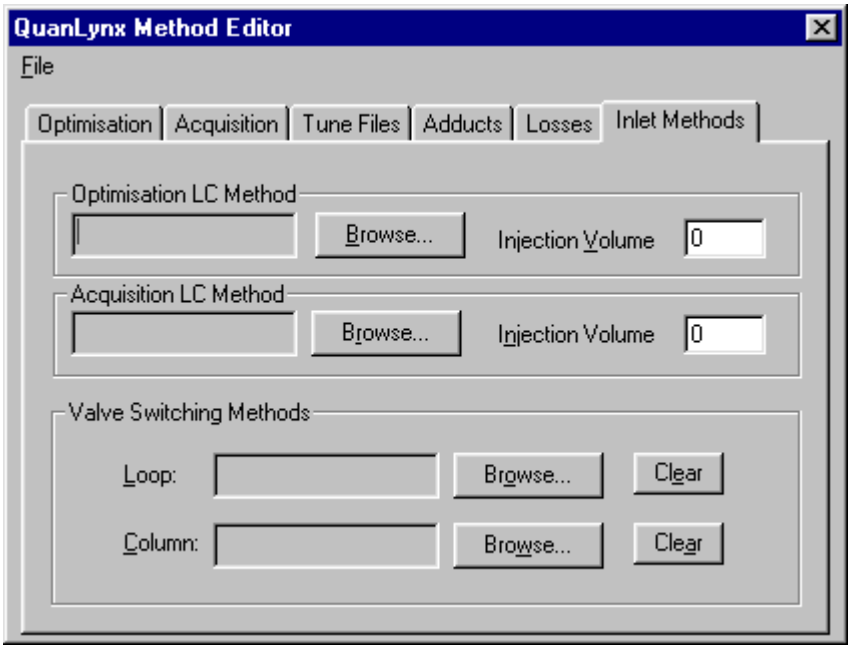


Figure 5.6 The Quan-Optimize Method Editor, Inlet Methods Page

Optimisation LC Method: Press the **Browse** button (which invokes the **Select LC File** dialog) and select the required Optimization LC Method file. This is the method used to control the loop injection that is required for the optimization of the standards. The method can also control any valve switching required to make sure that the LC column is bypassed, or this can be defined in the Valve switching methods below. The method should have sufficient run length to ensure that the sample alliquot injected has plenty of time to reach the mass spectrometer. The **Injection Volume** for the optimization loop injections should also be entered. The volume required will depend on the LC flow rate and tubing diameter, but 10 to 20 µl would be normal..

Acquisition LC Method Press the **Browse** button (which invokes the **Select LC File** dialog)and select the required Acquisition LC Method file. This is the method used to control the injection during acquisition of the Analysis Sample List. The method can also control any valve switching required to ensure that the LC column is selected, or this can be defined in the Valve switching methods below. The **Injection Volume** for the injections should also be entered.

Valve Switching Methods Press the appropriate **Browse** button (which invokes the **Select LC File** dialog) and select the required Loop and Column Valve Switching Method files. These methods are used to control any valve switching required to ensure that the LC column is bypassed or selected. These methods may also be used to equilibrate the LC system before and after switching (e.g. to avoid pressure pulses); refer to the "Create LC Method Files" section in Chapter 4, "Quan-Optimize", for further details. Pressing the **Clear** button will remove the selected file from this dialog

MUX Page

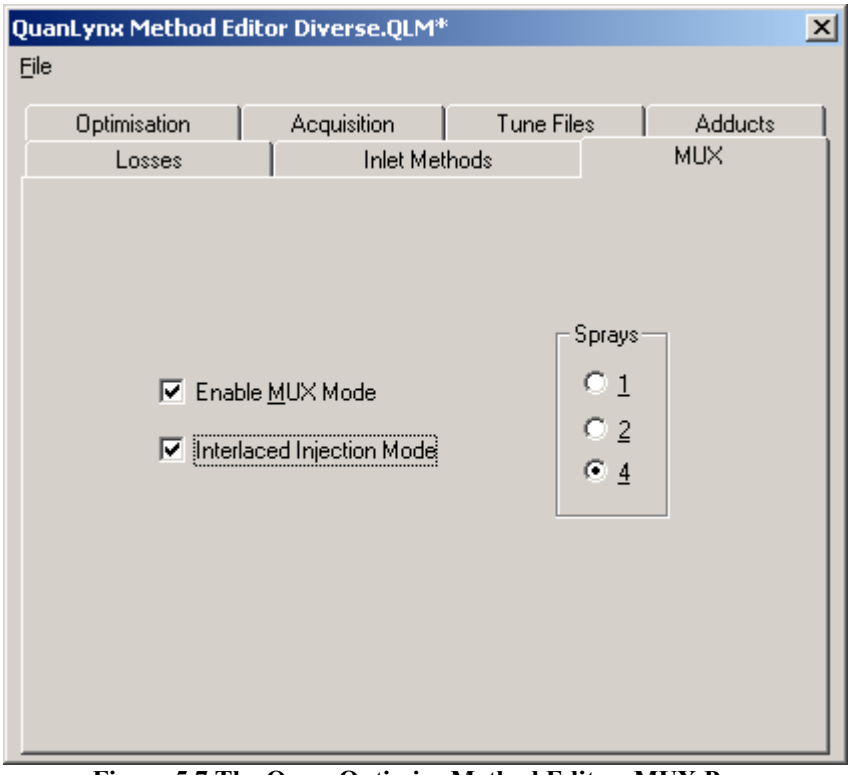


Figure 5.7 The Quan-Optimize Method Editor, MUX Page

This page is only available if a MUX system is installed.

Enable MUX Mode	Check this box to enable MUX Mode.
Interlaced Injection Mode	Check this box to enable Interlaced Injection Mode.
Sprays	Check 1,2 or 4 for the number of sprays.

Acquiring and Processing Quan-Optimize Data; the Quan-Optimize Wizard

To display the Quan-Optimize Wizard, **QuanLynx, Run Quan-Optimize** option from the MassLynx shortcut bar.

Methods

Reference files outside current project	Checking this box, allows QuanOptimize to use files outside the current project.
Quanlynx Method	Press the **Browse** button (which invokes the **QuanLynx Method** dialog) and select the required Quan-Optimize Method file.
Base Quan Method	Press the **Browse** button (which opens the **Select Quantify Method File** dialog); select the required acquisition method file and press the **Open** button. This method will be used as a template for the quantification method used to quantify the acquired samples. The integration and general parameters, as well as peak selection option, will be taken from the first entry in this file. The actual file used for quantitation will be saved in the same directory as this file and named after the convention described in Chapter 4.

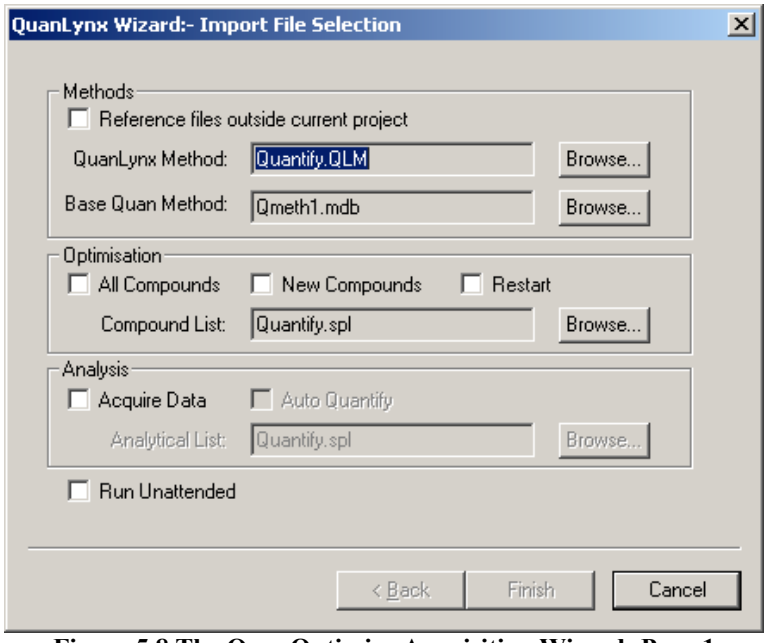


Figure 5.8 The QuanOptimize Acquisition Wizard: Page 1

Optimisation

All Compounds Check this option to optimize all the the compounds specified in the sample list, regardless of whether a compound acquisition method already exists (compound methods are overwritten in this case)

New Compounds Check this option to optimize only the new compounds in the sample list.

Restart Check to restart optimizations, without repeating data acquisition.

Compound List Press the **Browse** button (which invokes the **Select Compound List** dialog)and select the input file containing the details of the pure compounds. This can be in the form of a Sample List, tab or comma delimited text file, Excel file or Access file; see the MassLynx Interfacing Guide for more details.

Analysis

Acquire Data Check this option to activate the analysis stage of QuanOptimize. This enables the Analysis List option which must also be set

Auto Quantify Check this to automatically process the analytical sample list. This option also enables page 2 of the wizard.

Analytical List Press the **Browse** button (which invokes the **Select Analysis List** dialog) and select the input file containing the details of the compound mixes. This can be in the form of a Sample List, tab or comma delimited text file, Excel file or Access file; see the MassLynx Interfacing Guide for more details.

Run Unattended Selecting this option will allow any error messages produced during the Quan-Optimize processing to be ignored. The result files will need to be carefully examined to ensure that all compounds have been optimized, samples acquired and data analyzed. If this option is not selected, and an error or query occurs, a message box will appear and pause execution of the Quan-Optimize process, until such time as the error or query is addressed.

When the **Finish** button is pressed, the **Quan-Optimize Wizard:- Quantify Options** dialog is invoked.

Options

Integrate Samples Integrates all the Analysis Sample List data files. Leave this option deselected if integration has been performed, or if peaks have been manually changed

Calibrate Standards Uses Integration results to form Quantify calibration curves. Leave this option deselected if calibration has already been performed

Quantify Samples Uses Integration results and Quantify calibration curves to calculate compound concentrations. To change the **Main Method:** and **Curve:** files, press the **Browse** buttons and select new ones from the dialogs that are invoked.

Print Quantify Reports Produces hard copies of the results of integration and quantification.

Export Results to LIMS Produces a text file containing the quantification results details for use with LIMS systems. If this option is selected the **LIMS Export File Browse** button becomes enabled, press the **Browse** button, select a file or enter the name of a new one and press **Save**

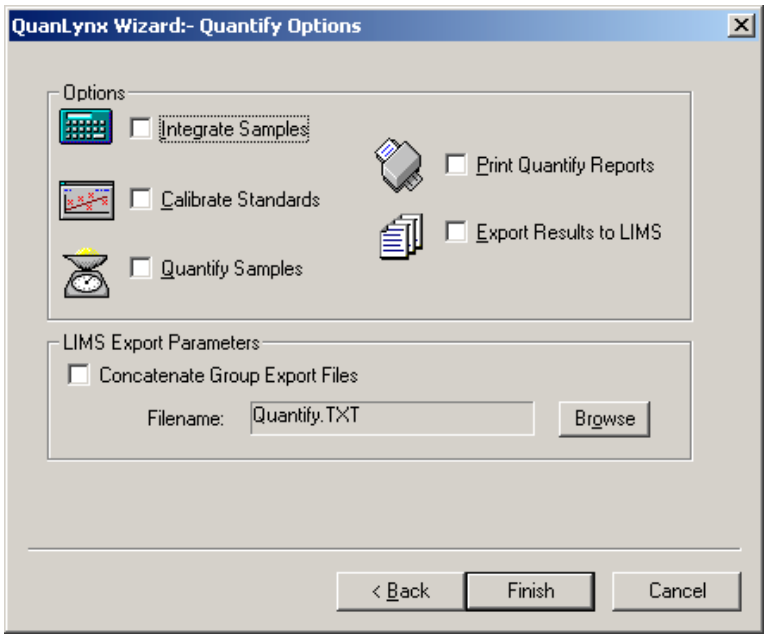


Figure 5.9 QuanOptimize Acquisition Wizard: Page 2

LIMS Export Parameters

Concatenate Group Export Files Selecting this option will cause all the LIMS export files created from running all sample groups to be concatenated together into one file

Filename The name of the concatenated export file

Press the **Finish** button to start the analysis.

Chapter 6 QuanLynx Security

Contents

Figures

Tables

Overview

This chapter provides a guide to the behavior of QuanLynx under the various possible security settings and permissions available under MassLynx. Further details and more comprehensive descriptions of the MassLynx Security features can be found in the MassLynx Security Users Guide.

On installation, Security can be selected with or without secure files. With security installed QuanLynx can be configured such that changes to QuanLynx Datasets have to be signed by the logged in user. With or without security installed all alterations to the data are recorded in the Audit Log (for further details see Chapter 3). There are three distinct signature policies that may be selected in the MassLynx Security Editor, **Don't Force Signatures, Force Signatures and Warn.**

There are several actions that are undertaken in QuanLynx that may require some form of security procedure, these are detailed in the MassLynx Users Security Guide. Each action will require a response either a comment detailing the change, permission by the currently logged in user (see Permissions page 6-8) or both. In addition Datasets can be Accepted, Reviewed and Unaccepted with in the QuanLynx Browser each requiring a comment and permission (see Chapter 3 Signing and Reviewing Datasets page 3-42 for a more detailed description).

No Security Installed

With no security installed, no permission checks are required. However, actions requiring comments will still prompt with the Alteration Comment dialog (Figure 6.1) and be recorded in the audit log.

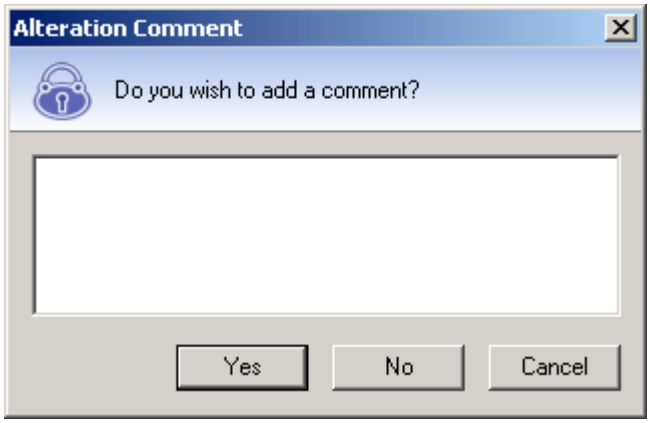


Figure 6.1 Alteration Comment dialog

This dialog may be enabled/disabled by selecting **Edit, Disable Comments** on the QuanLynx browser menu bar. This option is only available when no security is installed.

Security Installed

With Secure or Non Secure files installed the following options can be made available through the MassLynx Security Manager.

- Don't Force Signatures
- Force Signatures

- Warn Signatures

In each case further options “Allow Freeform Reason” and “All Fields Must Be Entered” can also be selected.

Don’t Force Signatures

This mode will prompt for a signature after every action that requires, but will accept a blank signature. If “Allow Freeform Reason” has been selected in the MassLynx Security Editor the Sign Alteration dialog (Figure 6.2) is invoked.

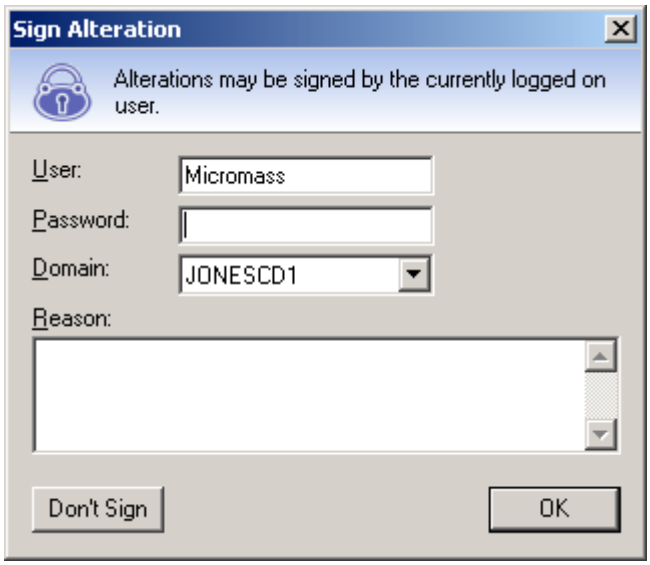


Figure 6.2 Sign Alteration dialog, with "Allow Freeform Reason" selected.

If a signature is entered a change will be entered in the audit log. If no signature is entered the change is still entered into the audit log but **No** is entered in the signed field and the following dialog is displayed.

Figure 6.3 The Signature Parameters Failed, warning dialog

If the “Allow Freeform Reason” option is not checked the following dialog is displayed, with the drop down list of predefined reasons. These reasons can be defined within the Security Editor and is explained in detail in the MassLynx Users Security Guide.

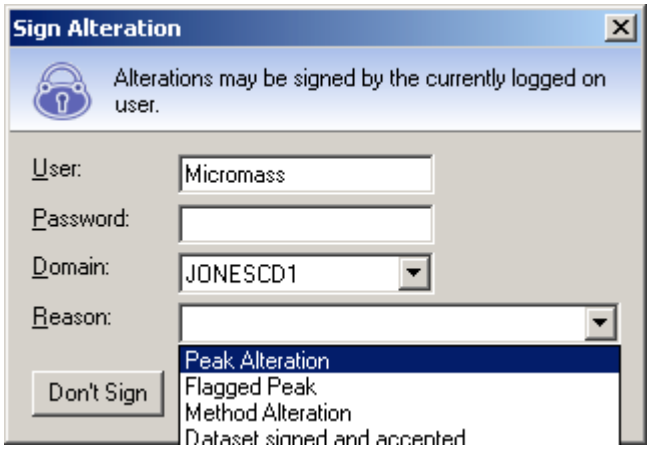


Figure 6.4 Sign Alteration dialog, with Predefined Reasons selected.

Selecting **Don't Sign** will remove the dialog, the audit log will not be updated and any changes will be cancelled.

Force Signatures

The following dialog (Figure 6.5) will be displayed when actions requiring a comment are attempted, assuming 'Allow Freeform Reasons' is selected in the Mass Lynx Security Editor. If this option is not selected, the reason box is replaced by a drop-down list containing pre-defined reasons, similar to the Don't Force Signatures mode described above.

Selecting **Sign It** will accept any changes and add an entry into the audit log, whilst **Don't Sign** will cancel any changes.

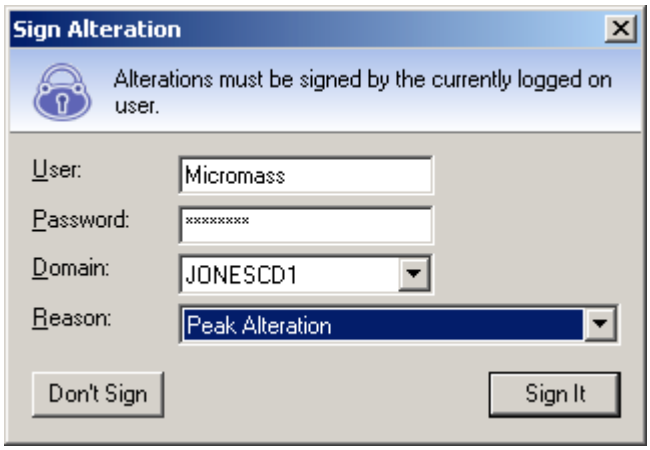
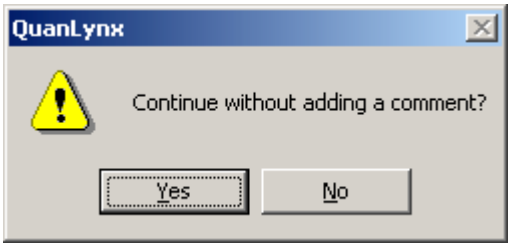


Figure 6.5 Sign Alteration dialog - Force Signatures Mode

Warn Signatures

This is similar to the force signatures mode except that the operation maybe completed without entering a signature, but the following warning dialog will be



If 'Yes' is selected, the changes will be saved and an entry added to the audit log. Otherwise the previous dialog is displayed.

Secure File Access

Under secure file access, a further two option are available on the File menu **Get From** and **Send To.** These allow for the sharing of secure files over a network. They are only available if the User Permissions for **BackLynx** Copy and Restore are checked in the MassLynx Security Manager. These actions are not recorded in the QuanLynx Audit Log but are recorded in LogLynx.

Note. The **Get From** and **Send To** options are only available if **Policies, Network File Access** has not been selected in the MassLynx Security Editor.

Send To

Selecting Send To prompts for a **Target for Network Copy** (Figure 6.6)and a file name, entering this information invokes an Administration Confirmation dialog (Figure 6.7) in which the currently logged on user and an administrator have to enter their passwords for the copy to proceed.

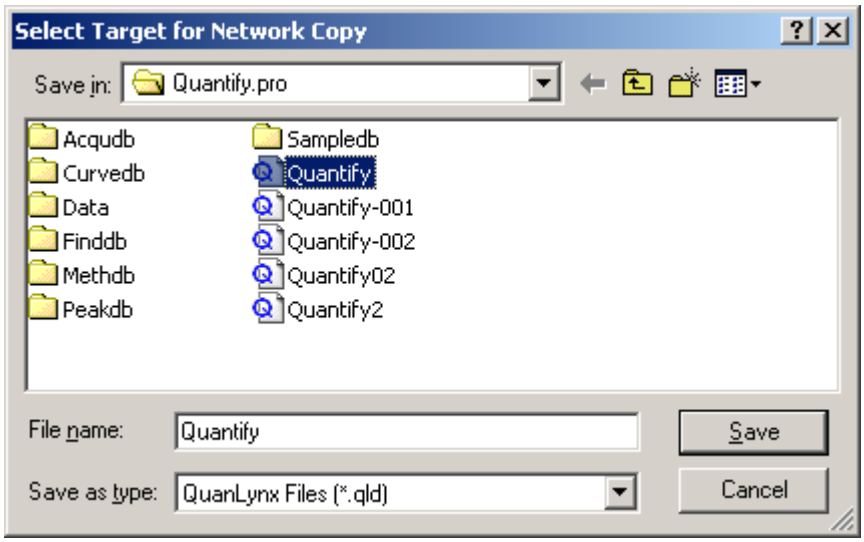


Figure 6.6 Select Target for Network Copy dialog

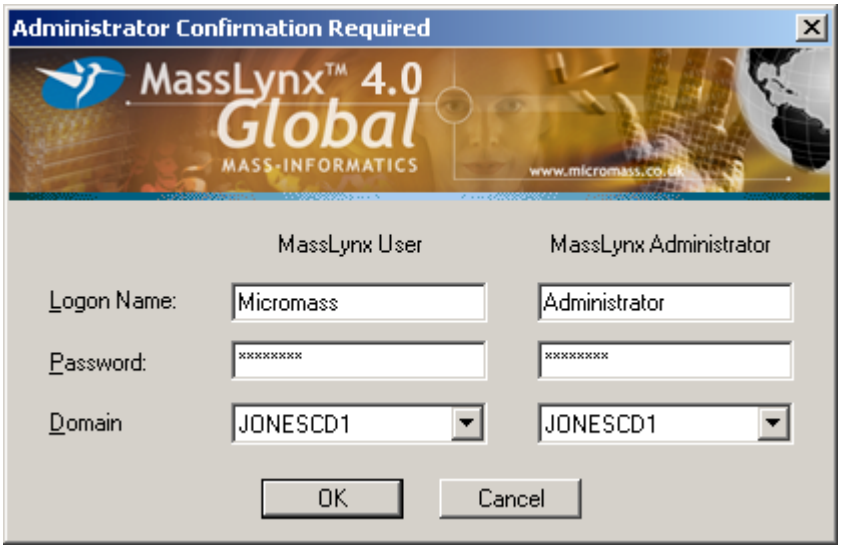


Figure 6.7 Administrator Confirmation Required dialog

This is followed by a confirmation dialog outlining the destination folder for the copy (Figure 6.8). If the signature parameters are incorrect a warning dialog is invoked that outlines the invalid fields (Figure 6.9). If Dual Authorization is not required it can be deselected under **Policies, Dual Authorization** in the MassLynx Security Editor.

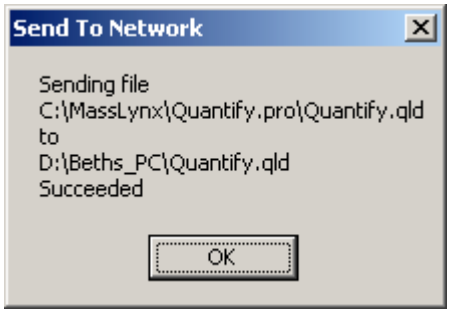


Figure 6.8 Send To Network Confirmation dialog

Figure 6.9 SecurityLogon Warning dialog

Get From

Get From prompts for **Source for Network Copy** followed by **Select Target for Network Copy** (Figure 6.6).

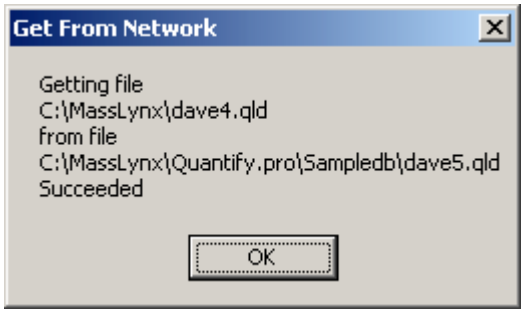


Figure 6.10 Get From Network Confirmation dialog

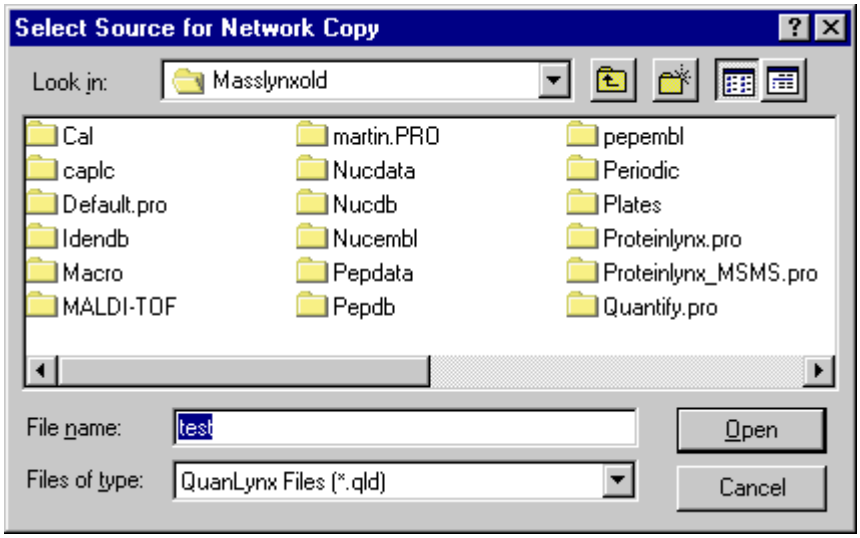


Figure 6.11 Select Source for Network Copy dialog

When the source and target have been chosen the Administrator Confirmation dialog is invoked (Figure 6.7) when signed this is followed by a Get From Network confirmation dialog box (Figure 6.10).

Permissions

The MassLynx Security Editor allows an administrator to set several permissions within QuanLynx. Initiate the Security Editor and logon as an administrator. The options associated with QuanLynx are listed in Table 6.1. For further information see the MassLynx Security Users Guide. Thus a user may be given permission to Accept a Dataset but not to Review it or Unaccept it.

If an action is attempted for which the right permissions are not held, the following dialog is displayed

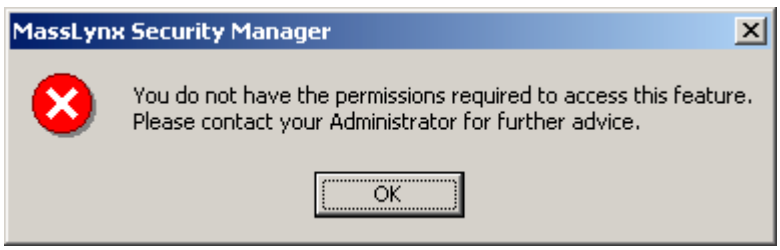


Figure 6.12 Denied Acces Warning dialog

Area	Function
Quantify Method Editor	Enter Editor Create new file on disk Alter existing file on disk
QuanLynx Method	Internal Alteration Internal RT adjustment
Quantify	Save new calibration curves to disk Alter existing calibration curves on disk Re/Integrate samples Add/Modify/Delete peaks
QuanLynx Dataset	Save new dataset to disk Alter existing dataset Accept a dataset Review a dataset Unaccept a dataset Use calculated calibration Include/Exclude calibration points Change individual curve fitting
Quantify	(legacy) Modify screen based report format Modify report format

Table 6.1 QuanLynx Group Right Options in Security Editor

Within all security enabled modes, a user is prevented from carrying out any of the above tasks for which they do not hold the required permission.

Events in the QuanLynx Audit Log

The following is a list of events that are recorded in the Audit Log, regardless of the security level selected.

- Method Changed
- Calibration Point Included
- Calibration Point Excluded
- Peak added

- Peak deleted
- Peak modified
- Process Extract
- Process Integrate
- Process Quantify
- Process Calibrate
- Update RT
- Dataset Created

Several of these events are also added to the LogLynx event log. The LogLynx event log may be viewed using the LogLynx event viewer. For a list of events that should be included in the External LogLynx audit trail, refer to the MassLynx Security Users Guide.

- Dataset Saved
- Accept Dataset
- Unaccept Dataset
- Review Dataset
- File Signed

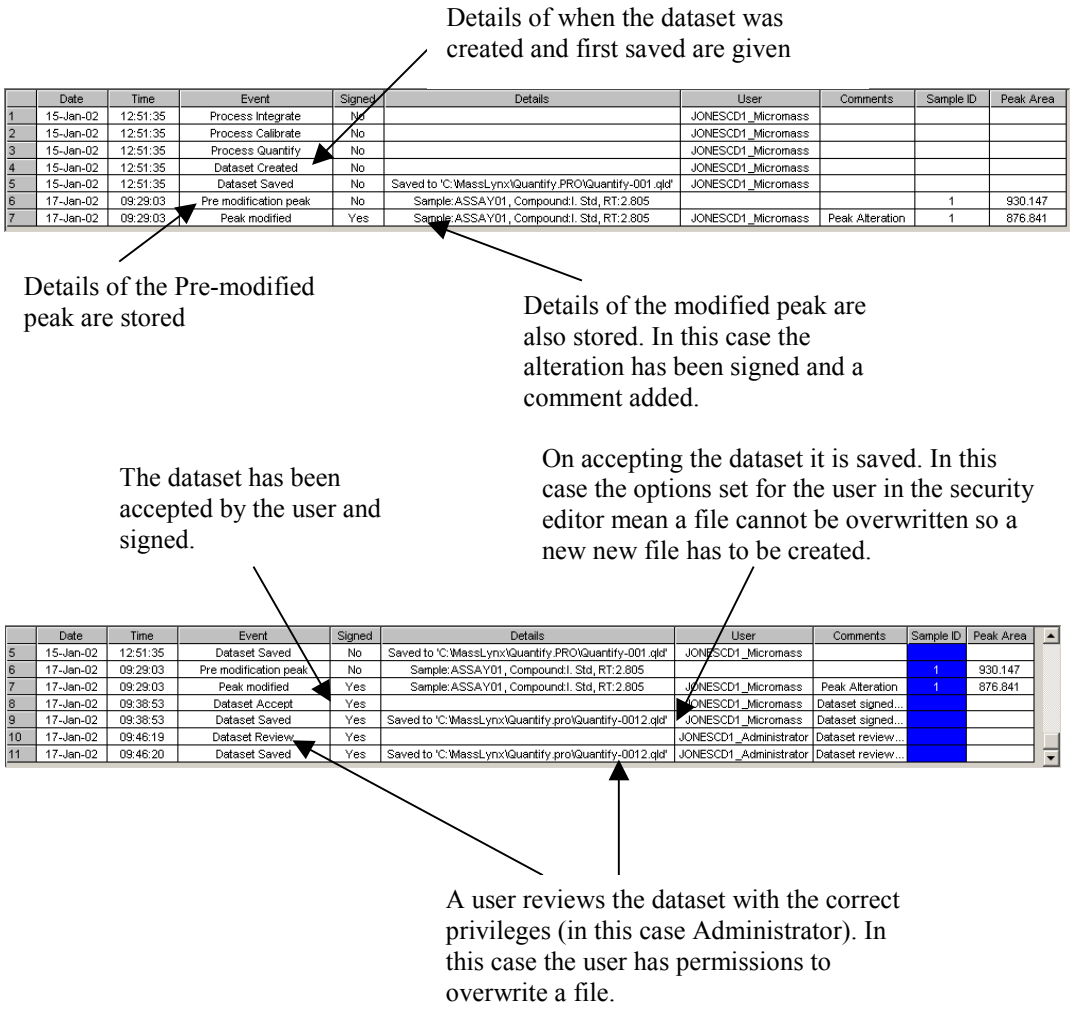
The QuanLynx Audit Trail

With all the above events recorded it is possible to trace the full history of a dataset and individual peaks within the audit log (see Figure 6.13), all of which is displayed in the Audit Log Bar (page 3-18). For details on columns available and how to display them see chapter 3 page 3-18.

In the example shown in Figure 6.13, the Force Signatures mode has been selected with predefined reasons. The user "Micromass" has created a dataset and made an alteration to the peak "Compound I. Std." from Assay01. The details of the original peak as well of those of the modified peak are recorded in the log. The peak alteration has to be signed for. In this case the column "Peak Area" is displayed, the difference between the modified and unmodified peak can be clearly seen.

The Dataset has then been accepted, again the user signs this. In this case the user permissions are such that the file "Quantify-001.qld" cannot be overwritten so a new file"Quantify-0012.qld" is saved. The user "Micromass" now logs off. This means that the original file "Quantify-001.qld" is still available should it be decided the changes to the peak are not OK.

A second user "Administrator", with permissions set to review datasets, logs on. The dataset is reviewed and approved. In this case "Administrator" also has permissions to overwrite existing files, so the file can be saved as "Quantify-0012.qld".



	Date	Time	Event	Signed	Details	User	Comments	Sample ID	Peak Area
1	15-Jan-02	12:51:35	Process Integrate	No		JONESCD1_Micromass			
2	15-Jan-02	12:51:35	Process Calibrate	No		JONESCD1_Micromass			
3	15-Jan-02	12:51:35	Process Quantify	No		JONESCD1_Micromass			
4	15-Jan-02	12:51:35	Dataset Created	No		JONESCD1_Micromass			
5	15-Jan-02	12:51:35	Dataset Saved	No	Saved to 'C:\MassLynx\Quantify.PRO\Quantify-001.qld'	JONESCD1_Micromass			
6	17-Jan-02	09:29:03	Pre modification peak	No	Sample:ASSAY01, Compound:I. Std, RT:2.805			1	930.147
7	17-Jan-02	09:29:03	Peak modified	Yes	Sample:ASSAY01, Compound:I. Std, RT:2.805	JONESCD1_Micromass	Peak Alteration	1	876.841

	Date	Time	Event	Signed	Details	User	Comments	Sample ID	Peak Area
5	15-Jan-02	12:51:35	Dataset Saved	No	Saved to 'C:\MassLynx\Quantify.PRO\Quantify-001.qld'	JONESCD1_Micromass			
6	17-Jan-02	09:29:03	Pre modification peak	No	Sample:ASSAY01, Compound:I. Std, RT:2.805			1	930.147
7	17-Jan-02	09:29:03	Peak modified	Yes	Sample:ASSAY01, Compound:I. Std, RT:2.805	JONESCD1_Micromass	Peak Alteration	1	876.841
8	17-Jan-02	09:38:53	Dataset Accept	Yes		JONESCD1_Micromass	Dataset signed...		
9	17-Jan-02	09:38:53	Dataset Saved	Yes	Saved to 'C:\MassLynx\Quantify.pro\Quantify-0012.qld'	JONESCD1_Micromass	Dataset signed...		
10	17-Jan-02	09:46:19	Dataset Review	Yes		JONESCD1_Administrator	Dataset review...		
11	17-Jan-02	09:46:20	Dataset Saved	Yes	Saved to 'C:\MassLynx\Quantify.pro\Quantify-0012.qld'	JONESCD1_Administrator	Dataset review...		

Figure 6.13 Typical Audit Trail in QuanLynx

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