

A Step by Step Guide to Quantification

1. Create a Sample List

The first thing that you must do when using MassLynx Quantify is to create a list of samples that you want to use to perform the analysis. These samples can be acquired manually, but more often they will be acquired automatically using an autosampler. The Sample List editor display has various columns such as Filename, Bottle Number and Sample Type that can be filled in for each sample. Each sample is displayed as one row in the Sample List. The Sample List editor is part of the MassLynx top-level screen.

We need to tell MassLynx everything that it needs to know about the samples in the list in order for it to perform a complete analysis. This really means that we must describe to the system what each of the bottles in the autosampler is, i.e. whether it is a standard, an analyte, a blank or a QC sample, how to acquire it and its concentration(s) if it is a standard. In addition we must give it a file name in which to store the data and we may want to add some management information such as Sample ID, the submitters name or a sample description.

For information on how to create a Sample List see Chapter 2 “Sample Lists”.

■ Projects

MassLynx gives you the option to organise your work into projects. Projects are a very useful way of organising all of the data files, methods and results for a particular assay into one directory structure on disk.

When you open a MassLynx project, MassLynx creates a new directory to hold all the different files associated with this project. The advantage of using projects is that it becomes very simple to archive everything associated with your assay because you do not have to hunt around the disk to find the files you need and the chances of you forgetting to save an important file are greatly reduced. The types of file that can be saved in a MassLynx Project are:

- Raw data files
- Peak lists
- Sample lists
- Quantify methods
- Quantify calibration curves
- Tuning files
- Scan methods
- Instrument calibration files
- Inlet methods.

Projects are created and selected from the MassLynx top level **File** menu. See Chapter 1, "Getting Started" for instructions on how to create or open a Project.

2. Create a Quantify Method

A Quantify Method must be created before Integration or Quantification can be performed.

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.
- Formation of a Quantify calibration curve.

■ Quantify Method Editor

The Quantify Method Editor creates new methods and modifies existing ones.

A method selected from within the Method Editor will become the current system method file and is used when performing Quantify operations.

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 quantification. This can be achieved by selecting the **File Save** menu item to update the current method file, or **File Save As** to save to a new method file.

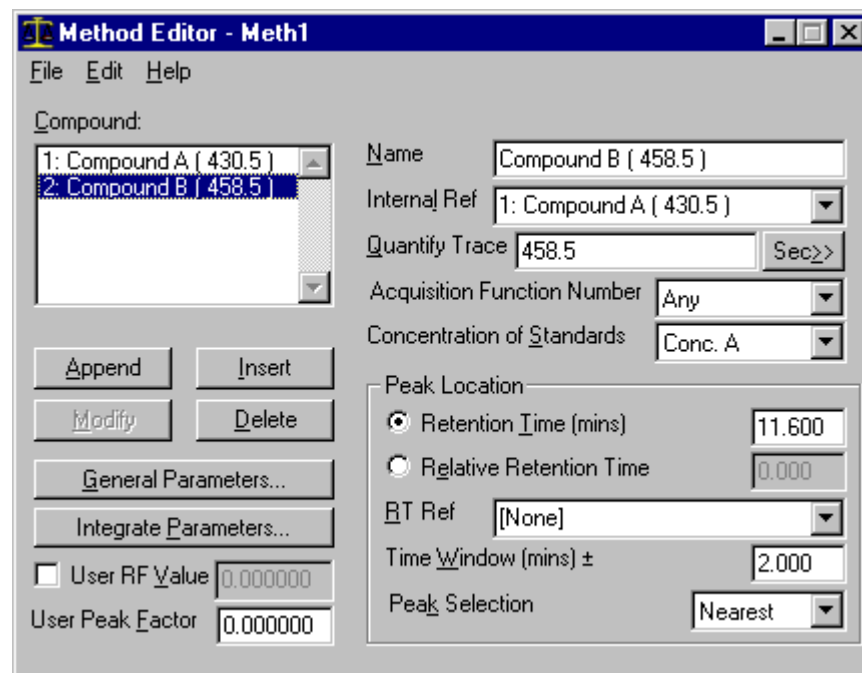


Figure 7.5 Quantify Method Editor

■ To access the Method Editor

Select **Edit Method** from the **Quantify** menu

-or-

Select **Quantify Method** from the **Quantify** service **Edit** menu.

When invoked the 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 Method Editor method becomes the current system method file that is used when performing quantification.

■ Setting Method Parameters

1. Enter the name of the compound in to the **Name** box. This can be up to 40 characters in length. The names of the compounds in the method appear in the **Compound List**.
2. 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 which appear in the compound list can be selected.
3. Set the **Quantify Trace** edit control to hold the trace descriptor of the chromatogram being used to quantify the compound. This should be
 - A single decimal number for mass chromatograms
 - Two decimal numbers separated by a ">" for an MRM function e.g. 274.10 > 182.10. The first number represents the parent mass and the second the daughter mass.
 - '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 DAD data.
 - Ch1, Ch2 etc for 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.

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.

Note: This value will be automatically entered if the Peak Location parameters are entered using the mouse.

- In specific cases, it may be necessary to specify a secondary ion. Press the **Sec>>** button. The Secondary dialog is displayed.

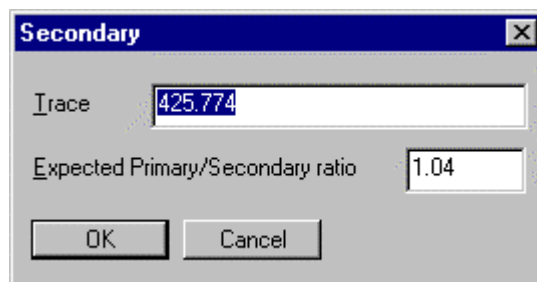


Figure 4.1 Secondary Ion dialog

- In the **Trace** field, enter the mass of the Secondary ion. If this field is left blank the Secondary ion will not be used during peak location.
- In the **Expected Primary/Secondary ratio** field, enter the expected ratio between the size of the Primary and Secondary peaks. If this field is set to zero the peak ratio will not be used for compound location. Press **OK** to accept the new settings.
- For multifunction data you can specify which function number is to be used to quantify the current compound in the **Acquisition Function Number** control.
- Set the **Concentration of Standards** box to the Sample List column that contains the compounds 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. If the compound is an Internal Standard and is at the same concentrations in all samples the **Fixed** option can be selected. The software allows up to 20 concentration levels within a single sample.
- Now set the **Peak Location** parameters. The location method determines how a peak within a Peak List is identified as matching a method compound.

Select the peak Location Method by clicking on the **Retention Time** or **Relative Retention Time** radio button. Alternatively, you can select a method compound to use as the retention time reference from the **RT Ref** drop down list. 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.

The Time Window and Retention Time or Relative Retention Time parameters can be entered with the keyboard or the mouse.

With the mouse, arrange the MassLynx display so that you can see both the Quantify Method Editor and the Chromatogram window showing the chromatogram you wish to use. Select the Compound for which you wish to set parameters in the Method Editor.

On the chromatogram press the right mouse button at one end of the chromatogram region of interest, and without releasing the button, drag the mouse horizontally to the other end. As you drag the mouse you will see a "rubber band" stretched out to indicate the range you have selected. The Quantify Method Editor window will be updated to show the new **Time Window** and the **Retention Time** or **Relative Retention Time** will be set to the middle point of the Time Window.

The **Quantify Trace** parameter will be set to the same type as the chromatogram selected with the mouse (TIC, BPI, mass chromatogram or MRM).

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

With the keyboard, if **Retention Time** was selected set it to the time in decimal minutes at which the compound is expected to elute. The **Time Window** parameter must also be set as described below.

If **Relative Retention Time** was 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.

7. If you have selected the **Location Method** to be **Retention Time** set the **Time Window** edit control to specify by how much the compound elution time may vary. The **Time Window** is applied either side of the predicted retention time to give a valid window. The **Time Window** also defines the chromatogram range that will be integrated.
8. Set the **Peak Selection** parameter to specify which peak should be located where more than one peak is detected within the 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.
9. If required set the **User Peak Factor**. This value is a multiplication factor which will be applied to all calculated concentrations for the current compound. If the **User Peak Value** is left at zero or set to 1 the concentration values will not be changed.
10. If required select the **User RF Value** box and enter a 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.
11. Now press the **General Parameters** button. To use these general parameters for all compounds in the method choose **Propagate General Parameters** from the **Quantify Method Editor Edit** menu. A tick mark will appear next to this option and the general parameters will be copied to all compounds in the method.

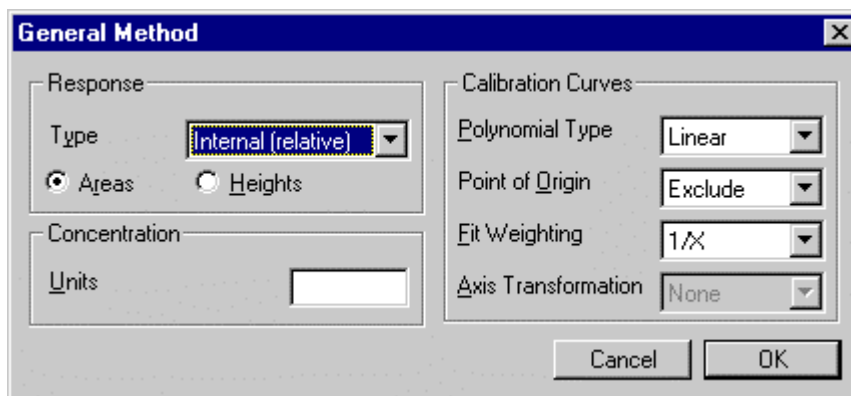


Figure 7.6 General Method dialog

12. 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. The Response **Type** box should be set to **Internal (relative)** or **External (absolute)**.

Internal (relative) should be selected if a compounds response is to be calculated using an Internal Standard, in which case the **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 **Heights** or **Areas** to specify if compound responses will be based upon peak heights or areas.
15. Next set the **Calibration Curves** Parameters. The calibration curve parameters determine how a compounds calibration curve is to be formed.

Select the type of calibration curve in the **Polynomial Type** control; **Average RF**, **Linear**, **Quadratic**, **Cubic** or **Quartic**.

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 with a Fixed concentration.

Linear Performs a linear regression on the compounds calibration points.

Quadratic Performs a second order regression on the compounds calibration points.

Cubic Performs a third order regression on the compounds calibration points.

Quartic Performs a fourth order regression on the compounds calibration points.

16. Set **Point of Origin** box 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.

17. Set the calibration **Fit Weighting** to **None**, **1/X**, **1/X²**, **1/Y** or **1/Y²**. 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.

18. 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.

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

19. 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.

■ **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 Users Guide.

To use the same integration parameters for all compounds in the method choose **Propagate Integration Parameters** from the Quantify Method Editor **Edit** menu. A tick mark will appear next to this option 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 **Time Window** parameter in the Quantify Method. If you wish to integrate over a larger window, select **Integrate Window** from the Quantify Method Editor **Edit** menu and specify a multiplication factor. This factor will be applied to the location window to calculate the integration window and is the same for all compounds in the method.

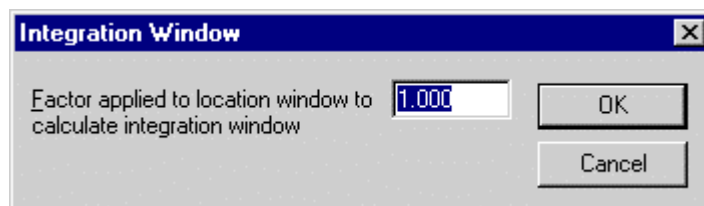


Figure 7.7 Integration Window Dialog

To define the integration parameters choose the **Integrate Parameters** button from the Quantify Method Editor to invoke the dialog box.

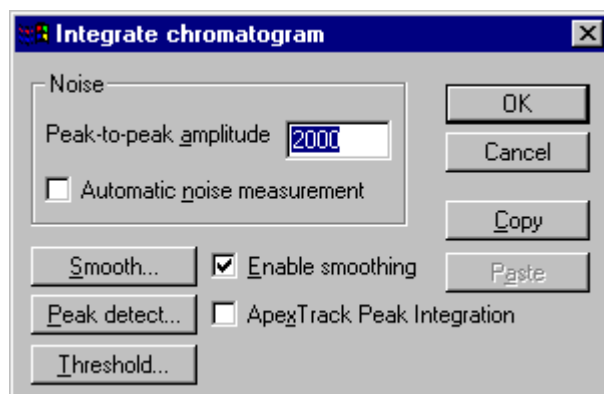


Figure 7.8 Peak Integration Dialog

The **Integrate chromatogram** dialog requires the user to enter the **Peak-to-peak noise amplitude**. This value is used by the integration software to prefilter the chromatogram. A suitable value can be measured directly from a chromatogram by clicking 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 Chromatogram and the Quantify Method. This can be useful when experimenting to find the correct integration parameters using chromatogram.

Check the **ApexTrack Peak Integration** box to use an alternative peak detection algorithm.

Smoothing

You may choose to smooth the chromatogram before integrating by selecting the **Enable smoothing** check box. The parameters for the smooth may be examined and altered by choosing the **Smooth..** button.

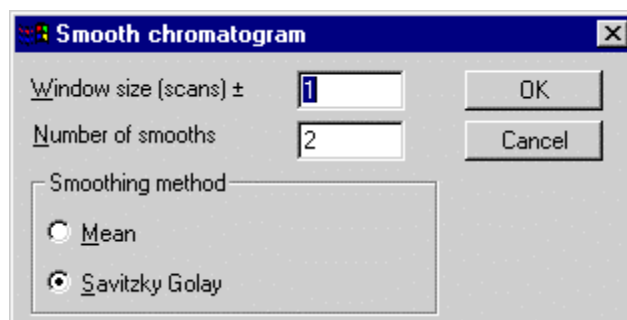


Figure 7.9 Smooth Chromatogram Parameters dialog

The **Window size** 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.

Peak Thresholding

Small peaks may optionally be removed by setting one of the four available threshold parameters. Choose the **Threshold...** button to examine or modify these parameters.

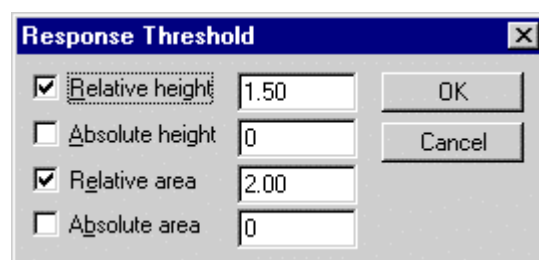


Figure 7.10 The Response Threshold dialog

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

Absolute height Check this box to remove peaks whose height is less than the specified value.

Relative area Check this box to remove peaks whose area is less than the specified percentage of the largest peak area.

Absolute area Check this box to remove peaks whose area is less than the specified value.

Peak Detection

You may examine and modify the parameters controlling the positioning of baselines and separation of partially resolved peaks by verticals (droplines) by choosing the **Peak detect...** button.

A brief description of each of the Peak Detection parameters is given below, for a more detailed discussion see the Peak Detection section of the "Chromatogram" chapter.

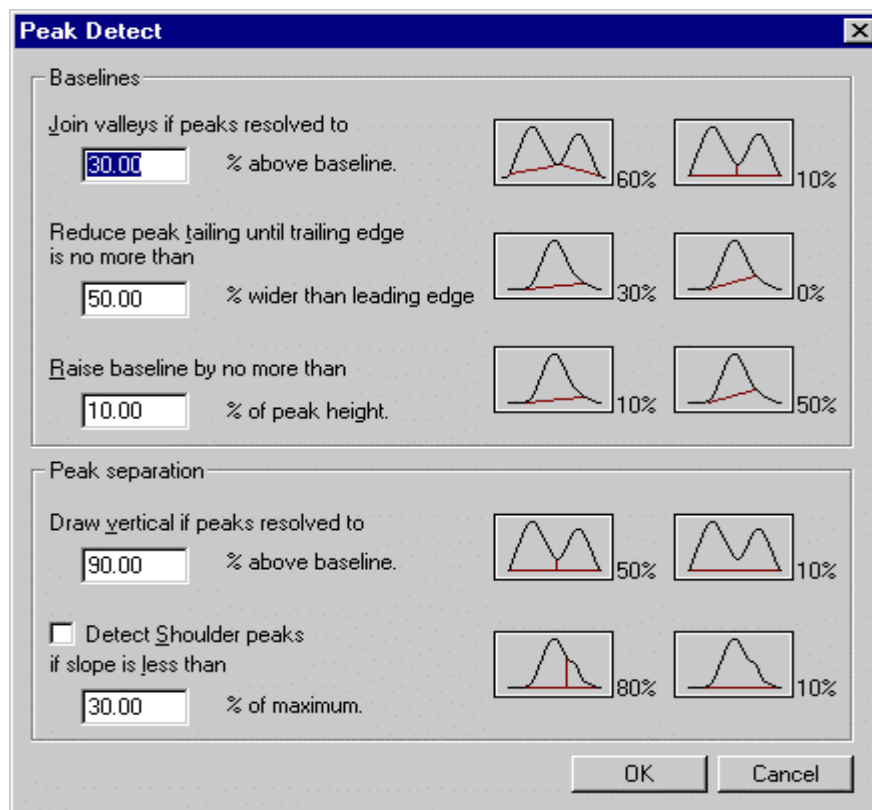


Figure 7.11 The Chromatogram Peak Detect dialog

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 normal operating range is 5%–75%.

Reduce peak tailing and **Raise baseline** These parameters allow control over the positioning of baseline end points. The default value for the reduce peak tailing parameter is 50%, and 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%–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). If you wish poorly resolved peaks to be separated, increase the value of this parameter. The default value is 90%, and normal operating range is 50%–100%.

Detect shoulder peaks Check this box to optionally attempt to detect completely unresolved peaks, or shoulders, by selecting the **Detect shoulder peaks** check box. 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%–90%.

■ **Creating a new method**

1. Select **New** from the **File** menu. The editor controls are set to default values and the compound list box is empty. The name of the current method in the editor title bar is set to [Untitled]
2. Add the desired compounds as described below.
3. Select **Save As** from the **File** menu. Enter the name of the new method into the Save As dialog.

■ **Selecting an existing method**

1. Select **Open** from the **File** menu.
2. Choose the required method file from the file selection dialog and press **Open**. The compounds held within the method are loaded into the editor compound list box. The first compound within the method is selected.

■ **To propagate general parameters to all compounds**

To use the same integration parameters for all compounds in the method choose **Propagate General Parameters** from the Quantify Method Editor **Edit** menu. A tick mark will appear next to this option and the general parameters will be copied to all compounds in the method.

■ **To propagate integration parameters to all compounds**

To use the same integration parameters for all compounds in the method choose **Propagate Integration Parameters** from the Quantify Method Editor **Edit** menu. A tick mark will appear next to this option and the integration parameters will be copied to all compounds in the method.

■ **To add a new compound**

1. Enter the required information for a new compound.
2. Press the **Append** button. The new compound will be added to the end of the compound list.

■ **To insert a new compound**

1. Select the entry in the 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.

■ **To modify information for an existing compound**

1. Select the entry in the compound list which is to be modified.
2. Enter the updated information.
3. Press the **Modify** button.

■ **To delete a compound**


1. Select the entry in the compound list which is to be deleted.
2. Press the **Delete** button or the <Delete> key.

■ **To delete all compounds in the method**

1. Choose **Delete All Compounds** from the Method Editor **Edit** menu. Choose **OK** to delete all compounds in the method.

3. Starting the Analysis

Before starting an analysis save any changes made to the Sample List by selecting **Save** from the **Sample List File** menu.

To begin acquiring data select **Start** from the MassLynx top-level **Run** menu or press the  toolbar button, this invokes the Start Sample List Run dialog.

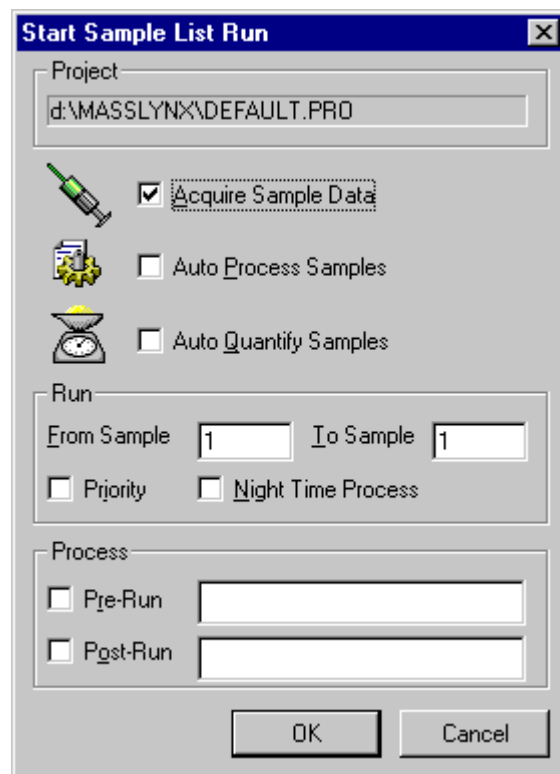


Figure 7.12 Start Sample List Run dialog

Project The name of the current project appears here. To acquire to a different project you must 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 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 then the current method will be used.

These three actions can be run together or independently. I.e. the user can acquire, process and quantify data in one go, or acquire data in one run and process or quantify it at a later date.

Priority Process Check this box to mark this entry as a Priority process. Note the **Pre-emptive Scheduling** box on the **Queue Properties** dialog must be checked. See the Getting Started chapter of the MassLynx manual for details.

Night Time Process Check this box to mark this entry as a night time process. Note the **Night Time Scheduling** box on the **Queue Properties** dialog must be checked. See the Getting Started chapter of the MassLynx manual for details.

Run From Sample n To Sample n Sets the range of samples in the sample list which will be acquired/and or analysed.

4. Quantify the Data

Once data has been collected it can be Quantified. Select **Process Samples** from the **Quantify** menu to display the Quantify Samples dialog. Check the boxes required and press **OK**.

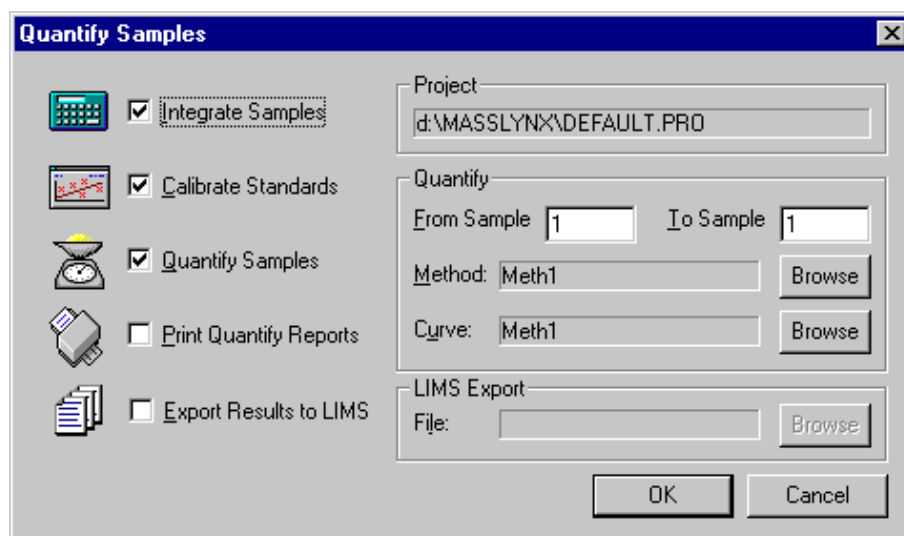


Figure 7.13 Quantify Samples dialog

Integrate Samples Integrates all the sample data files named in the Peak List.

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

Quantify Samples Uses Integration results and Quantify calibration curves to calculate compound concentrations. To change the Method and Curve files press the **Browse** buttons and select a new one.

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 box is checked 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**. See Export to LIMS File on page 328.








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

Quantify From Sample n To Sample n Sets the range of samples in the sample list which will be quantified.

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

5. Using the Quantify Window to Examine Results

■ The Quantify Toolbar

-  Press to show previous compound/sample in Summary window.
-  Press to show next compound/sample in Summary window.
-  Press to show previous peak in the Summary window.
-  Press to show current peak in the Summary window.
-  Press to show next peak in the Summary window.
-  Press to view previous Sample Group. This applies to data acquired by QuanLynx.
-  Press to view next Sample Group. This applies to data acquired by QuanLynx.

■ The Quantify Window

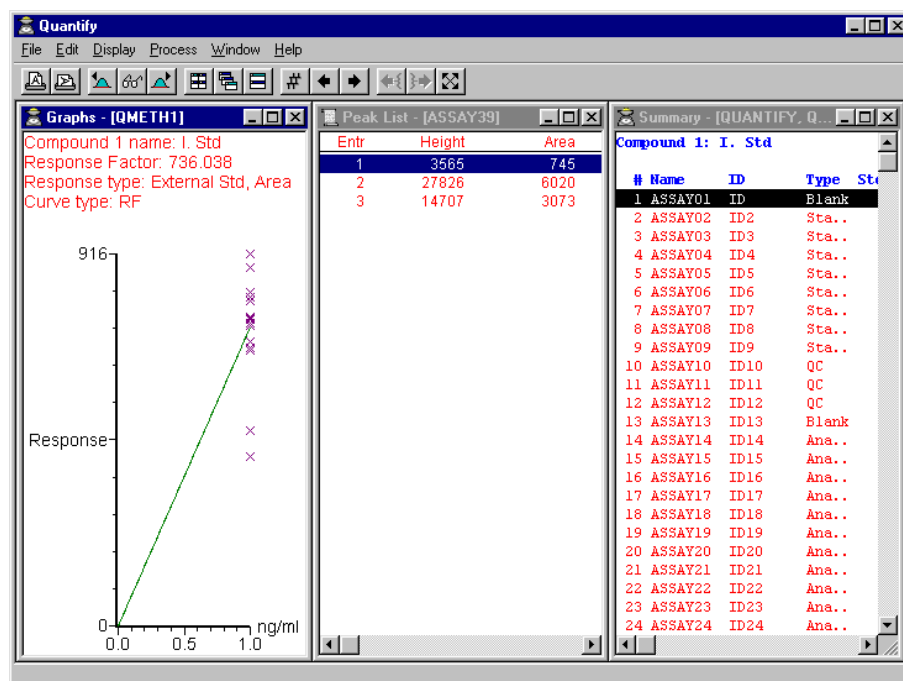


Figure 7.14 The Quantify Window

The Quantify window is displayed by selecting **View Results** from the top-level **Quantify** menu. It has its own menu bar and Toolbar and uses a Multiple Document Interface (MDI) display which allows multiple windows (called documents) to be displayed simultaneously.

There are three documents, the Summary document, the Graphs document and the Peak List document, you can turn each of these on and off as required.

■ Controlling the appearance of the Quantify display

1. Select **View** from the Quantify **Display** menu to enter the Quantify Display Parameters dialog.

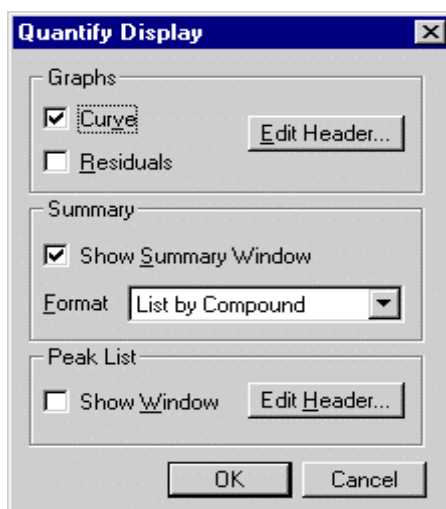


Figure 7.15 Quantify Display Parameters dialog

2. Select which Quantify windows you wish to display by checking the relevant boxes. You can choose to display any combination of the following: the Graphs window showing calibration curves, the Graphs window showing residuals, the Summary window and the Peak List window.
3. Choose whether you wish to display the Summary window listed by compound or by sample by selecting the relevant setting in the **Summary Format** control.
4. A user configurable document header can be displayed at the top of the Graphs window or the Peak List window. In the default display the document header is not displayed. To invoke the Edit Header dialog press the **Edit Header** button. Alternatively, the header editor can be invoked from outside the view dialog by double clicking, with the left mouse button, on an existing document header. See Chapter 1, "The Header Editor" for more information about using the Header Editor.

■ Controlling the appearance of the Quantify Chromatogram display

The Chromatogram window displaying a particular Peak List entry can be invoked by double-clicking with the left mouse button on the desired entry in the Summary window or the Peak List document entry. Calibration standard peaks can be selected by double clicking with the left mouse button on the desired calibration point in the Calibration Curve document. This allows manual adjustment of integration results.

The appearance of the Chromatogram window can be controlled by selecting **Chromatogram** from the Quantify **Display** menu.

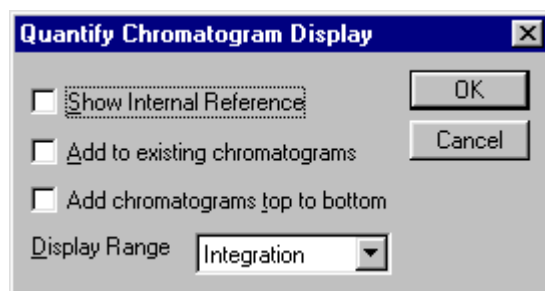


Figure 7.16 Quantify Chromatogram Display dialog

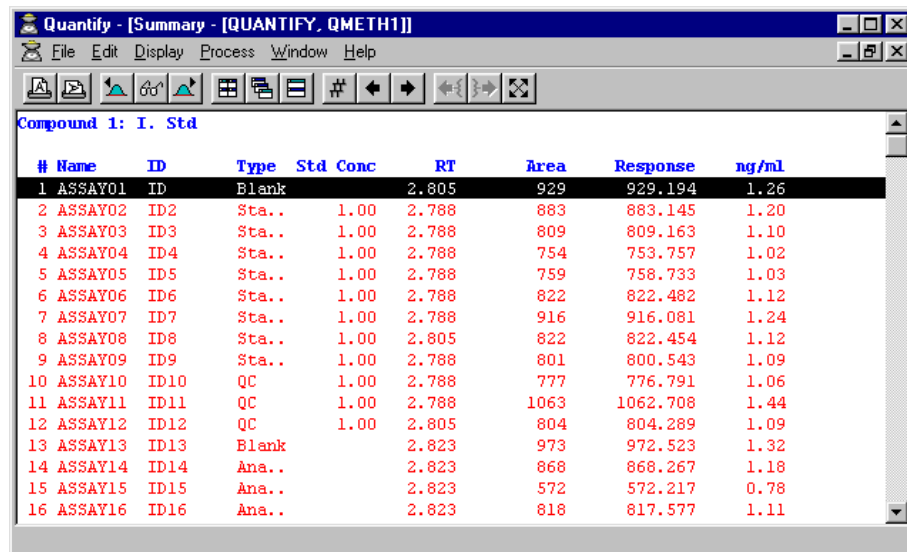
Show Internal Reference If this box is checked, the internal reference peak, if one is specified, will be shown with the current peak.

Add to existing chromatograms If this box is checked, each new chromatogram trace will be added to those already displayed.

Add chromatograms top to bottom If this box is checked, then when displaying chromatograms the compound primary chromatogram will appear at the top of the display with secondary chromatogram and IS chromatogram(s) below it. This is the reverse order to the default operation.

Display Range From the drop down list box, you can select **Integration** to use the range that was integrated over, **Keep Current** to keep range currently displayed or **Acquisition** to use the range acquired over.

- The Summary Document



Quantify - [Summary - [QUANTIFY.QMETH1]]

File Edit Display Process Window Help

Compound 1: I. Std

| # | Name | ID | Type | Std Conc | RT | Area | Response | ng/ml |
|----|---------|------|-------|----------|-------|------|----------|-------|
| 1 | ASSAY01 | ID | Blank | | 2.805 | 929 | 929.194 | 1.26 |
| 2 | ASSAY02 | ID2 | Sta.. | 1.00 | 2.788 | 883 | 883.145 | 1.20 |
| 3 | ASSAY03 | ID3 | Sta.. | 1.00 | 2.788 | 809 | 809.163 | 1.10 |
| 4 | ASSAY04 | ID4 | Sta.. | 1.00 | 2.788 | 754 | 753.757 | 1.02 |
| 5 | ASSAY05 | ID5 | Sta.. | 1.00 | 2.788 | 759 | 758.733 | 1.03 |
| 6 | ASSAY06 | ID6 | Sta.. | 1.00 | 2.788 | 822 | 822.482 | 1.12 |
| 7 | ASSAY07 | ID7 | Sta.. | 1.00 | 2.788 | 916 | 916.081 | 1.24 |
| 8 | ASSAY08 | ID8 | Sta.. | 1.00 | 2.805 | 822 | 822.454 | 1.12 |
| 9 | ASSAY09 | ID9 | Sta.. | 1.00 | 2.788 | 801 | 800.543 | 1.09 |
| 10 | ASSAY10 | ID10 | QC | 1.00 | 2.788 | 777 | 776.791 | 1.06 |
| 11 | ASSAY11 | ID11 | QC | 1.00 | 2.788 | 1063 | 1062.708 | 1.44 |
| 12 | ASSAY12 | ID12 | QC | 1.00 | 2.805 | 804 | 804.289 | 1.09 |
| 13 | ASSAY13 | ID13 | Blank | | 2.823 | 973 | 972.523 | 1.32 |
| 14 | ASSAY14 | ID14 | Ana.. | | 2.823 | 868 | 868.267 | 1.18 |
| 15 | ASSAY15 | ID15 | Ana.. | | 2.823 | 572 | 572.217 | 0.78 |
| 16 | ASSAY16 | ID16 | Ana.. | | 2.823 | 818 | 817.577 | 1.11 |

Figure 7.17 Quantify Summary Document

The Quantify Summary window gives a summary of the results of quantification. The results in the Summary window can either be listed by compound or by sample. If no peak has been located for a compound entry the peak information fields will be left blank.

The Quantify Toolbar buttons can be used to display information about a new compound/sample.

The format of the Summary window also determines the format of the Summary Reports that can be printed. Two Summary Reports can be printed - the Summary Report listed by sample and the Summary Report listed by compound.

There are many different columns of quantification information that can be displayed in the Summary window, the user can select which columns are currently displayed. Use the horizontal and vertical scroll bars, if available, to move around the Summary window display.

- **To select which fields will be displayed in the Summary window and Summary Reports**

The format of the Summary window listed by sample and listed by compound are changed independently. Double click with the left hand mouse button on one of the Summary window column headers or select **Output Compound Format** or **Output Sample Format** from the Quantify **Edit** menu.

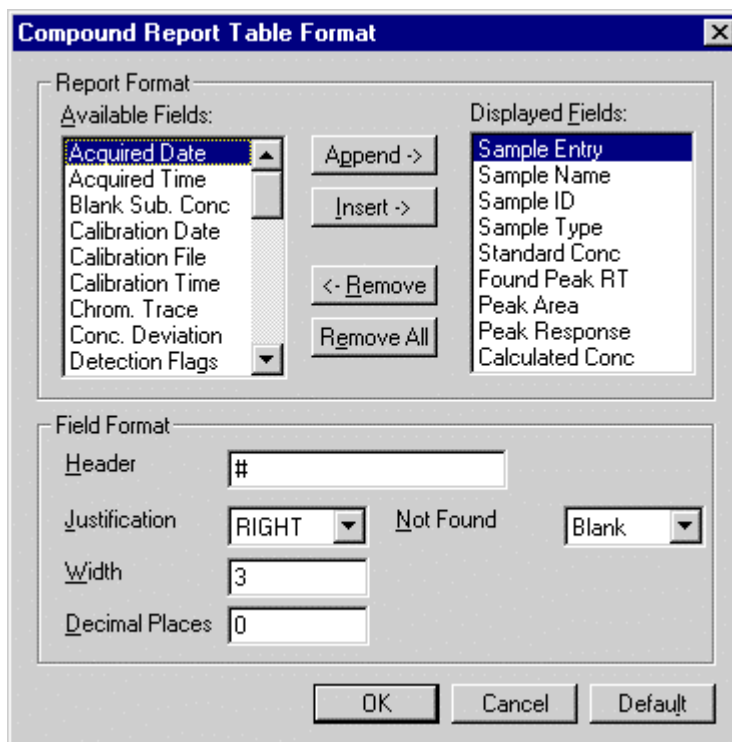


Figure 7.18 Quantify Compound Report Table Format dialog

The fields currently present in the Summary document are shown in the **Report Format** list on the right hand side. Other fields, which can be added to the Summary document, are shown in the **Available Fields** list on the left hand side.

Any changes made here will be reflected in the Summary window display and in the Summary Reports. The Summary Reports will show up to the maximum number of columns that will fit on one page. To include more columns print in landscape mode instead of portrait mode.

- **To append new fields to the Summary document**

1. Highlight the field you wish to append in the **Available Fields** list box.
2. Choose the **Append** button.
3. Repeat steps 1 and 2 as required.
4. Choose the **OK** button to save the changes and exit.

■ **To insert new fields in the Summary document**

1. Highlight the field you wish to insert in the **Available Fields** list box.
2. Highlight the field before which you wish to insert the new field in the **Report Format** list box.
3. Choose the **Insert** button.
4. Repeat steps 1 to 3 as required.
5. Choose the **OK** button to save the changes and exit.

■ **To remove a field from the Summary document**

1. Highlight the field you wish to remove in the **Report Format** list box.
2. Choose the **Remove** button. To remove all the fields in the Summary document choose the **Remove All** button.
3. Repeat steps 1 to 2 as required.
4. Choose the **OK** button to save the changes and exit.

■ **To format the display of a field in the Summary document**

1. Highlight the field whose display settings you wish to alter in either the **Available Fields** or **Report Format** list boxes.
2. Change the **Header** field to display the heading you wish to display above this column.
3. Change the **Justification** setting to **Left**, **Right** or **Centre** as required.
4. Change the **Field Width** and **Decimal Places** as required.
5. Change the setting of the **Not Found** control as required. The **Not Found** control determines what will be printed in the Quantify report for this field if the peak is not found. The options available are **Blank**, **Zero**, **Dash**, **Not found** or **n/a**.
6. Repeat steps 1 to 5 as required.
7. To change the settings for all fields back to default values choose the **Default** button.
8. Choose the **OK** button to save the changes and exit.

■ **To Save the Summary document**

From the Summary document select **Save Summary by Compound** or **Save Summary by Sample** from the **File** menu.

■ The Graphs Document

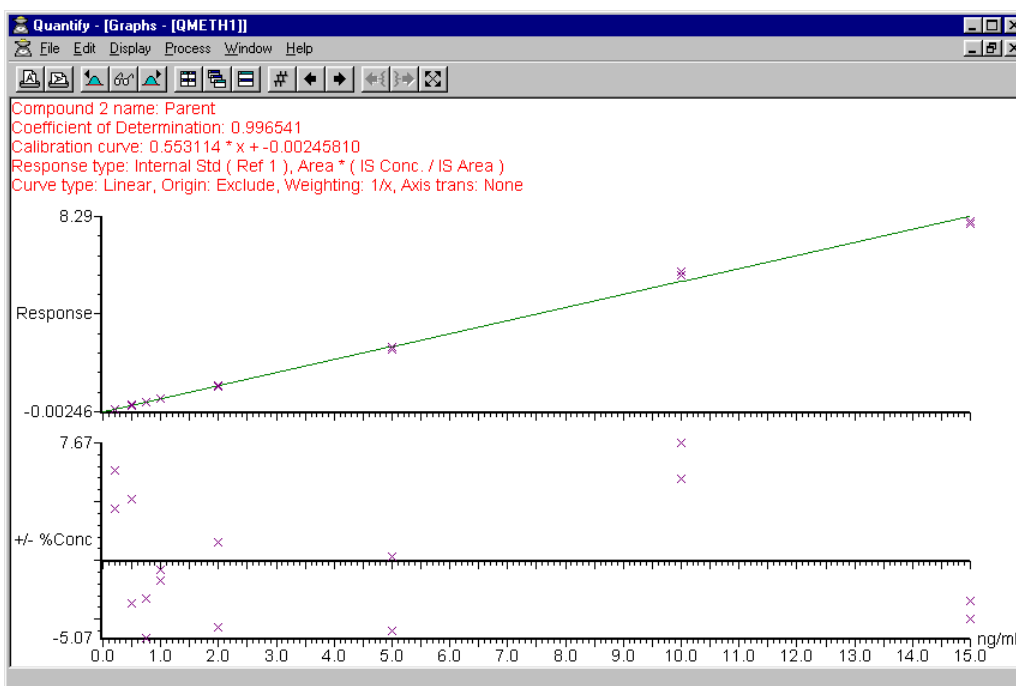




Figure 7.19 Quantify Graphs Document

The Quantify Graphs window contains a graphical display of the current calibration curve and/or its residuals plot. Statistical information on the calibration curve is displayed above the graphs. A user configurable document header can be displayed at the top of the window.


The current calibration curve file holds a calibration curve for each of the compounds being analysed. A Toolbar within the document allows other calibration curves to be easily selected by pressing the  or  buttons.


The calibration curve graph displays concentration against response value. The vertical axis is labeled as a percentage of the maximum response. The horizontal axis is labeled with the concentration units specified in the method. The displayed calibration curve shows the response value expected for particular concentrations. Crosses mark the calibration points used to form the curve.


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

■ **Selecting Another Calibration Curve**

To select another calibration curve, from within the current file, using the Toolbar.

Choose the  button to show the previous calibration curve.


Choose the  button to show the next calibration curve.

Choose the  button to invoke a dialog allowing the number of the desired calibration curve to be entered. Curve number 1 is for the first compound, curve number 2 the second and so on.

■ **Changing the display range of the Calibration Curve**

Both the horizontal and vertical display range of the Graphs window can be expanded. Press the left 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 you drag the mouse you will see a "rubber band" stretched out to indicate the range you have selected; don't go beyond the bounds of the axis. 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  button on the Toolbar restores the display to the default range.

■ **Changing Calibration Curve File**

To view another calibration curve file select **Calibration** from the **File** menu, the **File Open** dialog will appear. Select a file from the list box and press **Open**.

- **Displaying more information about a particular calibration point**

A single click with the left mouse button on a calibration point updates the Summary and Peak List windows to show the calibration point as the current entry.

Double clicking with the left mouse button on a calibration point displays the Peak List entry and shows the corresponding chromatogram. The **Edit Quantify Peak** dialog is automatically loaded allowing the user to make manual adjustments to the baseline assignment. A comment can also be stored in the peak list for this particular peak. For more information see "Manual Peak Integration" on page 321.

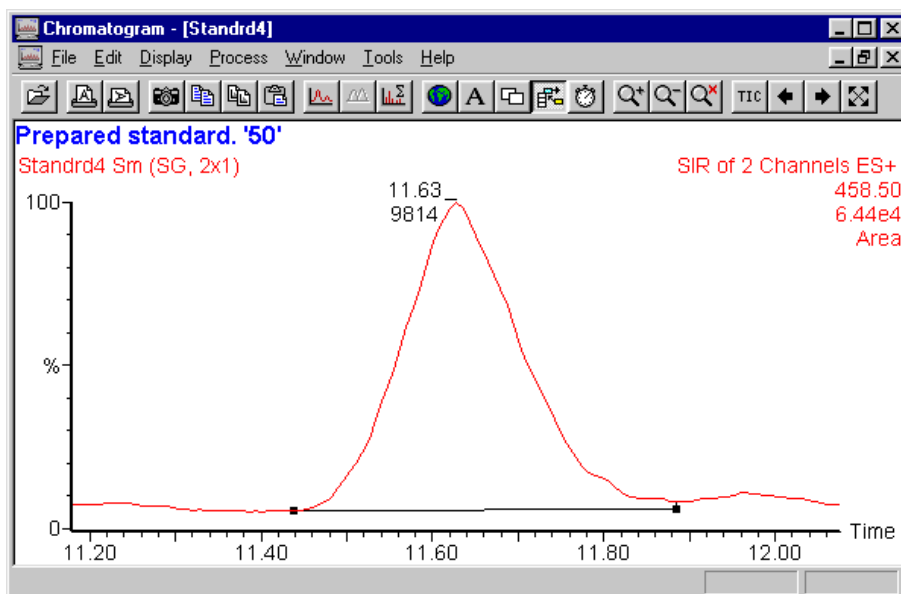


Figure 7.20 Chromatogram showing peak used for calibration point

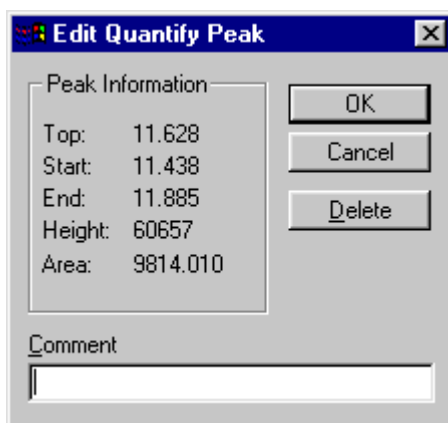
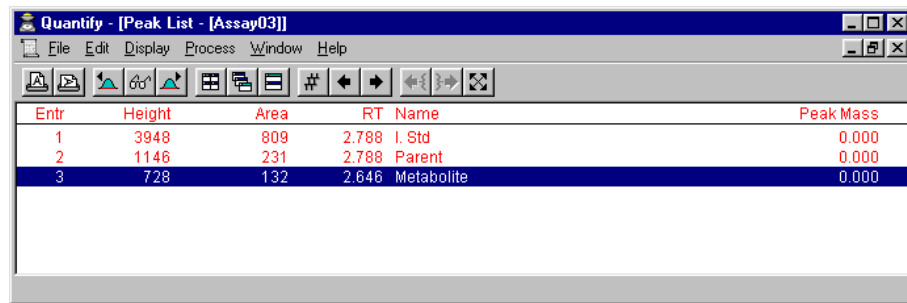


Figure 7.21 Edit Quantify peak dialog

■ The Peak List Document



| Entr | Height | Area | RT | Name | Peak Mass |
|------|--------|------|-------|------------|-----------|
| 1 | 3948 | 809 | 2.788 | I. Std | 0.000 |
| 2 | 1146 | 231 | 2.788 | Parent | 0.000 |
| 3 | 728 | 132 | 2.646 | Metabolite | 0.000 |

Figure 7.22 Peak List Document

The Quantify Peak List window contains a textual listing of all the peaks within the current peak list, the current peak is highlighted. Displayed Peak List columns are user configurable. Use the horizontal and vertical scroll bars, if available, to move around the peak list display.

A user configurable document header can be displayed at the top of the Peak List window.

■ Configuring Displayed Peak List Columns

The Peak List document allows all the information from a peak list entry to be displayed. Because of display space restrictions it is possible to select which columns are to be displayed and in which order they are to appear.

Select **PeakList display format** from the **Quantify Display** menu or double-click with the left mouse button on one the column headings in the Peak List window.

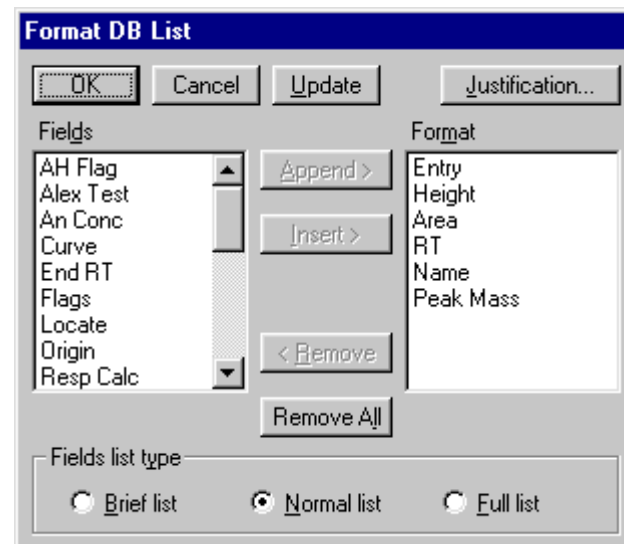


Figure 7.23 Peak List Display Editor

■ **To append new fields to the Peak List document**

1. Highlight the field you wish to append in the **Fields** list box.
2. Choose the **Append** button.
3. Repeat steps 1 and 2 as required.
4. Choose the **OK** button to save the changes and exit.

■ **To insert new fields in the Peak List document**

1. Highlight the field you wish to insert in the **Fields** list box.
2. Highlight the field before which you wish to insert the new field in the **Format** list box.
3. Choose the **Insert** button.
4. Repeat steps 1 to 3 as required.
5. Choose the **OK** button to save the changes and exit.

■ **To remove a field from the Peak List document**

1. Highlight the field you wish to remove in the **Format** list box.
2. Choose the **Remove** button. To remove all the fields in the Peak List document choose the **Remove All** button.
3. Repeat steps 1 to 2 as required.
4. Choose the **OK** button to save the changes and exit.

■ **To format the display of a field in the Peak List document**

1. Highlight the field whose display settings you wish to alter in either the **Fields** or **Format** list boxes.
2. Choose the **Justification** button.



Figure 7.24 Peak List Field Justification dialog

3. Change the **Field Name** control to show the heading you wish to display above this column.
4. Change the **Justification** setting to **Left**, **Right** or **Centre** as required.
5. Change the **Field Width**, **Significant Figures** and **Decimal Places** as required.
6. Choose the **OK** button to save the changes and exit.

■ **Changing Current Peak List File**

To view another Peak List select **Peak List** from the **File** menu, the **File Open** dialog will appear. Select a Peak List from the list box.

■ **Displaying Peak List Chromatograms**

To display the chromatogram and peak associated with a Peak List entry, double click with the left mouse button on the desired entry.

6. Manually Changing Quantify Results

Although MassLynx can perform a complete automated quantification analysis from setting up a Sample List and acquiring data to printing Quantify Reports, it is also possible to repeat individual Quantify processes and to manually edit results including:

- Manual editing of peak baselines.
- Editing calibration curves to exclude erroneous calibration points.
- Performing Quantify **Locate compounds**, **Calculate calibration curves** or **Quantify compounds** processes.

■ Manual Peak Integration

If the automated peak detection is not determining peak baselines satisfactorily it is possible to define the baselines manually. This can be achieved by modifying the peak information held in the Peak Lists or by creating them from scratch.

To display an integrated peak in Chromatogram double click with the left mouse button on the desired entry in the Summary window or the Peak List document entry. Calibration standard peaks can be selected by double clicking with the left mouse button on the desired calibration point in the Calibration Curve document.

The Chromatogram window will be displayed showing the relevant peak. Also the **Edit Quantify Peak** dialog is automatically loaded allowing the user to make manual adjustments to the baseline assignment.

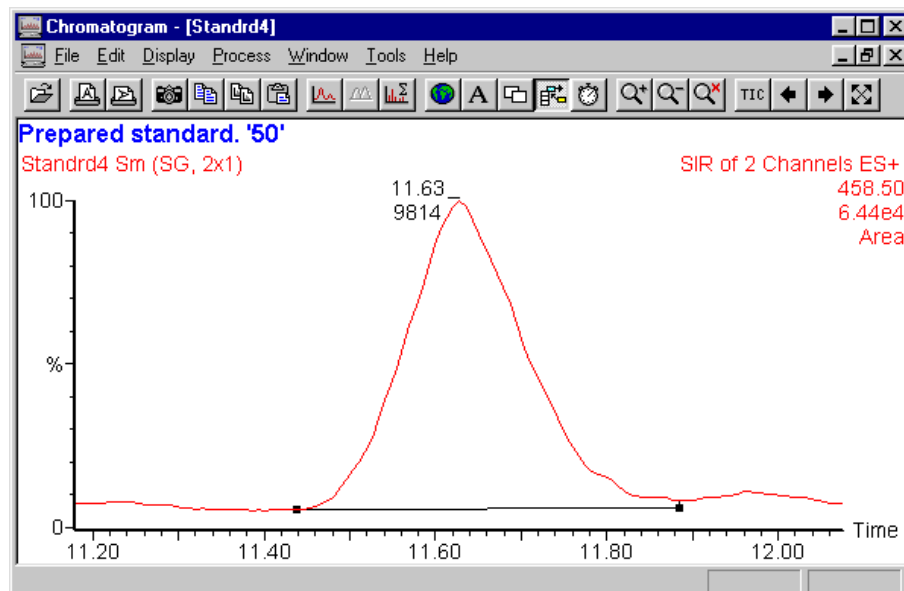


Figure 7.25 Chromatogram showing peak used for calibration point

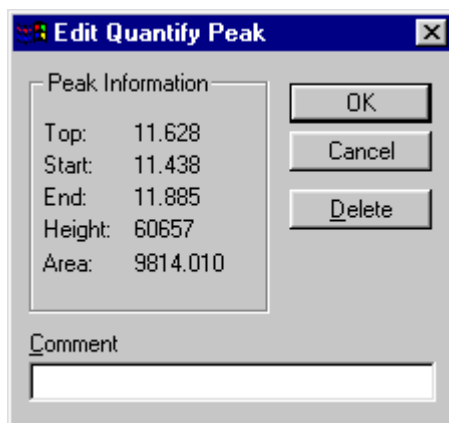


Figure 7.26 Edit Quantify peak dialog

The peak baseline can be modified by dragging the handles which appear at either side of the baseline with the left mouse button. The Peak Information will be updated. When you are satisfied with the manual integration choose the **OK** button to save the new peak integration information. A comment can also be stored in the peak list for this particular peak and this comment can be included in the printed report.

If no peak was detected, the chromatogram trace, which should have contained the peak, can be displayed by double clicking on the appropriate Summary window entry. A baseline can be added by pressing the right mouse button at one end of the chromatogram region of interest, and without releasing the button, dragging the mouse horizontally to the other end. As you drag the mouse you will see a "rubber band" stretched out to indicate the range you have selected and a baseline will be drawn.

To delete the current peak press the **Delete** button followed by the **OK** button.

The peak list and associated documents will be updated. If the peak is a calibration standard you will be asked if you want to recalculate the calibration curve. If a new curve is calculated all compounds will be re-quantified.

The Summary document can be formatted to include the **Detection Flags** for each peak. The Detection Flags give information about the start and end points of the peak and can have the following values :

- b** peak starts or ends on the baseline.
- d** peak starts or ends on a dropline.
- M** peak start or end point has been manually assigned.
- X** calibration point has been excluded from calibration curve.

The default Chromatogram display range can be controlled by selecting **Chromatogram** from the Quantify **Display** menu. For more information about setting the default Chromatogram display range see "Controlling the appearance of the Quantify Chromatogram display" on page 311.

■ To Exclude Erroneous Calibration Points

If once the calibration curves have been formed a calibration point is seen to be erroneous it can be removed from the calibration as follows.

1. Choose **Calibration Curve** from the Quantify **Edit** menu.

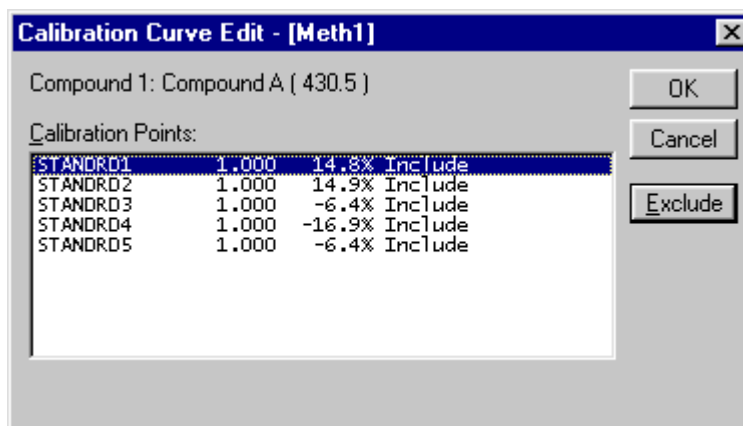


Figure 7.27 Calibration Curve Editor

2. The Calibration Curve Editor will be loaded displaying a list of the calibration points used to form the calibration curve. Each point is displayed with Peak List name, standard concentration, residual error % and a label to indicate whether the point has been included or excluded from the current calibration curve.
3. To exclude a point which currently being used to form the calibration curve, highlight the calibration point in the list and press the **Exclude** button. The label for the point will change from Include to Exclude.
4. To include a point which is not currently being used to form the calibration curve, highlight the calibration point in the list and press the **Include** button. The label for the point will change from Exclude to Include.
5. When you have finished making changes choose the **OK** button to save the changes. You will be asked if you wish to quantify compounds according to the new calibration curve. Choose **Yes** to quantify compounds or **No** to keep existing calculated concentrations.

The calibration curve will be re-plotted using only the included calibration points. Excluded points are denoted by a circle around the point. Excluded points are denoted in the Summary reports by adding an X to the Detection Flags column.

■ **To Exclude a complete sample from being used to form the calibration curve**

If once the calibration curves have been formed all calibration points from a particular standard sample are seen to be erroneous, the sample can be removed from the calibration as follows.

1. Determine which sample produced the erroneous calibration points.
2. In the Sample List Editor find the row containing the erroneous sample and set the **Type** field to **Blank**. Alternatively remove the row from the sample list.
3. Select the **Start** button and select the **Calibrate, Quantify** and **Print Report** options. There is no need to integrate again.
4. Select the **OK** button to commence the analysis.

■ **To perform any of the Quantify processes**

1. Choose **Calculate** from the Quantify **Process** menu.

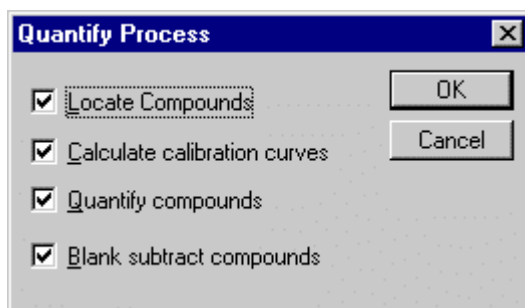


Figure 7.28 Quantify Process dialog

2. Choose which of the Quantify processes you wish to perform by checking the relevant check boxes.

Locate compounds Check this box to locate peaks for all compounds in the current method.

Calculate calibration curves Check this box to plot calibration curves for all standards.

Quantify compounds Check this box to calculate concentrations for analyte samples using the current calibration curves.

Blank subtract compounds When a sample defined as a blank is encountered, the value is saved and subtracted from subsequent samples until the next blank is encountered, this new value is saved and subtracted from the next set of samples.

3. Choose **OK** to exit.

7. Controlling Quantify Reports

Four printed reports of quantification results are available:

Quantify Compound Summary Report Displays quantification results for each of the Quantify compounds ordered by compound.

Quantify Sample Summary Report Displays quantification results for each of the Quantify compounds ordered by sample.

Quantify Calibration Report Gives calibration curve graph for each Quantify compound.

Quantify Sample Report Graphically displays all located chromatogram peaks and tables quantification results. Report is grouped by sample. Note: Chromatogram is invoked when producing the report.

■ To print Quantify Reports

1. Quantify Reports will be automatically printed at the end of a sample list analysis when the **Print Quantify Reports** field is selected when a sample list analysis is started.

-or-

Choose **Print Report** from the Quantify **File** menu.

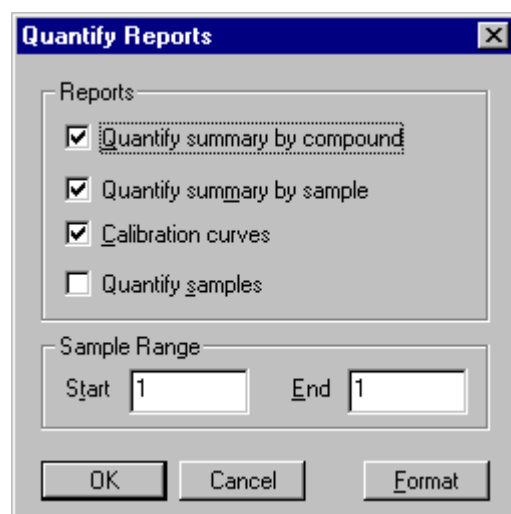


Figure 7.29 Quantify Reports dialog

2. Choose which reports you wish to print by selecting the relevant check boxes.
3. In the **Start** and **End** fields, enter the range of samples that you want the report to include.
4. Choose **OK** to save changes.

The **Print Report** dialog will be displayed.